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THE RAPID ESCAPE RESPONSE OF THE EARTHWORM *LUMBRICUS TERRESTRIS L.*: OVERLAPPING SENSORY FIELDS OF THE MEDIAN AND LATERAL GIANT FIBRES

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SUMMARY

1. In undissected, freely mobile earthworms the sensory input for tactile stimulation to the MGF and LGF shows a region of overlap occupying a number of segments behind the clitellum. The average number of overlapping segments from a sample of 14 worms was 13 (range 0–28).

2. The overlap zone consists of segments from which both LGF and MGF spikes can be elicited.

3. Increasing stimulus intensity in the LGF field reduces the latency of the first spike until it reaches a minimum of 6 ms. This value is used to calculate conduction velocity of afferent impulses along the sensory pathway.

4. It is suggested,on the basis of conduction velocity, that the large sensory neurones in the segmental nerves are those mediating afferent events in the rapid escape response.

INTRODUCTION

A rapid escape response occurs in animals from several phyla, for example the Annelida, Arthropoda and Mollusca. It has been well studied in the Annelida using the common earthworm, *Lumbricus terrestris* L. (Bullock, 1945; Rushton, 1946; Roberts, 1962*a*, *b*, 1966; Günther, 1972; Drewes, Landa & McFall, 1978). A focal point of these studies has been the activity, during this response, in the single median giant fibre (MGF) and the paired lateral giant fibres (LGF) of the ventral nerve cord.

One of the notable features of the escape response is that the associated muscular contractions are mediated from the anterior end by the MGF and from the posterior end by the LGF, (Bullock, 1945; Rushton, 1946). This apparent polarity of the giant fibres depends on the fact that the sensory connexions to the MGF occur only anteriorly whilst those to the LGF occur only posteriorly (Bullock, 1945; Rushton, 1946). An overlapping region between these two sensory fields has been reported but not previously described in any detail. The sensory fields of oligochaetes have been tested for only one other species, that of the Australian earthworm, *Megascolex* (Adey, 1951). He did not test specifically for overlap and reported an abrupt change over around segment 60 in worms of between 150–200 segments.

MATERIALS AND METHODS

Mature earthworms of the species *Lumbricus terrestris L.* were obtained from Gerrard Biological Supplies and kept at normal outdoor temperatures in fresh earth. Experiments were carried out at 21–23 °C. All records were obtained from intact animals, unrestrained except by the physical limits of the recording apparatus. This consisted of a rectangular box 20 cm long by 1 cm deep by 1 cm wide, the floor of which was interrupted by transverse recording wires at 5 mm intervals. Recordings could therefore be made from an adjacent pair of wires opposite any region of the worm, as the animal lay quiescent along the length of the box. The recording leads were taken to a unity gain amplifier (WPI Model 750), a Fenlow preamplifier and Telequipment DM63 storage oscilloscope. Photographs were taken either with a Polaroid camera or with a Cossor oscilloscope camera loaded with Kodak Ortho film. An audio amplifier enabled activity to be monitored aurally.

Mechanical stimulation was effected using a sharp brass needle fitted to a Derritron solenoid, solidly mounted on a Palmer type stand. Thus two types of movement were possible. Firstly, a rapid and constant 2 mm excursion of the needle – defined as the stimulus – which lasted for the duration of an applied current. Secondly, the starting position from which the stimulus was delivered could be established any distance from the worm by moving the solenoid up or down using the screwed stand. The solenoid was positioned so that the needle moved vertically and throughout the investigation the duration of the stimulus was maintained at 4 ms.

Exact quantification of this type of stimulus is difficult but the following standard procedure was adopted to establish a starting position for stimulation. The current was switched on to protrude the needle 2 mm and in this position it was lowered under a 10× binocular microscope until it just made contact with the cuticle of the worm directly above the central circumferential groove in the midline of the segment being stimulated. The current was switched off to retract the needle. If any movement of the animal occurred, the standard procedure was repeated to re-establish the starting position. If stimulation from this position did not evoke any response, the solenoid was gradually lowered until a response was just evoked. This was defined as a threshold stimulus. From this position the intensity of the stimulus could be increased by further lowering of the solenoid.

In experiments where a series of stimuli were delivered of increasing intensity, the solenoid was lowered 0·5 mm each time.

Fourteen worms were used for the investigation, one of which died through accidental damage before measurements were completed.

