

SOME STUDIES ON THE NERVE FIBERS OF CAMBARUS

by

RICHARD RILEY MARSH

A. B., Baker University, 1927

B. S., Kansas State Teachers College, Pittsburg, 1928

A THESIS

submitted in partial fulfillment of the

requirements for the degree of

MASTER OF SCIENCE

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1931

Docu-
ment
LD
2668
.74
1931
m33
C.2

TABLE OF CONTENTS

	page
INTRODUCTION AND REVIEW OF LITERATURE-----	3
PURPOSE AND STATEMENT OF THE PROBLEM-----	6
METHODS AND MATERIALS-----	9
RESULTS AND DISCUSSIONS-----	10
Dorsal Pair of Intermediate Size Fibers-----	10
Results-----	10
Discussion-----	13
Large Ventral Fibers-----	14
Results-----	14
Discussion-----	15
Giant Fibers-----	16
CONCLUSIONS-----	16
ACKNOWLEDGMENT-----	17
LITERATURE CITED-----	18
EXPLANATION OF PLATES-----	20

INTRODUCTION AND REVIEW OF LITERATURE

Since the middle of the nineteenth century the structure of the ventral nerve cord of invertebrate animals has been of interest to neurologists. The use of Paul Ehrlich's method of staining living nervous tissue with methylene blue, the development of the method of staining chrome hardened tissue with a silver solution (Glogi, 1885), and the application of vom Rath's fixative (1893) have done much to make a detailed study of the internal organization possible.

Schneider (1902) described the connectives of the cord of the crayfish (Fig. 1) as being elliptical in cross-section, paired, and somewhat flattened along the neighboring areas. There were separated by a double septum of connective tissue. This septum extended over the dorsal surface into and forming a part of the perineurium or binding tissue layer surrounding the cord. This perineurium was only weakly developed and contained the small cells of the second order of Leydig.

Krieger (1879-1880) described the abdominal ganglia as having great similarity one to another so that the morphological description of one would suffice for all, with the possible exception of the sixth ganglion or the last one of the cord. He found an abdominal ganglion had a length of approximately 0.9 mm. from the most anterior to the most posterior

cells, a total length of 1.4 mm. inclusive of the connective tissue. Its greatest width was 1.0 mm. and its greatest height was 0.85 mm.

Krieger as well as Schneider described two pairs of nerves going out from the ganglion, the more anterior pair leaving about 0.5 mm. posterior of the most anterior cells and about one fourth of the height above the ventral floor. Corresponding to this first pair of nerves were two fiber-bundles, the neuropile, within the ganglion, one on each side, which were connected with each other superiorly and anteriorly by a commissural strand of fibers. A strand of connective tissue also joined them across the lower anterior surface. Corresponding to the second pair of nerves, which left the ganglion approximately two thirds of the distance toward its posterior end, was another pair of bundles similar to the first but which were located slightly higher within the ganglion. A third pair of nerves went out from the cord about one millimeter back of the most posterior cells of the ganglion. Schneider as well as Krieger believed that the first pair of nerves went to the swimmerette while the second and third pairs went to the musculature of the abdomen, the second serving the muscles of the appendages and the third the other muscles of the region. The cells of the abdominal ganglia were of two sizes. The larger ones had a

diameter as great as 0.17 mm. while the smaller were usually about 0.04 mm. in size. The larger cells were found in the anterior position. Small cells were crowded in among these large cells. The thoracic portion of the cord contained five ganglia all very similar in structure to each other. The connectives separated above the suboesophageal ganglion to go around the oesophagus, beyond which they end in the brain.

