Quantification of neurotoxic effects on escape reflex response in earthworms

by

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GENERAL INTRODUCTION

Escape or startle reflexes are characteristically seen in many representatives of the Phylum Annelida. They occur in response to stimuli that have sudden or unexpected onset and result in short latency, fast motor acts involving avoidance or evasion. Escape responses are mediated by giant nerve fibers whose activity can be readily detected from the ventral surface of intact segments of the worm in response to tactile stimulation at either end of the animal. This leads to the possibility that neurotoxic effects of chemicals can be quantified by assessing alterations in the sequence of bioelectrical events underlying the worm's rapid escape response.

My thesis format involves two sections. The first section describes the quantification of neurotoxic effects of an agricultural pesticide, carbofuran, on escape reflex response in earthworms. The second section deals with the utility of spike time-course, in comparison to conduction velocity, as a measure for quantifying neurotoxic effects of chemicals on giant fiber activity.

SECTION I. NEUROTOXIC EFFECTS OF CARBOFURAN ON ESCAPE REFLEX RESPONSE IN EARTHWORMS

INTRODUCTION

There is an increasing concern over the toxic hazards caused by indiscriminate use of pesticides. Non-target organisms are affected by pesticides either directly through toxicity and/or indirectly through changes in their physical or biological environment. Earthworms, which are of considerable importance in improving soil aeration, drainage and fertility, are of great value as an indicator species representing the terrestrial environment of the biosphere. Use of the earthworm, Eisenia foetida, in conjunction with standardized protocols for chemical exposure (Goats & Edwards, 1988; Heimbach, 1988), has provided an important ecotoxicological tool for predicting field toxicity and for making interlaboratory and interagency comparisons for the relative toxicity of many pollutants. Additional efforts to incorporate sublethal parameters as end-points into these tests (Drewes et al., 1987, 1988; Venter & Reinecke, 1988) have also served to expand our understanding of the diverse range of biological effects of toxicants. Toxicity effects in earthworms can be assessed by tests involving biochemical assay (Stenersen, 1979), growth (Lofs-Holmin,1980; Karnak et al., 1982), segmental regeneration (Drewes et al., 1986), reproductive capacity (Lofs-Holmin, 1982) or

neurophysiological symptoms (Drewes, 1988).

Here, I have utilized test protocols that enable quantitation of sublethal neurophysiological effects of the agricultural pesticide, carbofuran, on Lumbricus terrestris, a prevalent species in many agricultural and urban settings. Carbofuran, a carbamate ester derivative, is a broad spectrum insecticide generally used on agricultural crops such as alfalfa, peanuts, rice, sugarcane, and especially corn. Specifically, the neurotoxic effects of carbofuran were quantified by assessing alterations in the sequence of bioelectrical events underlying the worm's rapid escape response. The reflex response is particularly conducive to such assessments because it is mediated by giant nerve fibers (axons of interneurons) which generate rapidly conducted impulses that are easily detected with electrodes placed along the ventral surface of the intact, unanesthetized worm (Drewes, 1984). In particular, short-term changes in medial (MGF) and lateral (LGF) giant nerve fiber conduction velocity and MGF-to-giant motor neuron (GMN) transmission time were studied using non-invasive recording procedures.

METHOD

Pesticide Selection

Carbofuran is a white crystalline solid, empirical formula $C_{12}H_{15}NO_3$, with a low vapor pressure and low water solubility. When used on crops such as field corn (i.e., for corn rootworm and European corn borer control), carbofuran (Furadan) is often applied, at planting, as covered bands or in seed furrows using a variety of mechanical applicators and one of several granular formulations. Granules may also be broadcast as foliar applications for corn borer control. For other crops, such as soybeans, granular Furadan is also used for insect and nematode control, by applying at planting and mixing with covering soil.

Species Selection

Lumbricus terrestris, and other coexistent earthworm species, are broadly distributed in agricultural and urban soils throughout the United States (Reynolds, 1977). However, L. terrestris is behaviorally distinguished from other species by vertical burrowing and nocturnal surfacing habits that facilitate successful foraging and mating.

In the present study, sexually mature L. terrestris (mean wt = 4.1 ± 0.7 g) were maintained at 10-15 °C in closed boxes containing a mixture of horse manure and soil. Before testing, worms were acclimated for 24 h at room temperature in covered boxes containing only moist paper towels.

Treatment Protocol

In view of agricultural application methods for Furadan and the surfacing behavior of L. terrestris, we used short-term and point-source exposures to either a single commercial granule (Furadan 15G), or an equivalent pre-weighed amount of pure carbofuran applied to the dorsal skin surface of individual earthworms. The size of a commercial granule was highly variable, but typically within a weight range of 350-1000 μg and diameter range of 0.5-1.0 mm. Two different dosages of pure carbofuran were used: 50 μg and 150 μg, values that correspond closely to the 15% carbofuran-content of typical, small and large Furadan 15G granules, respectively.

The site of application was approximately 10-20 segments posterior to the clitellum, corresponding to the center of the region from which electrophysiological

recordings were readily obtained. In control worms, a sand grain approximately equal in size to the chemical granule was placed in the same location on the worm's skin. The exposure period was one hour (21 °C). To ensure that the granules stayed in continuous contact with the skin, worms were placed on moist filter paper in a covered and darkened dish. This helped minimize crawling and searching behaviors (which tend to cause inadvertent loss of skin contact with the granule). After exposure and before testing, worms were rinsed briefly in distilled water.

Electrophysiological Testing

The medial and lateral giant nerve fibers of earthworms mediate startle withdrawal behaviors that are vital for avoiding predators. Non-invasive recordings of all-or-none action potentials (spikes) from these fibers are readily obtained from a wide variety of earthworm species, including L. terrestris (Drewes, 1984).

Giant fiber spikes were initiated by light touch to either end of the worm with a fire-polished glass probe. Signals from two pairs of recording electrodes (Fig. 1) were preamplified and filtered (Drewes et al., 1987). Evoked spikes were displayed as internally triggered, single sweeps

on a digital storage oscilloscope. For most analyses, two-channel recordings were obtained from sites 20 mm apart near the geometric middle of the worm. In treated worms, recording sites straddled the original site of application. In experiments requiring higher resolution of the spatial effects of carbofuran treatment, eight-channel, analog recordings were obtained using a Tektronix 5113 storage oscilloscope equipped with two four-channel amplifiers (5A14N).

Three neural parameters were measured: MGF conduction velocity, LGF conduction velocity, and time delay from the MGF spike to GMN spike. The latter time is essentially a measure of transmission time at the central synapse between MGF and GMN (Drewes, 1984). When determining conduction velocity, care was taken to ensure that body stretch in the recording region was similar for both pre-treatment and post-treatment measurements. Approximately 8-10 replicate measurements were obtained from each worm before, and again after, treatment. Pre-treatment and post-treatment means were then determined and expressed as relative values, with pre-treatment control values for each worm assigned as 1.0. Usually, 10-13 replicate measurements (to the nearest 0.01 ms) of the delay time from MGF-to-GMN spikes were obtained

from each worm. Statistical comparisons of relative velocities and delay times within each test group were made using a paired difference t-test. Eight worms were used in each of three test groups: 0 μg-control, 50 μg-treated, 150 μg-treated.

RESULTS

Behavioral Effects

Behavioral effects accompanying treatment with a single commercial granule of Furadan were generally indistinguishable from the effects of pure carbofuran. In all treated worms there were no obvious signs of intoxication or cutaneous irritation for approximately the first 15 min of exposure. Beginning about 15-20 min after exposure, however, all treated worms exhibited frequently repeated episodes of whole-body spasms and tight coiling, followed by partial relaxation. Such episodes occurred either spontaneously or in response to slight tactile stimulation. No coordinated or directed locomotion occurred during these episodes (i.e., worms were essentially paralyzed). Similar symptoms were also seen in earthworms (L. terrestris and Eisenia foetida) injected with carbofuran or exposed to carbofuran-treated soils (Stenersen et al., 1973). An important result noted by Gilman and Vardanis (1974) was that E. foetida, but not L. terrestris, burrowed effectively after injection and appeared to behaviorally discriminate between carbofuran-treated and untreated soils.

To determine whether behavioral effects of carbofuran

were long-lasting or lethal, 150 μg-treated worms were again observed after 4-6 h and 24 h. After 4-6 h, partial to no recovery was observed, with most worms showing the same symptoms of spasms and inability to crawl. By 24 h after treatment, however, complete recovery of behavioral effects was observed. No spasms or rigidity was observed, and worms exhibited normal crawling and responsiveness to tactile stimulation. Hence, it was concluded that the selected treatment levels were sub-lethal and that behavioral effects of carbofuran were reversible.

Effects on Giant Fiber Conduction Velocity

Medial Giant Fiber Responses A typical two-channel recording of a single-spike MGF response to a tactile stimulus, before treatment, is shown in Fig. 2. As previously noted (Drewes, 1984), this response has three distinct components: an all-or-none MGF spike, a compound GMN spike, and a small muscle potential. In the control group, these waveforms and the relative MGF velocity were unchanged when worms were retested after one hour.

No statistically significant change in MGF velocity was noted in the 50 μg-treated group (Fig. 3). However, a significant reduction in velocity, to approximately 80% of

pre-treatment, was noted in the 150 μg treated group. In addition, in four of eight worms in this group, the GMN spike and accompanying muscle potential were occasionally absent in waveforms obtained from one or both recording sites (Fig. 2).

