Chapter 2

Non-invasive Recording of Giant Nerve Fiber Action Potentials from Freely Moving Oligochaetes

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Charlie received his B.A. from Augustana College (South Dakota) and Ph.D. from Michigan State University. He is currently a Professor of Zoology and Genetics at Iowa State University. His research training and interests are in the areas of invertebrate neurophysiology and comparative physiology. He and his research students have extensively investigated the neurobiology of escape reflex behaviors in freshwater and terrestrial oligochaetes. On campus, Charlie teaches courses in neurophysiology, invertebrate biology, and bioethics. During summers, he instructs at Iowa Lakeside Laboratory where he leads workshops in freshwater biology for middle school, high school, and community college biology teachers. Throughout the year he is heavily involved in developing and sharing hands-on activities for biology education at all levels.

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Introduction

Action potentials are bioelectric events of fundamental importance to nervous system function. Initiation of a neuron action potential may occur when threshold-level depolarization is reached due to synaptic excitation arising from other neurons -- a process called *neural integration.* Since action potentials are *all-or-none* events, they represent a type of *digital electrical signaling*. Conduction of action potentials along nerve fibers is the sole means for rapid, long-distance electrical signaling in nervous systems. Many molecular and biophysical mechanisms underlying action potential initiation and conduction are commonly shared by neurons in all animals, including invertebrates.

Teachers encounter a variety of challenges in trying to provide introductory-level students with “hands-on, wet-lab” experiences in neurophysiology. Nerve fibers are small and rather inaccessible structures. Thus, experimental access to them often requires most or all of the following conditions: animal restraint, anesthesia, euthanasia, surgery, microscopic viewing, specialized recording electrodes, micromanipulators, expensive pre-amplifiers, significant preparation time before class, as well as considerable student time, practice, and good fortune during class. Additional complications for many teachers are increased ethical and budgetary concerns about utilizing vertebrates in laboratory education.

Here, I present an exercise that circumvents many of the aforementioned challenges and problems. In my view, it represents the simplest, fastest, most direct, and most reliable means of analyzing initiation and conduction of unitary, all-or-none nerve action potentials in any animal. The exercise is an outgrowth of the technical approaches I and my research students have developed over the past twenty years to study escape reflex function in oligochaetes. In this exercise, printed circuit board grids are used to obtain multi-channel, non-invasive recordings of spikes from the medial and lateral giant interneurons of a freshwater oligochaete, *Lumbriculus variegatus*, in response to tactile stimulation. The same technology may be used for many other freshwater and terrestrial oligochaete species. Within minutes, all-or-none giant fiber spikes, with exceptional signal-to-noise ratio, are reliably detected along the external body surface of intact worms without need for anesthesia, dissection, restraint, micromanipulation, or microscopy.

This investigation involves: (a) analysis of the waveform, directionality, and frequency of stimulus-evoked action potentials in the medial and lateral giant nerve fibers; (b) quantification of normal and "supernormal" conduction velocity in these fibers; and (c) mapping of the touch sensory fields for medial and lateral giant fiber systems.

Materials

California blackworms = *Lumbriculus variegatus* (Order Lumbriculida): recommended commercial source is KORDON-Novalek, Inc., 2242 Davis Ct., Hayward, CA 94545-1114;

phone: (510) 782-4058; FAX: (510) 784-0945 (reliable shipping, small quantities)

Spring water: 50-100 ml/group; in beaker or finger bowl (alternative is dechlorinated tap water)

Distilled water: 50-100 ml/group; in beaker or finger bowl

Printed circuit board recording grid: for source, see "Notes for the Instructor"

Two preamplifiers: capacity-coupled, differential inputs; x100 and x1000 gain

DC power supply: +/- 9V DC battery pack (or +/- 15V DC power supply) for pre-amplifiers

Foam block: about 20 cm x 20 cm x 5 cm; used as cushion for recording grid

Wooden block: about 5 cm x 13 cm x 13 cm; used as base for recording grid

Audio monitor: Grass AM7; or inexpensive alternative (see "Notes for the Instructor")

Oscilloscope: Tektronix TDS 210 digital storage oscilloscope (or equivalent)

Faraday cage: collapsible cardboard box with aluminum foil-lining, ground terminal, Velcro™ straps

Plexiglas strip: smooth sides, rounded ends (LxWxH = 4-5 cm x 0.5-1.0 cm x 0.5 cm)

Disposable plastic pipets: large bore, smooth wall; one per group

Plastic disposable Petri dish: 10 cm size, one per group

Tissue or Kim-wipes

Applicator sticks: two per group

Thin rubber band: size # 19, or thinner

Scissors

Paper tape

Calculator

Data sheet: see APPENDIX A

Cables/connectors: (for source, see "Notes for the Instructor")

Grid cables (2): socket end connects to pins on circuit board; banana ends go to amplifiers

BNC-to-BNC cable (2): with built-in notch filter positioned closest to oscilloscope

BNC T-connector (1): for splitting amplifier output to oscilloscope and audio monitor

BNC-to-audio cable (1)

Banana-to-banana cable (1): for grounding Faraday box to oscilloscope

Alligator clips (1-2): for connecting Faraday box to ground terminal

Y-connector (3): for splitting power supply outputs to power two amplifiers

[*Optional:* Depending on oscilloscope/display system and its interfacing, hard copies of recordings may be made with a Polaroid or digital camera, video camcorder, plotter, or printer.]

Notes for the Instructor

Contact me (C. Drewes) if you would like to receive detailed information about commercial sources, general biology, or laboratory culture of *Lumbriculus.* Also, I will gladly provide a complete listing of parts, detailed plans, and catalog numbers for all components of preamplifiers, filters, Faraday boxes, amplifier power supplies, special cables, connectors, and an inexpensive audio monitor. Also, contact me regarding purchase of any custom-made apparatus, such as preamplifiers and circuit boards. Inter-institutional sale of these items by Iowa State University is possible. This is done on a cost-recovery, non-profit basis. I am also happy to consult with you regarding implementation of this technology at your institution.

