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*J Neurophysiol* 8:55-71, 1945. ;

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ISSN: 0022-3077, ESSN: 1522-1598. Visit our website at  
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# FUNCTIONAL ORGANIZATION OF THE GIANT FIBER SYSTEM OF *LUMBRICUS*\*

THEODORE HOLMES BULLOCK †

Section of Neuro-Anatomy, Yale University, School of Medicine,  
New Haven, Connecticut

(Received for publication November 11, 1944)

## INTRODUCTION

MORE OR LESS elaborate systems of nerve fibers much larger than any others in the same animal have been evolved several times, apparently independently, in the animal kingdom. They exist among certain but not all groups of the cestodes, nemertean, annelids, crustaceans, cephalopods, enteropneusts, cephalochordates, and vertebrates. They exhibit a diversity of organization. Some are confined to the central nervous system, others are peripheral; some are simply very large ordinary neurons, others consist of compound fibers representing the fused axons of several cells. Complex combinations of these conditions exist and of course a variety of synaptic

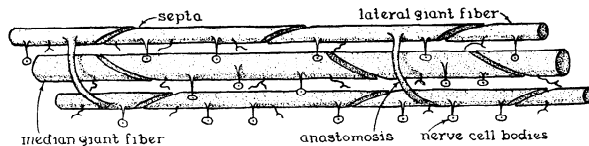


FIG. 1. Schematic representation of the giant fiber system in *Lumbricus*.

relations and functional patterns is represented. Probably, however, a common function is involved, that of mediating a rather specific stereotyped response which requires the rapid synchronous distribution of impulses to an extensive musculature. All of the giant systems which have been studied adequately are high speed, motor pathways (not necessarily final common paths) and those that are best known all mediate an escape response. The same or nearly the same movement may be used in normal locomotion (thus the tail flip of the crayfish, the jet-propelled backward dart of the squid, the quick twitch of the earthworm), but the extent to which the giant system is employed in life is not known. It is apparent from a survey of the known morphology and physiology that giant systems are particularly suitable for analysis of functional organization and it is the purpose of this paper to do so for one of the more primitive of these systems, that in the earthworm.

The giant fibers or neurocords of the earthworm have been known for

\* Aided by a grant from the Fluid Research Fund, Yale University School of Medicine.

† Now at the Department of Anatomy, University of Missouri School of Medicine, Columbia, Missouri.

nearly a hundred years and many studies of them have been made but the most significant neuroanatomical features were not described until the work of Stough (18). As now known the giant system of *Lumbricus* is made up somewhat as represented in the diagram (Fig. 1). There are three fibers, a median and two lateral ones. They run through the entire length of the ventral nerve cord, lying on its dorsal side (Fig. 2). In every segment sev-

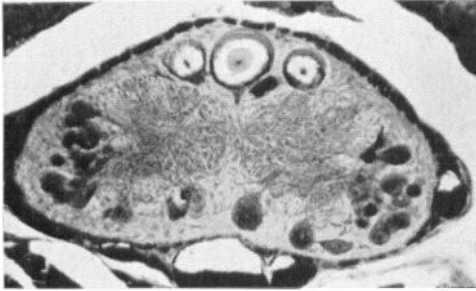


FIG. 2. Photomicrograph of nerve cord of *Lumbricus*, cross section, protargol stain  $\times 100$ . Note the three giant fibers dorsally with their thick connective tissue sheaths and central bundle of neurofibrils. Nerve cell bodies concentrated laterally and ventrally at the surface, the neuropile and fiber tracts in the center of the cord. One of the cells which contribute to the median giant fiber can be seen sending an axon towards that fiber. The lateral giant fiber on the left is sending one of its collateral processes into the neuropile (a fine black thread emerging from a cone-shaped outlet in the sheath).

eral cell bodies send axons into the neurocords and the neurocords or their cell bodies send small processes into the neuropile, presumably to make connection with sensory or motor neurons. The fibers are interrupted in every segment by oblique septa. These divide the neurocord into segmental units and provide a broad area of contact between adjacent units, representing essentially synapses of unusual proportions. The giant fibers are thus chains of short compound axons. The two lateral fibers are connected by frequent anastomoses.

The function of the giant fibers has been shown by Bovard (3), Yolton (13), Stough (19) and Ten Cate (21) to be related to the startle

reflex or twitch-like response of the entire worm to sudden mechanical stimuli—contact or vibration of the substratum. It was shown, furthermore, that the median fiber apparently conducted posteriorly and the lateral fibers anteriorly because Stough (19) was able to abolish the response of the posterior end to anterior stimulation without affecting the anterior response to posterior stimulation by cutting the median fiber without injuring the lateral fibers, and vice versa. It was concluded that the fibers are polarized in opposite senses.

The only reports of direct recording of the activity of the giant fibers appear to be the brief notes of Eccles, Granit and Young (8) and Rushton and Barlow (15). The first named authors showed the feasibility of such recording and gave evidence that the giant fibers are not polarized and that they conduct at very high rates—up to 25 meters per second at 12°C. Rushton and Barlow showed that the median fiber action potential could be detected from the surface of the intact animal.

#### MATERIAL AND METHODS

*Lumbricus terrestris* was used exclusively in this study. Specimens were kept in damp

moss at room temperature or in the refrigerator. It was found that, in general, better preparations were obtained from fresher specimens.

