Structure of the Giant Fibers of Earthworms

Abstract. *The median giant fiber and the pair of lateral giant fibers that run the length of the ventral nerve cord in earthworms were thought to arise by fusion of the axons of several nerve cells in each segment. The structure of these giant fibers has now been examined with a fluorescent dye injected into single fibers. Each giant axon connects to one cell body in each segment; the giant fibers are not fused axons. In each segment, the median giant fiber has three branches and each lateral giant fiber has five branches. These branches are presumably dendritic. No structural differences between the giant fibers in anterior and posterior regions of the worm seem to account for the functional polarity of the giant fiber system observed in behavioral studies.*

In the ventral nerve cord of earthworms there are three very large axons —a median giant fiber (MG) and a pair of lateral giant fibers (LG). This report describes the results of a reexamination of the detailed structure of these neurons. Two questions provoked the study. Do these fibers really result from the fusion of axons arising from several cell bodies, as had been described by earlier investigators (*1*)? Is there an obvious anatomical basis for the polarity of function observed when intact worms are touched at the anterior and posterior ends (*2*)?

Worms identified as *Lumbricus terrestris* were purchased from a biological supply house. They were anesthetized in 4 percent ethanol prior to dissection. Isolated pieces of ventral nerve cord about 20 segments long were kept moist in Rushton's saline (*3*) buffered with 0.01*M* tris to about *p*H 7.2 at 5°C. Procion Yellow M4RS was injected into individual giant axons through glass micropipettes as a 5 percent solution in distilled water (*4*). The pipette glass was cleaned in 3*M* HCl at 80°C before being forged, and the dye solution was filtered through Millipore filter (pore size, 0.45 μm) as it was introduced into the pipette. Each pipette was driven through the sheath that envelops the cord and then into the chosen fiber. Although many pipettes broke during attempted penetrations, this method gave much better results than did stripping the sheath off the cord. The flow of dye into the cell was regulated with a micrometer syringe. After a successful injection, the preparation was chilled overnight in the refrigerator in buffered Rushton's saline, and then fixed in Bouin's fluid. Fixed cords were dehydrated in ethanol, cleared in methyl benzoate, and examined with a fluorescence microscope (*5*). Successful preparations were then embedded in Parawax and sectioned at 15 μm. Interesting regions of individual cells were studied by photographing in color each 15-μm section and by drawing the relevant features of each section on an acetate sheet from a projection of the color slide. The drawings were stacked with appropriate spacers to make a three-dimensional scale model of the injected cell.

Three regions of the worm were examined: the subesophageal ganglion, the region anterior to the clitellum, and the posterior end of the animal. Segment 40, three segments posterior to the clitellum, is approximately the dividing line between the areas in which the MG and the LG's respond to tactile stimulation (*2*). I hoped that the giant fibers anterior to the clitellum would have structures different from those posterior to the clitellum. Six anterior MG fibers, three posterior MG, two anterior LG, six posterior LG, and one subesophageal MG were injected and studied in detail. Figure 1 shows the major results. The giant fibers are segmental interneurons conventional except for their size. Each giant axon has only one soma with one nucleus in each segment. No connections of MG and LG cells occur anywhere in the cord. The axons do not fuse.

The MG axon in each segment is connected by a long neurite to one cell body. This cell body is located on the ventral midline of the nerve cord, between the first and second segmental nerves. Every injected MG showed only one cell body per segment, and transverse sections through the cord showed only one nucleus in the injected cell body. No evidence of axonal fusion has been observed in any MG preparations. No differences in the location of the MG cell body appeared in anterior or posterior regions of the worm.

The MG axon has three branches in each segment which descend from the ventral surface of the axon (Fig. 2). Beginning at the anterior septum, the first branch (M1) occurs between the first pair of segmental nerves, the second (M2) is a short branch off the neurite between the cell body and the MG axon, and the third branch (M3) occurs just posterior to the second and third pairs of segmental nerves. The M1 and M3 branches divide once, giving rise to fibers that run along the axis of the cord beneath the MG axon. The MG branches show no evidence of bilateral symmetry. In the subesophageal ganglion, the first segment in which the MG occurs, the one successfully injected preparation showed the MG cell body at the base of the right circumesophageal connective. The first branch of the neuron in this ganglion was M2, the branch off the neurite. No more anterior extensions of the MG were observed. Contrary to Friedländer's report (*1*), the MG did not send branches out of any of the segmental nerves.

