Sensorimotor signal transmission through AIY interneuron in *C. elegans* during isothermal tracking

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INTRODUCTION

We introduce an original worm tracking epi-fluorescent microscope as a means for visualizing calcium dynamics in *C. elegans* AIY interneuron during isothermal behavior. On a temperature gradient, *C. elegans* exhibit high sensitivity to temperature by maintaining narrow tracks within 0.05˚C. This behavior is of interest as it reveals the fine degree of information must be transmitted to modulate downstream motor circuits in order to maintain specific tracking paths for both linear and circular temperature gradients. As AIY is the primary post-synaptic partner of AFD L/R thermosensory neuron pair, we expect to see a tight coupling of temperature sensed and AIY activity at Zone 2 where AFD synapses onto AIY.

Our novel automated worm tracking microscope is able visualize neural activity in unconstrained freely behaving worms, with real-time neuron tracking at 15fps. This enables us to capture neural activity using the ratiometric Cameleon calcium indicator with as few impositions on the worm in comparison to alternative methods of calcium imaging. The system is also flexible to accommodate various physical stimulations.

SAMPLE PREPARATION

10 young adult light insensitive *lite1(xu7)* mutants with AIY::CAM are washed and placed on a 12cm x 12cm NGM agar plate. The plate is allowed to equilibrate for 20 minutes prior to placing the worms on top of the assay plate. The linear temperature gradient is centered around the cultivation temperature 20˚C, with a gradient of between 0.5 and 0.6˚C/cm.

HARDWARE

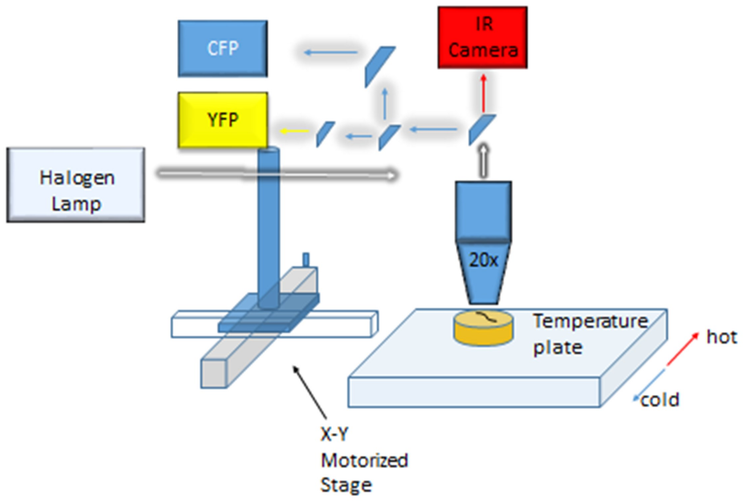
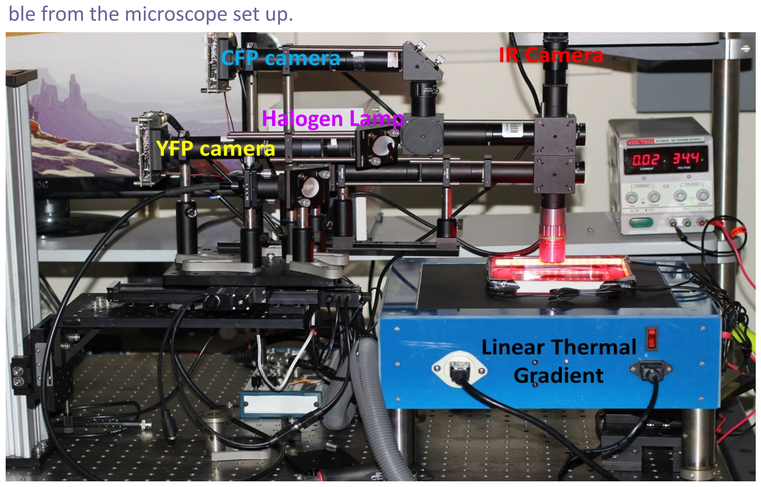


FIGURE 1: (Above) Schematic of worm-tracking microscope with optical path and (Below) actual microscope with thermal gradient. The light travels from the halogen lamp and passes through 433nm filter to a 20x objective. Light reflecting off of the worm via dark field illumination and fluorescence signal return through the same objective travelling through a variety of filters until they reach the camera with their respective wavelengths. YFP image is processed via LabVIEW program that updates the position of the stage at 15fps to re-center the neuron (not shown). Center of mass, worm skeleton, and velocity is extracted from IR data. The thermal gradient functions independently from the microscope and is removable from the microscope set up.



