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THE RAPID TAIL WITHDRAWAL REFLEX OF THE TUBIFICID WORM, *BRANCHIURA SOWERBYI*

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Summary

The rapid tail withdrawal of the tubificid worm, *Branchiura sowerbyi*, was studied using correlated electrophysiological and behavioural analyses. The minimal response latency (i.e. time from onset of mechanical stimulus to onset of withdrawal) was approximately 7 ms, faster than the escape responses of any previously studied invertebrate. Factors contributing to the speed and efficacy of this response include: (1) a sensitive mechanosensory system for detecting potential prey, (2) a short latency for excitation along afferent and efferent pathways, (3) a rapid intersegmental conduction of lateral giant fibre spikes, (4) a short coupling time from muscle excitation to the onset of shortening, and (5) the requirement of only a single lateral giant fibre spike for a complete (all-or-none) response. Videotaped sequences of predator–prey interactions showed that such reflex speed permits effective escape from the strike of the bluegill (*Lepomis macrochirus*).

Introduction

The aquatic oligochaete, *Branchiura sowerbyi* (Oligochaeta, Tubificidae), lives in aquatic sediments nearly devoid of oxygen (Brinkhurst & Jamieson, 1971; Naqvi, 1973), 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) uptake and anterior pumping of water by the rectum, and (3) rhythmic undulatory movement of posterior segments as the tail extends above the sediments. Although such behaviour clearly renders the animal's tail vulnerable to predation and thereby necessitates adaptations for rapid escape into the sediments, there has been little attention given to studying these adaptations.

A recent study has shown that *Branchiura* and other aquatic oligochaetes respond to posterior tactile stimulation with a lateral giant fibre-mediated withdrawal, or rapid escape reflex (Zoran & Drewes, 1987). In the present study, we have used videotape and high-speed cinematography for frame-by-frame documentation of *Branchiura* escape responses to applied stimuli and actual predatory attacks. This study contains the first account of the time requirements

Key words: escape reflex, giant fibre, neuroethology, oligochaete, predator–prey interaction.

for afferent, central and efferent excitation during escape responses of an aquatic oligochaete worm. The reflex is the fastest reported for an invertebrate escape reflex and the results reveal several features of tubificid escape behaviour which differ from escape behaviour in the more extensively studied terrestrial oligochaetes (i.e. earthworms).

Materials and methods

Animals and maintenance

Tubificid worms, *Branchiura sowerbyi* (Beddard), were obtained from established laboratory cultures. The aquaria were supplied with mud from a local lake, circulating fresh water, 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 behavioural 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, Vining & Drewes, 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 centimetres of sediment and several centimetres of water (Zoran & Drewes, 1987). After placement 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 within 1 mm of the tail, readily detected lateral giant fibre (LGF) 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 and d.c.-powered fluorescent light source), which simultaneously detected shadow movements of the worm's tail. Tactile stimuli were delivered electromechanically as single pulses (displacement $\leq 200\,\mu m$; duration = 1 ms) with a speaker probe ($100\,\mu m$ 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. Another advantage was that only the LGF sensory field was present in such preparations, thereby precluding inadvertent activation of the worm's medial giant fibre system. The tail-piece was submerged in a saline solution (Zoran

& Drewes, 1987) 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 fibres 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 mol l^{-1} KCl. Tactile stimulation was delivered either by the previously described electromechanically driven probe (<100 μm tip diameter to avoid significant water displacement) or by a hand-held glass probe (tip diameter approximately 200 μm). Electrical stimulation of giant fibres 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 curare were studied during escape responses in semi-intact preparations. Intracellular recordings from longitudinal muscle were first obtained in normal saline. These recordings were compared with those obtained during exposure to saline containing 10^{-4} mol l^{-1} *d*-tubocurarine chloride (Sigma) and after washing in normal saline. Similar procedures were used to study the effects of low-Ca^{2+}/high-Mg^{2+} saline. This saline contained 50 μmol l^{-1} Ca^{2+} and 10 mmol l^{-1} Mg^{2+}, compared with the normal 6 mmol l^{-1} Ca^{2+} and 1 mmol l^{-1} Mg^{2+}.