An alternative to battery-powered amplifiers is a +/- 15 V DC regulated power supply that runs from line voltage. In this case, the power supply must be kept outside the Faraday box to prevent 60 Hz electrical interference. If a power supply is rated at several hundred milli-amps, or more, it may be used to simultaneously power numerous amplifiers for a whole class.

Instructions here are for the Tektronix TDS 210 digital storage oscilloscope. Its cost was $995 -- by far the best quality, lightest weight, most compact, and versatile instrument for the price. An optional extension module has RS232, GPIB, Centronics communication ports, and software for computer and/or printer interface. Other companies, such as Tenma, B&K and Hitachi, make bulkier digital storage units, with prices ranging from $1,000-2,000. Non-storage oscilloscopes are not well suited for this exercise because they provide no means for capture and fixed display of transient, touch-evoked spikes. If a computer-based recording system (oscilloscope emulation) is used, it is imperative that the system have a sufficiently high digital sampling rate for detecting and faithfully displaying nerve action potentials with durations of about 1 msec. At a sweep speed of 2 msec/division, a minimum of 12 digital sampling points/msec/channel (i.e., one sample point per 0.08 msec) is needed for spike analysis, along with a total capture time of at least 20 msec/channel. Since giant fiber spiking on one of the recording channels will be used as the triggering event, it is imperative that the recording system have *internal triggering* capabilities, along with the ability to capture and display a few milliseconds of pre-trigger events. Finally, capabilities for rapid and fine adjustment of triggering sensitivity are critical.

This exercise involves comparisons in the waveform and timing of conducted nerve spikes recorded from two different sites along the worm’s body in response to touch at different loci. Therefore, students’ comprehension of spatiotemporal aspects of bioelectrical events will be challenged. Depending on the locus of the touch stimulus relative to the recording electrodes, students will observe that spike conduction in a given fiber may be unidirectional or bi-directional.

Students will observe that action potential waveform and velocity are highly stereotyped, provided that (1) replicate measurements are made from the same body region, (2) worms are oriented linearly on the recording grid, (3) temperature is constant, and (4) physiological integrity of the nervous system has not been altered by injury or toxicity effects.

Students should make special efforts to handle worms gently and keep them moist. Only the rubber widgets or hair widgets should be used to touch worms on the grid. Transfers of worms to and from the grid should always be made carefully, using a pipet and plenty of water. Rough handling will often cause worms to fragment -- a protective response called *segmental autotomy*. On the other hand, worms that are handled gently and kept moist will generate reliable giant fiber responses to touch for hours or days while still on the grid.

Student Outline

Introduction

The central nervous system of segmented oligochaete worms is the *ventral nerve cord*. It contains about 500-1000 neurons per segment, or about 100,000 neurons altogether. Three nerve fibers in the ventral nerve cord are exceptionally large compared to other fibers (Fig. 1). These so-called *giant nerve fibers* rapidly conduct action potentials from one end of the nerve cord to the other. The giant fibers function as key "decision-making" neurons that receive input from sensory neurons in the skin. When a sudden, threatening stimulus occurs, a series of action potentials may be initiated in the worm's giant nerve fibers. These spikes, in turn, trigger rapid shortening of the body and escape from predators. Such responses are highly stereotyped *reflexive* responses that fit into a general category of behaviors known as *startle responses* (Bullock, 1984).

A unique advantage for classroom study or research is that *all-or-none action potentials* (also called *spikes*) in a worm's giant nerve fibers may be detected non-invasively (Drewes, 1984). That is, electrical recordings of giant fiber spikes may be made without dissecting, anesthetizing or restraining worms. Two factors make this possible. First, giant fiber spikes produce large electrical currents due to their large diameter. Second, these currents easily pass through the worm's body wall and skin because these tissues have a low resistance to electrical current flow.

In this exercise non-invasive recordings are used to study action potential conduction and escape reflexes in a freshwater oligochaete, *Lumbriculus variegatus* (common name: California blackworm). Objectives are to (a) examine the waveform, directionality, and conduction velocity of giant fiber spikes, and (b) map the touch sensory fields for giant nerve fiber spikes.

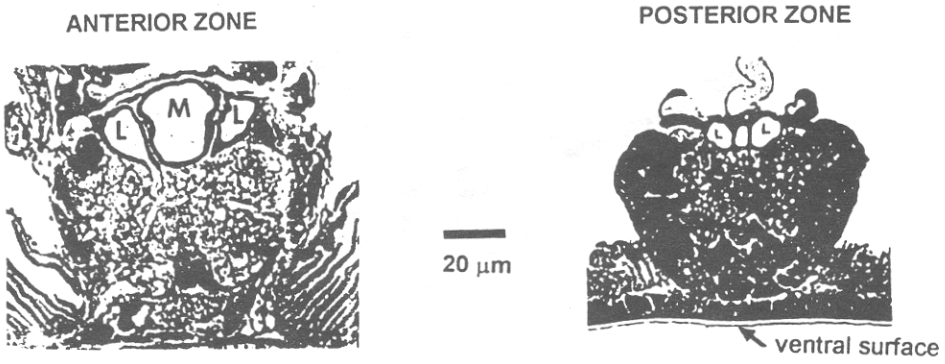

Figure 2.1. Cross-sectional views of the ventral nerve cord in anterior and posterior zones of the body in *Lumbriculus*. Note medial (M) and lateral giant nerve fibers (L).

