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SLOW ACTIVITY IN THE NERVOUS SYSTEM OF THE EARTHWORM, *LUMBRICUS TERRESTRIS*

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INTRODUCTION

The nervous system of the earthworm has received attention in recent years, but most of the investigations have focused on the giant fibres and the rapid escape response mediated by them. This is not surprising, for the giant fibres provide an excellent system for the analysis of reflex action. But this pre-occupation with the giant fibres has meant that few investigations have been carried out on the slow-conducting fibres in the nervous system of the earthworm. In fact information on the slow system of annelids generally is not very extensive. The classical studies of Friedlander (1894) suggested that transmission in the ventral nerve cord is decremental and this view is supported by the more recent studies of Gray & Lissman (1938). There is information on the sensory fields served by the segmental nerves in the earthworm (Prosser, 1935), and more recently giant fibre studies have provided incidental information on the part played by individual segmental nerves in producing muscular responses (Horridge, 1959; Horridge & Roberts, 1960; Roberts, 1962*a*, *b*, 1966). There is evidence of a fast and slow system in the peripheral nerves of *Nereis* (Wilson, 1960). On the anatomical front there are a number of descriptions of the neurone anatomy in the nervous system of earthworms but the accounts are confusing because of discrepancies between the different analyses.

The present enquiry was undertaken in order to throw light on the organization and functions of the neurones in the ventral nerve cord and segmental nerves of the earthworm.

MATERIAL AND METHODS

Mature specimens of the earthworm *Lumbricus terrestris* were used for all experiments. In experiments involving movement records a region of the worm 20–25 segments in length towards the posterior end was recorded kymographically using an isotonic or semi-isometric lever. Stimulating electrodes capable of moving freely with the preparation were inserted into the mid-dorsal body wall which was stimulated with condenser pulses. Full details of the techniques used for kymograph recording are described in a previous paper (Roberts, 1962*a*).

In experiments involving electrical recording, stimuli from a neon-lamp stimulator were used. These were delivered through platinum stimulating electrodes which were hooked under the nerve in question. Electrical records were made by placing platinum recording electrodes in contact with the nerve cord or longitudinal muscle whose

potentials were fed into a condenser-coupled pre-amplifier and thence into a double-beam cathode-ray oscilloscope fitted with a camera. In experiments involving only one pair of recording electrodes the lower beam of the oscilloscope was arranged to give a 50 cyc./sec. time trace. In cases where two pairs of recording electrodes were used these were connected to two separate pre-amplifiers and both beams of the oscilloscope were used. In these experiments the time relations were calculated from the known speed at which the recording paper moved through the camera. Further details of methods used are given in the accounts of individual experiments.

EXPERIMENTS AND RESULTS

Segmental nerves

A segmental nerve-muscle preparation was made by transecting the nerve cord on either side of a segmental nerve and lifting the piece of nerve cord on to a pair of stimulating electrodes. Muscle action potentials were recorded through a pair of electrodes placed in contact with the surface of the longitudinal muscle. In the earthworm each body segment has three pairs of segmental nerves, designated I (anterior), II (middle), and III (posterior). Nerves II and III are very close together and constitute the so-called 'double nerve'.

Two preparations were used:

(1) Segmental nerve I, separated from its neighbouring nerves as described above, was placed in contact with the stimulating electrodes (Fig. 1 *a*).

(2) Segmental nerves II and III, since they are difficult to separate without damaging nerve fibres, were stimulated together (Fig. 1 *b*).

With segmental nerve I it was found that below a certain threshold intensity no electrical response was recorded from the muscle (Fig. 2, record *a*). As the intensity was gradually increased a small response was recorded (Fig. 2, record *b*). With further increase in intensity a second threshold was reached giving a larger response (Fig. 2, record *c*) and at a slightly higher intensity a third threshold was reached (Fig. 2, record *d*). Further increase in the intensity of stimulation was found to have no effect on the size of this last response (Fig. 2, record *e*).

The three thresholds demonstrated in Fig. 2 are close to one another and in a number of preparations it was not possible to separate the first from the second, with the result that there appeared to be only two. In these cases the lowest effective intensity of stimulation produced muscle responses similar to the one recorded at the second threshold (record *c* in Fig. 2). On occasions the lowest threshold was brought in by slightly shifting the position of the stimulating electrodes.

The experiment was repeated with segmental nerves II and III ('double nerve'). In this case the majority of preparations showed two thresholds, a large response being recorded at higher intensities (Fig. 3, records *a*, *b*) and a smaller response at lower intensities (Fig. 3, records *c*, *d*). In some cases three thresholds were obtained though it was difficult to separate the first from the second, a very small response being recorded at the lowest threshold (Fig. 4).