The animals were prepared in various ways. In some cases they were pinned to cork blocks but usually they were suspended on a glass rod which was carefully passed through the alimentary canal from mouth to anus. With practice this can be done without injury to

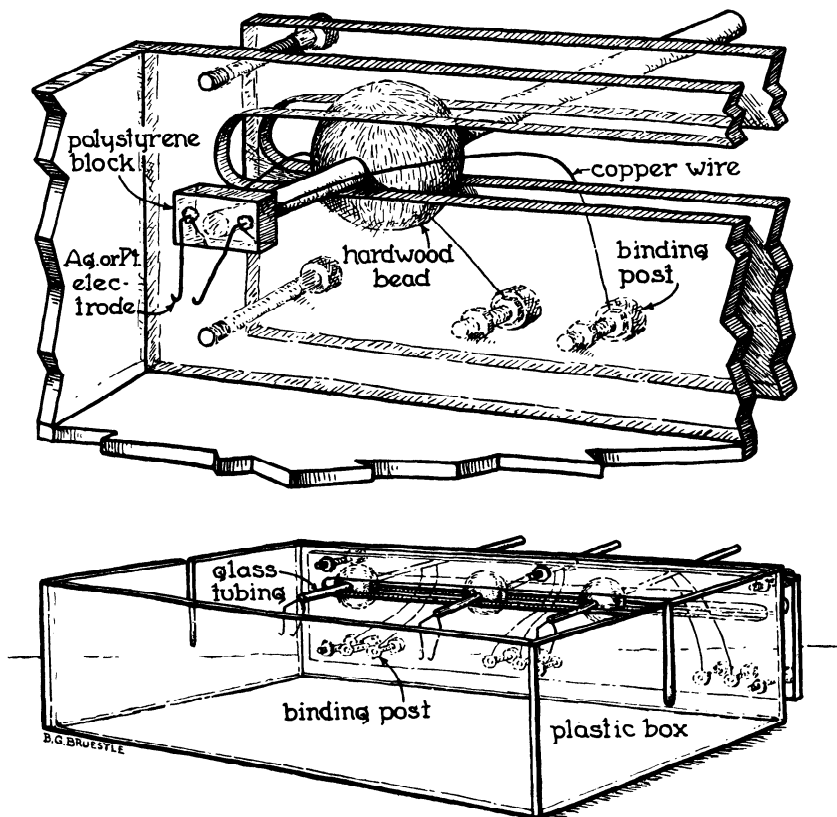


FIG. 3. Moist chamber and universal joint electrode holder. The specimen was suspended on a glass rod resting in the end-slots and pinned to a block of modeling clay which rose from the floor nearly to the level of the worm.

the specimen. The danger lies in the ease with which the rod may be forced through the wall of the gut. It is a convenient method of restraining the earthworm since the worm can do little but shorten and lengthen and a single pin through one point effectively fixes that point. The glass rod was suspended in a moist chamber whenever the experiment was to last more than a few minutes. Saline solutions were thus not necessary and none was employed except in the nicotine experiments when coelomic fluid was pooled from several specimens and used to dilute the drug. The moist chamber was constructed as shown in Fig. 3 with electrodes mounted in universal joints in the rear wall. The electrodes were Ag or Pt wires, in some cases chlorided, in some not. The electrodes were placed either on the external surface of the worm or on the exposed ventral nerve cord. For electrical stimulation of the giant fibers in the cord electrodes on the skin, when applied directly over the midventral line, were convenient and usually just as effective, especially in the posterior region. Threshold voltage under these conditions was however much higher than when stimulating the exposed cord. The action currents of the giant fibers similarly could be

picked up either from the exposed cord or from the surface of the intact worm, as pointed out by Rushton and Barlow (15). These spikes are of such amplitude that without extreme amplification they may be seen superimposed on large, slow muscle action potentials and artifactual potentials resulting from movements of the skin under the electrodes. If the sweep of the cathode ray is adjusted to a suitable rate and is not continuously recurrent but is triggered by the stimulus ("single sweep" operation) these large slow waves are minimized in effect and the giant spikes will appear regularly at the same place on the screen (see Fig. 4). Nevertheless, pickup was usually accomplished from the exposed cord because the amplification necessary was then much less and stimulus artifact in the record reduced. The high voltage of the spikes is a great advantage as it provides a physiological isolation of these few fibers, obviating the necessity of single fiber dissection. The central nervous system of the earthworm is in a state of continuous "spontaneous" activity, even in the absence of apparent stimulation and under anaesthetics. This activity is in the form of spikes, resembling single fiber action potentials. But it is easily eliminated from the records of giant fiber activity

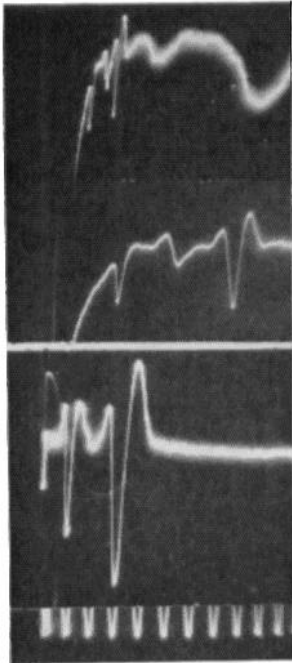


FIG. 4. General pattern of the giant response. Upper two records picked up from the skin of an intact animal. Lower, from exposed nerve cord. The stimulus, a brief condenser discharge, starts the sweep (left) and in the upper records, taken at higher amplification, causes a large stimulus artifact. The uppermost record is taken at a slow sweep speed and shows the slow action currents of muscle on which the two nerve spikes are superimposed. Successive sweeps would show the inconstant muscle pattern, the constant nerve pattern. Middle record, same with faster sweep. Both the median and lateral giant spikes are readily made out from skin recording. Bottom record shows characteristics described in text. Calibration signal = 1000 c.p.s., applies to lower record only.

by simply turning down the amplification, when the giant spikes will stand out of a relatively smooth baseline.

