

Electrotaxis of *C. Elegans* in Time-varying Fields

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Abstract

The nematode *C. elegans* actively crawls toward the negative pole of an electric field at some acute angle to the electric field lines. We sought to gain an understanding of the effects of time-varying electric fields on electrotaxis in *C. elegans*. Worms were subjected to rapid shifts in the electric field of varying sizes (30, 60, 90 and 120-degrees) We quantified the behavioral response to these shifts by grouping it into turns, reversals or stalls and a correlation between the size of the angular shift and reversal frequency was observed. To date, the correlation between sensory input and motile output in *C. elegans* has not been fully understood. We have succeeded in identifying specific interneurons and motor neurons that are involved in coordinating the worms' behavioral response during electrotactic navigation. This was done both by performing laser ablations on individual neurons in transgenic strains of the worms and by utilizing various mutant worms.

Introduction

Caenorhabditis elegans (*C. elegans*) is a nematode worm that is 1 mm in length. The structure and connectivity of its entire neural network has been mapped out by reconstructing serial sections of electron micrographs (1). Its network has been shown to contain only 302 neurons that are arranged in a very invariant pattern from worm to worm. *C. elegans* neural circuit consists of the sensory neurons, the interneurons that are involved in the computation of sensory input information, and the motor neurons that stimulate the muscles directly. The simplicity of this nematode's neural network has made it an ideal model organism for the study of behavioral and neural biology for many years. Despite its simple neural circuit, *C. elegans* display varying complex behavioral responses to different types of external stimuli, which include; chemotaxis, its response to chemical gradients, thermotaxis its response to temperature, barotaxis, its response to pressure and electrotaxis, its response to electric fields.

Sukul and Croll discovered Electrotaxis in 1978 when they observed that *C. elegans* actively crawl toward the negative pole of an electric field provided by a battery (2). Previous experiments conducted in our lab have both confirmed their observations and revealed a more complete understanding of how individual worms approach the negative pole. These initial studies delineate the worms' response to direct electric fields. Building on this, our investigation focused on gaining a more thorough understanding of electrotaxis by observing the worms' behavioral response to angular shifts or step rotations of the electric field. It is interesting that despite the simplicity of *C. elegans* neural network, it is able to display very complex behavioral responses to external stimuli. An understanding of how and why this happens is based on identifying the correlation between sensory input and observed motile output in the organism. A comprehension of this relationship, particularly the role the neural network plays in coordinating the observed behavioral responses, can serve as a useful model for

understanding similar correlations between behavior and sensory input in more complex organisms in the future. We strove to comprehend this relationship by studying the roles that specific neurons play in coordinating the observed electrotactic navigation of *C. elegans*, and this was done using both mutant worms and worms in which target neurons had been surgically ablated.

Materials and Methods

C. elegans were grown on cultivation plates, which are agar discs that had been coated with a lawn of the strain OP50 of *E. coli* bacteria (3). In each experiment, individual young adult worms were picked off the cultivation plates, rinsed in NGM buffer, (Nematode Growth Medium) and then placed on a foodless agar disc (1L distilled water, .25 mM NaCL, 4.6 g glycerol, 17 g Bacto-Agar, Difco). The worms were observed for several seconds while in the buffer and after initial transfer to the disc to ensure that there was normal motility and no obvious physical injury that could have occurred during transfer.

The agar disc was then placed on a platform in the middle of experimental apparatus shown in Figure 1. This platform supported the agar disc in a way that enabled it to be immersed in a salt solution up to its rim. The salt solution in the bath had the same salt and glycerol concentrations as the disc. The bath was constantly circulated during the experiments to prevent the temperature of the gel from rising due to Joule heating. The voltage was applied to the gel by four platinum wires that ran along the edge of the box. The direction and amplitude of the electric field during the experiments was monitored using four platinum electrodes that were inserted into the surface of the agar gel.

A CCD camera, equipped with a zoom lens that was placed above the apparatus recorded the worm movement on the disc surface during the experiments. A ring of superbright LEDs illuminated the surface of the disc producing a high contrast dark field image of the worms. Images were captured with a PCI frame-grabber (National Instruments). The recorded videos were analyzed by eye in slow motion in order to score the behavioral responses of individual worms using Lab View (National Instruments).

