CO-RELATION OF CELLULAR AND BEHAVIORAL RESPONSES OF *CAENORHABDITIS ELEGANS* TO PULSE DC ELECTRIC FIELDS

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ABSTRACT

Caenorhabditis elegans is a well-established model organism for neurobiology research. Neuronal circuitry of the worm and its correlation with behavioral responses has been investigated in significant detail. *C. elegans* responds to a variety of stimuli (chemical, mechanical, thermal) which has been studied by imaging the neuronal transient signals following stimulations. Electrically-induced neuro-behavioral response (electrotaxis) of *C .elegans* has not been studied in detail. Here, we describe the use of two microchips to study pulse DC electrotaxis of *C. elegans* at both behavioral and neuronal levels with correlations between the neuronal activity and the movement of the animal in the microchannel.

KEYWORDS

Caenorhabditis elegans, microfluidic, electrotaxis, neuron transient response, FRET, imaging, pulse DC

INTRODUCTION

Understanding fundamental biology of organisms, systems and disease processes is critical for tackling human health challenges and to enhance the quality of life. Live organisms or systems such as mammalian cells and whole-animals have been utilized to study conserved biological processes. Among several potential animal models, *C. elegans* [1] is attractive in biological and neurological research due to several reasons. It reaches a relatively small size (~1 mm long and ~50 µm thick) at a fully developed adulthood age and has a transparent body that allows observation of biological processes in live condition at single cell resolution. Combination of such benefits with the completely sequenced genome and simple mapped cellular system has made *C. elegans* an ideal model for performing biological assays both to understand human diseases as well as in drug discovery [2-3]. Behavioral analysis of worms can provide valuable information in these assays.

C. elegans sinusoidal movement behavior is one of the most important phenotypes that can provide valuable information about the neuromuscular state and capacity of the animal. Speed, turning time as well as the frequency of its body bending provides quantitative information of its movement. *C. elegans* locomotion is controlled by several neurons and their connections to body wall muscles. These include amphid neurons that sense a wide variety of external stimuli by activating specific pathways. These signaling pathways have been investigated through physical probing of neurons [4] as well as calcium transient imaging of intact animals *in vivo* [5-7] by using Föster resonance energy transfer (FRET). Although these methods have been used to investigate worms' chemo-, thermo- and mechano-sensation mechanisms, electric signal sensing has not been studied in great detail in worms.

We have demonstrated that nematodes respond to electric signals (DC and AC) by moving towards the negative pole in a stereotypic manner [8-9]. Recently, FRET imaging was used in a microdevice to study the worms' neuronal responses to DC electric currents [7]. However, electrotactic behavioral responses have not been correlated with neuron signaling. Recently, we introduced pulse DC electrotaxis of nematodes as a method to generate a variable turning response in worms with modulation in the applied pulse duty cycle and frequency [10]. In this paper, we have employed this technique along with FRET to correlate animals' behavioral responses to neuronal activities at various electric field conditions.

EXPERIMENTAL

The experimental setup used in this study is illustrated in Fig. 1a. It consisted of a function generator to produce the pulse DC signal (square pulses with a set amplitude, repetition frequency and duty cycle). The signal was amplified and applied through electrodes along the length of the microchannel. We have developed two devices, both made of PDMS by soft lithography, one for performing behavioral studies (Fig. 1b, *behavioral chip*) and the other for immobilizing and FRET imaging (Fig. 1c, *neuronal chip*) of the ASH amphid sensory neuron (one of the neurons mediating electrotaxis [11]). The worms' responses to pulse DC electric fields (3V/cm) of various duty cycles (10-90%) and frequencies (1-1000Hz) were studied.

Behavioral assay: This assay has been described previously [10]. Briefly, individual worms were loaded into the *behavioral chip* and positioned at the center. A pulse DC electric field with desired duty cycle and frequency was applied across the channel and animal's electrotaxis behavior was recorded in a video format. After animal's electrotaxis towards the cathode for 3 mm, the pulse DC signal direction was reversed and the time taken by the worm to perform a turn was measured and plotted from the video recording.

