J. exp. Biol. (1978), **72**, 217–227
With 5 figures
Printed in Great Britain

GIANT NERVE FIBRE ACTIVITY IN INTACT, FREELY MOVING EARTHWORMS

BY C. D. DREWES, K. B. LANDA* AND J. L. McFALL
Zoology Department, Iowa State University
Ames, Iowa, U.S.A. 50011

(*Received* 20 *June* 1977)

SUMMARY

1. An approach is described for recording and characterizing giant nerve fibre activity in intact, freely moving earthworms.

2. Medial giant fibre (MGF) spikes were conducted in an anterior–posterior direction at a mean rate of 32·2 m/s; lateral giant fibre (LGF) spikes were conducted in a posterior–anterior direction at a mean rate of 12·6 m/s.

3. Rates of giant fibre spike conduction and maximal frequencies of firing (up to 500/s) in intact animals were higher than values previously reported in isolated preparations.

4. MGF spikes were followed 1:1 by presumed giant motor axon spikes and facilitating muscle potentials.

5. Single MGF or LGF spikes evoked by applying tactile stimulation were not accompanied by longitudinal contraction, but a series of two or more MGF spikes or three or more LGF spikes were accompanied by such contractions.

6. MGF and LGF spikes occurred infrequently during locomotory movements in the absence of any experimenter-applied stimulation, suggesting that sensory inputs associated with normal locomotion over an irregular substrate are sufficient to excite giant fibres.

INTRODUCTION

Many animals escape from predators or noxious stimuli by means of specialized and stereotyped locomotory responses. In some arthropods and annelids these responses are very rapid, being mediated by giant nerve fibre activity that is well suited for electrophysiological recording at the level of individual nerve cells (Wiersma, 1947; Nicol, 1948; Kennedy, 1966; Zucker, 1972*a*, *b*; Parnas & Dagan, 1972). The recording of such activity, however, usually requires procedures (e.g. immobilization, dissection, and exposure to physiological saline solutions) which are invasive and potentially disruptive to both the neural functioning and behaviour being studied.

In some arthropods progress has been made in developing minimally invasive methods (e.g. implanted and tethered electrodes) for recording single neural unit activity and for correlating this activity with locomotory or escape behaviour (Stout, 1971; Delcomyn, 1976; Schrameck, 1970; Wine & Krasne, 1972). Also, in the marine

* Present address: Neurosciences Department, University of California – San Diego, La Jolla, California 92093.

annelid *Branchiomma vesiculosum* (Polychaeta), Krasne (1965) has non-invasively recorded giant axon and muscle responses to tactile stimulation using a pair of wire electrodes threaded into the animal's tube. The possibility of recording neural activity from undissected earthworms was first demonstrated by Rushton & Barlow (1943). Their brief study was restricted to recording, through the skin of restrained earthworms, the medial giant fibre response to an electrical shock. They also limited their recording to a single, central location on the worm.

In this study we have developed non-invasive methods for recording giant nerve fibre activity at two selected sites from unrestrained earthworms. This has permitted study of various giant fibre parameters *in situ* as well as correlation of giant fibre activity with locomotory and escape behaviour.

MATERIALS AND METHODS

Recordings from intact animals

Mature earthworms, *Lumbricus terrestris* L., were obtained from Mogul Corp. (Oshkosh, Wis., U.S.A.) and maintained at 15 °C in Buss Bed-ding (Buss Mfg., Lanark, Ill., U.S.A.). All experiments were carried out at 21–22 °C.

The experimental apparatus for recording giant fibre activity from intact, freely moving animals is shown in Fig. 1. Two concentric vinyl rings (78·1 and 81·9 cm circumferences; 5 cm high) formed a circular track (mean circumference 80·0 cm). The rings rested on a radiating array of 40 pairs of silver wire electrodes (0·405 mm diameter), each pair being spaced 20 mm apart. A nearly optimal signal: noise ratio for giant fibre spikes was obtained when the electrodes in a pair were spaced 3 mm apart. The electrodes rested on the track surface which consisted of wet-dry sandpaper (fine grit). This surface provided traction for the animal and was moistened with distilled water to prevent desiccation of the animals. The entire apparatus was mounted on sponge rubber to absorb extraneous vibrations.

