1987

The giant interneuron pathways and escape reflexes of the aquatic oligochaete, Branchiura sowerbyi

Mark Joseph Zoran
Iowa State University

Follow this and additional works at: https://lib.dr.iastate.edu/rtd
Part of the Zoology Commons

Recommended Citation
https://lib.dr.iastate.edu/rtd/8604

This Dissertation is brought to you for free and open access by the Iowa State University Capstones, Theses and Dissertations at Iowa State University Digital Repository. It has been accepted for inclusion in Retrospective Theses and Dissertations by an authorized administrator of Iowa State University Digital Repository. For more information, please contact digirep@iastate.edu.
INFORMATION TO USERS

While the most advanced technology has been used to photograph and reproduce this manuscript, the quality of the reproduction is heavily dependent upon the quality of the material submitted. For example:

- Manuscript pages may have indistinct print. In such cases, the best available copy has been filmed.

- Manuscripts may not always be complete. In such cases, a note will indicate that it is not possible to obtain missing pages.

- Copyrighted material may have been removed from the manuscript. In such cases, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, and charts) are photographed by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each oversize page is also filmed as one exposure and is available, for an additional charge, as a standard 35mm slide or as a 17”x 23” black and white photographic print.

Most photographs reproduce acceptably on positive microfilm or microfiche but lack the clarity on xerographic copies made from the microfilm. For an additional charge, 35mm slides of 6”x 9” black and white photographic prints are available for any photographs or illustrations that cannot be reproduced satisfactorily by xerography.
The giant interneuron pathways and escape reflexes of the aquatic oligochaete, *Branchiura sowerbyi*

Zoran, Mark Joseph, Ph.D.
Iowa State University, 1987
PLEASE NOTE:

In all cases this material has been filmed in the best possible way from the available copy. Problems encountered with this document have been identified here with a check mark ✓.

1. Glossy photographs or pages ✓
2. Colored illustrations, paper or print ✓
3. Photographs with dark background ✓
4. Illustrations are poor copy ✓
5. Pages with black marks, not original copy ✓
6. Print shows through as there is text on both sides of page ✓
7. Indistinct, broken or small print on several pages ✓
8. Print exceeds margin requirements ✓
9. Tightly bound copy with print lost in spine ✓
10. Computer printout pages with indistinct print ✓
11. Page(s) _______ lacking when material received, and not available from school or author.
12. Page(s) _______ seem to be missing in numbering only as text follows.
13. Two pages numbered _______. Text follows.
14. Curling and wrinkled pages ✓
15. Dissertation contains pages with print at a slant, filmed as received ✓
16. Other ________________________________

University
Microfilms
International
The giant interneuron pathways and escape reflexes of the aquatic oligochaete, *Branchiura sowerbyi*

by

Mark Joseph Zoran

A Dissertation Submitted to the Graduate Faculty in Partial Fulfillment of the Requirements for the Degree of DOCTOR OF PHILOSOPHY

Department: Zoology
   Major: Zoology (Neurobiology)

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

For the Major Department

Signature was redacted for privacy.

For the Graduate College

Iowa State University
   Ames, Iowa

1987
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>GENERAL INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>Evolution of Annelid Giant Fiber Systems</td>
<td>1</td>
</tr>
<tr>
<td>Biology of <em>Branchiura sowerbyi</em></td>
<td>3</td>
</tr>
<tr>
<td>Explanation of Dissertation Format</td>
<td>5</td>
</tr>
<tr>
<td>CHAPTER I. RAPID ESCAPE REFLEXES IN AQUATIC OLIGOCHAEITES: VARIATIONS IN DESIGN AND FUNCTION OF EVOLUTIONARILY CONSERVED GIANT FIBER SYSTEMS</td>
<td>7</td>
</tr>
<tr>
<td>Introduction</td>
<td>7</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>9</td>
</tr>
<tr>
<td>Animals and maintenance</td>
<td>9</td>
</tr>
<tr>
<td>Anatomy</td>
<td>10</td>
</tr>
<tr>
<td>Electrophysiology</td>
<td>11</td>
</tr>
<tr>
<td>Results</td>
<td>15</td>
</tr>
<tr>
<td>Giant fiber morphology</td>
<td>15</td>
</tr>
<tr>
<td>Electrophysiological correlates of rapid escape reflexes</td>
<td>27</td>
</tr>
<tr>
<td>Escape reflex function in the Lumbriculida</td>
<td>34</td>
</tr>
<tr>
<td>Escape reflex function in the Tubificida</td>
<td>38</td>
</tr>
<tr>
<td>Discussion</td>
<td>40</td>
</tr>
<tr>
<td>Conservation of giant fiber design and function</td>
<td>40</td>
</tr>
<tr>
<td>Divergence of aquatic and terrestrial escape systems</td>
<td>41</td>
</tr>
<tr>
<td>Adaptive significance of giant fiber systems</td>
<td>42</td>
</tr>
<tr>
<td>All-or-none versus graded escape responses</td>
<td>46</td>
</tr>
<tr>
<td>Acknowledgments</td>
<td>48</td>
</tr>
</tbody>
</table>
GENERAL INTRODUCTION

Study of the giant nerve fibers of many animals, most notably the squid (Young, 1936; Hodgkins, 1964), has extensively contributed to our knowledge of fundamental cellular neurophysiology. Nevertheless, the specific role of giant fibers in many animal groups is less well understood, but is generally presumed to involve the mediation of rapid escape or startle reflexes. The obvious survival advantage conferred by giant fibers is a more rapid impulse conduction along the body and, therefore, a faster and more effective escape.

Evolution of Annelid Giant Fiber Systems

Although giant fiber systems are found in nearly half of the major animal groups (phyla), these systems have evolved independently and are for the most part examples of convergent evolution. Also, giant fiber systems have arisen independently within single phyla (e.g., Annelida) and even within classes (e.g., Polychaeta).

Timm (1979) suggested that as the seas of the Carboniferous period withdrew, drying large marine marshes, some ancestral annelids began to adapt to more terrestrial conditions. The divergence of the sea-dwelling polychaetes and the terrestrial and freshwater oligochaetes (see Appendix A, Fig. 1) is reflected in comparative studies of modern
annelid giant fiber systems. The divergence and subsequent radiation of the polychaetes has produced considerable diversity in the number and design of giant nerve fibers (Nicol, 1948; Bullock, 1948; Bullock and Horridge, 1965). In contrast, there has been an evolutionary conservation in giant fiber number and arrangements among oligochaetes (Chapter I).

All free-living oligochaetes studied to date, including the Lumbricida, Tubificida, and Lumbriculida possess three dorsal giant fibers (see Appendix B, Table 1). In these worms, the giant fibers function as two distinct escape reflex systems: a medial giant fiber (MGF) system activated by sensory input from anterior segments and a lateral giant fiber (LGF) system consisting of two, electrically-coupled fibers activated by input from posterior segments (Drewes, 1984). This dichotomous arrangement of MGF and LGF systems provides the opportunity to investigate the influence of various factors such as habitat (aquatic versus terrestrial), lifestyle (errant versus sedentary), and predatory pressure (anterior vulnerability versus posterior vulnerability) on the design and function of oligochaete giant fiber systems.

The rapid escape reflexes of many terrestrial oligochaetes have been well documented (for reviews see Dorsett, 1978; Drewes, 1984). In addition, it is well known that many aquatic oligochaetes, such as tubificids, rapidly withdraw in response to vibrational or tactile stimuli (Stephenson, 1930);
however, there has been no correlated anatomical, physiological, and behavioral study of an aquatic oligochaete escape reflex. The present dissertation is the first correlated examination of such a reflex in the tubificid worm, *Branchiura sowerbyi*.

**Biology of *Branchiura sowerbyi***

*Branchiura sowerbyi* (Beddard, 1892) is a mud-dwelling tubificid worm widely distributed throughout freshwaters of the world. The species is generally limited to the benthos of shallow littoral zones of lakes, most often collected in water depths of 5 m or less (Causey, 1953). Burrowers like *Branchiura* invest the majority of their time foraging in the top 2-8 cm of sediment, rarely venturing into the substrate beyond a depth of 20 cm (McCall, 1982). They derive the bulk of their nutritional requirements from micro-organisms (e.g., bacteria) ingested along with organic sediments (Brinkhurst and Jamieson, 1971).

Aston (1968) reported a one year life cycle for *Branchiura* in British waters. Breeding began in early spring and continued throughout the summer months with maximum cocoon production correlating with highest water temperatures. Worms reached sexual maturity by March of the following year. The post-reproductive fate of *Branchiura* adults was unclear. Many tubificids die after the first breeding season, but a few species reabsorb their sexual organs and live to breed again.
(Brinkhurst and Jamieson, 1971). The existence of asexual reproduction in *Branchiura* remains unknown.

Optimal temperature for sexual reproduction and growth in the laboratory is 25°C, with minimal growth and no reproduction below 17°C (Aston, 1982). Worms are 3 mm in length when newly hatched and can exceed 7 cm when fully grown. Worms are easily cultured in the laboratory (see Appendix C).

*Branchiura* is exceptionally tolerant of organically polluted waters where oxygen is often in extremely low concentrations (Naqvi, 1973). It breeds during summer in eutrophic lakes where bottom sediments become devoid of oxygen for several weeks (Brinkhurst and Jamieson, 1971). In the laboratory, adults can withstand periods of complete oxygen deprivation for up to 4 weeks (Aston, 1966, as cited in Brinkhurst and Jamieson, 1971). Its success in these environments, which are too anoxic for other freshwater animals, suggests the presence of special respiratory adaptations of survival importance.

Three adaptations, in part, explain the success of *Branchiura* in such adverse habitats: (1) the presence of branchial gill filaments on the hindmost quarter of the body, (2) the rhythmic undulation of tail segments within the water column, and (3) the pumping of water along the gut epithelium. The majority of gas exchange in this species appears to take
place at the gill surface (Kawaguti, 1936). Brinkhurst and Jamieson (1971) reported that 90% of all oxygen uptake in *Brachiura* occurs through the gill region even though it comprises only 18% of the total surface area of the body. However, no physiological mechanisms have been described that can account for the facultative anaerobic nature of this animal.

Adult *Brachiura* possess several hundred segments and an impressive capacity to contract them, often shortening to one-tenth their normally elongated state (Coker, 1954). This contractility is obviously beneficial in light of this animal's ventilatory habits, since predatory threat imposed upon the exposed tail necessitates a rapid escape into the burrow. There is evidence that seasonal predation can be intense as worms extend from their burrows (Wisniewski, 1978; Samarakoon, 1981). Personal field observations revealed that 90% of adult worms collected during May and June were in some stage of tail regeneration.

**Explanation of Dissertation Format**

This dissertation is composed in the alternate format. Each of the three chapters are complete manuscripts modified to conform to the specifications of the Iowa State University Thesis Office. The first chapter presents comparative data supporting the hypothesis that giant nerve fiber structure and function is highly conserved among oligochaetes. The second
chapter deals with specific anatomical organizations and physiological functions of the lateral giant fibers of Branchiura sowerbyi. Finally, the third chapter documents several behavioral analyses of the Branchiura escape response including videotaping, cinematography, and electrical recordings from intact animals. Each chapter consists of an introduction, materials and methods, results, discussion, acknowledgments, and references. A general introduction precedes Chapter I and a general summary follows Chapter III.
CHAPTER I. RAPID ESCAPE REFLEXES IN AQUATIC OLIGOCHAETES: VARIATIONS IN DESIGN AND FUNCTION OF EVOLUTIONARILY CONSERVED GIANT FIBER SYSTEMS

Introduction

Giant nerve fibers mediate the rapid escape reflexes of many polychaete and oligochaete worms. Although there is considerable diversity in the number and design of giant fibers in polychaetes (Nicol, 1948a; Bullock, 1948; Bullock and Horridge, 1965; Dorsett, 1980), studies of terrestrial oligochaetes, specifically earthworms, have consistently revealed an arrangement of one medial giant fiber (MGF) flanked by two lateral giant fibers (LGF) (for reviews see Dorsett, 1978; Drewes, 1984). Early electrophysiological studies by Bullock (1945) and Rushton (1946) showed that the MGF and LGF in the lumbricid earthworm, *Lumbricus terrestris*, function as two distinct reflex systems; the MGF system is activated by sensory input from anterior segments and the two LGFs, functioning in electrical synchrony (Rushton, 1945; Wilson, 1961), are activated by input from posterior segments. More recent studies by Drewes et al. (1983) indicate that this functional dichotomy is applicable to numerous other terrestrial oligochaete families. However, their study did not compare the organization of giant fiber reflex systems in aquatic oligochaete groups. Except for briefly mentioning the presence of giant nerve fibers in early anatomical studies (Brode, 1898; Stephenson, 1912; Keyl, 1913; Isossimow, 1926),
there have been no detailed descriptions of giant fiber organization or neural correlates of escape reflex behavior in these worms. In view of this phylogenetic gap in the understanding of annelid escape reflexes, we have examined the design and function of giant fiber systems in representative species of the two major orders of aquatic oligochaetes, the Lumbriculida and the Tubificida.