In all records the stimulus is indicated by a small circular dot and the segment numbers at which stimulation (S) and recording (R) were carried out are indicated at the beginning of the Figure legend. In Table 1 a different numbering system is used because of the variability of the clitellum position, which usually occurs approximately between segments 32–37.

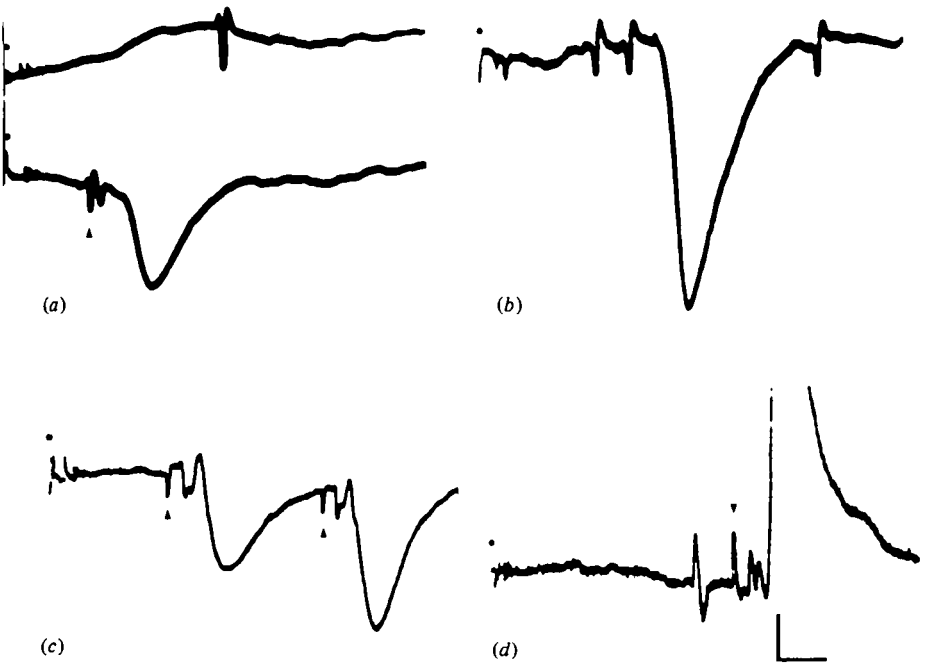

Fig. 1. Records from intact worms. (*a*) Upper trace, S76.R40. Threshold stimulus in territory served exclusively by the LGF elicits a single triphasic potential (see text for interpretation). Lower trace, S20.R40. As upper trace but stimulus in territory served exclusively by the MGF. The complex potential recorded is described in the text, but the arrow denotes the spike in the MGF. (*b*) Another preparation, S70.R40. Above threshold but not maximal stimulus. Three LGF spikes are seen with a much larger muscle potential between the second and third giant fibre spikes. (*c*) S20.R40. Same as (*b*) but in MGF territory. The MGF fires twice (arrows) with associated potentials (see text). Notice that the second muscle potential is larger and faster than the first. (*d*) S40.R85. Same as (*b*) but in the overlap zone. The first spike is from the LGF, the second is from the MGF (arrow) and the large body wall potential is not wholly shown. Scales for all records: 500 μV, 5 ms.

ABBREVIATIONS

GMN 1, giant motor neurone one. The specific motor neurone synapsing with the MGF centrally and the body wall musculature peripherally (Günther, 1972).

GMN 2, giant motor neurone two. The same as GMN 1 but synapsing centrally with the LGF.

RESULTS

Giant fibre action potentials can readily be recorded from intact worms (Rushton & Barlow, 1943), as can potentials from the body wall musculature (Drewes *et al.* 1978). The interpretation of externally recorded waveforms such as those presented in this study is based on the work of the latter authors, which accords with known morphological details of the neurones in the ventral nerve cord and segmental nerves of the earthworm (Günther & Walther, 1971; Günther & Schürmann, 1973), namely that stimulation of the posterior region of the worm elicits spikes attributed to the LGF whilst stimulation of the anterior region of the worm elicits a complex (and more variable) waveform made up of three separate electrical potentials. In order, these

