Pulse DC Electrotaxis of *C. elegans* in a Microdevice and its Application in Stimulus-evoked Neuronal Analysis

P. Rezai*, S. Salam**, B. P. Gupta**, P. R. Selvaganapathy*
Departments of *Mechanical Engineering and **Biology
McMaster University
phone: (1) 905-525-9140-21388
e-mail: rezaip@mcmaster.ca

***Abstract* — *C. elegans* is a roundworm that has been established as a model organism for neurobiological applications due to its simple neuronal system (302 neurons). These neurons respond to diverse stimuli such as electric signals and result in animals' behavioral responses through neural signaling pathways towards motor outputs. We have used a microfluidic device to establish accurately-controllable micro-environments to study the behavioral responses of *C. elegans* to pulse DC electric signals. Effect of frequency and duty cycle of the signal has been studied at the behavioral level.**

I. Introduction

Caenorhabditis elegans (*C. elegans*) is an important organism for biological researches [1] due to its simpler anatomy (~1000 cells, 302 of them being neurons), rapid development (2.5 days until adulthood through 4 larval stages) and the presence of many disease-related genes. The movement of the worm is highly stereotypic and provides a means to investigate the effect of drugs on the organism as a whole-animal model [2]. The use of movement as an indicator of worm's overall health requires that it could be manipulated on demand, in a repeatable manner inside a controlled environment. This paper reports a new method to study *C.elegans'* forward movement and turning behavior quantitatively in response to pulsed electric stimulus inside microchannels.

We have recently developed electrical techniques to stimulate worms locomotion (electrotaxis) and localization in a microfluidic channel setup by means of constant [3] and alternating [4] electric fields which have also been used to perform drug screening [5], sorting [6], and neuronal activity imaging [7] on worms. In this study, we find that *C. elegans* electrotaxis response to pulse DC signals is similar to constant DC fields in terms of forward motion speed. However, we investigated the effect of the pulse frequency and duty cycle on the turning time of the worm upon signal reversal. Turning time 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 signal along with turning time can be used to measure the dynamic response of the worms which was difficult to measure before.

II. EXPERIMENTAL

A 2-dimensional schematic of the experimental setup is illustrated in Fig. 1. It consisted of a power supply system (a function generator and an amplifier) and a microfluidic channel (300µm-wide, 100µm-deep and 5cm-long made of PDMS) with electrodes inside the two end reservoirs. All experiments were performed under a microscope and recorded with a digital camera in a video format.

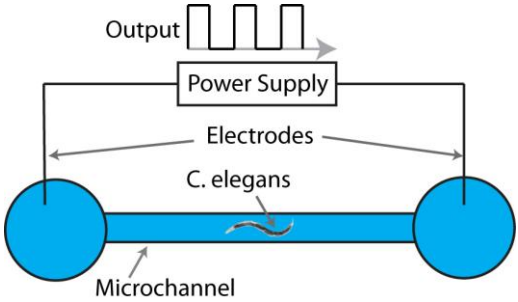

Fig. 1. Schematic of the experimental setup. Worms were loaded individually into the channel, pneumatic flows were eliminated, a desired pulse DC signal was applied and the worm response was recorded in a video format. The videos were then analyzed for speed and turning time measurements

After loading individual worms (n=7 adults pneumatically using syringes) into the microchannel, constant or pulse DC electric fields with various duty cycles (10%-90%) and frequencies (1-1000 Hz) (Fig. 2) were applied across the channel and the animal's response was recorded continuously. The direction of the electric field signal was reversed twice (every 40s) for each worm. Parameters such as swimming speed (in matching direction of motion with electric field direction) and reversal time (after electric field reversal) was quantitatively measure through the analysis of the recorded videos.

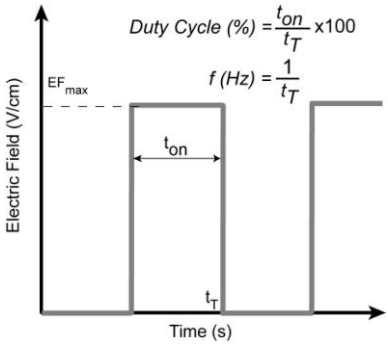

Fig. 2. Pulse DC electric field signal wave-shape. EF_{max}=3 V/cm in all experiments. Effect of frequency and duty cycle on forward motion speed and turning time was studied.

III. RESULTS

Fig. 3 demonstrates the average speed of worms in response to DC and 50% duty cycle pulse DC electric fields at various frequencies.

