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Probing the physiology of ASH neuron in *Caenorhabditis elegans* using electric current stimulation

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Electrical stimulation has been widely used to modulate and study the *in vitro* and *in vivo* functionality of the nervous system. Here, we characterized the effect of electrical stimulation on ASH neuron in *Caenorhabditis elegans* and employed it to probe the neuron's age dependent properties. We utilized an automated microfluidic-based platform and characterized the ASH neuronal activity in response to an electric current applied to the worm's body. The electrically induced ASH neuronal response was observed to be dependent on the magnitude, polarity, and spatial location of the electrical stimulus as well as on the age of the worm. © *2011 American Institute of Physics*. [doi:10.1063/1.3615821]

Electrical stimulation is known to trigger neuronal responses by directly affecting the neuronal membrane potential.[1,2] This has made electrical stimulation of neurons an exceptional technique for modulating and studying the functionality of the nervous system. Invasive as well as non-invasive electrical stimulation approaches have been utilized to create a functional map of the brain[3,4] and modulate sensory perception in monkeys.[5,6] Electrical stimulation has been used in cases of pathological conditions such as anxiety, depression, insomnia, and motoneuron deficiencies.[7] Furthermore, neural cells can respond to electric stimulation in the form of directional migration and growth, a phenomenon termed electrotaxis that has been extensively used in nerve regeneration studies.[8]

Electrical stimulation has also been shown to induce locomotory behavioral responses at the whole organism level.[9] This electrotactic behavior has been extensively investigated in the nematode *Caenorhabditis elegans (C. elegans)*, a widely used model organism in the neuroscience field. *C. elegans* navigates towards the negative terminal in the presence of a direct current (DC) electric field[10–12] while an alternating current (AC) electric field is known to constrain its navigation pattern.[13] It is speculated that this behavior is mediated by a network of amphid sensory neurons[11] and it has been used to robustly transport and sort *C. elegans* inside controlled microfluidic environments[12,13] as well as on electrophoretic gels.[14]

Here, we demonstrate that electric stimulation can be used to probe the age dependent functionality of the ASH neuron, a polymodal sensory neuron in *C. elegans* that has been implicated in electrotaxis.[11] We should emphasize that, we utilized a direct electric current to stimulate the ASH neuron and not electric field as previously described.[11–13] To do so, we microfabricated (as described in Ref. 15) a PDMS (Polydimethylsiloxane) microfluidic chip, the "e-chip" ("e" stands for electric) (Fig. 1(a)), that integrates a worm trap for immobilizing and imaging single worms and a set of transparent (indium tin 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 were also implemented, to facilitate automated operation.[16] In order to perform functional (calcium) imaging of a large number of worms, the operation of the e-chip and the image acquisition process were controlled by a custom-made graphical LabVIEW interface as previously described.[16] The experimental protocol consisted of four steps: (1) individual worms were 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), (2) the fluorescently labeled ASH neuron (Fig. 1(b)) was brought into focus using a piezoelectric stage, (3) an electric current of a particular magnitude and polarity was applied to the worm's body and the corresponding ASH response was recorded and, (4) the nematode was unloaded by pressurizing the flush channel.

To study the effect of aging on ASH functionality, we monitored electric current-evoked calcium transients in worms of 3 different ages (Fig. 2(a)): L4 + 1 day, L4 + 3

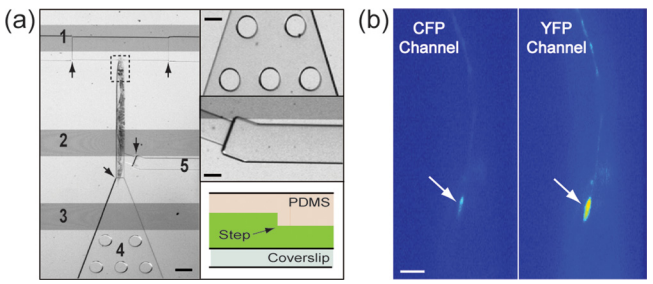

FIG. 1. (Color online) (a) The e-chip for electrically stimulating single worms. It consists of a worm trap, a set of ITO electrodes (labeled as 1, 2, and 3), an array of PDMS micropillars, (labeled as 4) and a flush channel (labeled as 5). Scale bar, 150 μm. Magnified views and the step architecture of the e-chip are shown on the right. Scale bars, 100 μm. (b) Pseudocolor-enhanced FRET (fluorescence resonance energy transfer) image of a trapped worm (marked by the dashed square in (a)). The ASH neuron is highlighted with the arrow in the two FRET channels (CFP and YFP). Scale bar, 5 μm.