Exposure of the cord for stimulation and pickup was usually done only in the few segments actually needed; the whole cord was not exposed, nor removed from the body. Anaesthetics were only occasionally used. None was found which provided an adequately long state wherein the animal

was quiet and the responsiveness of the giant fibers was not affected. Curare and beta-erythroidin were tried but neither quieted the animal without seriously affecting the central nervous system.

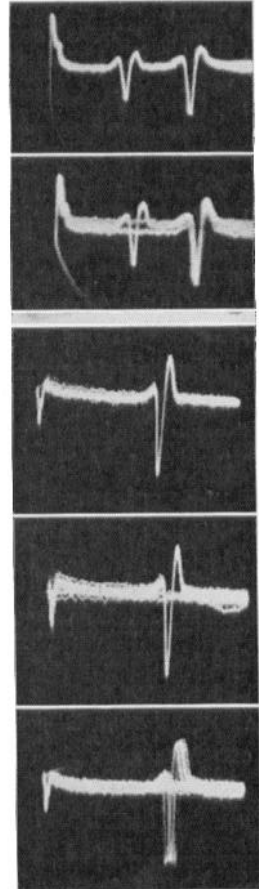
The amplifiers were Grass electroencephalographs, used in the high-pass filter position. Observation and records were made from a cathode ray oscillograph (modified Dumont 175A). A monitor speaker operated by a separate audio amplifier fed from the Grass output was proved useful. Stimulation was provided by a thyatron controlled, condenser discharge circuit, the pulses being of a time constant of 0.1 ms or less, but they were rounded off somewhat by the output transformer used, a GR #578A.

## RESULTS

*Electrical stimulation.* When condenser discharge shocks of appropriate strength are applied to any part of the earthworm there may be recorded from any other part of the animal the consistent and characteristic action potentials shown in Fig. 4. These are high potential spikes—from 20–100  $\mu\text{V}$

recorded from the skin of the intact worm, 100–1000  $\mu\text{V}$  recorded from the surface of the exposed nerve cord. Their duration, not counting slow components of the after potential, is about 0.5–1.5 ms. A just-threshold stimulus elicits one spike after a definite and consistent latent period (vanishingly small as the recording and stimulating electrodes approach each other). As stimulus strength is increased no change in the response occurs until at a new threshold another spike is added, with a different but also definite latency. Further increase in stimulus strength has no effect. The spikes have the characteristics of single fiber action currents: they are all-or-none, stimuli finely graded as to strength do not cause even small increments in amplitude, at increasing conduction distances the spikes do not become

FIG. 5. All-or-none and fatigue phenomena. Recording from the exposed cord and stimulating some distance away at 25 per second. One sweep of the cathode ray corresponds to each stimulus and its responses. The camera shutter was opened long enough to permit several successive sweeps to write over each other. Note the constancy of height and time of occurrence of the characteristic two spikes in the uppermost record. After a period of stimulation the first spike fatigues and drops out altogether (second record). The second spike (third record) reproduces itself perfectly hundreds of times but when fatigued drops out in all-or-none fashion (fourth record). Sometimes fatigue may also be shown by the slower conduction of the last few impulses (fifth record, note fraying out or lack of superposition of spikes). Sweep speed changed slightly for last three.



broader, with fatigue or high frequency stimulation the spikes drop out or persist in toto, they are smooth and simple in form and of appropriate duration. The absolute refractory period is rather long, from 2–4 ms at 24°C. and there is also a considerable period of relative refractoriness. The only occasion when the spikes are not of maximum amplitude is during this relative refractory period when they are still all-or-none but of considerably reduced height. Repetitive stimulation above threshold results in one spike for each shock (or two above the second threshold) at all frequencies up to about 200–250 per second. At moderate frequencies (20–50/sec.) firing of one spike for each shock may be maintained for many minutes. With fatigue the spike drops out, now and then—“missing,” at first occasionally then more often, always all-or-none but with a latency that increases up to 20 per cent just before complete cessation of the response (Fig. 5). After a few seconds’ rest

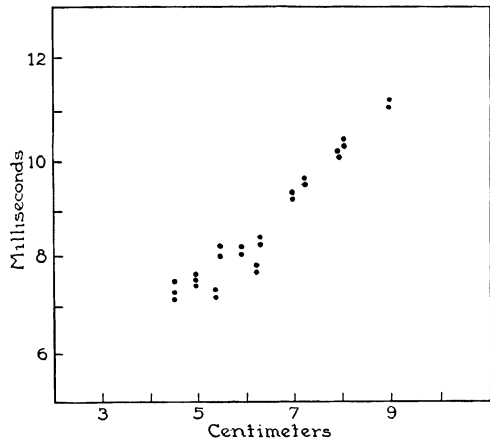
the original responsiveness and latency return and the stages of fatigue may be demonstrated again.

