Further experiments on insect competition

Pearl, R., Park, T. & Miner, J. R. 1941 Amer. Nat. 75, 5.
Stanley, J. 1932a Canad. J. Res. 6, 632.
Stanley, J. 1934 Canad. J. Res. 11, 728.
Stanley, J. 1941 Ecology, 22, 23.

Reflex conduction in the giant fibres of the earthworm

BY W. A. H. RUSHTON, Physiological Laboratory, Cambridge

(Communicated by C. F. A. Pantin, F.R.S.—Received 20 October 1944)

The present work confirms the conclusion of Friedländer and others that the giant fibres mediate the end-to-end shortening reaction in the earthworm. The chief concern has been to investigate Stough’s claim that the median giant fibre conducts impulses only in the direction from head to tail and the lateral giants only in the reverse direction. Two methods have been employed.

(a) The nerve cord was exposed at each end of the worm, and electrical records taken simultaneously from the two extremities when the surface of the worm was touched at different places. The results were usually a train of impulses in one or other giant fibre, and it was found that whenever an impulse appeared at one end of a given fibre, it always appeared at the other end of the same fibre. Each fibre, therefore, when it conducted at all, always conducted in both directions. Sensory nerves from the head appeared only connected to the median giant, since stimulation anterior to the citellum never resulted in lateral fibre activity. Similarly, the tail appeared only to join with the lateral giant fibres.

(b) Stough’s own method was used, and his observations confirmed, extended and re-interpreted. Either the median or both lateral fibres were divided in one segment. The success of this operation could be judged by leading off the giant fibre responses from the undissected worm (figure 5). Next day, when the worm had recovered, the shortening reflex was observed when the worm was touched at the head, the tail, or in the middle. The shortening was either throughout, or was arrested at the operation site, depending upon whether the active giant fibre was the intact or the damaged one. The results are summarized on p. 119. From both the head and the tail Stough’s observations are confirmed, and it is agreed that impulses from the head are conducted back by the median giant alone. The absence of impulses in the laterals might be due to contrary one-way conduction as Stough assumes, or to the absence of their sensory connexion with the head. But (a) above shows that the latter is correct, and the same must be concluded from touching the middle region of the worm, which apparently Stough did not do, for this part connects with the lateral giants, and thus affords a demonstration that these fibres may also conduct antero-posteriorly. The difference in function of the separate giant fibres, therefore, is probably related to their difference in sensory distribution.

From the time of Friedländer (1894), it has been generally accepted that the function of the giant fibres of the earthworm is to mediate the end-to-end reaction which is often seen when a worm is touched. Since, apparently, only one kind of shortening has been described, it is not obvious why there are three parallel giant
fibres instead of simply one, and the object of this paper is to see whether any light can be thrown on this matter by electrical records taken from the giant fibres during reflex activity. In particular, we shall examine the claims of Stough (1926, 1930) that the median giant can only transmit impulses towards the tail and the lateral giants only towards the head, as this appears to be the only hypothesis which differentiates between the functions of the three fibres. First, however, it was necessary to establish the reliability and significance of the electrical record obtained from the whole nerve cord (Rushton 1945).

If the isolated nerve cord be excited electrically, two potential waves of about 5 mV are recorded. These show an all-or-nothing relation to the stimulus and are conducted at about 10 and 20 m./sec. respectively. Various tests show that they are due to two independent excitable structures, the more slowly conducted wave being associated with a longer chronaxie. Micromanipulation allows the faster wave only to be abolished by damage applied to the median giant, and the slower wave only by damage to both laterals in the same segment. Further proof that the giant fibres conduct these waves is afforded by transection of the whole nerve cord except for the giant fibres, for this still allows both the waves to pass. Section of the lateral giants alternately at intervals of ten segments apart still allows the slower wave to be propagated at full size, and this and all other observations are independent of the direction of propagation.

These results, which confirm and amplify those of Eccles, Granit & Young (1933), strongly point to the conclusion that the fast wave is the action potential of the larger median fibre and the slow wave that of the two lateral fibres which must interconnect through a conducting bridge at least once every ten segments. Moreover, all these three fibres may conduct as effectively in one direction as the other.

