NEUROMUSCULAR PHYSIOLOGY OF THE CIRCULAR MUSCLE IN THE EARTHWORM, LUMBRICUS TERRESTRIS

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ABSTRACT

NEUROMUSCULAR PHYSIOLOGY OF THE CIRCULAR MUSCLE IN THE EARTHWORM, *LUMBRICUS TERRESTRIS*

By

Charles D. Drewes

In annelids the nerve-muscle relationships, particularly those involving the circular muscle layer, have not been extensively studied. In this study a suitable nerve-muscle preparation was obtained for the investigation of the physiological characteristics of the circular muscle of the earthworm, *Lumbricus terrestris*.

Stimulation of the segmental nerves using a suction electrode results in several distinct responses of the circular muscle layer. The first is a response to a single stimulus (rheobase, 2.5 V). The amplitude of the response is dependent on the stimulus strength. The single stimulus response summates, facilitates and rapidly fatigues. Recovery times for this response range from 15 to 40 minutes.

A second type of response is the slow response to repetitive stimulation at frequencies greater than 2 cycles/sec (rheobase, 0.2 V). At frequencies between 10 and 50 cycles/sec the slow response consists of two distinct phases of tension development.

A frequency-dependent inhibition of the slow response is observed with stimulation at frequencies greater than 50 cycles/sec. At frequencies of 20 cycles/sec and less, inhibition of the slow response is not apparent.

The excitation and inhibition of the circular muscle layer described in this study corresponds somewhat to the excitation and inhibition of

the longitudinal muscle layer. Such a reciprocal inhibition and excitation may be of functional significance during certain locomotor activities, such as burrowing.

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INTRODUCTION

The nerve-muscle relationships in the annelids have not been extensively studied. One of the reasons for this is that the annelid body wall musculature does not consist of separate and distinct bundles of fibers but rather consists of sheets of antagonistic (longitudinal and circular) muscles. This annelid feature has made it difficult to obtain discrete nerve-muscle preparations. In this thesis I describe a suitable nerve-muscle preparation which I have used for an analysis of some of the physiological characteristics of the circular muscle of the earthworm, *Lumbricus terrestris* L. (Class Oligochaeta).

Role of the Body Wall Muscle in Locomotion

The functional roles of the circular and longitudinal muscles in earthworm locomotion have been studied by Gray and Lissman (1938), Chapman (1950) and Seymour (1969). These studies have shown that forward crawling of the worm is accomplished by successive retrograde waves of circular and longitudinal muscle contractions. When burrowing the circular muscle of the worm functions in penetration of the soil, whereas the longitudinal muscle functions to press the walls of the burrow outward.

The escape reaction of the earthworm is characterized by a rapid longitudinal contraction of the body in response to tactile stimulation. Roberts (1962a, b, 1966) found that the longitudinal muscle gave this "twitch-like" response to a single electrical stimulus delivered to the giant axons of the ventral nerve cord. In these studies Roberts found that this giant fiber reflex was susceptible to rapid fatigue and also exhibited facilitation.

Physiology of the Body Wall Muscle

Some of the physiological characteristics of the earthworm body wall muscle were examined by Budington (1902). He described responses of the longitudinal muscle to direct electrical stimulation of a strip of the body wall musculature. The response to a single stimulus was dependent upon the stimulus strength. The response to multiple stimulation was a summating response, with tetanus occurring at frequencies of stimulation greater than 4 cycles/sec.

Botsford (1939, 1941) also examined longitudinal muscle responses to a short series of single shocks applied directly to the muscle. He found summation of the responses to successive single shocks, and a tetanic response similar to that described by Budington. In some experiments Botsford observed a facilitation of the successive responses to a series of stimuli. A facilitation of the electrical activity in the longitudinal muscle fibers, corresponding to facilitation of the mechanical responses observed by Botsford, was reported by Horridge and Roberts (1960) following stimulation of the segmental nerves at a frequency of 3 cycles/sec.

The mechanical properties of the longitudinal muscle of the earthworm, <u>Pheretima</u> <u>communissima</u>, were examined by Hidaka <u>et</u> <u>al</u>. (1969b). They found a "twitch-like" contraction of the longitudinal muscle in response to a single electrical stimulus applied directly to the muscle.

Summation of this "twitch" response was observed with repetitive stimulation. A tonic, or sustained, tension followed the "twitch" response but was not related to spike generation in the muscle fibers. The same investigators also presented evidence of peripheral inhibition of the longitudinal muscle.

Another type of contraction of the earthworm body wall muscle was first described by Straub in 1900. This type of contraction appeared to be spontaneous in origin since it was observed in strips of body wall muscle from which the ventral nerve cord had been removed. Chang (1969) supported Straub's observations by recording spontaneous electrical activity in the longitudinal muscle fibers of the earthworm *Pheretima hawayana*. Chang suggested that this electrical activity is myogenic.

The approaches used in many of the physiological investigations of the earthworm body wall muscle have had certain limitations. In the investigations of Straub (1900), Budington (1902), Botsford (1941) and Hidaka *et al*. (1969b) direct stimulation of the body wall muscle was used to determine the various muscle responses. It is difficult in this type of approach to separate the effects of direct stimulation of the muscle from the effects of stimulation of the peripheral nerves to the muscle.

In other investigations various anesthetizing agents were used during dissection of the worm, such as MS-222 (Roberts, 1962a, b), chloretone (Botsford, 1941), and magnesium chloride (Moore, 1923). The effects of such agents on the nervous and muscular activity of the animal are uncertain, although these investigators stated that the effects of the chemicals were reversible.

Nearly all of the physiological studies of the body wall muscle of the earthworm have been concentrated on the longitudinal muscle, thus leaving a gap in the understanding of the physiology of the antagonistic circular muscle. In order to determine the nerve-muscle relationships and patterns of innervation of the circular muscle, it would be beneficial to have a suitable nerve-muscle preparation. Once the physiological characteristics of such a preparation are determined, then we can better understand the neurophysiological bases of the locomotor activities of the earthworm.

Anatomy

The anatomical relationships between the nervous system and the musculature of the earthworm have been described by Hess (1925a, b), Smallwood (1927, 1930), Coonfield (1932) and Prosser (1934, 1935). These studies have shown that the outer portion of the body wall of the earthworm consists of an epidermis covered by a thin cuticle. Beneath the epidermis there is a thin layer of connective tissue forming a dermis. The circular (transverse) muscle layer lies just interior to the dermis and is relatively well developed (see Figure 1). The axes of the circular muscle fibers are at right angles to the body axis. The longitudinal muscle layer is located just interior to the circular muscle. The longitudinal muscle fibers are ribbon shaped and the entire layer has a feathery appearance. The axes of the longitudinal muscle fibers are parallel to the body wall axis.

A study of the fine structure of the longitudinal muscle has been made by Hanson (1957) using phase contrast light microscopy and electron microscopy. A more recent study of the fine structure of the circular

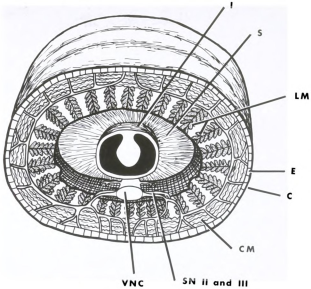

Figure 1. Diagramatic cross-section of the earthworm. The diagram shows the segmental nerves of a single segment and their relation to the body wall muscle. The section is viewed from a dorso-posterior direction. VNC, ventral nerve cord; SN II and III, second and third segmental nerves; CM, circular muscle; C, cuticle; E, epidermis; LM, longitudinal muscle; S, septum; I, intestine. (taken from Hess, 1925a)

and longitudinal layers was made by Mill and Knapp (1970). Both of the studies indicate that the fine structures of the longitudinal and circular muscle fibers are quite similar with respect to the arrangement of myofilaments and the organization of the tubule systems.

The segmental nerves arise from the ventral nerve cord and innervate the circular and longitudinal muscle layers of each segment. There are three pairs of segmental nerves per segment, each pair forming a ring around the body wall (Hess, 1925). The first pair of segmental nerves are small and located anteriorly in each segment. The second and third pairs of segmental nerves are located posteriorly in each segment. They arise so close together that the two nerves together are referred to by Bullock and Horridge (1965) as a "double nerve".

