

THE GIANT FIBRE REFLEX OF THE EARTHWORM, *LUMBRICUS TERRESTRIS* L.

I. THE RAPID RESPONSE

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INTRODUCTION

If an earthworm is stimulated by touching one end it responds by giving a rapid shortening of the body followed by peristaltic locomotion away from the stimulus. The initial shortening is characteristic of the escape reactions of many annelids though it is lacking in those species which do not possess giant nerve fibres. It is now well established that the rapid response is mediated through these giant fibres (Yolton, 1923; Stough, 1930).

An extensive literature on the morphological organization of the giant fibres in the central nervous systems of annelids is reviewed by Nicol (1948). Much is known also about their transmission characteristics (Eccles, Granit & Young, 1933; Bullock, 1945; Rushton, 1945, 1946; Nicol & Whitteridge, 1955; Kao, 1956; Kao & Grundfest, 1957), and recently physiological analyses have been made of their afferent and efferent connexions in several species (Horridge, 1959; Roberts, 1960, 1962*a*). However, there is little information on the muscular responses mediated by them beyond the fact that their high speed of transmission results in rapid synchronous contraction of the muscle.

Apart from observations on the genesis of tetanus, etc., in the sabellids *Branchiomma* (Nicol, 1951) and *Myxicola* (Roberts, 1962*b*) no work has been done on the muscular responses mediated through the giant fibres in polychaetes. Straub (1900), Budington (1902) and Botsford (1941) investigated the physiological properties of the longitudinal musculature of *Lumbricus*, but the preparations which they used were such that it would not be possible to say whether the responses obtained were produced through the giant fibres or through some other constituents of the nervous system. Botsford (1941) and Wu (1939) concluded that the neuromuscular apparatus of the earthworm is cholinergic. Von Holst (1932) found that the giant fibres can mediate a rapid response which need not necessarily occur over the entire length of the body but may be confined to the anterior end. He also presented evidence that the longitudinal contraction is accompanied by simultaneous contraction of the circular muscles. Apart from this no information is available on the muscular responses mediated through the giant fibres in oligochaetes.

The present series of papers reports on the properties of the muscular response and the means by which it is produced in the earthworm.

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MATERIAL AND METHODS

Mature specimens of *Lumbricus terrestris* were kept in earthenware pots containing a mixture of moist soil and leaf mould at a temperature of between 8 and 16° C. Under these conditions the worms will live actively for several months but normally they were replaced by a fresh stock after a few weeks.

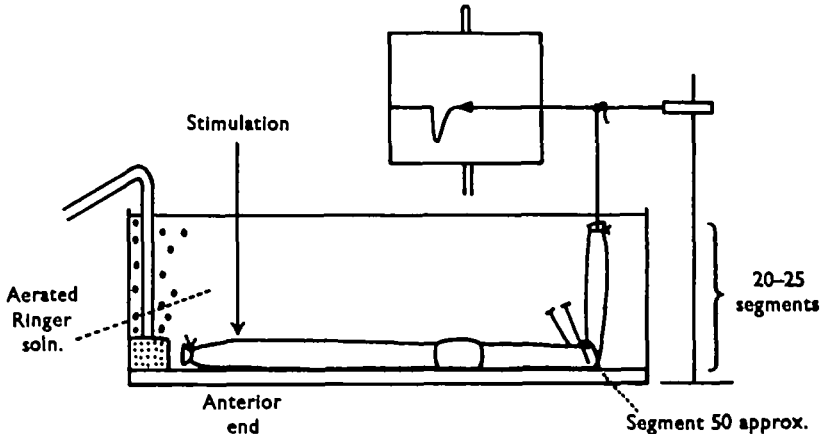


Fig. 1. Experimental procedure for kymograph recording of the earthworm. Further explanation in text.

Kymograph recording

This was carried out by transecting and ligating the worm at about segment 70 and fixing it to the wax floor of a dish 20–25 segments in front of the ligature (Fig. 1). The ligature thread was attached to a semi-isometric lever; the flexibility of the lever permitted some shortening of the preparation so that the muscular records do not represent the tension developed under strictly isometric conditions. For the majority of preparations a reasonably stiff lever was used, 1 g. tension being represented by a 1 mm. excursion of the writing point.

As indicated in Fig. 1 contractions of the posterior region of the worm were recorded in response to stimulation at the anterior end. This avoids direct stimulation of the recorded muscle, the responses being clearly separable into an initial rapid contraction, mediated through the giant fibre system, and slower contractions occurring later. Autotomy was overcome by using as short a length of worm as possible (the ligature rarely being posterior to segment 80), by setting up with the animal anaesthetized, and by removing the nerve cord from the three segments immediately in front of the ligature, thus preventing the autotomy reflex from occurring in this region. The prostomium and peristomium were removed since absence of these structures renders the animals more quiescent.