Background Anatomy and Physiology

Two separate escape reflex pathways are used by oligochaetes to escape from predators rapidly (Figs. 2.1, 2.2). Each pathway consists of an interconnected set of sensory neurons, giant interneurons, and motor neurons. Each pathway triggers a different escape reflex response. One response utilizes the middle, or *medial giant interneuron*. The axon of this interneuron is greatly enlarged and termed the medial giant nerve fiber (or MGF). Spikes in the MGF trigger rapid *head withdrawal*. The other response uses the two *lateral giant interneurons*, whose enlarged axons are termed the lateral giant nerve fibers (or LGFs). Spikes in the LGFs trigger rapid *tail withdrawal*. Because the two LGFs are cross-connected, they conduct spikes in unison and function as a single fiber. Figure 2.2 shows the three giant fibers and their segmentally arranged cell bodies. Note that the enlarged axons in each segment are connected in tandem (end-to-end fashion) along the worm's ventral nerve cord. Each of these segmental connections is actually an electrical synapse (gap junction) that allows uninterrupted spike conduction from cell-to-cell, and segment-to-segment, along the worm.

What triggers spikes in giant nerve fibers? Giant fibers are excited by *sensory neurons* in the worm's skin. Sensory neurons detect threatening stimuli, such as sudden touch or vibration (Drewes, 1984; Drewes and Fourtner, 1989). These neurons conduct spikes, via segmental nerves, into the ventral nerve cord where they excite the giant nerve fibers. Normally, MGF spikes are excited by touch to anterior segments, which are within the so-called *MGF sensory field*. In contrast, LGFs are excited by touch to posterior segments, which are within the *LGF sensory field*. Once excited, the MGF (or LGFs) produces one or a series of several spikes. These may be initiated anywhere in the giant fiber's sensory field, depending on the exact location of the stimulus.

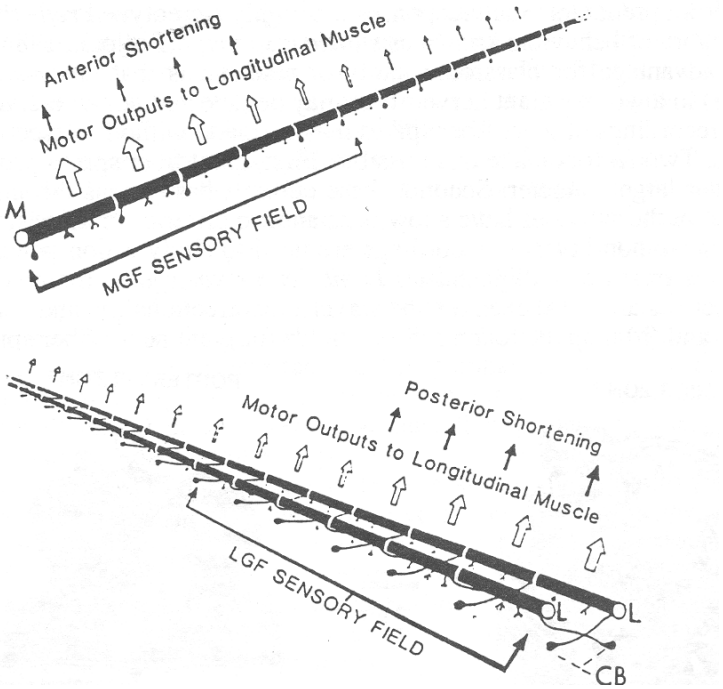

Figure 2.2. Side view of medial (M) and lateral (L) giant nerve fiber systems. This is a general plan for oligochaetes. Note the segmental cell bodies (CB) of giant fibers. Also, note the anterior sensory inputs and motor outputs for MGF, and the posterior inputs and outputs for the LGFs.

How do MGF and LGF spikes cause withdrawal of the worm? In each segment, giant fibers activate *excitatory motorneurons* whose axons extend into segmental nerves and synapse with longitudinal muscle fibers in the body wall. Contraction of these fibers shortens the body.

Recording Principles

As an action potential conducts along an axon, the inside of the axon becomes more positive (depolarizes) for about 1 millisecond, due to entry of a tiny amount of sodium through voltage-gated sodium channels that briefly open. At this instant, the *inside* the axon becomes about 100 millivolts (= 0.1 V) more positive than it was at rest. Just outside the axon membrane, at the same instant, an opposite electrical effect occurs -- the extracellular fluid briefly becomes more negative. Because the worm's body wall is a good conductor of electricity, some of this external negativity is instantaneously detectable on surface of the worm's skin. Even though this surface signal is very weak -- about 0.1 millivolt, or less -- it is readily detected with suitable electrodes and amplification. Thus, conduction of a giant fiber spike along a worm appears as a *moving wave of external negativity* that may be detected with metallic electrodes placed in contact with the underside of the worm (Fig. 2.3). Since the two electrodes in one recording pair detect the action potential at slightly different times, the recorded spike is *biphasic* in shape; that is, it has both negative and positive phases.