Lateral Giant Fiber Responses The LGF system consists of two, parallel, through-conducting pathways, connected to one another by a strong electrotonic junction (Drewes et al., 1980). As the two fibers conduct spikes synchronously, a unitary all-or-none LGF spike is detected from the body surface.

LGF spikes were readily detected in both control and treated groups at one hour after exposure. There were no changes in relative LGF velocity in control and 50 μg-treated groups, although a slight reduction was noted in the 150 μg-treated group (Fig. 4).

Effects on MGF-to-GMN Coupling

The medial giant fiber (MGF) system is closely associated with three pairs of segmentally homologous giant motor neurons (GMNs). Each MGF spike is coupled in a 1:1 fashion to a GMN spike (Gunther, 1972). Spikes in GMN axons are readily detected non-invasively (Drewes et al., 1980)

due to the large diameter of the axons.

The delay time from MGF to GMN spikes was unchanged after 1 h in the control group, but markedly increased in carbofuran-treated groups. In the 150 μg-treated group this increase was about 35%, and in the 50 μg-treated group about 15%, after 1 h exposure (Fig. 5). To test whether effects were spatially uniform or highly localized within the midbody, simultaneous recordings were obtained from eight different sites spaced 4 mm apart along the midbody of the worm (Fig. 6). Such recordings showed similar reductions in MGF-GMN delay times at each of the sites, suggesting that neural effects of carbofuran are relatively uniform, or systemic, rather than localized to the treatment site.

Recovery Studies Recovery of MGF-to-GMN transmission time was studied in eight, 150 μg-treated, earthworms. After 1 h, the delay time was increased by about 20%. But, worms observed after 4-6 h exhibited a MGF-to-GMN delay only 2% greater than pre-treated values (Fig. 7); i.e., complete recovery of MGF-to-GMN transmission time was observed 4-6 h after treatment.

DISCUSSION

Sites and Modes of Carbofuran Action

Previous studies by Stenersen (1979abc) have demonstrated that carbofuran treatment in earthworms is accompanied by inhibition of cholinesterase. Since neuromuscular transmission in earthworms is probably cholinergic (Gerschenfield, 1973), peripheral effects, such as muscle spasms and eventual loss of MGF-mediated muscle responses, may be indicative of cholinesterase-related action of carbofuran.

The results of this study indicate that carbofuran treatment is also accompanied by two distinctly different effects within the worm's central nervous system. These include reduced conduction velocity in giant nerve fibers and reduced efficacy of functional coupling between the medial giant fiber and its associated giant motor neurons.

The reduction in giant fiber conduction velocity raises important questions about carbofuran effects. Since each segmental unit, of the medial and lateral giant fiber system of the earthworm, is specialized for synaptic input and output as well as spike conduction, each segment has several potential sites for toxic actions on giant fiber membranes.

1) In each segment, the myelin-like sheath surrounding each giant nerve fiber is interrupted ventrally by collateral projections arising from the MGF and LGF. These ventral collateral membranes represent the main sites of synaptic inputs and outputs for the giant fibers (Killmann and Gras, 1988). 2) Another interruption in the myelin-like sheath is seen at the dorsal nodes (openings in the dorsal surface of the myelin sheath in each segment) that function in spike electrogenesis in the MGF (Gunther, 1976). 3) Gap junctional partitions are present at the intersegmental membrane boundaries of giant fibers in some segments (Drewes, 1984).

It is proposed that decreases in conduction velocity could result from changes in the biophysical properties of membranes at any of these three sites. For example, treatment-induced accumulation of neurotransmitter (e.g., acetylcholine) near synaptic inputs onto giant fiber collaterals might produce sustained increases in membrane conductance so that currents associated with giant fiber conduction would be "short-circuited" across these sites, thereby decreasing the effective space constant and conduction velocity of the fiber (Fig. 8). Our results do not eliminate the possibility that carbofuran effects could

involve other membrane sites, such as electrically excitable membranes at the dorsal nodes or electrically transmitting gap junctions in the membrane septa along the giant fibers.

The reduced efficacy of synaptic coupling between the MGF and the GMN raises the possibility that transmission at this synapse was directly affected by carbofuran. This might be expected if the synapse is cholinergic, but the question is open since supporting pharmacological data is non-existent.

Recovery studies on 150 μg-treated earthworms indicated complete recovery of the behavioral and neurophysiological effects of carbofuran. This implies that 1) treatment levels were sublethal and 2) effects of carbofuran are reversible. But, behavioral effects were found to persist longer than neural effects. This lack of correlation between recovery from behavioral effects and electrophysiological effects implies that effects on neural parameters, other than those tested, are longer lasting and, if detectable, would be a better indicator of persisting carbofuran effects on earthworms.

Ecological Significance

Since cutaneous exposure to granular doses of carbofuran can quickly, and without signs of behavioral avoidance, paralyze an earthworm, it seems important to consider granular application rates in assessing neurotoxic risk. Given that a typical granule of Furadan 15G weighs about 0.7 mg and that recommended application rates may be up to 13.3 lbs/acre, we estimate a concentration of 200 granules/ft^2 near the soil surface. Thus a worm's normal locomotor activities at or near the soil surface would very likely bring it into cutaneous contact with one or more granules. If the effective chemical concentration in each granule was not immediately and substantially reduced by water solubility and diffusion, we may assume that neurotoxicity effects would occur, leading to paralysis and eventual death by predation, dessication, or ultraviolet irradiation at the soil surface. If the contact occurred below the soil surface (e.g., by exposure to soil leachates), the question of recovery time from neural effects becomes critical for restoring earthworms' contributions to soil fertility.

In summary, this approach to neurotoxicity provides an "experimental window" through which we can detect a variety

of treatment induced lesions or "weak links" in the chain of electrophysiological events that underly rapid escape reflexes in intact earthworms. Effects on the functional properties of these reflexes may be useful in two ways. First, they may be indicative of the general performance, during and after treatment, of reflex function within the intact nervous system. Second, sublethal neurotoxic effects on escape reflexes may have implications in terms of increased vulnerability of earthworm to predation.

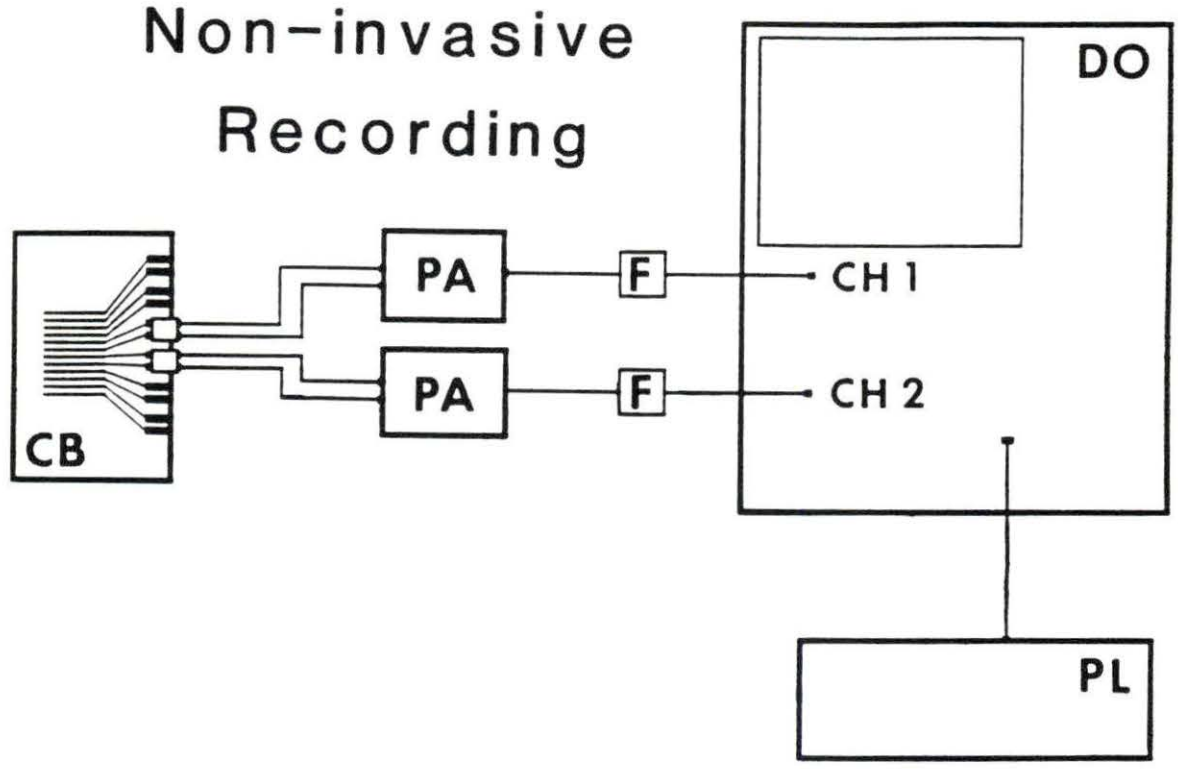

Figure 1. Recording apparatus. CB: Circuit Board, PA: Instrumentation Amplifiers, F: Filters, DO: Digital Oscilloscope, PL: Plotter.

