Effect of pulse direct current signals on electrotactic movement of nematodes *Caenorhabditis elegans* and *Caenorhabditis briggsae*

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The nematodes (worms) *Caenorhabditis elegans* and *Caenorhabditis briggsae* are well-known model organisms to study the basis of animal development and behaviour. Their sinusoidal pattern of movement is highly stereotypic and serves as a tool to monitor defects in neurons and muscles that control movement. Until recently, a simple yet robust method to initiate movement response on-demand did not exist. We have found that the electrical stimulation in a microfluidic channel, using constant DC electric field, induces movement (termed electrotaxis) that is instantaneous, precise, sensitive, and fully penetrant. We have further characterized this behaviour and, in this paper, demonstrate that electrotaxis can also be induced using a pulse DC electric signal. Worms responded to pulse DC signals with as low as 30% duty cycle by moving towards the negative electrode at the same speed as constant DC fields (average speed of *C. elegans* = $296 \pm 43 \mu\text{m/s}$ and *C. briggsae* = $356 \pm 20 \mu\text{m/s}$, for both constant and pulse DC electric fields with various frequencies). *C. briggsae* was found to be more sensitive to electric signals compared to *C. elegans*. We also investigated the turning response of worms to a change in the direction of constant and pulse DC signals. The response for constant DC signal was found to be instantaneous and similar for most worms. However, in the case of pulse DC signal, alterations in duty cycle affected the turning response time as well as the number of responding worms. Our findings show that pulse DC method allows quantitative measurement of response behaviour of worms and suggest that it could be used as a tool to study the neuronal basis of such a behaviour that is not observed under constant DC conditions. © 2011 American Institute of Physics. [doi:10.1063/1.3665224]

I. INTRODUCTION

Caenorhabditis elegans (*C. elegans*) and its close-relative *Caenorhabditis briggsae* (*C. briggsae*) are useful and widely studied model organisms for biological studies.^{1–3} Both animals possess orthologs of many disease-related genes that make them useful systems for investigating disease mechanisms and identifying potential therapeutic targets. One of the assessable phenotypes of *C. elegans*, frequently used in behavioural studies, is its sinusoidal pattern of movement. Observation of the movement provides valuable information about the functionality of the neuronal and muscular system of the worm and allows functional dissection of molecular mechanisms by means of mutations, chemicals, and drugs. Existing methods involve studying the movement behaviour of the worm in a Petri dish or multiwell plate in response to chemical stimuli.⁴ Although these methods provide rich information, they are labour-intensive, low

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throughput, and difficult to control precisely and modulate instantaneously. Therefore, a method to induce and quantify movement (forward motion and turning) on-demand in a rapid, sensitive, precise, and reversible manner would greatly facilitate biological studies.

Worms possess sensory and motor systems that enable them to perceive and move appropriately in response to a wide range of mechanical,^{5,6} thermal,⁷ chemical,⁸ light,⁹ magnetic,¹⁰ and electrical^{11,12} stimuli. The natural movement induced by any of these agents could be used as a useful tool to study how neuronal and muscular systems control movement behaviour. Alternatively, changes in its movement behaviour can be used to deduce the impact of drugs and other environmental factors on the neuronal and muscular system. Of these stimuli, electric field is the simplest to implement, can be rapidly switched on and off, and is easy to control. Therefore, it is ideally suited for these assays.

In recent years, microfluidic engineering approach has gained popularity in *C. elegans*-based assays through the use of low-cost microchips because of its potential to obtain high precision measurements in a rapid and automated manner.^{13–16} Typically, in order to visualize and manipulate worms within these microenvironments, their natural movement is suppressed by the use of hydraulic flows. In spite of being advantageous, this technique is not suitable for performing movement-related behavioural studies on worms. Some behavioural assays, such as chemotaxis¹⁷ and mechanosensation,^{18–21} have been performed using microfluidic and MEMS devices. The worms are not immobilized in such devices and are allowed to move freely, thereby allowing study of their behavioural responses. These methods are useful although there are limitations such as complexity in integration and control in a microscale device,^{18,19} inability to switch on and off instantaneously,¹⁷ and in some cases, lethality⁹ if used in microchannels. We have recently shown that the electric field can be used to control worm locomotion in a binary manner (i.e., start and stop) by means of direct, DC, and alternating, AC, current electric fields inside a microfluidic channel.^{22,23} The electrotactic response of *C. elegans* in a microchannel is instantaneous, highly sensitive, and robust and, therefore, can be used to perform movement-based behavioural studies.

