AN AUTOMATED MICROFLUIDIC PLATFORM FOR ELECTRICAL STIMULATION OF THE NERVOUS SYSTEM OF *C. ELEGANS*

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ABSTRACT

We demonstrate the use of an automated microfluidic platform for acquiring electrical stimulation-evoked neuronal calcium transients in the roundworm *C. elegans*. Our approach combines the use of a microfluidic chip for trapping single worms, microfabricated electrodes for applying electrical stimulation and genetically expressed calcium indicators for monitoring neuronal activity. We show that an electric current applied through the worm's body induces substantial calcium transients in the ASH sensory neuron. The automated platform also enabled us to characterize the impact of aging on the electrically-evoked ASH calcium transients. Our data supports the hypothesis that neuronal activity is altered with age and establishes the use of electrical stimulation as a tool to probe age-associated changes in the neuronal functionality of *C. elegans*.

INTRODUCTION

Electrical stimulation is known to trigger neuronal responses by directly affecting the membrane potential [1]. This has made electrical stimulation of neurons, an exceptional technique for modulating and studying the functionality of the nervous system [2]. Furthermore, neuronal cells can respond to electrical stimulation in the form of directional migration and growth, a phenomenon termed electrotaxis that has been extensively used in nerve regeneration studies [3].

Electrical stimulation has also been shown to induce locomotory behavioral responses at the whole organism level, such as in the nematode *C. elegans* – a widely used model organism in the neuroscience field. It is speculated that this electrotactic behavior is mediated by a network of amphid sensory neurons [4].

Here, we demonstrate the use of an automated microfluidic platform for electrical stimulation and calcium imaging of neuronal activity in *C. elegans*. We optically monitored the activity of the ASH sensory neuron using the genetically encoded FRET (fluorescence resonance energy transfer)-based calcium indicator TN-XL [5]. We further utilized the platform to characterize the effect of aging on the electrically induced ASH neuronal response. Our data allow us to hypothesize that electric current stimulation can be used to probe the age-dependent changes in physiology of the ASH neuron.

EXPERIMENTAL SETUP

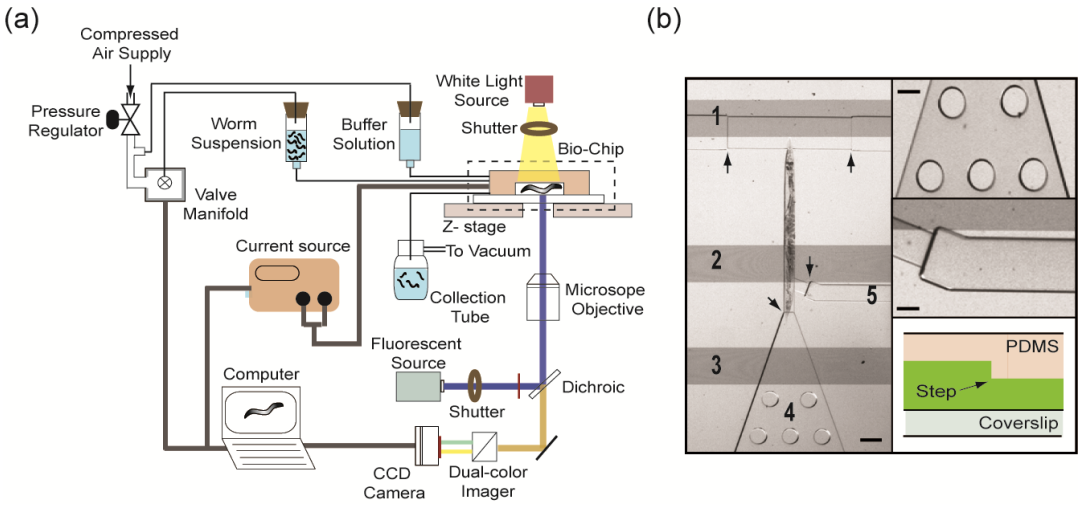

Figure 1: (a) The automated platform for electrical stimulation and calcium imaging. (b) The e-chip for electrically stimulating single worms. It consists of a worm trap, a set of ITO electrodes (labeled as 1, 2 and 3), an array of PDMS micropillars (labeled as 4) and a flush channel (labeled as 5). Scale bar, 150 µm. Magnified views of the micropillars and the flush channel as well as of the step architecture (indicated by the arrow on the picture of the e-chip) are shown on the right. Scale bars, 100µm.

