Photophobic Responses of Planaria Following Exposure to Cadmium

Chris Hartin*, Candice Sizer*, & Cassandra S. Korte, PhD

Lynn University, Boca Raton, FL

*These authors contributed equally to this work.

Abstract

Planarian Dugesia are often used in toxicological studies to model locomotor activity and neurobehavior upon exposure to toxicants. Cadmium, a heavy metal found in soils and marine ecosystems, is an environmental toxicant. Previous studies have shown the effects of cadmium on basic planaria locomotor and neurobehavioral activity (Wu, Lee, & Li, 2014). Dugesia demonstrate negative phototactic responses; meaning they tend to move away from light sources. However, there is a lack of research concerning the effects of environmental toxicants, like cadmium, on photophobia. As such, we plan to expose Dugesia to different concentrations of cadmium and compare these neurobehavioral effects using a photophobia assay. This assay consists of exposing Dugesia to cadmium chloride in square petri dishes. Following acclimation, they will be exposed to a gradient of light. Negative phototaxis will be recorded using a mounted DSLR camera and photophobic responses will be periodically counted (Paskin, Jellies, Bacher, & Beane, 2014). After 24 and 72 hours have passed, we will compare the rate of survival in both experimental and control groups. Previous studies have indicated that exposure to sub-lethal concentrations of cadmium can produce seizure-like motions, and ultimately lead to labored movement, depression, and unconsciousness (Wu, Lee, & Li, 2014). Therefore, we hypothesize that increased concentrations of cadmium may cause neurological impairments, decreasing and slowing their photophobic responses. This poster will present results from this experiment.

Introduction

Planaria are evolutionarily primitive organisms belonging to the phylum Platyhelminthes. They are free-living non-parasitic worms and are often used as model organisms to demonstrate locomotor activity and neurobehavior in toxicological studies.

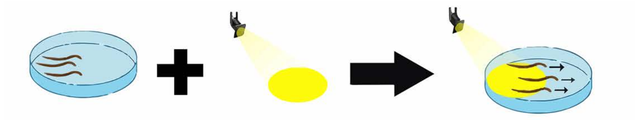


Fig. 1. Photophobic response in planaria flatworms.

Cadmium is a widely distributed heavy metal that is commonly used for industrial purposes. Since it cannot be degraded, cadmium is accumulating in the environment. Furthermore, increasing evidence shows that cadmium exerts neurotoxic effects on model organisms such as Planaria (Wu, Lee, & Li, 2014). Planaria exposed to sub-lethal concentrations of Cadmium for up to an hour and then recorded responses in morphology, neurology and morbidity (Wu, Lee, & Li, 2014).

Fig. 2. Summary of Planarian health effects following exposure to cadmium.

There is a lack of research regarding the effect of heavy metals on photophobic responses. As such, the goal of this research is to examine the impact of cadmium on photophobic responses.

Methods

Figure 3 A. A bird's eye view of the apparatus. Here we can see the Pasco sensor in the top left corner, the light which is directly above the template. Finally, the camera which is pointed down at the template, recording everything.

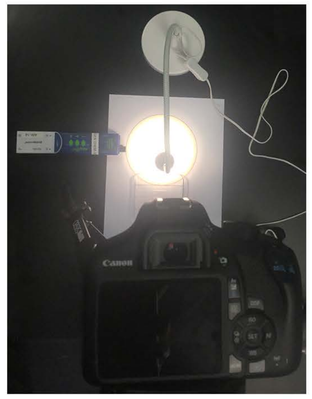
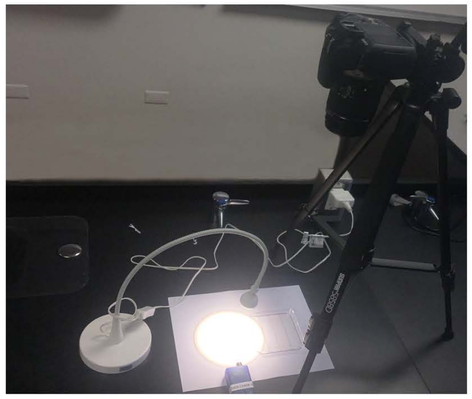


Figure 3 B. Side view of the apparatus. The Pasco sensor is measuring the light intensity from the LED light stand. While the light is shining on the template. The template is set up directly under the camera.



Acclimation: Dugesia worms were starved and acclimated to darkness for five days.

Initial preparation: Worms were randomly divided into 5 conditions (light only control, salt control, and the 3 cadmium treatment groups) with 15 worms per condition.

24-hour exposure: Worms were exposed overnight in the dark to each treatment.

Prior to experiment: Worms were placed on fresh water.