This is perhaps surprising in relation to the fact established histologically by Stough (1926) and confirmed by Smallwood & Holmes (1927) that the giant fibres are not single neurones. In every segment each giant fibre is in continuity with a cell body, and there is a transverse membrane interrupting its continuity with the fibre in the next segment. This harmonizes with the experiments of Bovard (1918) who cut the cord and found no degeneration of the giant fibres.

When two nerve fibres make functional union the junction is usually called a synapse, and this is the name given by Stough to his transverse membranes. It should be noted then that these synapses are unusual in that they conduct invariably and in both directions, and they have so little synaptic delay that 1 msec. may suffice to pass some forty of them in succession and to traverse 2 cm. of fibre as well.

Though the work just quoted substantiates many of the claims of Stough, it runs quite counter to his belief that the fibres can only conduct in one direction, a conclusion which was hardly deducible from his staining reaction, itself denied by Smallwood & Holmes. In quite a different category, however, is Stough's claim (1930), derived from experiment, that in reflex activity impulses always run anteroposteriorly in the median giant, and in the opposite direction in the laterals. This result would appear to be analogous to the familiar condition in vertebrate reflexes,
Reflex conduction in giant fibres

where both sensory and motor nerves conduct in a single direction though physically capable of conducting in both. It was therefore scarcely admissible to use this observation to support the claim that the giants can only conduct in one direction.

But on closer consideration there is an important difference between the vertebrate reflex and what is claimed of the giant fibre. For in the vertebrate arc in general the neurones are joined end to end, but in the worm the giant fibre response is obtainable by touching the body anywhere, and thus the sensory segmental nerves probably join a giant at various places along its course. If now, say, in the middle of the worm an impulse from a sensory nerve can pass to a lateral giant, and if, as is claimed, the junction is of a kind which will allow the impulse to turn towards the head and not towards the tail, then this junction will prove a very interesting phenomenon.

The first object then will be to study the effect upon the giant fibres of the reflex arising from touching various points upon the worm’s surface, and in particular to note which giant fibre is stimulated and in which direction or directions the impulse is conducted.

The direction of reflex conduction in the giant fibres

The aim of this experiment is to analyse the reflex activity of the giant fibres, by obtaining simultaneous records from both extremities of the ventral nerve cord. In general, the wave in the median giant will differ in appearance from that in the lateral and may be identified. It is easy then in every record to tell which fibre conducts the impulse from the stimulus site to the head, and which to the tail. There are a good many technical difficulties in getting satisfactory results, but these may usually be overcome as follows.

Dissection. The worm is anaesthetized by dropping into 10 % methylated spirit in water for 5–10 min. If the anaesthesia is too light, dissection is difficult and autotomy very liable to occur. If too deep, the giant fibre reflexes are hard to elicit in the subsequent experiment. After washing off the excess spirit from the skin, some 15 mm. of the posterior end of the cord is isolated, ligated and cut posteriorly, and then tucked back into the body cavity for protection whilst the anterior end is prepared. A mid-ventral incision is made just through the body wall from the 14th segment to the first, and the worm is pinned down on its back with nearly all its body floating on mercury, where it cannot get a grip to aid autotomy. The cord is ligated and cut just below the suboesophageal ganglion, and then very carefully isolated up to the point where it enters the mass of the seminal vesicles. It is important not to cut through one of the hearts which are apt to protrude suddenly through the incision. In favourable cases this dissection took 15 min.; the worm was then transferred to the recording turn-table.

Recording turn-table. It is clearly necessary to fix each end of the worm to a pair of recording electrodes, but apart from this it is desirable to allow the animal as
much freedom as possible. If it is pinned down and then stimulated to give reflex responses, it usually autotomizes, often in many places. A turn-table round which the worm could crawl (on the tread-mill principle) proved satisfactory.

![Diagram](http://rspb.royalsocietypublishing.org/)

**Figure 1.** Recording turn-table. Description in text.

The idea is indicated in figure 1. The turn-table is light and mounted on conical bearings which will turn very easily. The worm lies in the circular slot and occupies about half the circumference. Its posterior end rises from the slot to be attached, dorsal aspect uppermost, to a fixed platform A. The anterior end is attached to the cork B mounted on a radial arm freely movable about the axis independent of the turn-table. Suppose for a moment that there was no posterior fixation and that the tail just lay in the slot. Then the worm can obviously crawl round and round the turn-table pushing the arm B before it, and being thereby guided to keep in the slot. The only difference which results from the actual arrangement is that, owing to posterior fixation, the worm drags the turn-table round backwards instead of itself going round the turn-table forwards. This will hardly affect the freedom of the worm, but greatly simplifies the problem of electrical recording.