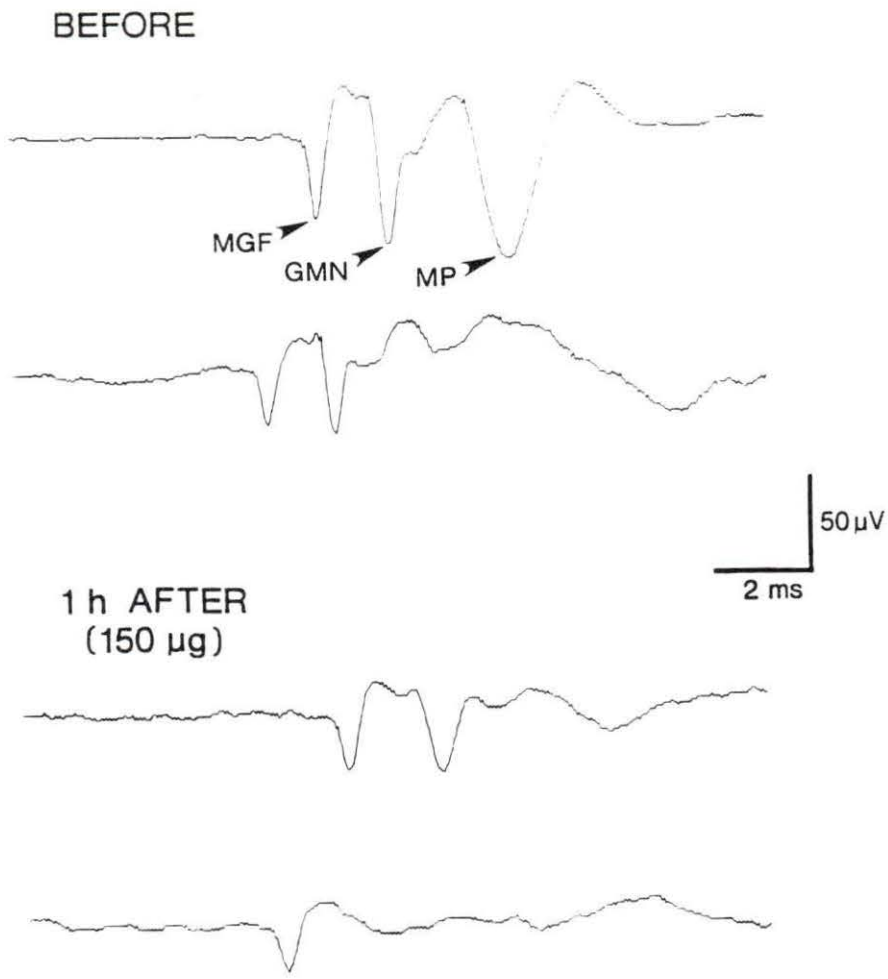

Figure 2. Before treatment, recordings from two sites (upper-posterior, lower-anterior) showed the three components in the MGF response: MGF spike, GMN spike, and muscle potential (MP). One hour after treatment, only the MGF spike was detected at the anterior site; the recording at the posterior site appeared more normal.

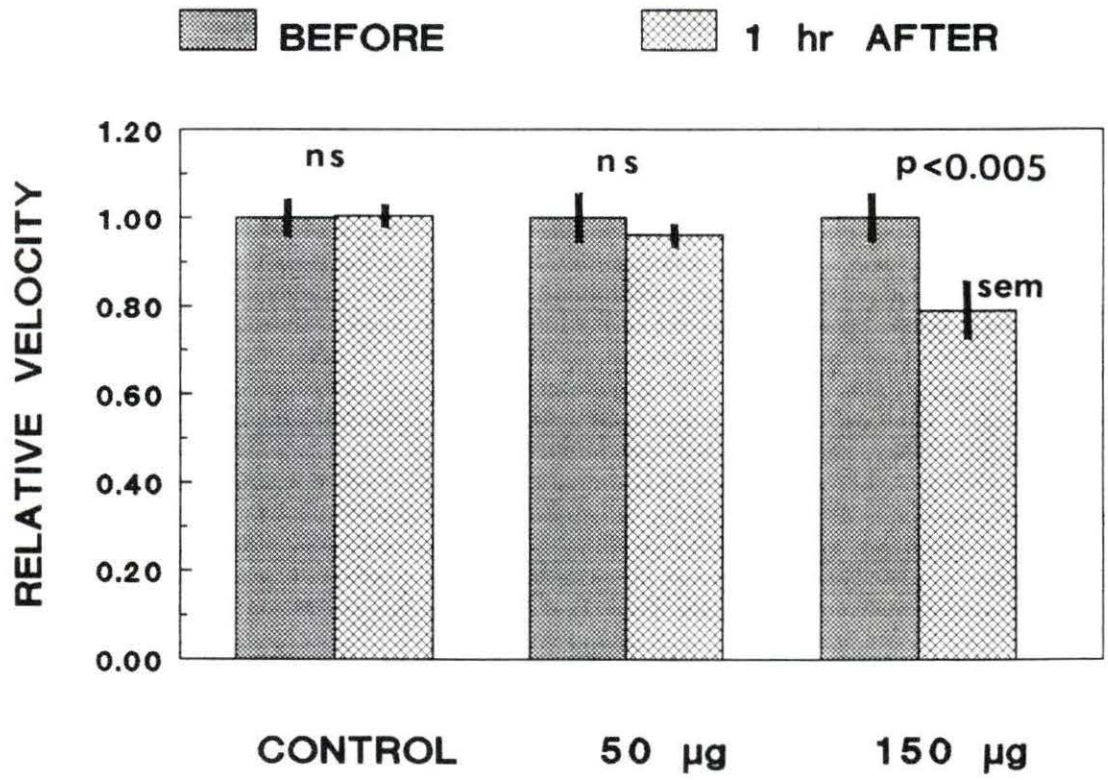

Figure 3. MGF conduction velocity was significantly reduced after 1 hr only in the 150 μg treated group; changes in velocity were not significant (ns) in other groups.

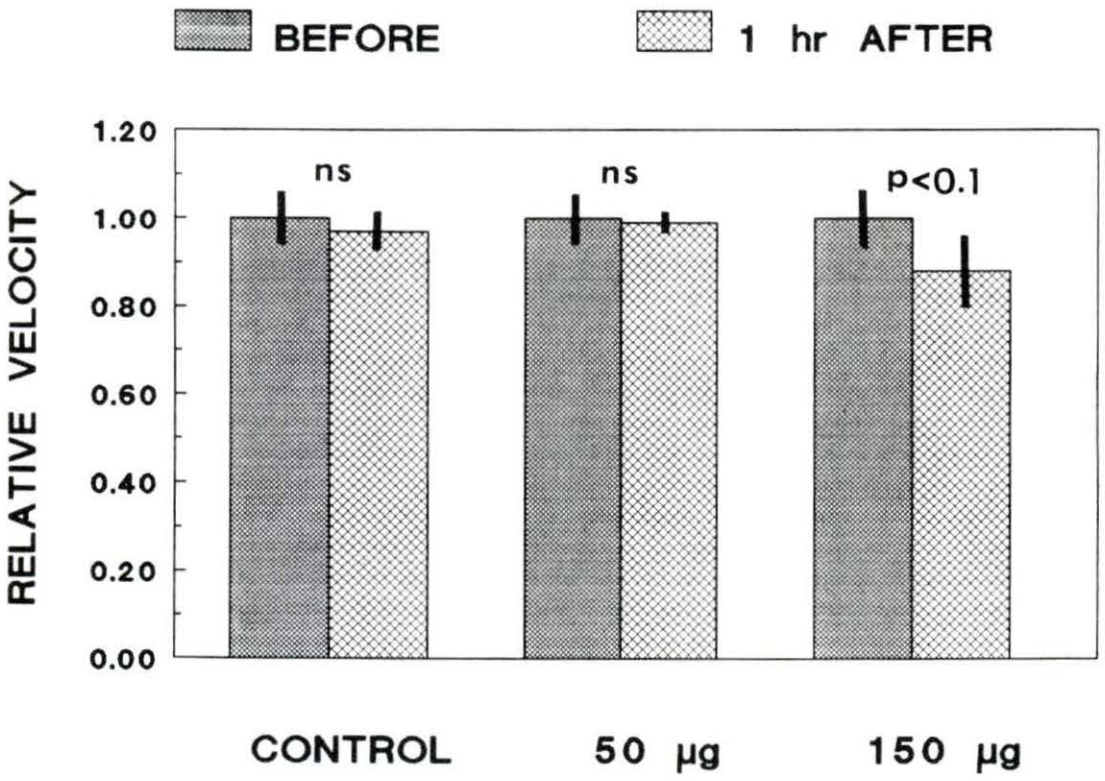

Figure 4. LGF velocity was reduced after 1 hr in the 150 µg-treated group.

