

THE ROLE OF THE SUBEPIDERMAL NERVOUS SYSTEM IN THE LOCOMOTION OF THE EARTHWORM

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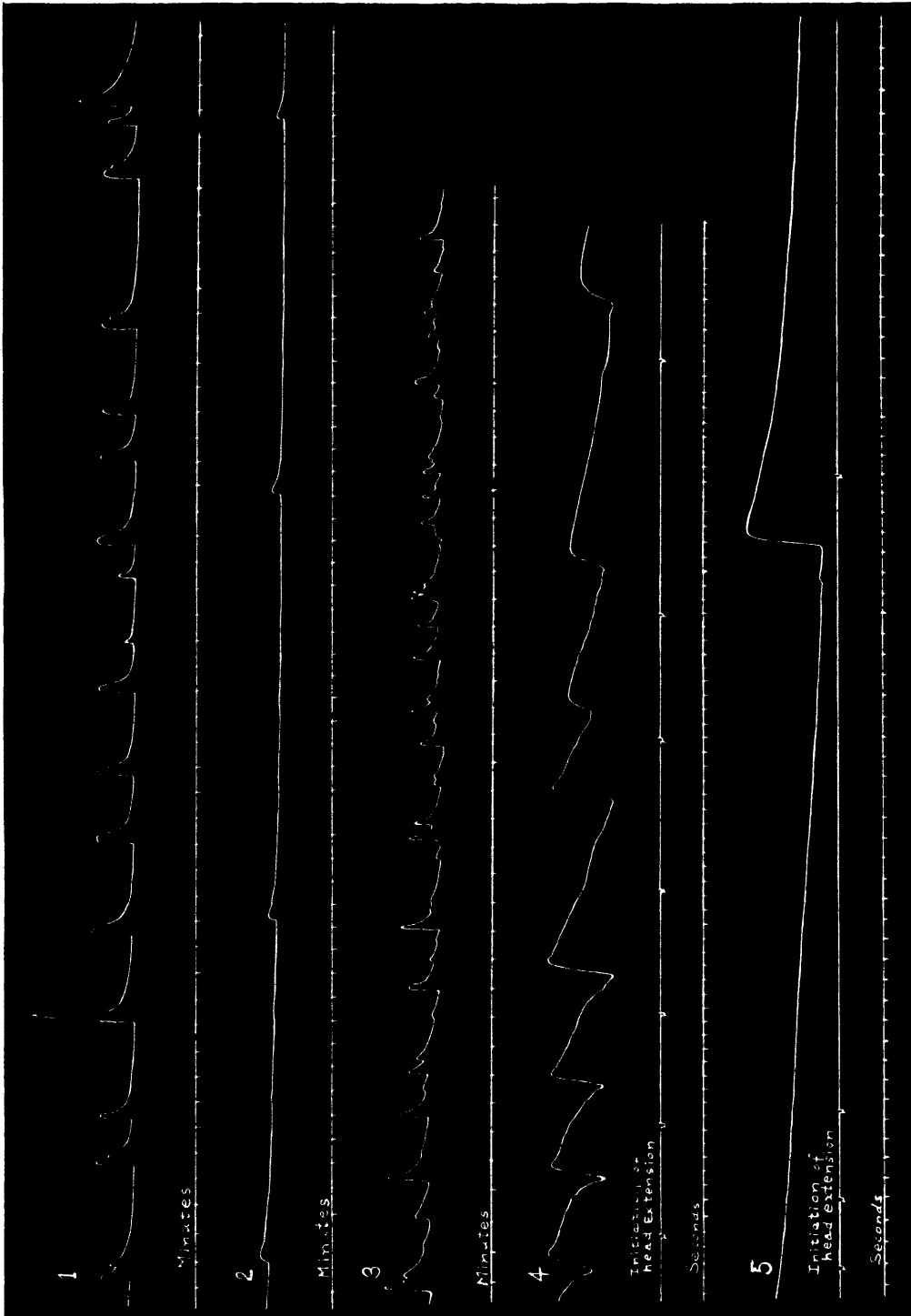
Friedländer (1894) was the first one to show that the central nervous system plays only a small part in the creeping movement of an earthworm. He concluded that the wave-like motion of the contractions proceeding the length of the worm was due first to the "pull" of segments on each other, and secondly to the sequence of reflex actions in each segment. This nervous mechanism, according to Friedländer, involves the individual segment alone without the passage of impulses through the cord. Biedermann ('04) found that when the middle region of a worm was anaesthetized, the posterior region acted in perfect coordination with the anterior part in creeping movements. In another experiment he observed that when the anaesthetized region was pinned to a cork plate, the posterior part still moved in perfect coordination with the anterior part. Thus, he demonstrated the transmission of impulses over considerable distance in the cord. Garry and Moore ('15) studied the coordination of the peristaltic waves. They verified the work of Biedermann to the extent that the peristaltic impulses are conducted by the cord. Straub (1900) observed that strips of body wall both with and without ventral nerve cord will give rhythmic contractions. However, the strips removed from denervated regions required several (eight) days for recuperation before such strips could produce rhythmic contractions. Budington ('02) found that when care was used to remove all nervous tissue and used only pieces of worm in which the whole ventral muscle had been removed they failed to demonstrate rhythmic contraction. An extensive study of the transmission of the nervous impulses in relation to locomotion was made by Bovard ('18). He showed that after the cord was cut at the middle region of the body, both ends would not act in perfect coordination. He disagreed with Straub's (1900) finding that a piece of the body wall without nerve cord is able to make rhythmic contraction.

