The Universal Two-Step Escape Response of *C. elegans* Induced by Blue Light and Touch Stimulation

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Background Information

- C. elegans are believed to live underground in the soil and be photo-insensitive.
- However, recent evidence (Ward et al, 2008) suggests that they are sensitive to short wavelengths of light corresponding to blue/violet/UV light, while remaining mostly insensitive to longer wavelengths like red light.
- When touched with a foreign object such as an eyelash (mechanical stimulation), C. elegans also exhibit escape behavior.
- Previous research (Donnelly et al, 2013) has indicated that head avoidance behavior in response to touch stimulation is characterized by a two-step process:
  1. Reversal, in which the worm backs up for a period of time.
  2. Omega Turn, in which the worm makes a 180° turn then moves in a forward direction away from the noxious stimulus.
- It was noticed in March 2014 in the Arisaka Lab at UCLA that C. elegans may exhibit the two process process in response to light stimulation.
- This avoidance behavior is NOT a commonly observed behavior in freely moving C. elegans.

Hypothesis

The locomotion pattern of C. elegans in response to stimulation of the head region by 405 nm light as well as to that of touch stimulation is characterized by the identical two-step avoidance response.

Materials and Methods

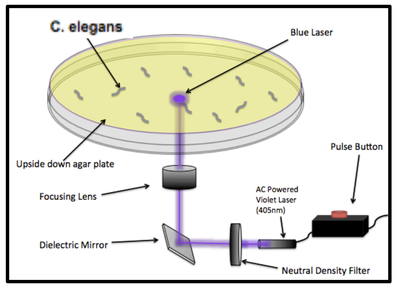

Figure 1a. Diagram of light setup

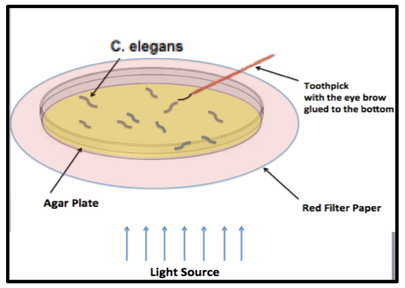

Figure 1b. Diagram of touch setup

- 15 C. elegans (N2) young adults were stimulated with the eyebrow pick and 15 were stimulated by two second light pulses.
- Only forward moving worms were stimulated and each worm was stimulated once.
- Red filtered light was used for both stimulations as illumination because it has been proven to have no effect on the worm's behavior; there was no other light sources in the room.
- A 405 nm laser with a Thorlabs NE05 neutral density filter was used and the measured intensity was 21 mW/mm^2. A 50 mm focusing lens was used.