All segmental nerves have both efferent and afferent elements. The third segmental nerve contains most of the efferent fibers, and the second segmental nerve contains most of the afferent fibers (Hesse, 1894).

An analysis of the sensory and motor fields of innervation of the earthworm body wall was carried out by Prosser (1935). His study suggests that sensory fibers extend for one segment on either side of the segment from which they arise, forming a sensory field of three segments. Similarly, the motor fibers extend into adjacent segments, but the overlap of motor fields is not quite as extensive as the overlap of sensory fields.

The branching of the sensory, and possibly the motor, fibers forms a complex sub-epidermal network. Although the organization of this network has been a controversial subject, the studies of Prosser (1935) discounted the possibility that this network is a true, anastomosing nerve net.

MATERIALS AND METHODS

Source and Maintenance of Animals

The earthworm, Lumbricus terrestris L., was used for this study. Specimens were obtained from E. G. Steinhilber and Co., Oshkosh, Wisconsin and Wholesale Bait Co., Hamilton, Ohio. During the warmer months some animals were obtained locally. The animals ranged in size from 12 to 18 cm in length, and from 0.5 to 1.0 cm in diameter. The animals were kept at 12 to 15° C and were maintained in wooden or styrofoam boxes containing Buss Bed-ding (Buss Manufacturing Co., Lanark, Illinois).

Dissection

Specimens were pinned dorsal side up into a shallow tray of paraffin. A middorsal incision just through the body wall was made for a length of 15 to 20 segments immediately posterior to the clitellum. The body wall was spread open and the septa were cut, thus allowing each side of the body wall to be flattened out and pinned to the tray. The intestine was dissected free from its septal connections to the ventral body wall. Care was taken to avoid cutting the intestine, since Prosser (1935) reported that the gut contents may affect nervous activity. Transverse cuts were then made through the entire body wall and nerve cord at the posterior and anterior limits of the dissected region. The undissected anterior and posterior regions of the animal along with the entire intestine were lifted away and discarded. The remaining preparation consisted of 15 to 20 segments of the body wall musculature with the intact ventral nerve cord and the segmental nerves of this region.

Three segments were then selected and the segmental nerves to these segments were severed as close as possible to the ventral nerve cord. The nephridia in each of the three segments were removed to facilitate viewing of the segmental nerves. Transverse cuts were then made through the body wall and nerve cord just anterior and posterior to the boundaries of the middle segment. The resulting strip of tissue consisted of the body wall muscle from slightly more than one segment and the segmental nerve stumps of this segment. A ventromedial cut was made through the body wall dividing the strip into left and right halves, each half being approximately 1.5 mm wide and 10 mm long. The nerve-muscle preparation therefore consisted of the body wall muscle from one lateral half of slightly more than one segment with the segmental nerve stumps from that half of the segment (see Figure 2).

During the entire dissection and throughout the experiment the nerve-muscle preparation was kept in the paraffin tray and bathed in the physiological solution of Pantin (1948), consisting of 135 mM NaCl, 2.7 mM KCl, 1.8 mM CaCl, 0.4 mM $MgCl_2$, 0.4 mM $NaSO_4$, and 1.0 mEq Na_2HPO_4 at pH 7.4.

Recording Muscle Tension Following Electrical Stimulation

A plexiglass muscle clamp was attached to the former dorsomedial end of the muscle strip, and the former ventromedial portion of the muscle strip was pinned securely as shown in Figure 2. The clamp was allowed to pivot freely on an insect pin which was stuck into the paraffin tray. A short thread connected the clamp to a microdisplacement myograph transducer (Narco Bio-Systems, Inc., Houston, Texas). The circular (transverse) muscle contractions were monitored on a Model Four Physiograph (Narco Bio-Systems, Inc., Houston, Texas).

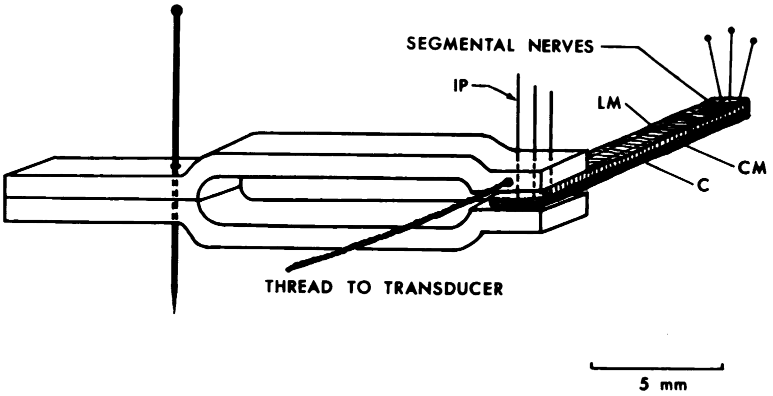

Figure 2. Diagram of plexiglass muscle clamp and strip of body wall muscle. CM, circular muscle; LM, longitudinal muscle; C, cuticle; IP, insect pins.

Spontaneous contractions were recorded for five to twenty minutes after the completion of the dissection. Experiments were not begun until nearly all of this spontaneous activity had ceased.

Prior to stimulation the adjoining segmental nerves II and III were drawn up into the tip of a glass suction electrode with an outside tip diameter of 0.2 mm. The tip was polished with an oil stone to ensure a tight seal around the nerve. Electrical stimulation was accomplished by using a Grass Model S-4 stimulator and a Grass stimulus isolation unit (Grass Instrument Co., Quincy, Mass.). Silver wires were used for both the stimulating and recording electrodes.

All recordings were made at 13 to 17° C. This temperature was maintained by means of an ice bath which surrounded the paraffin tray. Saline was replaced every 20 to 30 minutes. Under these conditions the nerve-muscle preparation remained in good condition for three to ten hours, although most preparations were not used longer than five hours.

RESULTS

Response to Single Stimulus

In nine of thirty animals a response to a single stimulus was observed (Figure 3). A strength-duration relationship for a just measureable single stimulus response, based on eight animals, is given in Figure 4. Rheobase and chronaxie were calculated from the graph and are 2.5 V and 4.0 msec, respectively.

The amplitude of the single stimulus response is dependent on the stimulus strength. The amplitude of the mechanical response at threshold was generally 10 to 20% of the maximum response obtainable at suprathreshold stimulus strengths. For example, in one animal the amplitude of the response at threshold (4.0 V, 3 msec) was only 18% of the amplitude of the response obtained at a higher stimulus strength (5.5 V, 3 msec). The mean tension for the maximum single stimulus response was 1.8 g (range 0.7 to 2.9 g; n=9).

Temporal aspects

The temporal aspects of the single stimulus response were examined at supramaximal stimulus strengths. Twenty-eight measurements were made from four preparations, allowing at least 15 minutes between successive stimuli. The mean time from stimulation to peak tension (rise time), based on these 28 measurements, was 3.8 sec ± 1.2 sec SD (range 2.0 to 7.0 sec). The mean rise time for each of the four preparations ranged from 3.2 to 5.1 sec.

The mean time interval from stimulation to relaxation to one half the peak tension was also calculated. This mean, also based on 28 measurements from four preparations, was 12.3 sec ± 6.2 sec SD. The mean in each of the four preparations ranged from 8.8 to 21.0 sec.

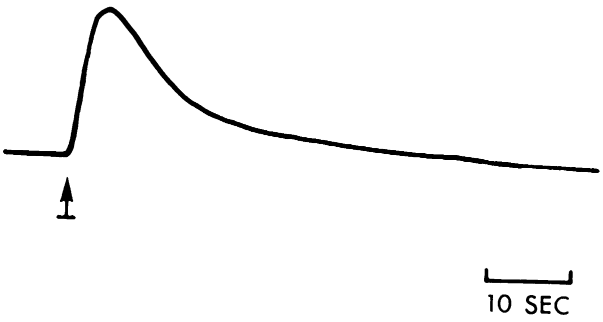

Figure 3. Single stimulus response. The stimulus strength is suprathreshold (15 V) and the duration is 5 msec. The stimulus is marked by the arrow.

