NEUROMUSCULAR PHYSIOLOGY OF THE LONGITUDINAL MUSCLE OF THE EARTHWORM, *LUMBRICUS TERRESTRIS*

II. PATTERNS OF INNERVATION

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INTRODUCTION

Nearly all information regarding nerve-muscle relationships in oligochaetes is derived from experiments on earthworm longitudinal muscle. The studies of Roberts (1962*a*, *b*, 1966) have defined the role of the giant fibres in the pathway of the rapid response of the longitudinal muscle of the earthworm. The efferent side of this pathway apparently involves several giant motor neurones whose activity is coupled to giant-fibre activity (Gunther, 1972). Although these studies have demonstrated some important functional components of the reflex pathway, they have not clearly demonstrated the functional properties of neuromuscular junctions nor have they indicated the overall pattern of innervation of the longitudinal muscle.

Intracellular recordings from longitudinal muscle fibres have recently been obtained in the earthworm, *Pheretima communissima*, by Hidaka *et al.* (1969*a–c*). These investigators recorded small spontaneous depolarizing and hyperpolarizing potentials in longitudinal muscle fibres. Such potentials were also obtained with peripheral nerve stimulation. Although inhibitory and excitatory inputs to the longitudinal muscle are indicated from their studies, no clear-cut conclusions can be drawn regarding the number of motor axons present or the distribution of motor axons in the segmental nerves.

Histological investigations, although numerous, have been of little help in clearly establishing the patterns of innervation in earthworm muscle. In some studies two structurally distinct nerve endings have been identified in the longitudinal muscle (Smallwood, 1926; Rosenbluth, 1972). However, in another study Mill & Knapp (1970) found only one morphological type of nerve ending in the longitudinal muscle. Thus neither physiological nor structural investigations have established clear-cut evidence for a specific pattern of innervation in earthworm muscle.

In the previous paper we have shown that many of the difficulties encountered in previous investigations of earthworm muscle physiology may be attributed to the use of an inappropriate physiological saline which causes rapid deterioration of the nerve-muscle preparation (Drewes & Pax, 1973). When using a newly developed saline with an ionic composition corresponding more closely to that of earthworm body fluids the functioning of nerve-muscle preparations is stable. In this study we have made use of

this new saline in examining the neuromuscular physiology of the longitudinal muscle of the earthworm, *Lumbricus terrestris*.

<center>MATERIALS AND METHODS</center>

Procedures for maintaining animals and methods for electrical stimulation and recording are described in the preceding paper. The dissection and all experiments were carried out using the saline developed by Drewes & Pax (1973).

For mechanical recordings the nerve-muscle preparation consisted of a strip of body-wall muscle approximately five to six segments in length. The lateral boundaries of the strip were the dorsal and ventral midlines, thus the strip was equivalent to one lateral half of the body wall. The preparation was attached to a Plexiglass muscle clamp (Drewes & Pax, 1971), and longitudinal contractions were monitored using a micro-displacement myograph transducer (Linear core F-50, Narco Biosystems Inc.). A resting tension of 0·5–1·0 g was applied to the preparation, thus stretching the muscle strip to a length about one and a half times the resting length.

<center>RESULTS</center>

<center>A. *Responses to single stimuli*</center>

External electrical responses

External electrical responses of the longitudinal muscle to segmental nerve stimulation were recorded from 12 preparations. The suction recording electrode was applied to the surface of the muscle 2·0 mm lateral to the segmental nerve being stimulated.

Typical external potentials recorded from the longitudinal muscle are shown in Fig. 1. The number of thresholds and the time course of the responses to stimulation of segmental nerve I (SNI) were indistinguishable from those of segmental nerve II–III (SNII–III). Also no differences were seen between responses in different segments of the same preparation.

The first electrical response of the muscle to gradually increasing stimulus strengths is a single and large negative potential, usually 1–5 mV in amplitude. The mean onset latency for the response to stimulation of SNI was 4·8 ± 0·7 msec s.e., with a mean time from stimulus to peak being 8·9 ± 1·0 msec s.e. For SNII–III the mean onset latency was 4·6 ± 0·8 msec s.e., with a mean time from stimulus to peak being 8·7 ± 1·3 msec s.e.

The threshold of this large initial response was sharp and the response was all-or-nothing, thresholds ranging from 1·8 to 5·1 V in different preparations. The all-or-none threshold and smooth time course of the responses suggest that the response is mediated by a single motor axon. Also, the similarity in the time course of the response mediated by the two nerves suggests that a similar motor axon exists in each segmental nerve.