Recording electrode pairs (numbered 1–40) were selected via a two-level switching system which permitted us to simultaneously record from two adjacent sites as the animal moved around the track. The first level of switching consisted of eight 10-position single-contact rotary switches allowing selection of two even-numbered and two odd-numbered electrode pairs. The second level consisted of two double-pole, double-throw toggle switches allowing the final selection of one odd-numbered and one even numbered pair of electrodes. Signals from each toggle switch were recorded differentially and amplified with a Grass P-15 preamplifier. Outputs from the two preamplifiers were led into separate channels of a Tektronix 5103N storage oscilloscope. All recording apparatus, except the oscilloscope, were contained in an electrically shielded cage. Neural spikes were easily identifiable on the basis of duration ($< 1{\cdot}5$ ms) and consistent amplitude during repetitive discharge. Optimal signal : noise ratios (up to 8:1) for giant fibre activity were obtained with a high-frequency filter setting of 1 or 3 kHz and a low-frequency filter setting at 300 Hz. Stored records of giant fibre activity were photographed either as recurrent, superimposed sweeps (e.g. Fig. 5) or as single sweeps triggered by the initial phase of the first giant fibre spike (e.g. Figs. 2–4).

Tactile stimulation was applied (> 5 min intervals between tests) by touching the

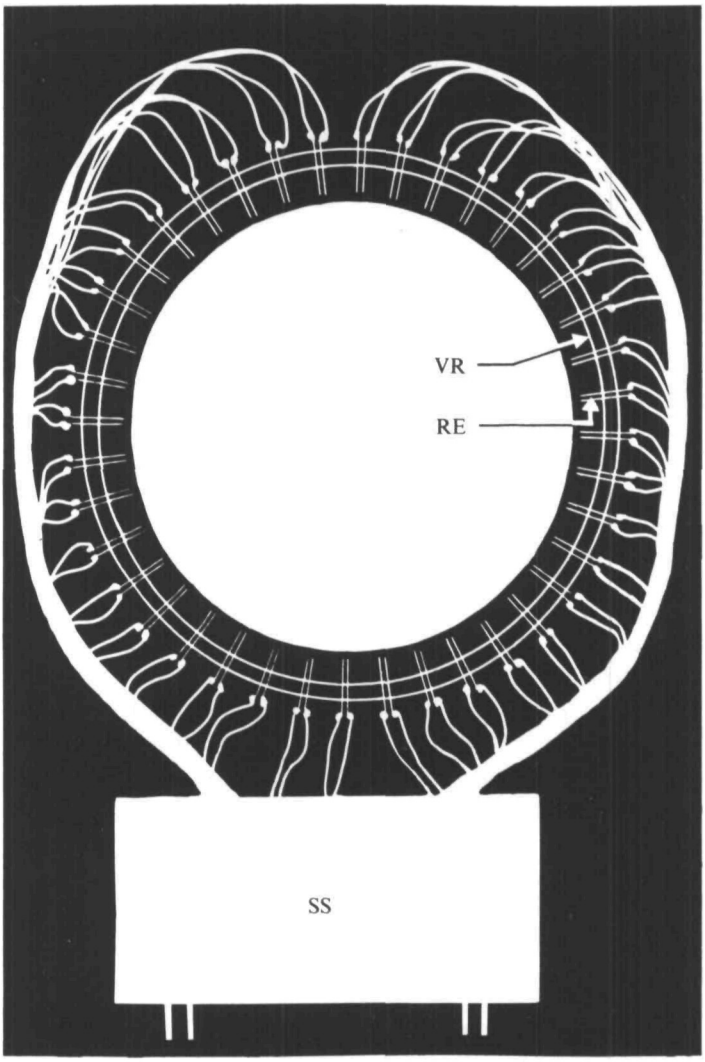

Fig. 1. Top view of apparatus for recording activity of giant fibres in intact earthworms. Animals were placed in between two concentric vinyl rings (VR) and were allowed to move freely over a radiating array of 40 pairs of silver-wire recording electrodes (RE). Giant fibre activity could be recorded from any two sites selected by the switching system (SS). Signals were led from the switching system to two pre-amplifiers.

animal with a hair mounted on a glass rod. Quantitative control of the stimulus strength was not necessary for the puposes of this study; after a few trials we found that with subjective control of stimulus strength we could elicit predictable giant fibre responses. No diminution of giant fibre responsiveness occurred during the usual testing period (4 h) and it appeared that testing could have continued for a considerably longer time.