For a typical trial, 10–12 worms were placed on a plate. Step rotations of the electric field by angles ranging from 30 to 120 degrees were applied to worms at 60–second intervals. Rotation of the shifts was alternated between the left and right to average out any directional bias in the worms' behavior. Typically, 15–20 field shifts were applied to each plate of worms. Worms that failed to produce the correct angle were discounted as these failed to do electrotaxis. In order to determine whether the mutants that we tested (see Table 1) performed electrotaxis, we simply applied a continuously rotating anti-clockwise electric field to them. The fields were rotated for one minute, two minute and three minute intervals.

During our experiments, we utilized various strains of *C. elegans* which include; the wild type strain N2, the mutant strain *ttx-3(ks5)* obtained from the *C. elegans* Genetics Center, a transgenic strain *nmr-1::gfp* which labels the AVA and RIM neurons (gift from A. Marcic), a transgenic strain *ttx-3::gfp* which labels the AIY neurons (gift from P. Sengupta), and the *GLR-1 (A/T)* mutant gift from A. Marcic). Additionally, we obtained a wide variety of mutant worms (see Table 1) from the *Caenorhabditis* Genetics Center.

Individual neurons were ablated using a cavity-dumping Ti:sapphire femtosecond laser (KM labs, Boulder, Col.). Using this laser, a single ablation pulse was delivered and the cell bodies and associated axons of the targeted neurons were destroyed. The neurons were identified and ablated in the L1 larvae and the behavior of the surgically operated worms was tested in the young adult stage no more than 72 hours after the surgery. Post surgery worms were tested individually and later re-imaged using microscopy to ensure that the targeted neurons were indeed destroyed. Controls that were subjected to the same conditions as the laser surgery worms with the exception that no ablations were performed, were also tested.