Videotaping and cinematography of escape sequences

Rapid tail withdrawals in response to attack by bluegill, *Lepomis macrochirus*, were studied in narrow aquaria (15 cm × 30 cm × 5 cm) supplied with a mud substrate, fresh water, 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 (30 frames s^{-1}). Single-frame images were photographed directly from a monitor.

High-speed cinematography of tail withdrawal sequences was filmed at 200 frames s^{-1} with a 16 mm, rotating prism camera (Hycam, model K2004E-115, Red Lake Laboratories, Santa Clara, CA). Tactile stimuli were applied with a glass rod (diameter = 3 mm).

Results

Analysis of videotaped escape sequences indicated that several types of 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). Abruptly-delivered water displacements and substrate vibrations could also 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

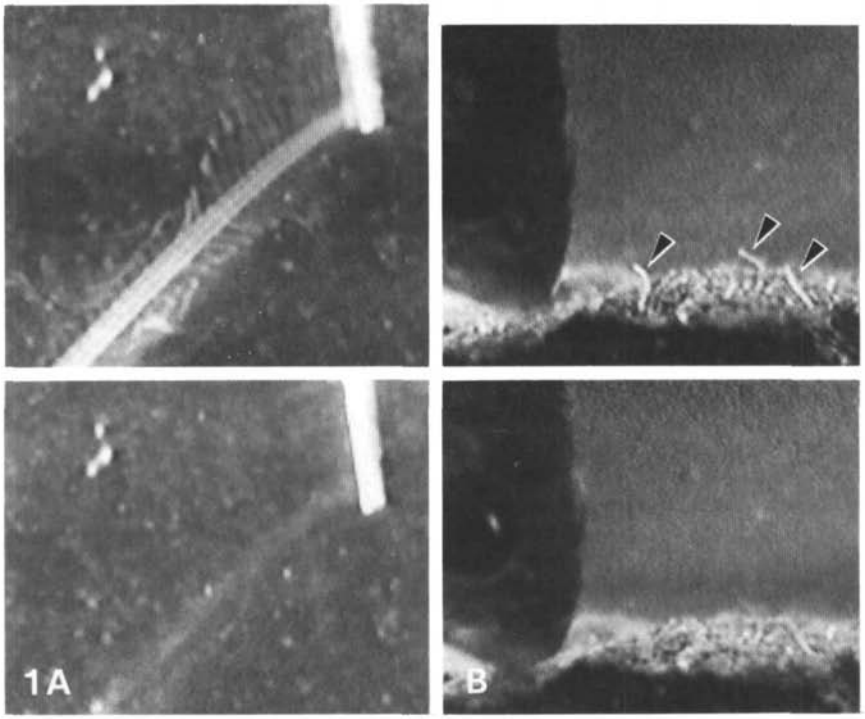

Fig. 1. Videotaped sequences of rapid escape behaviour of *Branchiura sowerbyi*. (A) Two consecutive frames illustrate a rapid tail withdrawal evoked by tactile stimulation with a hand-held probe. Note the gill filaments on the worm's dorsal and ventral surfaces. Magnification 7×. (B) Two consecutive frames show the synchronous responses of three worms to the strike of a bluegill that attacked the tail of a fourth worm (already withdrawn). All sequences were taped at 30 frames s^{-1}. Magnification 1·5×.

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. More often, escape responses were initiated in an individual worm, or simultaneously in several worms, during a strike at a worm's tail (Fig. 1B). In these cases, water displacement and/or substrate vibrations were probably the effective stimuli. In a few instances, worms also escaped rapidly in response to digging behaviour of fish in sediments at a considerable distance from the worm, suggesting that vibrational stimuli alone were adequate.