The anaesthetic substance MS 222 (tricain methanesulphonate), supplied by Sandoz Ltd., was found to be an ideal narcotic for the earthworm, being quite rapid in its action and fully reversible. Normally the worms were anaesthetized by immersion in a solution of 0.25 g. of MS 222 in 100 ml. of earthworm saline. The preparation was covered with aerated saline solution, both while recovering from the anaesthetic and also during the experiment. The saline solution generally used was that recommended

by Pantin (1948). In such a solution at a pH of about 7.4 the animals are active and give good responses for several days.

It will be shown in a subsequent paper that the escape response of the earthworm fatigues very rapidly unless a sufficiently long rest interval is given to the preparation between responses. In the present experiments at least 15 min. were allowed to elapse between successive recordings.

Electrical recording

Electrical records were made by placing a pair of fine platinum recording electrodes in contact with the nerve cord from which action potentials were fed into a condenser-coupled pre-amplifier and thence into a double-beam cathode-ray oscilloscope fitted with camera. In the present experiments involving only one pair of recording electrodes the lower beam was arranged to give a 50-cycle hum, thus providing a time trace. Giant fibre impulses were readily distinguished from other electrical activity in the nerve cord by their large size and high transmission speed.

Stimulation

In natural circumstances the escape response is initiated by brief tactile stimulation of the body surface. However, tactile stimuli are difficult to control experimentally so electrical stimulation has been used unless otherwise stated. Single shocks of controlled intensity and repetitive shocks at frequencies up to 3 per sec. were provided using the 'signal-marker-contact-maker' (Hall & Pantin, 1937). A neon lamp stimulator was used to achieve higher frequencies. For the experiment involving stimulation with controlled bursts of shocks a repetitive stimulator operated mechanically and fitted with a variable number of contacts was employed.

EXPERIMENTS AND RESULTS

The nerve-muscle preparation

Difficulty was encountered in making a reliable nerve-muscle preparation which was not suspected of suffering from post-operative fatigue. The preparation eventually used was set up as follows: the anterior end of the worm was pinned down, ventral surface uppermost, and the giant fibres were stimulated by inserting a pair of fine platinum electrodes, insulated almost to the tip, on to the nerve cord through the mid-ventral body wall. Bullock (1945) found that when the nerve cord is stimulated in this way a single shock of sufficient intensity will evoke a single impulse in the median and lateral giant fibres. I have confirmed this in the following way: the nerve cord was stimulated with repetitive neon lamp pulses at a frequency of 2 per sec. and the intensity was gradually increased. Recording electrodes were placed in contact with the nerve cord which was exposed over a short length of the worm immediately behind the clitellum. Below a certain threshold no giant fibre impulses are recorded (Fig. 2, record *a*). Above this threshold one impulse is produced by each shock (Fig. 2, records *b-d*). As the intensity is further increased another threshold is reached at which a second impulse, slower and slightly larger than the first, is recorded (Fig. 2, record *e*). Further increase in intensity does not increase the number of impulses recorded. From the work of Rushton (1945) and Bullock (1945) it is now established that the faster impulse with the lower threshold is transmitted in the median giant and the slower impulse with

the higher threshold in the two lateral giants. For the present purpose it is important to notice that only one impulse per shock is evoked in each giant fibre system however great the intensity of stimulation.