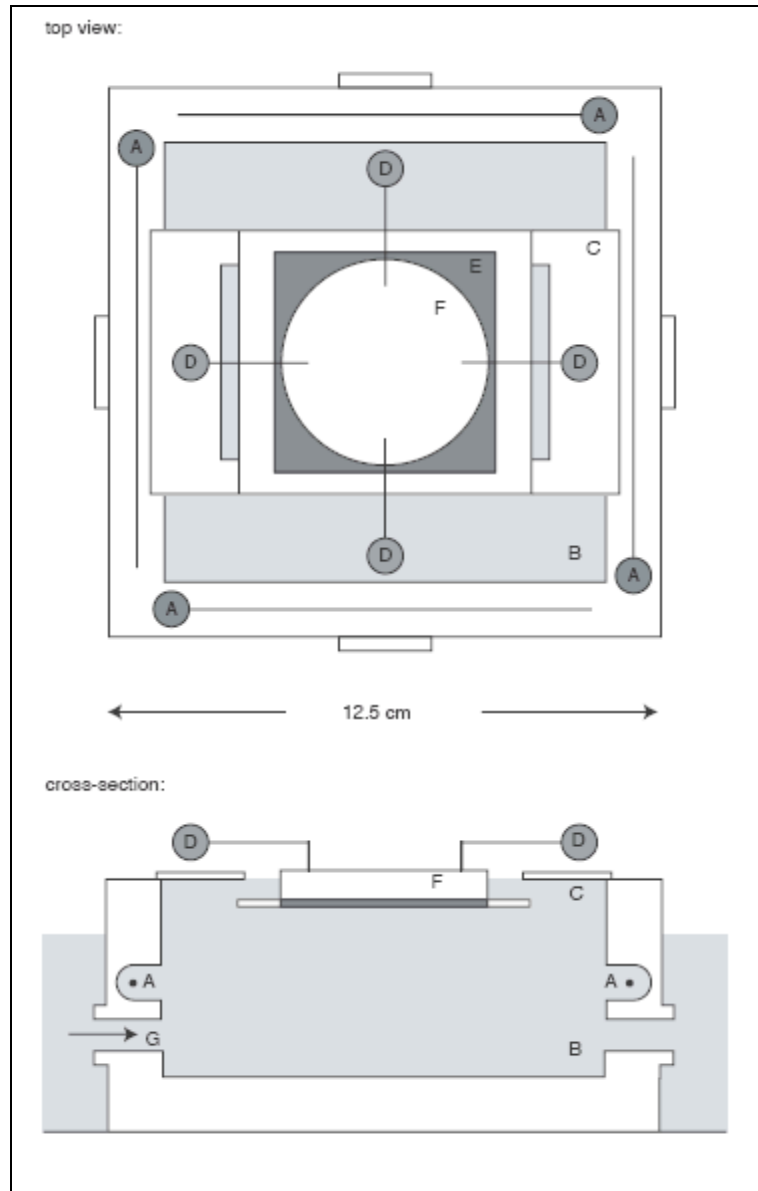


Figure 1: Diagram showing setup of experimental apparatus. A: Platinum wires, B: Salt solution, C: Supporting platform, D: Platinum electrodes E: Non-reflective surface, F: Agar disc, G: Opening for circulation of solution.

Results

The wild type N2 worms were subjected to step rotations of the electric field. This involved rotating the direction of the field by a set angle either clockwise or anti-clockwise at sixty-second intervals. The behavioral responses of the worms were grouped into two categories; turns or reversals. A turn was described as a rapid head swing that changed the direction of motion without decreasing the speed of the worm. A reversal was characterized by the worm retreating by at least half a body length in response to the shift and then continuing to move along its new trajectory. Tracks of worms showing a representative turn and reversal are shown in Figure 2. We investigated the effects of both decreasing and increasing the magnitude of the angular shift by testing angles of 30, 60, 90 and 120 degrees. We observed that as the step size of the shift increased, the frequency of turns decreased and the frequency of reversals increased. At 30 degrees, the worm employed turns more than 80% of the time, at 90 degrees, it employed turns and reversals in almost equal proportions and at 120 degrees, the worm reversed more than 80% of the time (Figure 3).

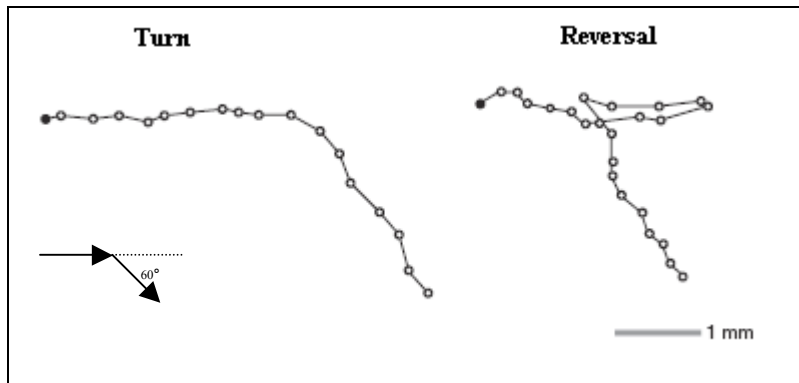


Figure 2: Diagram showing a representative turn and reversal. The worms were subjected to a 60-degree clockwise rotation of the field. The arrows below the diagrams delineate the 60-degree deviation from the original trajectory that occurred in both cases. The black circles indicate the starting position of the worm and the subsequent open circles are at one-second intervals. The 60-degree shift occurred after ten seconds.

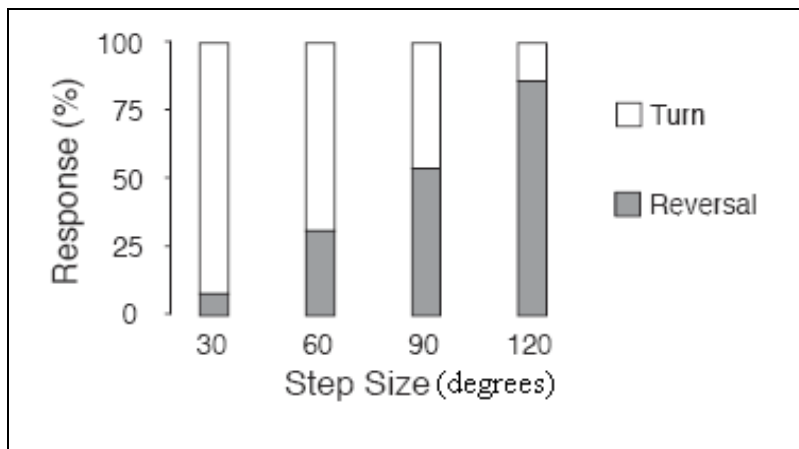


Figure 3: Bar graphs showing the relationship between the behavioral responses of the N2 worms and the step size of the field. The percentage implementation of turns and reversals as the step size was increased from 30 to 120 degrees is shown.