Recordings from dissected animals

In some experiments it was necessary to correlate giant fibre activity in undissected and dissected portions of the same animal. Dissected preparations were obtained by

Table 1. *Conduction rates of MGF and LGF spikes in intact earthworms*

	MGF			LGF		
Animal no.	Mean conduction rate (m/s)	S.D. (m/s)	No. of measurements	Mean conduction rate (m/s)	S.D. (m/s)	No. of measurements
1	38·1	5·1	24	13·7	2·0	21
2	31·4	6·8	17	13·4	1·8	13
3	29·2	4·7	13	11·4	2·2	19
4	29·7	7·5	17	11·7	2·8	12
5	29·7	4·1	6	11·4	1·6	16
6	36·1	5·8	23	13·6	2·1	16
7	30·1	4·9	17	12·8	2·7	19
8	33·5	4·4	16	11·9	1·5	18
9	32·2	5·3	23	13·7	2·1	21
10*	33·3	—	1	15·5	2·0	3
	Grand mean (m/s)	S.E.M. (m/s)	*N*	Grand mean (m/s)	S.E.M. (m/s)	*N*
	32·2	1·0	9	12·6	0·3	9

* Values not used in calculation of grand mean and S.E.M.

pinning down approximately 20 segments in the middle of the worm, exposing the ventral nerve cord in this region, and adding several drops of physiological saline (Drewes & Pax, 1974). Recordings of ventral nerve cord activity were obtained at the air–saline interface using a pair of silver-wire hook electrodes. Recordings were simultaneously obtained from the undissected posterior end of the same animal by looping the body over a similar pair of electrodes. Activity from each recording site was amplified and displayed as described in the previous section.

RESULTS

Electrical activity was recorded from ten intact and freely moving animals in both the presence and absence of experimenter-applied stimulation. Two types of spiking activity were clearly distinguishable on the basis of spike conduction rate and direction of spike propagation. Of 314 spikes recorded, approximately one half were conducted in an anterior–posterior direction at a grand mean rate of 32·2 m/s ± 1·0 S.E.M. (Table 1). The other spikes were conducted in a posterior–anterior direction at a grand mean rate of 12·6 m/s ± 0·3 S.E.M. These two rates are within the broad ranges given for the medial and lateral giant fibres, respectively, of isolated nerve cords in earthworms (Bullock, 1945; Bullock & Horridge, 1965). Also the opposite directions of spike conduction correspond to those of medial and lateral giant fibres of dissected preparations (Stough, 1930; Rushton, 1946; Bullock, 1945). This suggests that the activity we have recorded somehow involves the medial and lateral giant fibres.

Medial giant fibre activity

Activity evoked by tactile stimulation of the anterior few segments of intact animals was always conducted in an anterior–posterior direction at a conduction rate corresponding to that of the medial giant fibre (MGF). The activity had a complex waveform, always consisting of two similar monophasic spikes separated by an interval of