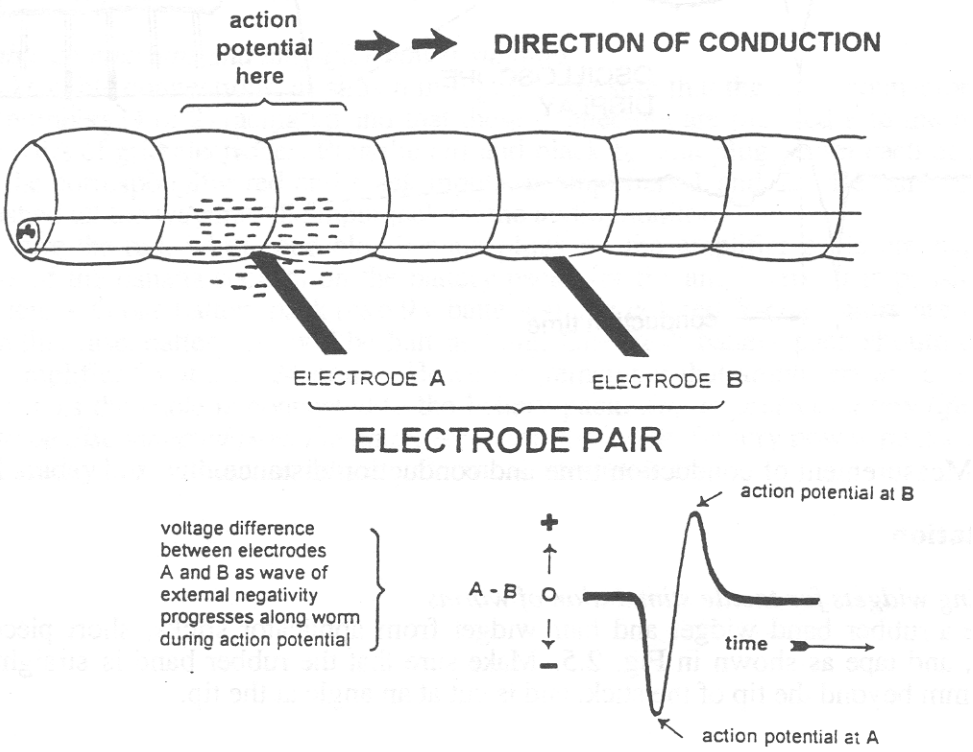

Figure 2.3. Non-invasive recording of a conducted action potential and polarity effects.

Two pairs of electrodes are used to record spikes. The electrodes are metallic lines etched onto a printed circuit board. The worm is positioned cross-wise on this electrode array and head or tail segments are lightly touched, thus triggering MGF or LGF spikes. As a spike is conducted, its electrical effect is detected first by the nearest pair of electrodes. Signals from this pair are amplified and displayed as one channel on the oscilloscope screen. The second pair of electrodes detects the spike a little later in time; its signal is amplified and displayed as a second channel on the screen (Fig. 2.4). The *conduction time* is then measured as the timing difference (from spike peak to spike peak) in the two traces on the screen. By dividing the *distance from the center of the first electrode pair to the center of the second electrode pair (= conduction distance*) by the conduction time, we obtain spike *conduction velocity* (see Equation 1, below).

Equation 1: $$\textit{conduction velocity (in meters/sec)} = \frac{\textit{conduction distance (in millimeters)}}{\textit{conduction time (in milliseconds)}}$$

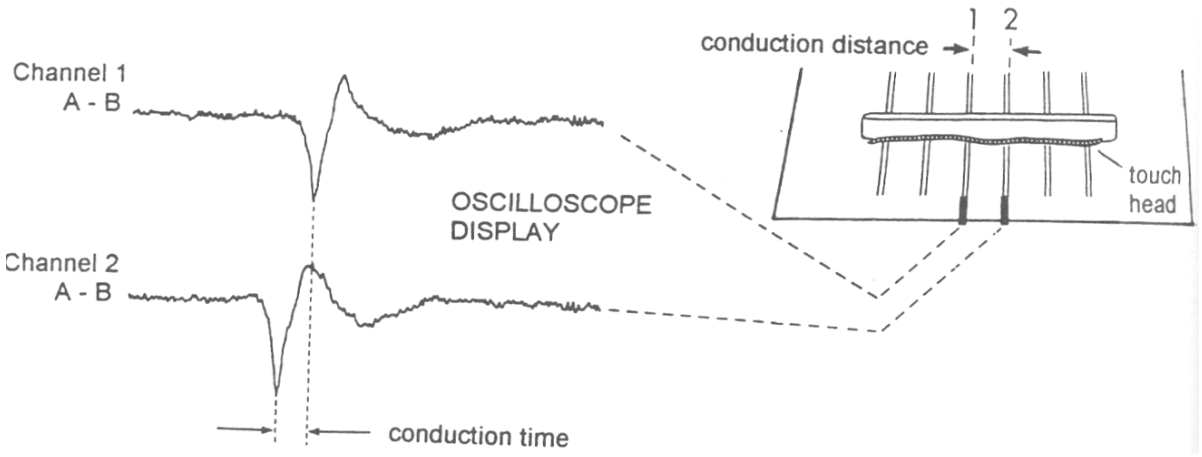

Figure 2.4. Measurement of conduction time and conduction distance.

Instrumentation

Making widgets for tactile stimulation of worms

Make a rubber band widget and hair widget from applicator sticks, short pieces of thin rubber band, and tape as shown in Fig. 2.5. Make sure that the rubber band is straight, extends about 12-15 mm beyond the tip of the stick, and is cut at an angle at the tip.

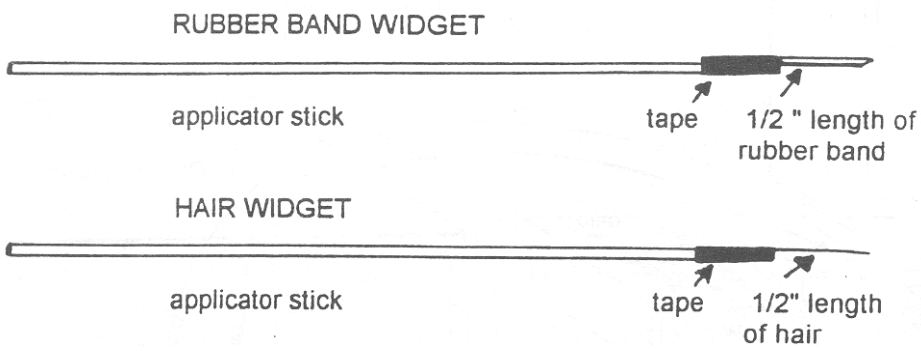

Figure 2.5. The worm widgets.