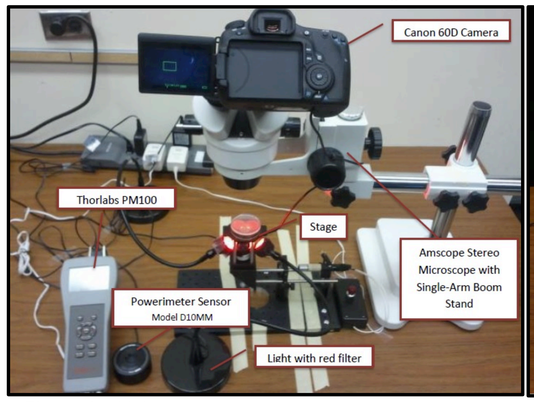

Figure 2a. Light response setup
Field of view is 10mm x 7mm

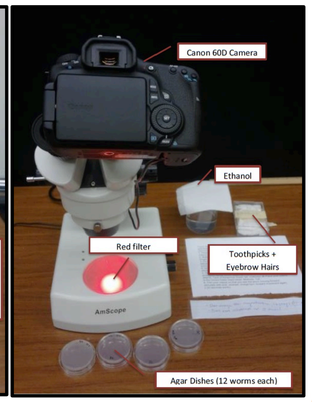

Figure 2b. Touch response setup
Field of view is 7mm x 4mm

Results

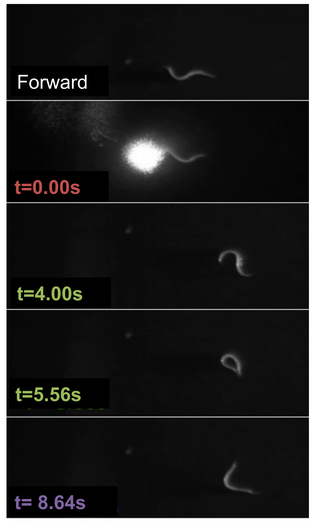

Figure 3. Two step light response

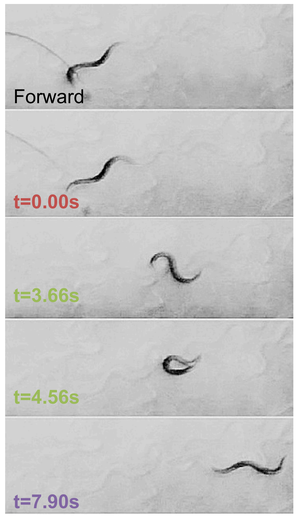

Figure 4. Two step touch response

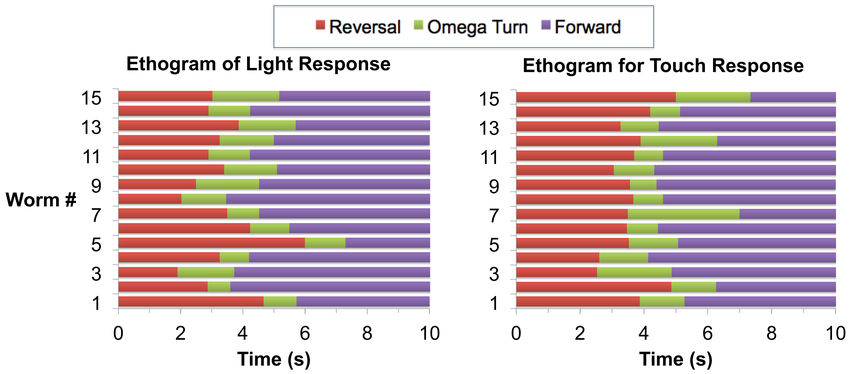

Figure 5. Ethograms of light and touch stimulation demonstrate the development of behavioral responses over time of 15 worms.

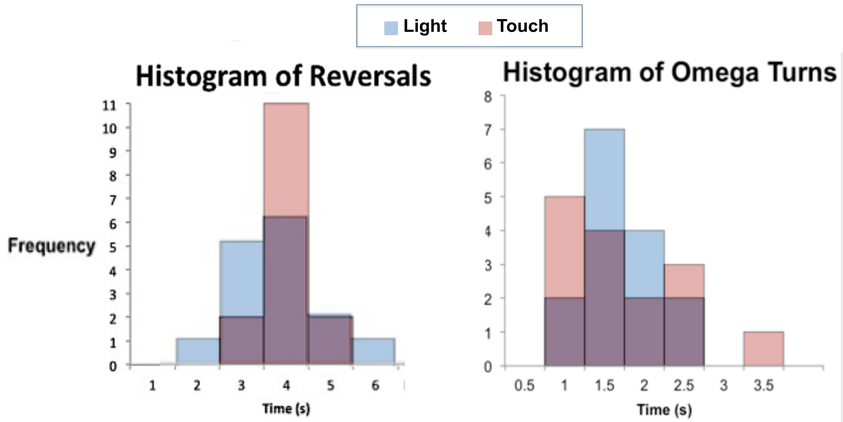

Figure 6. Histograms the length in time of reversals and omega turns in light and touch stimulation demonstrate the similarities between them. Reversals show 50% similarity and omega turns show 48% similarity.

Conclusions and Discussion

- Upon exposure to both the 405 nm light source as well as to the eyelash touch, the C. elegans worms immediately reversed direction.
- After a period of reverse motion, the worms produced an omega turn and began forward motion upon completion of the turn.
- Directional reversal was more immediate in response to touch stimulation versus the light stimulation.
- Freely moving C. elegans that were neither exposed to light nor touch stimuli were not observed performing the two-step process.
- As suggested by Edwards et al (2008), prolonged exposure to short wavelength light is lethal to C. elegans, thus necessitating a mechanism for detection and avoidance of such light, consistent with the behavior observed in this experiment.
- Nociceptors (ASH, ADL for phototaxis; ASH, PVD for mechanotaxis) are involved in both pathways, indicating that the head avoidance response is due to a painful interaction with the stimulus.

The identical two step process was observed in response to both light and touch stimulation.

Future Directions

- Neurons identified in head avoidance behavior for phototaxis include ASJ, ASK, AWB, ASH, ADL, AWC, ASI (ablation of individual neurons does not cause significant loss in light avoidance behavior, but ablation of the combination of ASJ, ASK, AWB, ASH causes significant impairment) yet the exact pathway is unknown.
- Due to response time delay in phototaxis (as opposed to mechanotaxis), it may imply that the sensory neurons activated in response to each stimulus are different yet project to the same interneurons and motor neurons that control the escape behavior.
- Future goal is to confirm or deny this by observing neuronal pathways that become active in response to light stimulation, using fluorescent protein markers to denote neuronal activity in response to stimulation.
- It has been noted that there is similarity in the neuronal pathways governing avoidance behavior in response to mechanical stimulation, thermal stimulation, and chemical stimulation, and it may be possible that the pathway governing that of phototaxis may exhibit similarities as well.

References

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