Krieger also showed four dorsal giant nerve fibers in his cross sections of the nerve cord of the crayfish. Their presence was further demonstrated by Retzius (1890) by means of inter-vitam methylene blue staining. Retzius also demonstrated giant fibers in the third pair of nerves. These were considered to be branches of the median giant fibers. The median and lateral giant fibers were thought to extend without breaks throughout the length of the cord. Johnson (1924) working on the crayfish (*Cambarus*) and the shrimp (*Palaeomonetes*) showed that the lateral giant fibers were segmented and that the giant fibers in the third pair of nerves sprang from cell bodies in the anterior ganglion. The latter were motor giant fibers evidently receiving impulses from the median and lateral giant fibers. By electrical stimulation it appeared that the giant fibers served to cause the vigorous flipping of the abdomen. (Johnson, 1926).

Since the giant fibers have been studied in annelids as well as in the crayfish, a brief statement of results of those studies may be considered for the sake of comparison. Friedlaender (1868) gave Leydig credit for the discovery of the giant fibers in the nerve cord of annelids. Friedlaender believed them to be neural in function and this was later proved by the experiments of Boverd (1918), Yelton (1923), and Stough (1926). The cord of the annelid differed from that of *Cambarus* in that ventral giant fibers were observed which appeared to have structure similar to the dorsal giant fibers. Another difference to be seen is that the median giant fibers of the earthworm are segmental while in *Cambarus* they seem to be continuous. However, there were places in that of the earthworm where the fiber failed to show segmentation regularly. Stough (1930) by physiological methods demonstrated the polarity of the dorsal giant fibers to be in agreement with the conclusions of Johnson (1924) for *Cambarus*, that is, the median fibers conducted posteriorly and the lateral fibers transmitted impulses anteriorly.

PURPOSE AND STATEMENT OF THE PROBLEM

The purpose of this paper is to further study the internal structure of the ventral cord of *Cambarus*, paying particular attention to two pairs of conspicuous dorsal

fibers that are intermediate in size between the giant fibers and the numerous small fibers of the cord; to reexamine the median giant fibers for possible septa indicating a segmental organization; and to observe fibers possibly corresponding to the ventral giant fibers of the Annelids.

The paired dorsal fibers have been illustrated by previous workers. They may be identified in drawings of cross sections by Krieger (1879-1880) lying immediately beneath the median giant fibers of *Astacus*. Johnson (1924) in his figures 28 to 31, of the abdominal ganglia of *Cambarus* in cross-section showed these fibers. He also refers to some methylen blue staining of one dorsal pair in *Palaemonetes*, but they are not clearly seen in his cross-sections of the cord of *Palaemonetes* and *Crangon*.

A search for ventral giant fibers in previous work on the crayfish revealed one medium sized fiber in a cross-section of a ganglion (Krieger, 1879-1880). Retzius (1890) included a figure of the first abdominal ganglion of *Astacus* bearing fibers marked "h" and "i" which might be similar to the fibers here being considered. However, since he did not indicate by diagram or by description, it was impossible to determine their plane within the cord and so to homologize them. This was also true of fibers h, i, j, k, and i' in figure 10 (Krieger, 1880) of the last abdominal ganglion of

atactous. These fibers have been inclusively designated by these investigators as merely "Nervenfasern".

An examination of Allen's (1894) diagram of the nervous system of the embryo lobster showed several long fibers. However, since he did not show their position in the cord it is not possible to determine if they are homologous to the fibers studied in this work. His fiber A (a) Ant. II, in figure 2, sent a process anteriorly as well as one posteriorly after having crossed in the region of the second thoracic ganglion to the other side of the cord. The posterior process extended into the sixth abdominal ganglion where it was lost. Fiber A (a) Th. I also branched, one process going posteriorly into the next ganglionic region and the other going to the other side of the cord and then into the sixth ganglion also. Fiber A (a) Th. II arose within the second thoracic ganglion, crossed the cord and proceeded posteriorly to the sixth ganglion. Allen was unable to trace the fate of any of these specific fibers after their entrance into the sixth ganglion.

No indication of fibers that would correspond to the dorsal paired fibers or the large ventral fibers of the crayfish was seen in the drawings of the fibers in certain insect larvae made by Hilton (1911, 1924).