Safety

All water containers should be small and kept well away from AC-powered equipment and outlets to prevent possibility of electrical shock.

Cable connections and amplifier power supplies

Make cable connections, as shown in Fig. 2.6. Be sure that the edge connectors to the grid have their numbers (1 or 2) facing up and that these connectors are plugged into the pins from the center two pairs of grid electrodes. Plug the red and black banana plugs from each edge connector cable into the corresponding red and black inputs of amplifiers 1 and 2. Be sure that the audio cable is pushed all the way into the input jack on the audio monitor.

Be sure the red, white, and blue banana plugs on the amplifier cable are matched to the same colors of the banana sockets on the battery-packs for the amplifiers. It is possible to power two amplifiers with one battery pack (two 9V batteries); the colored Y-connectors are used for this purpose. In this case, battery life will be half as long. One fresh battery pack should continuously power one amplifier for at least 24 hours. However, remember that amplifiers are drawing battery power as long as the cable is connected to the battery pack. *So, to protect battery life, the battery pack should be disconnected from the amplifier when not in use*. Battery power packs may be kept inside the Faraday box without introducing electrical interference.

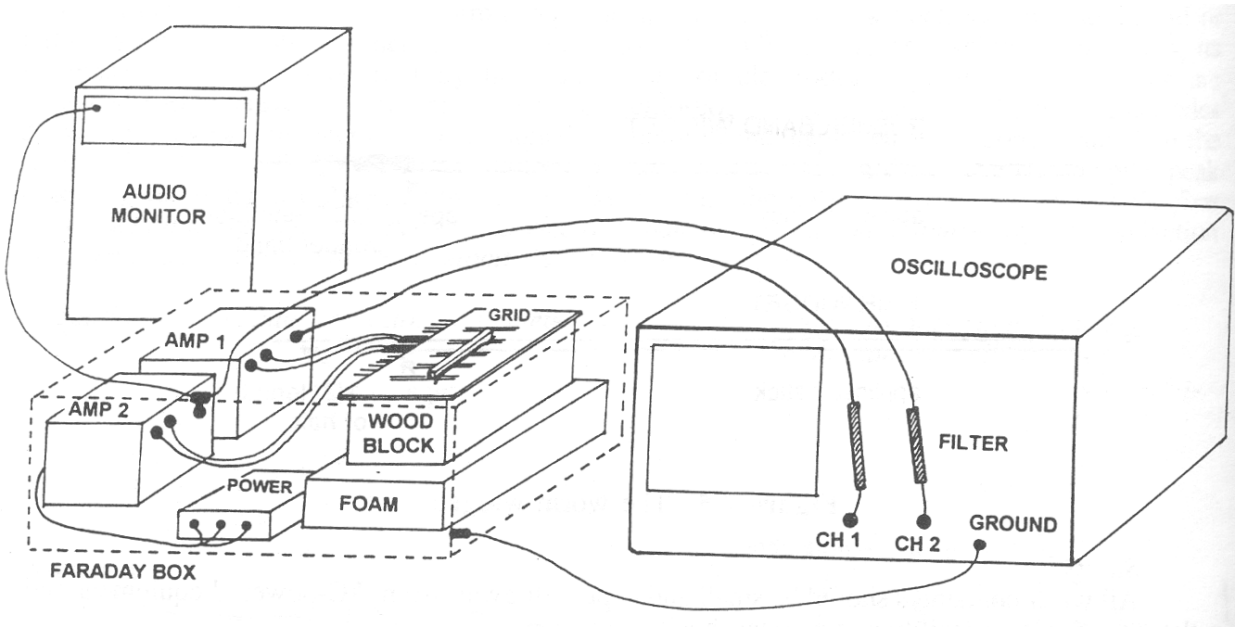

Figure 2.6. Recording set-up and cable connections.

Amplifier and Oscilloscope Settings

For Ch 1 and Ch 2 preamplifiers, start with coupling switch OFF and GAIN at 100X. On the audio monitor, start with settings as follows: input switch OFF, low filter at 300 Hz, noise clipper ON, high filter at 3 KHz, volume at 0 (minimum), and power ON.

Go to APPENDIX B and follow the "Initializing Procedures for Tektronix TDS 210" oscilloscope. Continue to refer to this appendix for diagrams of oscilloscope display area and oscilloscope front panel controls. Then do the following:

(a) Change sweep speed (HORIZONTAL - SEC/DIV) to 2.5 ms/div. The screen readout for sweep speed is labeled "J" in the Display Area diagram (APPENDIX B).
(b) Adjust sensitivity (VERTICAL - VOLTS/DIV) for CH1 and CH2 to 5 mV/div (see readout "K").
(c) Adjust TRIGGER LEVEL to about 5 mV (indicator arrow should be about one division above or below the CH1 baseline).
(d) At this point, two relatively flat traces should be seen on the oscilloscope screen. Use VERTICAL POSITION controls for CH1 and CH2 to adjust baselines to about 1-2 divisions above and below the horizontal centerline, respectively.

[For generic oscilloscope operation, recommended initial settings are: sweep speed = 2 msec/div; Channel 1 and 2 vertical sensitivity = 5 mV/div (assuming 100x preamplification); AC-coupled input for each channel; automatic sweep mode; two traces visible. Later, the sweep mode will be changed from automatic to internal triggering.]

Procedures

Step 1: Preparing the grid and handling worms

Place the Plexiglas strip in the middle of the grid, flat side down. Place one drop of distilled water on the grid next to the strip. Gently slide the strip back and forth over the drop so that electrodes under the strip are fully moistened.