The automated platform (figure 1a) consists of a PDMS microfluidic chip (the "e-chip" where "e" stands for electric), an epifluorescent microscope equipped with a piezoelectric z-stage, a dual-color imager attached to a back illuminated CCD camera, a current source and a valve manifold connected to a buffer and a worm-containing solution. The e-chip (figure 1b) integrates a worm trap for immobilizing and imaging single worms and a set of transparent (indium titanium oxide (ITO)) electrodes for applying current through the worm's body at different locations. An array of micropillars at the inlet, a "flush" microchannel and a "step" architecture are also implemented, to facilitate automated operation. The automated operation of the platform and the acquisition of calcium imaging data in response to electrical stimulation are implemented via a custom-made graphical LabVIEW interface as previously described [5]. The operational procedure consists of three steps (figure 2) : (i) a single worm is loaded inside the worm trap by a constant pressure-driven flow (a pressure of 10 psi was applied at the inlet of the e-chip), (ii) the fluorescently labeled ASH neuron is brought into focus using the z-stage and its activity is monitored in response to an electric current applied through the worm's body (e.g. by applying a current between electrodes 1 and 3 or 1 and 2), and (iii) the worm is unloaded by pressurizing the flush channel.

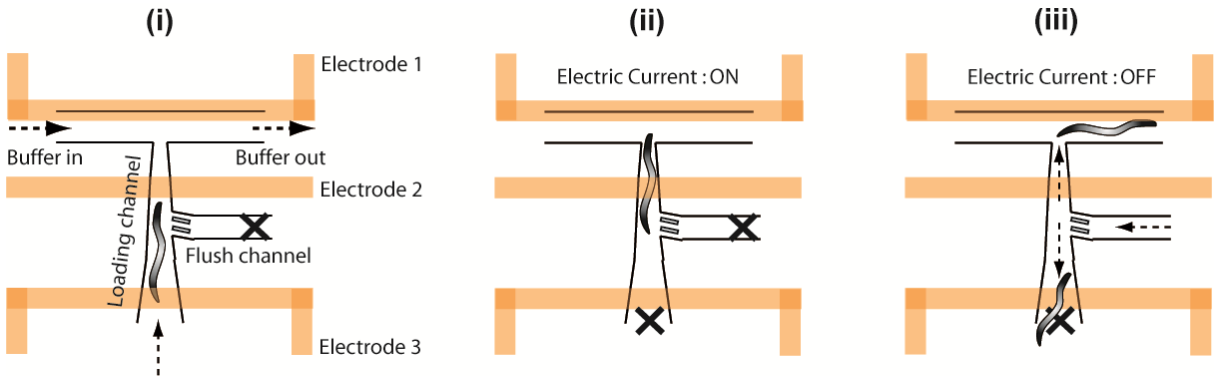

Figure 2: The operational procedure of the automated platform (X denotes a closed microchannel): (i) worm loading, (ii) electrical stimulation and optical recording of ASH activity, (iii) worm unloading.

RESULTS AND DISCUSSION

We first monitored ASH calcium transients in response to an electric current conducted through different segments of the worm's body. We noticed substantial ASH calcium transients only when the current was conducted through the entire body of the worm (i.e. between electrodes 1 and 3 in figure 1b) or between the worm's head and mid-body (i.e. between electrodes 1 and 2 in figure 1b). Next, we characterized the effect of aging on the electrically-evoked ASH neuronal functionality. To do so, we acquired ASH calcium transients in age-synchronous populations of worms of ages L4+1 day, L4+3 days and L4+5 days (denoted as 'Day 1', 'Day 3' and 'Day 5' worms, respectively) in response to a 10s current pulse of amplitude 0.01μA applied through the entire worm body (figure 3a). To quantify the stimulus-evoked calcium transients, we obtained the calcium dependent FRET signal and extracted the values of parameters that were characteristic of the observed transients. In particular, we calculated the slope and peak of the rising phase in the calcium transients at the presence of stimulus. These parameters, which are indicative of the rate (slope) and increase in the intracellular calcium level (peak), increased with age (figure 3b).