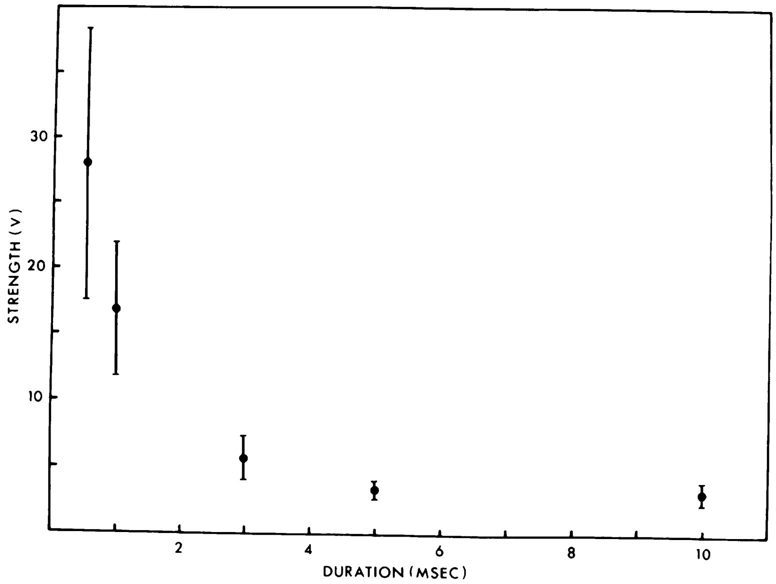

Figure 4. Strength-duration relationship for a just measurable single stimulus response. The vertical lines indicate 1 SD above and below the mean.

Direct stimulation of the muscle

Experiments were performed in three preparations to determine the effect of direct electrical stimulation of the muscle. In every experiment a single stimulus delivered directly to any part of the muscle strip with the suction electrode produced a small contraction, but only when stimulus strengths were 60 to 80 V and durations greater than 10 msec. Because these stimulus parameters were much greater than those used when stimulating the segmental nerves, it was concluded that the single stimulus muscle response is a result of stimulation of the segmental nerves, rather than a result of direct stimulation of the muscle.

Facilitation

In three preparations experiments were performed which demonstrated facilitation of the single stimulus response. In these experiments the mechanical responses to two closely spaced stimuli were examined. Two or three subthreshold stimuli (2.0 V, 5 msec) delivered at one or two second intervals never evoked a mechanical response. Two suprathreshold stimuli delivered several seconds apart resulted in a facilitation of the second response in all three preparations. The responses to pairs of suprathreshold stimuli (5 V, 5 msec) delivered at three, five and ten second intervals are given in Figure 5. When a pair of stimuli were delivered at either three or five second intervals, the response to the second stimulus was nearly twice the amplitude of the response to the first stimulus (Figure 5A, B). If the interval between stimuli was 10 sec (Figure 5C), the second response was slightly smaller than the first. In all three examples in Figure 5 the second response summates with the first, but a marked facilitation of the response was observed only at the three and five second intervals.

Figure 5. Facilitation of the single stimulus response. Intervals of 3, 5 and 10 sec were used between paired single stimuli. Each stimulus is marked by an arrow. The stimulus strength is 5 V (5 msec).

Figure 5A. The second stimulus was delivered 3 sec after the first. The second response shows facilitation and summation.

Figure 5B. The second stimulus was delivered 5 sec after the first. The second response shows facilitation and summation.

Figure 5C. The second and third stimuli were delivered at 10 sec intervals following the first stimulus. The second response shows slight summation, but no facilitation. The third stimulus resulted in only a small, summated response.

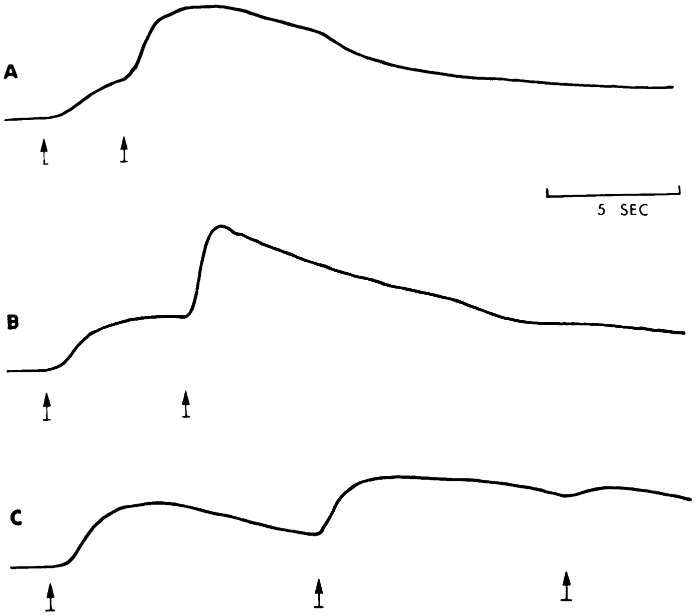

Figure 5

Fatigue and recovery

The single stimulus response is easily fatigued by a series of single stimuli. For example, in one preparation when four consecutive supramaximal stimuli were delivered at thirty second intervals the amplitude of the fourth response was reduced to 40% of the amplitude of the first response. In another preparation when four consecutive supramaximal stimuli were delivered at one minute intervals, the amplitude of the fourth response was 25% of the amplitude of the first response. More frequent stimulation often extinguished the response completely.

The fatigue of the single stimulus response is long-lasting. A determination of the time interval necessary for the response to recover completely from any effects of fatigue was made. Recovery times determined for two preparations ranged from 15 to 40 minutes when the stimulus strength was supramaximal (15 V, 5 msec). Recovery times were generally difficult to measure.

Response to Repetitive Stimulation

Responses to repetitive stimulation were found in all thirty animals examined. Such responses will be referred to as slow responses. The lowest frequency required to give an observable tension ranged from 0.5 to 2 cycles/sec. The threshold strengths for a just measurable slow response to repetitive stimulation at 20 cycles/sec determined in seven animals are plotted against the stimulus durations (Figure 6). Rheobase and chronaxie, as calculated from the graph were 0.2 V and 3.0 msec, respectively. At a given duration the stimulus strength for a threshold slow response was approximately one-tenth of the stimulus strength for a

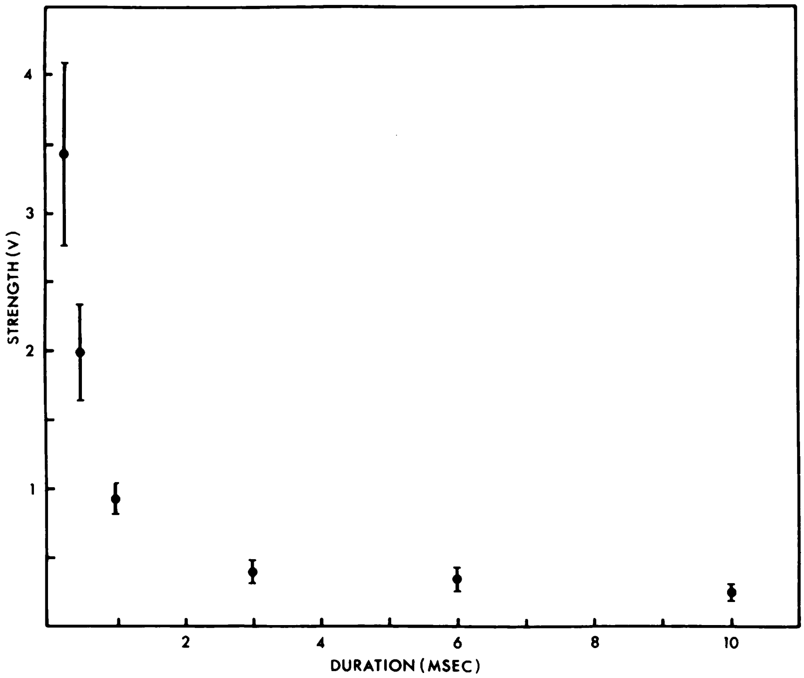

Figure 6. Strength-duration relationship for the slow response. The points indicate the thresholds for a just measurable slow response to repetitive stimulation at 20 cycles/sec. Vertical lines indicate 1 SD above and below the means.

threshold single stimulus response. In general the amplitude of the slow response was not significantly affected by increasing the stimulus strength to various suprathreshold values. This is in contrast to the single stimulus response whose amplitude was dependent on the stimulus strength.