This is made by a fixed pair of electrodes mounted on A, and a second pair mounted on B making contact with the fixed circuit through concentric mercury cylinders coaxial with the turn-table. In this way good contact is made with each end of the nerve cord despite fairly free movement of the worm. In some experiments the head was fixed to B and the tail to A.

**Electrical recording.** Each pair of electrodes could be connected to one of a pair of differential amplifiers (Matthews-Tönnies type), each deflecting one spot of a double-beam cathode-ray tube. Thus when both pairs of electrodes were so connected, simultaneous records could be taken from head and tail. Each pair of
electrodes could alternatively be connected to a stimulating circuit giving a brief condenser shock synchronized with the time-base.

A recording speed of 30 cm./sec. was used to enable the wave form to be distinguished. This was done economically by photographing single sweeps of the time-base operating synchronously on the two beams. Since, however, the time of traverse is only $\frac{1}{10}$ sec., it is clearly necessary to adjust the sweep nicely to synchronize with the reflex response. This was achieved by triggering the sweep from a thyatron whose grid was normally biased so as just to withhold the discharge. Thus when it was earthed even through 5 megohms, the thyatron discharged and a linear traverse flashed across the screen. Now the worm lies in an earthed metal slot, and its reflexes are to be elicited by touching the body surface with some object. Our problem is therefore solved by using as object the free end of this 5-megohm resistance, for it elicits the reflex by contact, and simultaneously earths the grid and triggers the sweep.

Despite the 5-megohm resistance there will be a small current flowing from the worm to the wire, but this is likely to be a negligible contribution to the stimulus for the following reasons:

(a) Though the current must be independent of the firmness of touching, discharges from the giant fibre were absent in very light touches and they increased with the force of contact.

(b) If the stimulating object was a non-conductor, e.g. a glass rod, the discharges were similar to those recorded. They could not be clearly photographed, but they could be appreciated by clicks in a loud speaker and by direct observation of the continuously running time-base with automatic fly-back.

(c) On touching my own (earthed) tongue or lips with the 5-megohm wire, I could not detect any trace of shock, though I naturally experienced the sense of touch.

**Results.** Figure 2 shows a typical series of results. Each frame shows two faint lines which are due to the fly-back of the sweep, and are to be disregarded, and two heavy lines, the upper of which is the record from the tail and the lower from the head.

Frame $a$ is the magnetic deflexion due to 500 cyc./sec. and shows the speed and degree of linearity of the time-base. In $b$ the head electrodes were switched from amplifier to stimulator, and the record from the tail shows the effect of a single shock. The result is precisely the same as in all the experiments with the isolated nerve cord (Rushton 1945) and may be similarly interpreted. The faster wave which travels in the median giant fibre is here almost monophasic, whereas the slower wave in the lateral giants is clearly diphasic. Frame $c$ shows the record from the head when the shock is applied to the tail. Both waves are nearly monophasic, but the median is easily recognized as being about three times the amplitude of the other.

Frames $d$, $e$ and $f$ are the responses from tactile stimuli applied approximately to segments 20, 25 and 30 respectively. It is clear that the responses are repetitive, that they appear at both extremities in the median fibre, and that the lateral giants are not involved. Frame $g$ shows no response in any giant fibre. As is well known, the middle region of the worm is the least sensitive, and no waves were
found in the present series between the clitellum and about 3 cm. from the posterior site of operation, i.e. 5 cm. from the tail. Frames $h$, $j$ and $k$ show the responses arising from 5, 4 and 3 cm. from the tail respectively. In these cases the repetitive responses appear at both extremities of the lateral giants and the median is not involved. Frames $l$ and $m$ are the final repetition of $a$ and $b$, to verify that the wave form has not changed.