In all segments a second threshold-dependent electrical response of the muscle was also found (Fig. 1). This response was obtained at stimulus strengths ranging from 0·1 to several volts above that of the first threshold. The threshold for this second response was also sharp, the response was all-or-nothing. Each of these responses consisted of a relatively small negative potential superimposed on the declining leg of
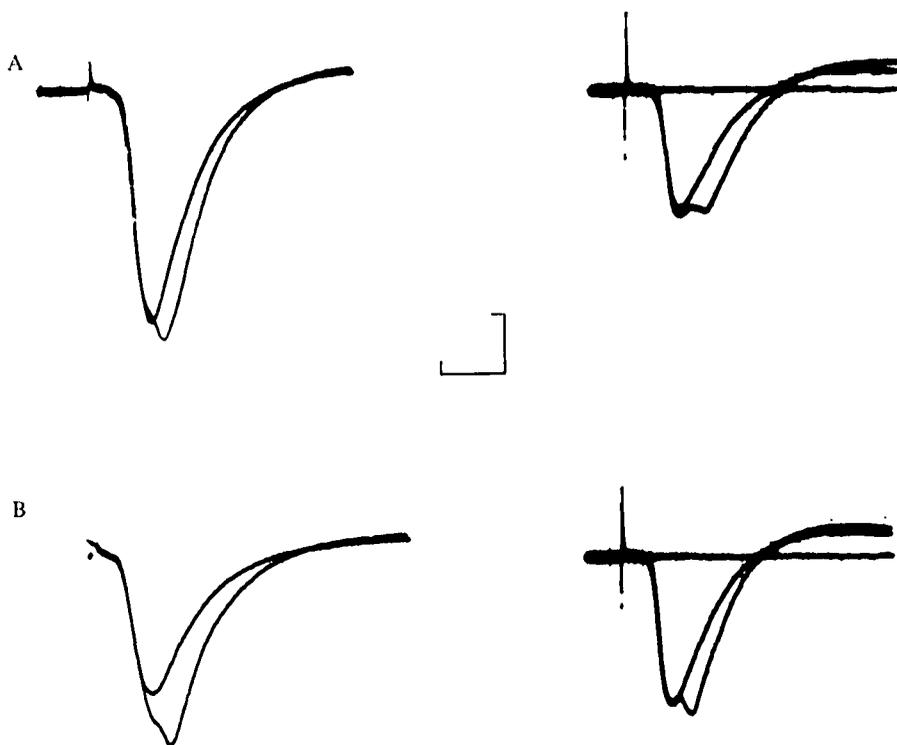
Fig. 1. External electrical responses of the longitudinal muscle to single stimuli. Upper traces (A) show responses to stimulation of SN I; lower traces (B) show responses to stimulation of SN II–III. In each record two responses are shown superimposed on one another. A large single-phased peak is recorded with stimulus strengths just above a sharp initial threshold. At higher stimulus strengths a second sharp threshold is reached, resulting in a second and later peak which is superimposed on the declining leg of the initial response. Voltage scale: 0·5 mV. Time scale: 10 msec.

the large initial peak, thus giving the appearance of a double-peaked potential. The second peak was generally reached 11–15 msec after the stimulus. No significant differences were found between the two segmental nerves with respect to the appearance of this second response.

The consistently sharp threshold and smooth appearance of this later response suggest the presence of a second, more slowly conducting, motor axon in each of the segmental nerves. For convenience and for reasons made clear later the two proposed axons will hereafter be termed fast and slow axons.

An accurate measure of the response mediated by the slow axon was difficult, since its threshold was nearly always above that of the fast axon. However, in one instance the threshold for the slow axon was slightly below that of the fast axon, thus allowing isolation and analysis of the muscle response mediated by the slow axon. In this case the electrical response consisted of a smooth negative wave approximately 300 μV in amplitude, or roughly one-tenth the amplitude of the faster response. The onset latency of the response was 10 msec with a peak at 14 msec following stimulation, about twice that of the fast axon.

In rare instances a third type of muscle response was recorded, occurring 10–50 msec
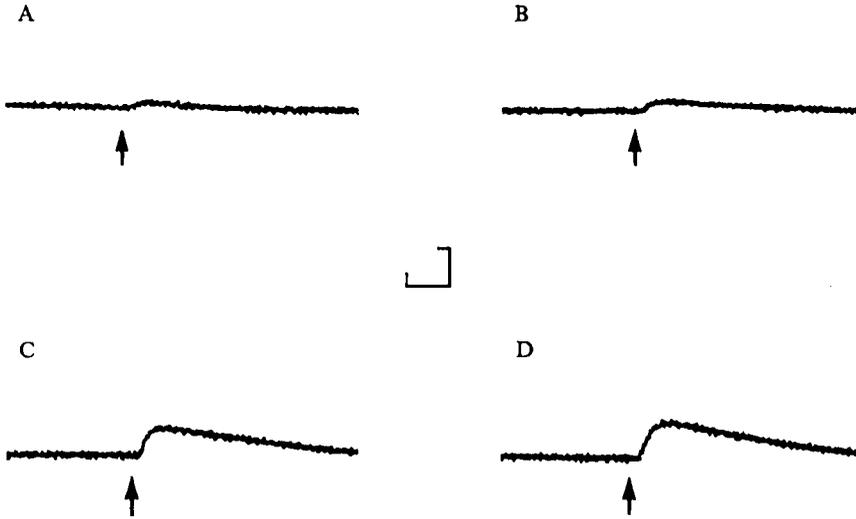
Fig. 2. Mechanical responses of the longitudinal muscle to single and paired pulses. In A a single stimulus to the fast axon in SN I resulted in a small twitch-like response. In B the single-stimulus response to fast-axon stimulation in SN II–III is shown. In C the response to paired-pulse stimulation of the fast axon in SN I is shown (stimulus interval, 15 msec). In D a similar response is obtained with paired-pulse stimulation of the fast axon in SN II–III. Vertical scale: 0·05 g. Time scale: 0·25 sec.