The latent period between the application of the stimulus and the appearance of the muscle response was found to be approximately 20 msec. for a preparation in

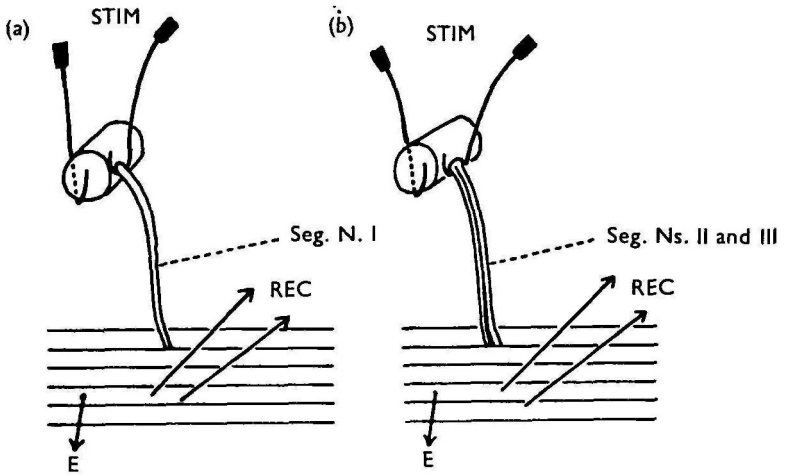

Fig. 1. Segmental nerve-muscle preparations. (*a*) Segmental nerve I; (*b*) segmental nerves II and III. Stimulating electrodes (STIM) placed in contact with nerve. Recording electrodes (REC) placed in contact with longitudinal muscle. E earth. Further description in text.

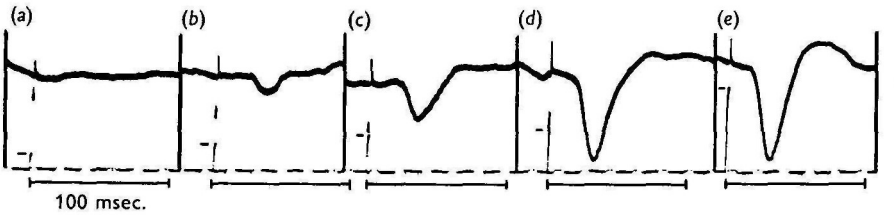

Fig. 2. Action potentials recorded from longitudinal muscle in response to stimulation of segmental nerve I with shocks of increasing intensity. Height of stimulus artifacts on lower trace indicates stimulus intensity. Note three thresholds.

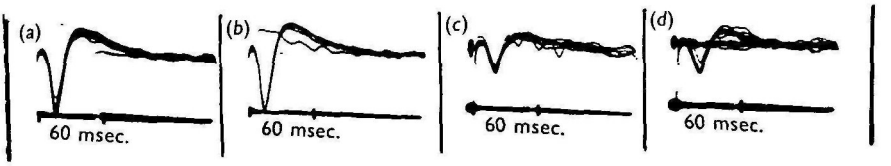

Fig. 3. Longitudinal muscle potentials in response to stimulation of segmental nerves II and III with shocks of decreasing intensity. Note two thresholds.

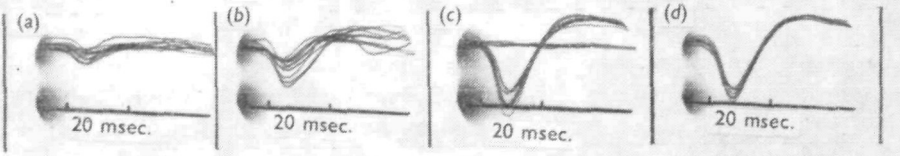

Fig. 4. Longitudinal muscle potentials in response to stimulation of segmental nerves II and III with shocks of increasing intensity. Note three thresholds.

which the recording electrodes were placed on the surface of the longitudinal muscle 8 mm. from the stimulating electrodes. On this basis the speed of conduction in the motor neurones was calculated to be 0·4 m./sec. which corresponds to the normal transmission speed found in small unmyelinated nerve fibres.