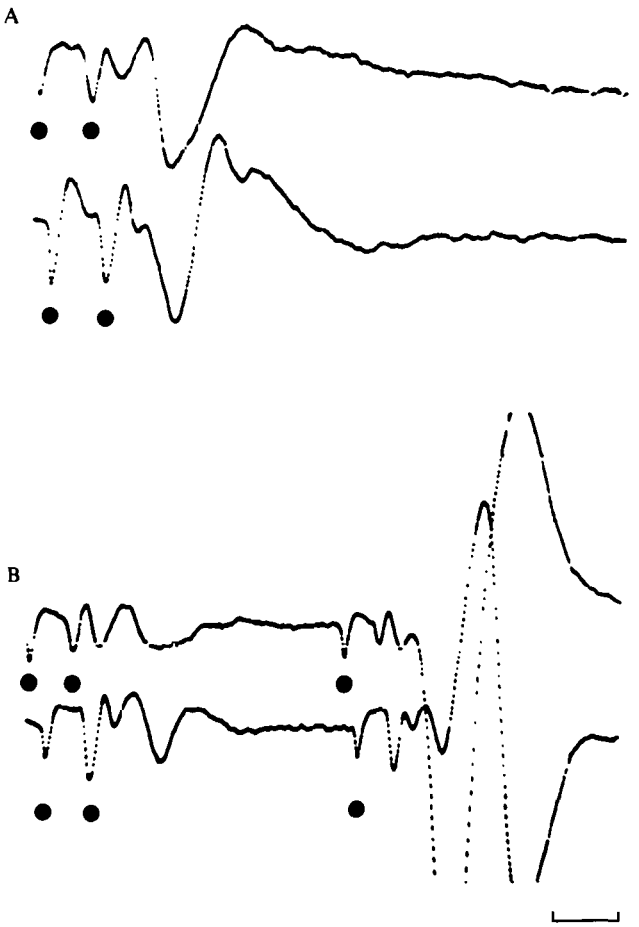

Fig. 2. Responses of the medial giant fibre (MGF) to tactile stimulation of the first few anterior segments. In each record the recording site for the upper trace was located 20 mm anterior to the site for the lower trace. (A) A light tactile stimulus evoked a response consisting of two spike potentials and a slower multiphasic potential. The initial MGF spike (first dot in each trace) was followed approximately 1·8 ms later by a second spike (second dot in each trace), probably from giant motor axons (Gunther, 1972). The larger, slower potential was probably of muscular origin. (B) With strong tactile stimulation two MGF spikes were evoked (first and third dots). Each MGF spike was followed by a motor axon spike (second dot). The slow potentials, presumably of muscular origin, showed marked facilitation in B. This response was accompanied by a rapid longitudinal contraction of the anterior end of the animal. Time scale: 2 ms.

1·2–2·0 ms (Fig. 2). These spikes were followed by a slower potential of variable waveform.

To demonstrate which, if any, of these potentials originated in the MGF we compared electrical recordings from undissected and dissected portions of the same animals. Responses to tactile stimulation of the head were identical whether recorded from the undissected posterior end of the worm (Fig. 3A; lower trace) or from the exposed ventral cord. If the segmental nerves were then cut close to their central connexions with cord, both the second spike and slow potential were no longer recorded from the ventral nerve cord, but were still seen in recordings from the undissected

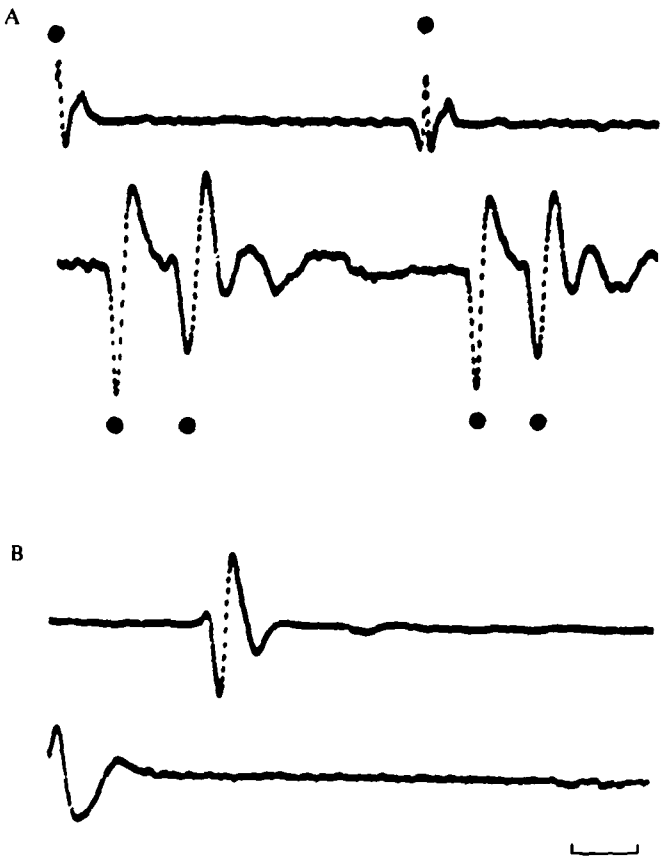

Fig. 3. Recording of giant fibre activity in partially dissected, restrained earthworms in response to tactile stimulation. (A) Strong tactile stimulation of the anterior end of the animal evoked a series of two MGF spikes. In the upper trace a pair of electrodes was in contact with the ventral nerve cord in the centrally dissected portion of the animal (all segmental nerves were severed in this region). The two spikes (dots) arise centrally from the medial giant fibre. In the lower trace a pair of electrodes simultaneously recorded activity (50 mm away) in the intact, posterior end of the animal. The two MGF spikes (first and third dots) were followed by spikes (second and fourth dots) presumably occurring in a peripheral motor axon. The smaller, slower potentials may be of muscular origin. (B) A strong tactile stimulus to the posterior end of the animal evoked a single spike, arising centrally from the lateral giant fibre. In the lower trace a pair of recording electrodes was in contact with the intact posterior end of the animal. In the upper record another pair of electrodes (50 mm away), made direct contact with the exposed ventral nerve cord in the central portion of the animal. Time scale: 2 ms.