The pattern of two spikes is quite characteristic. Not more than two spikes of this order of size and latency can be elicited by any strength of stimulation. The only exception to this statement occurs in the case of stimulation through the skin. As will be shown below the action currents under consideration are responses to direct stimulation of nerve fibers. When stimulating through the skin spikes identical in form with one of the two already mentioned (always a certain one from any one region of stimulation, see below under mechanical stimulation) may occur (Fig. 9) with a long and inconstant latency and sometimes with a lower threshold than the direct responses. These are interpreted as indirect responses, through sensory neurons. They never occur when the exposed cord is stimulated. And they do not form exceptions to the statement that only two spike *forms* occur. Although the actual values of amplitude and latency of the giant spikes vary with the position of the electrodes, it is characteristic that one is smaller, shorter in duration, faster (shorter latency) and of lower threshold, as recorded from most levels of the worm. The more slowly conducted giant response is of larger amplitude, longer duration and higher threshold. These relations are consistent from specimen to specimen and when a preparation for some reason, presumably injury, fatigue or condition of the worm, exhibits only one form of giant spike it is easy to recognize which of the two has been lost and which is still present.

The giant responses are undoubtedly the action currents of nerve fibers. They may be obtained from the isolated nerve cord, they are lost with damage to the nerve cord, they are conducted at rates and are of a form, duration and threshold incompatible with muscle action currents. The considerations mentioned in the preceding paragraph indicate that they represent the activity of two different nerve fibers. Evidence may now be cited that they represent the activity of the well known giant nerve fibers or neuro-cords. The fact that these are the largest nerve action potentials obtainable from this animal suggests that they must come from the nerve fibers of the largest diameter. The high speed of conduction indicates the same thing. Anatomically there are known to be three giant fibers (Fig. 2) and Stough (18) has shown that the two lateral fibers are connected by frequent anastomoses, so that the expectation from anatomy would be two forms of giant action currents. The giant fibers are on the dorsal side of the nerve cord. It is easy to cauterize the ventral side of the cord with a hot needle without impairing the giant spikes and conversely to eliminate the spikes by cauterizing the dorsal side of the cord without abolishing ordinary transmission through the ventral side. Furthermore, it is possible to puncture the median giant fiber with the cord exposed from the dorsal side, by the use of a glass needle under the dissecting binoculars. Continuously testing the giant response it can be shown that the faster, smaller spike disappears on the side of the preparation opposite to the stimulating electrodes immediately following the

puncture. It is still present up to the region of the operation and the slower, larger spike is unimpaired across that region. I have not succeeded in puncturing both lateral fibers without damaging the median, but it seems altogether likely from the evidence available that the faster giant action potential results from activity of the median and the slower from activity of the two lateral giant fibers. It has not been clearly established what are the consistent size relations of the giant fibers but usually the median is the largest in diameter while the cross sectional area of the median fiber may be

FIG. 6. Increase of conduction time with increase in length of worm. Each point is a determination, from a photograph of the cathode ray screen. The specimen was stretched and two or three photographs taken, then allowed to shorten the stimulation and recording repeated, the whole cycle of operations being repeated six times. Distances are the straight line measurements between nearest electrodes. Note that conduction time does not increase as fast as length so that conduction rate increases also (ca. 20 per cent for a 78 per cent increase in length). This experiment was the one with the least increase in rate.



more or less than that of the two laterals combined. There may be an explanation in these relations, as suggested by Eccles, Granit and Young (8), of the unusual situation wherein the faster conducting fiber has a smaller action current than the slower fiber.

If conduction rate is calculated from the total elapsed time between stimulus and recorded spike and the externally measured distance between the inside members of the two pairs of electrodes, figures for the faster fiber of 15–45 meters per second are obtained at 21–24°C. The slower fiber conducts at a half to a third of this rate. The actual figure depends on the state of contraction or extension of the specimen. Confirming Jenkins and Carlson (12) and Carlson (7) it was found that the elapsed time for conduction between any two points increases as the animal is stretched. This is in accord with expectation from the fact that the diameter of the fiber must be decreasing as the length of the animal increases, since there is no great amount of coiling or sinuosity of the nerve cord or giant fibers at moderate extension. (There is however marked coiling or zigzagging of the neurofibrils which disappears as the specimen is stretched, so that the present data constitute evidence that conduction is not mediated directly by the neurofibrils.) However, contrary to Jenkins and Carlson, the elapsed time does not increase fast enough to keep the *rate* (meters per second) constant. The rate of conduction increases as the animal is stretched. The limits of these relations



have not been explored but in the range tested (see Fig. 6) the changes were completely reversible and could be repeated on the same specimen many times, so that it is likely that the relations are normal ones and not inflicted by injury. The maximum rate given above is from specimens that were far from maximally stretched, so that still higher rates probably obtain under special conditions.

The conduction rates recorded in this study agree with those noted by Eccles, Granit and Young (8) but are considerably higher than those given