One behavioral feature shared by many aquatic species (including all those examined in this study) is their habitual extension of posterior segments above the sediments, a behavior which facilitates gas exchange across specialized respiratory surfaces or branchial appendages on these segments (Brinkhurst and Jamieson, 1971; Weber, 1978). This habit renders posterior segments vulnerable to predatory attack and contrasts with a habitual behavior of earthworms that imposes vulnerability on anterior segments as they extend from the burrow during surface feeding and copulation. In view of these fundamental differences in oligochaete lifestyle and behavior, we have given special attention in this study to comparisons of longitudinal variations in giant fiber form and function in aquatic versus terrestrial species.

Neurobiological studies of small bodied animals such as aquatic oligochaetes present several technical problems, including minimization of injury during microdissection and maintenance of microelectrode penetrations in small diameter
fibers. To avoid these problems most of our recordings of giant nerve fiber activity have been obtained through non-invasive methods, which previously were shown to reliably detect MGF and LGF spiking in very small specimens, such as embryonic earthworms, where giant fibers are only a few μm in diameter (O'Gara et al., 1982). In addition, we have adapted these methods for simultaneously recording the electrical and behavioral events associated with escape responses in situ (i.e., during normal ventilatory and burrowing movements in natural sediments).

Materials and Methods

Animals and maintenance

Brancliura sowerbyi (Beddard), Limnodrilus hoffmeisteri (Claparede), Tubifex tubifex (Lamarck), and Lumbriculus variegatus (Miller) were obtained from established laboratory cultures. The cultures were supplied with mud collected from a local lake. Prior to use, the mud was frozen at -15°C for several weeks to prevent contamination by unwanted species. Dero sp. (Oken) was purchased from Carolina Biological Supply (Burlington, NC) and Kincaidiana hexatheca (Altman) from Soil Biology Associates (McMinnville, OR). Naming of taxonomic orders is in accordance with Brinkhurst (1984).

Animals were kept at room temperature (22-25°C) and fed several times weekly with a ground mixture of Tetramin staple
flakes and trout chow. All aquatic cultures were aerated, but photoperiod was not regulated.

**Anatomy**

For histological studies, reproductively mature specimens were placed in culture water overnight for clearance of the digestive tract. Segmental amputations were made at multiple sites to obtain two pieces, 20-30 segments long, from two different body regions: one from the anterior one-third (excluding segments 1-10) and the other from the posterior one-third (excluding the 10 terminal segments). Each piece was stretched to a segmental length closely matching that of an animal extended from the burrow, then pinned to a small silicone rubber block with minuten pins (0.10 mm), and fixed overnight in 2.5% glutaraldehyde in 0.1 M cacodylate buffer. Each piece was then cut in 2 mm lengths which were washed in buffer and immersed for 2 hours in 1% OsO₄ in 0.1 M cacodylate buffer. Tissues were dehydrated in a graded series of ethanol ending in a 2 hour immersion in propylene oxide. The tissues were placed in a 1:2 propylene oxide/epon-araldite mixture overnight and then embedded in epon-araldite plastic at 60°C. Sections (0.5-1.0 μm) were cut with glass knives and stained with 0.5% toluidine blue in 0.5% borax for light microscopy.

Giant fiber diameters, exclusive of surrounding sheath, were measured to the nearest μm using an ocular micrometer. Mean diameters were obtained from a total of 300 randomly
selected sections (3-6 animals per species). In addition, transverse and longitudinal serial sections were used to make three-dimensional reconstructions of the LGF in posterior regions of some species.

**Electrophysiology**

Reproductively mature animals were also used for electrophysiological studies. Worms were rinsed briefly in distilled water and placed on the moistened surface of a printed circuit board grid designed for non-invasive, electrical recording (O'Gara et al., 1982). The spacing between the two electrodes in a recording pair varied from 0.5 to 1.0 mm depending on the species size. Outputs from each pair of electrodes were led into a capacity-coupled, differentially recording preamplifier. Amplified signals were filtered and displayed as multiple traces on an oscilloscope. Escape responses were evoked by gentle tactile stimulation to the head or tail of the worm with a fire-polished, glass probe. The resulting giant fiber spikes were used as an internal source for triggering the sweep.

Measurements of spike conduction time between adjacent pairs of recording electrodes were made on-line from the oscilloscope screen using the time-interval cursors of a Tektronix 5D10 Waveform Digitizer. Values of conduction velocity for MGF and LGF spikes were always obtained from within the anterior third and posterior third of the body.
(i.e., corresponding to those regions studied histologically). The velocity values were obtained by dividing the distance between the two electrode pairs by the spike conduction time (i.e., peak-to-peak time interval between giant fiber spikes at the two recording sites). Individual means, based on 3-5 measurements from each of six or more animals, were used in calculating a species mean.

Simultaneous extracellular and intracellular recordings of giant fiber spiking activity were made in the two largest aquatic species, *K. hexatheca* and *B. sowerbyi*. Extracellular recordings were obtained with polyethylene suction electrodes and intracellular recordings with glass microelectrodes (20-30 MΩ). With the body wall of the animal pinned to a silicone rubber dish and submersed in worm saline, the suction electrode was placed in contact with the dorsal surface of the exposed ventral nerve cord. Giant fibers were illuminated by a submerged fiber optics light-guide and impaled with the microelectrode. Giant fiber spiking was evoked by tactile stimulation to either head or tail ends and the resulting intracellular activity was recorded from the same segment as the extracellular recording. Amplified signals were stored on magnetic tape for later analysis.

*In situ* electrical recordings of escape reflex activity were obtained using the recording chamber shown in Fig. 1. A worm was placed into the chamber and within a few hours had
Figure 1. *In situ* apparatus for obtaining electrical and behavioral correlates of escape responses

A worm was placed within an 8 mm wide aquarium (A) containing sediment and water. Once a burrow was established, a pair of movable recording electrodes (RE) was positioned near the exposed tail of the ventilating worm to detect electrical activity evoked during escape. A light source (L) was positioned to cast the shadow of the tail on the window of a photocell (P).
established a burrowing system and begun ventilatory undulations of the tail above the sediments. Movable recording electrodes (fire-polished 30 gauge silver wires) built into the chamber wall were positioned to within 1 mm of the tail. Signals were amplified and displayed as described for grid recordings. Movements of the tail during escape were detected with a photocell (GE, Model 8PV1AAB) and a D.C. powered fluorescent light source (Fig. 1). The light was positioned to cast the shadow of the worm's tail on the window of the photocell. Mechanical stimuli were delivered to the tail with a hand-held, fire-polished glass probe (diameter less than 0.25 mm). Movement of the probe relative to the photocell window caused no detectable artifacts in electrical or photocell recordings. Withdrawal of the animal's tail in the sediment was indicated on the oscilloscope as an increase in light reaching the photocell. Photocell response times were less than 1 ms.

Results

Giant fiber morphology

Cross-sections of ventral nerve cords (VNC) from all six oligochaetes revealed three dorsal giant nerve fibers, a single medial fiber (MGF) flanked by two lateral fibers (LGF) (Figs. 2, 3, and 4). The MGF and LGFs of both species of Lumbriculida, *K. hexatheca* and *L. variegatus*, were surrounded
by a well developed glial sheath (Fig. 2), highly reminiscent of the myelin-like sheath which surrounds earthworm giant fibers (Coggeshall, 1965; Roots, 1984). In contrast, only one of the four species of Tubificida, *B. sowerbyi*, possessed a comparable myelin-like sheath (Fig. 3); no sheath was detectable in *L. hoffmeisteri*, *T. tubifex*, or *Dero* sp. at the light microscopic level (Fig. 4).

One consistent feature in all species was the large size of the MGF relative to the LGFs in anterior segments, but the opposite arrangement (i.e., larger LGF) existed in posterior segments (Table 1). Analysis of serial sections from different body regions indicated that these differences reflected a gradual longitudinal tapering of giant fiber diameters.

In addition to these gradients in diameter, the LGFs in two tubificid species, *L. hoffmeisteri* and *B. sowerbyi*, showed marked intrasegmental variations in diameter, thus deviating from a simple cylindrical design. In *L. hoffmeisteri*, segmentally-arranged expansions of the right and left LGFs were fused dorsal to the MGF to form a giant lateral commissure (Fig. 4). In thin sections of the VNC these commissures always lacked membrane partitions between contralateral fibers, suggesting an anatomical syncytium between right and left LGFs. A three-dimensional reconstruction of this arrangement is shown in Fig. 5.
The MGF was largest in anterior segments (left panels) and the LGFs in posterior segments (right panels). Note the well-developed neuroglial sheath surrounding the fibers and the interruption in the sheath at the level of a ventral collateral in the left panel of *L. variegatus*. Vertical bar: 50 μm (*K. hexatheca*) and 20 μm (*L. variegatus*).
Figure 3. Representative sections through the VNC of *B. sowerbyi* (Tubificida)

The MGF was largest in anterior segments (A) and the LGFs in posterior segments (B). In posterior segments, bulbous enlargements of the left LGF are also apparent in cross-section (C) and longitudinal section (D). All cross-sections are viewed from an anterior perspective. Vertical bar: 80 μm (A) and 65 μm (B-D).
Figure 4. Representative cross-sections through the VNC in posterior segments of *T. tubifex*, *Dero* sp., and *L. hoffmeisteri* (Tubificidae)

In *L. hoffmeisteri*, the two LGFs (arrows) were larger than the MGF and were joined by a giant lateral commissure (*). Vertical bar: 16 μm (*Dero* sp.) and 25 μm (*T. tubifex* and *L. hoffmeisteri*).
T. tubifex  Dero sp.

L. hoffmeisteri
Table 1. Giant fiber diameters of six representative aquatic worms from two oligochaete orders

<table>
<thead>
<tr>
<th></th>
<th>Giant fiber diameter ($\bar{x}$ μm ± SE)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anterior segments</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>MGF</td>
<td>LGF</td>
<td>MGF</td>
<td>LGF</td>
<td>MGF</td>
<td>LGF</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lumbriculida</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K. hexatheca</td>
<td></td>
<td>58.2 (+ 5.2)</td>
<td>18.9 (+ 5.0)</td>
<td>17.2 (+ 4.4)</td>
<td>31.8 (+ 5.4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. variegatus</td>
<td></td>
<td>21.2 (+ 3.7)</td>
<td>13.2 (+ 1.7)</td>
<td>7.0 (+ 1.3)</td>
<td>12.2 (+ 1.7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tubificida</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. hoffmeisteri</td>
<td></td>
<td>8.7 (+ 1.6)</td>
<td>4.6 (+ 1.6)</td>
<td>5.2 (+ 2.5)</td>
<td>15.9 (+ 4.7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T. tubifex</td>
<td></td>
<td>11.7 (+ 4.6)</td>
<td>3.9 (+ 1.4)</td>
<td>3.9 (+ 1.9)</td>
<td>12.6 (+ 4.3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dero sp.</td>
<td></td>
<td>4.0 (+ 0.9)</td>
<td>1.3 (+ 0.4)</td>
<td>2.1 (+ 0.7)</td>
<td>4.3 (+ 1.1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. sowerbyi</td>
<td></td>
<td>33.9 (+ 10.2)</td>
<td>11.9 (+ 3.2)</td>
<td>11.0 (+ 2.8)</td>
<td>24.8 (+ 10.1)</td>
<td>$^{a}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^{a}$Right LGF.

$^{b}$Left LGF.

$^{c}$Bleb of left LGF.
Figure 5. Three-dimensional reconstructions of tubificid VNC

Diagramatic reconstructions showing the giant commissure between left and right LGFs in posterior VNC of L. hoffmeisteri and the asymmetrical expansions of LGFs in posterior VNC of B. sowerbyi.
L. hoffmeisteri

B. sowerbyi
The LGF design in *B. sowerbyi* consisted of segmentally arranged, asymmetrical swellings of the right and left fibers (Fig. 3). In the right LGF, the diameter of the main longitudinal axis was approximately 15 \( \mu m \); however, twice per segment the fiber was expanded to approximately 30 \( \mu m \). The main longitudinal axis of the left fiber was approximately 20 \( \mu m \), but it also expanded twice per segment to form an anterior swelling of about 40 \( \mu m \) and a posterior, spherical bleb often exceeding 120 \( \mu m \) in diameter (Fig. 3). Longitudinal sections revealed no segmental partitioning in the left LGF, suggesting a true anatomical syncytium. Recent results from electron microscopy and dye (HRP and lucifer yellow) injections substantiate this conclusion (Chapter II). Figure 5 shows a three-dimensional reconstruction of the asymmetric design of the LGF system in *B. sowerbyi*.