Direct stimulation of the muscle

To ensure that the segmental nerves, rather than the muscle were being stimulated directly, the effects of repetitive stimulation of the muscle were also examined in five preparations. The suction electrode was moved from the nerve to various areas of the muscle strip. Stimulation in this manner resulted in recordable tensions only at stimulus strengths greater than 40 to 60 V and at frequencies greater than 20 cycles/sec. It was, therefore, concluded that this slow response was not a result of direct stimulation of the muscle, since the response was obtained and characterized with stimulus strengths of 10.0 V or less. The slow response could only have been a result of stimulation of the segmental nerves.

Responses to various frequencies of stimulation

The rate of tension development, the amplitude of the tension and the duration that tension is maintained during the slow response are all dependent on the frequency of stimulation. Therefore, the mechanical responses were examined over a range of five frequencies from 5 to 100 cycles/sec. For all measurements a stimulus strength of 10.0 V (3 msec) was used. This stimulus strength was supramaximal for the slow response and was also of sufficient strength to insure complete fatigue of the single stimulus response. An interval of three minutes was allowed between successive measurements. This interval was sufficient for recovery of the response.

The maximum tension developed by any preparation with repetitive stimulation ranged from 1.0 to 6.5 g. In order to compare the tension measurements of one preparation to those of another, it was necessary to express each tension measurement as a percentage of the maximum tension which was ever developed by that preparation during repetitive stimulation. Thus in one preparation, if five measurements were made at each of the five frequencies, then tension measurements for each of the twenty-five measurements are expressed as a percentage of the greatest tension which was ever developed during any of the measurements. This maximum tension was nearly always recorded with repetitive stimulation at 50 cycles/sec.

The slow responses to continuous supramaximal (10 V, 3 msec) stimulation at 5 cycles/sec were examined in five animals (15 measurements). Such stimulation resulted in a tension plateau which could be maintained for longer than five to ten minutes with little reduction in tension (Figure 7A). The rising phase of this slow response is smooth, and the mean time from the beginning of stimulation to the plateau (rise time) was 9.2 sec ± 1.7 sec SE (range 6 to 15 sec). The mean amplitude of this plateau, based on 15 measurements was 14.9% (range 8 to 22%) of the maximum mechanical response obtainable from that preparation.

The slow responses to stimulation at 10 cycles/sec were examined in six animals (15 measurements). The typical response consisted of a smooth rise in tension to a plateau (Figure 7B). Several features of the response to stimulation at 10 cycles/sec distinguish it from the

Figure 7. Slow responses to various frequencies of stimulation. Examples are given of the responses to stimulation at 5, 10, 20, 50 and 100 cycles/sec (10 v, 3 msec). The horizontal lines indicate the durations of repetitive stimulation.

Figure 7A. Response to stimulation at 5 cycles/sec.

Figure 7B. Response to stimulation at 10 cycles/sec. Note the faster rise time and greater amplitude of this response in comparison to that at 5 cycles/sec.

Figure 7C. Response to stimulation at 20 cycles/sec. Note the two distinct phases of the response.

Figure 7D. Type I response to stimulation at 50 cycles/sec. Note the single peak and rapidly declining tension.

Figure 7E. Type II response to stimulation at 50 cycles/sec. Note the two peaks.

Figure 7F. Response to stimulation at 100 cycles/sec. Note the rapidly declining response.

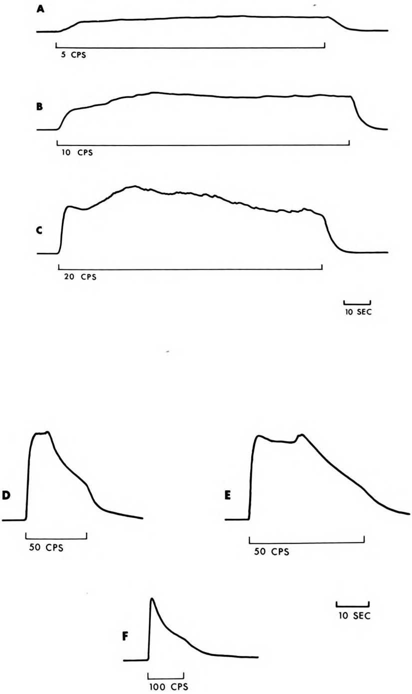

Figure 7

response to stimulation at 5 cycles/sec. First, the response at 10 cycles/sec has a shorter rise time than the response at 5 cycles/sec. Second, the mean relative amplitude of the plateau at 10 cycles/sec was 25.9% (range 19 to 41%), or approximately twice the mean amplitude of the plateau at 5 cycles/sec. Third, at 10 cycles/sec the tension was not always maintained as a plateau, but gradually increased above the level of the plateau resulting in a maximum tension in a mean time of 66.8 sec ± 25.8 sec SE (range 27 to 110 sec) after the beginning of stimulation. The mean amplitude of this maximum tension was 53.9% (range 35 to 74%). This gradual increase in tension above the plateau was not characteristic of the response to stimulation at 5 cycles/sec.

Nineteen measurements of the slow response to stimulation at 20 cycles/sec were made in eight animals. The response consisted of two distinct phases of tension development (Figure 7C). The first phase consisted of a smooth rise in tension to an initial peak, which had a mean amplitude of 42.6% (range 22 to 64%), n=8 animals). This peak was reached in a mean time of 4.5 sec ± 0.8 sec SE (range 2 to 8 sec). Thus the initial phase of the slow response to stimulation at 20 cycles/sec had a shorter rise time and a greater amplitude than the plateau recorded with stimulation at 10 cycles/sec. As seen in Figure 7C a second phase of the slow response was observed following the first. This second phase summated gradually and irregularly with the first phase to form a maximum tension in a mean time of 29.7 sec ± 9.4 sec SE (range 18 to 65 sec). The mean amplitude of this maximum tension was 82.8% (range 58 to 100%). The amplitude of the second phase is approximately the same amplitude as the first phase. The peak in tension was followed by a

gradual decrease in tension to below the value of the first plateau. In four cases the tension had been reduced to one half the peak tension in a mean time of 76.8 sec (range 70 to 82 sec).

Continuous stimulation at 50 cycles/sec resulted in two general types of slow responses; the first will be referred to as the Type I response. The Type I response was observed in 16 of 32 measurements from seven animals. The response consisted of a single, broad peak (Figure 7D) with a mean amplitude of 81.7% (range 56 to 100%). The time from the beginning of stimulation to the peak was difficult to measure because the peak was so broad, but generally the peak was reached approximately 3 to 10 sec after the beginning of stimulation. The Type I response was never sustained in the form of a plateau, but quickly fell to one-half the peak amplitude in a mean time of 13.9 sec (range 11 to 27 sec). The response continued to fall to near baseline even though stimulation was continued.

The second type of response to stimulation at 50 cycles/sec, referred to as the Type II response, was observed in 21 of 37 measurements from seven animals. This response consisted of two distinct peaks (Figure 7E). The mean rise time for the first peak was 3.3 sec ± 1.1 sec SE (range 1 to 10 sec), and the mean amplitude of the peak was 71.5 sec (range 35 to 100%). The second peak summated with the first, but was not always observed until the initial peak was declining. The two resulting peaks frequently appeared to have the same amplitude. It was, however, difficult to measure the actual amplitude of the second phase without taking into consideration the amplitude of the first phase. The mean rise time for the second was 18.7 sec ± 3.5 sec SE (range 10 to 34 sec). The Type II response declined to one-half the peak amplitude in a mean time of 34.8 sec (range 13 to 69 sec), which was more than twice the corresponding value for the Type I response. The response continued to fall to near baseline even though stimulation was continued (Figure 7E).

Slow responses to continuous repetitive stimulation at 100 cycles/sec were recorded from nine animals (41 measurements). The response always consisted of a single, sharp peak (Figure 7F), resembling the Type I response at 50 cycles/sec. The mean amplitude of the peak was 72.3% (range 23 to 100%), and the mean rise time was 5.4 sec (range 1 to 20 sec). The response rapidly and smoothly declined to one-half the peak tension in a mean time of 11.4 sec (range 7 to 24 sec). This value is slightly less than the corresponding value for the Type I response to stimulation at 50 cycles/sec.