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 postsynaptic potentials (EPSPs) within the

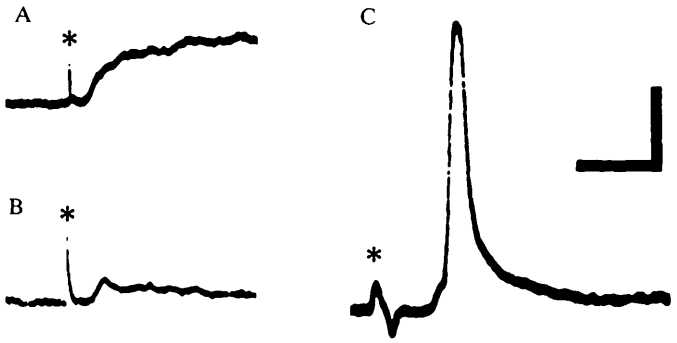

Fig. 2. Excitatory postsynaptic potentials (EPSPs) in the left lateral giant fibre (LGF). (A) An EPSP recorded during tactile stimulation of the body wall (* = stimulus artefact). Afferent latency (i.e. time from stimulus onset to onset of EPSP) was approximately 2 ms. (B) The effect of a 30 min exposure to 10^{-4} mol l^{-1} tubocurare on an EPSP evoked by tactile stimulation. Note that a small curare-resistant potential persists. (C) An EPSP and spike in the LGF following a stronger tactile stimulation of the body wall. Voltage scale: A,B, 5 mV; C, 20 mV. Time scale: A,B, 10 ms; C, 5 ms.

LGF (Fig. 2A). Mean afferent latency (i.e. the time from onset of stimulus to onset of EPSPs) was 2·2 ± 0·5 ms (±S.E.M.; $N = 5$). With slightly stronger stimuli, EPSPs were accompanied by LGF spikes occurring approximately 0·5–1·0 ms after onset of the EPSP (Fig. 2C).

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. Second, EPSPs produced by mechanical stimulation were reversibly reduced in amplitude by bathing semi-intact preparations in either low-Ca^{2+}/high-Mg^{2+} saline or normal saline containing the acetylcholine antagonist curare. In such preparations, a short-latency response of about 2 mV persisted (Fig. 2B), but the possibility that this persisting response was an electrically mediated PSP and that the afferent-to-giant pathway was monosynaptic was not investigated.

Central conduction

Non-invasive grid-recordings along the length of intact animals 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 100–200 μV) followed after less than 1 ms by a slower, multiphasic wave of variable amplitude (approximately 400–800 μV). Simultaneous intracellular 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