Now if Stough is right in supposing that in these reflexes each giant fibre conducts in one direction only, there is only one way to explain the invariable appearance of the wave at both extremities of the same fibre. It is that the impulse is conveyed by other nerves from the site of the stimulus to the anterior end of the median, or the posterior end of the laterals, and is then conducted down the whole length of the giant fibre. There are several reasons against this explanation, and it will suffice to note that the recorded reflex waves are similar to those in frames $b$ and $c$ and are not inverted. This must mean that every wave in figure 2 is propagated across the recording electrodes in the direction away from the centre of the worm.

If then one rejects, as clearly one must, the idea of one-way reflex conduction in the giant fibres, there no longer appears anything striking about the junction of the sensory segmental nerve with the giant fibre. Impulses pass from the former and are conducted in both directions along the giant, and arrive at the two ends with a latency difference depending upon the difference in conduction distance. In records $d$, $e$ and $f$ the worm was touched anterior to the clitellum and in each case the impulse reached the head before the tail. The reverse is the case in $h$, $j$ and $k$, where the stimulus was close to the posterior end. The latency difference is more obvious with the lateral giants because they conduct much more slowly as is seen from the shock-spike intervals in frame $b$, which give the time for conduction over the whole length of each fibre. There is thus a reasonably good correspondence between the latency difference observed and that calculated upon the assumption that the impulse arises in the giant fibre at the level of the stimulus and is conducted in both directions at the constant velocity shown in $b$.

This, however, applies only to the first wave of the series. In $d$, for example, though the first wave arrives earlier at the anterior end, the sixth wave appears earlier at the tail. It is known that when one impulse follows closely upon another its conduction speed is diminished, and Matthews has drawn attention to this in his records of sudden discharges in the nerve from a muscle spindle (1931). In the present case this slowing of conduction seems more pronounced at the anterior extremity of the median giant fibre than elsewhere.

It has been concluded from figure 2 that whenever an impulse appears at one end of a giant fibre it also appears at the other. This was found without exception in 500 pairs of waves which satisfied the condition that the final records ($l$, $m$, figure 2) reproduced the initial ones ($a$, $b$). When this failed the series was rejected. But further work has revealed one worm which showed three exceptions. As these run contrary to the conclusions of the present paper, they are given in their setting in figure 3.
Figures 2, 3. Action potentials from giant fibres. Upper line in each frame is the record from the posterior end of the cord; lower line is the record from the anterior end. Details of figure 2 are described on p. 113. Details of figure 3 are described on p. 116.
The experiment was essentially the same as that of figure 2 except that this time
the head of the worm instead of the tail was fixed to platform A, figure 1. Frame a
is a record from the tail when a shock is applied to the head; b is the corresponding
record from the head. In frames c to m the worm was prodded with the wire at
points starting near the tail, and each in succession some 8 mm. nearer the head,
m being just anterior to the clitellum. Points nearer the head gave no response
and are omitted. Frames p and n repeat a, b and q is the 500 cycle time. The nerves
were moistened and readjusted on the electrodes with the slight changes in wave
form seen in r and s, and t to x gives a further series due to prods starting this time
at the head and proceeding caudally (some records are omitted). Frames y and z
repeat r and s.

![Figure 4. Diagram of a worm with the median giant fibre divided at the arrow. Sensory nerves in the shaded region are connected chiefly to the lateral giant fibre system.](image)

![Figure 5. Records taken from the surface of an anaesthetized undissected worm. (a) 1000 cycles time scale, (b) just subthreshold shock, (c) shock just threshold for the median fibre response, (d) shock increased to stimulate lateral fibres also, (e) successful puncture of the median abolishing the median wave, but allowing the lateral to persist.](image)

This record differs from figure 2 in that the middle region is not inexcitable, and
frames g, h and j show that both median and lateral giants may be simultaneously
active, and conduct independently in both directions. Record k, however, taken
from about the posterior border of the clitellum, shows two median waves appearing
at the head which do not arrive at the tail. Frames n and p, nevertheless, prove
that conduction is possible throughout both fibres in both directions. Record u
again shows one-way conduction of the median, but this time the impulse arrives
at the tail only. It is natural to suspect a critical state of partial conduction in the region of this stimulus site. If \( k \) were anterior and \( u \) posterior to this place it might account for the two directions of one-way conduction. But, judging by the latency difference of the lateral fibre waves, \( k \) is nearer the tail and \( u \) nearer the head, so I can see no obvious explanation. Unfortunately, I was not aware of the anomaly until examining the record some hours after the experiment was finished.