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days, and L4 + 5 days (denoted as "Day 1," "Day 3," and "Day 5" worms, respectively). Recordings of intracellular calcium levels were performed using the genetically encoded FRET (fluorescence resonance energy transfer) indicator TN-XL,[16,17] while a 10 s pulse of electric current was applied to the worm's body with head-to-tail orientation (electric current of positive polarity).

To quantify the stimulus-evoked calcium (Ca^{2+}) transients, we established characteristic metrics that are representative of the ASH response and are physiologically relevant. Particularly, we extracted the peak, steepness of the rising phase, and the decay rate from the calcium transients at the presence of the stimulus (Fig. 2(b)). These parameters are indicative of the maximum calcium concentration (peak), the rate of calcium influx (steepness of the rising phase), and the rate of calcium efflux (decay rate) in the ASH neuron. We utilized a curve fitting modeling approach to derive a quantitative estimate of these parameters.[18] We first modeled the ratio transients with a first-order differential equation (1) that relates the rate of change of intracellular calcium concentration dCa(t) / dt to the rate of calcium influx and efflux (indicated by parameters α and β respectively)

$$\frac{dCa(t)}{dt} = \alpha * e^{-\tau.t} - \beta * Ca(t) \quad (1)$$

The above equation states that upon neuronal activation: (a) calcium enters the cell at a rate equal to "α" (calcium influx), which then decays exponentially with a time constant "τ" and (b) the neuron depletes itself of calcium at a rate proportional to the extra calcium present inside the cell due to depolarization. We curve fitted this differential equation to the experimentally obtained FRET ratio transients and extracted the values of the coefficients, "α" and "β".

All three parameters, the maximum calcium concentration, the rate of calcium influx and efflux were observed to increase with age (Fig. 2(b)). These trends can be attributed to several mechanisms. It is possible that electric current-evoked neuronal calcium transients are triggered directly by the activation of voltage gated calcium channels (VGCC's) such as the L-type calcium channels in the ASH neuron.[19] Studies have revealed an increase in the L-type Ca^{2+} channels and a subsequent increase in the voltage gated Ca^{2+} currents in aged rat hippocampal CA1 neurons.[20] Therefore, the observed age-dependent calcium influx might be due to an age-associated increase in the density of VGCC's in the ASH neuron, similar to the increase in hippocampal CA1 neurons.

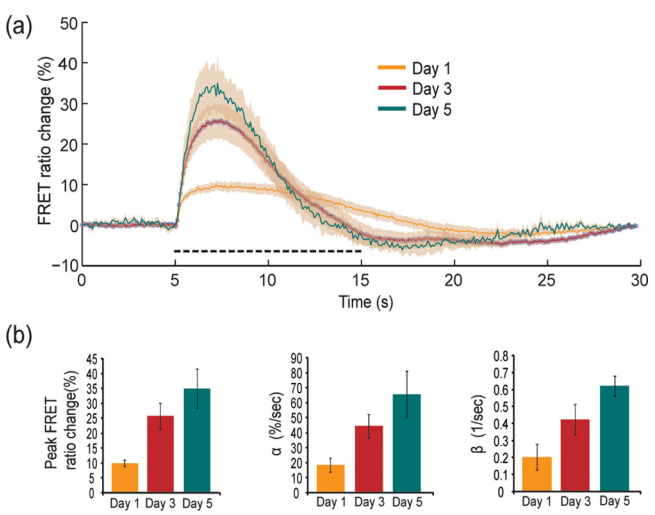

FIG. 2. (Color online) Age-dependent effects of electric current of positive polarity in ASH. (a) Individual curves represent an average of 15 recordings from Day 1, Day 3, and Day 5 worms. Shaded regions represent standard error of mean. The dashed line represents the presence of the stimulus. (b) Mean values of the peak, α, and β constants of the calcium transients for three different ages. Error bars represent standard error of mean.