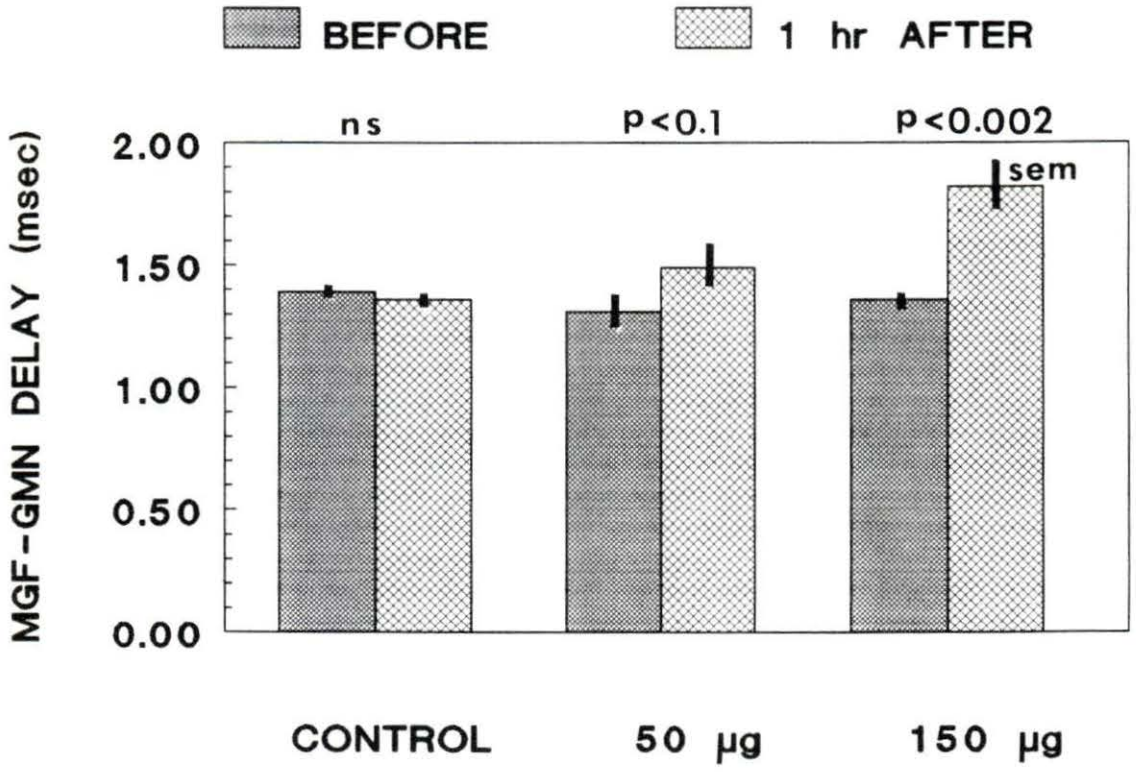

Figure 5. Concentration-dependent increases in the delay time from MGF to GMN spikes were seen in the two treated groups.

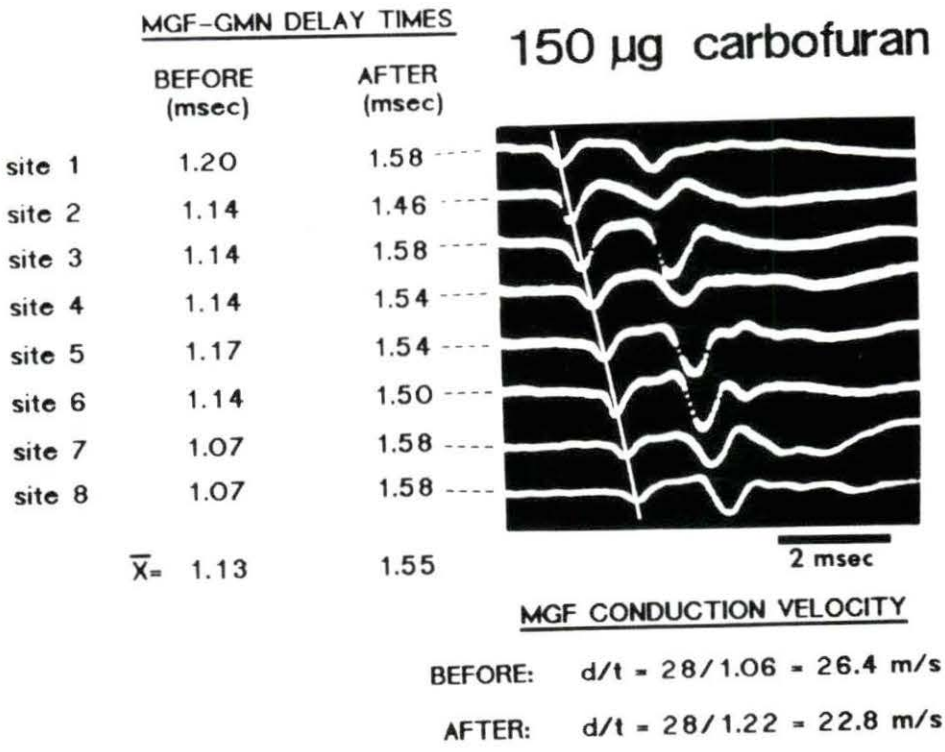

Figure 6. An eight-channel recording showed that the reduction in MGF velocity and the increase in MGF to GMN delay were uniformly distributed throughout the midbody region, rather than restricted to one or two recording sites.

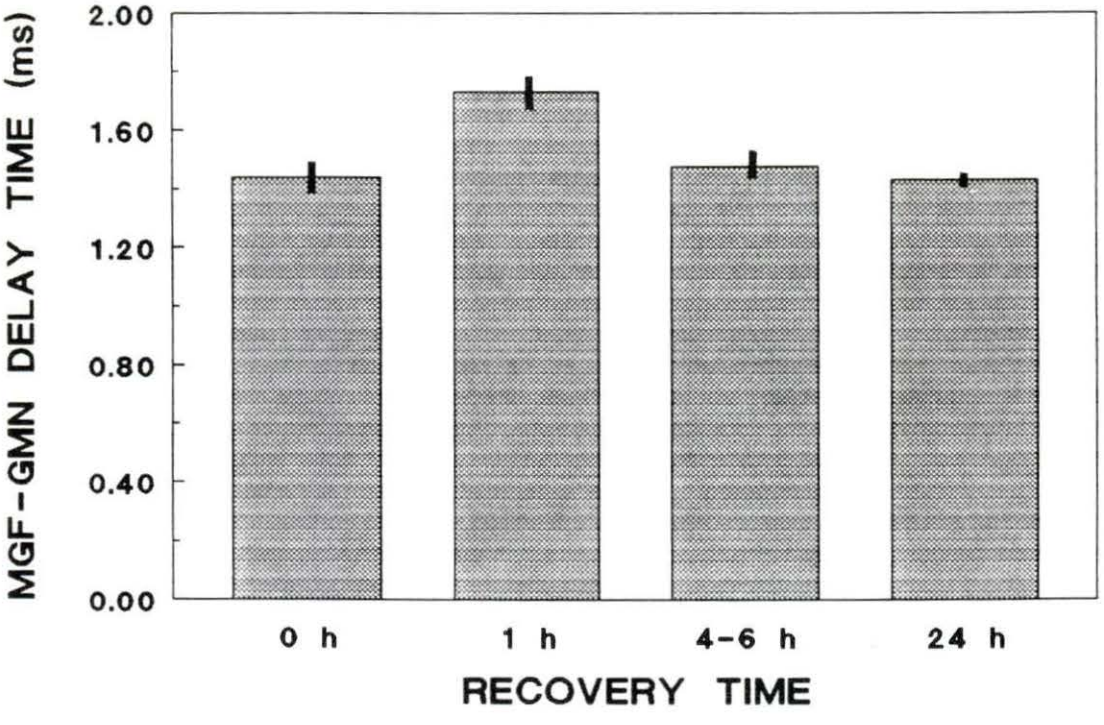

Figure 7. Recovery study on 150 μg-treated earthworms indicated complete recovery after 4-6 hrs.

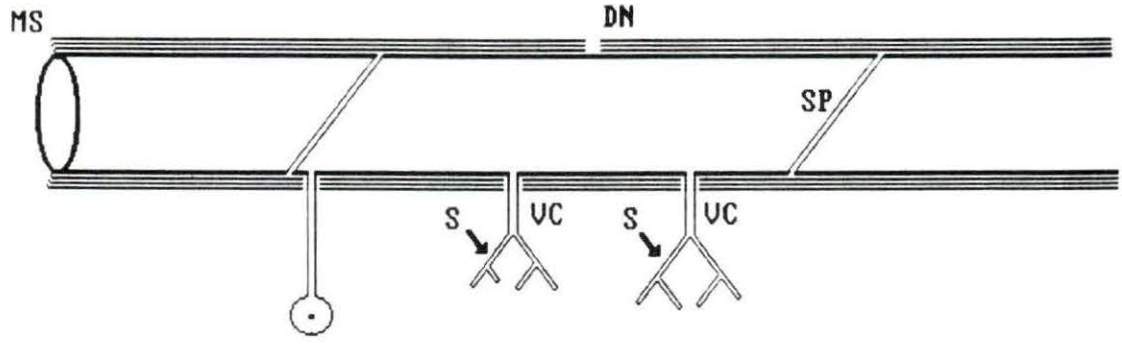

Figure 8. Slowing of giant fiber conduction velocity could occur by several cellular mechanisms, including: 1) decreased membrane resistance of ventral collaterals (VC), due to persisting effects at chemical synaptic inputs (S), 2) altered properties of voltage-sensitive spike channels at dorsal nodes (DN) that interrupt the myelin-like sheath (MS), or 3) increased resistance of gap junctions in septal partitions (SP).