Neuronal assay: To evaluate the response of the ASH neuron, we adopted a FRET imaging method described earlier [7]. The strain carrying ASH-specific TNXL calcium sensor was obtained from the Chronis lab [7] and constituted of cyan

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(CFP) and yellow (YFP) fluorescent proteins. Through binding with calcium ion flux due to neuronal activities, the calcium-specific sensor undergoes conformational changes that bring the two fluorescent proteins together at the scale of a few nanometers. Through dipole-dipole interactions between the two proteins, the energy is transferred non-radiatively from the donor (CFP) to the adjacent acceptor (YFP).

For this assay, a worm was immobilized pneumatically in the *neuronal chip* (Fig.1c) and imaged using FRET (under a dual-channel fluorescent microscope). Unlike the previous work [7], we applied defined voltage signals instead of current. After 5 seconds with no electric field, pulse DC electric field (50% duty cycle) was applied in the head-to-tail direction, while recording the FRET signal. Simultaneous CFP and YFP images were acquired, light intensities were extracted by post-processing the image using ImageJ software, and YFP/CFP ratio was obtained, which correlates to calcium flux from neuronal activities.

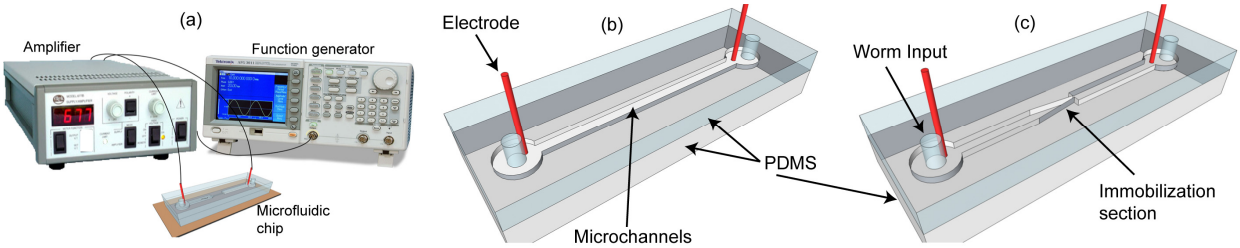

Figure 1: Experimental setup (a) and microfluidic devices for (b) behavioral studies and (c) immobilization and FRET neuronal imaging of C. elegans in response to pulse DC electric fields (3V/cm, 50% duty cycle) of various frequencies (f=1, 5, 10, 50, 100, and 1000Hz). (a) Channel size: 50mm-long, 0.3mm-wide, 0.1mm-deep, (b) side sections were 0.3mm wide and 0.1mm deep, immobilization section gradually narrowed down from 0.3mm to 0.02mm with a depth of 0.04mm (fabricated in 2 photolithography steps)

RESULTS

In the behavioral assay, worms (n=10-16 young adults for each frequency) were loaded individually into the channel (Fig. 1b) and exposed to the pulse DC electric field of interest as discussed in the Experimental section. Response of worms was found to be dependent on the frequency and duty cycle of the stimulus. Responding worms travelled towards the cathode similar to a DC electrotaxis behavior [8-9] while non-responders (not sensing specific pulse DC conditions) remained stationary or had random movement in the channel. For the responding worms, electrode polarity was reversed after 3 mm of travel and the time taken by worms to reverse their direction of motion was measured (Fig. 2). It was observed that worms responded faster to higher duty cycles at a fixed frequency. Interestingly, changing the frequency from 1 Hz to 5 Hz (at a fixed duty cycle) also resulted in an increased latency in turning response time. Subsequent increase in frequency beyond 5 Hz resulted in reduced latency and a fast turning response. Since electrotaxis is governed by neuronal signaling, we investigated the neuronal transient responses using FRET imaging.