later than the response mediated by the slow axon. The onset latency and amplitude of this response were extremely variable. The threshold of this response was also variable and usually several volts above that of the slow axon. The erratic nature of this response suggests that it may not represent a third motor axon, but is perhaps a result of double firing of one of the two motor axons, such activity being induced by the relatively high stimulus strength.

Mechanical responses

Mechanical responses of the longitudinal muscle to single, supramaximal stimuli (that is, to stimuli which gave both the fast and the slow axon responses) were recorded in eight preparations. Typical results are shown in Fig. 2A, B. Responses always consisted of very small twitches barely measurable even at maximum transducer sensitivities. In all cases the responses were clearly visible under the dissection micro-scope, with contraction being localized in the ventromedial aspect of the longitudinal muscle of one or two segments.

An analysis of these responses was difficult because twitches were highly labile, usually becoming too small to measure after 1 h of experimentation. A further com-plication was that twitch responses were subject to rapid and long-lasting fatigue. After only two or three single stimuli to a segmental nerve responses were often completely extinguished, seldom recovering within the duration of the experiment. Consequently all records of the twitch response were taken from previously unstimulated segments and were obtained only during the first hour of experimentation.

Experiments were performed to identify which of the proposed axons (fast or slow) mediates the twitch response described above. To do this mechanical responses were

Table 1. *Analysis of mechanical responses to single-pulse stimulation of fast axon in each segmental nerve.*

	Stimulation of fast axon	
	SN I	SN II–III
Percentage of trials giving a response (N)	24 % (17)	72 % (18)
Onset latency (msec ± S.D.)	60 ± 12	59 ± 15
Peak tension (mg ± S.D.)	4·7 ± 2·4	9·5 ± 3·4
Time from stimulus to peak (msec ± S.D.)	125 ± 20	128 ± 33

correlated with external electrical responses by simultaneously recording mechanical and electrical activity from the same segment.

The threshold for the twitch response always correlated exactly with that of the fast axon; that is, when the stimulus strength was raised just above threshold for the fast axon a single and large negative potential was recorded along with a small muscle twitch. Raising the stimulus strength above threshold for the slow axon, as indicated by the presence of the second and later electrical response, contributed no measurable addition to the mechanical response. Thus it appears that the fast axon in each nerve is excitatory and mediates a twitch response of the longitudinal muscle.

A quantitative analysis of the time course and amplitude of twitch responses to single stimuli is given in Table 1. Responses to stimulation of the fast axon in SN I were smaller than those of SN II–III. In fact, in many segments twitch responses mediated by SN I were not recorded even at maximum transducer sensitivities, probably because of their small size. Stimulation of the fast axon in SN II–III, however, nearly always produced a measurable twitch. As seen in Table 1 the twitch response to stimulation of SN I in all other characteristics examined was nearly identical to that of SN II–III.

B. *Response to twin pulses*

External electrical responses

To study the possibility of electrical facilitation the fast and slow axons in the segmental nerves were stimulated using paired pulses. Four preparations were examined and in each preparation the responses to stimulation of SN I were essentially the same as those of SN II–III. Examples of responses to twin-pulse stimulation of SN I are shown in Fig. 3.

When the twin pulses were just above threshold for the fast axon but below threshold for the slow axon, a large negative potential was recorded in response to the first stimulus, while a much smaller but otherwise similar potential was recorded in response to the second stimulus. This decrement in amplitude of the second response suggests an antifacilitation of the response to fast-axon stimulation.

In contrast to the decrement of responses mediated by the fast axon, responses to slow-axon stimulation involve an apparent facilitation (Fig. 3). In these cases the stimulus strength, already above threshold for the fast axon, was raised to just above threshold for the response mediated by the slow axon. Thus the responses to slow-axon stimulation are superimposed on those mediated by the fast axon. Though there is still obvious decrement of the faster response, there is a large increase in the amplitude of the second, slower response. This increase was difficult to quantify, but generally

Fig. 3. External electrical responses to twin-pulse stimulation of SN I. Each record consists of two superimposed traces (stimulus intervals: 18 msec, left; 27 msec, right). Each record shows the single-phased responses to fast-axon stimulation, in each case the response to the first stimulus being larger than that to the second. At higher stimulus strengths the slow axon is also stimulated, as indicated by a second peak superimposed on the fast-axon response. The slow-axon response to the second stimulus is considerably larger than that to the first, indicating facilitation. Voltage scale: 0·5 mV. Time scale: 10 msec.

the amplitude of the peak appeared to increase two to four times with stimuli spaced 20–30 msec apart. These results suggest a facilitation of the response mediated by the slow axon and tend to further substantiate differences in the functioning of the two motor axons.