Nerve impulses in the ventral nerve cord

The nerve cord was exposed from the ventral side for a distance of 6 cm. behind the clitellum. The preparation (Fig. 5) was pinned to a wax block, the ventral side being uppermost behind the clitellum and the dorsal surface uppermost towards the anterior end. Three pairs of electrodes were placed in contact with the nerve cord, the anterior pair (S1) for stimulating with low-frequency pulses, and the two posterior

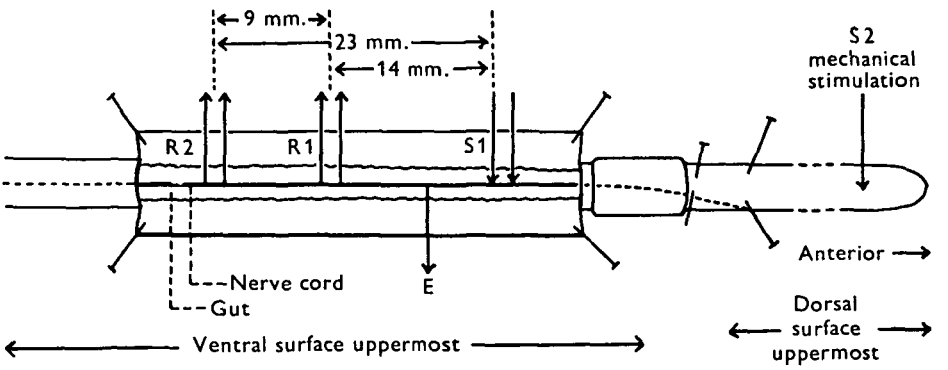

Fig. 5. Preparation for recording action potentials from exposed nerve cord through two pairs of electrodes in response to electrical stimulation of nerve cord and mechanical stimulation of mid-dorsal body wall. Further explanation in text.

pairs for recording electrical responses. Impulses recorded through the anterior pair of recording electrodes (R1) appeared on the upper oscillograph trace, those recorded through the posterior pair (R2) on the lower trace. The results of mechanical stimulation (S2) applied to the dorsal surface at the anterior end were compared with direct stimulation of the cord.

The burst of slow impulses following a single shock of maximal intensity lasts for 50–100 msec. It consists of several major peaks which appear on both the upper and lower oscillograph traces indicating that they are transmitted along the cord (Fig. 6). Each peak is composed of several partially fused but distinguishable spikes. Although equal in intensity some shocks produce larger peaks than others and not infrequently one peak was found to be absent altogether. Of the series of peaks the first was usually the largest and the most constant. No trace of facilitation was observed at any frequency of stimulation. Slow potentials can be elicited at a threshold below that required to evoke a giant-fibre response. This can be shown by lowering the intensity of stimulation to the point when neither lateral nor median giant-fibre impulses are produced. Slow potentials are still recorded (Fig. 7).

Peripheral stimulation

Repeated tactile stimulation of the dorsal surface at the anterior end of a preparation (S2 in Fig. 5) evokes median giant-fibre impulses which accommodate far more rapidly than the slow impulses which are also recorded. The slow impulses seen in Fig. 6 record *b* were elicited by a tactile stimulus applied to the dorsal surface at the anterior end long after giant-fibre impulses had ceased to be produced by peripheral stimulation. Their magnitude, shape and conduction speed are similar to those produced by direct electrical stimulation of the cord and it seems likely that the same nerve fibres are involved in both. The duration of the burst of slow impulses evoked by tactile stimulation of the body wall depends on the intensity and duration of the stimulus.

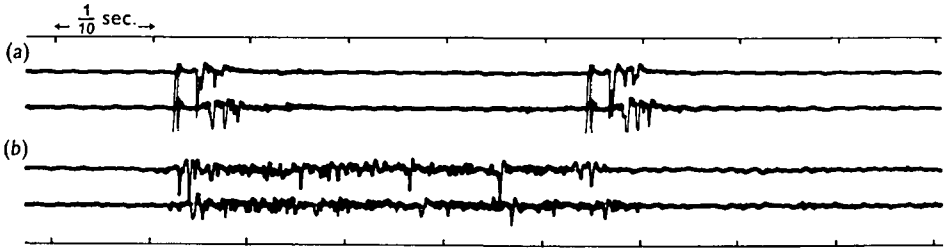

Fig. 6. Preparation Fig. 5. (*a*) Action potentials in response to two shocks sent in through S1. In each case burst of slow potentials is preceded by stimulus artifact, and a median and lateral giant-fibre impulse. (*b*) Potentials produced by mechanical stimulation of body wall (S2). Upper traces: recording electrodes R1; lower traces: recording electrodes R2. Time intervals, 100 msec.

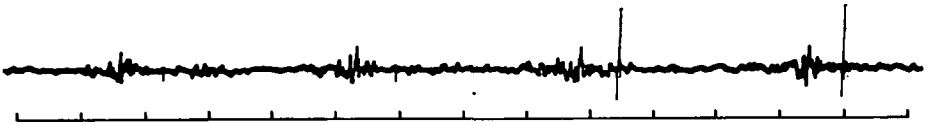

Fig. 7. Action potentials recorded from nerve cord in response to direct stimulation of cord. First two shocks above threshold for median giant fibre; intensity then slightly lowered. Note small upward-projecting stimulus artifacts. Time intervals, 100 msec.