We also sought to understand how *C. elegans* coordinates the observed behavioral responses during electrotaxis. Gray *et al.* (4) studied the behavior of the *C. elegans* in an

isotropic environment. They quantified the worms use of forward crawls, turns and reversals in their navigation and related these observed behaviors to specific sensory neurons, interneurons and motor neurons through laser ablations and mutant analysis. Though the behavior of the *C. elegans* will differ when a time-varying stimulus is applied, we used their findings as a starting point of our own investigation. In particular, we focused on identifying the interneurons that coordinate the worms' behavioral responses during electrotaxis.

Based on the results that Gray *et al.* (4) obtained, we also decided to study the role of the AIY, RIM and AVA neurons in electrotactic navigation. AIY and AVA are pairs of interneurons while RIM is a set of two motor neurons. Transgenic strains (*ttx-3::gfp* and *nmr-1::gfp*) that expressed the promoter GFP (green fluorescent protein), in the target neurons were used to facilitate our laser ablations. In order to serve as comparisons, we performed control experiments. 60-degree step rotations were chosen as the standard testing angle because roughly equal proportions of turns and reversals were observed in the N2 s at this angle. We measured the transgenic strains response to 60-degree step rotations of the field and observed that it was similar to the N2 s. Unlike N2 s, however, these transgenic strains were observed to stall for some portion of the time. A stall occurred when in response to a field shift, the worm simply bumbled in place for at least ten seconds, without any progressive forward or backward movement, when the shift occurred before continuing to move. These stalls were presumably due to an over expression of the GFP in the target neurons which may have affected the normal functioning of these neurons to some degree.

Ablation of the RIM neuron resulted in a decrease in the number of turns and a significant increase in the number of stalls when compared to the controls while the number of reversals remained roughly the same. The opposite effect was observed in the worms where the AVA neuron was ablated; a decrease in the number of reversals accompanied by a significant increase in the number of stalls. Additionally, we ablated both the AVA and RIM neurons at the same time and the results we obtained supported what we observed when we ablated the neurons individually; a decrease in both the number of reversals and turns accompanied by a significant increase in the number of stalls. Laser ablation of the AIY interneuron also led to a decrease in the number of reversals and an increase in the number of stalls when compared to the controls. These data are highlighted in Figure 4 below.