In each segment, each LG axon is connected by a long neurite to one contralateral cell body located ventrolaterally at the base of the double nerves (Fig. 2D). The neurite of each axon crosses the midline beneath the MG axon and joins, or rather becomes, the LG axon at the septum between it and the next posterior LG axon by expanding progressively into the full diameter of the giant fiber. The neurites of the two LG axons in each segment touch where they cross the midline. However, the neurites do not fuse. None of the dye injected into one LG ever appeared in processes of its contralateral homolog, except as noted below in the L1 synapse.

Each LG axon has five branches in each segmental ganglion (Fig. 3). The branches of contralateral pairs of LG axons are mirror images. Branch L1 occurs anterior to the intersegmental septum; L1 descends from the ventral side of the LG axon and splits into two extensions. One of these proceeds posteriorly beneath the LG axon. The other proceeds toward the midline to contact its contralateral homolog. The second branch (L2) is located just posteriorly to the first segmental nerve. It descends from the ventral surface of the LG axon and divides into two processes that are aligned along the axis of the cord beneath the LG. The third branch (L3) is similar to but longer than L2. It leaves the giant axon just anterior to the second segmental nerve and splits into two processes that run along the axis of the cord beneath the LG axon. The neurite of the MG runs anteriorly between the L3 branches. Two branches occur on each LG neurite—L4 ipsilateral to the axon and L5 contralateral to the axon. These are shown in Fig. 3D. Of all these LG branches, only the

median extension of L1 makes contact with its contralateral homolog. The large diameters of these LG branches suggest that each might conduct action potentials. I was unable to inject LG cells in the subesophageal ganglion. Contrary to Ogawa's report (*1*), no branches of the LG axon left the nerve cord via any of the segmental nerves in any of the preparations.

The two LG axons in each ganglion are connected by an electrotonic junction (*6*). Both the L1-L1 synapse and the neurite-neurite contact are possible sites of this junction. If one assumes that both the neurite and the L1 branch of each axon conduct action potentials and have a low internal resistance, the attenuation of current pulses observed by Wilson would occur principally at the junction itself. The estimated area of the L1 synapse is 400 μm^2 and of the neurite connection 25 μm^2. It seems likely that the L1 synapse illustrated in Fig. 3A is the main site of electrotonic coupling.

Friedländer (*1*) observed, at least in one case, that LG neurons were bipolar, that is, a second axon or set of branches extended anteriorly from the

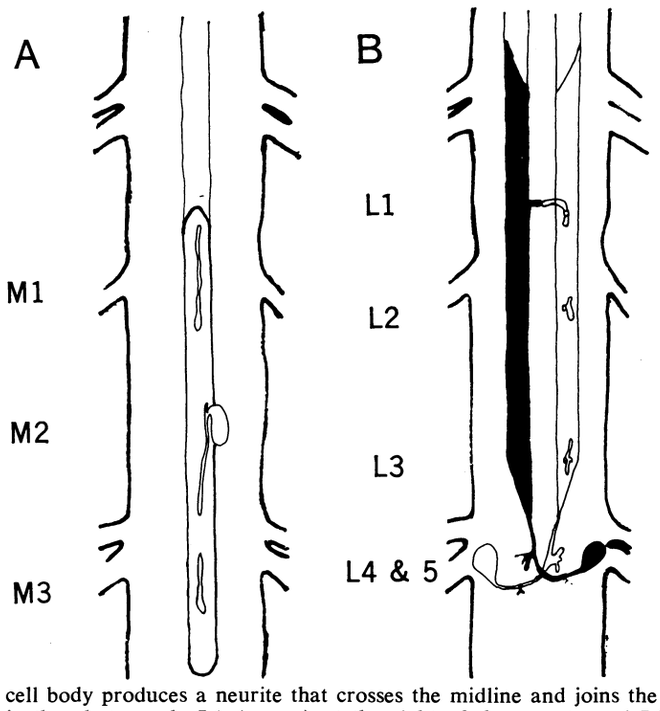
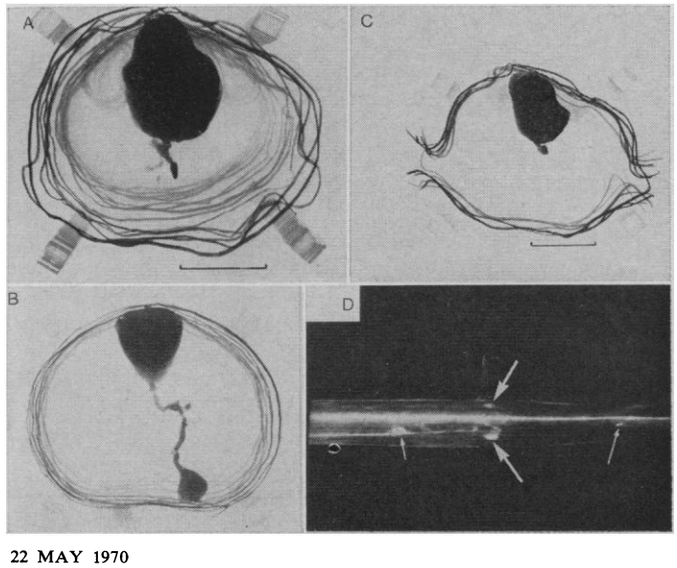
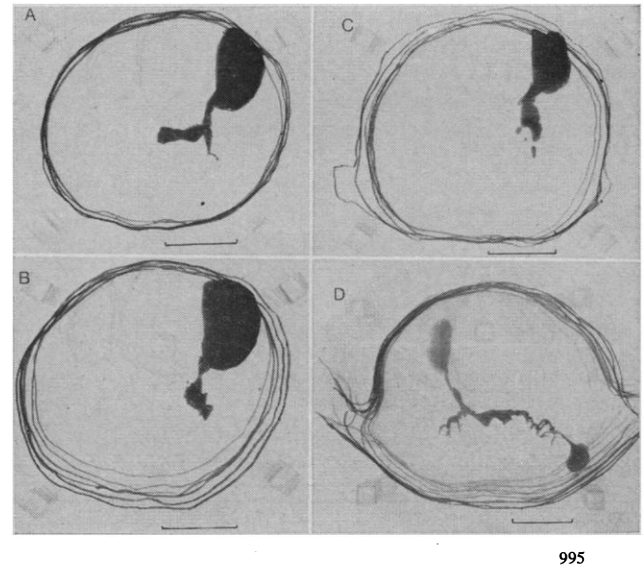