Effect of the intensity of direct stimulation

The nerve cord of a preparation illustrated in Fig. 5 was stimulated with a series of shocks of gradually increasing intensity. From Fig. 8 it will be seen that the first peak in the burst has the lowest threshold and, though small, is present in records *b* and *c*. As the intensity of stimulation is increased the magnitude of this peak suddenly increases. In the series of records (*a*) to (*j*) three heights, measured on the upper traces of the original records, can be recognized: an average height of 3·75 mm. in (*b*) and (*c*), 8·0 mm. in (*d*) to (*f*) and 15·2 mm. in (*g*) to (*j*). It is possible that records *b* and (*c*) represent two different heights but the difference is slight and it is difficult to be certain. However, the differences between records *c* and *d*, and between records *f* and *g* are unmistakable. It therefore appears that the first of the slow peaks is composed of at least three separate spikes conducted at the same velocity but evoked at different thresholds.

Transmission properties

In the records shown in Figs. 6 and 8 the peaks in the upper traces (recorded through the anterior pair of electrodes R 1) are larger than those in the lower traces (recorded through the posterior pair of electrodes R 2). Though possibly the result of decremental transmission in the nerve cord this could alternatively be due to differences in the properties of the two recording systems. The matter was settled by recording from one pair and stimulating through three different pairs of electrodes.

A nerve cord was isolated and laid across four pairs of platinum electrodes in a

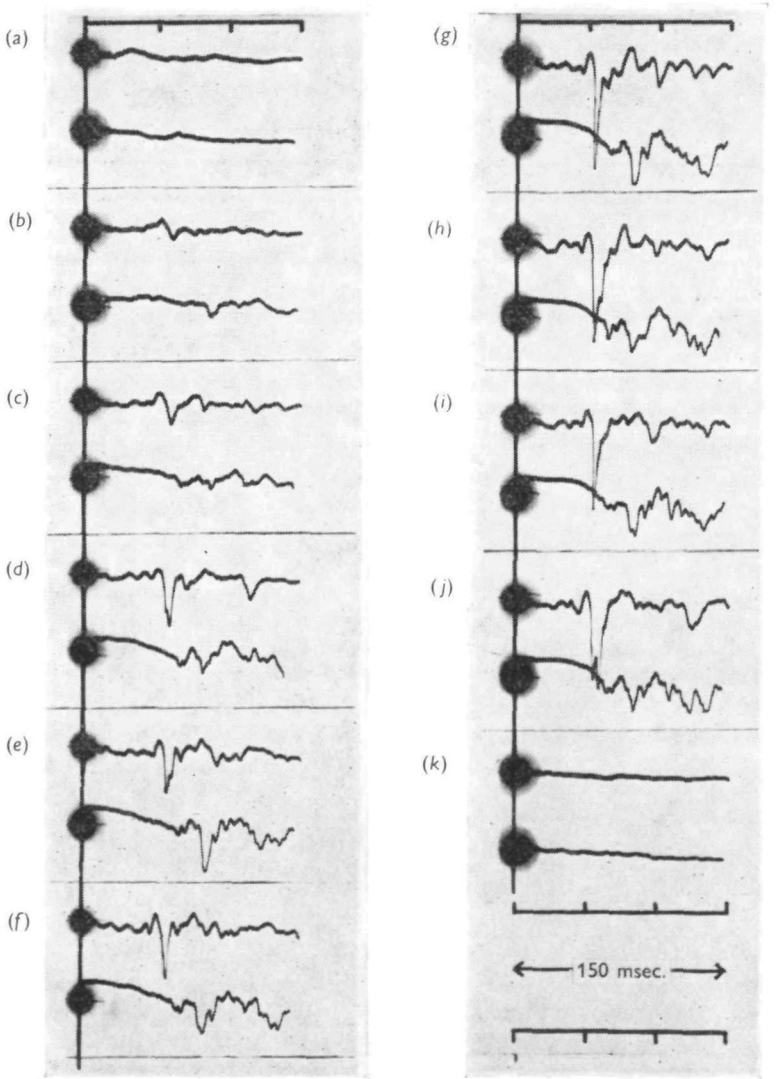

Fig. 8. Preparation Fig. 5. Slow potentials in response to stimulation of cord through S 1 with shocks of increasing intensity. (*a*) Subthreshold shock; (*b*) to (*j*) intensity increasing; (*k*) subthreshold shock. In each case upper trace R 1, lower trace R 2.

moist Perspex box (Fig. 9). Three pairs of electrodes, S 1 to S 3, were connected to the output of a neon-lamp stimulator via a switch which enabled any one of the three pairs to be coupled to the stimulator at a given time. The fourth pair of electrodes, R, was connected to a pre-amplifier for recording in the usual way. The intensity of stimulation was kept constant and well above threshold for evoking a maximal response.

