

Influence of Potential Difference and Current on the Electrotaxis of *Caenorhabditis elegans*

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Abstract: *C. elegans* responds directionally to a DC current. The response may be to the anode or cathode, depending on the current, potential difference, and ionic concentration of KCl. Tracks of the responding nematodes show that electrotaxes are genuine orientation phenomena. The directional movement is not due to the passive movement of nematodes or to the influence of currents on the muscular physiology; electrotaxes are mediated sensorily. Details of the response are described. **Key Words:** behaviour, galvanotaxes, electric current, electric potential.

When placed in an electric current, some nematodes show an electrotaxis (or galvanotaxis), accumulating at one electrode or the other (1, 2, 3, 17). Accumulation is usually at the cathode (1, 2, 8, 11, 14), though not always (3, 9, 17). While electrotaxes have been reported from a variety of nematodes, the phenomenon has not been examined in *Caenorhabditis elegans*, despite the wide use of that species as a behavioural model (4, 5, 16). Furthermore, nematode-tracking techniques have not been applied to the study of electrotaxes, so that the mechanism of orientation, if any, is unknown. Indeed, the apparent 'taxis' may in fact be a 'kinesis' (3).

Various theories about the nature of the phenomenon include: sensitivity to current strength (2, 10) or potential difference (3), or that affinity for an electrode depends on the density of cations (14). It may also be that passive electrophoresis or the direct effect of electric fields alters neuromuscular contraction (3).

This paper reports on the electrotaxis of *C. elegans* at different current strengths and potential differences. It also presents tracks of responding nematodes (4, 16).

MATERIALS AND METHODS

Five-day-old *C. elegans* (young adults) were used throughout. They were cultured on lawns of *Escherichia coli* (4) bacto-agar at 20 C (± 1). All experiments were conducted at 21 C (± 1.5 C). *C. elegans* were

exposed individually to currents on agar between two platinum electrodes placed 3 cm apart in a 5-cm-diameter plastic petri dish. The nematodes were exposed to currents from 0.02 to 0.04 mA and potential differences from 2 to 6V.

A DC current generated from three batteries connected in series to give 18V was regulated by a variable-resistance rheostat. The electrolysis medium was made of Difco Noble agar hydrated with distilled water or KCl solution in a proportion of 1.25 g per 100 ml solvent. Six-tenths ml of freshly prepared agar was poured into sterile plates to an average depth of 2.0 mm. The eight current strengths used under a fixed potential difference (PD) were obtained by varying the KCl concentration and the agar depths. KCl electrolysed for 6 min is not toxic to nematodes (6). Current strengths above 0.1 mA at 2V could not be obtained. KCl concentrations used for the PD values are given in Table 1. Before the test, the nematodes were incubated overnight on agar with bacteria that had been hydrated with a KCl concentration equal to that to be used in the test. This minimised sudden change in the ionic environment in an attempt to ensure that the only change felt would be from the current (15).

Nematodes were placed individually on the plates with a mounted bristle. There was often only a moment for them to recover from the transfer, but they quickly recovered and proceeded to move normally. That never took more than 5 min, and when they were moving normally the current was applied. Movement proceeded for 6 min and then the *C. elegans* was removed to a plate without current. The tracks on the plate were then quickly photographed (4, 16).

Twenty individuals were used for each combination of current and PD. In addition

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TABLE 1. Mean total electrotactic response of *C. elegans* to various combinations of currents and potential difference.^a

P D (V)	Current (mA)							
	0.02	0.04	0.06	0.08	0.1	0.2	0.3	0.4
2	+45 ^b	+ 9	-25 ^b	-51				
3	+47 ^b	+ 8	-72 ^b	-70 ^b	+21	- 78 ^b	-13 ^b	+30 ^b
4	+ 5	- 9	+20	+14	-47 ^b	-110	-18	-41 ^b
5	+ 9	+ 2	+17	+17	- 1	+ 29 ^b	-58	+10
6	+18	+27 ^b	+ 7	+11	-36 ^b	+ 26 ^b	+46	+15 ^b

^a20 individuals for each value.

