Comparative Analysis of Thermotaxis Tracking Behavior in Caenorhabditis elegans between Larval and Adult Life Stages

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ABSTRACT

- Organisms use multiple sensory systems in order to maintain homeostasis through making corrections to their normal body movements. Previous studies have established that adult *Caenorhabditis elegans* can exhibit isothermal behavior via the AFD thermosensory neuron to detect temperature differences as small as 0.01 °C in order to maintain their position along an isotherm within 0.1 °C per centimeter. (White)
- Adult *C. elegans* are able perform isothermal behavior through a side-to-side head movement known as klinotaxis. During this movement, they are able to compare the temperature as their head moves from one side to the next in order to track their preferred temperature. For this reason, *C. elegans* is a great model organism to study the development of memory in higher ordered organisms based on their ability to retain information from thermosensory neurons for use when comparing this preferred temperature to real time sensory information. (Luo)
- Although the genes required for this thermoregulatory network are activated during embryo development, isothermal behavior has only been recorded for adult behaviors. (Hobert, 1998) Preparing and analyzing isothermal behavioral assays with *C. elegans* while they are still undergoing neuronal development in their first larval stage allows us to better understand the development of a sophisticated memory system in a relatively simple organism.

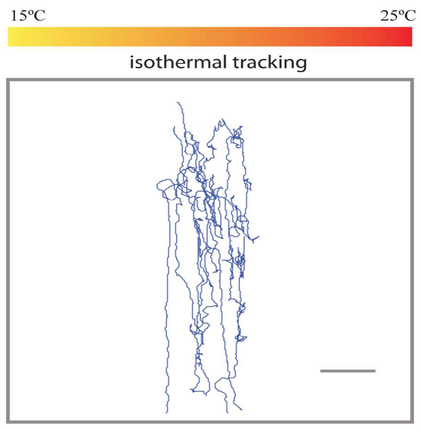
HYPOTHESIS

- C. elegans at the adult stage are able to correlate their head movement with a temperature gradient to a precision and do so by tracking isotherms on a steep thermal gradient (>0.2 degrees per cm) roughly 15%-20% of the time. (Luo)
- We hypothesize that L1 worms, which only have 222 neurons as opposed to 302 in the adult hermaphrodite, are not able to track isotherms to this degree of precision or for roughly the same percentage of time during the same experiment due to their incomplete neuronal development. (Hobert, 2010)

OBJECTIVES

Figure 1: (Right) isothermal tracking on a temperature gradient is linear along an isotherm. (Wormbook)

Figure 2: (Below) *C. elegans* move toward their cultivated temperature along a thermal gradient. (Mori)



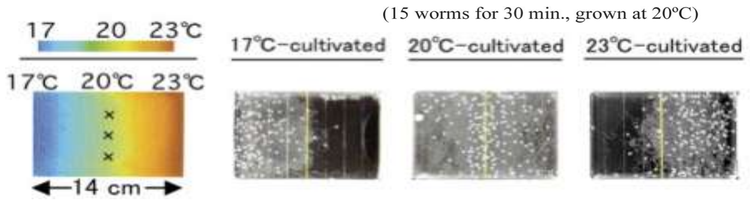
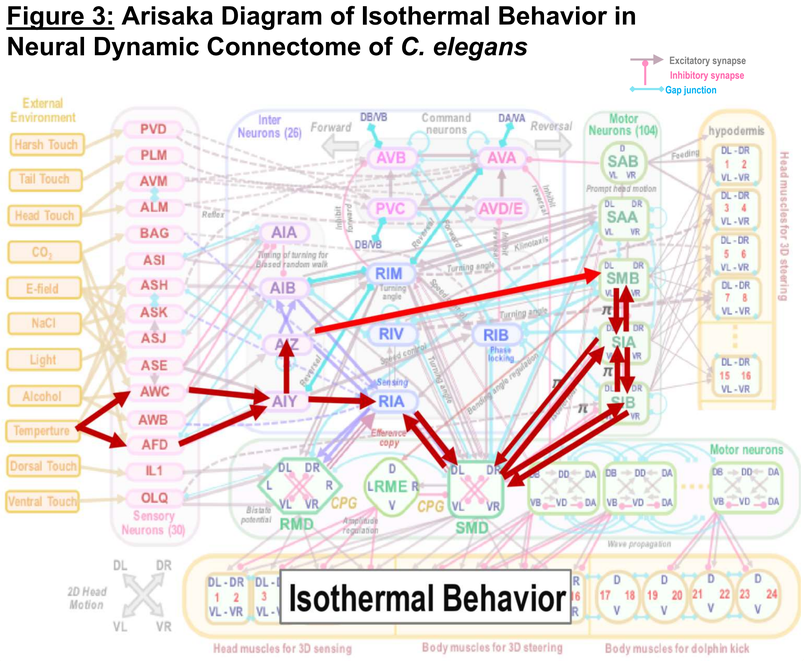