Typical results are shown in Fig. 10. The nerve cord was stimulated first through

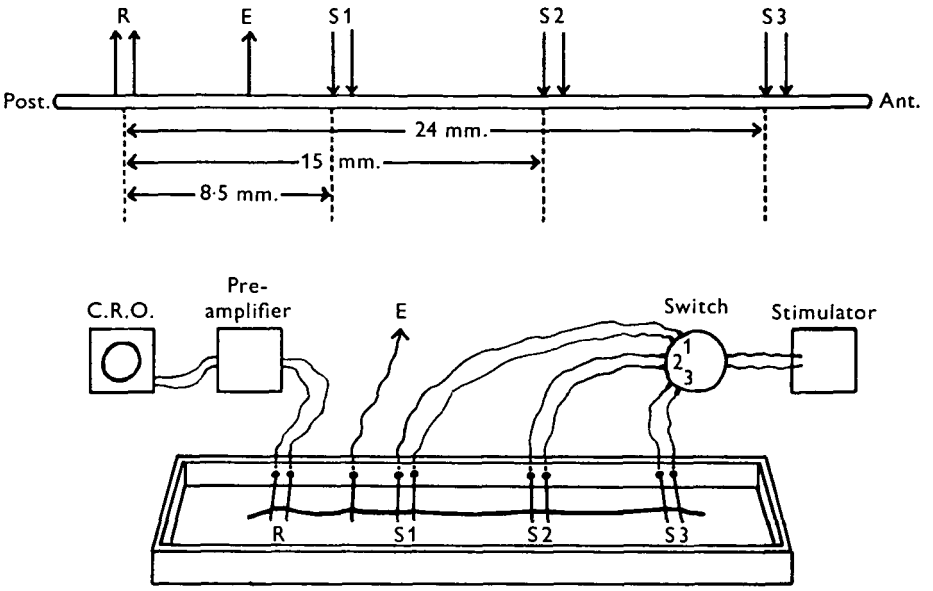

Fig. 9. Stimulation of isolated nerve cord through three pairs of electrodes S 1–3. R recording electrodes. E earth. Further explanation in text.

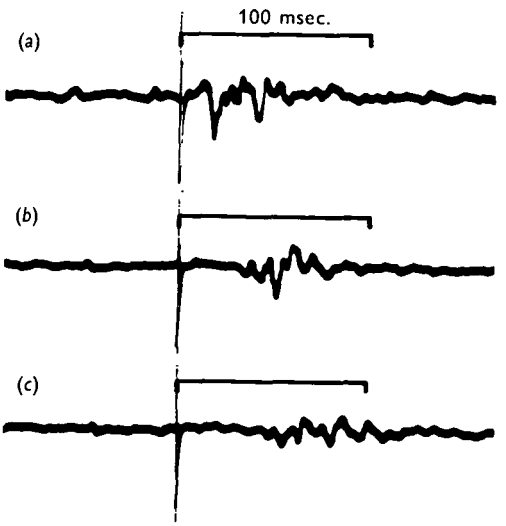

Fig. 10. Preparation Fig. 9. Slow potentials in response to stimulation of one end of nerve cord through three pairs of electrodes. Single shock sent through (*a*) S 1, (*b*) S 2 and (*c*) S 3.

S 1 (record *a*), then through S 2 (record *b*) and finally through S 3 (record *c*). It will be seen that the magnitude of the potentials diminishes as the distance between the stimulating and recording electrodes is increased. It therefore appears that transmission of at least part of the slow burst of electrical activity in the nerve cord is decremental.

Transmission speed

The technique described in the last section was used to measure the speed of transmission of the slow impulses in the cord. This was calculated from the known distance between the three pairs of stimulating electrodes and the recording electrode (see Fig. 9). For a number of different preparations tested this was found to vary

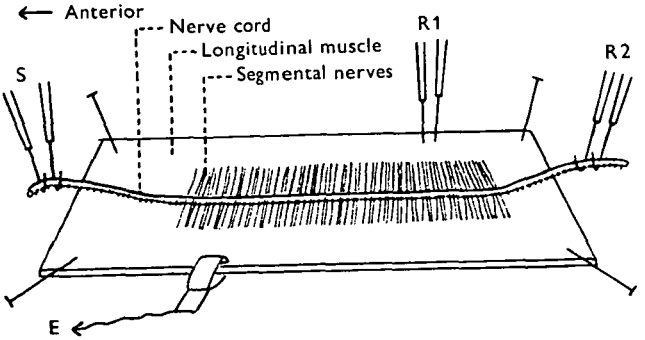

Fig. 11. Preparation for recording potentials from longitudinal muscle and nerve cord in response to direct stimulation of cord. E earth. Further explanation in text.