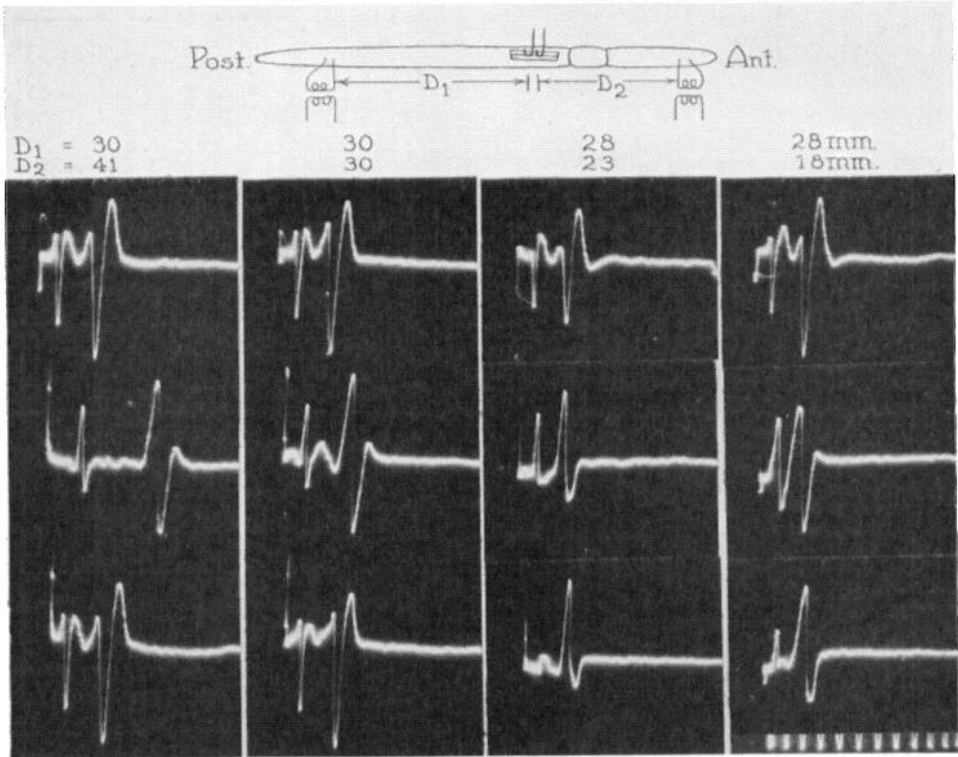


FIG. 7. Block of anterior conduction by posterior conduction in the same fibers, and vice versa. Upper row, stimulating posterior end only; middle row, stimulating anterior end only; lower row, stimulating both simultaneously. Calibration = 1000 c.p.s. When both ends are stimulated simultaneously the record does not show the sum of the responses to each stimulus alone, except when the impulses arrive at very nearly the same time (last two, bottom row, first spike). The results of stimulating both ends may be controlled at will by timing the arrival of the respective impulses at the recording electrodes, in this case by moving the stimulating electrodes.

by earlier authors (10, 2, 12). These workers used methods which included two unknown and possibly unequal synaptic and muscle delays. Bovard's figures, obtained at an unspecified temperature, have a mode of 1.5 m. per sec. and a maximum of 4.1. Jenkins and Carlson, using a number of annelids which might be expected to equal or surpass the earthworm in this respect recorded few rates higher than 5 m. per sec. and none above 10. Von Holst,

at summer room temperature, obtained a few figures as high as 12 m per sec., but mostly much less (5 m. per sec.). Many of his figures agree with ours for the slower, lateral giant fibers (6–15 m. per sec.) but von Holst's results show an apparent absence of any effect of stretching on conduction time. No obvious explanation of these discrepancies suggests itself.

As mentioned above, the pattern of two spikes is the same regardless of the position of the stimulating and recording electrodes. Thus between two given points conduction in either direction results in the same pattern, in fact in exactly the same latencies of the two familiar spike forms. This fact itself suggests that it is the same fibers that are conducting both ways. The following experiment adds weight to this probability. Recording electrodes are placed near the middle of the animal, stimulating electrodes are placed at both ends and arranged so that either end can be stimulated alone or both ends simultaneously. When the two ends are stimulated separately the just described similarity between anteroposteriorly conducted spikes and posteroanteriorly conducted spikes is confirmed (see Fig. 7). The exact latencies are purposely made different by making the conduction distances unequal. Now if spikes are started from both ends simultaneously it may be expected that (i) if there are separate fibers for conduction in the two directions and each fiber is polarized four distinct spikes would be recorded as though the records from anterior stimulation alone and from posterior stimulation alone were superimposed, but (ii) if the same two fibers conduct in both directions then the first arriving spikes would be recorded and the later ones, finding this region of the fiber refractory would be completely blocked. The latter alternative has been consistently realized in our experiments. Figure 8 shows what happens when, as in the former alternative, there are four fibers; this record was obtained from a specimen in which the true giants had ceased to function and the spikes shown are much smaller and slower than the true giants. They may represent activity in the ventral giant fibers, which are much smaller in diameter than the three dorsal fibers but are usually prominent. (Their number is not constant, but there may be four. Spikes of this character were not often seen, and cannot certainly be identified with any fibers, but they are clearly not related to the dorsal giant fibers.)

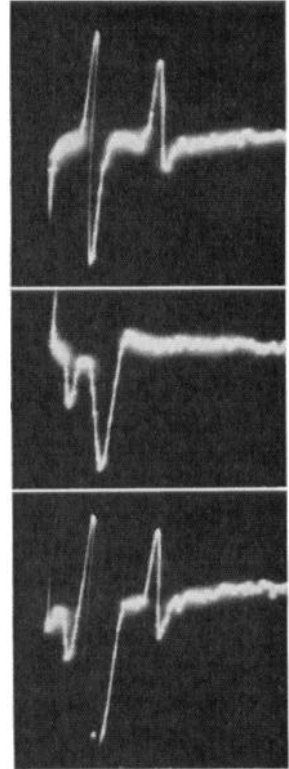


FIG. 8. Same experiment as Fig. 7 showing alternative result. Conduction in one direction does not block that in the other; there must be four different fibers. These are not the true giant spikes; they may represent activity of the ventral giant fibers.

Apparently, then the giant fibers comprise two high speed conducting systems which are unpolarized throughout their length.