METHODS AND MATERIALS

The specimens used in this study were obtained some from Wildcat Creek and some from Pillsbury Crossing on Deep Creek, both near Manhattan, Kansas. Methylene blue stain was injected according to the method of Retzius (1890) but no staining of the fibers studied was obtained. For the greater part sections of the nerve cord prepared according to the von Rath (1895) method (Johnson, 1924) were studied.

The cords were studied by serial sections under a microscope. Not all the series were complete nor were all the slide of each series of a nature that would permit study because of shrinkage or faulty staining. However, by making serial drawings of sections showing changes, a fair picture of the location and courses of the fibers could be secured by reconstructed diagrams.

Because of the difficulties encountered in the tracing of branching fibers within the sixth abdominal ganglion, series of this were cut to a thickness of 60 microns in the case of three cords. This was, however, too great a thickness in one case to allow study and with the other two cords the staining had been too light to enable their use.

RESULTS AND DISCUSSIONS

Dorsal Pair of Intermediate Size Fibers

Results. Study of the course of the large paired dorsal fibers of the two species of crayfish, *Cambarus virilis* and *Cambarus limosa*, indicated that they continued through the cord into the sixth ganglion of the abdominal region showing neither septa nor branches. In the connectives these fibers (Fig. 2, Y and Y') lie slightly ventral to the median giant fibers until past the third pair of nerves when they resume a position adjacent to the ventral surface of the median giant fibers. This position was retained into the ganglion to the region of the second pair of nerves where they descended slightly to the position found in the anterior portion of the connectives. (Fig. 3)

Within the ganglia the Y and Y' fibers rotated so that 0.15 mm. from the anterior cells the Y fiber may lie ventrally or even medially to the Y' fiber. (Fig. 3) While the degree of rotation was not consistent yet more or less rotation occurred in each ganglion. Beyond the second pair of nerves, at about the place where the pair of fibers descended from the median giant fibers, the Y fiber again shifted so as to lie laterally and sometimes dorso-laterally to the Y' fiber. This is the relationship found in the con-

nectives for these fibers.

In their course through the cord the dorsal fibers maintained a fairly constant diameter, being of about the same size within the ganglion as within the connectives. It was noticeable, however, that their diameter increased as they extended posteriorly. Within the ganglia, accompanying fibers increased in size to such a degree that it was often difficult to distinguish the paired fibers that were being traced. (Fig. 3) These fibers were segmental, however, and usually disappeared within the ganglion. Through the connectives these additional dorsal fibers maintained a diameter much less than that of the dorsal paired fibers, again allowing them to be seen as a distinct pair.

The sixth abdominal ganglion or the most posterior one of the cord disclosed changes both in the non-branching character and in the constancy of position of the dorsal paired fibers. Four out of five series of the sixth ganglion of the abdomen clearly showed lateral branching of the Y' fiber (Fig. 4) to occur in this ganglion. In the first series the lateral branches were given off at about 0.22 mm., in the fifth series at 1.10 mm. for the right Y' fiber and 1.24 for the left one, in the seventh series at 0.44 mm. on the right side and 0.62 mm. on the left side, and in the thirteenth the lateral branch of Y' fiber on the right side

was sent out at 0.37 mm. and for the left side at 0.22 mm. These distances were measured from the anterior cells of the ganglion. In all four instances the Y' fibers lay adjoining the mid-line of the ganglion and slightly ventral to the level of the median giant fibers while the Y fibers had moved to a lateral plane with the Y' fibers and had migrated outward until they lay almost directly below the lateral giant fibers. The lateral branch of the Y' fibers in all cases passed dorsally over the Y fiber and could be traced only a short distance beyond the Y fiber. (Fig. 4)

In the first series there could be observed, in addition to the lateral branching of the Y' fibers, a branch from each one extending medially and ventrally. (Fig. 5) These inward directed branches could only be traced a short distance downward along the median line of the ganglion, and seemed to originate in about the same region of the fiber as the lateral branches.