Photophobic assay: Each exposure group was then randomly subjected to the photophobic assay. For each trial, five worms were transferred from their original container into a fresh petri dish placed over a template. This template divided the dish into 4 different quadrants, each representing a distance from the light source. All worms were placed in quadrant 1, the quadrant containing the brightest intensity of light. Five seconds after the camera started recording, we switched on the lamp. After two minutes, the assay was concluded. After each trial, the worms were transferred into fresh containers according to their condition. A total of three trials were conducted for each group.

Screenshots: Screenshots were taken every 30 seconds per trial to count the number of worms per quadrant.

Survivability: The following day we counted how many worms survived in each condition.

Qualitative analysis: We observed the worms for any neurobehavioral responses. This included contraction and depression, irregular appearances, and whether death took place (dissolution of head).

Results

Time course for photophobic response amongst the controls

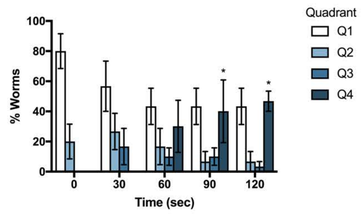


Fig. 4 A. Photophobic responses of *Dugesia* following exposure to white light for 0, 30, 60, 90, and 120 seconds. *, Significantly different by two-way ANOVA with Tukey post-tests compared to Q4 at t=0 secs (p=0.027 at 90 seconds and p=0.0072 at 120 seconds). 15 worms were assayed per time point.

Worm location after 120 seconds

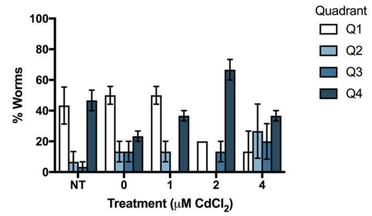
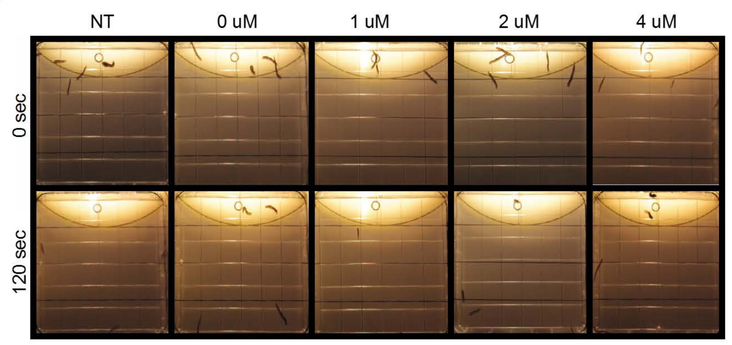


Fig 4 B. Lack of photophobic responses of *Dugesia* following exposure to cadmium (1 µM, 2 µM, and 4 µM). The concentration listed as 0 µM represents the NaCl control, included to control for any chloride effects. NT (no treatment) refers to flatworms exposed to light only. 15 worms were assayed per treatment.

Results



Representative images of *Dugesia* treated with different concentrations of cadmium at 0 or 120 seconds into the assay.

In the experiment, we observed morphological changes in *Dugesia* such as depressed morphology and contraction (red circles).

The experiment also yielded changes in neurological behavior. Snake-like movement is one of the neurological behaviors associated with cadmium exposure.

Conclusion

The results of this experiment did not support our hypothesis. There appeared to be no statistically significant differences concerning the overall effect of cadmium chloride when comparing treatments.

Before coming to any conclusions about whether cadmium chloride may have an effect on neurological responses, we need to implement new protocols to optimize our assay.

Modifying our experiment would include finding effective ways to transfer the worms by substituting nylon paintbrushes for bristles made from camel or horsehair with an angulated tip. This would make transferal easier and could reduce the trauma that worms experience. And perhaps reduce the contraction/depression response that we observed.

As demonstrated by previous studies, different wavelengths of light may alter their phototactic responses, thus we would experiment with different colors of light as a positive control for this experiment. In addition, we should adopt a quantifiable scoring system for measuring stress behavioral responses.

References

Paskin, T. R., Jellies, J., Bacher, J., & Beane, W. S. (2014). Planarin Phototactic Assay Reveals Differential Behavioral Responses Based on Wavelength. PLoS ONE, 9(12), e114708. http://doi.org/10.1371/journal.pone.0114708

Wu, J. P., Chen, H. C., & Li, M. H. (2011). The preferential accumulation of cadmium in the head portion of the freshwater planarian, Dugesia japonica (Platyhelminthes: Turbellaria). Metallomics, 3(12), 1368-1375.

Wu, J. P., Chen, H. C., & Li, M. H. (2012). Bioaccumulation and toxicodynamics of cadmium to freshwater planarian and the protective effect of N-acetylcysteine. Archives of environmental contamination and toxicology, 63(2), 220-229.

Wu, J. P., Lee, H. L., & Li, M. H. (2014). Cadmium neurotoxicity to a freshwater planarian. Archives of environmental contamination and toxicology, 67(4), 639-650.