On the other hand the presence of a subepidermal network of nerves was observed by Retzius (1892), Smirnow (1894), Langdon (1895), and Smallwood ('23, '26) and was carefully studied morphologically by Hess ('25). Hess found that the subepidermal network of nerves in the earthworm is a true nerve plexus composed of anastomosing nerve fibers which form a network in the region between the bases of the epidermal cells and the basement membrane. He concluded that this plexus is not segmental in its arrangement. Coonfield ('32) disagrees with Hess and Smallwood regarding the continuous nature of the plexus. His experiments on the secretion of mucus and the discharge of coelomic fluid show that impulses are not transmitted from somite to somite without the presence of the ventral nerve cord. He concluded that the peripheral nervous system is not a continuous nerve net.

Because of these controversies of opinions, it is the purpose of this paper to present the results obtained from further investigations employing physiological methods. The earthworm used in the following experiments is *Lumbricus terrestris*, found in Columbus, Ohio. The room temperature during the experiments was 26-27° C.

BEHAVIOR FOLLOWING REMOVAL OF VENTRAL NERVE CORD

Sections consisting of 40-60 segments exclusive of the head were cut from healthy unanaesthetized worms. Following the removal of the intestine in saline



solution, the body wall was turned inside out and the ventral nerve cord was completely removed with the aid of the binocular microscope. The body wall was then reversed (skin side out) and placed in a Petri dish containing normal saline solution. It was seen under the binocular microscope that this operated section exhibited only slight constriction movements. When these movements were watched carefully, we could see that each was confined to an individual segment and was independent of the remaining segments. After one to two hours, the above described involuntary movements ceased to occur. The section as a whole, however, was more sensitive to touch. If such a section was touched or pinched, a wave of longitudinal muscle contraction could be initiated at any segment and could move in either or both directions. The sequence of contraction was segment to segment. Measuring with a stop watch, it was determined that the rate of contraction wave varied from 3.0 segments per second to 4.3 segments per second. The average rate was 3.8 segments per second. The distance traveled by the contraction wave in the pieces tested varied from 11-43 segments. Stimulation of a section in the middle would initiate waves of contraction in both directions. The speed of the contractions thus initiated was the same in both directions. The contraction of the circular muscle was not observed when the body wall was pinched or touched.