DATA & RESULTS

Below are the preliminary results from the application of our system upon a linear thermal gradient. The following data is from a single 20 second isothermal track with a reversal midway (t ≈ 12s). Fluorescence data was obtained for the soma (not shown) and Zone 2 of the neurite process, which showed more robust signals. Forward traversal is slower and shows slight head undulations with an amplitude of A = 0.07 mm.

The temperature perceived at the head, obtained by translating the x-position from stage data with the temperature gradient (0.56˚C/cm), is shown to be tightly correlated with AIY Ca^{+2} changes at Zone 2. As noted, there is a slight delay in calcium dynamics in the neurite and with the temperature sensed. However, our data differs from that previously reported in AIY literature that we do not see a sharp decrease in AIY Zone 2 during reversal initiation.

When looking at the individual CFP and YFP normalized traces from Zone 2, we can see that there are few parallels between the individual traces and the position of the head, eliminating the fluorescence as a motion artifact. The soma is identified by isolation a region of interest which contains the brightest pixel and encapsulates the whole soma; Zone 2 fluorescence is then extracted by identifying the local maximum within a region outside of the soma. To normalize CFP and YFP data, the fluorescence for each frame is compared to background brightness and then divided by the average fluorescence for the sample time sequence.

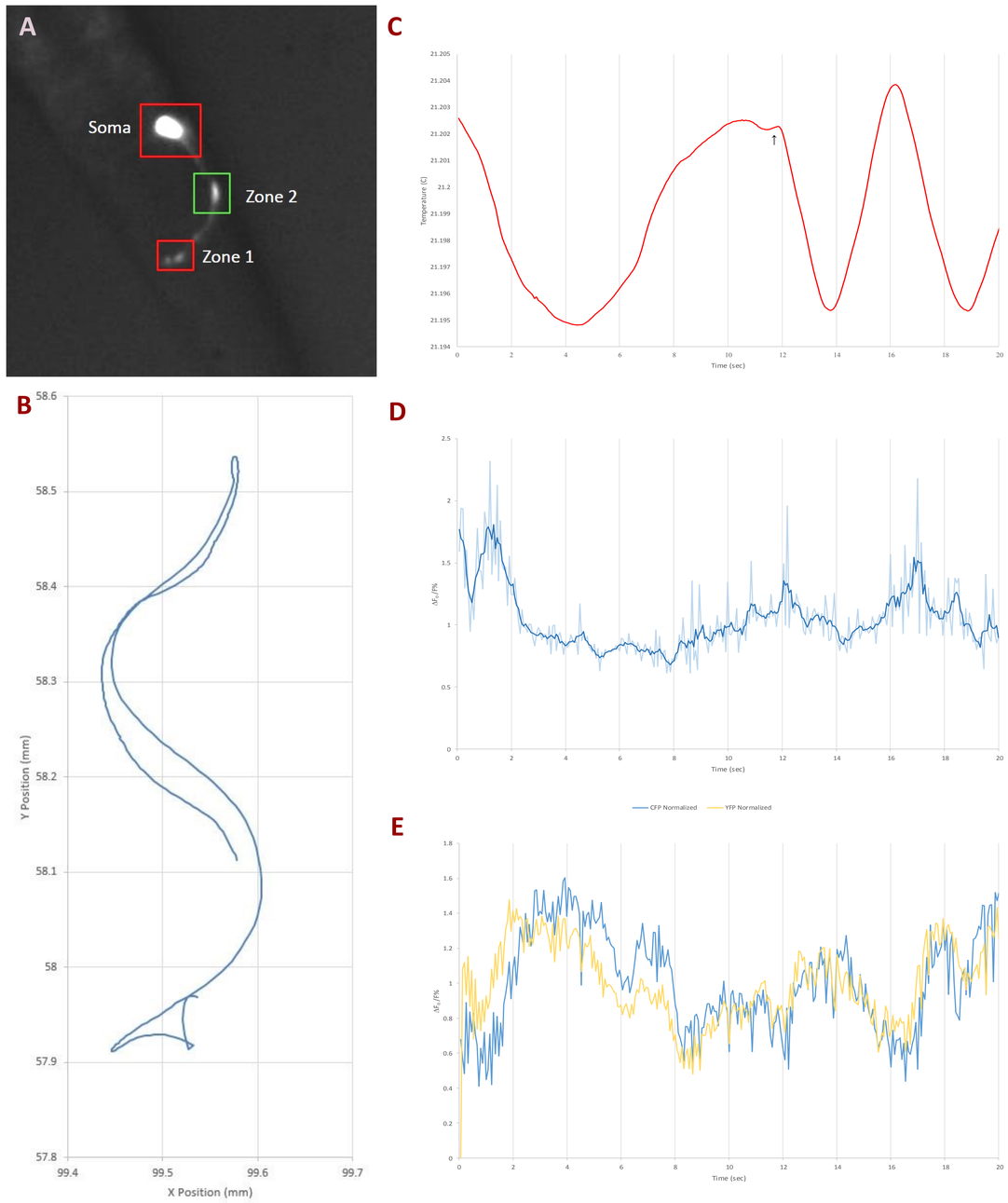


FIGURE 2: Data for 20 second segment of isothermal data along linear gradient of 0.56°C/cm

(A) YFP fluorescence image for AIY neuron. Three regions of brightness are highlighted, the soma, Zone 1, and Zone 2. Process Zone 2 (green) is analyzed for changes in fluorescence, as it is the location at which AFD synapses on to AIY. (B) Position of head extracted stage data during isothermal behavior. (C) Temperature sensed at head via gradient conversion (0.56°C/cm). Arrow denotes beginning of the reversal. (D) YFP/CFP normalized fluorescence ratio of AIY Zone 2, with smoothing function superimposed in dark blue. (E) Individual traces from CFP and YFP from AIY Zone 2.

CONCLUSIONS & DISCUSSION