posterior end (Fig. 3A). We infer from these results that only the first spike occurs in the ventral nerve cord and, given its rate and direction of conduction, that it arises from the MGF. The second spike appears to occur in a peripheral nerve and may represent activity in a large motor axon. Using dissected earthworms, Gunther (1972) demonstrated 1:1 coupling of MGF spikes with spikes in identifiable giant motor axons in the segmental nerves. These motor axon spikes when recorded extracellularly from a peripheral nerve had a large amplitude and followed the MGF spike by approximately 1·5 ms. This value is within the range of interspike intervals in our recordings. The

slow potential which follows the presumed motor axon spike (Figs. 2, 3A) may be that of a muscle in the body wall.

The number of MGF spikes evoked in intact animals in response to a tactile stimulus was roughly proportional to the strength of stimulation. Light tactile stimulation evoked a single MGF spike along with the presumed motor axon spike and muscle potential (Fig. 2A). In 29 tests single MGF spikes were never accompanied by observable longitudinal contraction of the animal. Stronger tactile stimulation evoked a series of two or more MGF spikes (Fig. 2B). Each series was accompanied by facilitation of the slower potentials and marked longitudinal contraction.

An interesting characteristic of MGF activity was that the second and subsequent spikes in a train were always propagated at a faster rate than the initial spike. Bullock (1951) observed similar increases in conduction rate in isolated nerve cords. He found that this 'facilitation of conduction' was maximal 6 ms after a previous spike, but was still perceptible after 100–200 ms. In our study paired MGF spikes were recorded from eight animals. The mean rate for the second MGF spike was 39·0 m/s ±2·1 S.E.M. (preceding interspike interval ⩽15 ms). Using a paired-difference *t*-test this value was significantly greater ($P < 0{\cdot}001$) than the mean rate of the first spike (mean = 31·9 m/s ±1·7 S.E.M.).

Lateral giant-fibre activity

Activity evoked by tactile stimulation of the terminal few segments of intact animals was always conducted in a posterior–anterior direction at a rate matching that of the lateral giant fibres (LGF). The activity invariably consisted of simple triphasic spikes (Fig. 4).

To demonstrate that these spikes arose from the lateral giant fibre we compared recordings from dissected and undissected portions of the same animal using procedures described in the previous section. Spikes recorded from the exposed ventral nerve cord in the central portion of the animal were identical to those recorded in the undissected posterior end (Fig. 3B). Cutting the segmental nerves had no effect on the waveform of the spikes. Therefore we conclude that the spikes occur in the ventral nerve cord and, considering conduction parameters, that they arise from the LGF.

The number of LGF spikes evoked in intact animals by tactile stimulation was roughly proportional to the stimulus strength. With strong stimulation high-frequency trains of LGF spikes were evoked (spike frequencies up to 500/s). Single spikes, evoked in 33 tests of eight animals (Fig. 4A), were never accompanied by longitudinal contraction of the animal. In 12 tests paired spikes were evoked (Fig. 4B). In six of these tests slight longitudinal contraction was observed; in the other six no contractions were observed. Trains of three or more spikes (Fig. 4C) were invariably accompanied by large and slower potentials (presumably of muscular origin) and by rapid longitudinal contraction of the animal.

As with the MGF spikes, the second and subsequent LGF spikes in a train were propagated at a faster rate than the initial spike. In nine animals the mean rate for the second spike was 14·0 m/s ±0·4 S.E.M. (preceding interspike intervals ⩽15 ms). Using a paired difference *t*-test this value was significantly greater ($P < 0{\cdot}001$) than the rate of the initial spike (mean = 11·9 m/s ±0·4 S.E.M.).