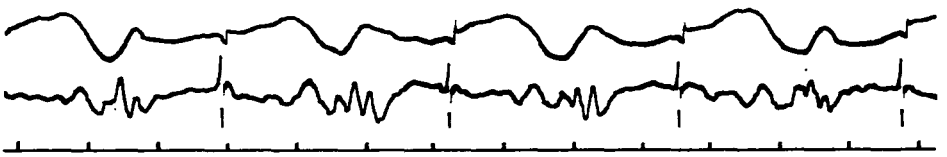

Fig. 12. Preparation Fig. 11. Slow potentials recorded from nerve cord (upper trace) and longitudinal muscle potentials (lower trace) in response to direct stimulation of cord with four shocks. In each case slow nerve potentials preceded by median giant-fibre impulse. Downward-projecting stimulus artifacts appear on lower trace. Time intervals, 50 msec.

between 0·4 and 0·6 m./sec., a value which, like the one found for transmission in the segmental nerves, conforms to the transmission speed normally found in small unmyelinated nerve fibres.

Muscular responses initiated by the slow impulses

The burst of slow impulses transmitted along the cord contains components which evoke activity in the longitudinal muscle. This was shown by stimulating the nerve cord and recording action potentials from the muscle. The preparation is shown in Fig. 11. One end of the nerve cord was stimulated with neon-lamp pulses at a frequency of 3/sec. Muscle potentials were recorded through a pair of electrodes placed in contact with the surface of the longitudinal muscle (R 1). Nerve impulses were recorded simultaneously through a second pair of electrodes placed in contact with the other end of the nerve cord (R2). The results shown in Fig. 12 show the nerve and

muscle potentials evoked at the lowest intensity of stimulation required to produce a maximum response in the muscle. Slow activity in the nerve cord appears approximately 50 msec. after the stimulus artifact, and corresponding diphasic responses are recorded in the muscle. Each burst of slow impulses in the nerve cord is preceded by a median giant-fibre impulse but this fails to produce a muscle response because of accommodation at the giant-to-motor junctions (see Roberts, 1962*b*).

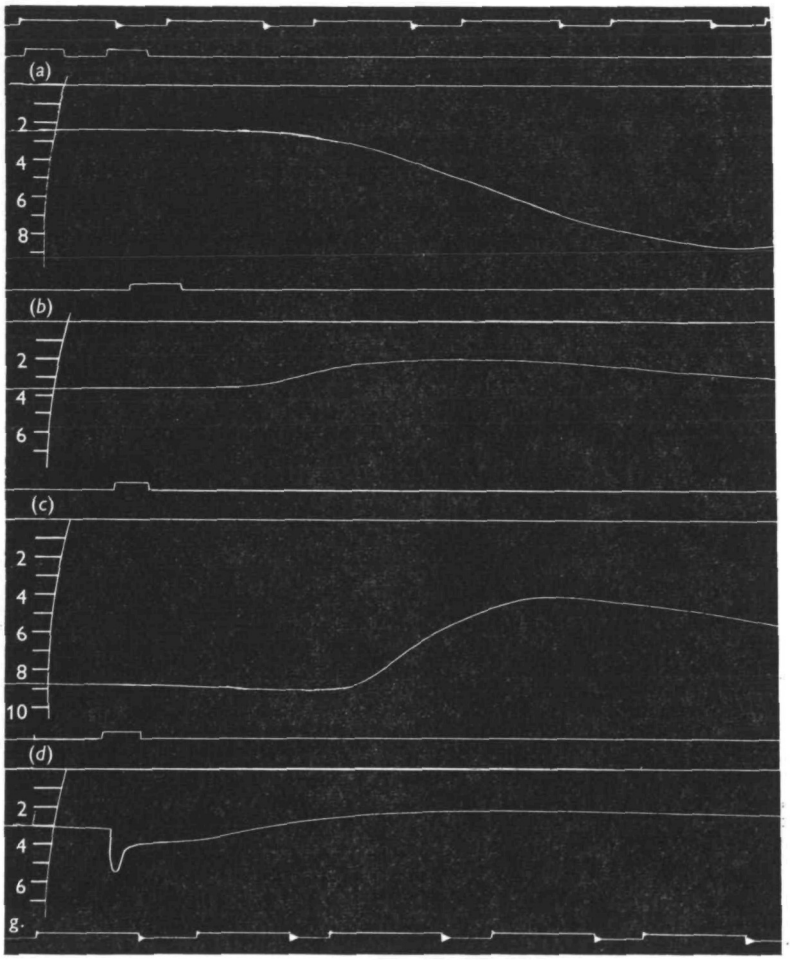

Fig. 13. Muscular contractions of posterior region in response to stimulation of mid-dorsal body wall at anterior end with shocks of increasing intensity. Semi-isometric lever. Longitudinal contraction indicated by downward movement. Stimulus marks above each base line. Time signal, 1 sec.