Table 1 summarizes diameter data for the axial portions, as well as segmental expansions, of giant fibers in anterior and posterior regions of the six aquatic species. In all species, the MGF was larger than the LGF in anterior segments but, due to longitudinal tapering, the reverse was always true in posterior segments. In fact, LGF diameters in posterior segments of tubificids were greater than MGF diameters in anterior segments.
**Electrophysiological correlates of rapid escape reflexes**

As shown in Figs. 6 and 7, two stereotyped and functionally distinct escape reflex responses were recorded non-invasively from all six aquatic species. One response, evoked by tactile stimulation of anterior segments, was propagated posteriorly along the entire animal. The other was evoked by stimulation of posterior segments and propagated anteriorly. In middle segments, the sensitivity for both responses was diminished. Simultaneous extracellular and intracellular recordings were obtained from two of the six species (*K. hexatheca* and *B. sowerbyi*) and indicated that the responses to anterior and posterior touching were mediated by separate giant fiber systems (Fig. 8). In both species, a light touch to anterior segments evoked MGF spiking with each intracellular spike corresponding to the initial spike-like potential in extracellular records. Likewise, intracellularly recorded LGF spikes, initiated by light touch to posterior segments, correlated with the extracellularly detected spikes. Although no intracellular recordings were obtained from the giant fibers of the other aquatic species due to small fiber size, we infer from the stereotyped waveforms of extracellularly recorded giant fiber spikes (Figs. 6 and 7) and sensory field arrangements that a functional dichotomy into MGF and LGF systems exists in all six species.
Figure 6. Non-invasive recordings of giant fiber spiking in two lumbriculid worms

In *K. hexatheca*, a pair of posteriorly conducted MGF spikes (M) and a large muscle potential were evoked by anterior tactile stimulation. With the animal in a reversed orientation on the recording grid, three LGF spikes (L), followed by a large muscle potential, were evoked by posterior tactile stimulation. Similar response patterns were evident in *L. variegatus*, a much smaller species. Voltage scale: 200 μV (*K. hexatheca*) and 50 μV (*L. variegatus*). Time scale: 5 ms.
K. hexatheca

L. variegatus
Figure 7. Non-invasive recordings of giant fiber spiking in three tubificid and one naidid worm

In *B. sowerbyi*, touch to the anterior end evoked a posteriorly conducted MGF spike (M) and a large muscle potential. With the animal in a reversed orientation on the recording grid, an anteriorly conducted LGF spike (L) and large muscle potential were evoked by tactile stimulation to the tail. Similar response patterns were evident in the other species. Note in *L. hoffmeisteri* the reduction in the amplitude of the muscle potential following the second MGF spike compared to the first. In *T. tubifex*, MGF spikes were evoked by direct electrical stimulation to the anterior end (* = stimulus artifact). Voltage scale: 200 μV (*B. sowerbyi*), 100 μV (*L. hoffmeisteri* and *T. tubifex*), and 50 μV (*Dero* sp.). Time scale: 2 ms (*B. sowerbyi*) and 5 ms (*L. hoffmeisteri*, *T. tubifex*, and *Dero* sp.).
Figure 8. Simultaneous extracellular and intracellular recordings of giant fiber spiking in *K. hexatheca* (Lumbriculida) and *B. sowerbyi* (Tubificida)

Giant fiber spikes recorded intracellularly (bottom traces) were coincident with the initial spike potentials in extracellular records (top traces). In both species, MGF spiking was evoked by light touch to the head and LGF spiking by touch to the tail. The large, slower potentials which follow the giant fiber spikes are muscle potentials. Voltage scale: 200 µV (top traces) and 20 mV (bottom traces). Time scale: 5 ms.
Escape reflex function in the Lumbriculida

Figure 6 shows a comparison of non-invasive grid recordings of MGF and LGF spiking in the lumbriculid worms, *K. hexatheca* and *L. variegatus*. In both species, the velocity of MGF spikes (evoked by tactile stimulation of the head) was faster in anterior than posterior segments (Table 2). A single MGF spike was usually followed after approximately 2.0 ms by a small, slower potential of variable amplitude but no behavioral response was evident. However, following two closely spaced MGF spikes, evoked by a slightly stronger touch, the amplitude of the second, slower potential was usually facilitated and a twitch-like shortening of the animal was evoked. These electrical and behavioral response patterns, therefore, parallel those of the MGF system in earthworms (Drewes et al., 1980).

Light tactile stimulation of the tail in both species invariably evoked anteriorly conducted LGF spikes. These spikes were always conducted faster in posterior than anterior segments (Table 2). Single spikes were not accompanied by any detectable muscle potentials or shortening. However, slightly stronger stimuli evoked repeated firing of two or three LGF spikes which triggered a large muscle potential and twitch-like shortening.

In situ recordings (Fig. 9) of rapid escape responses were obtained from the posterior segments of normally ventilating
Table 2. Conduction velocities of giant fiber spikes in six representative aquatic worms from two oligochaete orders

<table>
<thead>
<tr>
<th></th>
<th>Conduction velocity ((\bar{x}) m/s ± SE)</th>
<th>Anterior segments</th>
<th>Posterior segments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MGF</td>
<td>LGF</td>
<td>NGF</td>
</tr>
<tr>
<td><strong>Lumbriculida</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K. hexatheca</td>
<td>19.0 (+ 1.5)</td>
<td>12.3 (+ 1.3)</td>
<td>13.4 (+ 2.3)</td>
</tr>
<tr>
<td>L. variegatus</td>
<td>13.5 (+ 1.8)</td>
<td>6.4 (+ 0.7)</td>
<td>6.4 (+ 0.8)</td>
</tr>
<tr>
<td><strong>Tubificida</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. hoffmeisteri</td>
<td>1.5 (+ 0.1)</td>
<td>1.3 (+ 0.1)</td>
<td>1.4 (+ 0.1)</td>
</tr>
<tr>
<td>T. tubifex</td>
<td>1.3 (+ 0.4)</td>
<td>0.9 (+ 0.3)</td>
<td>0.6 (+ 0.2)</td>
</tr>
<tr>
<td>Dero sp.</td>
<td>1.4 (+ 0.5)</td>
<td>0.8 (+ 0.1)</td>
<td>0.9 (+ 0.1)</td>
</tr>
<tr>
<td>B. sowerbyi</td>
<td>12.9 (+ 1.7)</td>
<td>8.2 (+ 1.9)</td>
<td>5.7 (+ 1.9)</td>
</tr>
</tbody>
</table>
Figure 9. Electrical and behavioral correlates of LGF-mediated escape responses in three aquatic oligochaetes

The bottom trace in each pair is the extracellular, electrical recording. The top trace is the photocell record indicating onset of withdrawal (i.e., downward deflection). In *K. hexatheca*, at least two or three closely spaced LGF spikes (dots) were required for initiation of rapid escape. The magnitude and onset of the escape were variable depending on the number and frequency of LGF spikes. In both *B. sowerbyi* and *L. hoffmeisteri*, a single LGF spike (dot) was followed by a large, longitudinal muscle potential and a rapid, complete withdrawal of the tail. Voltage scale: 200 μV (*K. hexatheca*), 50 μV (*B. sowerbyi*), and 20 μV (*L. hoffmeisteri*). Time scale: 5 ms (*K. hexatheca* and *B. sowerbyi*) and 10 ms (*L. hoffmeisteri*).
K. hexatheca

B. sowerbyi

L. hoffmeisteri
K. hexatheca. The responses evoked by posterior touch always consisted of one or a series of LGF spikes with waveforms essentially identical to those obtained in grid recordings. A single LGF spike was insufficient in evoking any detectable shortening in situ. However, following a closely spaced pair of spikes, or a high frequency train of spikes, large muscle potentials and rapid tail withdrawal were seen (Fig. 9). The speed and intensity of this withdrawal response, as indicated by the onset and slope of the photocell traces, were graded and depended on the number and frequency of LGF spikes. Consequently, efferent latencies (i.e., time from first LGF spike to the onset of shortening) were relatively variable in K. hexatheca (mean = 14.2 ms + 5.3 SD; n = 8). The small size and transparent tail of L. variegatus precluded comparable in situ recordings.

Escape reflex function in the Tubificida

Non-invasive, grid recordings from three tubificid worms, B. sowerbyi, L. hoffmeisteri, and T. tubifex, as well as the naidid worm, Dero, are shown in Fig. 7. Following a light touch to the head, an all-or-none, posteriorly conducted MGf spike was evoked. The conduction velocity of these spikes was fastest in anterior segments and slower in posterior segments (see Table 2). In each species, a single MGF spike was accompanied by a large muscle potential and twitch-like longitudinal contraction. In addition, if a closely spaced
pair of MGF spikes was evoked by a stronger stimulus, the muscle potential associated with the second MGF spike was always reduced in amplitude compared to the first.

Responses to tail stimulation consisted of all-or-none, anteriorly conducted LGF spikes. LGF conduction velocity was faster in posterior than anterior regions of the body (Table 2). A single LGF spike was always accompanied, in a 1:1 fashion, by a large muscle potential and a rapid shortening of the tail (Fig. 7). With paired or repetitive LGF spiking, successive muscle potentials decreased in amplitude (antifacilitation).

In situ recordings of rapid escape responses of freely behaving B. sowerbyi paralleled those obtained with the grids. A single LGF spike elicited a rapid and complete tail withdrawal into the sediments (Fig. 9). Efferent latency values were short and relatively invariant (mean = 6.2 ms ± 1.3 SD; n = 22) compared to those of K. hexathea. The small body size and transparent tails of the other tubificid species usually precluded electrical recordings and resolution of behavioral responses in situ. However, a few records were obtained from L. hoffmeisteri and these were similar to the all-or-none withdrawals of B. sowerbyi.
Discussion

Conservation of giant fiber design and function

Species selected for this study represent the two major orders of aquatic oligochaetes (Lumbriculida and Tubificida). Consequently, descriptions of their giant fiber systems and escape reflexes, together with similar descriptions from previous studies of terrestrial oligochaetes (Order Lumbricida; for reviews, see Dorsett, 1978; Drewes, 1984), provide sufficient perspective for theorizing about the evolutionary history and adaptive significance of giant fiber systems in the Oligochaeta.

A common feature in virtually all oligochaetes is the presence of three dorsal giant fibers, one medial and two laterals, throughout the length of the ventral nerve cord. One known exception is a parasitic species, Branchiobdella pentodonta, in which giant fibers are absent (Vagnetti and Farnesi, 1978). Electrophysiological studies from a wide variety of terrestrial (Drewes et al., 1983) and aquatic species (Figs. 6 and 7) indicate that the fibers function dichotomously. The medial giant fiber (MGF), activated by sensory input from anterior segments, excites segmentally arranged motor elements leading to anterior shortening; whereas, the lateral giant fibers (LGF), activated by input from posterior segments, initiate a distinctly different motor response (usually tail withdrawal). These similarities in
escape reflex design and function suggest that (1) the MGF and LGF systems of modern aquatic and terrestrial oligochaetes are evolutionarily conserved features, and (2) similar systems must have been extant in a common ancestral oligochaete prior to the divergence of aquatic and terrestrial lines of descent.

Divergence of aquatic and terrestrial escape systems

Notwithstanding such evolutionary conservation, comparison of MGF and LGF diameters in different body regions of aquatic and terrestrial species indicate that fiber caliber proportions have diverged in these groups. More specifically, the LGFs of aquatic worms are disproportionately large, often twice as large as the MGF in posterior segments (Table 1). However, the LGFs of terrestrial worms are always smaller than the MGF throughout the length of the animal (Adey, 1951; McFall et al., 1977; O'Gara et al., 1982).

These size differences correlate well with differences in burrowing habits. Many aquatic species (including all in this study), even during feeding behavior, continuously extend their tails out of burrows and perform ventilatory tail-beating to extract oxygen from the water above the muddy sediments. On the other hand, terrestrial species frequently extend their anterior ends out of the burrow, especially during nocturnal feeding and copulation. Therefore, posterior segments of aquatic worms (with prominent LGFs) would appear more vulnerable to predatory attack, whereas anterior segments
of terrestrial worms (with a prominent MGF) would seem more vulnerable. This correlation between behavior and relative giant fiber size leads to the hypothesis that large fiber diameter somehow confers an advantage in escape.

**Adaptive significance of giant fiber systems**

One possible advantage of large fiber systems may be that the associated large spike currents overcome current loading imposed by electrical junctions with numerous post-synaptic cells (Kennedy, 1966; Dorsett, 1980). Bennett (1966) has argued that such electrotonically transmitting junctions are more effective when large presynaptic elements are involved. Since some of the giant-to-motor junctions in earthworms may involve electrical synapses (Gunther, 1972; Schurmann and Gunther, 1973; Jamieson, 1981), large diameter giant fibers may confer increased reliability and effectiveness in synchronously driving segmentally-arranged, motor elements (Bennett, 1984).

Another advantage of large diameter is the resultant increase in spike conduction velocity (Table 2). Figure 10 illustrates the relationship between MGF and LGF velocities in various terrestrial and aquatic species. A biasing toward proportionately faster LGF velocity is clearly evident in posterior segments of aquatic species. Arguments have been made that the time-savings associated with rapid velocity can represent a substantial fraction of the total reflex time,
Figure 10. Relationships between MGF and LGF conduction velocities in anterior and posterior segments of seven oligochaete species.