In three of the five series of sections of the last abdominal ganglion studied it was possible to follow the dorsal paired fibers to their posterior termination. These showed a crossing over of the Y' fibers into the opposite side of the ganglion. In one case (Figs. 6 and 7) it was possible to trace the crossed fibers as they continued anteriorly along the Y' fiber of the other side. These con-

tinuation fibers were lost, however, without their showing cellular connections. The crossing-over of the fibers occurred slightly posterior to the point of the dividing of the lateral giant fibers and occupied a horizontal space equal to the distance from the lateral line of the one median giant fiber to the extreme lateral border of the one on the other side of the ganglion. In one series the Y fiber in the right side of the ganglion crossed over to the left side and continued anteriorly for a very short distance. (Fig. 6) The Y fibers in all other series diminished to non-traceable size slightly posterior to the place of the crossing over of the Y' fibers. No cellular connections were observed for the Y fibers at the posterior ending.

Discussion. Johnson (1924) found this pair of fibers in the thoracic region of the cord of *Cambarus* and suggested their being the same as the fibers lying directly ventral to the median giant fiber in the abdominal region. He pointed out that they appeared to be non-segmental. Johnson also described a single non-segmented dorsal fiber extending from the posterior part of the thoracic nerve cord to the third abdominal ganglion in *Palaemonetes*. In his paper dealing with the function of the giant fibers, Johnson (1926) showed that though the flipping of the abdomen occurred in animals whose cord had been cut and whose giant fibers had regener-

ated, the uropods did not fold over the telson as completely as in the control animals. This held true even under stronger stimuli. Apparently this was due to the loss of function of fibers other than the giant fibers. Because the dorsal paired fibers are non-segmental and because they lie in close proximity to the median giant fibers, the control of the folding of the uropods might be delegated to them. The dorsal paired fibers may be associative in function in that direct contact with extending nerves was not indicated throughout this investigation.

Large Ventral Fibers

Results. Each connective throughout the cord possessed at least one ventral fiber that was consistently larger than the median giant fiber. These fibers, unlike the dorsal paired fibers, were strictly segmental. The number of these fibers decreased toward the posterior end of the abdominal region so that only one to two such fibers extended from the fifth to the sixth ganglion, while the more anterior connectives had from two to four of these large fibers. The large ventral fibers, in their course through the connective lay in the central region and slightly below the mid-point in a dorsal-ventral line. (Fig. 2) Each ganglion showed these fibers becoming reduced to a size that prohibited

tracing in the anterior neuropile opposite the first pair of nerves. Rather than changing location near or at their ending, these fibers seemed to terminate in the same plane in which they entered the ganglion. The fibers appeared to be rising from the floor in the region of the second pair of nerves as one came to the posterior region of the ganglion. It was of interest to note that both at the points of ending and of re-appearance of these fibers, conspicuously large ventral cells could be seen, the posterior group usually being in pairs occurring along the ventral half of the lateral wall of the ganglion. It was in this region that the fibers first appeared though no connections were to be seen between these cells and the fibers under discussion. (Fig. 3) Investigation of two series of thoracic portions of the cord showed the ventral fibers to be present in much larger numbers than in the abdominal region, and to be segmental there.

Discussion: The large ventral fibers apparently are segmental throughout the cord and seem to be variable as to the number in any segment of the cord. Though they might be called giant fibers because of their diameter, they hardly compare with the dorsal segmental giant fibers because of the intervening distances between fibers of each segment. Because they appear to end in the region of the first pair of nerves and to originate at about the exit of the second pair of nerves, they seem to be associative motor fibers

joining one segment with the next posterior segment.

Giant Fibers

At no place in this investigation were septa seen to occur in the median giant fibers. Close observation failed to disclose any one fiber ventrally located that compared with the ventral giant fiber of the earthworm.