Movement records

Apart from the rapid response produced by giant-fibre activity (Roberts, 1962*a*), two types of muscular response can be produced by peripheral stimulation at the anterior end of *Lumbricus*. These are illustrated in Fig. 13.

(1) *A slow longitudinal contraction.* Extremely weak mechanical or repetitive electrical stimulation of the body surface at the anterior end, weaker than that required to evoke any other type of muscular response, produces a slow longitudinal contraction which

starts at the head end and passes backwards along the body as the first of a series of peristaltic contractions. With tactile stimulation a continuous gentle stroking of the integument is required to bring about this response; a single touch is rarely effective. With electrical stimulation the shocks must be weak and usually it requires two or three in succession to produce a response (Fig. 13*a*). If stimulation is continued the longitudinal muscle frequently undergoes a slow contraction which may be maintained for a considerable time. If stimulation is very weak and brief the wave of longitudinal contraction fails to pass along the entire length of the body if the animal is resting in Ringer's solution or on a smooth surface. Stronger or more prolonged stimulation, however, will frequently bring about a response which is propagated throughout the length of the worm.

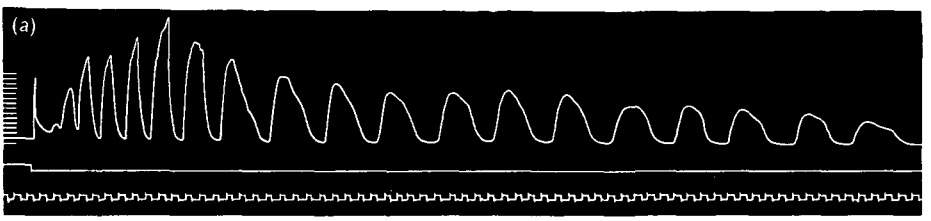

Fig. 14. Muscular contractions to stimulation of mid-dorsal body wall with single shock. Isotonic lever. Longitudinal contraction indicated by upward movement. Stimulus mark below muscle record. Time signal, 1 sec.

(2) *The elongation reflex.* Aroused by weak tactile or electrical stimulation at the anterior end, this consists of a wave of circular contraction which starts at the posterior tip and moves forward as the first phase of a series of antiperistaltic contractions. Its threshold is higher than that required to produce the slow longitudinal contraction, but lower than that required to initiate the rapid response from which it is therefore independent (Fig. 13*b*, *c*). At higher intensities of stimulation it is preceded by the rapid giant-fibre response, the elongation reflex following the initial longitudinal contraction after a variable period of time (Fig. 13*d*). The latent period preceding posterior elongation is very variable and depends on the intensity of stimulation. When produced by strong tactile stimulation at the anterior end it follows the initial rapid contraction almost immediately, but with weak stimulation the latent period may exceed 1 sec.

If the initial stimulus is sufficiently strong posterior elongation is followed immediately by antiperistalsis involving alternate contractions of the longitudinal and circular muscles. Gradually becoming smaller and slower, these rhythmical contractions may continue for over a minute (Fig. 14).

The elongation reflex will still occur after the efferent side of the giant fibre reflex has been fatigued. A strong stimulus applied to the body wall at the anterior end of a fatigued preparation produces no rapid longitudinal contraction, but the elongation reflex still occurs after a latent period of approximately one second.

DISCUSSION

The electrical records described in the first part of this paper may provide an explanation of the nervous mechanisms underlying the two types of movement response. For example, the fact that the elongation reflex produced by anterior stimulation commences at the posterior tip of the worm indicates that it must involve the use of a through-conduction system in the nerve cord. Yet the fact that this reflex is elicited at a threshold lower than that required to excite the rapid response indicates that the giant-fibre system is not involved in the elongation reflex. The elongation reflex involves contraction of the circular muscles at the posterior end, and it is likely that some of the component spikes in the slow activity recorded from the nerve cord are involved in bringing about this response. This supports the observation of ten Cate (1938), who showed by partial transection of the cord that the elongation reflex does not require the giant fibres and can be mediated through the ventral chain of the nerve cord.