^bDiffers ($P = 0.05$) from the unstimulated control using the Mann-Whitney non-parametric test.

to recording the tracks, the exact position of the nematode was quantified in each test. The entire field area between the electrodes (3 cm) was divided into 10 parallel zones of 3 mm width. The two middle zones, where the individuals were placed was considered to be a 'no-worm's land'. Those nematodes moving into all other zones were scored + or - 5 for every zone, depending on whether they moved towards the anode or cathode end. Thus each combination could score both a cathodal and an anodal response. These could be used to calculate a total response (Table 2).

RESULTS

At certain current/PD combinations electrotaxes were observed. Eight significant anodal (0.05 using Mann-Whitney non-parametric test) and 13 cathodal migrations occurred (Tables 1 and 2). Lower currents and also high current with high PD stimulated anodal responses (Table 1). Medium current strengths caused cathodal electrotaxes. A few individuals were irreversibly immobilised at 6V or 0.4 mA, presumably electrocuted.

Figs. 1 to 6 show a selection of tracks which illustrate additional points; 7 is a

control. Unstimulated controls in sterile conditions move forward in loops and spirals; the spontaneous reversal rate is usually low (Fig. 7), but can occasionally be higher. The other tracks show individuals responding with direct movements to the electrodes (4). Disruptive reversals during orientation are very low with only infrequent spontaneous reversals. None of the tracks lead all the way to the electrodes; about 2 mm from the electrode each individual increased reversing (Figs. 5, 6), or reversed continuously at a certain constant and critical distance from the electrode (Figs. 3, 6). Once in this position, *C. elegans* did not migrate to the pole but kept moving at the same distance from the electrode. In many cases (e.g., Figs. 3, 6) strangely-shaped tracks were inscribed that were composed of straight sections and obtuse angles caused by reversal movements. The initial response was not characterised by high reversal rates and if the initial direction was 'wrong', the individuals moved forward in a smooth circle (e.g., Figs. 3, 5).

DISCUSSION

C. elegans adults move directionally at selected combinations of voltage and po-

TABLE 2. Total mean cathodal (C) and anodal (A) responses which were significantly different ($P = 0.05$) from the unstimulated control.

P D (V)	Current (mA)							
	0.02	0.04	0.06	0.08	0.1	0.2	0.3	0.4
2	A		C	C	C			
3	A	A	C	C		C	C	A
4					C	C	C	C
5						A	C	
6		A			C	A	A	A

TABLE 3. Potassium chloride concentrations (g/litre) used to obtain various combinations of currents and potential differences used in Tables 1 and 2.