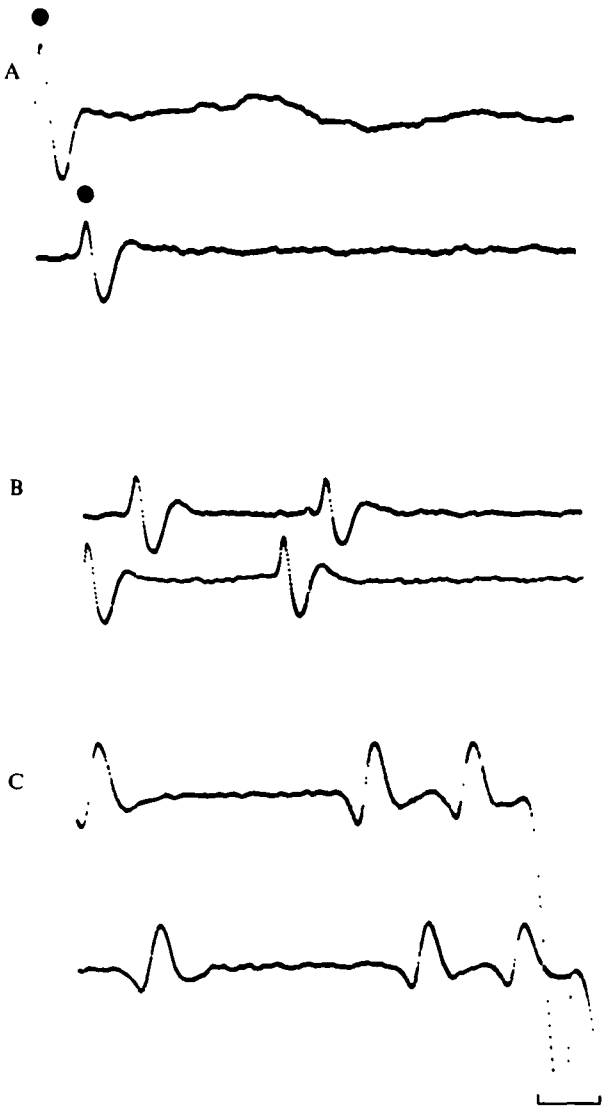

Fig. 4. Responses of the lateral giant fibre (LGF) to tactile stimulation of the tail. (A) A light tactile stimulus evoked a single spike (dot) which is recorded at two different sites in the posterior half of the animal. The recording site for the upper trace was 20 mm more posterior than that for the lower trace. (B) A tactile stimulus evoked a pair of LGF spikes, the interspike interval being approximately 7 ms. The recording site for the upper trace was located 20 mm anterior to the site for the lower record. (C) A strong stimulus evoked three LGF spikes. The recording site for the upper trace was 20 mm posterior to the site for the lower trace. In each trace the third spike was followed by a very large downward deflexion (not shown), presumably a muscle potential from the body wall. The animal responded with a rapid longitudinal contraction. Time scale: A–C = 2 ms.

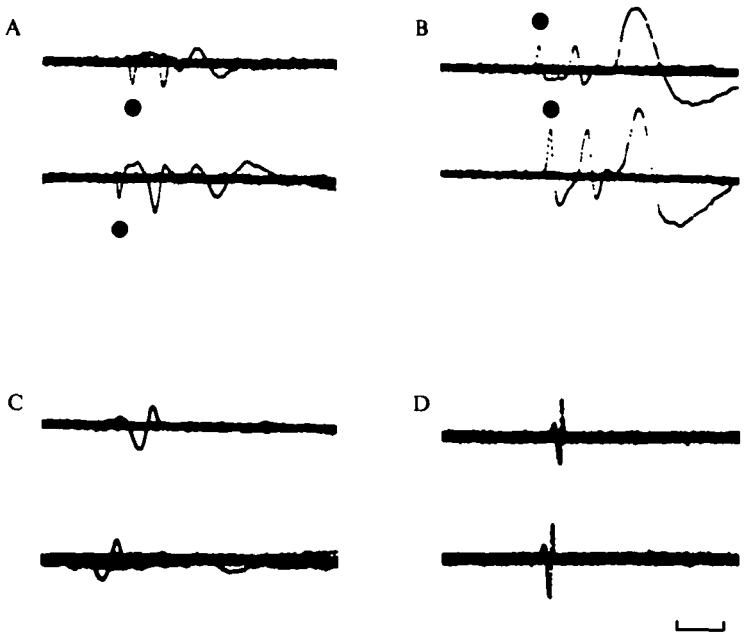

Fig. 5. Giant fibre activity in the absence of any experimenter-applied stimulation. (A, B) MGF spiking activity (dot) was recorded at two different sites (20 mm apart) during locomotory movements. The lower trace in (A) and the upper trace in (B) are from anterior recording sites. In both records the activity, consisting of a single MGF spike, was conducted in an anterior–posterior direction. (C, D) LGF activity was recorded at two sites (upper traces, anterior; lower traces, posterior). Time scale: A–C = 2 ms; D = 5 ms.