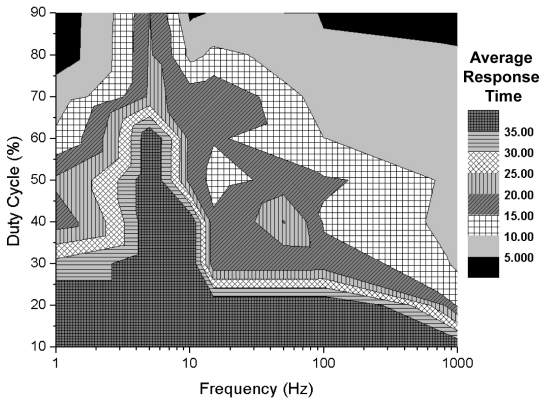

Figure 2. Behavioral response of the worm to pulse DC under various frequencies and duty cycles. Average reversal response time of young adults (n=10-16 worms at each frequency) to a change in the direction of the pulse DC electric fields of various frequencies and duty cycles.

The ASH neuron is one of the neurons that is involved in mediating electrotaxis behavior in *C. elegans*. The worms expressing TNXL sensor in ASH (n=14 young adult) were immobilized in the *neuronal chip* (Fig. 1c) and Ca^{+2} transients under exposure to pulse DC electric fields of various frequencies was studied. After the animal was immobilized, FRET images were acquired for 30 s (Fig. 3), the first 5 s with no electric stimulus and the rest with the application of a pulse DC signal at one of the frequency levels of 1, 5, 100 or 1000 Hz. It was observed that the FRET ratio (which indicates the presence of Ca^{+2} ions and activation of the neuron) was approximately zero for the first 5 s. Application of a signal resulted in a rapid increase in the FRET ratio in 5-10 s followed by a gradual decline which was on average different for various frequency levels. Fig. 3 shows that the FRET ratio change following the stimulus exposure was the lowest at *f=5Hz* compared to other frequencies on average indicating that the neurons are not robustly

activated at that frequency. This results support our electrotaxis behavioral data in Fig. 2, showing that worms have slowest turning response at *f=5Hz* followed by 1, 100 and 1000 Hz.

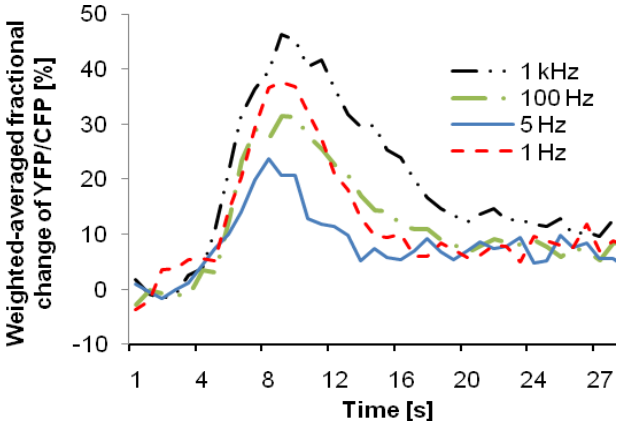

Figure 3. FRET ratio change in young adults (n=14) in response to pulse DC electric fields of various frequencies at 50% duty cycle (data from 2 independent trials).

CONCLUSION

These results show that our electrotactic behavioral and correlating neuronal assays can be used to investigate neuronal signaling in a quantitative manner. This is the first demonstration of transient electrotactic behavioral studies correlated with neuronal signaling. Our methods can be used for more elaborate studies of *C. elegans'* neuronal circuitry and development. These findings demonstrate that ASH neuron is required for sensing the direction of the electric field. Furthermore, our work suggests that the electrosensory response of ASH, and possibly other electric field-sensing amphid neurons, requires roughly 100ms processing time (based on 5Hz frequency at 50% duty cycle). Electric-field based microfluidic devices are well amenable to parallelization and higher throughput analysis and therefore more suited for future biological assays on worms.

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