The slow longitudinal contraction evoked by weak peripheral stimulation, on the other hand, starts as a wave of contraction at the anterior end which passes slowly backward. This would suggest a nervous mechanism similar to that involved in normal locomotion (Gray & Lissmann, 1938). That peripheral stretch receptors are necessary is supported by the observation that, when elicited by very weak stimulation, the wave of longitudinal contraction will not pass along the entire length of the body. It appears that this response is the result of decremental transmission in the cord reinforced by stretch reflexes. Since some of the component spikes in the slow activity of the cord are transmitted decrementally it would seem reasonable to suppose that these spikes are associated with the propagation of such antero-posterior locomotion waves.

The experimental data presented in this paper allow us to make certain predictions concerning the neurone anatomy in the nervous system of the earthworm. The three thresholds demonstrated in segmental nerve I suggest that there are at least three motor neurone tracts innervating the longitudinal muscle in that nerve. The two (or three) thresholds found in the 'double nerve' suggest a corresponding number of motor neurone tracts in nerves II and III together.

At present there is no histological proof of these findings, since the motor axons entering the segmental nerves have not been traced with certainty to the particular muscles which they supply. However, there is some information on the total number of motor axons entering each segmental nerve, though there are discrepancies between the accounts of individual investigators. From the early investigations of Cerfontaine (1892), Retzius (1892), Krawany (1905) and Haller (1889, 1910) it appears that there are about three motor axons in segmental nerve I, and between 5 and 10 in the 'double nerve'. With the exception of Krawany, who used *Eisenia*, these workers used *Lumbricus* for their histological analyses. Ogawa (1939), working on *Pheretima*, claims a much larger number of motor axons in each segmental nerve. Thus she figures a minimum of nine principal motor axons entering segmental nerve I and at least fourteen in the 'double nerve'. What proportion of these innervates the longitudinal muscles is unclear, but the evidence presented here suggests that those that do so are organized into tracts, three in segmental nerve I and at least two in the 'double nerve'.

The size, duration and transmission speed of the spikes recorded from the ventral nerve cord are consistent with the view that they represent impulses transmitted in the small nerve fibres of the ventral chain. The major peaks, of which the burst of slow impulses is composed, may correspond to tracts or pairs of tracts within the cord. Each peak consists of several partially fused spikes which suggests that the tracts are themselves composed of a number of neurones all conducting at approximately the same speed. The spatial separation of the peaks in the electrical records indicates that the transmission speeds differ in the different tracts. However, the speed of transmission is the same in the constituent neurones of any one tract. The three thresholds demonstrated in the first of the three peaks suggest that the most rapidly conducting tract is composed of at least three neurone pathways.

To what extent is this experimental model of the nerve cord supported by anatomical studies? Again, the existing histological information is confusing because of differences of opinion between various investigators. Ogawa, whose analysis of the oligo chaete nervous system remains the most extensive to date, recognize three types of internuncial neurone in the nerve cord of *Pheretima*:

(1) Large, mainly unipolar, types whose processes give off numerous collaterals. The cell bodies lie in the median and lateral regions of the cord and their processes either pass to the other side or remain on the same side as the cell bodies.

(2) Very small unipolar cells with short ipsilateral processes. The latter occupy a lateral position in the nerve cord along which they extend longitudinally.

(3) Large multipolar cells occupying a median position in the cord.

There is no evidence that these three types of neurone are organized into well-defined tracts within the nerve cord. It is known, however, that afferent nerve fibres are arranged into discrete longitudinal bundles, three on each side of the cord (Retzius, 1892, on *Lumbricus*; Ogawa, 1939, on *Pheretima*). According to Ogawa each side of the nerve cord contains an inner, middle and outer bundle of such nerve fibres, the outer bundle being the largest. Whether or not these correspond to the tracts demonstrated experimentally is a problem for future investigation.

SUMMARY

1. Three thresholds are demonstrated in the first segmental nerve and two (sometimes three) in the second and third segmental nerves together.

2. Slow potentials recorded from the ventral nerve cord consist of several peaks. The first peak is composed of three spikes which make their appearance at different thresholds. Transmission of at least some of the slow potentials is decremental.

3. Transmission speeds in the nerve cord and segmental nerves range from 0·4 to 0·6 m./sec.

4. Action potentials in the longitudinal muscle are recorded in response to slow potentials in the nerve cord.

5. Two slow reflexes, one involving elongation, the other longitudinal contraction, are described. The latter has the lower threshold with peripheral stimulation.

6. Slow activity in the nervous system is discussed in relation to reflex activity of the earthworm and the neurone anatomy of the nerve cord and segmental nerves.

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