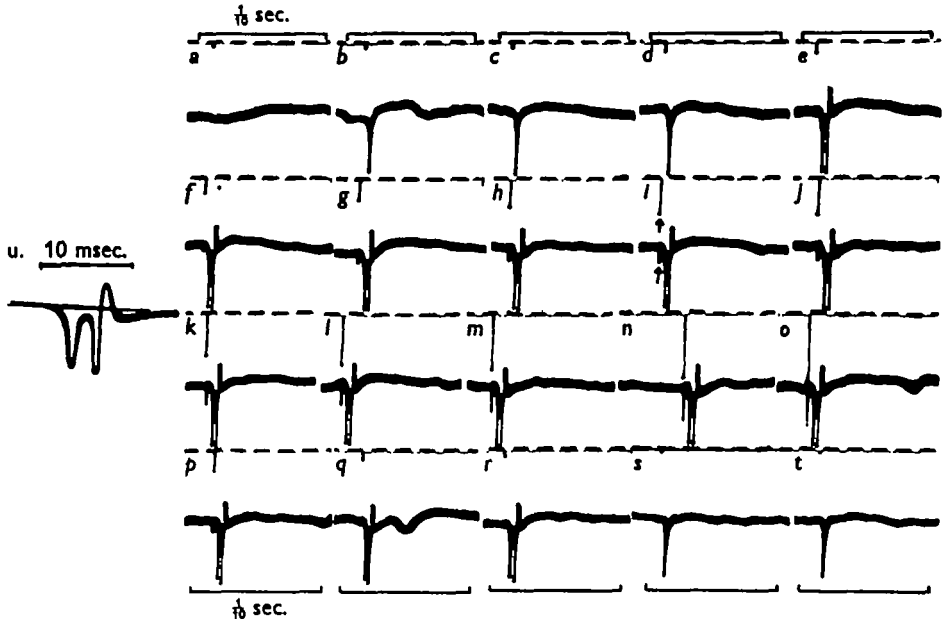


Fig. 2. *Lumbricus terrestris*: nerve-muscle preparation. Median and lateral giant fibre impulses in response to direct stimulation of nerve cord with shocks of varying intensity. Action potentials preceded by downward-projecting stimulus artifacts on both traces of oscillograph (arrowed in record i) whose magnitude indicates relative intensity of stimuli. Record u: median and lateral giant fibre impulses on a wider time base.

The rapid response produced by direct stimulation of the nerve cord

The same type of preparation was used for stimulating the nerve cord with shocks of increasing intensity and recording the longitudinal contractions produced in a region, 20 segments in length, towards the posterior end of the worm (Fig. 1).

The results (Fig. 3) indicate that as the intensity of stimulation is increased two responses are recorded. The first, at the lower threshold, is a very small twitch (Fig. 3, records a-g). At a higher threshold a slightly larger twitch is produced (Fig. 3, record h) and a considerable increase in the intensity of stimulation does not increase the magnitude of this response (Fig. 3, record i).

The supposition that the smaller twitch is produced by a median giant fibre impulse and the larger twitch by single impulses in the median and lateral giant fibres was tested as follows.

(1) The nerve cord of the preparation used in the above experiment was repeatedly stimulated at the lower threshold until no rapid contractions were given (Fig. 3, record j). The intensity was then increased to the higher threshold and a twitch, smaller than was formerly obtained at this intensity, was produced by a single shock (Fig. 3, record k). It is probable that this represents the muscular response evoked by an impulse in the lateral giant fibres, the median giant fibre response having been

fatigued. Subsequent repetitive stimulation at the higher threshold was found to abolish this lateral giant fibre response too (Fig. 3, records *l-n*).

(2) The intensities of stimulation required to evoke the larger contractions were found as accurately as possible. A short length of the nerve cord was then exposed at the posterior end of the preparation. Giant fibre activity was recorded from the nerve cord and the intensities required to evoke (*a*) a median, and (*b*) a lateral giant fibre impulse were found. In all cases tested the smaller contraction with the lower threshold was found to have been produced at approximately the same intensity as that required to evoke an impulse in the median giant fibre, and the larger contraction with the higher threshold corresponded with the intensity required to evoke a lateral giant fibre impulse.

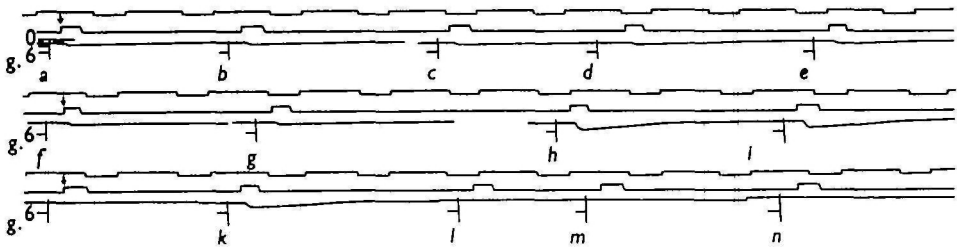


Fig. 3. *L. terrestris*. Rapid longitudinal contractions in response to direct stimulation of nerve cord with single shocks of increasing intensity. Upper trace: 1 sec. time signals; middle trace: stimulus marks (arrowed in record *a*); lower trace: muscle records, each preceded by excursion of the lever recorded on stationary drum. Potentiometer readings (intensity scale): *a*, 2.0; *b*, 3.0; *c*, 3.5; *d*, 4.0; *e*, 4.25; *f*, 4.75; *g*, 5.0; *h*, 5.25; *i*, 10.0; *j*, 4.0; *k*, *l*, *m*, 6.0; *n*, 10.0. Tracing of original kymograph record.

