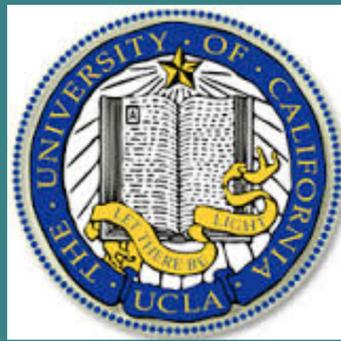




# Comparative Locomotory Behaviors between Starved and Non-starved *C. elegans* under Blue Light Stimulation

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## Introduction

The nervous system is a key component in the motility of animals, and though the human brain is the most developed and complex, others are nevertheless equipped with this beautifully complex system. We choose the nematode *Caenorhabditis elegans* as our model organism to study because it exhibits such behaviors (as shown in Figure 1 below) in the in the freemotion state, which illustrates a sense of awareness of external factors within the environment. This tells us the worm also has the ability to move around and locate food when it is hungry. But what happens if a starved worm is presented with an obstacle, such as blue light? We know from recent studies<sup>2</sup> that *C. elegans* exhibits a negative response immediately after being exposed to a pulse of blue light. But what can we say about a worm whose ability to move around in a healthy manner is affected by starvation? We hypothesize that response time (reversal) under blue light stimulation decreases as starvation time progresses, as the worm loses sensitivity to anything other than food.

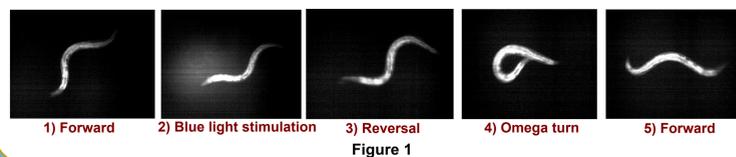
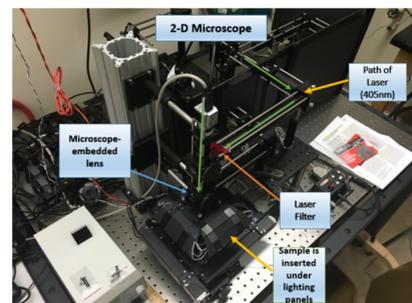


Figure 1

## Methods and Materials



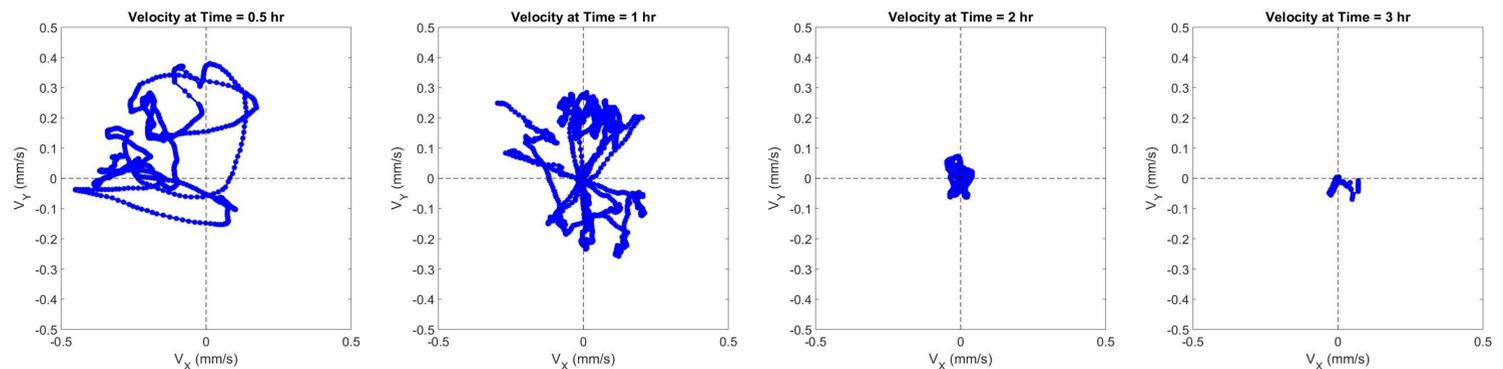
**Figure 2** – 2-D microscope (with IR camera) used with x/y motorized stage at 30.3 frames per second to produce a 320 mm/pixel image. Red light to illuminate the sample of *C. elegans* and tracked by center of mass using a *Wormtracker* feature.