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SECTION II. SPIKE DURATION IN OLIGOCHAETE GIANT NERVE FIBERS: SPECIES VARIATION AND TOXICANT EFFECTS

INTRODUCTION

Conduction velocity has proven to be a sensitive and reliable measure for assessing sublethal neurotoxic effects of pesticides and other pollutants on earthworms (Drewes et al., 1988). Importantly, this parameter can be assessed non-invasively, thus permitting the use of environmentally relevant methods of exposure, while maintaining the anatomical and physiological integrity of body compartments.

Several mechanisms have been suggested for conduction velocity effects in vivo. One mechanism involves a decrease in the membrane space constant, possibly resulting from decreased membrane resistance at any or all of the abundant synaptic inputs and outputs that occur sequentially along the axon membrane (refer to Section I). Such effects on membrane resistance could arise from 1) direct action of the toxicant on giant fiber membrane (e.g., opening of chemically-gated channels), or 2) indirect action of the toxicant (e.g., toxicant-induced release of neurotransmitter by neurons that are presynaptic to the giant nerve fibers). An alternative mechanism, for direct toxicant action, is an effect on voltage-gated channels on giant fiber membrane (Narahasi, 1980), resulting in altered kinetics of spike channel function. However, since conduction velocity

measurements would not necessarily reflect effects on spike channels, a measure that addresses this parameter, *in vivo*, is needed.

In this study, the utility of measuring the time-course of non-invasively detected spikes, from earthworm giant fibers, was analyzed. Specifically, the half amplitude duration of the all-or-none, medial and lateral giant fiber spikes was analyzed using modifications in previously described non-invasive recording procedures.

The objectives of this study were two-fold: 1) to determine whether there are species variations in half amplitude duration that may be related to known differences in conduction velocity (Drewes et al., 1983) and 2) to observe the effects of a selected compound (formaldehyde), that is known to alter the kinetics of spike channel function in giant fibers (Hille, 1984), in *Lumbriculus variegatus*, an aquatic oligochaete.

METHOD

Species Selection

Seven species belonging to the three orders of the Class Oligochaeta were selected (Table 1). This selection ensured not only a diverse grouping of taxonomic and ecological types, but also a wide range of non-invasively detected signal characteristics, such as spike amplitude and conduction velocity.

For large earthworm species (Order Haplotaxida), 8-10 sexually mature worms in each group were acclimated for 24 h at room temperature in covered boxes containing moist filter paper. For small aquatic species (Order Lumbriculida and Order Tubificida), worms were isolated for 24 h in distilled water before testing.

The aquatic oligochaete, Lumbriculus variegatus, was used for studying the effects of dilute formaldehyde on spike time-course. Some of the important considerations in selecting L. variegatus are the following: 1) Small body size and relatively thin body wall facilitates penetration of toxicant and minimizes treatment time; 2) Large numbers of specimens are obtainable from laboratory colonies; 3) Giant fiber spike amplitude is exceptionally large and

spikes are easily detected using non-invasive electrophysiological recordings (Drewes et al., 1990), thus permitting intra-animal comparison before and after treatment; and 4) Half amplitude duration of MGF spike can be assessed without interference from GMN spike potentials that follow each spike in most earthworm species (Drewes, 1984).

Conduction Velocity Measurements

Conduction velocity measurements were obtained from diphasic recordings, using an amplifier with capacitor-coupled, differential inputs, as described in the previous chapter. Measurements of MGF and LGF spike conduction velocity were made in the mid-body region of all worms; 8-10 replicate measurements were obtained per worm. In two species (Diplocardia sp.) lacking any previously published taxonomic and electrophysiological descriptions, a conduction velocity profile was also obtained by measuring the MGF and LGF conduction velocities in the anterior, middle, and posterior regions of the worm (Appendix).

Half Amplitude Measurements

In theory, each electrode in a diphasically recording

pair sequentially detects the moving wave of extracellular negativity caused by the inward positive currents associated with the depolarization phase of the propagating action potential. The resultant spike then appears diphasic when electrodes in a given pair are spaced at sufficient distance. However, the degree of temporal separation between these two phases varies in relation to both electrode spacing and conduction velocity. If the electrodes are relatively close, the activity recorded by the first electrode overlaps that of the second, resulting in partial cancellation of the two phases in the diphasic wave (Fig. 1A). If, however, electrode separation is sufficiently increased, this overlapping of phases does not occur (Fig. 1B), allowing measurements of spike amplitude and half-amplitude duration at each recording site. For optimal recordings from the various worm species, the two pairs of electrodes were separated 6-40 mm, depending upon species differences in body size, conduction velocity, and behavior.

To determine whether the half-amplitude measure from the extracellular record is similar to the time course of the intracellular spike, a comparison of extracellular and intracellular waveforms was made. An intracellularly

recorded MGF spike waveform (obtained from Fig. 2 by Kao et al., 1957) was superimposed, using a common time base, upon a non-invasively recorded extracellular spike (Fig. 2). Both waveforms had similar time courses. Though there appeared to be a slight attenuation of the rising slope of the extracellular recording (presumably due to low-pass filtering characteristics of the worm body wall), half amplitude measurements of the two spikes were nearly identical, indicating that half amplitude duration of non-invasively recorded spikes may be a useful measure for toxicant effects on spike generation processes.

Treatment Protocol

The chemical was selected for its known action on the inactivation process of Na channels. Formaldehyde inhibits the inactivation process of Na channels that occurs during the repolarization phase of the action potential. Toxicity studies were performed on L. variegatus by immersion exposure in treated water. Two different concentrations (0.075% and 0.025%) of formalin, with exposure time of 10 min, were used to study effects on conduction velocity and half amplitude duration. Both concentrations of formalin were eventually lethal to the worms.

RESULTS

Species Variation in Half-Amplitude Duration

Typical recordings used for measuring conduction velocity and half amplitude duration in L. terrestris are shown in Figs. 3 and 4. The recordings for a few other species are shown in the Appendix. The LGF spikes of all species showed a clear separation of the two phases of opposite polarity, making measurements of half amplitude duration relatively unambiguous. In MGF records, two monophasic waves were usually evident, though the phases were not as widely separated and the second phase was often truncated or obscured by the giant motor neuron spike potential that normally follows each MGF spike in earthworms.

The oligochaetes selected showed a wide variation in conduction velocity, ranging from 1.22 m/s (LGF in D. digitata) to 24.70 m/s (MGF in L. terrestris). Nevertheless, the half-amplitude duration of all MGF and LGF action potentials was always within a narrow range (0.43-0.64 ms), and appeared independent of conduction velocity (Fig. 5).

To determine whether the half amplitude duration of

spikes was related to spike amplitude, half amplitude measurements were compared to spike amplitude in L. terrestris, a species in which the spike amplitude can vary from 12-60 μV. It was evident that the half amplitude duration was independent of the spike amplitude (Fig. 6).

Toxicity Studies

Typical MGF and LGF waveforms in Lumbriculus variegatus, before and after treatment with formalin, are shown in Fig. 7. Table 2 summarizes the effects of formalin on half amplitude duration and conduction velocity, at two different concentrations. No statistically significant change in half amplitude duration of MGF and LGF spikes, was noted in the 0.025%-treated group. However, the MGF conduction velocity was significantly reduced by 10% in comparison to pre-treatment values. In the 0.075%-treated group, MGF velocity was reduced by 28.4% and LGF conduction velocity showed a 21.4% reduction (Fig. 7). Also, the half amplitude duration of MGF and LGF spikes was reduced by 20.0% and 18.6%, respectively.

DISCUSSION

Species Variation in Half Amplitude Duration

Despite the wide taxonomic variation in giant fiber conduction velocity, no species variation in half amplitude duration was noted (Fig. 5). Assuming that the half amplitude duration in all species accurately reflects the time course of the intracellular spike (as was the case in L. terrestris, Fig. 2), then these results suggest a fundamental similarity in the spike generating mechanisms (e.g., spike channel kinetics) in the seven worm species. Such an evolutionary conservation of physiological properties is not unexpected since the medial and lateral nerve fibers in these species are viewed as anatomically conserved homologs throughout the Class Oligochaeta (Drewes, 1984).

Toxicity Studies

Effects of low concentrations of formalin on conduction velocity, in Lumbriculus variegatus, occurred without any significant effect on half amplitude duration, indicating that conduction velocity is a more sensitive parameter for detecting the central nervous system effects of formalin.

This result further supports the idea (Fig. 5) that the two parameters (conduction velocity and half amplitude duration) are independent of one another.

How could formalin change conduction velocity without affecting half amplitude duration? One possibility is that treatment with low concentrations of toxicants could induce a decrease in membrane resistance due to direct effects (e.g., opening of chemically-gated channels) or indirect effects on presynaptic neurons (e.g., release of neurotransmitter that opens chemically-gated channels). A reduced membrane resistance would then account for a reduced membrane space constant and conduction velocity.

The additional effect of formalin (at higher concentrations) on the half amplitude duration might be explained by a direct effect on the active membrane properties (i.e., voltage-gated channels on giant fiber membrane). This combination of results suggests that spike channels are less vulnerable, or perhaps less accessible, to toxicant action than chemically-gated channels on the giant nerve fibers.