CONCLUSIONS

The following conclusions may be drawn from these results:

1. That the pair of dorsal fibers of intermediate size are non-segmental, having neither septa nor lateral branches except in the last abdominal ganglion.
2. That the pair of dorsal fibers of intermediate size branch laterally in the last abdominal ganglion and that the more ventral and median fiber of the pair crosses over to the opposite side of the cord in the last ganglion.
3. That the large ventral fibers are segmental and segments of the cord may contain a variable number of these fibers. These fibers arise from the ventral portion of the ganglion opposite the second pair of nerves and proceed posteriorly into the next ganglion.
4. That the ventral fibers may be termed giant fibers

because of their size only and not because of arrangement or similarity to other giant fibers.

5. That the median giant fibers are not segmental, having neither branching fibers nor septa within the abdominal region, and that there are no giant fibers in the crayfish that correspond to the ventral giant fibers of the annelids.

ACKNOWLEDGMENT

I wish to express my appreciation to Dr. George E. Johnson for his unceasing interest and aid in conducting this study, for his suggestion of the problem, his placing his collection of prepared slides of the nerve cords of *Cambarus limosus* at my disposal and for his help in writing this paper.

LITERATURE CITED

- Allen, E. J., 1894. Studies on the nervous system of crustacea. I. Some nerve elements of the embryo lobster. *Quart. Jour. Mic. Sci., N. S.*, 36:461-497.
- Bathe, A., 1895. Studien ueber das Centralnervensystem von *Carcinus Maenas* nebst Angaben ueber ein neues Verfärben Methylensblaufixation. *Arch. f. Mikro. Anat.*, 44:579-622.
- Bovard, J. P., 1918a. The transmission of nerve impulses in relation to locomotion in the earthworm. *Univ. Calif. Pub. Zool.*, 18:103-134.
- _____, 1918b. The function of the giant fibers in the earthworm. *Univ. Calif. Pub. Zool.*, 18:136-144.
- Friedlaender, B., 1889. Ueber die markhaltige Nervenfasern und Neurochorde der Crustaceen und Anneliden. *Mittheil. Zool. Sta. Neapel*, 9:205-265.
- Hilton, W. A., 1911. Some remarks on the motor and sensory tracts of insects. *Jour. Comp. Neur.*, 21:238-295.
- _____, 1924. Afferent and efferent pathways in an abdominal segment of an insect. *Jour. Comp. Neur.*, 36:299-308.
- Johnson, C. E., 1924. Giant nerve fibers in crustaceans, with special reference to *Cambarus* and *Palaemonetes*. *Jour. Comp. Neur.*, 36:323-373.
- _____, 1926. Studies on the function of the giant nerve fibers of crustaceans, with special reference to *Cambarus* and *Palaemonetes*. *Jour. Comp. Neur.*, 42:19-33.
- Krieger, K. R., 1879-1880. Ueber das Centralnervensystem des Flusskrebses. *Zeitschr. f. Wissen. Zool.*, 33:557.
- Retzius, G., 1890. Zur Kenntnis des Nervensystems der Crustaceen. *Biol. Unterauch.*, N. F., 1.
- Schneider, 1902. Lehrbuch der Vergleichenden Histologie der Tiere. Jena, Verlag von Gustav Fischer.

Stough, E. B., 1926. Giant fibers of the earthworm. Jour. Comp. Neur., 40:409-463.

_____, 1930. Polarization of the giant nerve fibers of the earthworm. Jour. Comp. Neur., 50:217-230.

Yolton, L. W., 1923. The effects of cutting giant fibers of the earthworm. Proc. Nat'l Acad. Sci., 9:383-385.

EXPLANATION OF PLATES

Figure 1. Diagram showing the major structures of two abdominal ganglia and connectives from the dorsal surface.