*Mechanical stimulation.* The giant fibers respond readily to mechanical stimuli applied to the skin. We have used various kinds of contact, simulating as closely as possible normal stimuli such as the earthworm encounters in nature. The most convenient for electrical recording because of the minimization of artifacts is effected by a clean, dry glass rod. Vibration, as by tapping the table top, is also effective in many preparations. Responsiveness varies widely between preparations but certain features of the response are entirely consistent. Thus the tail end is more sensitive and responds by a greater burst of giant activity than the head end or middle of the worm. A maximum response is of the order of 20 spikes at a frequency declining from about 200 to 50 per second. More usual responses consist of six or eight spikes at frequencies from 120–50 per sec. Adaptation occurs and commonly the normal response consists of a single spike, especially to anterior stimulation. Characteristically and consistently only one spike form results from any one stimulus and the spike form is always the same from any one site of stimulation. As shown in Fig. 9 which relates the spike resulting from mechanical stimulation to those elicited by direct electrical stimulation, the smaller, faster spike, *i.e.* the median giant fiber is activated by mechanical stimuli to the anterior end, the slower, larger spike, *i.e.* the lateral fibers by stimuli to the skin in the posterior end. This relation is apparently definite and mutually exclusive—only the median fiber is excited by anterior stimulation and this fiber can be fired only from the anterior end—by normal mechanical stimulation of the skin. But it is fired by stimuli applied anywhere from the head back to a definite level a few segments behind the clitellum. The lateral fibers are fired by stimuli anywhere from this level back, only these fibers are fired by such stimuli and only in this region can these fibers be excited. (Vibration of the whole animal stimulates the lateral fibers.) There is apparently no significant overlapping. Within each zone the only differences from level to level appear to be in threshold and intensity of response obtainable with maximal stimulation.

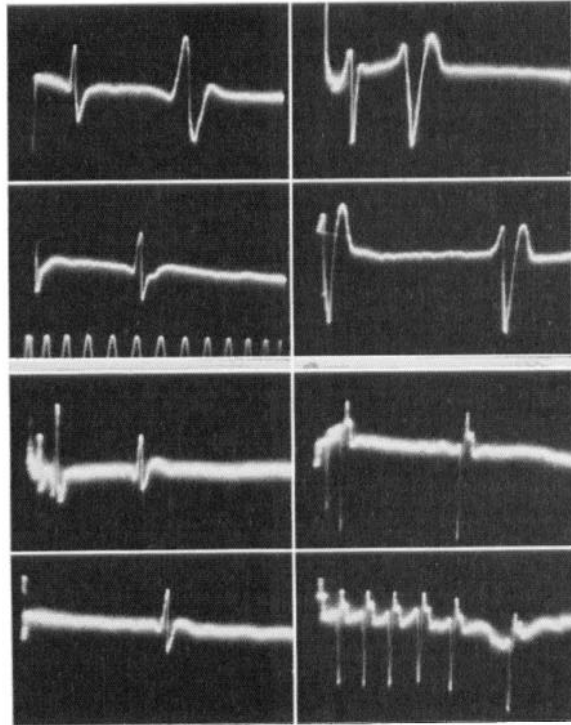
Finally it is significant that whichever spike form has been elicited it may be recorded from any part of the worm, *i.e.*, is conducted in both directions from the site of stimulation and reaches all levels of the cord. This means that not only under experimental conditions but under normal conditions the giant fibers conduct both ways, for, if a stimulus is applied to the tail end for example, the lateral fibers conduct a spike anteriorly while in response to a stimulus in the middle of the worm, just behind the boundary of the median fiber zone, the same fibers will fire and conduct not only anteriorly from this point but posteriorly over the same stretch of fiber as previously conducted forwards. The same is true of the median fiber, *i.e.*, it is not only unpolarized experimentally but in normal life conducts both ways at least over that part of the fiber within its zone of connection with sensory cells.

The latency of the first spike to mechanical stimulation has not been

carefully determined but appears to be variable and of the order of 3–15 ms., *i.e.*, there is time for one or several synapses and probably temporal summation is involved at least sometimes.

*Effect of nicotine.* In three experiments nicotine was applied to the exposed nerve cord with the idea of finding out whether the supposed synapses in the giant fibers possess the property characteristic of vertebrate synapses of specific susceptibility to this drug. Fresh solutions of nicotine in water, diluted

FIG. 9. Electrical versus mechanical stimulation. Two experiments. Upper row in each case, electrical stimulation; lower row, mechanical stimulation. Left column, stimulus applied near anterior end; right column, stimulus applied near posterior end. Time signal, 1000 c.p.s., applied to first experiment only. In both note that electrical stimuli elicit responses from both median and lateral giants, whereas mechanical stimuli only excite the median (small, fast spike) from anterior stimulation or the laterals (large, slow spike) from posterior stimulation. Not shown is the fact that whichever spike is elicited, the same one is recorded at all levels of the cord. The lower experiment also shows (i) the indirect response, of one of the giants only, to electrical stimuli delivered to the skin (third spike). It is the same fiber as that excited by mechanical stimuli to the same region and is presumably mediated through sensory neurons. (ii) A normal burst of impulses to a natural stimulus of moderate intensity is shown in the lower right; the lateral giants typically fire more easily and vigorously to mechanical stimuli than the median fiber (cf. lower left).