This much is clear. First, the median fibre was not in a normal state of conduction, because by the time records \( y \) and \( z \) were obtained it had lost through transmission altogether, and it never regained it thereafter. Secondly, the phenomenon, whatever it may be, is exceedingly rare in the conditions of my experiments, for I have never seen it before or since. Thirdly, it lends no support to Stough's concept of one-way conduction, for the very anomaly has presented itself once as conduction forwards and once backwards. In the absence of further evidence, therefore, it seems best to treat this as some unexplained imperfection of conduction, and ignore it.

**Reflex contraction with a divided giant fibre**

The foregoing observations place us in a better position to understand the conditions of Stough's experiment (1930). He cut through (say) the median giant fibre and, after the worm had recovered from the operation, he found that the shortening reaction elicited by touching the tail was normal, but when the head was touched, the shortening extended only as far as the operation site. His immediate conclusion, which I confirm, is that the 'head reflex' is conducted by the median giant fibre (up to the damaged point), and the 'tail reflex' is conducted by the laterals (throughout).

There are two possible explanations of this. Either the sensory nerves of the head are in physiological connexion with the median and not the lateral giants, or they are connected to both, but impulses can only pass backwards in the median and forward in the laterals. Stough accepts the latter interpretation without, apparently, considering the other, and does not mention touching the worm in the middle, which allows us to settle the matter. But from figures 2 and 3 it appears that the head is only connected to the median giant, and that impulses can pass in both directions, hence the first interpretation above is clearly true, and the second is certainly false.

It can now be seen how Stough's own experiment could have been conducted so as to distinguish between the two interpretations. For if the median giant (say) is divided half-way between the clitellum and the tail (figure 4), then in the shaded area between the clitellum and the cut, according to figure 3, stimuli should affect chiefly the lateral giants and hence elicit a shortening reaction throughout the full length of the worm just as if the tail were touched. According to Stough, on the other hand, the backwards conducted impulses must necessarily flow in the median,
and hence the shortening must be confined to the region anterior to the site of operation, just as if the head were touched. The experiment now to be described, therefore, is to elicit the shortening reflex from the head, the tail, and the middle region of the worm after dividing the median or lateral giant fibres. In anticipation it may be stated that in the reflexes from the head and the tail the observations of Stough are confirmed, but the reflex from the middle region refutes his interpretation.

Operation. Stough took great care to see that his giant fibre was completely divided and that no other structure was damaged. I was less exact and employed one of two methods.

(a) After the usual alcohol anaesthesia an incision was made about 1 cm. long in the line of the ventral setae in the region shown in figure 4. The nerve cord was exposed and isolated for several mm. with care to avoid damage to the ventral blood vessel. The tiny electric bulb of an ophthalmoscope was placed below the nerve cord which was viewed, dorsal side uppermost, at binocular magnification of 15 diameters. This transillumination allowed excellent observation of the giant fibres, and the median or both laterals were damaged by pricking and tearing the upper surface with a fine needle. The incision was then closed by suture, and the worm left in the dark in a clean closed jar containing wet cotton-wool. Experiments were made during the next few days.

(b) After anaesthesia the worm was floated on mercury (electrically earthed), with the head and tail raised above the surface. A strong electric shock applied to the tail stimulated the giant fibres, and in the absence of much muscular response (due to deep anaesthesia) the action potentials of the giant fibres may be led off from the surface of the worm by electrodes applied to the anterior end (Rushton & Barlow 1943). With the shock synchronized with the time base each action potential appears in a fixed place on the tube, and may be identified by shape and position. Now a transverse incision, 3 mm. long, is made in the appropriate place on the ventral surface of the worm, to expose the nerve cord for 1 mm., but the giant fibres cannot be seen since they run on the dorsal aspect of the cord. A fine needle is plunged through the centre of the cord and out beyond, and the worm replaced on the electrodes. Sometimes both waves were still present, whereupon another puncture was made. Sometimes both waves were abolished, and the worm was rejected. In favourable cases the median wave was abolished while the lateral persisted. No suture was applied, and the worm kept as in (a).