Frequency-tension relationships

A graph summarizing the relationship between the frequency of stimulation and the relative amplitude of the initial phase of the slow response to repetitive stimulation is given in Figure 8. Each point represents a mean of at least 15 measurements from five or more animals. Figure 8 shows that the relative tension of the initial phase of each slow response is greater at higher frequencies of stimulation. In this figure the point at 50 cycles/sec represents the mean relative tension for the first phase of the Type II response, and its value is nearly twice that measured at 20 cycles/sec. The Type I response and the response at 100 cycles/sec are not plotted because the amplitude of the single peak for each of these responses does not necessarily correspond to the actual amplitude of the initial phase of the slow response. The single peak recorded with stimulation at 50 cycles/sec (Type I) or 100

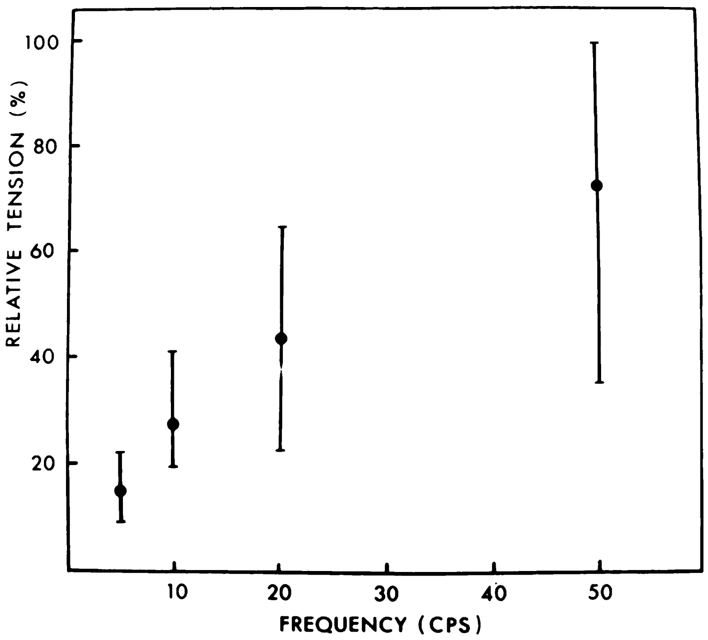

Figure 8. Relationship between frequency and tension. This figure shows a direct relationship between the mean relative tension of the initial phase of the slow response and the frequency of stimulation. The point at 50 cycles/sec is the mean value for the initial phase of the Type II response (see text for further explanation). The vertical lines indicate the ranges.

cycles/sec is apparently the result of a summation of the two phases of the slow response, the phases being more distinct at lower frequencies of stimulation.

Frequency-rise time relationships

In Figure 9A, B the mean rise times for the two phases of the slow response are plotted as a function of the frequency of stimulation. Each point in both A and B represents a mean of at least 15 measurements from five or more animals. Figure 9A shows the inverse relationship between the rise time of the first phase of the slow response and the frequency. The point plotted at 50 cycles/sec is the mean for the Type II response. The rise times for the initial phases of the Type I response at 50 cycles/sec and for the response at 100 cycles/sec could not be determined with accuracy.

The relationship in Figure 9B indicates that there is also an inverse relationship between the rise time for the second phase of the slow response and the frequency of stimulation. The rise time for the second phase of the response to stimulation at 5 cycles/sec is not plotted since the response consisted only of a plateau with no visible second phase. In Figure 9B the point plotted at 50 cycles/sec is the mean for the second phase of the Type II response. The rise times for the second phase of the Type I response at 50 cycles/sec and for the response at 100 cycles/sec could not be determined with accuracy, since the two phases of the slow response were not clearly separable at these frequencies. At frequencies where the two phases were separable the rise time of the second phase was about ten times that of the initial phase.

Figure 9. Relationship between frequency and rise time.

Figure 9A. This graph shows an inverse relationship between the mean rise of the initial phase of the slow response and the frequency of stimulation. The vertical lines indicate 1 SE above and below the means.

Figure 9B. This graph shows an inverse relationship between the mean rise time of the second phase of the slow response and the frequency of stimulation. Note that the rise times in Figure 9B are approximately ten times those in Figure 9A. The vertical lines indicate 1 SE above and below the means.

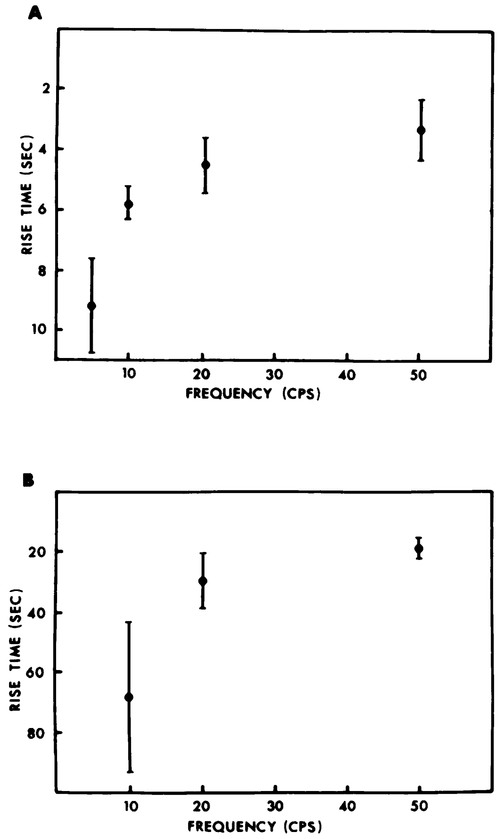
A
RISE TIME (SEC)
2
4
6
8
10
10
20
30
40
50
FREQUENCY (CPS)
B
RISE TIME (SEC)
20
40
60
80
10
20
30
40
50
FREQUENCY (CPS)

Figure 9

Thresholds for the two phases of the slow response

In two preparations distinct thresholds for the two phases of the slow response were found. A frequency of 20 cycles/sec was used to examine these thresholds, since the two phases of the slow response are clearly observed at this frequency. In Figure 10 separable thresholds for the two phases of the slow response are shown. The threshold for the initial phase, or plateau, was 0.5 V (3 msec, 20 cycles/sec). The initial phase was maintained until the stimulus strength was raised to 0.6 V. Then the tension increased gradually to a maximum and only slowly declined. Thresholds for these two phases were so close to one another in other preparations that the thresholds were not clearly separable.

Effects of changing the frequency

To demonstrate the possible presence of a frequency-dependent inhibition of the slow response of the nerve-muscle preparation, the effects of changing the frequency during continuous repetitive stimulation were examined in 21 animals. Invariably, a sustained tension at a frequency of 20 cycles/sec was sharply reduced by an increase in frequency to 50 cycles/sec (Figure 11A). If the frequency was then increased to 100 cycles/sec no change in tension was observed and the tension remained near baseline. If, however, the frequency was reduced to 20 cycles/sec the original tension was quickly and smoothly re-established. In general the frequency of stimulation at which this tension reduction was first observed was 30 to 40 cycles/sec.

Such responses to changes in the frequency of stimulation were not altered by changing from a monophasic to biphasic mode of stimulation

Figure 10. Thresholds for the two phases of the slow response.

Figure 10A. The slow response consists of two phases. The stimulus strength (0.6 V) is suprathreshold for both phases. The frequency of stimulation (20 cycles/sec) and the stimulus duration (3 msec) remain constant during stimulation.

Figure 10B. The threshold for the second phase of the slow response is shown. The initial stimulus strength (0.5 V) is suprathreshold for the first phase (plateau) of the slow response. The threshold for the second phase of the response is 0.6 V. The frequency of stimulation is 20 cycles/sec (3 msec). The horizontal lines indicate the duration of repetitive stimulation in both 10A and 10B.