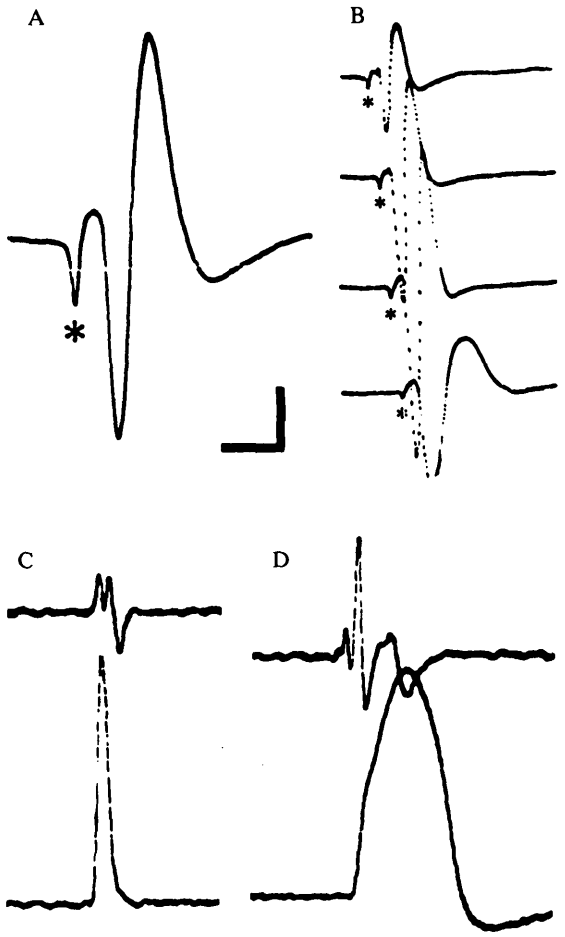

Fig. 3. Extracellular and intracellular recordings of lateral giant fibre (LGF) and longitudinal muscle activity. (A) Non-invasive grid recording from the tail consisted of two components: an initial LGF spike (*) followed by a multiphasic, longitudinal muscle potential. (B) A four-channel recording shows the LGF spike (*) was conducted anteriorly at a velocity of approximately 15 m s^{-1}. The top trace is the most posterior recording and the distance between each recording pair was 8 mm. (C) An LGF spike (intracellular recording; bottom trace) was coincident with the initial spike in extracellular suction electrode recordings (top trace). (D) A longitudinal muscle potential recorded intracellularly (bottom trace) was coincident with the multiphasic potential recorded extracellularly with suction electrodes (top traces). Voltage scale: 100 μV (A), 500 μV (C, top trace), 200 μV (D, top trace), 20 mV (C,D, bottom traces). Time scale: 2 ms (A), 5 ms (B,C), 10 ms (D).

(Fig. 3B), showed that such LGF spikes were conducted anteriorly at 15–20 m s^{-1}. However, because of an associated longitudinal gradient in LGF diameter (Zoran & Drewes, 1987), velocities in mid-body and anterior segments were reduced to 10–15 and 5–10 m s^{-1}, respectively.

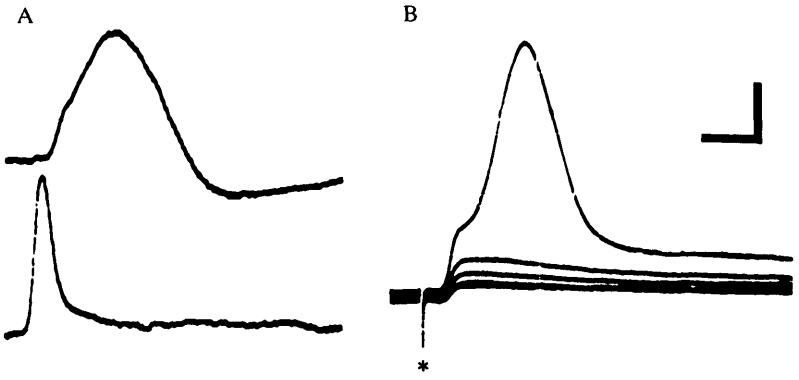

Fig. 4. Intracellular recordings of longitudinal muscle activity. (A) Simultaneous intracellular recordings from the lateral giant fibre (LGF) (bottom trace) and a longitudinal muscle fibre (top trace) illustrate the short efferent latency. (B) The shoulder-like prepotential or excitatory junctional potential (EJP) decreased in amplitude during repetitive electrical stimulation of the LGF at 1 Hz (* = stimulus artefact). Voltage scale: 20 mV (A), 10 mV (B). Time scale: 5 ms.

Efferent timing and transmission

Simultaneous intracellular and extracellular recordings showed that the onset of the large, slow potential in non-invasive recordings corresponded to the onset of an action potential in longitudinal muscle fibres of the body wall (Fig. 3D). As shown in Figs 3D and 4A, these muscle potentials were composed of an initial shoulder-like prepotential, with a steep rate of rise (20–30 mV ms^{-1}), and an overshooting spike with a slightly slower rate of rise (15–20 mV ms^{-1}). Two lines of evidence suggest that the prepotential is an excitatory junctional potential (EJP). First, occasional spontaneous longitudinal muscle spikes, not associated with escape responses and presumably caused by electrode penetration, lacked the prepotential. Second, during repetitive firing of the LGF, muscle prepotentials were gradually reduced in amplitude (antifacilitated) and soon failed to initiate action potentials (Fig. 4B). 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 curare. Curare rapidly and reversibly reduced the amplitude of the EJPs, thus preventing muscle spiking and the concomitant rapid contraction that usually followed each LGF spike (Fig. 5).