Figure 3: Arisaka Diagram of Isothermal Behavior in Neural Dynamic Connectome of *C. elegans*



MATERIALS & METHODS

Figure 4: Young adult and synchronized L1 worms are fed in separate plates for 2 hours at their cultivated temperature of 20°C for comparative analysis. (Image from Wormbook)

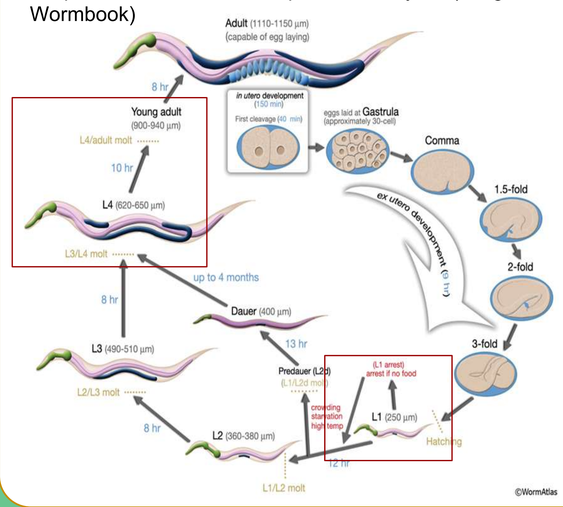


Figure 5: Thin 2% agar sheet of approximately is made as a 2D surface for worms to move on. This agar sheet is transferred then to temperature plate.

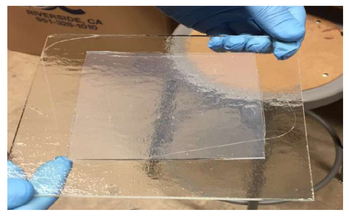


Figure 6: Linear temperature plate controlled by custom built software and allowed to reach thermal equilibrium with agar sheet before worms are dropped around cultivated temperature for behavioral analysis.