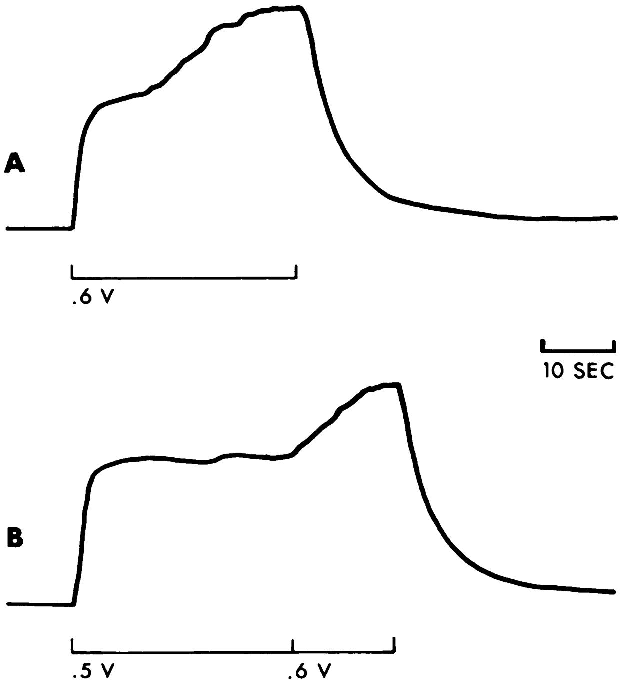

Figure 10

Figure 11. The effect of changing frequency during repetitive stimulation.

Figure 11A. The mode of stimulation is monophasic (10 V, 3 msec). The horizontal lines indicate the duration of repetitive stimulation. The numbers below the lines indicate the frequency in cycles/sec (cps). Note that a decrease in the frequency from 50 to 20 cycles/sec causes a rapid increase in tension.

Figure 11B. The mode of stimulation is biphasic (10 V, 3 msec). Note the similarity of these responses to those obtained using a monophasic mode (Figure 11A).

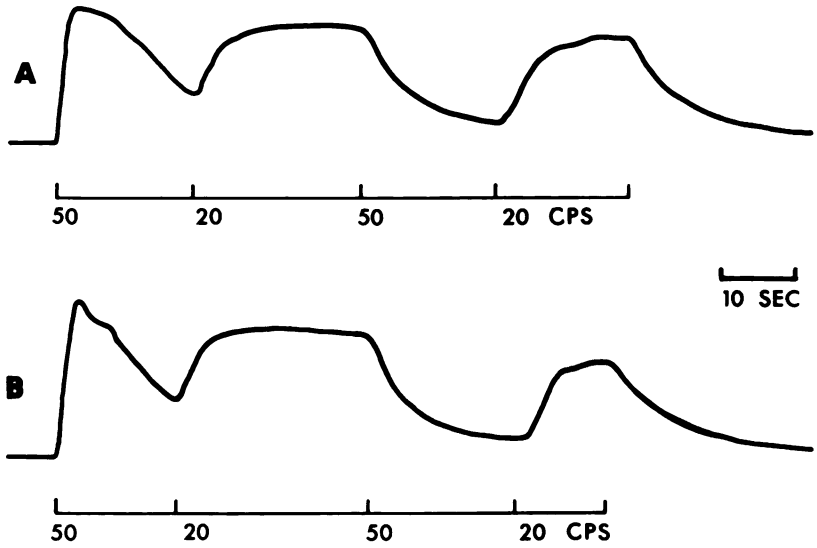

Figure 11

(Figure 11B). This indicates that the responses to changes in frequency are not a result of polarization at the stimulating electrode.

Thresholds for tension reduction

In three preparations thresholds were found for the decline in tension observed at high frequencies of stimulation (Figure 12), providing further evidence for inhibition of the slow response of the circular muscle. In this figure stimulation at a frequency of 50 cycles/sec and a stimulus strength of 0.8 V resulted in little reduction in tension, but if the stimulus strength was raised slightly the tension rapidly and smoothly dropped to near baseline. If the stimulus strength was then reduced to 0.8 V the tension was again established. Inhibitory thresholds ranged from 0.8 to 1.0 V (50 cycles/sec, 3 msec). Inhibitory thresholds were generally very close to the slow thresholds, making it very difficult to separate thresholds in other preparations.

Separation of the slow and single stimulus responses

In one preparation it was demonstrated that the single stimulus response and the slow response are each the result of stimulation of separate motor systems. A single stimulus response was obtained with a suprathreshold stimulus strength of 10 V (3 msec). Approximately five minutes after this a typical slow response to repetitive stimulation (20 cycles/sec, 3 msec) was obtained with a stimulus strength of 1.0 V. This stimulus strength was subthreshold for the single stimulus response (see Figure 4), but was suprathreshold for the slow response (see Figure 6). Approximately five minutes later a single stimulus response was again obtained, which was similar to the previous single stimulus response. Then a typical slow response was again obtained, this time

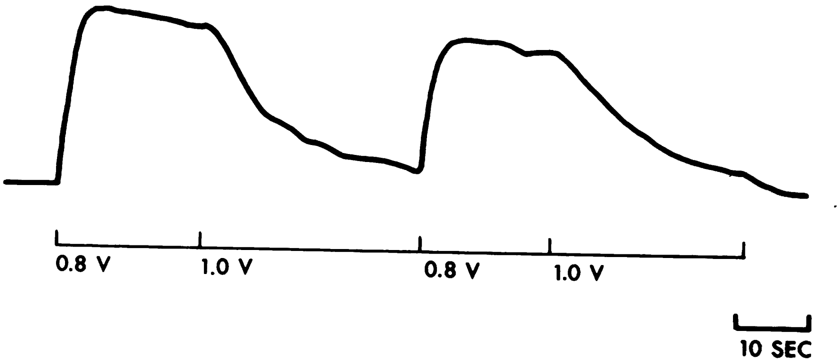

Figure 12. Threshold for inhibition of the slow response. The horizontal line indicates the duration of repetitive stimulation. The stimulus strength is changed during continuous stimulation as indicated; the frequency of stimulation (50 cycles/sec) and the stimulus duration remain constant. Note that the slight increase in the stimulus strength (from 0.8 V to 1.0 V) causes a rapid reduction in tension.

using a stimulus strength of 10.0 V (20 cycles/sec, 3 msec). This stimulus strength was suprathreshold both for the slow and single stimulus responses. Following this stimulation, no single stimulus response could be obtained, indicating that the single stimulus response had been completely fatigued. Any subsequent slow responses appeared similar to previous slow responses. These results provide further evidence for the existence of distinct motor responses of the circular muscle layer.

DISCUSSION

The Nerve-Muscle Preparation

The structure of the annelid body wall is such that the nerve-muscle relationships are difficult to study. However, a narrow transverse strip of the body wall along with corresponding segmental nerves serves as an adequate nerve-muscle preparation for studying the neuromuscular physiology of the circular muscle of the earthworm. This preparation has certain advantages over those used in previous investigations of the body wall muscle. First, the segmental nerves rather than the body wall muscle are stimulated electrically. This stimulation is made possible by using a small-tipped suction electrode. Second, the segmental nerves are severed from their central connections to the ventral nerve cord. This procedure prevents input from the central nervous system to the motor elements of the segmental nerves. Third, the preparation consists of only that portion of the body wall musculature innervated by the segmental nerves of a single segment. Thus the width of the preparation is limited to slightly more than one segment, corresponding to the size of the motor field of the peripheral nerves of an individual segment as described by Prosser (1935).

The Single Stimulus Response

My study indicates that there are several distinct motor responses of the circular muscle of the earthworm. The single stimulus response is somewhat similar to the rapid response of the longitudinal muscle of the earthworm (Roberts, 1962a). The time course of the single stimulus response of the circular muscle, however, is considerably slower than that of the "twitch" response of the longitudinal muscle. The single stimulus response of the circular muscle relaxes to one half peak tension in about nine seconds (Figure 3), whereas the "twitch" response examined by Roberts relaxes to one-half peak tension in approximately one second.

Although the "twitch" response of the longitudinal muscle is mediated by the giant fibers of the ventral nerve cord, no one has presented structural or physiological evidence concerning the relationship between the central nervous system and the motor axons innervating the circular muscle. Von Holst (1932) did present some evidence that the rapid response of the longitudinal muscle is accompanied by a simultaneous contraction of the circular muscle. The function of such a response remains unclear.