An example is shown in figure 5. Frame a is 1000 cycles time scale, b shows the effect of a just subthreshold shock, in c it is just threshold for the median wave, and in d for the laterals. Frame e shows the effect of a successful median puncture.

Observation. The best way of testing the reflexes was to leave the worm in a moist flat dish in the dark for an hour. The motor response to touching is thereby greatly enhanced as it is when worms are found in the open in the night time. Then, after dark-adapting oneself, the contraction is observed by the light of a photographic safe-lamp. The worm is touched once and then allowed a further period of
Reflex conduction in giant fibres

119

a quarter of an hour to rest before touching again. In these circumstances the shortening reaction is usually very definite, and may be described as follows.

Median divided. (i) Touching the tail gives the normal shortening of the whole worm with the exception of segments denervated at the operation site in method (a). In particular, the segments just posterior to the operation site become more crowded.

(ii) Touching the head gives shortening of the worm down to the operation site, posterior to which the body remains flaccid and is passively extended by the anterior pull, so that, in contradistinction to (i), these segments become less crowded.

(iii) Touching the middle of the worm is apt to give no response at all, consistent with the relative insensitivity of this region. When there is a brisk response, however, it is like (i), and there is no increased spacing of the posterior segments. The observation is thus inconsistent with Stough's interpretation.

Laterals divided. (i) Touching the head gives the normal shortening of the whole worm except for denervated segments.

(ii) Touching the tail gives a shortening only posterior to the operation site.

(iii) Touching the middle of the worm gives shortening only anterior to the operation site.

Here again touching in the middle discriminates against the explanation of Stough, for reflexes in both (ii) and (iii) are transmitted only as far as the operation site and hence are carried by the divided laterals but in opposite directions, while reflexes (i) and (iii) though both transmitted antero-posteriorly are affected differently at the point of nerve division, showing conduction in (i) to be median and (iii) lateral.

In some experiments I 'listened in' to the giant fibre responses accompanying these reflexes. A slab of paraffin wax had a circular groove cut in it 5 cm. in radius and 8 mm. wide and deep. Upon the floor of this groove were embedded twelve equally spaced radial copper wires with the upper surface scraped bare, each of which passed outwards to enter a mercury cup open to the surface. So any pair of radial wires could be connected to an external circuit without disturbance, by placing the external leads into the appropriate mercury cups. The groove was moistened, and the worm introduced and imprisoned by placing a glass cover on top. After leaving in the dark a suitable time, it was viewed by dim light. If the worm was extended at rest the cover was removed, and two pairs of leads connected through the mercury, one pair to a region of the worm anterior and one pair posterior to the operation site. Each pair connected to a separate amplifier and the output went one to each ear-piece of a pair of head telephones.

The reflexes were elicited in the ordinary way by touching some part of the worm (with an electric insulator) and the nature of the reflex contraction noted by eye. At the same time the two earphones gave information of the giant fibre responses by a series of distinct clicks amidst a good deal of other noise. If the particular fibre involved was the damaged one, the clicks were heard in one ear only. If it
was the intact one, they were heard in both ears. It thus was possible simultaneously to perceive both the identity of the fibre, the nature of its discharge, and the type of motor response resulting.

As these results were consistent with the interpretations which have been set forth earlier in this paper, they need no further comment.

References

Eccles, J. C., Granit, R. & Young, J. Z. 1933 *J. Physiol.** **77**, 23P.
**Figures 2, 3.** Action potentials from giant fibres. Upper line in each frame is the record from the posterior end of the cord; lower line is the record from the anterior end. Details of figure 2 are described on p. 113. Details of figure 3 are described on p. 116.
Figure 4. Diagram of a worm with the median giant fibre divided at the arrow. Sensory nerves in the shaded region are connected chiefly to the lateral giant fibre system.

Figure 5. Records taken from the surface of an anaesthetized undissected worm. (a) 1000 cycles time scale, (b) just subthreshold shock; (c) shock just threshold for the median fibre response, (d) shock increased to stimulate lateral fibres also, (e) successful puncture of the median abolishing the median wave, but allowing the lateral to persist.