Mechanical responses

Although the electrical responses mediated by the fast axon show characteristics of antifacilitation in response to closely spaced stimuli, the mechanical events mediated by the fast axon do not appear to reflect this situation. Instead, twitch responses to closely spaced pulses appear to be facilitated, facilitation in these cases involving a large increase in the amplitude of the mechanical response to the second stimulus.

To examine this mechanical facilitation the fast axon in each nerve was stimulated with paired pulses (15–20 msec stimulus interval). Occasionally both fast and slow axons were stimulated, but in these cases there appeared to be no additional contribution to the mechanical response due to slow-axon stimulation.

Fig. 2 C, D shows examples of mechanical responses to twin-pulse stimulation of the fast axon in each segmental nerve. The individual responses to each stimulus are not apparent because of the close spacing of stimuli. Facilitation is indicated, however, since the amplitude of the response to twin pulses is much more than twice the response to a single stimulus, suggesting mechanical facilitation of the response mediated by the fast axon. For both SN I and SN II–III the mean amplitudes of the mechanical responses to twin-pulse stimulation were more than three times those of the responses to single stimuli.

C. *Responses to repetitive stimulation*

Correlation of mechanical and electrical responses

In order to further characterize the responses mediated by the fast and slow axons, recordings of the electrical and mechanical responses of the longitudinal muscle to repetitive nerve stimulation were made. The most suitable range of frequencies for these experiments was 5–10 Hz. At frequencies higher than 10 Hz thresholds of the two axons became increasingly difficult to separate, and at frequencies below 5 Hz mechanical responses were very small and therefore difficult to analyse.
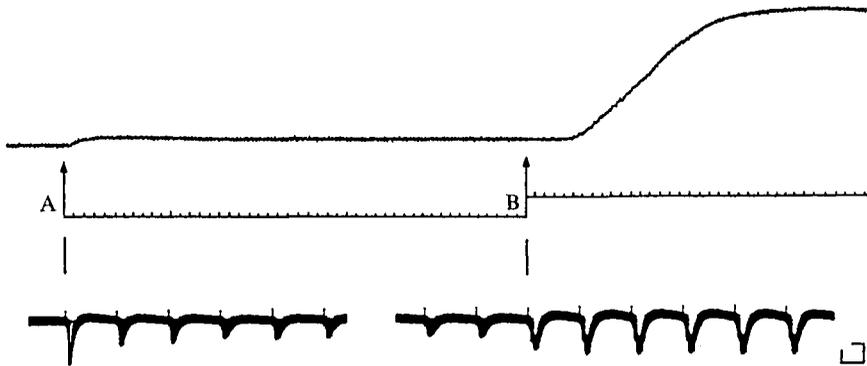
Fig. 4. Responses to repetitive stimulation of fast and slow axons. Mechanical responses (upper record) and external electrical responses (lower record) are shown in response to stimulation of SN I at 10 Hz. At A the stimulus strength is raised just above threshold for the fast axon, resulting in a small twitch-like response and rapid decrement in muscle potentials. At B the stimulus strength is raised just above threshold for the slow axon, as indicated by a second, later peak in the electrical record. This results in a large, slowly developing tension plateau. Vertical scale: 0·05 g (upper); 0·5 mV (lower). Time scale: 250 msec (upper); 50 msec (lower).

When either segmental nerve was stimulated at a frequency of 10 Hz and at a stimulus strength just above threshold for the fast axon, but below threshold for the slow axon, the external electrical response to the first stimulus was the typical fast-axon response, a large negative potential. With successive stimuli there was a rapid decrement of electrical responses. In a series of 10 such experiments at a stimulus frequency of 10 Hz the mean amplitude of the fast-axon electrical response to the second stimulus was only 61 % of the initial response, while by the tenth stimulus the response was only 39 % of the initial response. With further stimulation responses decreased only slightly and then remained at a stable but low amplitude.

Correlated with the first electrical response to fast-axon stimulation there occurred a small twitch-like response similar to the response to a single stimulus. With successive stimuli no other twitch responses were seen and the tension gradually returned to baseline.

Repetitive stimulation at a stimulus strength above threshold for the slow axon gave considerably different results. In these experiments the fast-axon response was first reduced by repetitive stimulation below threshold for the slow axon, but above threshold for the fast axon. The stimulus strength was then raised so that the slow axon was also stimulated. When the slow-axon threshold was reached a smooth and large increase in mechanical tension followed. This large and slow mechanical response will hereafter be termed the slow response. In contrast to the fast response, slow responses are maintained well above baseline for at least a minute of repetitive stimulation.