Roberts (1966) suggested that the site of the most pronounced facilitation of the rapid response was central rather than peripheral. He also found that this facilitation was long-lasting with enhanced contraction occurring as long as 9 sec after the first stimulus. In the case of the circular muscle I have shown that the single stimulus response can facilitate considerably if a second stimulus is delivered within five seconds following the first stimulus. The site of this facilitation could not be central, but must involve peripheral elements, such as neuromuscular junctional events or elastic elements in the body wall.

The fact that the single stimulus response of the circular muscle was observed in only one-third of the preparations possible indicates that the response was either completely fatigued during dissection, or that the motor fibers mediating the response were easily damaged during dissection.

The possibility does exist that the response to a single stimulus does not actually involve nervous components apart from those responsible for the slow response. It is possible that the motor fibers in the segmental nerves II and III possess a high degree of excitability and that the response to a single stimulus is actually a result of repetitive discharge of the slow motor axons to the circular muscle. The threshold for the single stimulus response is nearly ten times that for the slow response (Figures 4 and 6), and this relatively high stimulus strength may be of sufficient intensity to cause multiple discharge of the motor fibers in the segmental nerves and contraction of the circular muscle. Such an explanation is difficult to support if one considers the fact that the single stimulus response was obtained following a slow response to repetitive stimulation (20 cycles/sec) with a stimulus strength of 1.0 V, but was not obtained following a slow response to repetitive stimulation with a stimulus strength of 10.0 V.

These possibilities could be further investigated by externally recording the activity in the segmental nerves and intracellulary recording the activity in the circular muscle fibers following a single stimulus to the segmental nerves.

The Slow and Inhibitory Responses

The slow response of the circular muscle has two distinct components, each with thresholds considerably less than the single stimulus response. The first component appears as a plateau at frequencies of 0.5 to 20 cycles/sec. At higher frequencies, such as 50 and 100 cycles/sec, this component is not sustained in the form of a plateau, and the tension rapidly declines to near baseline. The second

component of the slow system has a slightly higher threshold than the first component. This second component is observed in a frequency range of 10 to 50 cycles/sec. It consists of a rise in tension which is neither as smooth, nor as rapid, as the first component.

The fact that the two components of the slow response have separate thresholds (Figure 11A, B) indicates that there are separate motor fibers which mediate each of the phases of the slow response. It is not possible from this study to determine whether a single motor fiber, or a family of fibers, mediates each component of the slow response. If, in fact, there is a family of fibers mediating each component, then all these fibers have nearly the same thresholds, and are probably of the same size. In future studies it would be helpful to subdivide the "double" segmental nerve and stimulate each subdivision separately. Thus one could possibly determine the locations and numbers of motor fibers mediating the two components of the slow response.

Both components of the slow response show a rapid decline in tension at frequencies of 50 cycles/sec and above. It has been shown that this decline could not be a result of polarization effects at the stimulating electrode since the biphasic mode of stimulation gave the same responses to changes in frequency as the monophasic mode (Figure 10).

It has also been demonstrated that this decline in tension is not simply a result of fatigue, but more likely a result of a frequency-dependent inhibition of the slow response. The rapid re-establishment of tension after the frequency is reduced from 50 to 20 cycles/sec (Figure 10) would not be expected in a system which fatigues rapidly at high frequencies of stimulation but would be expected in a frequency-

dependent inhibitory system. In addition, the thresholds which were found for this decline in tension (Figure 12) further substantiate the presence of efferent inhibitory elements in the segmental nerves. In future investigations it would be interesting to attempt to block this inhibition with various drugs.

The functional roles of the slow and inhibitory responses in the circular muscle need further investigation. It is possible that this slow system is used by the worm as an efficient means of penetrating the soil during burrowing activities. A muscle system which is not easily fatigued would be particularly useful to the animal during such locomotor activities. The two components of the slow response (Figure 11) are features that have not been described for the slow systems of other annelids, and the functions of these components remain unclear.

Inhibition of the slow response may also have functional significance during burrowing activities. Perhaps the longitudinal shortening of the worm during burrowing is accompanied by inhibitory output to the antagonistic circular muscle. Inhibition of the longitudinal muscle has been found in the earthworm, *Pheretima communissima* (Ito *et al.*, 1969; Hidaka *et al.*, 1969). They recorded inhibitory and excitatory junction potentials, as well as, miniature inhibitory and miniature excitatory junction potentials from the longitudinal muscle fibers. The electrical activity of the circular muscle fibers has not been studied, but such studies would perhaps help substantiate a reciprocal excitation and inhibition of the antagonistic muscle layers of the earthworm body wall.

Comparative Aspects of Annelid Muscle

The muscle responses of certain polychaetes parallel the single stimulus response and slow response of the earthworm. A thorough description of the nervous system of nereid worms (Class Polychaeta), as given by Smith (1957), provided a basis for the analysis of the rapid responses of two polychaetes, Nereis and Harmothöe, by Horridge (1959). Both worms responded to tactile stimulation with a rapid jerk caused by contraction of the longitudinal muscles. This shortening of the body is preceded by giant fiber activity in the central nervous system and by impulses in the large motor fibers of the segmental nerves. As in the earthworm, the sensory to giant fiber junction shows rapid habituation so that after several tactile stimuli to the anal cirri the characteristic rapid contraction of the whole worm does not occur. Horridge also presented evidence that the longitudinal muscles are capable of slower movements mediated by smaller motor fibers. The circular muscles in nereids are not well developed and have not been studied in detail. In contrast to the innervation of the circular and longitudinal muscle layers of the earthworm, no traces of inhibitory fibers to the body wall muscles of these polychaetes has been found.

Wilson (1960) further examined the nerve-muscle relationships in the longitudinal muscle of nereids. Using a nerve-muscle preparation he demonstrated that stimulation of segmental nerve IV resulted in a low threshold fast response, which rapidly decreased in magnitude with repetitive stimulation at 1 cycle/sec. A second response, or slow response, of higher threshold showed summating and facilitating characteristics and was obtained with repetitive stimulation of the same nerve above a frequency of 10 cycles/sec.

Dorsett (1964a, b) has provided structural evidence for the presence of two distinct types of innervation of the longitudinal muscle of nereids. These studies suggest that each type of muscular response may be mediated through morphologically distinct nerve terminals. It would be of significance to know whether such distinctions could be made with respect to the nerve terminals in the earthworm circular muscle.

The pattern of innervation of the leech (Class Hirudinea) musculature differs from those of the earthworms and polychaetes. The studies of Gaskell (1914) suggested a reciprocal excitatory and inhibitory relationship between the longitudinal and circular muscle of the leech body wall. Later investigations by Beritov (1945) confirmed the presence of efferent inhibitory nerve fibers to the antagonistic muscle layers of the leech body wall muscle.

The efferent excitatory fibers in the leech have been shown by Wilson (1960) to mediate only a slow response. Single pulses delivered to the segmental nerve stumps resulted in a slight response, which was graded with increasing stimulus strengths, indicating numerous motor axons. Facilitation and slow fatigue of the response were evident with stimulation at frequencies greater than 10 to 15 cycles/sec. This slow response is in slight contrast to the slow response of the earthworm circular muscle, since slow responses of the earthworm circular muscle were detectable only with repetitive stimulation at frequencies of 0.5 to 2 cycles/sec and above.

In a more recent investigation (Stuart, 1969) intracellular activity in inhibitory and excitatory motor neurons in the leech ganglion were recorded. The function of these neurons was determined by simultaneously recording externally from the segmental nerves and intracellularly from longitudinal muscle fibers.

The slow and single stimulus responses of the circular muscle of the earthworm circular muscle show some resemblance to the dual innervation pattern of the sipunculid retractor muscle (Prosser and Melton, 1954; Prosser and Sperelakis, 1959). Two systems, fast and slow, were differentiated on the basis of (1) muscle fiber structure, (2) differential effects of various drugs (atropine, veratrine and procaine) on the electrical activity of the muscle fibers, (3) different motor fiber thresholds, (4) analysis of the nerve fiber diameters, and (5) results from nerve degeneration experiments. Thus it appears that dual motor innervation is characteristic not only of most annelids, but also of the related Phylum Sipunculida.