Studies have also reported an increase in neural calcium level in aged rat's adrenergic neurons due to an impairment of intracellular calcium regulatory/buffering mechanisms.[21] Ca^{2+} buffers regulate calcium transients and modulate the amplitude and duration of Ca^{2+} influx, by mechanisms that involve Ca^{2+} binding proteins and calcium sequestering organelles such as the mitochondria and the endoplasmic reticulum. Strong calcium buffering slows the rate of intracellular calcium clearance from the cytoplasm, which is depicted by an increase in the duration of the intracellular calcium transients and a sustained Ca^{+2} influx. On the other hand, weak calcium buffering results in an increased calcium influx and in a limited duration of the calcium rise, which is observed in the calcium transients from aged worms. Thus, it is possible that the age-dependent characteristics of the ASH calcium transients might be also associated with the age-dependent decline in the calcium buffering mechanisms.

We should point out that the age-dependent ASH functionality has been previously studied using glycerol as a chemical stimulus.[16] 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). While, glycerol-evoked ASH calcium transients also involve the participation of VGCC's, those channels are triggered by upstream signaling pathways involving olfactory G-protein coupled receptors (GPCR's).[22] GPCR's can recognize a specific chemical stimulus or an osmotic shock (e.g., glycerol), but might not be activated at the presence of an electrical stimulus. Thus, we speculate that observed age-dependent glycerol evoked ASH neuronal responses are due to age-associated changes in the glycerol induced signaling cascade that acts upstream of the VGCC's.

The electrotactic behavior of *C. elegans* has been reported to be sensitive to the polarity of the applied electric field.[11,12] To investigate the effect of electrical polarity, we applied an electric current to the worm's body with a tail-to-head orientation (electric current of negative polarity). We obtained ASH calcium transients from Day 1 worms, in response to three different electric current magnitudes (Fig. 3). A preferential bias with respect to the polarity of electric current was observed. Increasing the magnitude, reduced neural depolarization while magnitudes higher than 0.01 μA hyperpolarized ASH. This trend was also observed for Day 3 and Day 5 worms (data not shown).

We also obtained ASH calcium transients from Day 1 worms, in response to electric current applied between the head and the mid-body (electrodes 1 and 2 in Fig. 1(a)) as well as between the tail and the mid-body (electrodes 2 and 3 in Fig. 1(a)) of the worm. The above mentioned polarity

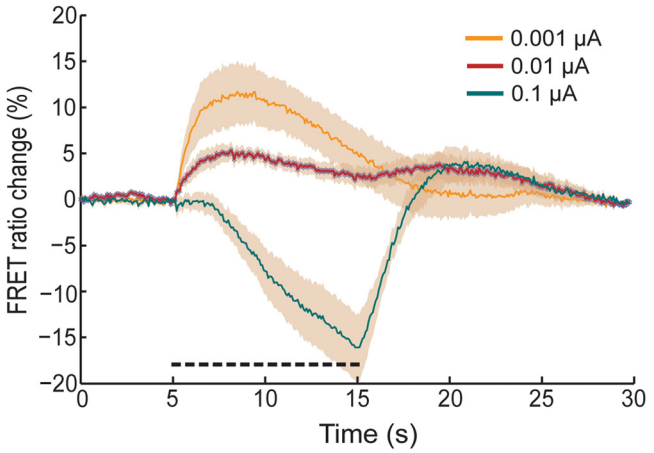

FIG. 3. (Color online) Effect of electric current of negative polarity in ASH in Day 1 worms. Individual curves represent an average of 15 recordings corresponding to electric current magnitudes of 0.001 μA, 0.01 μA, and 0.1 μA, respectively. ASH is hyperpolarized when stimulated with a current of 0.1 μA. Shaded regions represent standard error of mean.

dependent ASH response was only observed in the former case. No ASH response was observed in the latter case.

In conclusion, this study reveals that the electrically induced ASH neuronal response in *C. elegans* is not only dependent on the magnitude and polarity of the electric current but also on the age of the worm. 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 into the physiology of the nervous system of *C. elegans* and study its age-dependent properties. 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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