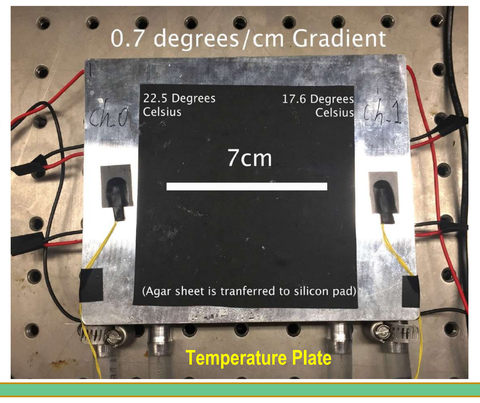
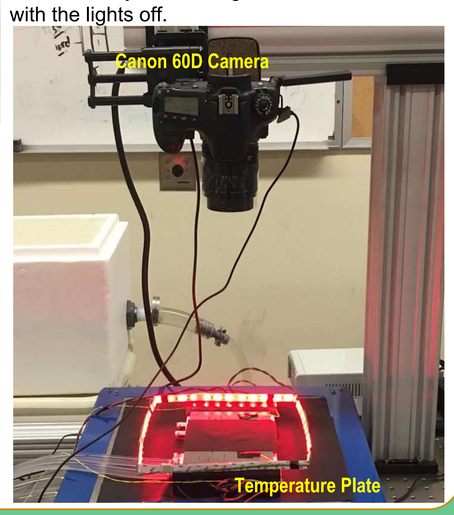
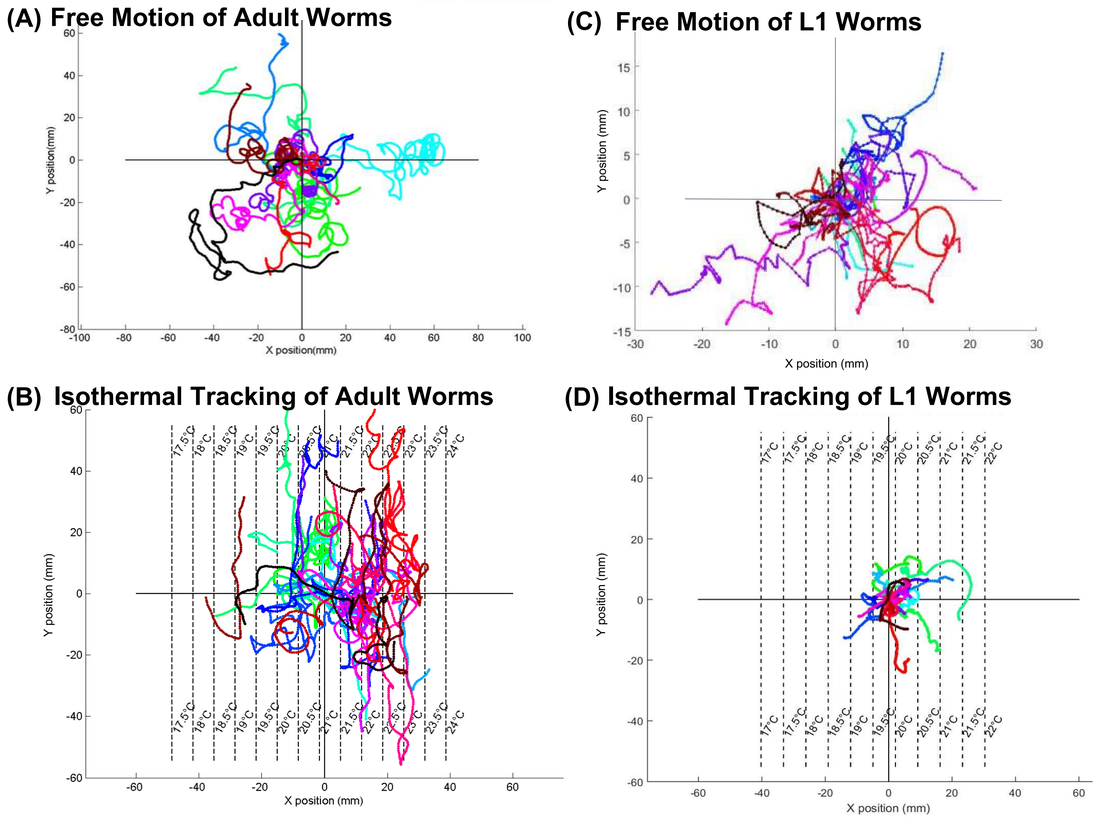


Figure 7: Behavior is observed by a Canon Camera with a 50mm macro lens. Worms are illuminated by red LED lights while data is taken with the lights off.



RESULTS

Figure 8



8A) Free Motion of Adult Worms
- Colored lines representative of individual adult *C. elegans* in 2% agar medium without stimulus as a control.
- All tracks are analyzed by custom MatLab program used to mitigate human bias.
- N=10-15

8B) Isothermal Tracking of Adult Worms
- Colored lines representative of individual adult *C. elegans* in 2% agar medium on a linear temperature plate.
  - Gradient: 0.07 °C/mm
  - C. elegans dropped at 20°C
  - Data taken for 30 minutes
  - N=20-25

8C) Free Motion of L1 Worms
- Colored lines representative of individual L1 *C. elegans* in 2% agar medium without stimulus as a control.
- N=10-15

8D) Isothermal Tracking of L1 Worms
- Colored lines representative of individual L1 *C. elegans* in 2% agar medium on a linear temperature plate.
  - Gradient: 0.07 °C/mm
  - C. elegans dropped at 20°C
  - Data taken for 20 minutes
  - N=10-15

CONCLUSIONS

- We have not been able to observe any statistically significant isothermal behavior in L1 stage *C. elegans*. L1 worms do not spend 15%-20% of their time tracking isotherms on steep thermal gradients like adults have shown in our data.
- This behavioral data could indicate the fact that the L1 stage *C. elegans* cannot perform isothermal behavior because sensorimotor integration is not established yet.
- Specifically, more research must be done on the development of neuroplasticity in the isothermal pathway shown in **Figure 3**.
  - Although the AFD thermosensory neuron may be able to sense temperature change, L1s are possibly unable to relay this information to motor neurons due to ongoing development in the interneurons (AIY→ RIA←→ SMD) that ultimately command isothermal tracking.
- Importantly, further behavioral assays at steeper and shallower gradients must be performed to understand the true capability of L1 thermotaxis and the development of sensorimotor integration.

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