Giant fibre activity in the absence of experimenter-applied stimuli

Previous approaches have not allowed investigators to determine if giant fibre spikes occur in earthworms in the absence of experimenter-applied stimulation. The possibility that such spikes may occur during locomotor movements was tested in the freely moving animals. Approximately 15–20 min of recordings were obtained for each animal, during which both medial and lateral giant fibre spikes were seen.

A total of 49 MGF spikes were recorded (Fig. 5A, B). These usually occurred during forward peristalsis or local movements of the head, though most locomotory movements were not accompanied by MGF activity. Conduction was in an anterior–posterior direction at a grand mean rate of 30·5 m/s (± 1·4 S.E.M.). We observed 45 instances of single MGF spikes and two instances of paired spikes (interspike intervals less than 20 ms). Each MGF spike was followed by a presumed motor axon spike and muscle potential.

A total of 22 LGF spikes were also recorded in the absence of experimenter-applied stimulation (Fig. 5C, D). Conduction was in a posterior-anterior direction at a grand mean rate of 12·7 m/s (± 1·4 S.E.M.). These spikes, though infrequent, usually occurred during reverse peristalsis and rarely during forward peristalsis. We observed 18 instances of single LGF spikes and two instances of paired spikes.

DISCUSSION

Prior to undertaking any detailed investigation of how giant fibre activity is expressed in behaviour, it was necessary to characterize, for intact animals, giant fibre parameters previously described only in isolated or semi-intact preparations. We have confirmed that the difference in the direction of spike conduction, characteristic of the medial and lateral giant fibres in dissected preparations (Stough, 1930; Rushton, 1946; Bullock, 1945), also occurs in the intact animal. We have also confirmed that there are increases of up to 20% in conduction rate of closely spaced giant fibre spikes (Bullock, 1951).

Several parameters of giant fibre spikes in intact animals differ from those in dissected preparations. The conduction rates of giant fibres in intact animals (MGF, 32·2 m/s; LGF, 12·6 m/s), although within the very broad range given by Bullock (1945), are considerably greater than values given in detailed studies of conduction rates in isolated cords. Lagerspetz & Talo (1967), who have done an extensive study of the effect of temperature on giant fibre conduction rates, give means of about 16 and 9 m/s for the medial and lateral giant fibres, respectively, in isolated cords (22·4 °C). They also tabulated values obtained by other workers (range 11·2–27·8 m/s for MGF; 5·5–12 m/s for LGF). In work preparatory to this study we have obtained values similar to Lagerspetz and Talo in isolated cords from our animals. These are relatively low rates, compared to values obtained from intact animals, and may be attributed to the trauma (e.g. disruption of circulatory system or change in chemical environment) associated with dissection. Similarly, the capability of intact animals to discharge giant fibre spikes at high frequencies (up to 500/s) in response to tactile stimulation exceeds that seen in isolated cords using electrical stimulation (absolute refractory period = 2–4 ms; Bullock, 1945).

The relationship of giant fibre activity to longitudinal contraction in intact animals appears similar to that seen in dissected preparations (Roberts, 1962; 1966). Roberts's studies showed that a single giant fibre spike can evoke a very small longitudinal twitch and that large contractions are seen only in response to repetitive giant fibre discharge. Therefore, Roberts suggested that the escape response of the earthworm may be graded, its magnitude depending on the number of giant fibre spikes which occur. Our studies of intact animals support this idea. Single spikes were never accompanied by observable longitudinal contraction. Paired spikes, on the other hand, were sometimes adequate in evoking observable contractions, and three or more spikes consistently evoked such contractions.

The occurrence of single and paired giant fibre spikes in freely moving animals in the absence of experimeter-applied stimulation suggests that either sensory inputs associated with normal locomotion over an irregular substrate are sufficient to excite giant fibres or giant fibre activity may occur as part of an internally generated motor programme. The infrequency of giant fibre spikes relative to locomotor cycles argues against the latter; current data, however, are insufficient for a final determination.