Using a disposable pipet, transfer a large *Lumbriculus* specimen (> 5 cm long) into a small dish of distilled water. Note that the head end of the worm is more darkly pigmented, thicker, and more active than the tail end. Use the pipet to transfer the worm, along with a little water, onto the center of the recording grid right next to the Plexiglas strip. *Without touching the worm, use a pipet to carefully remove excess water from the grid*. The idea is to keep the worm confined beneath the surface tension of a thin bridge of water that forms around (and is limited to) the base of the Plexiglas strip where it contacts the grid. Add distilled water as needed. However, *excessive water on the grid will reduce signal strength*. Also, do not allow water to contact the edge connectors on the grid; this will lead to significant electrical interference.

You should use only the hair or rubber band widgets to touch the worm while it is on the grid. Do not touch the grid with fingers or other electrically conductive materials while the amplifiers are operating! Use a widget to coax the worm to straighten out and crawl around the base of the Plexiglas strip. When the worm is straightened and positioned cross-wise over the desired electrodes, recordings of giant fiber spikes can be made.

Step 2: Preparing to make recordings

Move the coupling switches on the two preamplifier boxes from OFF to AC (up position). Move the audio monitor INPUT switch from OFF to CH1. Then, slowly increase volume until a weak crackling sound is heard.

Now press the TRIGGER MENU button. Change the menu "Mode" window to "Single." Press the RUN/STOP button (upper right of front panel). The message in readout "B" in the display area should be "R Ready." If "R" immediately disappears and "Stop" appears, adjust the TRIGGER LEVEL so that a slightly larger trigger value is shown in "F" of the display area. Press the RUN/STOP button again. This button resets the trigger for capturing a single sweep.

The duties of each student group may be divided as follows:

Worm Widgeteer: This person uses the widgets to keep the worm aligned along the Plexiglas strip and informs the group when the worm is (or is not) properly aligned across both pairs of electrodes (see Fig. 4). When the worm is properly aligned, the Widgeteer holds the tip of the rubber or hair widget just above the worm, and asks -- "Ready?"

Oscilloscope operator(s): This may be one or two people -- a *Trigger Person* and a *Cursor Person*. The Trigger Person lets the Widgeteer know that the "R Ready" message is displayed just before the worm is touched. Since the oscilloscope sweep may trigger inadvertently, the Trigger Person may often need to reset the trigger (press RUN/STOP button). This person also confirms that the "Stop" message appears at the same instant when an audible spike is heard and the worm is touched. The Cursor Person then presses the CURSOR button and uses the two VERTICAL POSITION controls to measure the peak-to-peak timing difference, in milliseconds, between events in the two traces. This difference is read in the middle ("Delta") window of the screen menu.

Recording Data: Another person may be the Data Recorder. This person records data and makes sure that conduction times are properly ascribed to MGF or LGF data sets.

Step 3: Initial observations

When the Widgeteer and Trigger Person are ready, the Widgeteer should lightly touch the worm with the widget and carefully note whether the head end or tail end is touched. The group should hear one or a few crisp pops (giant fiber action potentials) on the audio monitor. The oscilloscope operators should confirm that the evoked action potentials are indeed captured by the oscilloscope and that the "Stop" message appeared when the pop(s) occurred. If pops are heard, but the "Ready" message is still seen, the TRIGGER LEVEL is too high and should be reduced.

[*NOTE: It works best if the Widgeteer touches the worm so lightly that just one spike (pop) occurs, rather than a burst of spikes. Bursts of spikes may cause the worm to rapidly shorten and crawl away, resulting in temporary loss of electrode contact with the worm. When just one spike is evoked by very light touch, no body shortening occurs.*]

The oscilloscope operators should confirm that the captured spike was conducted in the expected direction past both electrode pairs. The spike should have the same polarity sequence in both traces and should be detected first by the electrode pair closest to the point of touch. Under these conditions, responses to head touch should consist solely of MGF spikes and responses to tail touch should consist solely of LGF spikes. Carefully note, record, and explain differences in the amplitude and waveform of MGF and LGF spikes. Describe and explain changes in polarity and timing of MGF and LGF spikes that occur when a worm's head-tail orientation reverses on the grid; this will occur frequently as the worm crawls around the Plexiglas strip.

Step 4: Measuring conduction velocities of giant fiber spikes

With the two pairs of recording electrodes *straddling the center of the worm's body* (see Fig. 4), lightly touch the worm's head or tail end. Use CURSOR controls to measure the conduction time of the first spike in each response. This is done by measuring the *difference in time (select "Delta" window in Cursor menu) between the first sharp peak in one trace and the corresponding first sharp peak in the other trace*. Depending on whether the head or tail is touched, record the data as MGF or LGF conduction time (mid-body).

Obtain 8-10 measures of conduction time for both MGF and LGF responses. Record these on the data sheet (APPENDIX A). Then, using Equation 1, calculate a conduction velocity value for each conduction time. Express this in units of m/sec. [Note: mm/msec = m/sec.] Calculate the mean velocity, range, and standard deviation for each giant fiber system. Which giant fiber pathway (MGF or LGF) has the fastest velocity? Explain the likely basis of conduction velocity differences.

How much does MGF velocity vary? Explain possible sources of variation and error in measurements. What is the precision of your velocity measures? [Note: For digitized data, the precision of measurements may vary with the sweep speed.] Compare mean conduction velocities of different worms by using another worm or comparing results between groups. Explain why velocities may vary in different worms.

Make detailed drawings of how the worm was positioned on the grid, where the touches occurred, and how the resulting spikes appeared on the oscilloscope screen. On these drawings label: worm's head end, channel 1 and 2 electrodes, location of touch, channel 1 and 2 traces, conduction distance, and conduction time. If possible, obtain a permanent record of the oscilloscope trace (e.g., Polaroid oscilloscope, digital camera, video camcorder, or waveform transfer to printer).