In a DC electric field, *C. elegans* moves towards the negative electrode in a spontaneous and continuous manner.^{11,12,22,24} The worm uses its amphid neurons^{11,22} to sense the amplitude and direction of the field and initiates a highly stereotypic movement response. We have determined that the specific range of electric field (termed “active range”), which is required to stimulate this response, is lower (2–4 V/cm) for adult worms compared to younger L3 stage larvae (4 V/cm to more than 10 V/cm).²² We have also shown that worms gradually lose sensitivity to the electric field stimulus below the active range and become paralyzed beyond this range. But the response to the electric field within the active range is fully penetrant and robust, and each stage of worms has a constant speed (regardless of the field strength) which, on average, is higher in adults compared to larvae.²² However, as animals age, tissues start to degenerate^{25,26} and as expected, aging has been shown to cause a significant reduction (70%) in speed within a week’s time after adulthood.²⁷ We also demonstrated that by exposing worms to symmetric AC electric fields at frequencies between 1 Hz and 3 KHz, it is possible to localize them at one location in a microchannel.²³ By using a symmetric sinusoidal AC electric field on a Petri dish (frequencies governed by spinning electrodes around the dish at different speeds), Gabel *et al.*²⁴ demonstrated that electric fields with $f > 16$ Hz are perceived similar to DC signals. Taken together, these findings demonstrate that the electrotaxis can be used as a reliable and faithful indicator of neuronal and muscular activities that modulate movement in worms.

Under a constant DC field, *C. elegans* exhibits a steady state movement response with a characteristic speed towards the cathode.²² When the field direction is reversed, a switch in the direction of worm’s movement in the microfluidic channel (turn) is almost instantaneous, typically happening in less than 10 s, irrespective of its age or size. Thus, constant DC electrotaxis is a good method to assay for the effect of a chemical or drug on the movement of the organism.²⁸ However, in order to understand the effect of the electric field on the neurophysical system of the worm and consequent changes in movement, it is important to develop a method that could induce a proportionally variable and dynamic response in movement to a variation in the stimulus, either in an individual worm or in a population of them. Electric field is an ideal

stimulus to modulate as it is easy to control at the microsecond timeframe. In this paper, we investigated the use of pulsed electric field (pulse DC) and its reversal on the speed and turning response time of the worm. In addition, we compared the constant and pulse DC electrotaxis behaviours of *C. elegans* with that of *C. briggsae*. *C. briggsae* is very similar to *C. elegans* in terms of morphology, development, and genetic makeup.³ The two species are likely to have been separated around 30 million years ago.²⁹ *C. briggsae* is frequently used in comparative studies to understand conserved mechanisms of gene function and biological processes.²

II. EXPERIMENTAL

A. The setup

The device used to perform electrotaxis experiments on worms consisted of a 100 μm -deep, 300 μm -wide, and 50 mm-long polydimethylsiloxane (PDMS) microchannel with platinum wire electrodes and fluidic access tubes inserted at its end reservoirs (Figs. 1(a) and 1(b)). The experimental setup utilized in this work was previously reported by our group for characterizing the DC and AC electrotaxis behavior of *C. elegans* in microchannels.^{22,23} Soft lithography technique³⁰ was utilized for the fabrication of the microchannel in PDMS material. The electrodes were connected to an electric signal generation unit for the application of electric fields and current in the channel as shown in Fig. 1(a). This unit consisted of a AFG3022B function generator (Tektronix Inc., OR, USA) with a maximum voltage output of 5 V, a 677B amplifier (TREK Inc., NY, USA) with a 400 gain, a switch to reverse the polarity of electrodes, and copier wires to connect the setup to the device electrodes.