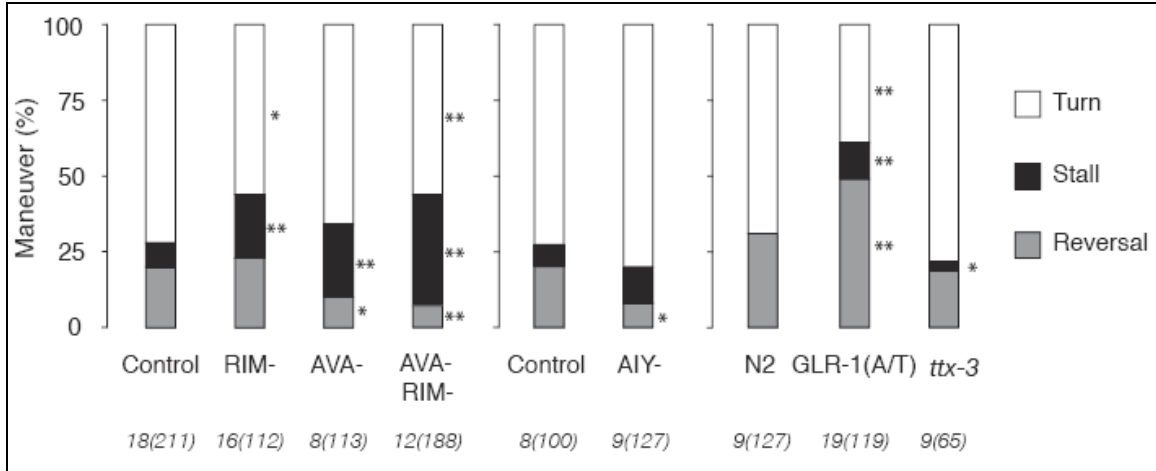


Figure 4: Shows the percentage implementation of turns and reversals in response to 60-degree shifts of the field. The laser ablated worms are compared to the respective controls for each strain and the mutants were compared to the wild type, N2 s. Two of the mutants tested, *glr-1(A/T)* and *ttx-3* are shown on the right. The controls were subjected to the same conditions as the laser surgery worms, with the exception that no neurons were ablated. The number of worms tested is shown below each bar with the total number of shifts obtained from those worms shown in bracket. P-values for percentage usage of each maneuver were calculated for significant difference with the mock surgical control indicated by asterisk. (* $p < 0.05$; ** $p < 0.005$).

In order to increase our understanding of the correlation between sensory input and observed motile output, we tested various mutant strains with known defects. The *GLR-1 (A/T)* mutants display a hyper-reversal tendency, which impedes their ability to produce substantial forward or backward movement when observed in the absence of a stimulus. However, when an electric field was applied to these worms, their hyper-reversal tendency was overcome and they actively crawled toward the negative pole of the electric field. The application of a field shift to the *GLR-1(A/T)* worms resulted in a decrease in the frequency of turns and a significant increase in the frequency of reversals and stalls, much like their behavior in the absence of an electric field stimulus. The *ttx-3* mutation results in a disruption of the formation of the *AIY* interneuron. Like the *GLR-1(A/T)* these mutant worms successfully performed electrotaxis and we observed a decrease in the number of reversals and an increase in the number of stalls. These results were similar to those obtained from the laser surgery worms where *AIY* was ablated.

In addition to studying the aforementioned mutants we tested a wide variety of mutants that are known to be defective in specific sensory neurons to determine whether these neurons are involved in the sensation of the electric field. A major characteristic that we used for choosing the mutants we tested was a defect in the formation of their cilia, as these cilia may be involved in sensing the external electric field. Table 1 shows mutant strains that we tested, the phenotype and/or expression patterns, and their various responses to the electric field. In order to test the worms, we applied a continuously rotating clockwise field to them. We ran four one-minute circles, two two-minute circles and one four-minute circle for each worm. Worms that displayed wild type behavior were able to keep up with the field by producing well-defined circles (Figure 5a), whereas worms that did not do electrotaxis, bumbled around randomly (Figure 5b). Only three of the mutants, *che-13*, *che-2* and *tax-6* failed to perform electrotaxis, all three of which are defective in a large number of ciliated sensory neurons.

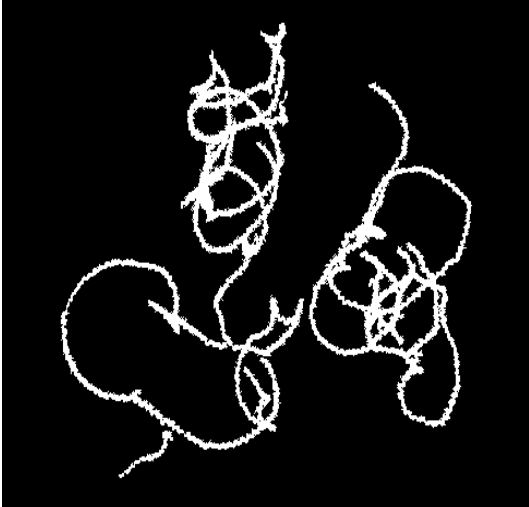


Figure 5a: Representative track of a wild type response of *osm-9* to a continuously rotating anti-clockwise field at 3°/sec. The tracks represent 4min of movement