The center of each rectangle represents the mean MGF and LGF velocities for adults in a given species. The boundaries of each rectangle indicate ± 2 SEM. The species are representative of three oligochaete orders as indicated by the key. In all seven species, MGF velocity is greater than LGF velocity in anterior segments. However, in aquatic species (d-g), the LGF conducts faster than the MGF in posterior segments. The dotted lines indicate equivalence points for MGF and LGF velocity.
Anterior segments

Lumbricida - □
  a - L. terrestris
  b - E. fetida
  c - Sparganophilus sp.

Lumbriculida - □
  d - K. hexatheca
  e - L. variegatus

Tubificida - ■
  f - B. sowerbvi
  g - T. tubifex
especially in animals with highly elongate bodies (Bullock, 1952; Gunther, 1975; Drewes, 1984).

Large fiber diameter is not the only factor that may contribute to increased conduction velocity. The elimination of septal boundaries in segmentally arranged giant fibers, as seen in the syncytial MGF of *L. terrestris* (Gunther, 1971; Gunther, 1975) and LGF of *B. sowerbyi* (Chapter II), may be another means of optimizing conduction velocity (Brink and Dewey, 1980). An additional factor which enhances velocity is the presence of an insulating neuroglial sheath. For example, the myelin-like sheath of some lumbricid giant fibers, together with nodal openings in the dorsal surface of the sheath (Gunther, 1973; Gunther, 1976; Roots, 1984), function to focus spike currents during saltatory-like impulse conduction (Gunther, 1976). Although well developed sheaths are found in several lumbricids (Coggeshall, 1965; Roots and Lane, 1983; Roots, 1984), at least two lumbriculids (*L. variegatus* and *K. hexatheca*; Fig. 2), and one tubificid (*B. sowerbyi*; Fig. 3), their presence is not universal among oligochaetes. The absence of a conspicuous sheath in at least three tubificids (*T. tubifex*, *Dero* sp., and *L. hoffmeisteri*; Fig. 4) is indicative of this heterogeneity. The consequence of lacking a well developed neuroglial sheath is illustrated by a comparison of diameters and velocities in the LGFs of *L. variegatus*, *L. hoffmeisteri*, and *T. tubifex*. Although the
LGFs in posterior segments of these animals are of comparable diameter (Table 1), conduction velocity in the ensheathed LGFs of *L. variegatus* is more than four times faster than those of *L. hoffmeisteri* and *T. tubifex* (Table 2).

Apart from enhancing giant fiber conduction velocity, another means of optimizing reflex speed is by coupling a single giant fiber spike with a large-amplitude muscle potential that is sufficient to trigger rapid escape shortening (Fig. 9). The time-savings of such an all-or-none response are substantial. For example, in *B. sowerbyi*, the efferent latency (i.e., time from a single LGF spike to the onset of tail withdrawal) is usually less than 7 ms. In contrast, the minimal efferent latency for the graded escape of the earthworm *L. terrestris* (i.e., time from the first MGF spike to the onset of behavior) is 15 ms (Pallas and Drewes, 1981).

**All-or-none versus graded escape responses**

If speed is a primary consideration in annelid escape reflexes, then why do earthworms utilize longer latency, graded responses? The answer is probably related to the animal's lifestyle and habitat. Earthworms make burrows within relatively hard and particulate substrates. A graded escape would seem appropriate if irrelevant stimuli, occasionally encountered during burrowing, were sufficiently strong to activate giant fiber systems, but not sufficiently
threatening to warrant escape. On the other hand, an all-or-none escape reflex may be more advantageous for tubificid worms that are relatively sedentary and live in soft-aqueous sediments where locomotory movements are much less likely to inadvertently trigger giant fiber spiking. Therefore, stimuli capable of eliciting giant fiber spikes are more likely to represent real threats that warrant an immediate and complete response. Interestingly, similar differences with respect to the gradedness of escape behaviors exist in certain polychaetes. For example, errant polychaetes, such as *Nereis*, possess graded escape responses (Dorsett, 1964); whereas, only a single giant fiber spike is needed for rapid and complete withdrawal in certain sedentary and tubicolous polychaetes such as *Myxicola* (Nicol, 1984a,b; Roberts, 1962). Despite such parallels between polychaete and oligochaete escape responses, the conserved arrangement of three giant fibers in the Oligochaeta contrasts with the considerable diversity of giant fiber number, organization, and function that exists in the Polychaeta (Nicol, 1948a; Bullock and Horridge, 1965; Krasne, 1965; Dorsett, 1978).

The assumption that escape reflex speed is selectively advantageous in relation to predation pressures is implicit in any discussion of escape reflexes. In fact, there is evidence that predation pressures on both terrestrial and aquatic oligochaetes are intense, particularly with respect to
seasonal predation by many vertebrate species that prey on the worms as they extend from their burrows (Wisniewski, 1978; Samarakoon, 1981; MacDonald, 1983). Therefore, the various anatomical and physiological specializations that contribute to reflex speed in the LGF systems of aquatic and MGF systems of terrestrial oligochaetes provide clear-cut examples of how known predatory pressures, together with fundamental differences in lifestyle and habitat, can modify the design and function of evolutionarily conserved sets of serially homologous giant interneurons.

Acknowledgments

I thank Dr. B. Christensen for supplying *L. hoffmeisteri* and Dr. A. Nebeker for *L. variegatus*. I also thank Dr. D. Emery for use of histological facilities and Ms. M. Nims for typing the manuscript.

References


CHAPTER II. THE LATERAL GIANT INTERNEURONS OF THE TUBIFICID WORM, BRANCHIURA SOWERBYI: STRUCTURAL AND FUNCTIONAL ASYMMETRY OF A PAIRED GIANT FIBER SYSTEM

Introduction

Giant nerve fiber systems are found in nearly half of the major animal phyla (Bullock, 1984). The large diameter axons of these diverse groups are for the most part independently evolved and examples of convergent evolution (Bennett, 1984). The expediency of escape (i.e., the survival advantage) conferred by giant fibers is undoubtedly responsible for this convergence. Enhanced impulse conduction along giant axons can be achieved in several ways including: (1) large diameter which is the definitive characteristic of all giant fiber systems, (2) myelin and pseudo-myelin sheaths which have been described in vertebrates (Celio, 1976; Yasargil et al., 1982), arthropods (McAlear et al., 1958; Heuser and Doggenweiler, 1966), and annelids (Hama, 1959; Gunther, 1976; Roots and Lane, 1983), (3) saltatory or nodal conduction which is found in a wide variety of animals such as shrimp (Kusano and LaVail, 1971), earthworms (Gunther, 1976), and fish (Yasargil et al., 1982; Funch et al., 1984), and (4) syncytium formation in multisegmental systems such as crayfish (Wiersma, 1947; Kondoh and Hisada, 1983) and earthworms (Gunther, 1975).

One common feature in virtually all annelid and arthropod giant fiber systems composed of paired units is bilateral symmetry. However, a recent report described the occurrence
of a bilaterally asymmetric pair of giant fibers (LGFs) in the
tail segments of the tubificid worm, *Branchiura sowerbyi*
(Chapter I). The present study, via correlated
neuroanatomical and electrophysiological results, provides
evidence as to the functional significance of giant fiber
asymmetry during spike initiation and propagation.

Materials and Methods

Animals and maintenance

Tubificid worms, *Branchiura sowerbyi* (Beddard), originally
collected from a local lake (Ames, Iowa), were subsequently
raised for many generations in laboratory aquacultures.
Aquaria were supplied with circulating freshwater, a mud
substrate, and constant aeration. Worms were reared at
22°-25°C and fed several times weekly with a mixture of
Tetramin flakes and trout chow.

Histological procedures

Reproductively mature worms were placed in culture water
overnight for clearance of the digestive tract. The posterior
one third of the animal was amputated and stretched to a
length closely matching that of an animal extended from its
burrow (= 0.15 mm/segment). The tail piece was pinned to a
silicone rubber block and fixed overnight in 2.5%
glutaraldehyde in 0.1 M cacodylate buffer. Tissues were
washed in buffer and immersed for 2 h in cacodylate buffered
1% osmium tetroxide. The fixed tails were cut into smaller pieces, dehydrated in a graded ethanol series, and then immersed for 2 h in propylene oxide. The tissues were then embedded in epon-araldite plastic at 60°C. Sections (0.5–1.0 μm) were stained with 0.5% toluidine blue in 0.5% borax for light microscopy. Thin sections were processed using standard procedures for electron microscopy.

Microdissection procedures

The gilled posterior region (100 segments) of mature worms was amputated and used for electrophysiological and micro-iontophoretic studies. Such tail-piece preparations were advantageous because they exhibited little forward peristaltic crawling characteristically seen in whole worms, and yet survived in culture water for several weeks following amputation.

Tail-piece preparations were pinned to a small, silicone rubber dish with minuten pins and bathed in a modified earthworm saline (Drewes and Pax, 1974) in which the sucrose concentration of 55 mM was reduced to 10 mM. A longitudinal incision of approximately 20–30 segments was made just above the ventrolateral set of setae. The body wall and gut were then pinned into a position that allowed access to the ventral nerve cord and giant axons. The terminal 10–20 segments of the tail remained intact and freely ventilated in the bath for more than 24 h. Giant fiber resting and action potentials in
these preparations were comparable to those in dissected, whole worms and were stable for at least 6-8 h after dissection.

**Lucifer yellow procedures**

Lateral giant fibers (LGFs) were impaled with borosilicate microelectrodes (20-40 MΩ) filled with the fluorescent dye, lucifer yellow (LY-CH), dissolved in 1% LiCl (Stewart, 1978). Dye was injected using hyperpolarizing current pulses of 3-10 nA (100 ms duration at 5 Hz) for 20-40 min. Since it was technically infeasible to excise the ventral nerve cord from the closely apposed body wall, the entire tail preparation was fixed in 4% formaldehyde in 0.1 M cacodylate buffer (6-12 h at 4°C). Glutaraldehyde fixation was avoided because of high background fluorescence produced by body wall tissues in this species. After dehydration, whole mounts were cleared in methyl benzoate and viewed. Paraffin embedding procedures were similar to those described by Strausfeld et al. (1983). Briefly, dehydrated tissues were rinsed in xylene and infiltrated with three changes of paraffin wax for 1 h each at 60°C. Blocks were serially sectioned (0.5-1.0 μm) and the wax ribbons were then placed on a slide. The wax was dissolved by xylene and coverslips were mounted with fluoromount.
Horseradish peroxidase procedures

Lateral giant axons were also impaled with microelectrodes containing a 4% solution of horseradish peroxidase (HRP, sigma type VI) in 0.2 M KAc. HRP was injected with positive current pulses of 1-5 nA (100 ms at 5 Hz) for 20 min. Following a diffusion time of 2-4 h, the unfixed tissue was covered with 10 ml of 0.2 M Tris buffer (pH 7.6) containing 5 mg of p-phenylenediamine, 10 mg of pyrocatechol, and 0.01 ml of 30% hydrogen peroxide (Hanker et al., 1977; Purves and Hume, 1981). The tissues were incubated for 20 min and then washed with saline. Fixation, embedding, and sectioning procedures were as described earlier for histological studies.

Ferric ion-ferrocyanide staining procedures

The ferric chloride-potassium ferrocyanide staining technique, originally developed by Quick and Waxman (1977a,b), specifically stains regions of membrane associated with a high density of Na⁺ channels. This procedure was used in their studies to specifically stain nodes of Ranvier in vertebrate myelinated nerve fibers (Quick and Waxman, 1977a,b) and has subsequently been used to stain nodal regions of giant axons in fish (Yasargil et al., 1982) and earthworms (Roots, 1984). In the present study, tail pieces were pinned to a silicone rubber block and a dorsal incision was made along the length of the tail. The tissue was fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer and post-fixed in buffered 1% OsO₄.
Tissues were then washed in three changes of double distilled water and immersed in 0.01 M FeCl₃ (pH 1.5-2.0) for 60 min. Following another series of washes in distilled water, the tissues were placed in K₄Fe(CN)₆·3H₂O for 20 min. The tissues were then processed for araldite embedding and sectioning as described earlier. The ferric ion-ferrocyanide reaction yields a Prussian blue staining product. For comparison, identical procedures were performed using 0.1 M phosphate buffer. Although the reaction sites were similar, preparations processed with cacodylate-buffered fixative produced a darker reaction product.

**Electrophysiological procedures**

Intracellular recordings were made with borosilicate microelectrodes (5-20 MΩ) filled with 3 M KCl. Tactile stimulation of the tail was produced by either a hand-held or electromechanically-driven probe. Resting and action potentials were amplified by a dual-channel microprobe system (W. P. Instruments Inc., Model KS-700), displayed on a Tektronix 5113 dual beam oscilloscope, and recorded on magnetic tape. Current injection was delivered through a bridge circuit in one channel of the microprobe system. The bridge was balanced before cell impalement and, when necessary, readjusted after cell penetration. All experiments were conducted at 22°-25°C.
Results

Anatomy of the LGF system

Histological sections and intracellular injections of lucifer yellow (LY-CH) revealed numerous anatomical features of the lateral giant interneurons of Branchiura sowerbyi. These included cell body locations, giant axon geometry, and ventral collateral projections. Two remarkable features of the LGF geometry were (1) a marked asymmetry between the left (≈ 80 μm) and right (≈ 20 μm) LGF diameter (Fig. 1) and (2) conspicuous segmentally-arranged swellings (blebs) along the left LGF (Figs. 1 and 2). This gross enlargement was associated exclusively with the left LGF and was found in all experimental preparations (intact or tail pieces). In addition, blebbing was present even when preparations were subjected to wide variations of ventral nerve cord stretch (Figs. 1 and 2A). Therefore, blebbing was clearly not an artifact of segmental shortening.