FIG. 1. Pulse DC electrotaxis, (a) experimental setup consisting of the microfluidic channel with electrodes connected to an amplifier and a function generator, (b) close schematic of the microchannel with a worm and the reservoir with the electrode and fluidic port, (c) Pulse DC electric field signal wave-shape, and (d) sequential snapshots of a young adult *C. elegans* electrotaxis in response to a pulse DC electric field: $EF_{max} = 3$ V/cm, $f = 1000$ Hz, Duty Cycle = 30%. Scale bar = 0.3 mm.

B. Generation of electric field

The function generator was set to output a pulse DC electric field with adjustable characteristics such as the frequency ($f = 1/t_T$), maximum pulse electric field strength (EF_{max}), as well as the duty cycle calculated using Eq. (1). The waveform of the pulse DC signal is shown in Fig. 1(c). The signal rises in a step-like manner from zero to a maximum set point for a controllable duration of time (duty cycle) and decays to zero for the rest of the signal cycle which is then repeated at a fixed frequency

$$Duty Cycle(\%) = f \times t_{on} \times 100, \quad (1)$$

where t_{on} is the on-portion time of the pulse signal. Since the maximum pulse electric field strength (EF_{max}) output of the function generator did not exceed 1 V/cm, the signal was subsequently amplified and applied to the microdevice. The rise and decay time of the pulse signal were 50 μ s each, which restricted the experimental frequency range to less than 1 kHz and the duty cycle to more than 10% in order to produce a rectangular-shape pulse waveform.

C. Animal culture and maintenance

The *C. elegans* N2 and *C. briggsae* AF16 strains were grown on standard nematode growth (NG) agar plates previously seeded with OP50 strain of *Escherichia coli* and maintained at 20 °C.³¹ Synchronized worms were used in all the experiments. For this, gravid adult hermaphrodites were treated with bleach solution (Commercial bleach and NaOH (4N) in the ratio of 3:2). After bleach treatment, dead worms were washed with M9 solution (3 g KH_2PO_4 , 6 g Na_2HPO_4 , 5 g NaCl, 1 ml $MgSO_4$ (1 M) in 1 l). Embryos were allowed to hatch in M9 for 24 h resulting in growth arrest of L1 larvae. The animals were plated on NG agar containing Petri Dishes and grown till adulthood.

D. Pulse DC electrotaxis methodology and turning response

For all experiments, synchronized worms were individually loaded into the microchannel from a reservoir of diluted worm suspension (approximately 1 worm in 20 mm³ of M9) and positioned at the center of the channel by applying a manual suction (using a 5 ml syringe and an approximate flow rate of 0.25 ml/s) at the outlet tube while inserting the inlet tube into the suspension. The microscope was focused on at the inlet region of the channel. As soon as a worm was observed entering the channel with the flow, the outlet and the inlet tubes were disconnected from the syringe and pulled out of the worm suspension. A manual hydrostatically driven flow was used to bring the worm to the center of the channel. Tubes were then leveled to the same height to prevent any flow in the channel. The head orientation of the worm was then determined in the microscope. The worms moved randomly in either direction (left or right) or remained stationary within the channel. The electric field (either constant or pulse DC) with a specific duty cycle and frequency was then applied along the channel (parallel to worm's body) in a direction opposite to its head orientation. The resulting turning movement response was digitally recorded using a camera (Coolpix P6000, Nikon Inc., Tokyo, Japan) connected to the microscope for the entire duration of the experiment. The applied stimulus either induced the worm to turn inside the microchannel and move towards the cathode at its rear or did not generate such a response. For the responding worms, the time taken to reverse their direction (when field was reversed) and the movement speed (when field matched the worm motion direction) was measured from videos (Fig. 1(d)). Since the turning response time was variable among individual worms (mostly within 40 s), we chose the 40 s cut-off time to observe whether a worm as a responder or non-responder. We then used the aggregate population response time for our data analysis. Within 40 s time window, we also quantified responders for 5 s, 10 s, and 20 s durations. The responder worms were allowed to swim towards the cathode for a distance of 5 mm after which the polarity of the electrodes across the channel was reversed and the worm's behavior was continuously recorded. This process was repeated thrice

for each animal to rule out a random electrotactic turning response. Fig. 1(d) illustrates sequential time-lapse images of a *C. elegans* worm turning response to a change of direction in the pulse DC electric field of 1000 Hz frequency and 30% duty cycle ($EF_{max} = 3 \text{ V/cm}$). In the case of non-responding worms in the 40 s time window after signal reversal, the field was switched off, the worm was delivered to the center of the channel again pneumatically, and the same experiment was repeated for two more times. The signal characteristics (either frequency or duty cycle) were then set to a different value and the experiment on the same worm was repeated for the desired spectrum of frequency (1–1000 Hz) and duty cycle (10%–90%). After this set of experiments, the worm was washed off the channel and new experiment was conducted on a newly loaded animal.