The electrical events occurring during the slow response correlate well with the mechanical events. Just as there is little mechanical fatigue, there is also no reduction in the amplitude of the corresponding muscle potentials. In fact for at least the first few potentials there is a significant increase in their amplitude, suggesting facilitation of the electrical responses. The amplitude of potentials occurring during the next few seconds is relatively stable. Fig. 4 gives the results of a typical experiment.
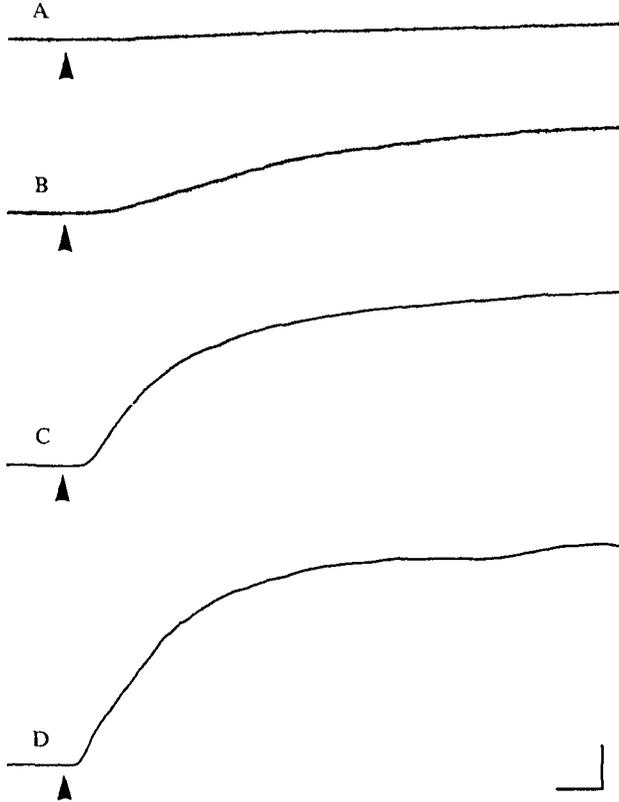
Fig. 5. Slow mechanical responses of the longitudinal muscle to repetitive stimulation of SN II–III. Frequencies of stimulation are: 2 Hz (A), 5 Hz (B), 20 Hz (C), and 50 Hz (D). The beginning of stimulation in each record is marked by an arrow and stimulation is continued for the remainder of the record. Note decreases in the onset latency of mechanical responses as the stimulus frequency is increased. The slope and peak amplitude of the responses also increase with increases in the stimulus frequency. Vertical scale: 0·13 g (A, B); 0·25 g (C, D). Time scale: 0·5 sec.

Frequency dependence of slow mechanical responses

The frequency-dependent characteristics of slow muscle responses were examined in nine preparations using the following stimulus frequencies: 2, 5, 10, 50 and 100 Hz. This range of frequencies was chosen because stimulation at lower frequencies (e.g. 1 Hz) seldom resulted in any measurable tension. At higher stimulus frequencies, such as 50 or 100 Hz, the responses appeared to reach limits with respect to rate of tension development and amplitude. As standard procedure in these experiments the twitch-like responses to fast-axon stimulation were reduced to a minimum by prior repetitive stimulation.

Fig. 5 shows typical responses to stimulation of SN II–III at 2, 5, 20 and 50 Hz. As can be seen from this figure onset latencies become less at higher frequencies of stimulation (0·82 sec ± 0·26 sec S.D. at 5 Hz; 0·11 ± 0·06 sec S.D. at 50 Hz). The peak tension developed also varied with the frequency of stimulation in the range from 2 to 20 Hz, while above these frequencies little further tension increases were seen. For SN I the responses were very nearly the same except that the maximum tension developed at any frequency was only about one half to one fourth that developed for SN II–III.
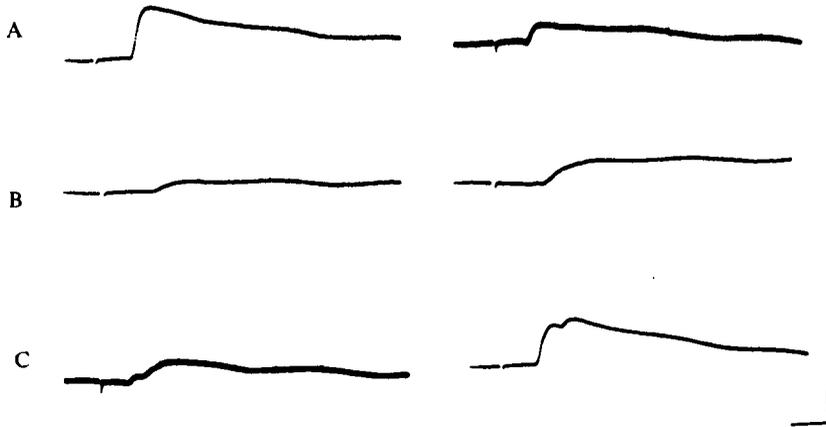
Fig. 6. Excitatory postsynaptic potentials in longitudinal muscle fibres. Intracellular records from six different muscle fibres are shown in response to single stimuli to SN II–III. Muscle fibres in A are innervated only by the fast axon; those in B are innervated by the slow axon. Note that potentials in B have a longer onset latency and slower time course than those in A. Muscle fibres in C are innervated by both fast and slow axons, as indicated by the two distinct phases of depolarization. Voltage scale: 5 mV. Time scale: 5 msec.