This thesis has described some of the physiological characteristics of the circular muscle of the earthworm. A thorough investigation of the fine structure and physiology of the neuromuscular junctions in the circular muscle, as well as, an analysis of the structure of individual circular muscle fibers are needed. Such studies may further substantiate the presence of various excitatory and inhibitory axons to the circular muscle. The relationship of such efferent fibers to the central nervous system is unclear. No one has related the activity of neurons in the central nervous system to the efferent activity in the segmental nerves or to the activity in the circular muscle fibers. From investigations of this kind we could begin to learn more about the role of the circular muscle in the locomotor activities of the earthworm.

SUMMARY

1. A suitable nerve-muscle preparation was obtained for the investigation of the physiological characteristics of the circular muscle of the earthworm, *Lumbricus terrestris*.

2. Stimulation of the segmental nerves using a suction electrode results in several distinct responses of the circular muscle layer.

3. The first response is a response to a single stimulus. Rheobase for this response is 2.5 V. The amplitude of the response is dependent on the stimulus strength, the amplitude at threshold being only 10 to 20% of the amplitude at supramaximal stimulus strengths.

4. The single stimulus response summates and facilitates when three or five second intervals are allowed between paired stimuli, but no facilitation is observed when a ten second interval is allowed between a pair of stimuli.

5. The single stimulus response fatigues rapidly. Recovery times for the single stimulus response ranged from 15 to 40 minutes.

6. The second type of response is the slow response to repetitive stimulation at frequencies greater than 2 cycles/sec. Rheobase for this slow response is 0.2 V, or approximately one-tenth the rheobase value for the single stimulus response.

7. At frequencies between 10 and 50 cycles/sec the slow response consists of two distinct phases of tension development. In some preparations separate thresholds for the two phases were found.

8. A frequency-dependent inhibition of the slow response is observed with stimulation at frequencies greater than 50 cycles/sec. At frequencies of 20 cycles/sec and less, inhibition of the slow response is not apparent. In some preparations thresholds for this inhibition

were separated from the thresholds for the slow response.

9. The excitation and inhibition of the circular muscle layer described in this paper corresponds somewhat to the excitation and inhibition of the longitudinal muscle layer. Such a reciprocal excitation and inhibition may be of functional significance during certain locomotor activities.

LIST OF REFERENCES

Beritov, I. 1945. On the excitation and inhibition of the reflex reactions of the nerve muscle preparation of the middle segments of a leech. Trud. Tbilissk. Univ. 27a:29-52.

Botsford, E. F. 1939. Temporal summation in neuromuscular responses of the earthworm, Lumbricus terrestris. Biol. Bull. 77:328-329.

Botsford, E. F. 1941. The effect of physostigmine on the responses of earthworm body wall preparations to successive stimuli. Biol. Bull. 80:299-314.

Budington, R. A. 1902. Some physiological characteristics of annelid muscle. Am. J. Physiol. 7:155-179.

Bullock, T. H. and G. A. Horridge. 1965. Structure and Function in the Nervous Systems of Invertebrates, Vol. I. W. H. Freeman and Co., San Francisco.

Chang, Y. C. 1969. Membrane potential of muscle cells from the earthworm Pheretima hawayana. Am. J. Physiol. 216:1258-1265.

Chapman, G. 1950. Of the movement of worms. J. Exp. Biol. 27:29-39.

Coonfield, B. R. 1932. The peripheral nervous systems of earthworms. J. Comp. Neurol. 55:7-17.

Dorsett, D. A. 1963. The motor axon terminations of annelids (Lumbricus and Nereis). Nature 198:406.

Dorsett, D. A. 1964. The sensory and motor innervation of Nereis. Proc. Roy. Soc. London 159:652-677.

Gaskell, J. F. 1914. VI. The chromaffine system of annelids and the relation of this system to the contractile vascular system in the leech Hirudo medicinalis. Philos. Trans. Roy. Soc. Lond. (B) 205:153-211.

Gray, J. and H. W. Lissman. 1938. Studies in animal locomotion VII. Locomotor reflexes in the earthworm. J. Exp. Biol. 15:506-517.

Hanson, J. 1957. Structure of the smooth muscle fibers in the body wall of the earthworm. J. Biophys. Biochem. Cytol. 3:111-122.

Hess, W. N. 1925*a*. Nervous system of the earthworm, *Lumbricus terrestris* L. J. Morph. *40*:235-261.

Hess, W. N. 1925*b*. The nerve plexus of the earthworm, *Lumbricus terrestris*. Anat. Rec. *31*:335-336.

Hesse, R. 1894. Zur vergleichende Anatomie der Oligochaeten. Zeit. f. wiss. Zool. *58*:394-439.

Hidaka, T., Y. Ito, H. Kuriyama and N. Tashiro. 1969*a*. Neuromuscular transmission in the longitudinal layer of somatic muscle in the earthworm. J. Exp. Biol. *50*:417-430.

Hidaka, T., H. Kuriyama and T. Yamamoto. 1969*b*. The mechanical properties of the longitudinal muscle in the earthworm. J. Exp. Biol. *50*:431-443.

Holst, E. von. 1933. Weitere Versuche zum nervösen Mechanismus der Bewegung beim Regenwurm (*Lumbricus terrestris* L.). Zool. Jb. *53*:67-100.

Horridge, G. A. 1959. Analysis of the rapid responses of *Nereis* and *Harmothoe* (Annelida). Proc. Roy. Soc. London (B) *150*:245-262.

Ito, Y., H. Kuriyama and N. Tashiro. 1969. Miniature excitatory junction potentials in the somatic muscle of the earthworm, *Pheretima communissima*, in sodium free solution. J. Exp. Biol. *51*:107-118.

Mill, P. J. and M. F. Knapp. 1970. The fine structure of obliquely striated body wall muscles in the earthworm, *Lumbricus terrestris* Linn. J. Cell. Sci. *7*:233-262.

Moore, A. R. 1923. Muscle tension and reflexes in the earthworm. J. Gen. Physiol. *5*:327-333.

Pantin, C. F. A. 1948. Notes on Microscopical Technique for Zoologists. University Press, Cambridge.

Prosser, C. L. 1934. The nervous system of the earthworm. Quart. Rev. Biol. *9*:181-200.

Prosser, C. L. 1935. Impulses in the segmental nerves of the earthworm. J. Exp. Biol. *12*:95-104.

Prosser, C. L. and N. S. Melton. 1954. Nervous conduction in smooth muscle of *Phascolosoma* proboscis retractors. J. Cell. Comp. Physiol. *44*:255-275.

Prosser, C. L. and N. Sperelakis. 1959. Electrical evidence for dual innervation of muscle fibers in the sipunculid *Goldfingia* (=*Phascolosoma*). J. Cell. Comp. Physiol. *54*:129-133.

Roberts, M. B. V. 1962*a*. The giant fiber reflex of the earthworm, *Lumbricus terrestris* L. I. The rapid response. J. Exp. Biol. *39*:219-227.

Roberts, M. B. V. 1962*b*. The giant fiber reflex of the earthworm, *Lumbricus terrestris* L. II. Fatigue. J. Exp. Biol. *39*:229-237.

Roberts, M. B. V. 1966. Facilitation in the rapid response of the earthworm, *Lumbricus terrestris* L. J. Exp. Biol. *45*:141-150.

Seymour, M. K. 1969. Locomotion and coelomic pressure in *Lumbricus terrestris* L. J. Exp. Biol. *51*:47-58.

Smallwood, W. M. 1926. The peripheral nervous system of the common earthworm, *Lumbricus terrestris*. J. Comp. Neurol. *42*:35-55.

Smallwood, W. M. 1930. The nervous structure of the annelid ganglion. J. Comp. Neurol. *51*:377-392.

Smith, J. E. 1957. The nervous anatomy of the body segments of nereid polychaetes. Philos. Trans. Roy. Soc. London (B) *240*:135-196.

Straub, W. 1900. Zur Muskelphysiologie des Regenwurms. Pflüg. Arch. ges. Physiol. *79*:379-399. As quoted in Stephenson, J. 1930. The Oligochaeta. Clarendon Press, Oxford.

Stuart, A. E. 1969. Excitatory and inhibitory motoneurons in the central nervous system of the leech. Science. *165*:817-819.

Wilson, D. M. 1960. Nervous control of movements of annelids. J. Exp. Biol. *37*:46-56.