III. RESULTS AND DISCUSSION

The electrotactic responses of *C. elegans* and *C. briggsae* to pulse DC fields were investigated as described in Sec. II. Two different movement phenotypes, turn time after signal reversal and forward motion speed during signal application, have been studied. The results were compared to DC electrotaxis of these animals and are described below.

A. Speed and turning response of *C. elegans* and *C. briggsae* to pulse DC stimulus

With a pulse DC signal, the duration of the electric field stimulation of the worm can be adjusted by changing the duty cycle of the signal as shown in Fig. 1(c). In addition, this method has the potential to provide information about the transient response time of a population of worms to electrical signals. For instance, if the pulse width of the signal is less than that needed to evoke a neuronal response, then that signal will not be likely to affect movement even when applied for indefinite number of cycles. In this experiment, *C. elegans* and *C. briggsae* worms (62 h post-L1, young adults) were loaded individually into the channel. The time for worms to turn around in response to reversal of the pulse DC signal, at various duty cycles, was recorded for a population of worms. Simultaneously, the speed of worms in response to pulse DC signal at various frequencies and duty cycles was also recorded. For all pulse DC experiments, the maximum electric field of the pulse signal was set to $EF_{max} = 3 \text{ V/cm}$ as measured previously in the DC electrotaxis²² experiments for a robust response in young adult animals.

In order to examine the effect of the duty cycle on electrotactic response, we exposed *C. elegans* ($n = 14$) to a range of duty cycles (from 10% to 90%) at a constant frequency of 1000 Hz and analyzed their forward motion speed (Fig. 2) and turning response time (Fig. 3) to the directional change of the signal. We observed that there was no response of the worms to a 10% duty cycle signal. At 20%, a few responded but the majority did not. The range from 30% to 90% duty cycle provided a more robust response. The speed of worms remained constant with average values between a minimum of $290 \pm 37 \mu\text{m/s}$ and a maximum of $360 \pm 24 \mu\text{m/s}$ at

FIG. 2. Effect of duty cycle on *C. elegans* ($n = 14$) pulse DC electrotaxis forward motion speed. Frequency for all the experiments were 1000 Hz.

FIG. 3. Effect of duty cycle on *C. elegans* ($n = 14$) pulse DC electro taxis: (a) turn response time and (b) percentage of responders at $f = 1000$ Hz. Each curve in (b) shows the maximum time consumed by the worms to respond to the electric field reversal, turn, and initiate movement in the opposite direction.