Step 5: Bi-directionality of spike conduction

With the worm's body centered over the two pairs of recording electrodes (Fig. 4), very lightly touch the worm at a mid-body position *exactly between the two pairs of electrodes*. Examine the polarity and timing of the evoked spike(s) on Channels 1 and 2. Is an LGF or MGF spike evoked? How do you know? In what direction is the evoked spike conducted? What is the peak-to-peak difference in timing

between the spike in Channel 1 and 2? Where is the spike initiated? Explain your answers. Compare these records to those obtained in Step 5.

Step 6: Mapping giant fiber sensory fields

Refer to APPENDIX A and note the diagram showing the worm's body length sub-divided into ten equal zones (head end = 1, tail end =10). While the worm is in contact with both pairs of recording electrodes, touch the worm at a location corresponding to one of these zones and note whether an MGF or LGF spike is evoked. Use clues from spike waveform (and velocity, when possible) to determine which giant fiber system responds to touch within each of the ten zones. Below each zone on the sketch, mark M or L to indicate which giant fiber system actually responds to touch. The result is a *map of the sensory fields* for each giant fiber system. What are the boundaries of MGF and LGF sensory fields? Do the fields overlap? Explain, in neurophysiological terms, how differences in sensory field organization could arise along the body. Explain how overlapping of fields could occur. Discuss the possible adaptive significance in having two different escape reflex responses with opposing sensory field boundaries.

Step 7: Graded nature escape

During previous steps you should have noted that the number of giant fiber spikes varies in direct relation to the strength of the touch stimulus. Only one or two spikes are evoked in response to a very light touch, but many spikes are evoked in response to stronger touch. Use your understanding of neurophysiological events to explain how such differences in the number of evoked spikes could arise. What differences did you see in the strength of the worm's behavioral responses to touch and how did these correlate with the number of evoked spikes? Are giant fiber-mediated escape responses in this worm *graded* in strength, or all-or-none? In the worm's natural environment, what are potential advantages of utilizing graded, as opposed to all-or-none, escape responses?

Step 8: "Supernormal" conduction velocity

Set the oscilloscope HORIZONTAL SEC/DIV to 1 msec/div. Obtain a response in which two (or more) spikes are evoked. Note whether they are MGF or LGF spikes. Locate the *first spike* in the response. Measure its conduction time, calculate its velocity, and record values on the data sheet. Now, focus attention on just one of the traces and measure *the time interval from the first spike to the second spike in the same trace*. This is the interspike interval (ISI). Next, measure the conduction time and calculate velocity for the second spike. Compare these to the first spike. Is the second spike conducted at a faster velocity than the first? Such a phenomenon occurs in certain nerve fibers of certain animals, and is referred to as "facilitation of conduction velocity" (Bullock, 1951; Drewes, 1984; Turnbull and Drewes, 1996). The second spike conducts faster than the first because there is a brief period of "supernormal" membrane excitability that follows an initial spike. The underlying biophysical basis of this phenomenon is not known.

How much increase in velocity occurs? Express the velocity of the second spike as a percentage of the first. Is supernormal conduction seen in both MGF and LGF? If possible, examine numerous responses in which ISI varies between 10 and 20 msec. To do this, it will be useful to decrease the HORIZONTAL-SEC/DIV to 2 msec/div. Does increased velocity vary in relation to ISI? If so, describe and discuss.

Suggestions for Further Investigation

A. Regional differences in conduction velocity: Obtain a data set of MGF and LGF velocities when *both pairs* of electrodes are positioned in the anterior half of a worm (see data sheet in APPENDIX

A). Then, obtain another data set with electrodes positioned in the posterior half of the worm. Compare these data sets to each other and to values obtained with electrodes in a mid-body position (Step 4). Explain the results in terms of giant nerve fiber anatomy and principles of neurophysiology. Estimate how long it should take for a giant fiber spike to conduct the entire length of the worm. Try to confirm this estimate by measuring conduction time when inter-electrode spacing is at the maximum possible [See Drewes, 1984; Zoran and Drewes, 1987; Drewes and Fourtner, 1989, 1990.]

B. Plasticity and morphallactic reorganization of escape reflexes: Place a large, fresh *Lumbriculus* specimen on wet filter paper and cut the worm in half with a razor blade. Place one of the fragments on the recording grid. Are giant fiber spikes still detected in response to touch? Are they MGF or LGF spikes? Test the other fragment in the same way. Map day-to-day changes in giant fiber sensory fields in each fragment during stages of segmental regeneration. Compare to results from fragments to those from whole worms. [See Drewes and Fourtner, 1990; Drewes, 1996.]

C. Species specificity in giant nerve fiber function: Try to record giant fiber action potentials from other oligochaete species, such as an earthworm, *Eisenia foetida* (redworm). Most earthworm species are so active that it is necessary to use a long, narrow Plexiglas enclosure (with cover) to restrict worm movements. Describe species differences in touch sensitivity, conduction velocity, spike waveforms. [See Drewes et al., 1983; Drewes, 1984; Zoran and Drewes, 1987.]

D. *Growth-related differences in giant fiber conduction velocity*. Measure MGF and LGF velocity in the redworm, *Eisenia foetida.* These are easy to culture in the lab. Newly hatched worms (≈ 1 cm long) grow to maturity (≈ 10 cm long) in a few weeks. [See O'Gara *et al*., 1982.]

E. Sublethal neurotoxicity effects: Study neurotoxicity effects of sublethal exposure to pharmacological agents, pesticides, or other environmental toxicants on escape reflex functioning in *Lumbriculus*. Compare electrical properties of the worms' escape reflexes before and after treatment. [For exposure protocols, see Rogge and Drewes, 1993; Drewes, 1997.]