Dye injections also showed unrestricted longitudinal diffusion of LY-CH for many (> 20-30) segments along the LGFs. The absence of any abrupt segmental transitions in fluorescence intensity (Fig. 1) suggested the possibility that the segmental constituents of the LGF were syncytial. This idea was clearly substantiated by results showing a similar unrestricted longitudinal diffusion of HRP, as well as, results showing the absence of segmentally-arranged...
LY-CH was injected iontophoretically into the LGF of posterior body segments. Each panel represents approximately three segments of ventral nerve cord. LY-CH diffused into the right LGF and along the longitudinal axis of both fibers. Note the marked asymmetry in size between the left fiber (top) and the right fiber (bottom) in each panel. Also note the striking discontinuities in axonal diameter, particularly in the left LGF. Several LGF cell bodies are visible ventral to the fibers. The bottom panel (B) was photographed at the left LGF's level of focus. Note that a different set of cell bodies is now visible below this fiber. The preparation was stretched, pinned, and fixed in an elongate state. Horizontal bar: 140 μm (A,B). The bar designates the length of one segment of VNC.
Figure 2. Spherical enlargements (blebs) of the left LGF

Panel A shows approximately three segments of VNC in posterior segments of a living preparation. LY-CH was injected into the left LGF and the fluorescent image was photographed while focused on the spherical blebs of that fiber. In this preparation, the tail segments were pinned in a contracted state. Panel B shows a light micrograph of a longitudinal section through the left LGF of another preparation. Note the extreme variations in axonal diameter between the bleb and non-bleb regions of the LGF. Also note the lack of any membranous partitions along the length of the axon. Vertical bar: 100 μm (A), 80 μm (B).
septal-partitions in serial longitudinal sections (Fig. 2B). Likewise, intracellularly injected LY-CH (Fig. 1) and HRP readily diffused between the left and right LGFs indicating that connections between the fibers are also syncytial.

LY-CH injections also demonstrated cell body locations of the LGFs. Figure 2 shows that each posterior body segment contained a bilaterally symmetrical pair of LGF cell bodies. Cross-sections of LY-CH filled LGFs revealed the ventrolateral positions of the cell bodies within the ventral nerve cord (Fig. 3A). The neurites that projected dorsally from each cell body branched near the middle of the neuropile. One branch projected ipsilaterally, without interruption, to form one of the ventral collaterals of the LGF. The other branch projected medially and united with an identical branch of the contralateral neurite to form a small diameter (≤ 2 μm) lateral crossbridge. Together, the neurites, collaterals, and crossbridge formed an H-configuration (Fig. 3A) that was repeated once per segment at the level of smallest LGF caliber (i.e., between left LGF blebs). Since these crossbridges appear to be the only direct connections between the left and right fibers, they probably are the anatomical basis for bilateral dye coupling.

LY-CH injections also revealed four other pairs of LGF collaterals per segment in addition to the pair that
Figure 3. Ventral collaterals of the LGFs in posterior body segments

Panel A, a reconstruction based on serial paraffin sections of a preparation injected with LY-CH, shows that one pair of ventral collaterals forms a crossbridge that connects the left and right LGFs. In addition, this crossbridge is continuous with the neurites of the LGF cell bodies that are located in the ventrolateral periphery of the VNC. Panel B shows the LGF collaterals projecting ventrally into the neuropile of the VNC. Panel C is a micrograph of a frontal section through the neuropile of the VNC in a posterior segment. Note that four pairs of collaterals, with similar diameters, project into the neuropile between each set of crossbridges. Vertical bar: 50 μm (A), 120 μm (B), 72 μm (C).
constitute the lateral crossbridge. These collaterals projected ventrally from the axon for a length of 10 to 30 \( \mu m \) and terminated in the neuropile of the VNC (Fig. 3B). Serial frontal sections, viewed at the light level, revealed a regular spacing of about 20 \( \mu m \) between each pair of ventral collaterals along the axon's length (Fig. 3C). No obvious asymmetries were apparent in comparisons of geometry (i.e., length and caliber) between the collaterals of the left and right LGFs.

Electron microscopic observations showed that these ventral collaterals represent myelin-free extensions of the axolemma (Fig. 4A) and may be sites of both synaptic input and output. Terminals containing both large, dense-core vesicles and smaller, electron-lucent vesicles were found often contacting the collaterals close to their origin from the axons (Fig. 4C). In addition, ventral collaterals contained large clusters of electron-lucent vesicles and membrane specializations (e.g., apparent thickening) suggesting that collaterals are sites of synaptic output (Fig. 4D).

Some of the non-junctional surface of the collateral plasmalemma had an electron dense undercoat appearing as a thickened membrane. This undercoating was particularly obvious at membrane regions apposing extracellular spaces. Results from ferric ion-ferrocyanide staining (Quick and Waxman, 1977a,b) consistently showed a positive Prussian blue
Figure 4. Electron micrographs of LGF collaterals and sheath

Panel A shows a longitudinal section through the LGF and neuropile of the VNC. The arrows indicate an interruption in the sheath by a ventral collateral. Panel B, a higher magnification, shows the residual cytoplasm within the sheath membranes and the occasional spaces within the lamellae. Panel C shows the close apposition of the collateral membrane with a vesicle-containing terminal (arrows) and the thickening of the junctional membrane between the two cells. Panel D shows the large quantities of vesicles contained within the ventral collateral near its apposition with another neuron (arrows). Magnification: X7500 (A), X45,000 (B), X36,000 (C), X57,000 (D).
Figure 5. Ferric ion-ferrocyanide staining of the LGF ventral collaterals

Panels A and B show two consecutive cross-sections. Ferric ion reaction products (arrows) are evident at the tips of the collaterals that have arisen from the left and right LGFs. Panels C and D (a higher magnification) show that reactions also occurred along the length of some collaterals. Sections were photographed without additional general tissue staining. Vertical bar: 25 μm (A,B), 30 μm (C), 15 μm (D).
reaction at these myelin-free regions of the collaterals (Fig. 5). No other reactive sites were found along the length of the axons.

Comprehensive examination at light and electron microscopic levels showed that the ventral collaterals were the only interruptions in the 2-4 μm thick, myelin-like sheath surrounding the giant axons (Fig. 4A). This sheath, like that of earthworms (Gunther, 1976; Roots and Lane, 1983), consisted of glial membranes separated by residual cytoplasm (Fig. 4B). A total of approximately 50 membranes were present in sections of sheath possessing a regular interlamellar spacing (approximately 250 Å). However, irregularities in membrane arrangements, such as redundant lamellar loops and organelle-filled spaces, occurred frequently. In addition, sheath membranes near the origin of the ventral collaterals contained many interlamellar attachments, or desmosomal stacks, similar to those described at the nodal branches of shrimp giant axons (Kusano and LaVail, 1971) and the dorsal nodes of earthworm medial giant fibers (Gunther, 1976; Roots, 1984).

Electrophysiology of the LGF system

Intracellular recordings were obtained from the right and left LGFs of tail preparations. Resting potentials of both LGFs ranged from -65 to -75 mV. Overshooting action potentials (ranging from 75 to 85 mV) were readily evoked by tactile stimulation of the tail’s body wall (Fig. 6A) or by
depolarizing current injection (Fig. 6B). Spike durations at half amplitude were approximately 1 ms. LGF spikes were abolished in Na^+^-free saline.

Dual microelectrode penetrations at different sites along the left LGF were made to experimentally measure the length constant (λ) of the axon. In these experiments, hyperpolarizing current pulses were passed into the giant axon and the resultant displacements of the membrane potential were simultaneously recorded at the site of current injection and at sites 5, 10, 15, and 20 segments away (Fig. 7). A length constant of 1.5 mm or approximately 12 segments was calculated for the left LGF.

Since anatomical studies revealed a marked asymmetry between the left and right LGFs, current clamp studies were conducted to see if corresponding differences existed in biophysical properties of the fibers. Input resistances of both the right and left LGFs were determined from standard I/V plots (Fig. 8) produced by current injection using a single, bridge-balanced microelectrode. The small right LGF had an input resistance of 3.3 MΩ, while the larger left LGF had an input resistance of 1.5 MΩ. Correspondingly, the rise-time of voltage changes in the left fiber was much shorter than that of the right (Fig. 8, insets). An applied current of 4-6 nA was sufficient to initiate a spike in the right LGF; whereas,
Figure 6. Intracellular recordings of LGF spikes

LGF spiking was recorded intracellularly from the left fiber of semi-intact preparations following stimulation of the posterior body wall with an electromechanically-driven probe (A) or intracellular injection of a depolarizing current pulse (B). In both cases, resting membrane potentials were 74 mV and overshooting action potentials of 80 mV were evoked (* = stimulus artifact). Current/voltage scale: 20 mV (A), 10 nA (B, top trace), 20 mV (B, bottom trace). Time scale: 5 ms (A), 10 ms (B).
Figure 7. Experimental measurement of spatial decay of membrane potential changes along the left LGF

Membrane potential changes at various sites expressed as the percentage of the voltage change at site 0 were plotted against the distance between recording sites. The estimated length constant ($\lambda$) of the left LGF was 1.5 mm. The inset illustrates a dual recording at segment 0 ($S_0$) and 10 segments ($S_{10}$) from the stimulus site. Vertical bars: $\pm$ S.D.; $n$ = five preparations. Inset current/voltage scale: 5 nA (top trace), 10 mV (middle and bottom trace). Inset time scale: 20 ms.
Figure 8. Resting current-voltage relationships of the right and left LGFs

The input resistance or slope of the current/voltage curve for the right LGF (open circles) was 3.3 MΩ (n = 8 preparations). The input resistance of the left LGF (closed circles) was 1.5 MΩ (n = 13 preparations). The insets show current steps and voltage changes recorded from both fibers and the relative differences in time constants. The duration of the current pulses was 100 ms.
10–15 nA of injected current was needed to initiate an action potential in the left LGF.

These results raised the possibility that membrane potential changes, caused by synaptic inputs, might differ in left and right fibers. To test this hypothesis, dual-intracellular recordings were made with microelectrodes inserted into each of the two giant axons in a single tail segment. A light tactile stimulation of the mid-ventral gill filament, on the same segment as the recording electrodes, produced subthreshold excitatory post-synaptic potentials (EPSPs) in the right LGFs that were consistently greater in amplitude than those recorded from the larger left LGF (Fig. 9A, B). Similar dual recordings, made in response to stronger tactile stimuli sufficient to evoke LGF spikes, showed that action potential initiation in the right fiber preceded that in the left by approximately 0.5 to 1.0 ms (Fig. 9C). However, these action potentials were always synchronous when recorded at locations 5 or more segments from the site of tactile stimulation (Fig. 9D).

Discussion

This study demonstrates the neuronal cell body positions, axonal geometry, and several ultrastructural features of the paired lateral giant fiber (LGF) system in the ventral nerve cord of the tubificid worm, *Branchiura sowerbyi*. Pronounced bilateral asymmetry, segmentally-arranged axonal enlargements
Figure 9. Simultaneous intracellular recordings from the left and right LGFs

Following light tactile stimulation of a gill filament, EPSPs recorded in the right LGF (bottom traces in A and B) were always larger than those recorded in the left LGF (top traces in A and B). When tactile stimulation was sufficiently strong to elicit LGF spiking, the action potentials were always initiated first in the right LGF (bottom trace in C) and then in the left (top trace in C). However, when similar dual recordings were made five segments from the site of stimulation, the paired LGF spikes were in synchrony (D). Voltage scale: 5 mV (A,B), 20 mV (C, top trace), 10 mV (C, bottom trace), 20 mV (D). Time scale: 5 ms (A-D).
(blebs), intersegmental and intrasegmental (i.e., between fibers) syncytia, and a myelin-like sheath are among the most conspicuous specializations of this system. Electrophysiological results, together with cytological staining properties, provide evidence as to the functional significance of these specializations, especially with respect to spike initiation and propagation.

**LGF spike initiation**

The average caliber of the left LGF in tail segments is at least four times greater than the right (Fig. 10). Correspondingly, electrophysiological results show that the right LGF has a higher input resistance and is more strongly excited by sensory inputs than the left LGF. Spikes evoked during tactile stimulation are initiated earlier in the right fiber than the left (Fig. 9C). Thus, the small, right LGF, at the segmental level of stimulation, functions as the locus for spike initiation.