Although the motor neurones involved in the efferent pathway were not identified, it was possible to make accurate measurements of efferent latency. Dual intracellular recordings (Fig. 4A), made simultaneously from the LGF and longitudinal muscle fibres in the same segment, revealed that the latency from the peak amplitude of the LGF spike to the onset of muscle potentials was 0·7 ± 0·2 ms (±S.E.M.; $N = 8$). Since the latency was essentially the same in all body regions,

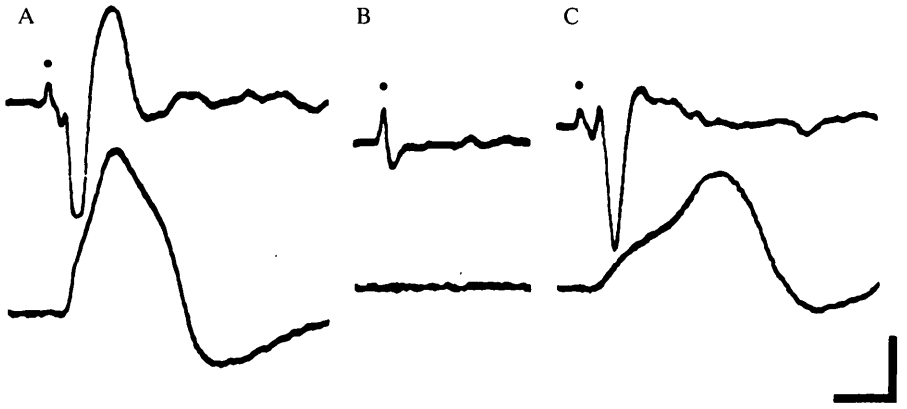

Fig. 5. Effects of curare on the lateral giant fibre (LGF)-mediated response. (A) Simultaneous recordings are shown from the ventral nerve cord (top trace; extracellular suction electrode) and longitudinal muscle (bottom trace; intracellular recording) in normal saline. (B) After bathing in 10^{-4} mol l^{-1} tubocurare for 10 min, the longitudinal muscle response was abolished. The dot denotes the persisting LGF spike. (C) After 20 min of washing with normal saline, the muscle response partially recovered. All responses were evoked by tactile stimulation of intact tail segments situated 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 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*

Animals ventilating in aquaria were stimulated with a probe and movements associated with rapid escape were filmed at high speed. Analysis of these sequences showed 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 an adequate stimulus. Initially tails were extended approximately 5–10 mm above the sediment and the disappearance of the tail into the sediment was completed within 15–20 ms (3–4 frames). Thus, an estimated rate of shortening during these escape episodes was 0·40 mm ms^{-1}.

Non-invasive electrical recordings were then obtained from a worm's tail as it extended into the water column of a small aquarium; simultaneously, tail movements were detected with a photocell to correlate directly the timing of behavioural and electrical events during escape. The waveforms of these *in situ* electrical recordings, although somewhat attenuated in amplitude, were essentially identical to those obtained in grid recordings (cf. Figs 3A, 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 onset of an electromechanically driven stimulus to LGF spike) was 2·8 ± 0·9 ms (±S.E.M.; $N = 8$). The mean efferent latency (i.e. time