Fig. 1 (left). (A) Diagram of an MG axon in one segment, aligned correctly relative to the structures of the LG axons. The cell body is displaced somewhat from its midventral position to permit branch M2 to be drawn in place. The septa, which occur near the intersegmental boundaries, mark the terminations of the MG axon. The scale is the same as in (B). (B) Diagram of a pair of LG axons in one segment. One cell is outlined, the other solid. The septa are the anterior and posterior boundaries of each cell. One of the five branches of each LG (L1), and the neurite, cross the midline and contact its contralateral homolog. L1, L2, and L3 are the first, second, and third branches of the LG. L4 and L5 are branches off the neurite. Each LG axon is 50 μm in diameter. Fig. 2 (below left). Reconstructions of regions of MG axons. (A) The M1 branch in a posterior segment, photographed from its anterior end looking posteriorly along the cord. The anterior extension of M1 branches again, sending one branch ventrally in the neuropile. The scale is 50 μm. (B) The neurite, cell body, and M2 branch of the MG axon in an anterior segment, photographed from its anterior end looking posteriorly along the cord. The M2 branch appears as a short process above the cell body. (C) The M3 branch of the MG axon in a posterior segment, photographed from its posterior end looking along the cord. The M3 branch does not divide again after it splits near its connection with the main axon. The scale is 50 μm and is the same for (B) and (C). (D) A photograph of a ventral nerve cord in a posterior segment in which both LG axons have been injected with dye. Two LG cell bodies are visible (large arrows), one near the base of the right double nerve, and the other at the base of the left double nerve. Anterior is to the left. Each cell body produces a neurite that crosses the midline and joins the contralateral LG axon. Two branches of these axons can be seen in the photograph, L1 (arrow) at the right of the somata and L3 (arrow) at the left. The right double nerve and its blood vessel are in the upper middle of the photograph. The septum of the upper (right) LG fiber ruptured during injection, and dye can be seen through the entire course of this fiber in the photograph. Dye did not diffuse beyond the septum of the lower (left) LG fiber. Fig. 3 (below right). Reconstructions of regions of LG axons. (A) The L1 branch in a posterior segment, photographed from its anterior end looking posteriorly along the cord. One part of L1 makes contact with its contralateral homolog at the midline. (B) The L2 branch, photographed as in (A). (C) The L3 branch, photographed as in (A). The reconstructions in (A), (B), and (C) were made from the same segment. Neither L2 nor L3 approach the midline. (D) The neurite, cell body, and L4 and L5 branches of the LG axon in an anterior segment, photographed from their posterior end looking anteriorly along the cord. The cell body and the L4 branch are in about the same transverse plane, while L5 is posterior to both of them. The neurite expands into the LG axon anterior to the cell body. The scale marker is 50 μm, and is the same for all four photographs.





cell body. He was unable to trace the course of this axon. I have observed short, blunt processes extending from the anterior side of the cell body for very short distances—less than 15 μm. If these extensions synapse with other cells, the LG axons are bipolar.