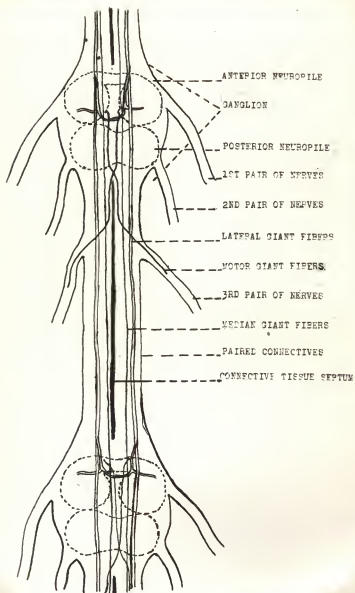
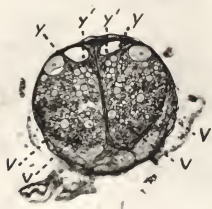


Figure 2. A cross section of the cord through the connectives between the third and the fourth abdominal ganglia showing the dorsal pair of fibers of intermediate size, Y and Y', and two large ventral fibers, V.

Figure 3. A cross section of the fourth abdominal ganglion in the region of the second pair of nerves showing fibers Y and Y', the dorsal pair, and the large ventral fibers, V.

Note. Figures 2-6 are photomicrographs. Magnification is 67 diameters.



2.

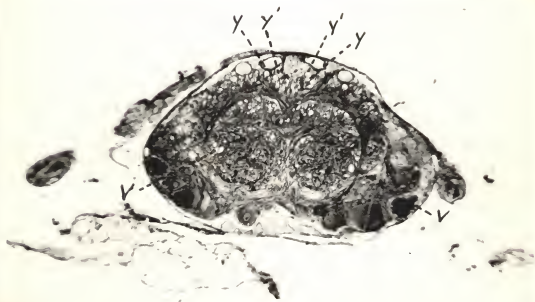
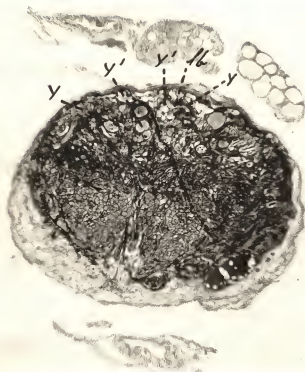
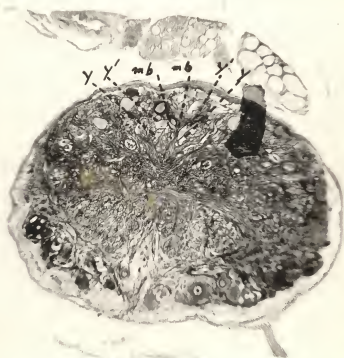


Figure 4. A cross section through the sixth abdominal ganglion of series 1, 0.7 mm. posterior to the most anterior cells of the ganglion showing the dorsal pairs of fibers, Y and Y', with Y' of the right side giving off the branch 1b.

Figure 5. A cross section through the sixth abdominal ganglion of series 1, 0.16 mm. back of the most anterior cells, showing fibers Y and Y', the Y' fibers on each side giving off branches, med. b, extending medially and ventrally.



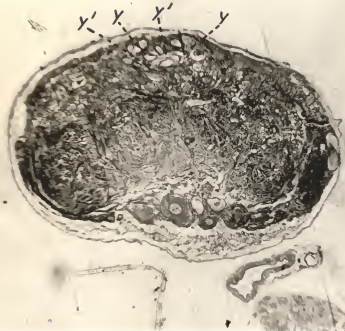
4.



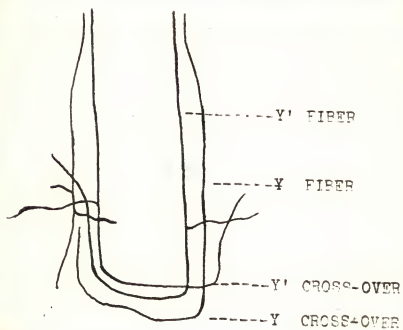
5.

Figure 6. A cross section through the sixth ganglion of series 6, showing the crossing over of Y fibers and Y fiber of the right side of the cord to the opposite side of the cord.

Figure 7. A diagrammatic reconstruction of the crossing over of the fibers seen in figure 6 in the sixth abdominal ganglion. There are no anastomoses of the fibers or of their branches.



6.



7.