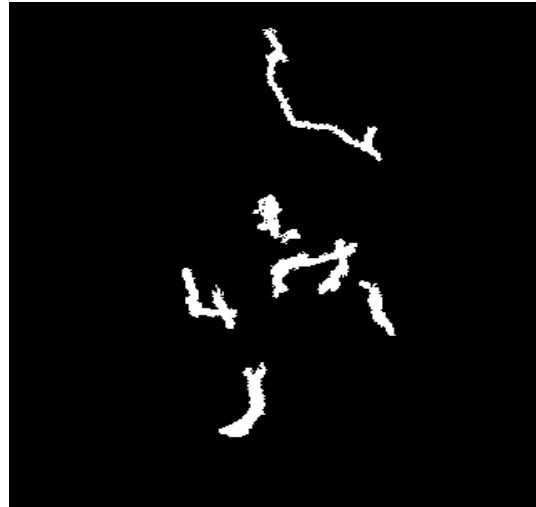


Figure 5b: Representative track of *che-2* worms that did not respond to the electric field. Tracks shown are of worms in an anticlockwise field rotating at 3°/sec for 4min.

Genotype	Phenotype and/or expression pattern	Response to field
Odr3	Osmotic advance defective. Cilia of sensory neuron AWC stunted	Wild-type
Osm-3	Eliminates distal segment of amphid channel	Wild-type
Osm-9	Defective in osmotic advance, nose touch I ASH	Wild-type
Osm-10	Defective in osmotic advance in ASH	Wild-type
Osm-6	Ectopic assembly of ciliary structures and microtubules in many sensory neurons	Wild-type
Osm-5	Ectopic assembly of ciliary structures	Wild-type
Che1	ASE and chemotaxis	Wild-type
Ttx1	Abnormal structure of AFD neuron	Wild-type
Che2	Abnormal formation of ciliated neurons.	Defective
Tax6	Affects AFD, ASE, AWA, AWC, ASH neurons	Defective
Che13	Affects development of ciliated neurons	Defective

Table 1: Shows the genotype of the mutant strains, the phenotype and/or expression pattern and the response to the electric field. A wild-type response was recorded as one in which well defined circles were seen in the tracks of the worms in the rotating field. A defective response was scored as one where the worm was unable to follow the field and did not produce obvious circular tracks.

Conclusion

The goal of behavioral and neural sciences in *C. elegans* is to understand every facet of the behavioral responses from the neural mechanisms involved in sensing of external stimuli to those involved in eliciting the observed behavioral response. The simple neural network of *C. elegans* and the deterministic nature of electrotaxis make this an ideal model system for investigating these correlations. The nematode *C. elegans* actively crawls toward the negative pole of both fixed and time varying fields. We observed that worms respond to rapid shifts in the direction of the field by displaying different behavioral responses which include, turns, reversals and stalls. From our results, we can see that *C. elegans* find it easier to utilize reversals to bring about a large change in directional motion and turns to produce a smaller change in their direction of forward crawling. The reason for this preferred behavior is uncertain, however, it is another facet of the worms complex behavior that can be explored in the future.

We have succeeded in shedding some light on the correlation between the sensory input and the motile output in the *C. elegans*. We studied the three neurons AIY, AVA and RIM, and we have gained some understanding of the roles that they play in coordinating electrotactic navigation. AVA seems to be involved in coordinating the worms' decision to reverse. This can be inferred from the decrease in the frequency of reversals and corresponding increase in the occurrence of stalls that we observed when the laser-ablated worms were tested. We can surmise that for a portion of the time when the laser ablated worms tried to reverse, they were unable to carry out this behavior, got confused and stalled instead. The opposite assumption can be made of the RIM motor neuron. A decrease in the number of turns was accompanied by a significant increase in the number of stalls in these worms. We can therefore assume that for a portion of the time when these worms attempted to turn in response to the step rotation, they were unsuccessful and stalled instead. This suggests that the RIM is involved in executing turns.

To date, the mechanism for electric-field sensation in *C. elegans* is unknown. So far, we have identified three mutants that do not do electrotaxis, *che-13*, *che-2* and *tax-6*. All three of these mutant worms are defective in ciliated neurons that are exposed to the exterior environment through pores in the nose of the worm. It is possible that these ciliated neurons are the mechanisms employed by the worms to sense the stimulus. By further identifying the mutants with defects that do not do electrotaxis, we can then determine which sensory neurons are actually sensing the electric field. In addition, a more in depth analysis of the neurons that are affected by the mutations through techniques such as laser ablations will facilitate a better understanding of the correlation between neurons and behavior.

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