This study represents the first phase of a study of neural correlates of behaviour in intact, freely moving earthworms. Further development of the apparatus will provide recording of behavioural and electrophysiological data under the control of a microcomputer. Since the recording technique allows monitoring of electrophysiological

events and behaviour for relatively long periods, it may be particularly useful in studying developmental or possible daily/seasonal variations.

We thank Y. McFall and G. Kutish for contributing their photographic expertise and Dr C. Fourtner for critical reading of the manuscript.

REFERENCES

BULLOCK, T. H. (1945). Functional organization of the giant fiber system of *Lumbricus*. *J. Neurophysiol.* **8**, 55–71.

BULLOCK, T. H. (1951). Facilitation of conduction rate in nerve fibers. *J. Physiol., Lond.* **114**, 89–97.

BULLOCK, T. H. & HORRIDGE, G. A. (1965). *Structure and Function in the Nervous Systems of Invertebrates*. San Francisco: W. H. Freeman.

DELCOMYN, F. (1976). An approach to the study of neural activity during behaviour in insects. *J. Insect Physiol.* **22**, 1223–1227.

DREWES, C. D. & PAX, R. A. (1974). Neuromuscular physiology of the longitudinal muscle of the earthworm, *Lumbricus terrestris*. I. Effects of different physiological salines. *J. exp. Biol.* **60**, 445–452.

GUNTHER, J. (1972). Giant motor neurons in the earthworm. *Comp. Biochem. Physiol.* **42**A, 967–973.

KENNEDY, D. (1966). The comparative physiology of invertebrate central neurons. *Adv. comp. Physiol. & Biochem.* **2**, pp. 117–184.

KRASNE, F. B. (1965). Escape from recurring tactile stimulation in *Branchiomma vesiculosum*. *J. exp. Biol.* **42**, 307–322.

LAGERSPETZ, K. Y. H. & TALO, A. (1967). Temperature acclimation of the functional parameters of the giant nerve fibers in *Lumbricus terrestris*. I. Conduction velocity and duration of the rising and falling phase of action potential. *J. exp. Biol.* **47**, 471–480.

NICOL, J. A. C. (1948). The giant axons of annelids. *Q. Rev. Biol.* **23**, 291–323.

PARNAS, I. & DAGAN, D. (1972). Functional organizations of giant axons in the central nervous systems of insects: new aspects. *Adv. Insect Physiol.* **7**, 95–144.

ROBERTS, M. B. V. (1962). The giant fibre reflex of the earthworm *Lumbricus terrestris* L. I. The rapid response. *J. exp. Biol.* **39**, 219–227.

ROBERTS, M. B. V. (1966). Facilitation in the rapid response of the earthworm, *Lumbricus terrestris* L. *J. exp. Biol.* **45**, 141–150.

RUSHTON, W. A. H. (1946). Reflex conduction in the giant fibres of the earthworm. *Proc. R. Soc.* B **133** 109–120.

RUSHTON, W. A. H. & BARLOW, H. B. (1943). Single-fibre response from an intact animal. *Nature, Lond.* **152**, 597–598.

SCHRAMECK, J. E. (1970). Crayfish swimming: alternating motor output and giant fiber activity. *Science, N.Y.* **169**, 699–700.

STOUGH, H. B. (1930). Polarization of the giant fibres of the earthworm. *J. comp. Neurol.* **50**, 217–229.

STOUT, J. F. (1971). A technique for recording the activity of single interneurons from free-moving crickets (*Gryllus campestris* L.). *Z. vergl. Physiol.* **74**, 26–31.

WIERSMA, C. A. G. (1947). Giant nerve fiber system of the crayfish. A contribution to comparative physiology of synapse. *J. Neurophysiol.* **101**, 23–38.

WINE, J. J. & KRASNE, F. B. (1972). The organization of escape behaviour in the crayfish. *J. exp. Biol.* **56**, 1–18.

ZUCKER, R. S. (1972*a*). Crayfish escape behavior and central synapses. I. Neural circuit exciting lateral giant fiber. *J. Neurophysiol.* **35**, 599–620.

ZUCKER, R. S. (1972*b*). Crayfish escape behavior and central synapses. II. Physiological mechanisms underlying behavioral habituation. *J. Neurophysiol.* **35**, 621–637.