The usefulness of any parameter, for quantifying neurotoxic effects, can be assessed by considering both the relative sensitivity to toxicity effects and inherent

statistical variability in the parameter. In control worms, the variability of half amplitude duration measurements was low (usually < 5%) as compared to 12% for conduction velocity. However, for detecting and quantifying sublethal neurotoxic effects of formalin as well as other chemicals, half amplitude duration may not be as sensitive a measure as conduction velocity.

Table 1. Species selected for half-amplitude measurements

SPECIES	SOURCE
Order Haplotaxida	
Family Lumbricidae	
Lumbricus terrestris	USDA Tilth Lab
Aporrectodea trapezoides	USDA Tilth Lab
Family Megascolecidae	
Perionyx excavatus	Drewes, C. D.
Family Acanthodrilidae	
Diplocardia sp. (medium)	Iowa
Diplocardia sp. (small)	Oklahoma
Order Lumbriculida	
Family Lumbriculidae	
Lumbriculus variegatus	Drewes, C. D.
Order Tubificida	
Family Naididae	
Dero digitata	Drewes, C. D.

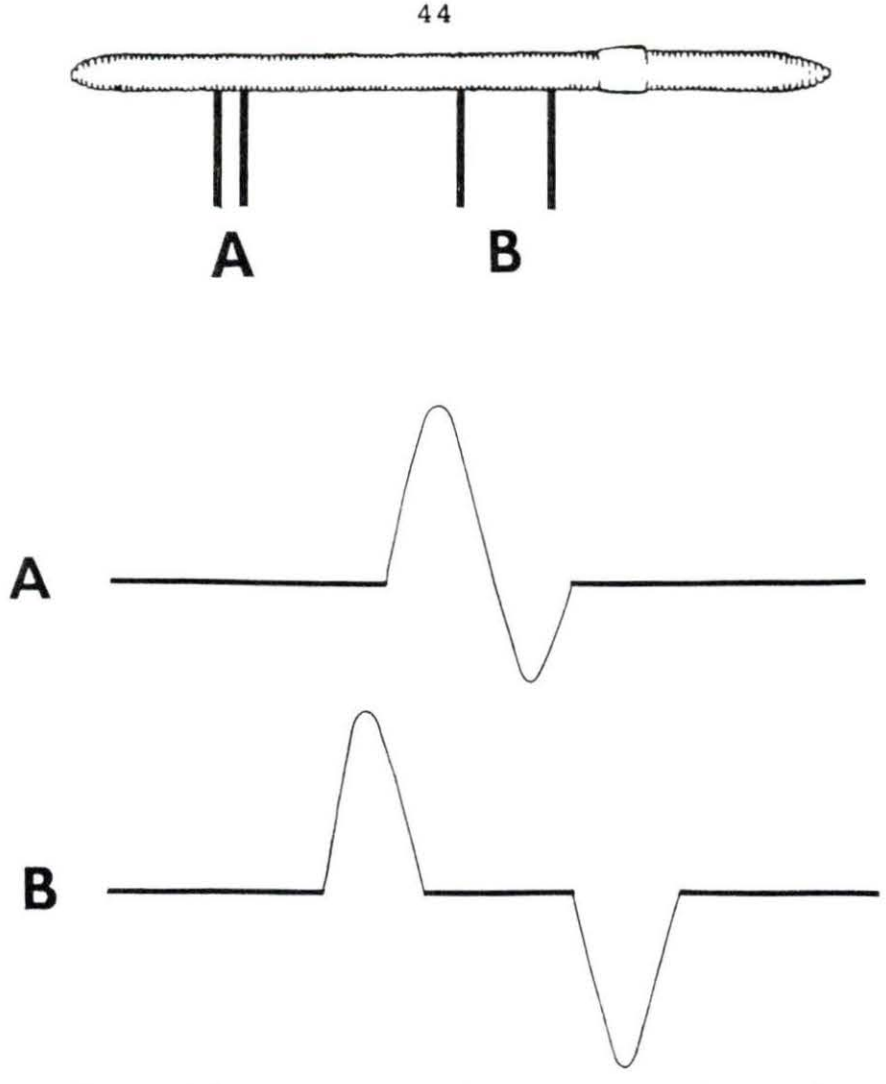

Figure 1. Theoretical alternative for configuration of extracellular recording electrodes. A) Recording electrodes are closely spaced to give a nearly diphasic recording of the action potential. B) Recording electrodes are spaced further apart to give a recording that appears as two separated monophasic waves of opposite polarity.

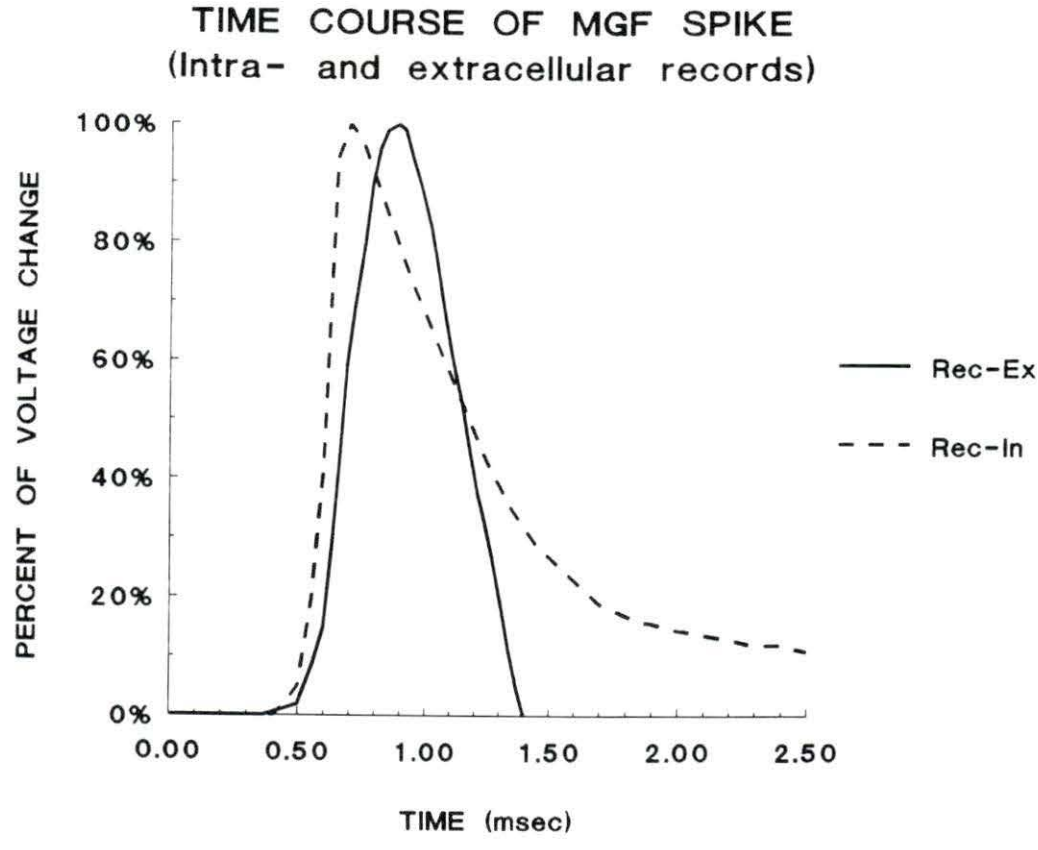

Fig. 2. Comparison of extracellular (Ex) and intracellular (In) waveforms. Note that half amplitude durations of the two waves are similar and the greatest difference between the two waves is that the depolarizing after-potential in the intracellular record is not reflected in the extracellular record.

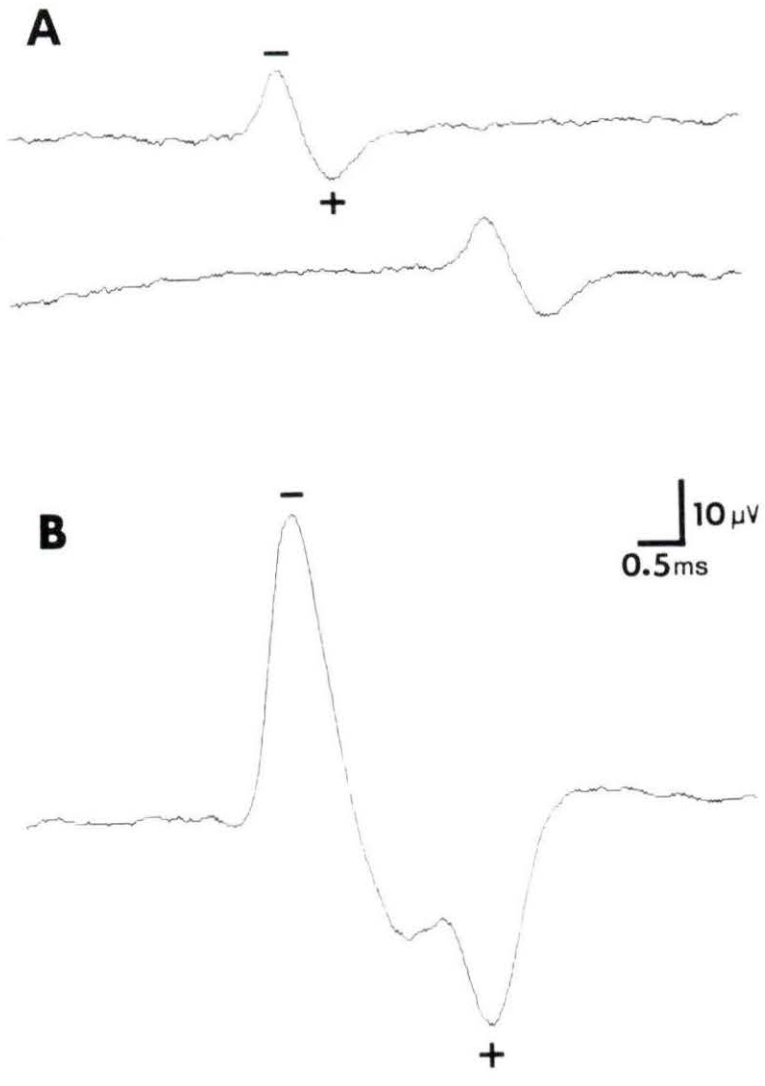

Fig 3. Lumbricus terrestris LGF records. A. Waveforms used for conduction velocity measurements. B. Waveforms used for half-amplitude duration measurements.