different duty cycles in this robust response range, as shown in Fig. 2. These speeds were statistically independent of duty cycle values ($p > 0.1$, using a single factor analysis of variance method, ANOVA) and were averaged at $315 \pm 37 \mu\text{m/s}$. This result implies that even when the electrical stimulus is not continuous, it is sufficient to induce movement response in the worm beyond a certain threshold duty cycle. Next, we investigated the turning response of worms to DC and pulse DC signals following a sudden change in field direction. Here, the worms were exposed to a constant or pulsed DC signal (with a specific pre-set duty cycle at a fixed frequency of 1000 Hz) that was reversed suddenly. The turning response time of the worm was measured as described in Sec. II. The signal reversal was repeated two more times to confirm that the turning response was genuine. The average turning response time for 3 V/cm DC electric field was 6.6 ± 2.6 s for all responding young adult (YA) worms (100% response). However, we found that the time for the worms to reverse their direction in response to pulse DC signals varied significantly (Fig. 3(a)) for certain duty cycles, even though the experiments were done on a homogenous population of young adults. This variation in turning response time was greatest when the duty cycle was the smallest (large standard deviations at low duty cycles in Fig. 3(a)) and reduced as the duty cycle increased and the signal approached constant DC. In addition to this, the number of worms showing turning response at lower duty cycles dramatically decreased such that none responded at 10% duty cycle in less than 40 s. In order to understand this phenomenon better, we used population statistics as shown in Fig. 3(b), which plots the percentage of worms that responded to the pulse DC signal (at 1000 Hz frequency) of a certain duty cycle in a specific time window (i.e., 5, 10, 20, and 40 s). We observed that at lower duty cycles, comparatively fewer worms responded quickly to the reversal of the pulse DC signal. For instance, only 20% of worms responded to 20% duty cycle signal with a turning response time of 5 s or less. However, 60% of them were able to respond to a change in the direction within 40 s. These results show that at lower duty cycles, worms in aggregate were unable to efficiently sense the direction of the pulse DC signal and, unlike constant DC signal, take much longer time to respond. This could be due to an inherent limitation of amphid sensory neurons and/or other neurons involved in turning behavior to respond to lower duty cycles of pulse DC signal. The cellular and molecular basis of this phenomenon is currently under investigation.

Fig. 3(b) also shows that at higher duty cycles of the signal, greater number of worms showed turning response and in a faster manner. For instance, the proportion of the worms responding to the reversal of signal within 5 s increased from 20% at 20% duty cycle to $\sim 80\%$ at 90% duty cycle. Therefore, the turning time can be used as a measure of the dynamics of sensing by the worm. The variation of the duty cycle can provide a method to modulate the dynamic response in a population of worms. In summary, variation in duty cycle of pulse DC signals and turning response time to field reversal together provide a unique method to elicit varying movement response to correspondingly varying electrical stimulus.

FIG. 4. Effect of frequency on *C. elegans* ($n=7$) and *C. briggsae* ($n=7$) pulse DC forward electrotactic swimming speed at $EF_{max} = 3 \text{ V/cm}$ electric field and 50% duty cycle. Average DC electrotaxis speed is also plotted.

The effect of the frequency of pulse DC signal on the forward-motion electrotactic movement speed was also studied for *C. elegans*. A duty cycle of 50% that induced robust response in worms (more than 80% responders in less than 10 s in Fig. 3(b)) was used for the pulse DC field with frequencies ranging between 1 and 1000 Hz. The speed of the electrotactic forward movement is shown in Fig. 4. Each data point corresponds to an average of 7 worms (62 h post-L1 young adult, $719 \pm 37 \mu\text{m}$ long) examined. Average DC electrotactic speed is also depicted in the same figure for comparison. It can be seen clearly that the frequency does not have a significant effect on the pulse DC forward electrotactic movement speed ($p > 0.1$, using ANOVA) which was also similar to their DC electrotactic movement speed. Such behaviour has been previously observed in *C. elegans*.²⁴ The *C. briggsae* worms ($n=7$) responded in a similar fashion (Fig. 4), suggesting that the mechanism of electrotactic response is conserved between the two species.

B. Field strength-based DC electrotaxis of *C. briggsae* and comparison to *C. elegans*

In addition to pulse DC assays, we also tested the response of *C. briggsae* worms to constant DC field similar to what was done earlier for *C. elegans*.²² Considering that the two species have a large number of conserved genes and appear morphologically similar,³ we wanted to compare their electrotactic responses to DC fields. For this, adult *C. briggsae* animals ($n=5$, $845 \pm 40 \mu\text{m}$ length, 62 h post-L1 stage) were loaded individually into the microchannel device, and DC electric fields (1–6 V/cm) were applied across the length of the channel. All tested animals responded to the electric field and moved towards the cathode spontaneously after the application of the signal. The direction of the field was reversed twice for each worm, and every time, it was allowed to rotate and travel for a distance of 5 mm. The entire movement response was recorded and analyzed (Fig. 5) as described previously.²²