Each operated section was then attached to a recording lever. The section was so placed that it hung vertically with only very slight stretching by the lever. The whole piece was kept submerged in normal saline solution and the contractions were recorded in a slowly moving kymograph drum. It was found that of the many pieces tested, some of them recorded autonomic contractions during the first few hours after their preparation (see figure 1). Those sections which did not give immediate contractions were kept in refrigeration at 5°C for a day and then were allowed to stay at room temperature for a few hours before they were tested again. It is shown in the kymograph record (see figure 3) that active autonomous contractions of shorter intervals were initiated by such treatments.

However, both the magnitude and rhythm of contractions of a single piece or of the different pieces varied a great deal. It was observed that the magnitude of the contractions depends in part upon the total length of the section and the vitality of the piece tested. It is also related to the distance of the contraction waves traveled, i.e., the farther the waves travel, the greater will be the magnitude, because of the fact that the relaxation phase is much shorter than the contraction phase as the contraction waves travel along each of the successive somites. As to the rhythm, the intervals of contraction were irregular, varying from two to five minutes.

In order to determine whether the magnitude or the interval of contractions in a given section could be changed by splitting, a previously tested section was split longitudinally along the middorsal line. It was observed that immediately after splitting, the length of the section became much shorter. A section thus

EXPLANATION OF PLATE I

FIGURE 1. The rhythm of contractions of a section (42 segments) whose nerve cord has been completely removed.

FIGURE 2. The decrease of frequency and magnitude of contractions following a longitudinal split along the middorsal line. (The same section used in the illustration Figure 1.)

FIGURE 3. Showing the occurrence of contractions of a section (40 segments) which has been kept in refrigeration for a day.

FIGURE 4. Illustrating the coordinated movement of the tail after each extension of the head when ten segments of the middle region of the body were fixed by pinning. Each peak represents maximum contraction.

FIGURE 5. Demonstrating that no coordinated movement of the tail took place when the nerve cord was cut in the fixed region.

operated lengthened in time but never regained its initial length. The magnitude of contractions of such operated pieces diminished while the interval of contractions increased (see figure 2). This is interpreted as the result of the loss of tone of the circular muscle layer.

DISTANCE OF IMPULSE TRANSMISSION THROUGH THE SUBEPIDERMAL NERVOUS SYSTEM

An unanaesthetized worm was split longitudinally along the middorsal line for a distance of ten segments in the midbody region. The intestine was removed from this region exposing the ventral nerve cord. The operated portion was then fixed firmly in a dissecting tray with small-sized insect pins (about three to four pins on each segment). The pinning process was done under the dissecting microscope to avoid injury to the segmental nerves. Both the anterior and posterior regions were free to move. Only the posterior tip was connected to the recording lever. The initiation of each extension of the head was marked by a signal magnet. The record (figure 4) shows that the impulses from the head region could be transmitted through the fixed middle region to the tail in an average of four seconds when the ventral nerve cord was intact. When the nerve cord was severed between the seventh and eighth segment of the fixed operated region without injury to the lateral nerves, there was no movement of the tail following head extension in this experiment (figure 5). It was necessary that the nerve cord be cut near the caudal margin of the exposed area in order that all "pull" produced by the unattached anterior region would be eliminated. Furthermore, movement of the tail region could not be induced by applying Faradic shocks to the cord in the head region. This clearly shows that the subepidermal nervous system cannot transmit locomotor impulses from one segment to the next if the segments are prevented from movement. The experimental evidence further substantiates the contention that the subepidermal nervous system is segmental in arrangement.

DISCUSSION

The occurrence of irregular contractions of a section lacking the ventral nerve cord is worthy of discussion. These experiments have been repeated several times and consistent results obtained. It is also necessary to mention here that extreme care was taken to eliminate the entire ventral nerve cord. It has been shown that when the body wall was split longitudinally, the tone of the circular muscle layer was diminished, and the whole piece became shorter in length. As a result both the frequency and magnitude of contractions decreased. That Budington ('02) and Bovard's ('18) isolated strips could not produce contraction might be due to the loss of tone of the circular and longitudinal muscles. In addition, sufficient time for recovery from operative shock or injury due to overstretching was necessary. At all times the section should be kept in a saline solution bath even though attached to the recording lever. In addition to the above requirements, the writers have found that a slight stretching of the section by the recording lever may be an important factor in initiating contraction. Such contraction waves were not observed when the section was laid unattached in normal saline solution. Zyeng ('30) has found that there are nerve cells located between longitudinal and circular muscles, between the epidermis and the circular muscle layer and sometimes within the circular muscle layer itself. The presence of these cell bodies may account for the autonomic movement of the isolated piece in the absence of the ventral nerve cord.