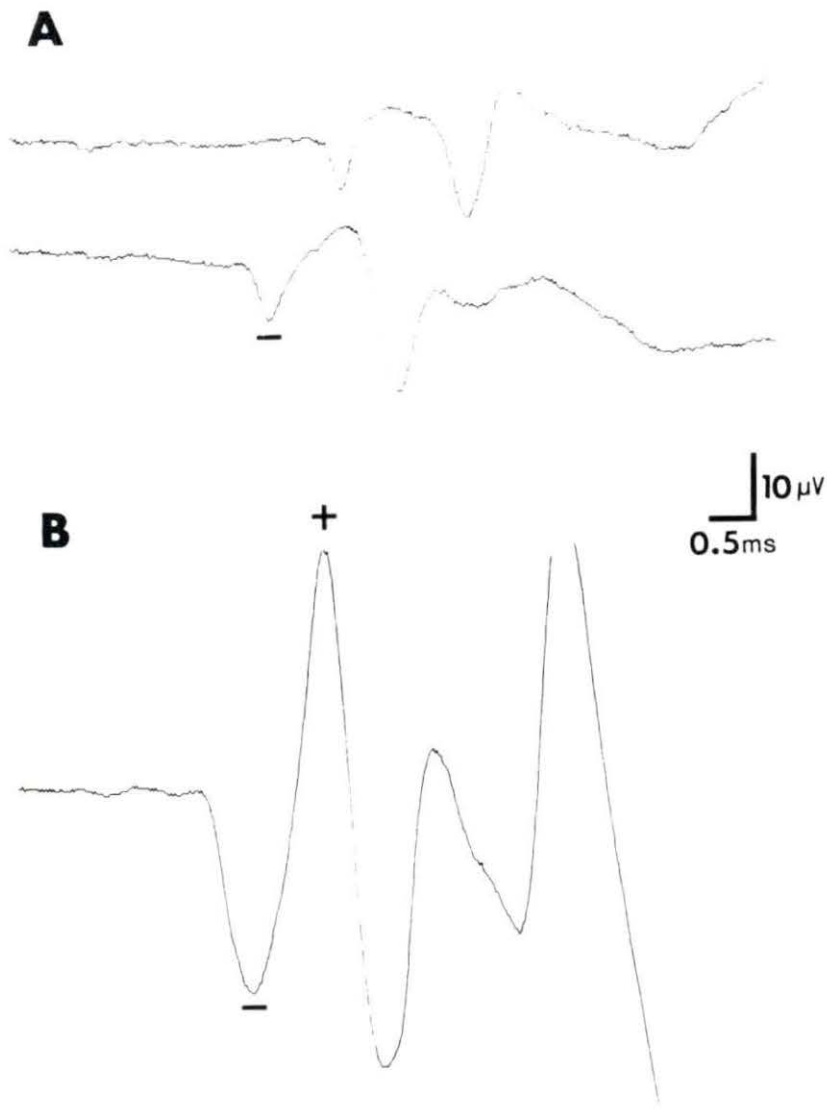

Fig 4. Lumbricus terrestris MGF records. A. Waveforms used for conduction velocity measurements. B. Waveforms used for half-amplitude duration measurements.

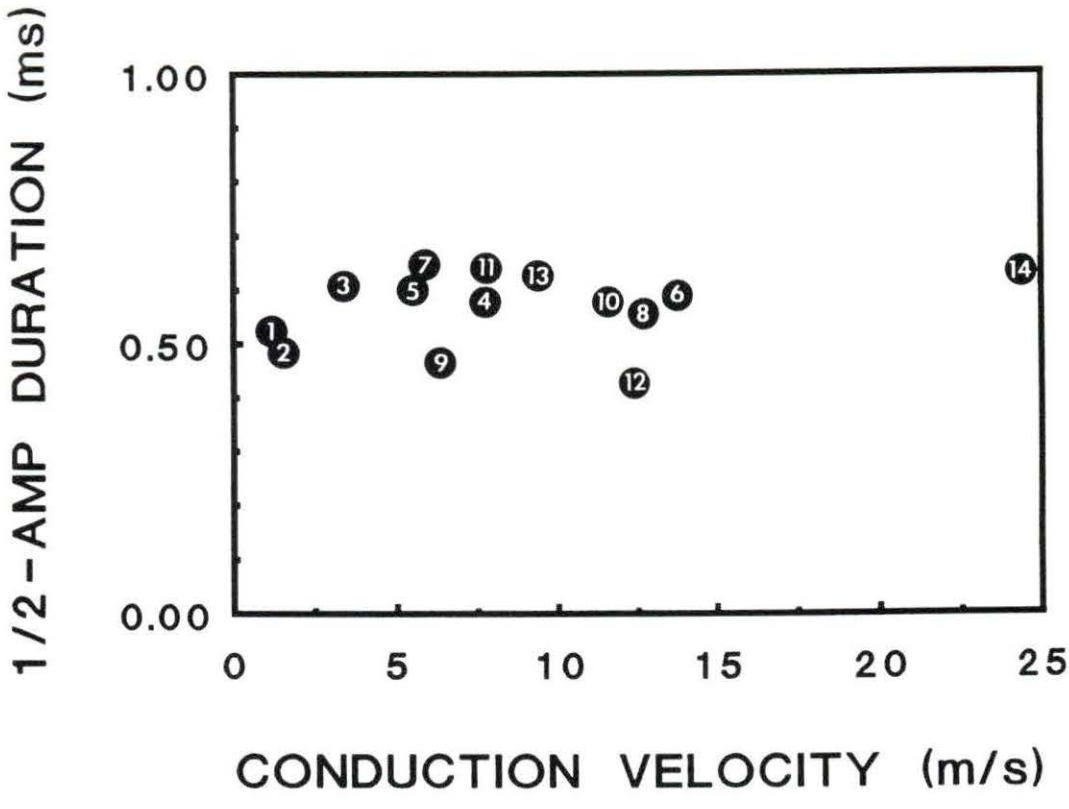

Fig 5. Half amplitude duration versus conduction velocity for seven species of oligochaetes. Dero digitata (1,2); small Diplocardia sp. (3,4); medium Diplocardia sp. (5,6); Aporrectoda trapezoides (7,8); Lumbriculus variegatus (9,10); Perionyx excavatus (11,12); and Lumbricus terrestris (13,14). Odd numbers correspond to LGF and even numbers correspond to MGF. Each point represents a mean of 8-10 worms.

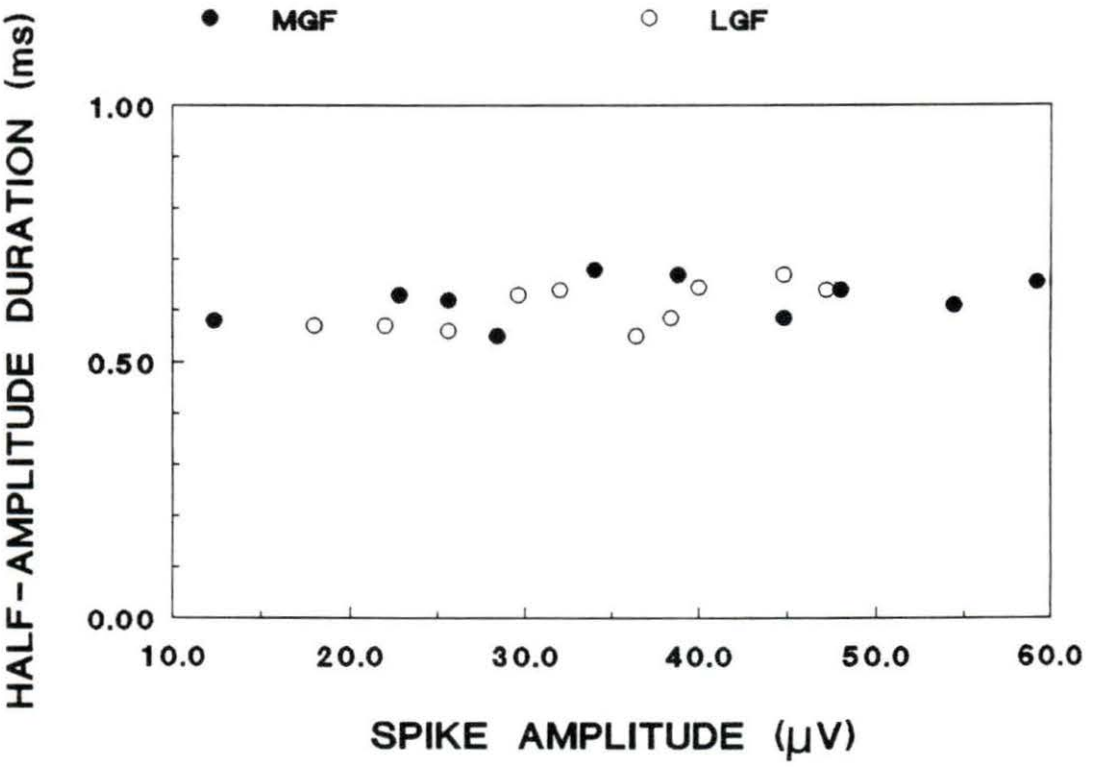

Fig 6. L. terrestris half amplitude duration versus spike amplitude.