The branches of both the MG and LG fibers were very similar in different segments. The lengths of branches M1 and M3 varied, but no systematic differences in branching patterns between anterior and posterior regions of the worm occurred. No identified structures of the MG or LG fibers explain the different thresholds in the anterior and posterior regions. Bullock and Horridge (*2*) mention that the cross-over point of the behavioral response (normally segment 40) may change with repeated stimulation, so the polarizing mechanism may be the distribution of sensory input to the giant fibers.

The failure of dye to cross septa except where they had been ruptured by the pressure of injection supports Coggeshall's conclusion (*7*) that the septum is a complete cell boundary in which no pores or gaps occur. This is unlike the segmental synapses of *Procambarus*, which are slightly permeable to Procion Yellow (*8*). Procion Yellow is believed to bind covalently with carbohydrates and proteins (*4*), and dye molecules so bound would diffuse more slowly than would molecules in solution, but even after 12 hours there was no visible fluorescence past earthworm septa that had not ruptured. However, the synapse between the two L1 branches in each segment is very slightly permeable to the dye. I do not think this permeability can be dismissed as an artifact, but rather it indicates that the segmental synapses of earthworms have a structure different from that of the L1-L1 synapse. Otherwise, the LG fibers of earthworms are remarkably similar to the LG fibers of *Procambarus clarkii* (*5*). Both are formed by chains of segmental axons which make electrotonic junctions (*3*, *6*) with the corresponding axons in the adjacent segments. They have one cell body per axon located on the ventrolateral edge of the ganglion contralateral to the axon. In each segment, they both have major branches on the neurite on the side ipsilateral to the axon and make electrotonic connections with the contralateral LG axon.

It would be very interesting to know if the giant axons of earthworms make electrotonic connections with motor neurons similar to those in crayfish, but like many other basic features of the physiology of these everyday animals, the connections between the giant fibers and motor neurons are unknown.

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Harderian Gland: Development and Influence of Early Hormonal Treatment on Porphyrin Content

Abstract. *The porphyrin content of the rat Harderian gland remains low until 12 days of age at which time both porphyrin content and concentration rapidly increase. Intraperitoneal administration of tetraiodothyronine (thyroxine) into newborn animals advances the appearance of porphyrin in the gland. Conversely, a single injection of cortisol acetate into newborns retards the appearance of porphyrin. The time of porphyrin appearance in the gland parallels the time for maturation of the evoked cortical response to visual stimulation in normal and hormone-treated animals.*

The Harderian gland is a large, bilobed, saccular gland located in the orbit of reptiles, birds, and mammals. It is rudimentary or lacking in Anthropoidea (*1*). In some rodents the gland has a characteristic appearance due to its high porphyrin content (*2*). Information on the development of the Harderian gland and on its function is sparse. Müller has studied the histological changes in the gland from birth to 13 weeks (*3*). He reported a rapid, pronounced alteration in cellular morphology and increased secretory activity at about the 14th day of age which he correlated to eye-opening. He also found evidence of porphyrins in the gland at 8 days and the appearance of yellowish-brown pigments at day 9. Boas and Scow (*4*) reported atrophy of the Harderian gland in thyroidectomized or hypophysectomized adult rats, but Figge and Davidheiser (*5*) failed to observe any effect of hypophysectomy on porphyrin synthesis of the gland. Rohonyi and Kelényi (*6*) found the porphyrin content of the Harderian gland of young rats to be lower than that of adult animals. Klüver (*7*) found that a fluorescent spectrum similar to that of porphyrin first appears in the nervous system during a period coincident with vascularization and development of motor activity and suggested a possible relation between porphyrins and myelinization.

We have suggested that the Harderian gland may act as a secondary phototransducer in blinded suckling rats, because removal of the gland affects the light-dependent variation of serotonin in the pineal gland (*8*). The normal maturation of evoked cerebral cortical potentials in response to sensory stimuli in the rat can be altered by early hormonal treatment (*9*). Such treatment particularly affected the following response in the visual cortex. This study was undertaken to determine the normal development of porphyrin in the rat Harderian gland, the influence of early hormonal treatment on such development, and the relation of the development of the Harderian gland to the maturation of the visual cortex.

Harderian gland weight, porphyrin content, and porphyrin concentration were determined in 153 male and female Sprague-Dawley rats bred in our laboratory and ranging in age from 4 to 26 days. The infant rats were divided