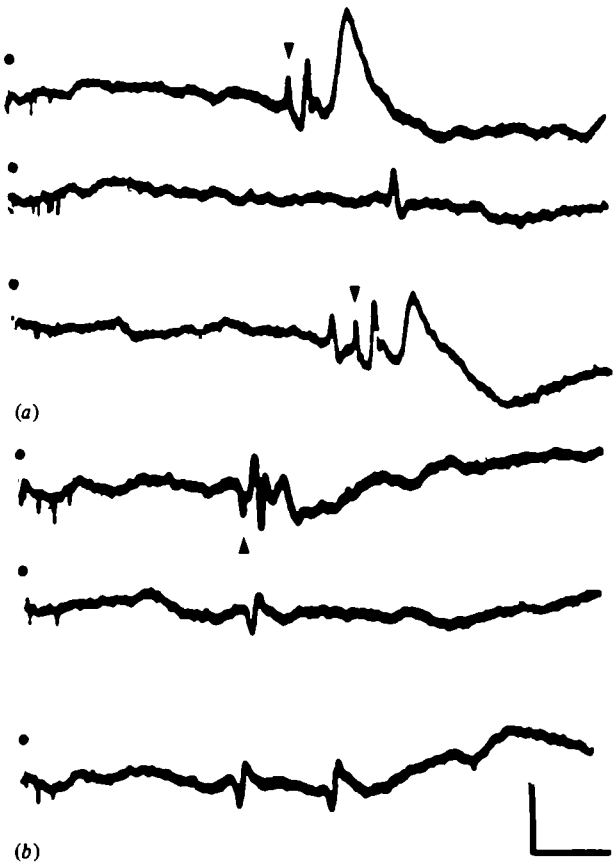

Fig. 2. Characteristics of the anterior and posterior change over points. (*a*) Anterior, upper trace, S6.R81. A complex potential evoked by the MGF is seen after a threshold stimulus. Middle trace, S7.R81. A single LGF spike is seen after a threshold stimulus. Notice that this is one segment posterior to the record above. Lower trace, S7.R81. Same as middle trace but an above threshold stimulus. In this case one LGF spike has been evoked followed by one MGF spike (arrow). (*b*) Posterior, upper trace, S35.R76. A threshold stimulus elicits an impulse in the MGF (arrow). Middle trace, S36.R76. One segment posterior to the above record, a threshold stimulus elicits a single LGF spike. Lower trace, S36.R76. Stimulus of greater intensity to same segment as above (middle trace) elicits two spikes, both from the LGF. Notice the reduced latency of the first spike. Scales for all records: 500 μV, 5 ms.

represent (i) a spike from the MGF, (ii) a spike from GMN 1 and (iii) a potential generated by the body wall musculature. The MGF spike is marked by a small arrowhead in every appropriate record in the results given here.

The reason why the MGF spike is always followed by a visible GMN 1 spike whilst the LGF does not give a GMN 2 spike is not clear (see below for Discussion) but it provides a most convenient and reliable marker by which the two giant fibre spikes may be distinguished.

In verification of much previous work it was found that stimulation at the posterior end elicits LGF spikes whilst stimulation at the anterior end elicits MGF spikes. For a variable distance behind the clitellum however, there is a zone of overlap where both MGF and LGF fire together (Fig. 1 *d*). Threshold stimuli evoke single giant fibre spikes in all regions outside the overlap zone (Fig. 1 *a*), but stimuli of greater intensity

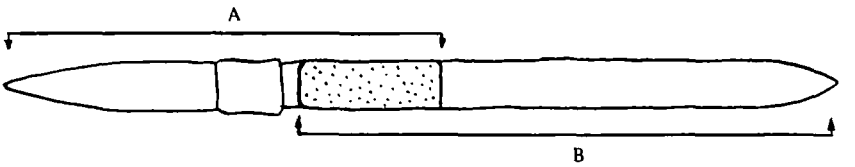

Fig. 3. Diagram of the overlapping zone. (A) The territory served by the median giant fibre; (B) that served by the lateral giant fibres. The stippled region behind the clitellum is the overlapping zone where either giant fibre type may fire after tactile stimulation. In the worms tested the clitellum was never found to have sensory connections to the lateral giant fibre.

Table 1. *Overlap of MGF and LGF fields in individual worms. Because the clitellum does not occur between the same segments in all worms numbers in this table refer to segments behind the clitellum*

Worm no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Most anterior segment eliciting LGF response	8	6	12	7	1	2	1	7	6	8	10	19	16	8
Most posterior segment eliciting MGF response	23	22	20	35	20	25	12	14	25	16	22	20	15	20
No. of segments behind clitellum	97	85	88	109	64	93	93	73	106	72	105	111	95	88
No. of overlapping segments	15	16	8	28	19	23	11	7	19	8	12	0	0	12

evoke more than one (Fig. 1 *b*, *c*). The two changeover points, one anterior and one posterior, occur in both cases at adjacent segments and their characteristics are shown in Fig. 2. These points do not vary upon increasing the stimulus intensity. The zone is shown diagrammatically in Fig. 3.