We should highlight that the age-dependent ASH functionality has been previously studied using an osmolarity shock (glycerol)) [5]. In contrast to the electric current evoked responses, glycerol evoked ASH responses indicated an increased peak and rate of calcium influx in younger ages (up to Day 3), followed by a decrease in older ages (Day 5). We speculate that the glycerol and electric current evoked ASH responses are due to the activation of different signaling pathways in the worm's nervous system. For example, signaling pathways associated with G-protein coupled receptors (GPCR's) are believed to be involved in glycerol-evoked ASH responses. However, these GPCR pathways might not get triggered by an electrical stimulus. Therefore we hypothesize that the differences in the age-dependent responses might be due to age-associated changes in these distinct signaling pathways.

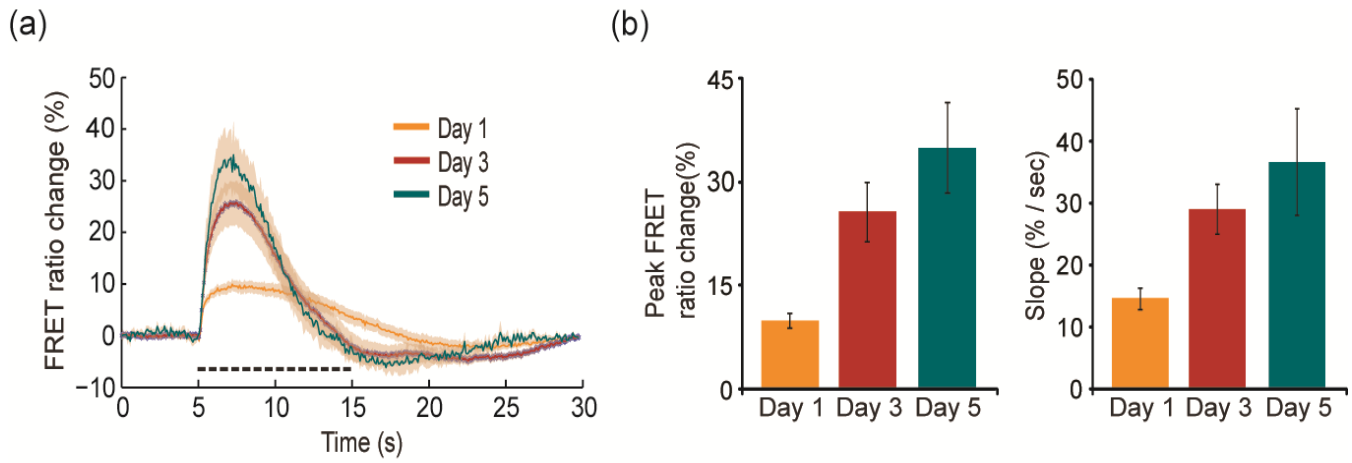

Figure 3: (a) Electrical stimulation-evoked ASH calcium transients in worms of different ages (L4 worms are taken as Day 0 worms). Each curve represents an average of 15 recordings. Shaded regions represent standard error of the mean. (b) The peak (left) and slope (right) of the rising phase of the FRET ratio curves shown in (a). Error bars indicate standard error of the mean.

CONCLUSION

We demonstrated the use of an automated microfluidic platform for studying the age-dependent, electric current-evoked activity of the ASH sensory neuron in *C. elegans*. Our results support the hypothesis that physiological properties of neurons are altered with age and establish electric current stimulation as a new approach for understanding the process of aging of the *C. elegans* nervous system. Furthermore, compared to other forms of stimuli (chemical, mechanical and thermal), the ease with which an electrical stimulus can be precisely controlled in terms of delivery, strength and spatial location, makes it a powerful tool to probe the physiology of the nervous system *in vivo*. We envision the use of electrical stimulation as a well controllable and highly tunable stimulus for performing *in vivo* functional imaging as part of a high-throughput anti-aging drug screening assay.

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REFERENCES

[1] R. W. Doty, Annual Review of Psychology, 20, 289 (1969).
[2] A. Selimbeyoglu, *et al.*, Frontiers in Human Neuroscience, 4, 46 (2010).
[3] C. D. McCaig, et al., Experimental Physiology, 76, 473-494 (1991).
[4] C. V. Gabel, et al., The Journal of Neuroscience, 27, 7586-7596 (2007).
[5] T. V. Chokshi, *et al.*, Lab Chip, 10, 2758-2763 (2010).

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