D. *Intracellular responses to segmental nerve stimulation*

Intracellular muscle responses to single supramaximal stimuli delivered to individual segmental nerves were examined in 180 cells (five preparations). In all cases intracellular recording sites corresponded closely to those from which external recordings were taken in earlier experiments. Thus a direct correlation of intracellular and extracellular records was possible.

In two-thirds of the muscle fibres examined, recognizable postsynaptic potentials were recorded in response to stimulation. Three main types of postsynaptic potentials were seen (Fig. 6).

The first type of postsynaptic potential, found in about 40% of all muscle fibres, consisted of a single smooth depolarization (Fig. 6A). Responses such as this were obtained with stimulation of SN I as well as SN II–III. The mean onset latency of the response mediated by SN I was 6·0 ± 0·8 msec S.D. The potential developed rapidly, reaching a peak in a mean time of 1·6 ± 0·6 msec S.D. following the onset of the potential. Similar responses were recorded with stimulation of SN II–III, the mean onset latency being 5·6 msec ± 0·8 msec S.D. and the time to the peak being 1·4 ± 0·7 msec S.D.

The amplitude of these potentials was quite variable from one cell to another, and much variation was also seen within a cell if single stimuli were delivered every few seconds. The mean amplitude of responses mediated by SN I was 2·3 mV (range 1–6 mV) and that for SN II–III was 2·7 mV (range 1–10 mV).

The threshold for this first type of response was determined by gradually decreasing the stimulus strength and observing responses to single stimuli. In all cases the threshold for this response was sharp and coincided exactly with the threshold of the external electrical response mediated by the fast axon. The above information along with the close correlation between the time course of this response and that for the externally recorded fast-axon response make it appear likely these muscle fibres are innervated by the fast excitatory axon.
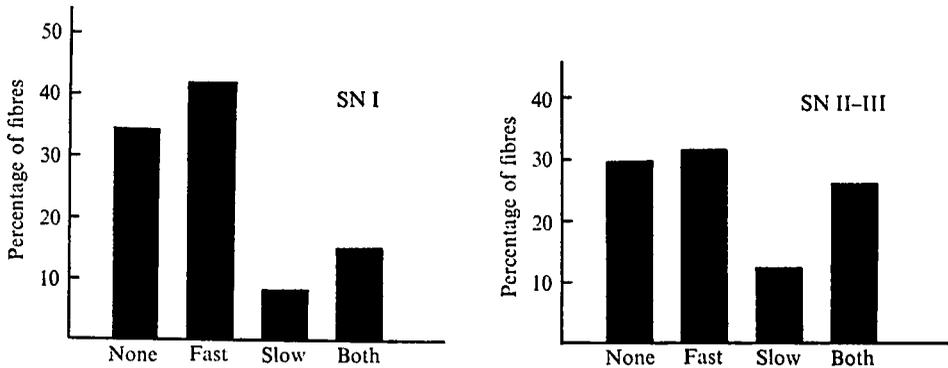
Fig. 7. Innervation patterns of longitudinal muscle fibres. Results were obtained by sampling intracellular muscle responses to segmental nerve stimulation in two populations of muscle fibres, one population lateral to SN I ($N = 90$) and the other lateral to SN II–III ($N = 90$). For both nerves innervation by the fast excitatory axon was most common. A small percentage of muscle fibres was innervated by the slow excitatory axon alone, and a somewhat larger percentage was innervated by both fast and slow axons. Some muscle fibres received no apparent excitatory input from the segmental nerve.

A second type of postsynaptic potential was recorded in about 10 % of all muscle fibres. This response consisted of a single, smooth depolarization which occurred later and had a slower time course than the response mediated by the fast axon (Fig. 6 B). For stimulation of SN I the mean onset latency of this response was $10\cdot3 \pm 1\cdot2$ msec S.D. and the mean time from the onset of the potential to its peak was $2\cdot3 \pm$ $0\cdot5$ msec S.D. Similar responses were obtained with stimulation of SN II–III, the mean onset latency being $10\cdot2 \pm 1\cdot1$ msec S.D. and the mean time to the peak being $3\cdot6 \pm$ $1\cdot0$ msec S.D.

The amplitude of these potentials was variable from one cell to another. The mean amplitude of the response to stimulation of SN I was $2\cdot3$ mV (range 1–3 mV), but the responses to stimulation of SN II–III were somewhat larger, the mean amplitude being $3\cdot9$ mV (range 2–9 mV).

Thresholds for these responses were determined by gradually lowering the stimulus strength. In every case the threshold was sharp, all-or-none, and coincided exactly with the threshold of external electrical responses mediated by the slow axon. This similarity in threshold along with the similar time course for intracellular and extracellular responses make it appear likely that these muscle fibres are innervated solely by the slow excitatory axon.