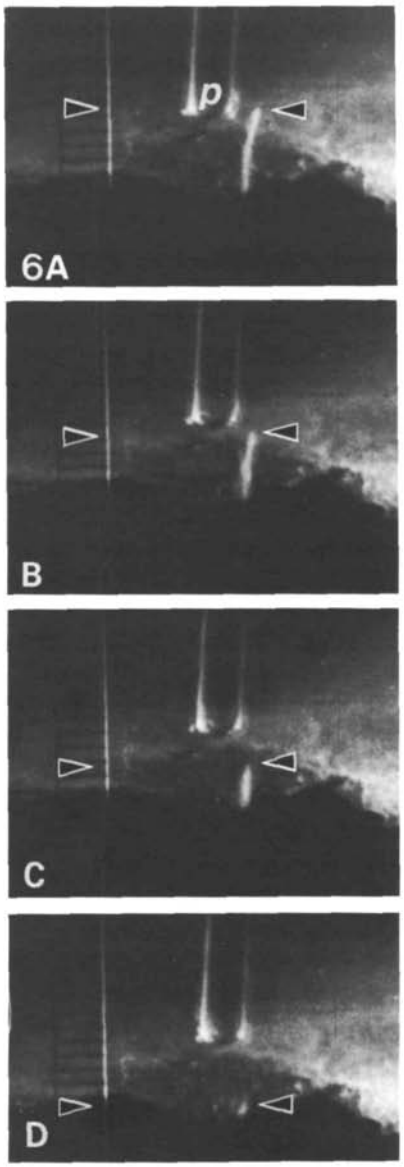

Fig. 6. High-speed cinematography of rapid tail withdrawal. The tail of a ventilating worm (tip of tail indicated by right-hand arrowhead) was stimulated by the approach of a hand-held glass probe. Tail withdrawal was filmed at 200 frames s^{-1}; A–D are consecutive frames. The tail, originally extended from the burrow approximately 5 mm, was completely withdrawn in three frames (= 15 ms). The left-hand arrowhead indicates tail position on a millimetre scale.

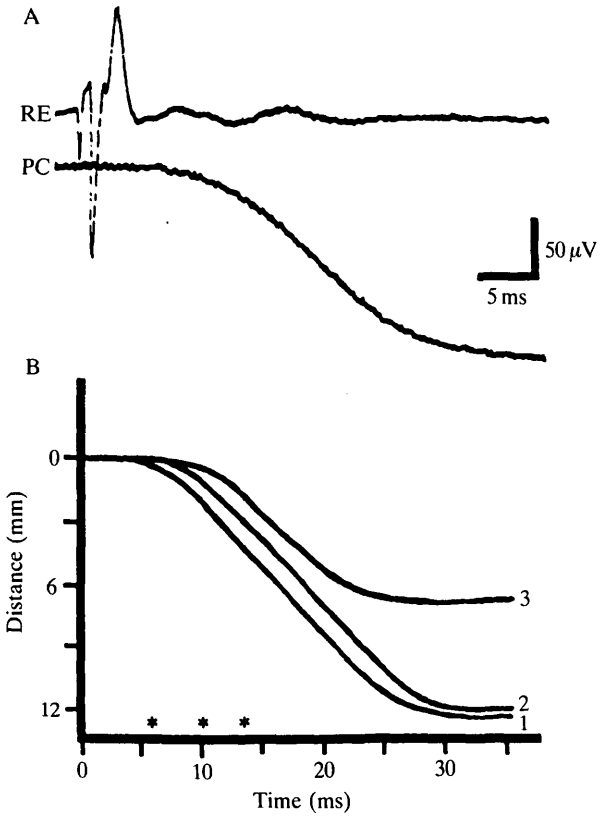

Fig. 7. *In situ* recordings of rapid escape. (A) Extracellular recording (RE) from the tail of an intact worm (top trace) and a photocell recording (PC) of tail movements (bottom trace). The onset of the trace coincides with the onset of the electromechanically driven tactile stimulus. The lateral giant fibre (LGF) spike and the onset of tail withdrawal occurred approximately 2 and 7 ms, respectively, after the onset of the stimulus. (B) Three photocell records are superimposed on the same time scale. Record 1 was evoked by a single LGF spike occurring at time 0. Record 2 was evoked by four spikes, the first occurring at time 0 and the others as indicated by the asterisks. Record 3 (single spike response) shows the shortening of a tail extended about 6 mm out of the burrow. In records 1 and 2, the tails were extended approximately 12 mm out of the burrow. The shorter length of extended tail in record 3 reflected fewer segments exposed, rather than shorter segment length in comparison with records 1 and 2.

from peak of LGF spike to onset of muscle potentials) was $0{\cdot}9 \pm 0{\cdot}2$ ms (±S.E.M.; $N = 8$). 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 $5{\cdot}7 \pm 1{\cdot}4$ ms (±S.E.M.; $N = 8$). Therefore, the

mean behavioural latency (i.e. time from onset of tactile stimulus to 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 ($=0{\cdot}54 \pm 0{\cdot}04\,\text{mm}\,\text{ms}^{-1}$; $\pm$S.E.M.; $N = 4$), 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 for the photocell records.