Equipment Shut-down

Turn the INPUT knob on audio-monitor to OFF.
Switch POWER on audio-monitor to OFF.
Move the COUPLING switch on preamplifiers 1 and 2 to the OFF position.
Disconnect battery packs from preamplifiers to prevent batteries from running down.
Turn the POWER switch on the oscilloscope to OFF.
Use a pipet to add water to the base of the Plexiglas strip.
Use a pipet to remove the worm from the grid.
Dry off the recording grid and Plexiglas strip.

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APPENDIX A

Data for Step 4
Recordings from **Mid-Body**

	M G F		L G F	
trial #	cond time ms	cond vel m/s	cond time ms	cond vel m/s
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
mean				
range				
st dev				

OPTIONAL - Investigation A
All electrodes in **Anterior** zones

	M G F		L G F	
trial #	cond time ms	cond vel m/s	cond time ms	cond vel m/s
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
mean				
range				
st dev				

OPTIONAL - Investigation A
All electrodes in **Posterior** zones

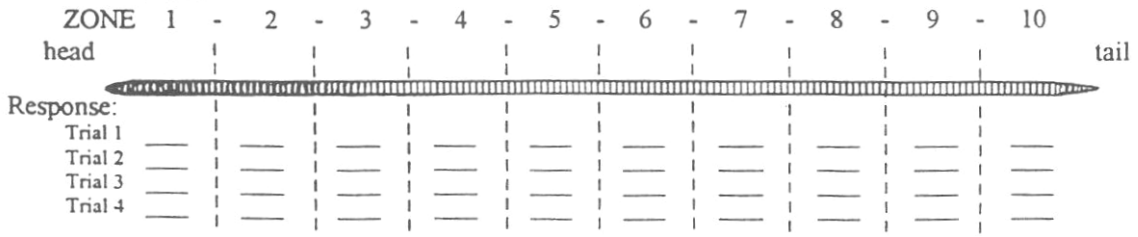
	M G F		L G F	
trial #	cond time ms	cond vel m/s	cond time ms	cond vel m/s
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
mean				
range				
st dev				

Data for Step 8: Facilitation of Giant Fiber Conduction Velocity

	MEDIAL GIANT FIBER						LATERAL GIANT FIBER					
	1st SPIKE			2nd SPIKE			1st SPIKE			2nd SPIKE		
trial #	cond $time_1$ ms	cond vel_1 m/s	ISI ms	cond $time_2$ ms	cond vel_2 m/s	ratio cv_2/cv_1 x100	cond $time_1$ ms	cond vel_1 m/s	ISI ms	cond $time_2$ ms	cond vel_2 m/s	ratio cv_2/cv_1 x100
1												
2												
3												
4												
5												
6												
7												
8												
9												

Data for Step 6: Giant Fiber Sensory Fields

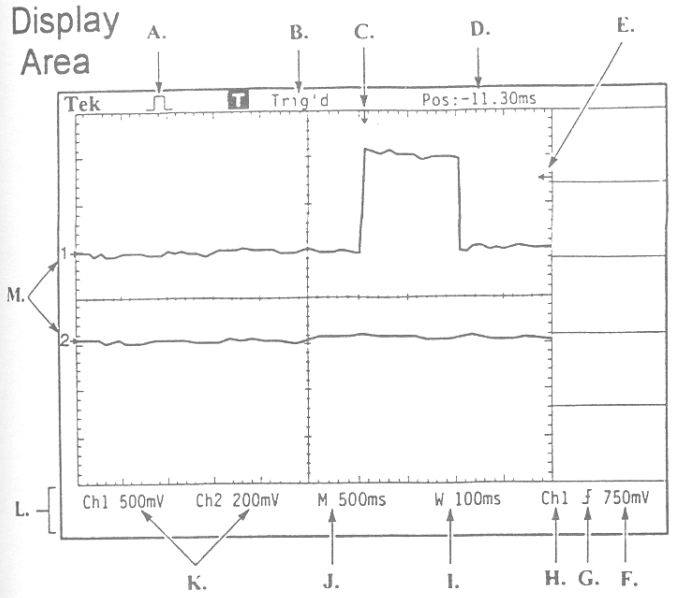
Location of touch:



APPENDIX B

Initialization Procedure for Tektronix TDS 210

1. Press the POWER On/Off button on the top left. Then push any button to see display.
2. Identify each of the labelled display settings and symbols, **A** through **M** shown below:



A. Icon display shows acquisition mode.
- Sample mode
- Peak detect mode
- Average mode

B. Trigger status shows if there is an adequate trigger source or if the acquisition is stopped.

C. Marker shows horizontal trigger position. This also indicates th horizontal position since the Horizontal Position control actuall moves the trigger position horizontally.

D. Trigger position display shows the difference (in time) between the center graticule and the trigger position. Center screen equal zero.

E. Marker shows trigger level.

F. Readout shows numeric value of the trigger level.

G. Icon shows selected trigger slope for edge triggering.

H. Readout shows trigger source used for triggering.

I. Readout shows window zone timebase setting.

J. Readout shows main timebase setting.

K. Readout shows channels 1 and 2 vertical scale factors.

L. Display area shows on-line messages momentarily.

M. On-screen markers show the ground reference points of the displayed waveforms. No marker indicates the channel is not displayed.

3. Refer to the diagram below showing front panel controls. Then, press the **TRIGGER** button on the far right of the front panel. Note that a column of five menu boxes appear on the right of the screen. **Use menu control buttons to the right of each menu box to highlight and change desired settings. Selected menu settings are highlighted in black on the screen. Choose the settings shown in bold lettering in the TRIGGER menu diagram on the next page.**