Temperature experienced at the head and calcium dynamics in AIY neurite process Zone 2 activity are tightly correlated, with a slight delay in calcium signals. This delay can be attributed to time in which temperature is sensed at AFD and received by AIY, as well as other potential motor relays back to AIY.

The role of AIY is multifaceted as it receives sensory input from various modalities and also plays a role in motor output and feedback. As exhibited in the RIA interneuron, neuronal compartmentalization in AIY may differentiate sensory input signals as well as motor input and output signals. Our data indicates that while there is salient thermal input to Zone 2 of AIY and significant correlation between the two, calcium activity in Zone 1 or the soma may correspond to alternative sensory or motor information. In order to elucidate the function of AIY, it is important to differentiate and partition the functions of AIY within a network, not as a solitary unit. To address this issue, it is crucial undergo this type of data taking with various other interneurons and motor neurons within this circuit, and eventually move to multi-neuron worm tracking.

The application of this microscope enables a high degree of fidelity for neural activity in behavioral assays. It can be used to uncover more about the role of neurons in ways that ablation and mutant studies are unable to address, and adapted to employ optogenetic interrogation techniques as well. Additionally, the set up is easily manipulated to accommodate a wide variety of stimulations.

FUTURE DIRECTIONS

We will continue data taking with *lite-1(xu7)* mutants with AIY::CAM and AFD::CAM, as well as various downstream interneurons (RIA, RIB, AIZ).

Within the next year, we hope to make the following adjustments to the worm-tracking microscope:

- Addition of a Piezo Z scanner for autofocus and increased neuron resolution
- Improve adaptability of microscope to interconvert between dark field and light field setups to facilitate various behavioral stimulations
- Larger field of view for IR camera to incorporate center of mass tracking along with neuron tracking for increased stabilization of the microscope

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