The extent of the overlap is consistent in a given worm from day to day (maximum tested, 4 days) but a variable number of segments is involved in different worms. The results are summarized in Table 1. It was notable, in the majority of cases in the overlap zone, that the LGF would fire at threshold. Variations of this general observation were that the MGF and LGF spikes would be elicited together and could not be separated, or that the MGF would fire alone at threshold (Fig. 2 B, upper trace).

In most cases, the MGF dropped out first upon repeated stimulation, as shown in Fig. 4. Threshold response is of both spike types but the following identical (as near identical as testing conditions permitted) stimuli have produced only LGF spikes. The reason for believing that nearly identical stimuli have been applied is that in a series of stimuli such as that shown in Fig. 5 the latency of the first evoked spike is quite a sensitive indicator of stimulus strength. Nevertheless it is noted that no certainty can be expressed about this proposition. In Fig. 4 the LGF appears after a nearly constant latency in each case.

If stimuli of increasing intensity are applied to segments posterior to the overlap zone, the resultant response is of the kind shown in Fig. 5. As the intensity is increased the latency of the first spike becomes shorter until more than one is produced and finally multiple spikes appear with associated muscle potentials. A muscle response of the size shown by this record does not produce a visible movement of the worm. It is difficult to obtain similar series for the MGF because even the small muscle contractions produced by one or two spikes lead to movement by the worm, with consequent impossibility of producing a regular series of stimuli of increasing magnitude.

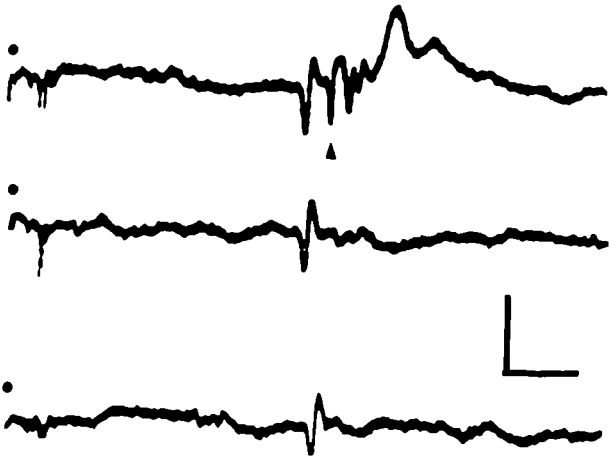

Fig. 4. Effect of threshold stimulus applied repeatedly to a single segment. Upper trace, S52.R40. Stimulus evokes a single LGF spike followed by a single MGF spike (arrow). Middle trace, S52.R40. Stimulus of same intensity as above applied 10 s later. Only the LGF spike is recorded. Lower trace, S52.R40. As middle trace but 10 s later. A single LGF spike is evoked. Scale for all records: 500 μV, 5 ms.

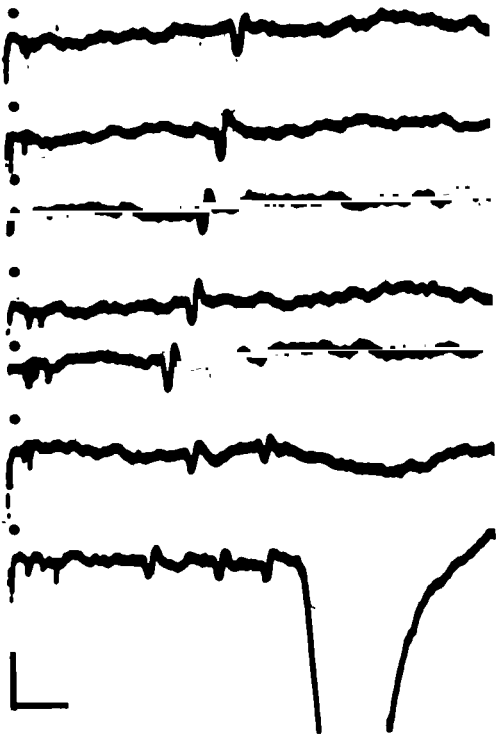

Fig. 5. Increasing intensity of stimulus to a single segment which is served exclusively by the LGF. S95.R40. The stimulus intensity was increased from the first trace to the seventh. The single LGF spikes in the first five traces showed reduced latency. On increasing intensity still further more than one spike is evoked until a muscle response is seen (seventh trace). The slightly increased latency in trace six could have been caused by a small movement of the worm not visible to the eye. The recording electrodes were 7 cm away from the point of stimulation and the latency of the first spike in the seventh trace is 13 ms. The circumference of the worm at the point of stimulation was 2 cm. (see text for details). Scale for all records: 500 μV, 5 ms.