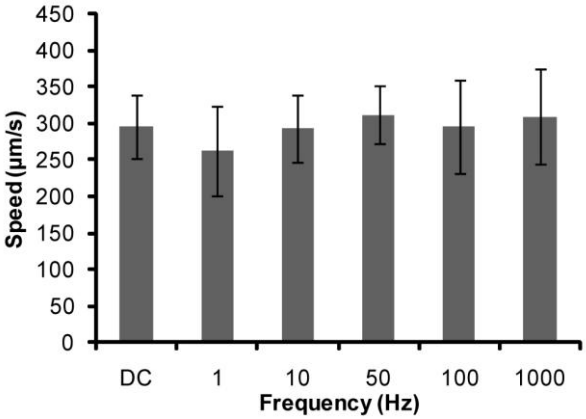

Fig. 3. DC electrotaxis speed of *C. elegans* and comparison to pulse DC electrotaxis speed at E_{max}=3V/cm electric field, 50% duty cycle, and various frequencies. Worms moved towards the cathode. Frequency did not have a significant effect on forward motion speed.

Worms showed electrotactic movement towards the negative electrode with a constant speed which was quantitatively similar to the constant DC electrotaxis speed. We found that the frequency of pulse DC signal had no significant effect on the speed ($p>0.1$, ANOVA) when the direction of the field was matching the worm forward motion direction, suggesting that a discontinuous electrical stimulus is sufficient to induce movement response in the worm.

To examine the duty cycle effect, we exposed worms (n=14) to a range of duty cycle (10-90%) at a constant frequency (1000 Hz). Turning time of worms upon electric field reversal, up to a maximum of 40s duration, was measured (Fig.4).

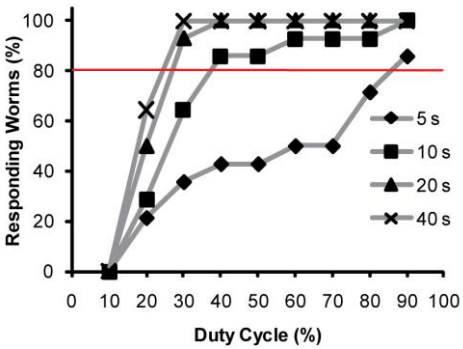

Fig. 4. Turning time (legend) and % of *C.elegans* (total n=14) responders to pulse DC electric fields at 1000 Hz frequency and various duty cycles. The legend shows the time windows provided to the worms to respond to the electric field reversal, rotate, and start moving towards the opposite direction

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 (Fig.4). Robust electrotactic responses (>80% responders, turn time<10s) could be obtained using a *f*=1000Hz signal with 30% and higher duty cycles. This could be due to an inherent limitation of amphid sensory and/or other neurons [8] 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. A second similar microchip has been designed that can immobilize the worms in the center of the channel (narrowing channel) for cellular imaging. Individual neurons can be functionalize using fluorescent protein molecules and their transient response to diverse ranges of electric signals can be studied using this device. We have discovered that worm's ASJ neuron responds to the electric pulses in a manner that well describes our observations at the behavioral level. While we discovered that less number of worms respond to 5Hz frequency pulses (50% duty cycle) in our behavioral analysis chip, the neuronal imaging trends also confirmed a less significant ASJ neuron signaling at the same frequency.

IV. Conclusion

Our work demonstrates that the pulse DC signal provides a unique way to modulate the temporal response of *C. elegans* to the electrical stimulus and generates a characteristic response that varies with the duty cycle of the signal. These findings should enable researchers to use the electrotactic turning response as a phenotype to perform quantitative analysis of neuronal signaling in *C. elegans*. In addition, the use of pulse DC signals at a high frequency (1000Hz) and low duty cycle (30%) reduces the exposure time of worms to the electrical stimulus by up to 70% yet generating a response similar to constant DC fields.

References

[1] L. Segalat, "Drug discovery: here comes the worm," *ACS Chem Biol*, vol. 1, pp. 277-8, Jun 20 2006.
[2] P. Rezai, *et al.*, "Microfluidic systems to study the biology of human diseases and identify potential therapeutic targets in C. elegans," in *Integrated Microsystems*. vol. In Press, K. Iniewski, Ed., ed: CRC Press, 2010, pp. 581-608.
[3] P. Rezai, *et al.*, "Electrotaxis of Caenorhabditis elegans in a microfluidic environment," *Lab Chip*, vol. 10, pp. 220-6, Jan 21 2010.
[4] P. Rezai, *et al.*, "Behavior of Caenorhabditis elegans in alternating electric field and its application to their localization and control," *Appl Phys Lett*, vol. 96, p. 153702, 2010.
[5] J. A. Carr, *et al.*, "A microfluidic platform for high-sensitivity, real-time drug screening on C. elegans and parasitic nematodes," *Lab Chip*, vol. 11, pp. 2385-96, Jul 21 2011.
[6] X. Manière, *et al.*, "Running Worms: C. elegans Self-Sorting by Electrotaxis," *PLoS One*, vol. 6, p. e16637, 2011.
[7] T. V. Chokshi, *et al.*, "Probing the physiology of ASH neuron in Caenorhabditis elegans using electric current stimulation," *Appl Phys Lett*, vol. 99, pp. 53702-537023, Aug 1 2011.
[8] C. V. Gabel, *et al.*, "Neural circuits mediate electrosensory behavior in Caenorhabditis elegans," *J Neurosci*, vol. 27, pp. 7586-96, Jul 11 2007.