Action potentials in the larger, left LGF occurred approximately 1 ms after those in the right. Initiation in the left LGF presumably occurred by a combination of the depolarizing sensory inputs from its crossbridge ventral collaterals and by electrotonic inputs from connections with the right LGF.

Given the differential in caliber between left and right LGFs, one might expect that action potential currents
generated during spike initiation in the right fiber would be significantly loaded by the much larger, left fiber. However, the small diameter (\(\approx 2 \, \mu m\)) of the lateral crossbridges probably protects the system from excessive resistive and capacitative loading. Similar loading-protection arguments have been made for the restriction zones located between the somata and major axons of crayfish sensory neurons (Mellon and Kaars, 1974). In addition, the lateral crossbridges are always strategically located at the level of closest size matching between the paired LGFs (Fig. 10). This arrangement likely provides the best possible impedance matching between the asymmetric fibers. Therefore, one functional consequence of blebbing might be that segmental constrictions of the left LGF, at the level of the crossbridges, confer the impedance matching necessary for effective electrical cross-connections during spike initiation.

**LGF spike propagation**

Action potentials that have been conducted only a few segments from the site of stimulation (i.e., spike initiation) occur synchronously in both LGFs (Fig. 9D). Therefore, it appears that the speed of impulse conduction within the paired LGF system is predominantly determined by the larger, left fiber.

The left LGF possesses several specializations, aside from its conspicuous diameter, that likely contribute to rapid
The LGF system was reconstructed from LY-CH injections and light microscopy of frontal, sagittal, and cross-sections. Note the ventrolateral positions of the paired cell bodies, the gross asymmetry between the giant axons, the spherical enlargements (blebs) of the large left LGF, the regular positions of the lateral crossbridges, and the positions of the other ventral collaterals.
conduction. One of these is a syncytial design. The fact that each body segment contains two LGF cell bodies suggests that the axons are a fused chain of multisegmental units. Evidently, the membrane partitions (i.e., septa) that surely separate the segmental units early in development, subsequently degenerate creating a syncytial fiber. Gunther (1971) demonstrated that 60% of these septa are eliminated from the medial giant fibers of earthworms. Brink and Dewey (1980) have shown that the electrical junctions associated with each septum, in an earthworm giant fiber, produce a conduction delay of about 34 μs. Assuming that similar delays are avoided by the complete elimination of septa in the tail of Branchiura (♂ 100 segments in length), a conduction time savings of 3 to 4 ms is achieved. This is a substantial fraction of the total reaction time (Chapter III).

Yet another adaptation for rapid conduction in Branchiura is a well-developed, myelin-like sheath that surrounds the giant axons and has structural characteristics (e.g., lamellae number and spacing) similar to those reported for earthworms (Hama, 1959; Gunther, 1976; Roots and Lane, 1983). This LGF sheath is interrupted five times in each segment by a pair of ventrally projecting collaterals (Fig. 10), representing the only regions of myelin-free axolemma along the axons. These ventral collaterals not only appear to be the sites of synaptic inputs and outputs, but also the
probable sites of spike electrogenesis. The possibility that the collaterals function as nodal regions for action potential initiation is supported by their positive reaction with ferric ion-ferrocyanide staining. This procedure has been shown to specifically stain membrane regions of high sodium channel density such as vertebrate nodes of Ranvier (Waxman, 1974; Quick and Waxman, 1977a,b; Waxman and Quick, 1978). The staining characteristics in the ventral collaterals of Branchiura are strikingly similar to those described at the tips of nodal collaterals in Mauthner cell giant axons (Yasargil et al., 1982). Apparently such reactions are stronger than the diffuse staining seen at ventral collaterals and dorsal nodes of earthworm medial giant fibers (Roots, 1984). Although electrophysiological recordings of focal spike currents support the idea of saltatory-like nodal conduction in Mauthner axons (Celio et al., 1979) and earthworm medial giant fibers (Gunther, 1976), comparable studies have not been done in Branchiura and are likely to be difficult because of the inaccessibility of the ventral collaterals.

Paired giant fiber systems, which function as a single unit, must somehow maintain a left-right synchrony during action potential propagation. This synchrony is likely achieved by segmental crossbridges (probably non-syncytial) in the paired giant axons of the polychaete worm, Protula
(Hagiwara et al., 1964) and the lateral giant fibers of the earthworm, *Lumbricus* (Wilson, 1961; Mulloney, 1970). Similarly, commissural processes (probably syncytial) synchronize the paired giant axons of the polychaete *Sabella* (Mellon et al., 1980). Likewise, the syncytial crossbridges of *Branchiura*, while ensuring left-right spike synchronization and the execution of a symmetrical escape, are unique with respect to the asymmetric geometry of the LGF system.

Acknowledgments

I thank Dr. C. Fourtner and Mr. A. Siegel at SUNY, Buffalo, for the production of electron micrographs in this manuscript. I also thank Dr. D. Emery for his assistance and advice concerning histological techniques.

References


CHAPTER III. THE RAPID TAIL WITHDRAWAL REFLEX OF THE TUBIFICID WORM, BRANCHIURA SOWERBYI: ADAPTATIONS FOR SPEED AND EFFICACY IN AN ALL-OR-NONE ESCAPE RESPONSE

Introduction

The tubificid worm, Branchiura sowerbyi (Class Oligochaeta), lives in aquatic sediments nearly devoid of oxygen (Naqvi, 1973; Brinkhurst and Jamieson, 1971), its survival depending on several respiratory adaptations found only in posterior segments. These include: (1) a pair of movable gill filaments on each segment, (2) an uptake and anterior pumping of water by the rectum, and (3) a rhythmic undulatory movement of posterior segments as the tail extends above the sediments. Clearly, such behaviors render the animal vulnerable to predation, thereby necessitating neuro-behavioral adaptations for rapid escape into the sediments.

Recent studies (Chapter I) have shown that Branchiura, as well as other tubificid worms, respond to posterior tactile stimulation with a lateral giant fiber-mediated withdrawal, or rapid escape reflex. In the present study, we have used videotape and high-speed cinematography to document escape responses to experimenter-applied stimuli and actual predatory attacks. We have also made a precise accounting of the time required for afferent, central, and efferent excitation during escape responses in intact animals. The results demonstrate that this reflex is among the fastest in the animal kingdom and reveal several features of tubificid escape behavior which
differ from escape behavior in terrestrial oligochaetes (i.e., earthworms).

**Materials and Methods**

**Animals and maintenance**

Tubificid worms, *Branchiura sowerbyi* (Beddard), were obtained from established laboratory cultures. Culture aquaria were supplied with mud from a local lake, circulating freshwater, and constant aeration. Cultures were kept at room temperature (22°-25°C) and fed twice weekly with a ground mixture of Tetramin staple flakes and trout chow. Photoperiod was not regulated.

**Non-invasive recordings**

Reproductively mature worms were used for all electrophysiological and behavioral studies. For some non-invasive recordings, worms were placed on the moistened surface of a grid of recording electrodes engraved on a printed circuit board (O'Gara et al., 1982). Outputs from pairs of electrodes were differentially amplified, filtered, and displayed as multiple traces on an oscilloscope. Escape responses were evoked by tactile stimulation with either a hand-held or electromechanically-driven probe. Non-invasive recordings were also made in situ using a miniature aquarium which contained several cm of sediment and several cm of water (see Chapter I). After being placed into the aquarium, the
worm quickly burrowed into the sediments, protruded its tail into the water column, and began ventilatory movements of posterior segments. Movable recording electrodes, positioned to within 1 mm of the tail, readily detected giant fiber spiking and muscle potentials associated with escape. These signals were amplified and displayed on an oscilloscope, along with the outputs of a photocell (G.E. Model 8PV1AAB with D.C. powered fluorescent light source), which simultaneously detected shadow movements of the worm's tail. Tactile stimuli were delivered as single pulses (1.0 ms duration) with an electromechanically-driven (speaker) probe (100 μm tip diameter) placed in contact with the tail.

**Semi-intact preparations**

The posterior 100 segments (approximately one-third of body length) of mature worms were amputated and used for microelectrode studies of escape reflex activity. These tail-piece preparations were advantageous because they exhibited few spontaneous forward peristaltic movements, but survived for many days after amputation (see Chapter II). Another advantage was that only the LGF sensory field was present in such preparations, thereby precluding inadvertent activation of the worm's medial giant fiber system. The tail-piece was submerged in a saline solution (see Chapter II) and pinned, left side up, to a silicone rubber dish. A longitudinal incision was made along the left ventrolateral
set of setae. The opened body wall and gut were secured with minuten pins to allow access to the lateral giant fibers and longitudinal musculature. Extracellular recordings were obtained with polyethylene suction electrodes placed in contact with the dorsal surface of the ventral nerve cord and/or the ventrolateral musculature of the body wall. Intracellular recordings were made with borosilicate microelectrodes (5-20 MΩ) filled with 3 M KCl. Resting membrane potentials of lateral giant fibers and muscle fibers were amplified by a microprobe system (W. P. Instruments Inc., Model KS-700) and displayed on a Tektronix 5113 dual beam, storage oscilloscope. Tactile stimulation was delivered by either the previously described electromechanically-driven probe or by a hand-held glass probe (tip diameter approximately 200 μm). Electrical stimulation of giant fibers was delivered through a suction electrode. Electrical activity associated with escape was stored on magnetic tape for later analysis.

The effects of the acetylcholine antagonist, curaré, were studied during escape responses in semi-intact preparations. Intracellular recordings from longitudinal muscle, were first obtained in normal saline. These recordings were compared to those obtained during exposure to saline containing 10⁻⁴ M d-tubocurarine chloride (Sigma) and after wash in normal saline. Similar procedures were used to study the effects of
low Ca\(^{++}\)/high Mg\(^{++}\) saline. This saline contained 50 \(\mu\)M Ca\(^{++}\) and 10 mM Mg\(^{++}\), compared to the normal 6 mM Ca\(^{++}\) and 1 mM Mg\(^{++}\).

**Videotape and cinematography of escape sequences**

Rapid tail withdrawals in response to attack by bluegill, *Lepomis macrochirus*, were studied in narrow aquaria (15 cm x 30 cm x 5 cm) supplied with a mud substrate, freshwater, and aeration. A partition separated the bluegill from the ventilating worm during an initial 15 min acclimation period. Following removal of the partition, strike/withdrawal sequences were videotaped with a Panasonic WV-1400 TV camera equipped with a Cannon 100 mm lens. These episodes were recorded on a Panasonic PV-1560 recorder and displayed on a Hitachi TP-300 videomonitor. Single-frame images were photographed directly from the monitor with a Polaroid oscilloscope camera.

High-speed cinematography of tail withdrawal sequences was done with a 16 mm, rotating prism camera (Hycam, Model K2004E-115, Red Lake Laboratories, Santa Clara, CA). Sequences were filmed at 200 frames/s with Eastman Ektachrome 7250, tungsten film. Still photographs of individual frames were made directly from film prints.
Results

**Videotaped escape responses**

Analysis of videotaped escape sequences indicated that several types of experimenter-applied stimuli were adequate for eliciting escape in *Branchiura*. Invariably, direct tactile stimulation of the tail (either body wall or gill filaments) with a small, hand-held probe evoked rapid shortening (Fig. 1A). It was also evident that either abruptly delivered water displacements or substrate vibrations could readily elicit escape. In contrast, abrupt delivery of light or shadow stimuli was always ineffective in eliciting rapid escape.

Videotape analysis of worm movements in the presence of bluegill sunfish provided further support for the adequacy of mechanosensory stimuli in evoking escape. In some episodes, rapid escape was elicited, without actual attack, by the water displacements caused by fin movements as the fish hovered or swam near the worm (Fig. 1B). More often, escape responses were initiated in an individual worm, or simultaneously in several worms, during a strike at a worm's tail (Fig. 1C). In these cases, water displacement and/or substrate vibrations were most likely the effective stimuli. In a few instances, worms also rapidly escaped in response to digging behavior of fish in sediments at considerable distance from the worm, suggesting that vibrational stimuli alone were adequate.
Figure 1. Videotaped sequences of rapid escape behavior of *Branchiura sowerbyi* in response to experimenter-applied and natural stimuli.

The two consecutive frames in A illustrate a rapid tail withdrawal evoked by tactile stimulation of posterior segments with an experimenter-held probe. Note the gill filaments on the dorsal and ventral surfaces of the tail. The three consecutive frames in B (lower magnification) show the response of a ventilating worm (arrow) to the water movements created by the fins of a hovering bluegill. Note the pelvic fin of the fish in the upper right corner of the top frame. The two consecutive frames in C illustrate the synchronous responses of three worms to the strike of a bluegill that attacked the tail of a fourth worm (already withdrawn in these frames). All sequences were videotaped at 30 frames/s.
Afferent timing and transmission

Since mechanosensory stimuli were effective in initiating escape, intracellular recordings from the LGF system in semi-intact preparations were obtained following direct electromechanical stimulation of the body wall. Stimulation of any tail segment reliably evoked excitatory post-synaptic potentials (EPSPs) within the left LGF of the stimulated segment (Fig. 2A). Mean afferent latency (i.e., the time from onset of stimulus to the onset of EPSPs) was 2.2 ms (± 0.6 S.D.; n = 9, from five animals). With slightly stronger stimuli, suprathreshold EPSPs were evoked. The initiation of an LGF spike occurred approximately 0.5 to 1.0 ms after onset of the EPSP (Fig. 2B).