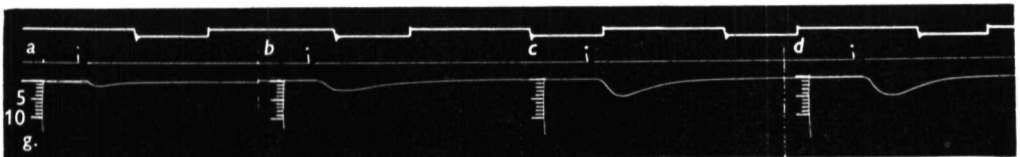


Fig. 4. *L. terrestris*: nerve-muscle preparation. Rapid longitudinal contractions in response to direct stimulation of nerve cord with: *a*, single shock; *b*, 2 shocks; *c*, 3 shocks; *d*, 4 shocks. Approximate interval between successive shocks in records *b*, *c*, and *d*: 10 msec. Upper trace: 1 sec. time signals; middle trace: stimuli (dotted); lower trace: muscle records.

The rapid response produced by peripheral stimulation

The rapid contractions produced by direct stimulation of the nerve cord with single shocks are very small. Larger contractions are recorded only when the nerve cord is stimulated with short bursts of repetitive stimuli, the size of the resulting contraction depending on the number of shocks in the burst (Fig. 4).

When the nerve cord is stimulated with a single shock of sufficient intensity, a single impulse is recorded from the median giant fibre. However, tactile stimulation of the body surface at the anterior end of the worm produces a burst of up to 20 or 30 impulses, the number of impulses depending on the intensity and duration of the stimulus. Large rapid contractions in the earthworm are thus the result of not one impulse but of a burst of giant fibre impulses evoked when the animal is stimulated peripherally.

Grading of the rapid response

The effect on the rapid muscular response of varying the intensity of peripheral stimulation was investigated as follows. The earthworm was anaesthetized and set up as shown in Fig. 1. A pair of platinum stimulating electrodes was inserted superficially into the mid-dorsal region of the body wall at the anterior end. These electrodes, soldered to the ends of a pair of fine copper coils, were free to move with the preparation. The longitudinal contractions of 20–25 segments at the posterior end were recorded in response to single condenser shocks of gradually increasing intensity.

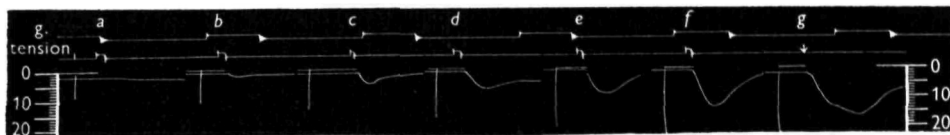


Fig. 5. *L. terrestris*. Rapid longitudinal contractions in response to stimulation of anterior mid-dorsal body wall with single condenser shocks of increasing intensity. Upper trace: 1 sec. time signals; middle trace: stimulus marks; lower trace: muscle records, each preceded by excursion of lever recorded on stationary drum. Potentiometer readings (intensity scale): a, 3.0; b, 3.25; c, 3.5; d, 4.5; e, 5.0; f, 6.0; g, brief mechanical stimulus.

The records obtained (Fig. 5) indicate that the greater the strength of the shock the larger is the rapid contraction obtained. The relationship between the intensity of stimulation and the magnitude of the responses was investigated in a number of preparations. In all cases the thresholds for the rapid contraction were approximately the same and increasing the intensity of the stimulus resulted in a corresponding enhancement of the muscular response.

It may be supposed that increasing the intensity of peripheral stimulation results in the production of more numerous impulses in the median giant fibre and this was confirmed by stimulating the mid-dorsal body wall with shocks of increasing intensity as before and recording the giant fibre impulses from a short length of the nerve cord exposed behind the clitellum.

The results (Fig. 6) indicate that a single shock of sufficient strength evokes a burst of impulses at a frequency of between 140 and 180 per sec. It is clear that the number of impulses in the burst depends on the intensity of the stimulus, a weak shock producing only one or two, and a strong shock as many as eleven impulses. The relation between stimulus intensity and the number of impulses produced was explored in a variety of preparations and it was found that the intensity required to evoke a single impulse corresponded to the intensity required to produce the smallest contractions in the muscle records.