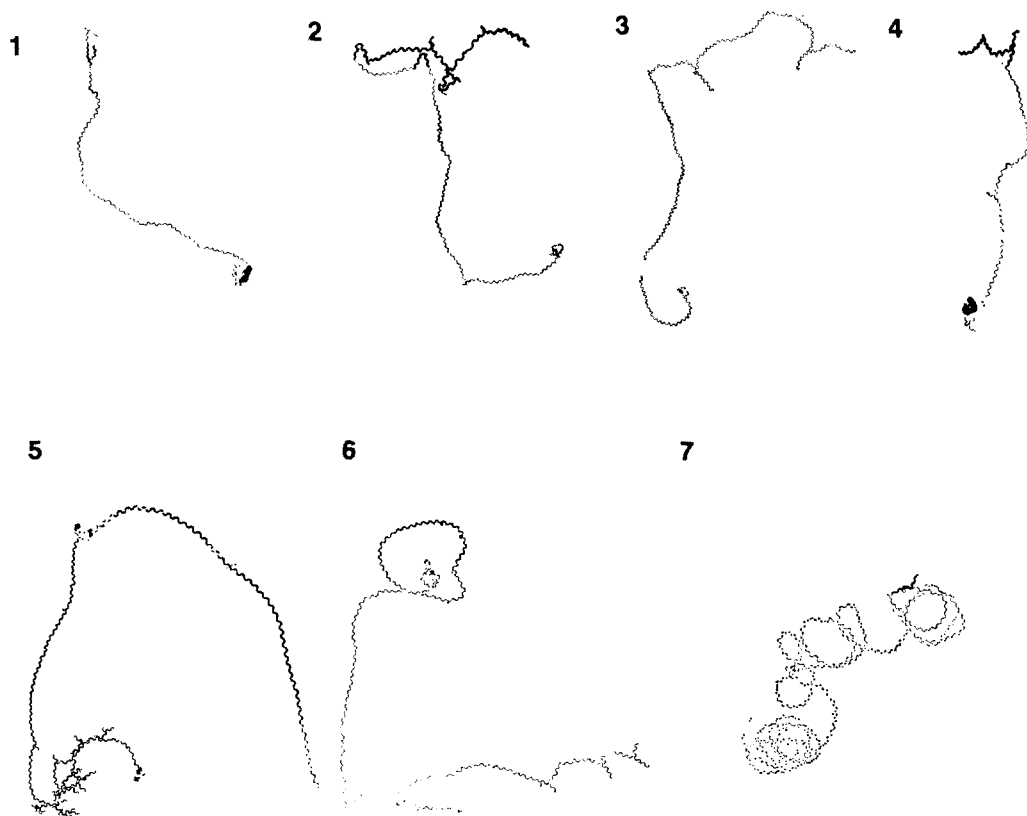
P D (V)	Current (mA)							
	0.02	0.04	0.06	0.08	0.1	0.2	0.3	0.4
2	1	3	4	6	6			
3	0.5	0.8	0.8	2.0	3.0	4.0	4.5	5.0
4	0.1	0.2	0.2	0.4	1.0	2.0	3.0	4.0
5	0.05	0.05	0.1	0.1	0.2	0.5	1.5	2.0
6	H ₂ O	0.1	0.1	0.1	0.2	0.5	1.0	2.0

tential difference. This is the first study in which nematodes responding to currents have been tracked, and it is clear that electrotaxes are genuine phenomena (14, 15) and that electrophoresis of the nematodes can be ruled out. Furthermore, because normal rhythmic waves are used to propel responding individuals, electrotaxes are not due to the action of a direct current on neuromuscular contractions of the nematodes. The results indicate that the response is a sensorily-mediated orientation response

to the current or some product of the current.

The role of ions, particularly the cations, as mediators of electrotaxes was proposed by Sukul *et al* (14). Stringfellow (13) reported that hydroxyl ions attract males of *Pelodera strongyloides* and *C. elegans* responds to a series of salts (16). The muscle larvae of *Trichinella spiralis* showed 'tactic' responses to both K⁺ and Cl⁻ or to both (10).

At lower currents, the anode goes into solution and then positively charged metal



FIGS. 1-7. Tracks of individual *Caenorhabditis elegans*. All tracks on agar for 6 min at 21°C, between platinum electrodes. Electrodes 3 cm apart. Fig. 7 is an unstimulated control without current, all others have current, cathode at the top, anode at the bottom.

ions may attract the nematodes, hence anodic electrotaxis at lower currents. Klingler (12) observed that copper anodes were more attractive than platinum (the former also dissolve more rapidly). The agar is known to contain traces of SO_4^{-2} which evolves O_2 at the anode which may also be an attractant at high currents for the anode. Furthermore, the water molecules are carried to the anode by the chloride ions (electroendosmosis). A moving nematode would experience a lower concentration gradient of the electrolyte towards the cathode and would move to the anode.

Cathodal movements occurred most significantly at 3 to 4 volts. That may be due to the decomposition potential of KCl, or 4V (7). At that potential there is a steady discharge of K^+ and Cl^- at the electrodes. The nematodes may be able to sense a steady mobility of the ions under these potential differences and may be guided by the cations to the cathode (14). At different currents those factors alter, and that may be perceived by the nematodes. The extent of ionic mobility is altered also by the concentration of electrolytes, which, in these experiments, was altered to obtain various voltages and current strengths. The close association of the ionic and electric parameters suggests that electrotaxes are associated with ions. We have found nothing which contradicts Sukul et al. (14) that the ionic changes may be perceived by the amphids.

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