Two lines of evidence suggest that at least some fraction of these excitatory inputs to the LGF was mediated by chemical synaptic transmission. First, hyperpolarization of the LGF, during the generation of excitatory input, enhanced the amplitude of EPSPs (Fig. 2A). Second, intracellular responses to mechanical stimulation were reversibly reduced to subthreshold levels by bathing semi-intact preparations in either low Ca²⁺/high Mg²⁺ saline or normal saline containing the acetylcholine antagonist, curare. Interestingly, in such preparations, a short-latency response of about 2 mV persisted (Fig. 2C). Since the amplitude of this persisting potential
Figure 2. Excitatory post-synaptic potentials (EPSPs) recorded from the left LGF following tactile stimulation of the body wall

Panel A (next to bottom trace) shows the EPSP recorded during tactile stimulation of the tail (* = stimulus artifact). Afferent latency (i.e., time from stimulus onset to the onset of EPSP) was approximately ms. Hyperpolarization of the LGF during an identical stimulus resulted in an enhancement of EPSP amplitude (bottom trace). The upper traces show the current monitor. Panel B shows a supra threshold EPSP and a pike in the LGF following a stronger tactile stimulation of the body wall. Panel C shows the effect of a 30 min exposure to $10^{-4}$ tubocurarine on the EPSPs evoked by tactile stimulation. Note that a small curarine-resistant potential persists and that hyperpolarizing current injection was ineffective in modifying the amplitude of this potential. Current/voltage scale: 10 nA (A and C, top traces), 5 mV (A and C, bottom traces), 20 mV (B). Time scale: 10 ms (A and C), 5 ms (B).
was unaffected by LGF hyperpolarization, it seemed likely that the response was an electrically-mediated PSP.

Central conduction

Non-invasive grid-recordings showed that light tactile stimulation of the tail evoked a highly stereotyped and rapidly conducted electrical response. At each recording site, the response consisted of two distinct components (Fig. 3A): an initial spike-like potential of invariant waveform (approximately 100 to 200 µV) followed after less than 1 ms by a slower, multiphasic wave of variable amplitude (approximately 400 to 800 µV). Dual intra- and extracellular recordings revealed that the initial component was always coincident with an intracellularly recorded LGF spike (Fig. 3C). Non-invasive recordings, made at multiple sites along the animal's tail (Fig. 3B), showed that such LGF spikes were conducted anteriorly at 15-20 m/s. However, because of an associated longitudinal gradient in LGF diameter (Chapter I), velocities in mid-body and anterior segments were reduced to 10-15 m/s and 5-10 m/s, respectively.

Efferent timing and transmission

Dual intra- and extracellular recordings showed that the onset of the large, slow potential seen in non-invasive recordings corresponded to the onset of an action potential in longitudinal muscle fibers of the body wall (Fig. 3D). As
Figure 3. Extracellular and intracellular recordings of LGF and longitudinal muscle activity during rapid escape

A non-invasive grid recording from posterior segments made during an escape response to touching the tail consisted of two components: an initial LGF spike (dot) followed by a multi-phasic, longitudinal muscle potential. A four channel recording shows the LGF spike was conducted anteriorly along the animal at a velocity of approximately 15 m/s (B). The top trace is the most posterior recording site and the distance between each site was 8 mm. In panel C, an LGF spike recorded intracellularly (bottom trace) was coincident with the initial spike in extracellular suction electrode recordings (top trace). In panel D, a longitudinal muscle potential recorded intracellularly (bottom trace) was coincident with the multi-phasic potential recorded extracellularly with suction electrodes (top trace). Voltage scale: 100 μV (A), 500 μV (C, top trace), 200 μV (D, top trace), 20 mV (C and D, bottom traces). Time scale: 2 ms (A), 5 ms (B,C), 10 ms (D).
Figure 4. Intracellular recordings of longitudinal muscle activity during rapid escape

Dual intracellular recordings from the LGF (A, bottom trace) and a longitudinal muscle fiber (A, top trace) illustrate the short efferent latency (from 0.6 to 1.0 ms). The shoulder-like prepotential characteristic of muscle kpotentials evoked during rapid escape (B, top trace) was not present in spontaneous action potentials in the same fiber (B, bottom trace). Panel C shows the decrease in excitatory junctional potential (EJP) amplitude, or antifacilitation, during repetitive electrical stimulation of the LGF at 1 Hz (* = stimulus artifact). The dots denote LGF spikes; the intracellular recording was approximately 30 mm from the extracellular recording site. Voltage scale: 20 mV (A,B), 10 mV (C), 200 μV (D, top trace), 20 mV (D, bottom trace). Time scale: 5 ms (A-D).
shown in Figs. 3D and 4A, these muscle potentials were composed of an initial shoulder-like prepotential, having a steep rate of rise (20-30 mV/ms), and an overshooting spike with a slightly slower rate of rise (15-20 mV/ms). Two lines of evidence suggest that the prepotential is an excitatory junctional potential (EJP). First, occasional spontaneous longitudinal muscle potentials, not associated with escape responses and presumably caused by electrode penetration, lacked the prepotential (Fig. 4B). Second, during repetitive firing of the LGF, muscle prepotentials were gradually reduced in amplitude (antifacilitated) and quickly failed to initiate action potentials (Figs. 4C and 4D). Muscle EJPs normally recovered from such stimulation within seconds.

Assuming that neuromuscular transmission might be cholinergic as in other oligochaetes (see Gerschenfeld, 1973), muscle responses to LGF spiking were studied in the presence of the acetylcholine antagonist, curaré. Figure 5 shows that curaré rapidly and reversibly reduced the amplitude of the EJPs, thus, preventing muscle spiking and the concomitant rapid contraction that usually follows each LGF spike.

Although the motor neurons involved in the efferent pathway remain unidentified, it was possible to make accurate measurements of efferent latency. Dual intracellular recordings (Fig. 4A), made simultaneously from the LGF and longitudinal muscle fibers in the same segment, revealed that
Figure 5. Effects of curare on the LGF-mediated response

Panel A shows simultaneous recordings from the ventral nerve cord (top trace; extracellular suction electrode) and longitudinal muscle (bottom trace; intracellular recording) in normal saline. In panel B, after bathing in 10 tubocurare for 10 min, all longitudinal muscle activity associated with rapid escape was abolished from both extracellular and intracellular recordings. The dot in panel B denotes the persisting LGF spike. After 20 min of washing with normal saline, the longitudinal muscle response was partially recovered (C). All responses were evoked by tactile stimulation of intact tail segments many segments from the dissection site. Voltage scale: 500 μV (A-C, top traces), 20 mV (A-C, bottom traces). Time scale: 5 ms (A-C).
the latency from the peak amplitude of the LGF spike to the onset of muscle potentials was 0.7 ms (+ 0.1 S.D.; n = 17, from eight animals). Since the latency was essentially the same in all body regions, the timing difference between the onset of muscle potentials recorded in any two body segments was equal to the LGF conduction time between those segments (Fig. 3B).

In situ escape reflex timing

High-speed cinematography was used to estimate the response latency of rapid withdrawal. Animals ventilating in a miniature aquarium were stimulated with a hand-held, glass probe (3 mm diameter) and movements associated with rapid escape were filmed at 200 frames/s. Frame-by-frame analysis of escape sequences revealed that withdrawal was often initiated just prior to direct physical contact with the probe (Fig. 6); apparently, the near-field water displacement created by the moving probe was the adequate stimulus. Measurements from individual frames indicated that initially tails were extended approximately 5-10 mm above the sediment and that disappearance of the tail into the sediment occurred within 15-20 ms (3 to 4 frames). Thus, an estimated rate of shortening during these escape episodes was 0.40 mm/ms.

Non-invasive electrical recordings were obtained from a worm's tail as it extended into the water column of a small aquarium; simultaneously, tail movements were detected with a
The tail of a ventilating worm was stimulated by the rapid approach of a hand-held, glass probe. Tail withdrawal was filmed at 200 frames/s. The tail, originally extended from the burrow approximately 5 mm, was completely withdrawn in three frames (≈ 15 ms).
photocell to correlate behavior with electrical events during escape. The waveform of these in situ electrical recordings, although somewhat attenuated in amplitude, were essentially identical to those obtained in grid recordings (Figs. 3A and 7A). The recordings showed that a single LGF spike, and the associated muscle potential, were sufficient to evoke a complete tail withdrawal (Fig. 7A). The mean afferent latency (i.e., time from the onset of an electromechanically-driven stimulus to the LGF spike) was 2.7 ms (± 0.5 S.D.; n = 22, from eight animals). The mean efferent latency (i.e., time from the peak of the LGF spike to the onset of muscle potentials) was 0.9 ms (± 0.2 S.D.; n = 22, from eight animals). These in situ latencies were in close agreement with latencies calculated from semi-intact preparations.

Since photocell records provided a sensitive means for detecting the onset of movement, estimates of excitation-contraction coupling time (i.e., time lag between onsets of muscle electrical and mechanical responses) were also obtained. The mean coupling time was 5.6 ms (± 2.1 S.D.; n = 22; from eight animals). Therefore, the total response latency (i.e., time from onset of tactile stimulus to the onset of escape withdrawal) was approximately 9 ms.

In situ photocell recordings also provided an accurate measure of the speed of segmental shortening. The mean rate of shortening (= 0.54 mm/ms; ± 0.06 S.D.; n = 12, from four
Figure 7. In situ recordings of rapid escape behavior

An extracellular recording (RE) from the tail of an intact worm (A, top trace) was correlated with a photocell recording (PC) of tail movements (A, bottom trace). The onset of the oscilloscope trace coincides with the onset of the electromechanically-driven tactile stimulus. The LGF spike and the onset of tail withdrawal occurred approximately 2 ms and 7 ms, respectively, after the onset of the stimulus. In B, three superimposed photocell recordings are shown. The rate of tail shortening, determined from the slope of the recordings, was not effected by the number or frequency of LGF spikes eliciting the response. The bottom trace is a response evoked by a single LGF spike occurring at time 0. The middle trace is another response involving an additional three spikes occurring at the times indicated by the asterisks. In addition, the rate of shortening was independent of the distance originally extended from the burrow as indicated by the slope of the top trace response. Voltage scale (A): 50 μV. Time scale (A): 5 ms.
animals), calculated from the slope of photocell records, was slightly faster than the estimate obtained by high-speed cinematography. However, it should be noted that the temporal resolution from cinematography was substantially less than the photocell records.

Figure 7B shows that the rate of shortening, as well as excitation-contraction coupling time, was independent of the number and frequency of LGF spikes evoked during escape. Thus, the slope of photocell traces (i.e., rate of shortening) was identical following a single LGF spike or a train of closely-spaced spikes. Likewise, the rate of shortening was independent of the distance that the tail was extended above the sediments (Fig. 7B).

Discussion

The stimulus modalities utilized by invertebrates to initiate rapid escape behavior can be as diverse as shadows (Nicol, 1950), wind (Camhi and Nolen, 1981), or vibration (Nicol, 1950). However, one feature shared by all stimuli that elicit giant fiber-mediated responses is a sudden onset. Among annelid worms, many abrupt stimuli (i.e., touch, substrate vibration, and light or shadow) trigger escape responses that invariably involve a rapid longitudinal withdrawal (Bullock, 1945; Nicol, 1948, 1950; Dorsett, 1964; Drewes et al., 1978). In aquatic oligochaetes such as *Branachiura sowerbyi*, direct touch of the body wall is
certainly adequate for initiating withdrawal (Fig. 1), but as shown in Figure 6, some responses may begin while an inanimate stimulus probe or living predator is approaching, but not yet in contact with the animal's tail. These escape responses are most likely triggered by associated water displacements. A high sensitivity to water movement cues may ensure earlier activation of the giant fibers, thereby increasing the probability of effective escape.

One annelid in which a comparable sensitivity to water movement has been demonstrated is the leech. Stimulation of the body wall with water waves can activate the S-cell interneuronal network which mediates a relatively rapid shortening response (Mistick, 1978; Friesen, 1981). Water motion is detected by a set of sensillar movement receptors localized near middle annuli of mid-body segments of Hirudo (Friesen, 1981). The S-cell system of the leech, like many giant-fiber systems in polychaetes, can also be activated by photic stimulation (Kretz et al., 1976). However, the lateral giant fiber (LGF) system of Branchiura is not activated by photic stimulation.