In all records the latency can be divided into two components: (i) time taken for the impulse to travel from the point of stimulation to the sensory/giant fibre junction and (ii) time taken for conduction along the giant fibre to the recording electrode.

In the present experiments the conduction velocity of the LGF was about 1 cm.ms^{-1} and about 2·5 cm. ms^{-1} for the MGF. This is in agreement with previously published estimates (Rushton, 1945; Günther, 1976; Drewes *et al.* 1978). Exact figures are difficult because in an intact worm the length of nerve cord over which velocity has been measured cannot be determined with certainty. Whenever the animal assumes a resting length shorter that the possible maximum the velocity is likely to be underestimated. From this 13 ms latency (Fig. 5, lowest trace) therefore, 7 ms is accounted for by the LGF conduction, leaving 6 ms for the sensory pathway. This latter value was found to be the same (or less) for many records. The same time is required in the case of the MGF (Fig. 1*a* lower trace), using the figure of 2·5 cm.ms^{-1} for calculating the time taken to travel the 7 cm to the recording electrode.

Since the stimulus was applied in the dorsal midline and occupied a very small area, the distance travelled in 6 ms must closely approximate to half the circumference of the worm (measured as 2 cm). A calculation for conduction velocity gives approximately 2 m.s^{-1}.

DISCUSSION

External records from intact earthworms were first reported by Rushton & Barlow (1943) and later by Drewes *et al.* (1978). One puzzling feature of their recordings and those presented here is the absence of a GMN 2 spike, after activity in the LGF. Like the GMN 1 spikes, they may be easily recorded by electrodes in direct contact with the segmental nerves. If the giant motor neurones are of comparable size and position in the segmental nerves a GMN 2 spike should be recorded before the onset of muscle activity. According to Günther (1972) there are three neurones in the category GMN 1 but only one (which bifurcates) in GMN 2. This may be sufficient to reduce the current density to a level where externally recorded potentials cannot be made. An alternative explanation could be found in the architecture of the GMN 2 neurones, which bifurcate in the nerve cord, one branch turning forwards and one backwards, which might lead to a cancelling effect by the action currents. An explanation of this absence must await further investigation.

Two worms in the present series appeared to have no overlap. Both were very large diameter, not very sensitive and responses were difficult to evoke. Possibly the LGF response nearer the clitellum was being masked because strong stimuli were needed to evoke any response at all.

An anatomical basis for the change over may reside in the morphology of the afferent connexions, which differ for the LGF and MGF. Günther & Walther (1971), in a detailed description of the ventral cord and segmental nerves, could detect no abrupt change of sensory connexions in this region. However, Günther & Schürmann (1972) found that the afferent connexions to the LGF probably occurred via its collaterals which make contact with sensory fibres in the ventrolateral part of the cord. These collaterals were better developed in the posterior region of the worm. Describing the MGF, the same authors found that afferent connexions were made indirectly via intrasegmental interneurones and that these were better developed anteriorly.

Clearly, there exists the possibility on morphological grounds for both giant fibre types to receive information from all parts of the worm, but the afferent polarization is probably in part accounted for by these structural differences.

Günther & Walther (1971) first drew attention to the specific fast giant motor neurones concerned with the escape response. These had a conduction velocity of over 1 $m.s^{-1}$ and were identified morphologically within the segmental nerves. They also demonstrated large afferent fibres but did not implicate these in the escape response. It seems reasonable to propose that a response utilizing a fast intermediate (MGF and LGF) and a fast efferent (GMN 1 and GMN 2) pathway might have fast afferent pathways. The present results strongly suggest this. The figure of nearly 2 $m.s^{-1}$ calculated in the results for the sensory fibres is considerably in excess of the 0·04–0·08 $m.s^{-1}$ previously reported by Prosser (1935). It is close to the figure given by Günther (1971) for the giant motor neurones. Thus it seems likely that the large sensory fibres which have been reported by Günther & Walther (1971) are involved in the mediation of the escape response.

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