**Figure 3** – 3cm x 4cm copper tape. Each copper tape is 180  $\mu$ m thick. 2% gelatin was used as the medium over this plate and later transferred onto the microscope panel in Figure 1. About 3-5 adult worms at 1 mm length were starved for 30 minutes before transfer onto plate.

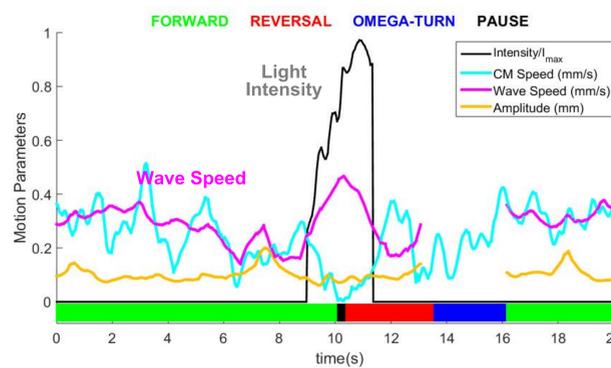


**Figure 4** – An image taken during data collection. This worm is being hit with a 405nm pulse of blue light (14.13 mW/mm<sup>2</sup> intensity) for 2 seconds. Softwares *Fiji*, *ImageJ*, and *MATLAB* used for image analysis.

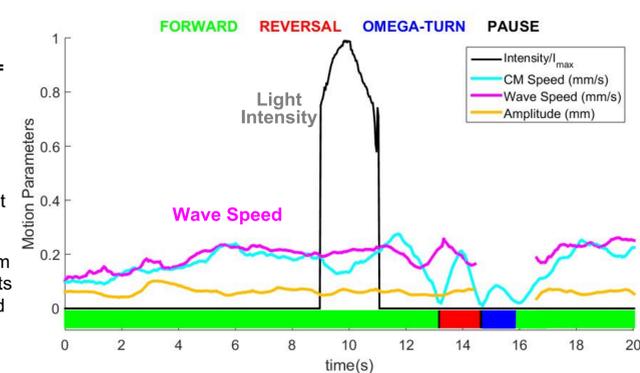
## Starved Worm Behavior under Blue Light



**Figure 5** – Center of mass velocities as starvation progresses. A drastic decrease is evident as the worm loses energy, exhibiting atypical avoidance behavior.



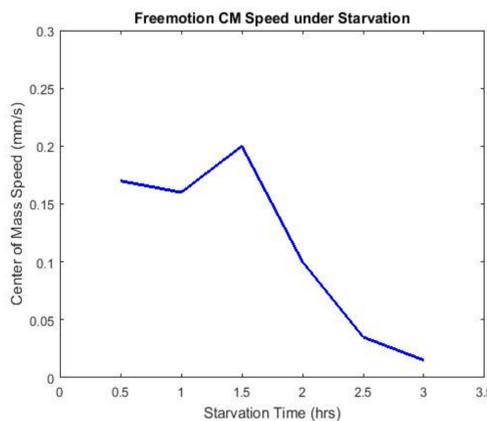
**Figure 6 (Left)** – A *C. elegans* behavior plot at  $t = 30$  minutes. The worm exhibits typical avoidance behavior as shown in the immediate reversal and center of mass velocity after 2 seconds of blue light stimulation.



**Figure 7 (Right)** – A worm starved for 2 hours exhibits atypical behavior, reflected in its lower CM speed and delayed response time.