DISCUSSION

In the giant fibre system of the earthworm a single impulse evokes an 'all-or-nothing' twitch in the longitudinal muscle. However, the response is very small and in normal circumstances the muscle gives larger contractions by receiving more than one impulse. In *Myxicola*, by contrast, the response resulting from a single giant fibre impulse is large and repetitive discharge of impulses in the giant fibre is rare (Roberts, 1962*b*). Preliminary observations suggest that the latter situation also exists in a number of other

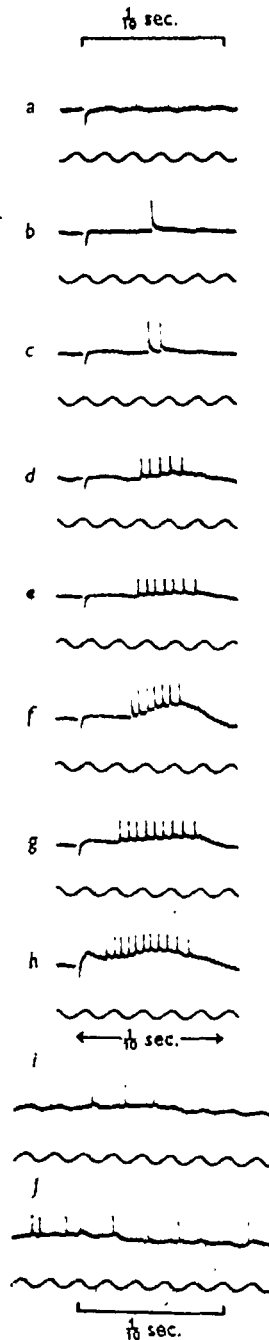


Fig. 6. *L. terrestris*. Median giant fibre impulses in response to stimulation of anterior mid-dorsal body wall with single condenser shocks of increasing intensity. Upward-projecting action potentials preceded by a downward-projecting stimulus artifact. Lower traces show 50 cycle hum. Potentiometer readings (intensity scale): a, 2.0; b, 2.5; c, 3.0; d, 3.3; e, 3.5; f, 3.7; g, 4.0; h, 5.0; i and j, brief mechanical stimuli.

tubicolous polychaetes such as *Branchiomma* (Sabellidae), *Hyalinoecia* (Onuphidae), *Filigrana* (Serpulidae) and a number of eunicids and terebellids. Bullock (1948) implies that, with tactile stimulation of the body wall, single giant fibre impulses are transmitted in *Diapatra* (Onuphidae), *Lepidametria* (Polynoidae) and several other forms, and these produce extensive escape responses. This situation is not confined to annelids. Young (1938) has shown that a single shock above threshold applied to the stellar nerve of *Loligo* produces a very large twitch of the circular muscle fibres of the mantle, further increase in the intensity of stimulation not leading to larger responses. Since each stellar nerve contains one giant axon it seems likely that the large contractions are the result of single impulses transmitted in these.

The position in the earthworm contrasts sharply with this. Here a single giant fibre impulse evokes a very small twitch and repetitive impulse discharge, as normally produced by peripheral stimulation, is necessary for the production of larger responses. A similar situation appears to exist in *Nereis* in which graded escape responses can be observed and repetitive discharge of impulses frequently occurs when the animal is stimulated peripherally. Bullock (1948) has recorded repetitive impulses in the bamboo worm, *Clymenella torquata* (Maldanidae), and in the errant burrower, *Haploscoloplos bustorus* (Orbinidae) in which a weak stimulus frequently evokes a long-lasting, high-frequency discharge in the faster-conducting giant fibre. As a broad generalization it seems that single impulses are transmitted in the giant fibres of tubicolous polychaetes whereas repetitive discharge is characteristic of the oligochaetes and errant polychaetes.

The adaptive value of repetitive discharge would seem to be that it enables the animal to grade its escape reaction. This is important for errant and burrowing forms whose life habits would be expected to bring them into contact with a variety of tactile and other stimulus situations, many of them unimportant and not requiring a maximum withdrawal response. The tubicolous habit of a polychaete such as *Myxicola*, however, would render graded responses unnecessary and in such cases a large 'all-or-nothing' contraction would seem to be the more appropriate escape response.

SUMMARY

1. A nerve-muscle preparation including the longitudinal musculature and the giant fibres in the nerve cord of the earthworm is described.

2. Direct stimulation of the nerve cord with single shocks of increasing intensity results in two types of response: (a) a low threshold, very small twitch, resulting from a single impulse in the median giant fibre, and (b) a higher threshold, slightly larger twitch, resulting from single impulses in the median and lateral giant fibres. Both responses are highly susceptible to fatigue.

3. Stimulation of the body surface evokes a much more powerful contraction which is associated with a burst of impulses in the giant fibre. The strength of the contraction depends upon the number of impulses in the burst and this in turn upon the intensity and duration of the stimulus.

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