1:10 with earthworm coelomic fluid, to concentrations of 1:100,000 and 1:10,000 were dropped on the cord and transmission of giant spikes across the area observed. No effect was noted even with the stronger solution acting for periods of thirty minutes. Spikes were conducted in a normal fashion throughout this period. The septal junctions of the giant fibers in *Lumbricus* do not appear to be nicotine sensitive.

#### DISCUSSION

The experiments described provide an example of the analysis of functional and anatomical organization of the nervous system by tracing action

currents as signs of the course of nerve fibers. Independently of histological evidence it can be shown by this means that two giant fibers, with defined functional properties, exist and conduct through defined regions of the central nervous system. Furthermore, their connections with sensory and (not yet analyzed) with motor neurons can be described. We may now explain the apparent polarization of the giant fibers found by Stough (19). The fibers are not polarized but the median giant is only excited by anterior stimulation and thus mediates the reflex response of the tail to stimulation of the head while the laterals are excited by posterior stimulation and thus mediate the response of the head to tail stimulation. But with the methods used at that time it was not noted that the median fiber mediates the posteroanterior reflex response of the head to stimulation in the region of the clitellum and that the lateral giants mediate the anteroposterior reflex response of the tail to stimulation in the mid-region of the body, about segment 50.

Stough (19) and others recognized that the whole worm reacts to stimulation of the middle region and believed that this meant impulses are conducted posteriorly in the median fiber and anteriorly in the lateral fibers from the point of stimulation. This reasonable expectation leads to a functional conception and predicates anatomical connections (11) with sensory and motor cells quite different from those arrived at in this paper. But the evidence from direct tracing of action potentials clearly eliminates this possibility.

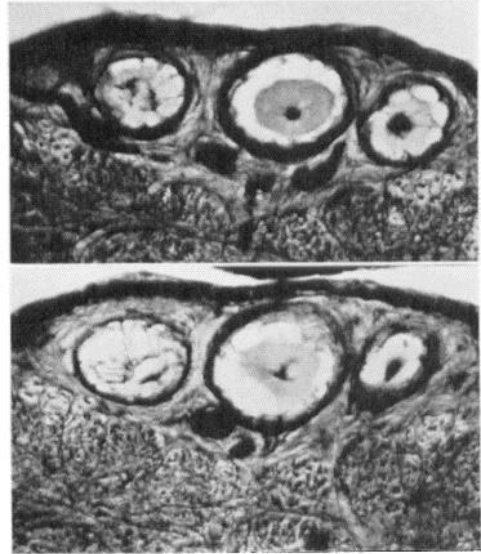
From the physiological evidence we can anticipate that critical histological analysis will reveal differences in the connections of the giant fibers in front of and behind a line approximately through segment 40. Anterior to this point the median fiber will probably have connections with sensory neurons as well as motor, the lateral giants only with motor cells—probably different motor cells; posterior to the boundary the laterals should have connections with sensory and with motor neurons, the median giant with other motor cells and not with sensory cells. The physiological data confirm the reality of the anastomoses between the lateral giants described by Stough (18) and do not confirm the general occurrence of anastomoses between the lateral and median fibers, described by others (18). They suggest that there is some significant and general relation between the diameters and the area of cross section of the fibers that distinguishes them from most other nerve fibers and accounts for the unusual relation of spike amplitude and speed of conduction. The high speed of conduction in a fiber smaller than, for example, many giant fibers in the squid suggests the importance of the thick sheath.

The present evidence confirms previous ideas of the general function of the giant system. It does not apparently fire "spontaneously," but must be set off by a certain pattern of activity of sensory neurons. It is admirably adapted to mediate a rapid, unsustained, synchronous, nondiscriminatory response of a large musculature to stimuli of a particular sort—suddenly applied, considerable or even slight mechanical disturbance of the body wall

or substratum, thus local or general. The response is quickly adapting and quickly recovering, *i.e.*, sustained stimulation causes a brief response of this system but another stimulus may be effective a short time later. These characteristics fit particularly an avoidance reflex to "startle stimuli," a function shared by many other widely different and independently evolved giant systems in other animal groups.

The question arises: if the giant fibers in the earthworm are unpolarized and each fiber always conducts to all parts of the worm, what difference in

FIG. 10. Photomicrograph of nerve cord, cross section, protargol stain,  $\times 300$ . The sections pass in each case through one of the oblique septa of the lateral giant fiber on the left. The septum is unstained but the neurofibrils fray out near it and do not cross it. In the upper picture the two sets of fibrils are shown, like chromosomes in metaphase; in the lower picture one set of fibrils has frayed out but the other, ventromedial to the septum, is still a compact bundle.



function may there be between the lateral and the median fibers? It would seem probable that they bring about different muscular responses, one appropriate to anterior stimulation, the other appropriate to posterior stimulation, *i.e.*, that they are anatomically connected with different efferent neurons. This may be demonstrable by a continuation of the present technic as well as by histological means. It may be as suggested by von Holst's work (10) that the same giant brings about different responses at different times, depending on the state of contraction existing in the longitudinal and circular muscles.

The presence of histologically demonstrated septa across the giant fibers is of especial interest in view of the physiologically demonstrated properties of these fibers. The septa are conspicuous, low power, von Rath-staining structures, well documented by photomicrographs (18). They have been seen in the fresh state under polarized light and it has been shown that ". . . The optical properties of the transverse segmental partitions of the giant fibers are similar to those of the myelinated axon sheath, indicating similarity in their composition and ultra structure . . ." *i.e.*, they contain oriented

lipid and protein molecules (20). Long before the septa were known Boule (1) showed apparent discontinuities in the neurofibrils by silver impregnation. The same thing was seen by Smallwood and Holmes (17). I have examined Bodian preparations which show the neurofibrils beautifully, noted these periodic interruptions in the neurofibrils and searched carefully, but in vain, for clear cut fibrillar continuity across these regions. I am unwilling to deny categorically the occurrence of such exchange but in general it is conspicuous by the difficulty of demonstrating it (see Fig. 10). The septa then seem real enough and, although on a vastly different scale of size, these junctions have histologically the essential character of synapses.