## Free Motion Behavior

For the first experiment, we observed starved adult worms under freemotion for 3 hours. We chose the start of starvation  $t=0$  hours following standard starvation protocol (30 min). Our worm size measured to be 0.9 mm, and data was taken every 10 minutes beginning each time interval.

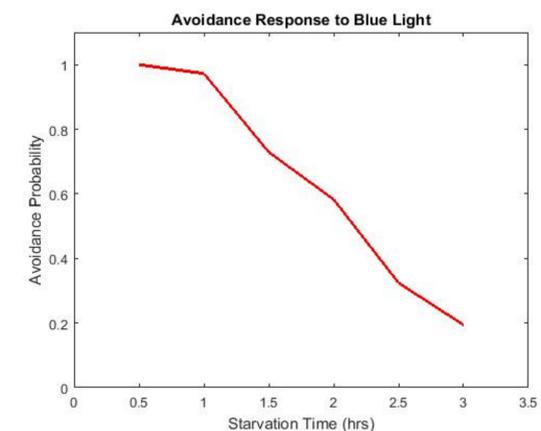


**Figure 8 (Left)** – A plot of a worm's center of mass velocity vs time. As hypothesized, we see a peak after starvation has begun, and then a sharp decline in the worm's velocity. The lack of food leads to a lack of energy, leaving the worm near motionless.

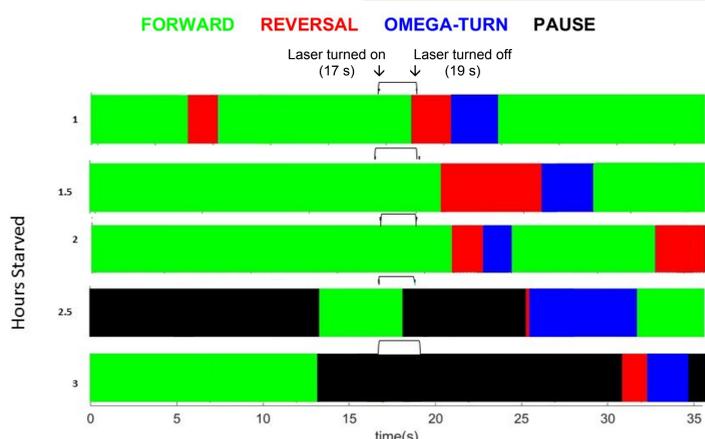
**Figure 9 (Right)** – A plot of avoidance probability as starvation time progresses. Analyses of starved worms showed a trend: a dramatic decrease in reversal time after blue light stimulation at each time interval. This aligns with our hypothesis: as time progresses and along with decreasing energy, the worm loses sensitivity to stimuli other than food, including the danger of blue light.

## Phototaxis Behavior

Following the standard starvation protocol, the worms were analyzed under freemotion surveillance for 1 minute prior to light stimulation, which lasted 2 seconds, followed by 1 minute of free motion. Data was taken every 10 minutes beginning each time interval.



## Discussion



**Figure 10** – A cumulative ethnogram of worm behavior under blue light stimulation due to starvation. As predicted, response time decreased sharply over time, as seen in the duration of and delay in reversals and omega turns following stimulation.

Under starvation for up to 3 hours, we predicted *C. elegans* to become hyperactive shortly after starvation begins, as it searches for food (around 30 minutes – 1 hour of starvation). As the lack of food (and energy) begin to take its toll on the worm, its velocity plateaus (around 1.5-2 hours of starvation) and it becomes less sensitive to blue light as it continues its search for food. By 2.5-3 hours of starvation, the worm has lost much energy and will not respond to any other stimuli other than food, including dangerous/obstructive stimuli such as blue light.

Our data has supported our hypothesis, indicating *C. elegans* loses sensitivity to stimuli other than food. This suggests it prioritizes its search for food over danger – its neural pathway for nutrition acquisition is more active than other survival necessities. Further research will have to be conducted to determine *C. elegans*' sensitivities and how it perceives and responds to stimuli in order to survive. With this, we may be able to discover the connection to survival and consciousness to stimuli/space/time in our own brains.

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