It was observed (Fig. 5) that *C. briggsae* responded to electric fields in a range of 1–4 V/cm and showed partial paralysis starting at 5 V/cm. Consistent with this, the electrotactic movement speed at 5 and 6 V/cm was significantly lower compared to 1–4 V/cm ($p < 0.01$, ANOVA). The paralysis was initially observed in the tail region as animals appeared to gradually form coiled-shape configuration in the tail region which extended to the whole body if the signal persisted, eventually leading to coiling. Although the higher threshold of response (4 V/cm) of young adult *C. briggsae* was similar to that of *C. elegans*,²² we observed that these animals, unlike *C. elegans* (which had 2 V/cm lower threshold²²), responded to DC electric fields as low as 1 V/cm in a robust manner (100% responders, $n=5$). However, similar to *C. elegans*, swimming speed of *C. briggsae* did not vary significantly with electric field strengths in the active range (1–4 V/cm) ($p > 0.01$, ANOVA) and had an average value of $356 \pm 20 \mu\text{m/s}$. We also repeated the DC electrotaxis experiment for *C. elegans* ($n=7$, $719 \pm 37 \mu\text{m}$ length, 62 h post-L1 young adult) and observed an average speed of $296 \pm 43 \mu\text{m/s}$ in the 2–4 V/cm range. Since

FIG. 5. *C. briggsae* ($n = 5$) DC electrotaxis speed at various electric field strengths. Based on the analysis of variance (Ref. 32) comparing the groups *: significant ($p < 0.01$) reduction in speed at 5 and 6 V/cm electric fields compared to 1–4 V/cm.

the forward movement of both species is a result of the motion of the body in a sinusoidal waveform propagating from head to tail, we hypothesize that their speed is directly dependent on the body stroke frequency of motion and their sinusoidal wave's peak-to-peak value, which is primarily influenced by the channel width design in microfluidic devices rather than the electric signal characteristics. The body stroke frequency for both *C. briggsae* and *C. elegans* was on an average 2 ± 0.1 Hz and resulted in slightly higher speed for *C. briggsae* (Figures 4 and 5). An electric field with proper strength (i.e., EF_{max} in pulse or signal strength in constant DC) and exposure duration (minimum required duty cycle in pulse DC signal) induces electrosensation and the locomotory behaviour in the animal. After this, it seems that the animal uses its mechanosensory system to optimally choose a constant body stroke frequency that depends strongly on environmental parameters (e. g. specific channel geometry) and, hence, demonstrate a constant speed in it. This hypothesis is supported by different speeds reported for *C. elegans* in experiments within various swimming or crawling environments.^{20,21}

IV. CONCLUSIONS

We have reported *C. elegans* and *C. briggsae* electrotaxis responses to constant and pulse DC electric field stimuli. In agreement with our previous findings,^{22,23} both these species moved towards the negative electrode upon exposure to DC and pulse DC electric field stimuli, although *C. briggsae* exhibited somewhat greater sensitivity to signals and higher speed compared to *C. elegans*.

The pulse DC-induced electrotactic speed of animals did not vary significantly with changes in the frequency and the duty cycle of the signal and was similar to their DC electrotaxis speed. However, the duty cycle of the signal had a direct effect on the proportion of worms responding to the stimulus and their turning response time. Higher duty cycles resulted in a gradual increase in the proportion of responding worms and an overall decrease over their turning time and variability. Robust electrotactic responses ($> 80\%$ responders with turn time of 10 s or less) could be obtained using a 1000 Hz frequency signal and 30% or higher duty cycles. These findings demonstrate that the pulse DC signal provides a unique way to modulate the temporal exposure of the worm to electrical stimulus, resulting in a characteristic response that varies with the duty cycle of the signal. Future experiments will reveal the neuronal basis of the relationship between the duty cycle and the turning response of animals.

The pulse DC method, when characterized in sufficient detail, could potentially be more useful to study subtle changes in responses of the worm to environmental and chemical influences as compared to constant DC electrotaxis. This will in turn enable researchers to use the electrotactic turning response as a phenotype to perform quantitative analysis of neuronal signalling in *C. elegans*. In addition, the use of pulse DC signals at high frequencies (1000 Hz)

and duty cycles as low as 30% reduces the exposure time of worms to electric signals by up to 70%, yet generating a response similar to constant DC fields. This should benefit certain experiments where it is desirable to minimize worms' exposure to the electric field.

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