The rate of shortening, as well as excitation–contraction coupling time, was independent of the number and frequency of LGF spikes evoked during escape (Fig. 7B). 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 response latencies and rate of shortening were 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 behaviour can be as diverse as shadows (Nicol, 1948, 1950), wind (Camhi & Nolen, 1981) or vibration (Bullock, 1945; Nicol, 1950). However, one feature shared by all stimuli that elicit giant fibre-mediated responses is a sudden onset. In the aquatic oligochaete *Branchiura sowerbyi*, abrupt stimuli such as direct touch of the body wall (Fig. 1) and near-field water displacements are adequate for initiating withdrawal. A high sensitivity to water movement, in addition to tactile cues, ensures earlier activation of the giant fibres, thereby increasing the probability of effective escape.

A comparable sensitivity to water movement has been demonstrated in another annelid, 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 some giant-fibre systems in polychaetes, can also be activated by photic stimulation (Kretz, Stent & Kristan, 1976). In contrast, the lateral giant fibre (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 remarkably fast reflex response. When compared with other invertebrate escape behaviour (see Table 1), the minimal latency of the response ($=7$ ms) is the fastest ever reported for an invertebrate escape reflex. Some of this speed is undoubtedly due to the negligible distance and times involved in peripheral conduction along afferent and efferent pathways; the length of these paths is probably no more than 0·5 mm. Notwithstanding the time savings from these considerations, many other factors contribute to minimizing the response

Table 1. *Comparison of total escape reflex latencies in selected invertebrate species*

Species	Stimulus	Minimal response latency* (ms)	Reference
Oligochaetes			
Lumbricus	tactile	25	Pallas & Drewes, 1981; Drewes, 1984
Branchiura	tactile	7	Present study
Polychaetes			
Branchiomma	tactile	14	Krasne, 1965
Crustaceans			
Crayfish	tactile	15	Wine & Krasne, 1982; Krasne & Wine, 1984
Insects			
Cockroach	wind	11	Camhi & Nolen, 1981

* Time from stimulus onset to onset of movement.

time of this escape. These include: (1) a short latency for afferent-to-giant transmission (Fig. 2), (2) a rapid intersegmental conduction of LGF spikes which confers a near synchronous muscle excitation in all tail segments (Fig. 3B), (3) an extremely short efferent latency from giant fibre spike to muscle potential (Fig. 4A), (4) a very short coupling time from muscle potential to the onset of movement (Fig. 7A), (5) a fast rate of segmental shortening (Fig. 7B), and (6) the requirement of a single LGF spike, 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 (Drewes, 1984). In *Branchiura sowerbyi*, an LGF spike elicits a large-amplitude muscle EJP and multisegmental shortening of longitudinal muscle. The effects of any subsequent LGF spikes are inconsequential with respect to the speed or efficacy of the escape. This contrasts with the requirement of multiple giant fibre spiking in the graded escape reflexes of earthworms (Drewes, Landa & McFall, 1978; Drewes, 1984). For example, the graded head withdrawal reflex of *Lumbricus terrestris* is evoked by a closely spaced pair or train of medial giant fibre spikes that, in turn, elicit facilitated muscle responses (Gunther, 1972; Drewes, McFall, Vining & Pallas, 1980). These differences appear to be neurobehavioural adaptations related to lifestyle and habitat (Zoran & Drewes, 1987).

A likely focal point for future investigations is the possibility that, as in some insects, the efficacy of tubificid escape responses may be modulated by behavioural or environmental factors. For example, Camhi & Nolen (1981) demonstrated that the response latency of the cockroach escape is less during walking than during standing. Recent studies have also shown that the efficacy of the escape in certain cockroach species can be markedly influenced by ambient

temperature (Simpson, Ritzmann & Pollack, 1986). In a freshwater worm such as *Branchiura*, some environmental factors that may be relevant to the efficacy of its escape are dissolved oxygen concentration, temperature and pollutants.

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