A third type of response was recorded from about 20 % of all muscle fibres. This response differed from the other two in that it consisted of two distinct phases of depolarization (Fig. 6 C). The first phase of the response was a rapid and smooth depolarization closely resembling the fast-axon response shown in Fig. 6 A. The second phase was a slower and later depolarization closely resembling the slow-axon response shown in Fig. 6 B. The occurrence of this two-phased response suggests that it is composed of two different excitatory postsynaptic potentials superimposed on one another.

This idea was substantiated by determining the thresholds for the potential. In nearly all cases thresholds for the two phases of the response were clearly separable, and these thresholds coincided exactly with those of extracellular responses mediated

by the fast and slow axon. Thus these muscle fibres appear to receive dual excitatory innervation, that is, innervation from both fast and slow axons.

In the remaining 30 % of all muscle fibres no measurable postsynaptic responses to nerve stimulation were recorded. These fibres did not appear to be damaged since resting potentials in these fibres were not significantly different from resting potentials in innervated fibres. These results may indicate that such muscle fibres were not innervated by the nerve being tested and perhaps are innervated by adjacent segmental nerves.

A quantitative analysis of the pattern of innervation of longitudinal muscle fibres is given in Fig. 7. These figures represent results obtained from only one specific region of the longitudinal muscle (about 2 mm lateral to the segmental nerve). Thus they may not reflect the pattern of innervation for the entire muscle layer.

DISCUSSION

In earthworm longitudinal muscle there appear to be two functionally distinct excitatory systems, a fast system mediating a small, rapid twitch-like response and a slow system mediating a more slowly developing and sustained response. Each of these responses appears to be mediated by one excitatory axon of a single type, a fast axon mediating the rapid response and a slow axon mediating the sustained response. Each segmental nerve, SN I or SN II–III, contains one fast and one slow axon which innervate the longitudinal muscle. In many ways this functional differentiation of motor axons resembles that seen in other annelids as well as in other invertebrate phyla.

Fast motor systems

The fast system of earthworms resembles that seen in other annelids, particularly nereid polychaetes. Horridge (1959) and Wilson (1960a) have shown a fast response in the longitudinal muscle of *Nereis*, the response being mediated by a single axon in SN IV. Stimulation of this axon results in a large external muscle potential which shows characteristics of antifacilitation. Thus the properties of the polychaete fast system are similar to those I have described for the earthworm fast system. A major difference is that in the earthworm each side of a segment is innervated by two fast axons rather than one as in *Nereis*.

The fast motor system of earthworms is also comparable to the fast systems of other invertebrates, such as the proboscis retractor muscle of sipunculid worms (Prosser & Melton, 1954) and the mantle muscle of cephalopods (Wilson, 1960b). Fast motor systems have been extensively studied in arthropods, particularly in insects and crustaceans (cf. Usherwood (1967) and Atwood (1967) for reviews). In these groups motor systems have reached a high degree of specialization and diversity, this diversity being reflected in the wide range of mechanical and electrical responses obtained with fast-axon stimulation.

The fast system of earthworm longitudinal muscle differs from the fast systems seen in most arthropods. Generally fast systems in arthropods are relatively stable, significant fatigue occurring only after prolonged periods of repetitive stimulation. Also in some arthropods fast responses to repetitive stimulation may summate and undergo facilitation, reaching a large and distinct tension plateau (tetanus). In contrast the fast reponse

of earthworm longitudinal muscle is highly labile, complete mechanical fatigue appearing after only a few stimuli. This rapid fatigue is accompanied by a significant decline in the amplitude of external muscle potentials.

The weak mechanical responses to fast-axon stimulation obtained in these experiments suggest that relatively few muscle fibres produce such responses. Visual observations also indicate these responses are localized, appearing to involve only muscle fibres in the ventrolateral region of the segment. However, experiments involving intracellular recordings suggest that many muscle fibres in a segment are innervated by the fast axon. Therefore, many muscle fibres, though innervated by the fast axon, do not contribute to the mechanical response to a single stimulus. It is possible, however, that such fibres respond mechanically to several closely spaced stimuli in the fast axon or possibly to simultaneous stimulation of fast axons in two segmental nerves innervating the same muscle fibres. Alternatively, the lack of responsiveness to a single stimulus may indicate that fast responses were partially fatigued as a result of the dissection of the animal.

From the results it is not clear what type of intracellular response is required to initiate the twitch response. Such a response in a muscle fibre could be initiated by either a large postsynaptic potential or by an active membrane response (spike). Furthermore, it is possible that the level of depolarization necessary for contraction may vary from one cell to another, a situation seen in many arthropods (Atwood, 1967).

Slow motor systems

The slow motor system in earthworm longitudinal muscle closely resembles the slow systems found in other annelids. In nereid polychaetes, for example, Wilson (1960a) has demonstrated a slow electrical response of the longitudinal muscle following stimulation of SN IV. This response is smaller than the fast response, occurs after a longer latency, and shows summating and facilitating characteristics with repetitive stimulation. These characteristics are very similar to the electrical characteristics of the slow muscle responses we have identified in the earthworm.