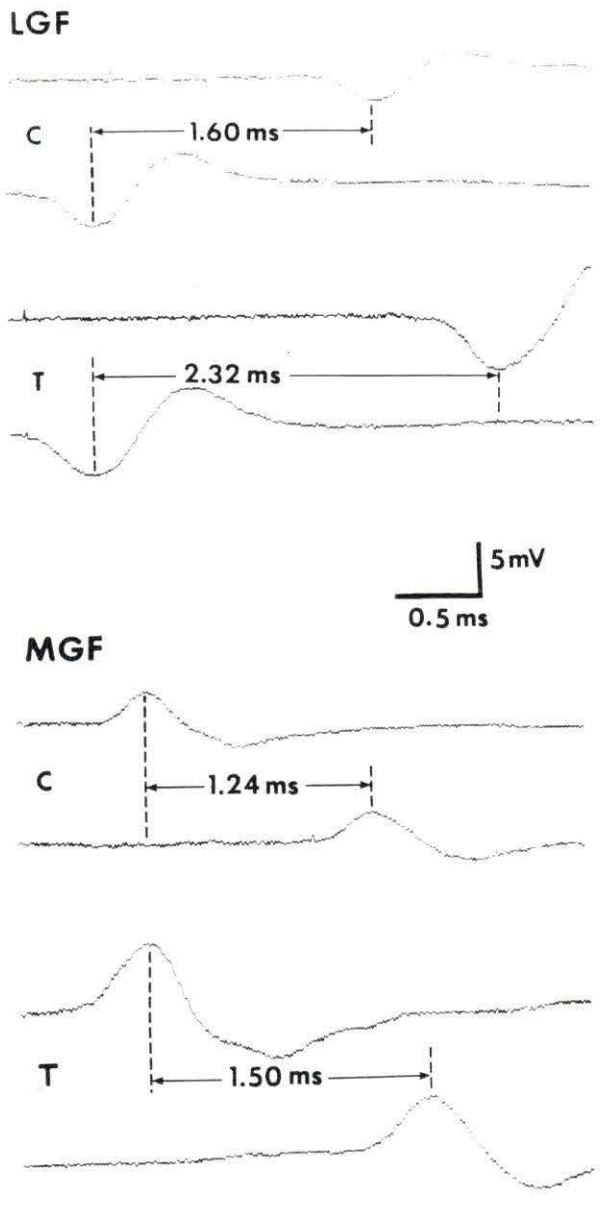

Fig 7. Diphasic records showing MGF and LGF conduction velocity measurements. C-control (before treatment) and T-after treatment.

Table 2. Summary of effects of two concentrations of formalin on conduction velocity (CV) and half amplitude duration (1/2 AD) in L. variegatus.

1) Formalin (0.025%)

	MGF		LGF	
	CV (m/s) mean ± SEM	1/2 AD (ms) mean ± SEM	CV (m/s) mean ± SEM	1/2 AD (ms) mean ± SEM
BEFORE	8.580 ± 0.209	0.557 ± 0.005	5.700 ± 0.250	0.436 ± 0.007
AFTER	7.725 ± 0.192	0.565 ± 0.005	5.750 ± 0.182	0.438 ± 0.007
prob	$p \leq 0.01$	ns	ns	ns

2) Formalin (0.075%)

	MGF		LGF	
	CV (m/s) mean ± SEM	1/2 AD (ms) mean ± SEM	CV (m/s) mean ± SEM	1/2 AD (ms) mean ± SEM
BEFORE	8.420 ± 0.250	0.550 ± 0.004	5.270 ± 0.102	0.435 ± 0.004
AFTER	6.030 ± 0.290	0.660 ± 0.034	4.146 ± 0.150	0.516 ± 0.017
prob	$p \leq 0.001$	$p \leq 0.02$	$p \leq 0.001$	$p \leq 0.02$

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GENERAL SUMMARY

Results from section I indicate that single-granule, one-hour treatment of Lumbricus terrestris with carbofuran results in two distinctly different and reversible effects within the worm's central nervous system. These include reduced conduction velocity in giant nerve fibers and reduced efficacy of functional coupling between the medial giant fiber and its associated giant motor neurons.

Results from section II indicate that half amplitude duration is a highly conserved parameter, that is independent of conduction velocity differences, in a wide variety of oligochaete species. Although this parameter was found to increase in Lumbriculus variegatus after 10 min treatment with dilute formalin, conduction velocity effects occurred at even lower concentrations and therefore appeared to be a more sensitive indication of formalin neurotoxicity effects.

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I would also like to thank my parents and Naresh who made this task worthwhile and meaningful.

APPENDIX

Diplocardia sp. velocity profile in the anterior (A), middle (M), and posterior (P) regions of the worm:

1. Diplocardia sp. (small)

MGF velocity (m/s) mean ± SEM			LGF velocity (m/s) mean ± SEM		
A	M	P	A	M	P
7.15 ± 0.227	5.97 ± 0.229	3.88 ± 0.193	2.78 ± 0.279	3.19 ± 0.209	3.28 ± 0.144

2. Diplocardia sp. (medium)

MGF velocity (m/s) mean ± SEM			LGF velocity (m/s) mean ± SEM		
A	M	P	A	M	P
14.99 ± 0.355	13.60 ± 0.500	10.21 ± 0.407	5.06 ± 0.204	5.53 ± 0.294	5.56 ± 0.255

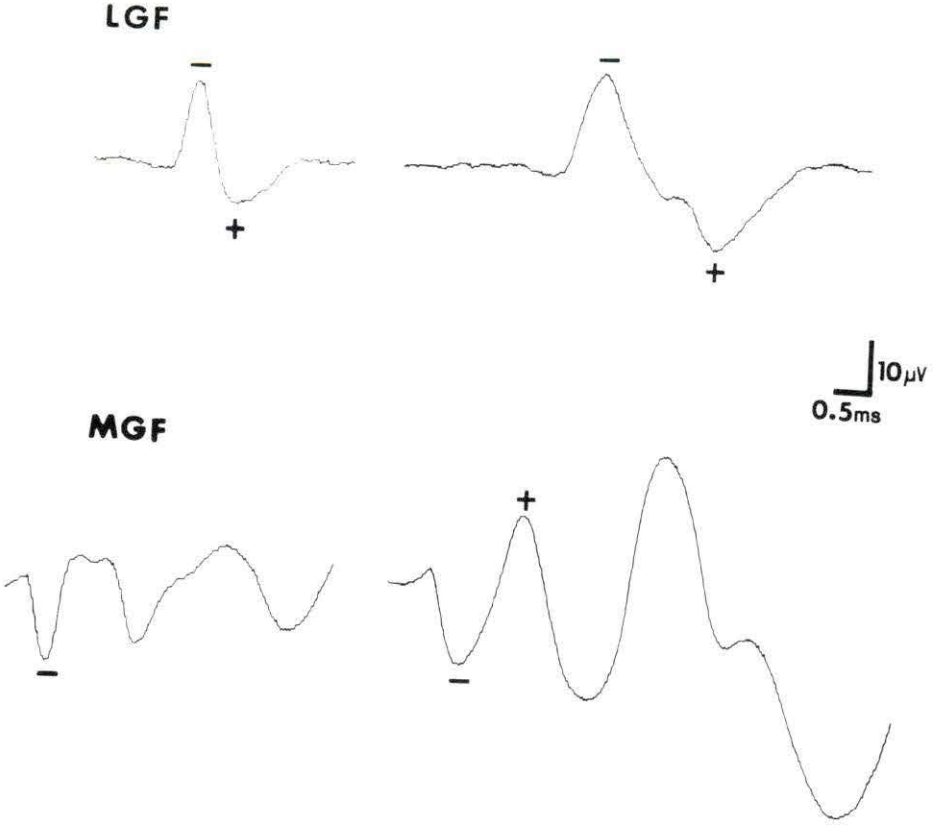

Fig 8. Diplocardia sp. (medium) LGF and MGF records. Conduction velocity measurements (left) and half amplitude duration measurements (right).