Bovard ('18) in interpreting the rhythmic contractions described by Straub suggested that such phenomenon may be due to the regeneration of nerve fibers and cells. However, according to our observations, such contractions occurred either shortly after the operation or within twenty-four hours after recovery under

refrigeration. This strongly suggests that regeneration is not a factor in explaining this phenomenon.

The results obtained by the writers using completely different physiological methods from those previously employed are in agreement with Coonfield's ('32) findings concerning the segmental arrangement of the subepidermal nervous system in the earthworm. The impulses which are transmitted from segment to segment are not through the "subepidermal nerve net" but are conveyed through the peripheral nerves and ventral nerve cord. In locomotion, impulses are initiated in successive segments by the pulling action of the adjacent segments.

According to the writers' observations, the most probable functions of the subepidermal nervous system are the regulation of the contraction of the longitudinal muscle and, as a reinforcement of locomotor impulses. Bovard ('18) found that the locomotor impulses travel short distances of ten segments very readily, and more difficultly as the number of segments increases; but, impulses are unable to pass through a distance of thirty segments unless there is reinforcement from the muscle. Let us make some mathematical calculations based on Bovard's and the writers' data to see whether our conclusion concerning the function of the subepidermal nervous system as a reinforcing mechanism can fit the actual situation. The rate of the peristaltic waves of the earthworm under the conditions tested was found by the writers to be about 3 cm. per second. If a worm is 14 cm. long, it will take 4.7 seconds for a contraction wave to travel from the head to the tail. The time consumed in this reaction far exceeds the time required for actual impulse transmission. But if we assume that in every ten segments a reinforcement takes place, then there will be fourteen reinforcements. As found in one of the previous experiments, the rate of contraction wave in the body wall in absence of the nerve cord is approximately 3.8 segments per second (1 segment per 0.26 second). For fourteen reinforcements, it will take about 3.6 seconds which is $\frac{3}{4}$ of the total time needed for the propagation of the wave, and only 1.1 second is left for the transmission of the impulses through the nerve cord and the delay in the various synapses. The synapses involved include those in the ventral nerve cord and those between the segmental nerve endings and the subepidermal neurons. It seems that this shorter time fits better the faster conduction rate of the nerve impulses occurring in the central nervous system.

The activity of the isolated section from which the ventral nerve cord was removed shows that the circular and longitudinal muscles are governed by two different sets of nerves. Stimulation of the epidermis results in the contraction of only the longitudinal muscle. The contraction of the circular muscle should therefore be controlled by the central nervous system. This interpretation seems to fit very well with the mechanism of locomotion. As we know, when a worm begins to move forward, the circular muscle of the head region contracts first. The initiation of the contraction of the circular muscle is due to impulses from the central nervous system. The contraction of the circular muscle in turn stretches the longitudinal muscle. As a result, reinforcing impulses are sent into the central nervous system, and at the same time, a contraction of the longitudinal muscle is initiated.

SUMMARY

1. In the absence of the ventral nerve cord, impulses can be initiated in the body wall and transmitted in both directions.
2. The initiation of such impulses can be accomplished by mechanical stimulation.
3. The impulses initiated in such a denervated section are conducted within the limits of a single segment through the subepidermal nerve net. Such impulses originate in successive segments as a result of the pulling action of the adjacent segments.

4. The contraction wave is 3.8 segments per second of a section without a ventral nerve cord.
5. The subepidermal nervous system of the earthworm is shown through physiological analyses to be segmental in arrangement.
6. The most probable role of the subepidermal nervous system is that of controlling the contraction of the longitudinal muscle and acting as a reinforcing agent in the peristaltic waves.

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