Videotape analysis and high-speed cinematography of the tubificid escape response, in combination with non-invasive electrophysiological recordings, revealed a remarkable speed with respect to reflex timing. In fact, when compared to other invertebrate escape behaviors (see Table 1), the total
Table 1. Comparison of escape reflex latencies in selected invertebrate species

<table>
<thead>
<tr>
<th>Species</th>
<th>Sensory latency&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Motor latency&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oligochaetes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lumbricus</td>
<td>5 ms</td>
<td>15-30 ms</td>
<td>Pallas and Drewes, 1981</td>
</tr>
<tr>
<td>Branchiura</td>
<td>2-3 ms</td>
<td>6-7 ms</td>
<td>present study</td>
</tr>
<tr>
<td>Polychaetes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myxicola</td>
<td>-</td>
<td>8-15 ms</td>
<td>Roberts, 1962</td>
</tr>
<tr>
<td>Branchiomma</td>
<td>7-8 ms</td>
<td>10-42 ms</td>
<td>Krasne, 1965</td>
</tr>
<tr>
<td>Crustaceans</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crayfish</td>
<td>3-7 ms</td>
<td>≥ 13 ms</td>
<td>Krasne and Wine, 1984</td>
</tr>
<tr>
<td>Insects</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drosophila</td>
<td>24 ms</td>
<td>-</td>
<td>Wyman et al., 1984</td>
</tr>
<tr>
<td>Cockroach</td>
<td>14-20 ms</td>
<td>20-30 ms</td>
<td>Westin et al., 1977; Plummer and Camhi, 1981</td>
</tr>
</tbody>
</table>

<sup>a</sup>Time from stimulus onset to the onset of initial giant fiber spike.

<sup>b</sup>Time from initial giant fiber spike to onset of movement.
latency of the response (≈ 9 ms) is among the fastest ever reported. Some of this speed undoubtedly is due to the negligible distance and times involved in peripheral conduction along afferent and efferent pathways; the length of these paths probably is no more than 0.5 mm. Notwithstanding the time saving from this consideration, many other factors contribute to minimizing the response time of this escape. These include: (1) an electrical component within the afferent to giant pathway (Fig. 2C), (2) the rapid intersegmental conduction of the LGF which confers a near synchronous muscle excitation in all tail segments (Fig. 3B), (3) an extremely short efferent latency from giant fiber spike to muscle potentials (Fig. 4A), (4) a very short coupling time from muscle potentials to the onset of movement (Fig. 7A), (5) a fast rate of segmental shortening (Fig. 7B), and (6) the requirement of a single firing of the LGF, rather than multiple spiking, for initiating a complete (all-or-none) escape.

The all-or-none escape in tubificid worms contrasts with the graded nature of escape in earthworms (Chapter I). In Branchiura sowerbyi, an LGF spike elicits a multisegmental excitation of longitudinal muscle via junctions capable of relatively high transmitter output, as indicated by the large amplitude of muscle EJPs (Fig. 4). The effect of any subsequent LGF spikes are inconsequential with respect to the
speed or efficacy of the escape. This contrasts significantly with the requirement of multiple giant fiber spiking in the graded escape reflexes of earthworms (Drewes, 1984). The graded head withdrawal reflex of Lumbricus is evoked by a closely spaced pair or train of medial giant fiber spikes that, in turn, elicit facilitated muscle responses (Gunther, 1972; Drewes et al., 1980).

A likely focal point for future investigations is the possibility that, as in some insects, the efficacy of escape responses may be modulated by behavioral or environmental factors. For example, Camhi and Nolen (1981) demonstrated that the response latency of the cockroach escape is less in a walking, rather than a standing, behavioral state. Recent studies have also shown that the efficacy of the cockroach escape can be markedly influenced, in certain species, by ambient temperature (Simpson et al., 1986). In a freshwater worm such as Branchiura, some environmental factors that may be especially relevant to the efficacy of its escape are dissolved oxygen concentration, temperature, and pollutants.

Acknowledgments

I thank Dr. G. Atchison for supplying the bluegills used in this study. I also thank Dr. J. Redmond, Mr. T. Baldus, and Mr. E. Rearick for technical assistance during videotaping and cinematography.
References


GENERAL SUMMARY

The rapid withdrawal reflex of the tubificid worm, *Branchiura sowerbyi*, has a response latency ($\approx 9$ ms) that is one of the fastest in the animal kingdom. In addition, the reflex was found to be an effective escape from the strike of bluegill. Several features of this behavior ensure the efficacy of escape. These include: (1) a sensitive mechanosensory system for detecting the approach of potential predators, (2) short latency afferent, interneuronal, and efferent pathways, and (3) an all-or-none withdrawal associated with a single spike within its lateral giant fiber (LGF) system.

The LGF system in *Branchiura* consists of a pair of bilaterally asymmetric axons, highly adapted for rapid impulse conduction. Intersegmental syncytia and a myelin-sheath are among the most conspicuous LGF specializations for rapid conduction. The pronounced asymmetry between the left and right LGFs is unique among invertebrate systems. Evidence suggests that the small, right fiber functions as a high input resistance locus for spike initiation; while, the large, left fiber predominates impulse propagation along the animal. Impulse traffic between the fibers is likely accomplished by segmentally-arranged crossbridges.

Neuroanatomical studies, revealing a pair of LGF cell bodies within each segment, indicate that the syncytial LGFs
of Branchiura are derived from segmental origins. This result, together with comparative neurobiological studies of several aquatic species, indicate several evolutionary trends. These studies suggest that an arrangement of three dorsal giant fibers, functioning as two distinct and dichotomous conduction pathways, has been evolutionarily conserved within the three major orders of aquatic and terrestrial oligochaetes. The medial giant fiber (MGF), activated by afferents of anterior segments, initiates anterior shortening; whereas, the two lateral giant fibers (LGFs), activated in synchrony by afferents of posterior segments, initiate a different response (usually tail withdrawal). Notwithstanding these common features, the design and function of LGF systems differ considerably in aquatic and terrestrial groups. In posterior segments of aquatic species, LGFs are disproportionately larger and conduct faster than MGFs. This contrasts with posterior segments of earthworms in which LGFs are smaller and conduct slower than MGFs.

In addition, in aquatic tubificids, a single LGF spike is sufficient to evoke rapid and complete tail withdrawal, whereas a pair of closely-spaced LGF spikes are needed to elicit posterior shortening in earthworms. The graded nature of earthworm escape seems appropriate for worms that burrow in relatively hard substrates and may frequently encounter inanimate stimuli that evoke meaningless giant fiber spiking.
On the other hand, the all-or-none nature of the tubificid escape appears advantageous for relatively sedentary worms that are vulnerable to intense predation but reside in aqueous sediments where triggering of giant fiber spikes by non-threatening stimuli is infrequent.

These studies suggest that anatomical and physiological modifications of giant fiber pathways in aquatic and terrestrial worms have occurred during the evolution of oligochaete nervous systems. I hypothesize that differential predation pressures, together with fundamental differences in lifestyle and habitat, have led to this divergence in the structure and function of evolutionarily conserved sets of homologous giant interneurons.


Figure 1. Evolutionary relationships of the Annelida (modified from Brinkhurst and Jamieson, 1971)
## APPENDIX B

Table 1. Literature survey of anatomical studies of oligochaete species in which giant fiber numbers were indicated

<table>
<thead>
<tr>
<th>Taxonomy</th>
<th>Species</th>
<th>Giant fiber number</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Branchiobdellida</td>
<td>Branchiobdella pentodonta</td>
<td>0</td>
<td>Vagnetti and Farnesi, 1978</td>
</tr>
<tr>
<td>Aeolosomatida</td>
<td>Aeolosoma tenebrarum</td>
<td>0 (?)</td>
<td>Brace, 1901</td>
</tr>
<tr>
<td>Haplotaxida</td>
<td>Phreoryctes emissarius</td>
<td>0 (?)</td>
<td>Forbes, 1890</td>
</tr>
<tr>
<td>Moniligastrida</td>
<td>Moniligaster grandis</td>
<td>1 (?)</td>
<td>Bourne, 1894</td>
</tr>
<tr>
<td>Moniligastrida</td>
<td>Moniligaster indicus</td>
<td>3</td>
<td>Benham, 1893</td>
</tr>
<tr>
<td>Lumbriculida</td>
<td>Lumbriculus variegatus</td>
<td>3</td>
<td>Isossimow, 1926; Zoran and Drewes, 1987</td>
</tr>
<tr>
<td>Lumbriculida</td>
<td>Kincaidiana hexatheca</td>
<td>3</td>
<td>Zoran and Drewes, 1987</td>
</tr>
<tr>
<td>Lumbricida</td>
<td>Lumbricus terrestris</td>
<td>3</td>
<td>Bullock, 1945</td>
</tr>
<tr>
<td>Lumbricida</td>
<td>Eisenia foetida</td>
<td>3</td>
<td>Stough, 1926</td>
</tr>
</tbody>
</table>

*Order and family.*
Table 1. Continued

<table>
<thead>
<tr>
<th>Taxonomy^a</th>
<th>Species</th>
<th>Giant fiber number</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glossoscolecidae</td>
<td><em>Criodrilus lacuum</em></td>
<td>3</td>
<td>Honig, 1910</td>
</tr>
<tr>
<td>Glossoscolecidae</td>
<td><em>Alma nilotica</em></td>
<td>3</td>
<td>Keyl, 1913</td>
</tr>
<tr>
<td>Megascolecidae</td>
<td><em>Pheretima communissima</em></td>
<td>3</td>
<td>Ogawa, 1928</td>
</tr>
<tr>
<td>Megascolecidae</td>
<td><em>Megascolex musicus</em></td>
<td>3</td>
<td>Adey, 1951</td>
</tr>
<tr>
<td>Megascolecidae</td>
<td><em>Perichaeta coerulea</em></td>
<td>3</td>
<td>Keyl, 1913</td>
</tr>
<tr>
<td>Alluroididae</td>
<td><em>Standeria transvaalensis</em></td>
<td>3</td>
<td>Jamieson, 1968</td>
</tr>
<tr>
<td>Tubificida</td>
<td><em>Tubifex tubifex</em></td>
<td>3</td>
<td>Zoran and Drewes, 1987</td>
</tr>
<tr>
<td>Tubificida</td>
<td><em>Branchiura sowerbyi</em></td>
<td>3</td>
<td>Zoran and Drewes, 1987</td>
</tr>
<tr>
<td>Tubificida</td>
<td><em>Limnodrilus hoffmeisteri</em></td>
<td>3</td>
<td>Zoran and Drewes, 1987</td>
</tr>
<tr>
<td>Naididae</td>
<td><em>Dero vaga</em></td>
<td>3</td>
<td>Brode, 1898</td>
</tr>
<tr>
<td>Naididae</td>
<td><em>Dero sp.</em></td>
<td>3</td>
<td>Zoran and Drewes, 1987</td>
</tr>
<tr>
<td>Enchytraeidae</td>
<td><em>Pachydrilus litoreus</em></td>
<td>3</td>
<td>Hesse, 1894</td>
</tr>
</tbody>
</table>
Oligochaetes are maintained in glass aquaria (20 gallons) containing a mud medium and freshwater. Mud is obtained from local lakes with high organic sediments, screened through a wire mesh, and autoclaved for 1 h and/or frozen at -15°C for several weeks. All cultures contain 3 to 6 inches of mud and a circulating water system. The cultures are continuously aerated and covered to prevent evaporation. Air is bubbled into the large water reservoir of the circulation system to avoid turbulence of the soft sediments. The cultures are fed twice weekly a mixture of Tetramin staple flake food and trout chow. It is beneficial to harvest a small group of animals periodically and maintain them in smaller containers (at a much lower density) to facilitate growth and reproduction. Five cubic cm of sediment are scooped from the culture and screen through wire mesh to harvest worms. A fire-polished, glass hook is used to transfer worms from the screen to a dish containing freshwater. Cocoons can be harvested in a similar fashion using a fire-polished pipette to remove the cocoons by suction. Periodically water and mud medium should be changed. However, cultures of tubificid worms can survive remarkably long periods of neglect. Optimal temperature for growth is 23°-25°C.
GENERAL ACKNOWLEDGMENTS

I sincerely thank my major professor, Dr. Charles D. Drewes, for his guidance, friendship, and generous provision of his time and energy to my success. I also thank Charlie and his family for the many joys they have given me during my years in Ames.

I wish to thank the members of my committee, Dr. Kenneth C. Shaw, Dr. Gary J. Atchison, and Dr. Ronald H. Peters, for their many helpful suggestions. I especially thank Dr. Dennis G. Emery for his technical assistance and frequent use of his laboratory facilities.

I also thank Dr. Philip G. Haydon and Dr. Sheldon S. Shen for their friendship and valuable advice. I shall fondly remember the many times Sheldon and I walked up the 18th fareway at Augusta National together.

I am also appreciative of the many friendships I have developed with many of my fellow graduate students at Iowa State. I especially want to thank Russ Dunn, Tom Baldus, and Tom McMullin (along with H.B.) for their companionship, encouragement, and the pleasure of their friendship. I also thank Tom Heppner, Bruce O'Gara, Beth Vining, and Maggie Welch for putting up with my music in the lab.

Finally, I am and always will be most indebted to my parents, Mr. and Mrs. Joseph Zoran, and my four sisters for their continued love and support.