If these junctions are as real as they seem and are to be regarded as a peculiar form of synapse, then their properties become of primary interest as indications of what is fundamental and universal and what on the other hand is derived and secondary in the evolution of the synapse. This is the more true because of recent speculation that many of the apparently fundamental physiological properties of synapses, properties commonly used in the definition of this entity, are really not inherent in the nature of the junction but are the result of the anatomical arrangement usual in vertebrates (14). According to this idea polarization is the result of the asymmetrical, many-to-one relation of axonic telodendria and the post-synaptic cell, and synaptic delay is chiefly the conduction time along the very fine telodendria. Lloyd (13) has come to the conclusion that “. . . once a full accounting is made for all the factors contributing to the so-called minimum synaptic delay it seems possible that synaptic time in the strictest sense will prove vanishingly small.” It might be anticipated on the basis of these ideas that a synapse which exhibits a one-to-one symmetrical anatomical relation and no great reduction in diameter of the terminal fiber, would be unpolarized and without apparent synaptic delay. The earthworm giant fibers are perhaps just such a case. The physiological evidence is quite definite that these fibers, which appear to be chains of compound axons separated by macrosynapses, are unpolarized, not only experimentally but in normal functioning. Furthermore, there must be no significant synaptic delay since there is about five milliseconds total latency to provide for conduction over some ten or twenty centimeters of minimum path and 50–100 of the supposed macrosynapses (one in each segment in each fiber).

To pursue this idea, if it is true that synapses under appropriate anatomical arrangements may be unpolarized and without delay, then few properties are left by which the synapse, as a general entity in comparative neurology, may be defined. Essentially it is simply an anatomical discontinuity in a normal nervous pathway, a potential barrier to conduction. Thus conceived it may have no necessary or universal special physiological properties but its importance is still great as a site for the development of special properties in particular cases: facilitation, summation, polarization, integration and selective transmission of impulses from different sources, for example. Many of

these properties probably depend on particular anatomical arrangements not on unique inherent physiological properties of the membranes in each case.

The notion that the primitive synapse is unpolarized is in accord with the probabilities created by modern studies of the nerve net. Here, as in the earthworm, the positive physiological evidence is that conduction takes place in both directions and the inconclusive histological evidence is in favor of the existence of real neuronal discontinuities (see 9, 4, 22, 5, 6).

#### SUMMARY

1. The giant fiber system of the earthworm is a favorable object for the study of functional organization by means of action potential recording since its potential spikes are large and simple, few in number, detectable from the surface of the intact animal or from the exposed, intact nerve cord, readily and dependably excitable by electrical stimulation of the surface of the intact animal and by normal mechanical means.

2. A consistent and characteristic pattern of two spikes is elicited by direct electrical stimulation of the cord. The two spikes are of different threshold, conduction rate, height and form, the faster one typically having the lower threshold and the lower amplitude. Each occurs in an all-or-none fashion and stimulus strength has no effect above threshold. They behave as single fiber action currents, and are shown to represent the activity of the giant fibers, the smaller, faster spike belonging to the larger, median fiber.

3. The overall, effective speed of conduction (not counting any utilization time or synaptic delays at the segmental septa) is very high—up to 45 meters or more per second (24°C.)—probably the fastest conducting fibers yet recorded among invertebrates, although they are far from the largest. The time for conduction from head to tail increases as the worm is stretched, but not proportionately, so that the rate also increases. This is a normal, reversible phenomenon.

4. The pattern of two spikes is the same, when elicited by direct electrical stimulation of the fibers, regardless of the site of stimulation, site of recording electrodes or direction of conduction. Evidence is presented that this is because the giant fibers are unpolarized.

5. The pattern of response of the giant fibers to normal mechanical stimulation, as it probably occurs in nature, is described. They are apparently on the efferent side of the reflex arc but are not probably the final common path. A normal response may consist of a single spike or a dozen or two at a frequency of 50–200 per second. Only a single spike form, *i.e.*, a single fiber is activated by any one stimulus. The median fiber fires as a result of stimulation anterior to about the fortieth segment, the laterals fire to stimuli posterior to this point. But in either case the spikes are conducted in both directions, to all parts of the worm. The apparent polarization of the system



noted by earlier workers is due to regional localization of sensory connections capable of firing the giant fibers.

6. Nicotine in concentrations as high as 1:10,000 did not abolish conduction over the giant fibers within 30 minutes.

7. From the physiological evidence certain predictions of histological findings are made. The typical anastomoses described by Stough are confirmed and those of Haller and others are denied on this basis.

8. The high speed of conduction and its unpolarized character are significant in view of the apparently synaptic nature of the system as demonstrated histologically. These properties are compatible with and perhaps constitute a demonstration of recent theories which suppose that the synapse is not inherently polarized nor delaying but is only so as a result of the particular anatomical relations prevalent in vertebrates and that these properties should not be a part of the definition of the synapse. This interpretation is in harmony also with recent opinions to the effect that the nerve net of lower invertebrates exhibits unpolarized synapses.

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