Rapid escape reflexes in aquatic oligochaetes: variations in design and function of evolutionarily conserved giant fiber systems

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Accepted April 25, 1987

Summary. This study provides neuroanatomical and electrophysiological evidence 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.

Our 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. We 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.

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 descrip-
tions 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 that 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. Branchiura sowerbyii (Beddard), Limnodrilus hoffmeisteri (Claparède), 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 (McMinville, OR). Naming of taxonomic orders is in accordance with Brinkhurst (1984).

Animals were kept at room temperature (22–26 °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 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 and 1 M cacodylate buffer. Each piece was then cut in 2 mm lengths which were washed in buffer and immersed for 2 h in 1% OsO₄ in 0.1 M cacodylate buffer. Tissues were dehydrated in a graded series of ethanol ending in a 2 h 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 3-dimensional reconstructions of the LGF in posterior regions of some species.

Electrophysiology. Repродuctively 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 noninvasive, 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 species means.

Simultaneous extracellular and intracellular recordings of giant fiber spiking activity were made in the two largest aquatic species K. hexatheca and B. sowerbyii. 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 submerged 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.