The slow system of the earthworm is also somewhat similar to that of the leech longitudinal muscle. In the leech responses to stimulation of slow axons consist of summating and facilitating excitatory postsynaptic potentials which give rise to slow and summating mechanical contractions (Stuart, 1970).

Similar slow systems are seen in the muscle of other invertebrate phyla such as the sipunculid proboscis retractor muscle (Prosser & Melton, 1954; Prosser & Sperelakis, 1959) and molluscan mantle muscle (Wilson, 1960b). In these instances slow responses are characterized by facilitating electrical responses and slowly developing mechanical responses to repetitive nerve stimulation.

Slow motor systems are also common in arthropods, having been extensively studied in crustaceans and insects. The slow responses of earthworm muscle are similar to those of arthropods. In the earthworm longitudinal muscle, as in many arthropod muscles, the slow response is mediated by only a few axons. Also, the amplitude of the slow response is clearly dependent on the frequency of stimulation, frequencies as low as 2 Hz giving responses which are just measurable and frequencies of 20–50 Hz giving maximal responses.

Inhibition

No evidence for peripheral inhibition in the longitudinal muscle of the earthworm could be found. In no cases were threshold-dependent decreases in mechanical or electrical responses of the longitudinal muscle observed. Thus the innervation pattern of earthworm muscle appears similar to that in nereid polychaetes in which fast and slow, but no inhibitory, axons have been demonstrated (Wilson, 1960a).

This lack of inhibition is in contrast to the apparent presence of peripheral inhibitory systems in other annelids. For example, Hidaka *et al.* (1969c) and Ito, Kuriyama & Tashiro (1969) have recorded inhibitory postsynaptic potentials from longitudinal muscle 'fibres in the earthworm, *Pheretima communissima*. Stuart (1970) also has clearly demonstrated postsynaptic inhibition in the longitudinal muscle of the leech.

The results of the present study, though giving no support for inhibition, do not exclude the possibility that peripheral inhibition exists in the longitudinal muscle. It is possible, for example, that inhibitory axons exist in the segmental nerves, but thresholds of these axons are not clearly separable from the thresholds of excitatory axons. In this situation stimulation of a segmental nerve might simultaneously excite both inhibitory and excitatory axons, with the net result still being a depolarization in the muscle. An alternative possibility is that a presynaptic inhibitory mechanism is involved. With such a mechanism no inhibitory postsynaptic potentials would be seen.

Functional significance of innervation patterns

In the earthworm there are two well-known locomotor activities involving the longitudinal muscle. The first is the rapid escape response of many oligochaetes and polychaetes. The response consists of a powerful multi-segmental and twitch-like contraction of the longitudinal muscle. The central components of the reflex pathway of the rapid response are the giant fibres (Stough, 1930; Rushton, 1945, 1946; Roberts, 1962a). In the earthworm, giant fibres make functional contact with several motor neurones in each segment and at least some of these motor neurones appear to innervate the longitudinal muscle (Gunther, 1972). It is possible that the axons arising from these motor neurones correspond to the fast axons we have identified in each segmental nerve. Also it is possible that the activation of these fast axons by the rapidly conducting giant fibres may result in a rapid and synchronous shortening of the animal.

Another locomotor activity in earthworms is the slow peristaltic movements of the worm observed during crawling or burrowing. These movements actually involve successive retrograde waves of circular and longitudinal muscle contractions. In any one segment there is a reciprocal relationship between the timing of circular and longitudinal muscle contractions (Seymour, 1969, 1971). Events occurring in the central nervous system during peristaltic contractions are not well understood. Roberts (1967) has identified a slow-conducting pathway in the ventral nerve cord which seems to be multisynaptic in nature and which may represent the central pathway for slow peristaltic movements of the animal.

The slow and sustained longitudinal muscle contractions seen during peristaltic movements are comparable to the contractions we have recorded in response to slow-axon stimulation. Perhaps the slow axons may be important in mediating these loco-

motor movements. If this is the case, then sequential bursts of activity in the slow axons of successive segmental nerves could bring about the wave-like contraction of the worm.

SUMMARY

1. Patterns of innervation of the longitudinal muscle of the earthworm, *Lumbricus terrestris*, were examined electrophysiologically.

2. The longitudinal musculature of a segment is innervated by relatively few axons, a fast and slow axon being present in segmental nerve I and in the double nerve, segmental nerve II–III.

3. Single-pulse stimulation of the fast axon produces large external muscle potentials and small twitch-like contractions, which with repetitive stimulation are antifacilitating.

4. Repetitive stimulation of the slow axon produces large, slowly developing and sustained mechanical responses, with electrical and mechanical responses showing summation and facilitation.

5. The amplitude and time course of slow mechanical responses are related to the frequency of stimulation.

6. Individual longitudinal muscle fibres are innervated by either the fast or slow axon in a segmental nerve, or by both fast and slow axons.

7. No evidence was found for peripheral inhibitory innervation of the longitudinal muscle.

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