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The Effects of Aluminum Ion Exposure on Planarian Photophobic Response to Different Wavelengths of Light

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by

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Abstract

Aluminum exposure has been linked to the development of neurological diseases (Kim et al., 2021). It has increased in quantity in the environment due to industrial activity in producing personal care products and fast food (Tietz et al., 2019). The freshwater invertebrate planarian flatworm *Giardia dorotocephala* (*G. dorotocephala*) serves as a model organism. Humans and planarians both express the ion channel transient receptor potential ankyrin 1 (TRPA1), which is responsible for thermotaxis, locomotive response to heat, and pain reception (Arenas et al., 2017). Aluminum exposure can exert ion channel-related toxicity via the TRPA1 protein. It has been found that planarian TRPA1 activates when exposed to light. The planarians have a photophobic reaction to light sources, meaning that when exposed to a light source they will instinctually swim away and towards the darkest area they can reach. This experiment addresses the question of what effect aluminum has on planarian photophobic behavior. We hypothesize that aluminum ion exposure increases photophobic behavior in planarians. The study was split into different parts that built off each other. First, planarians were exposed to various concentrations to determine their aluminum to reference dosage. It was determined that 300 mg/L aluminum was the highest dosage without acute behavioral toxicity. Next, we observed their reaction to different wavelengths of light to observe natural photophobic behavior in *G. dorotocephala*. The results suggest that all light sources garnered a photophobic response, while the no light control did not. The next experiment co-exposed the planarians to 300 mg/L aluminum and different wavelengths of light. It was found that white exposure did not elicit photophobic behavior, while UV light did cause photophobia, though this was dependent on the duration of aluminum exposure. To observe the planarians' behavior to light when TRPA is modulated, they were exposed to HC-030031 dissolved in DMSO and DMSO alone. For both

white and UV light, there was a steady decrease in their photophobic behavior. Taken together these results suggest that aluminum does not amplify their photophobic behavior. We suggest aluminum may instead inhibit planarian motility.

Acknowledgments

I would like to first thank Dr. Cassandra Korte who mentored me, helped me develop my research and gave me feedback when needed. She was always there with helpful advice whenever I felt stuck. A thanks to my committee members, Dr. Alanna Lecher and Dr. Kimberly Rowland, who were always there to help in guiding me my data analysis and my writing. I wish to thank Dr. Melissa Lehman for helping with data analysis as well. A thanks to my fellow lab assistants Jonathan Newman and Natalie Gonzalez who aided running every part of this experiment. Jonathan was there during every step of this experiment providing support and a helping hand when it was needed. A thanks to my good friend Anthony Franklin who helped me with my data analysis. Finally, I must express my gratitude to my parents and to my siblings for providing me with unfailing support and continuous encouragement while researching and writing this thesis.

Dedication

This study is in dedication to my grandmother Judith Kent Sessa. She has been a supporter of my passion for science and an inspiration to keep moving forward with my dreams.

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The Effects of Aluminum Ion Exposure on Planarian Photophobic Response to Different Wavelengths of Light

Introduction

Aluminum is one of the most common metals on Earth, and it can be toxic to humans (Centers for Disease Control and Prevention, 2015). It is the third most abundant element after oxygen and silicon, respectively. Recently, its quantity in the environment has increased through industrial activity such as in producing personal care products and fast-food packaging (Tietz et al., 2019). Unfortunately, aluminum provides no biological benefit to the human body (Exley & Mold, 2015). Though the Agency for Toxic Substance and Disease Registry (ATSDR) says it is relatively harmless, toxicity occurs when levels of aluminum in the body are high (Centers for Disease Control and Prevention, 2015).

The route of aluminum exposure includes ingestion or inhalation of substances containing aluminum. Examples of sources of exposure by ingestion are from processed foods and drugs like antacid agents. Examples of inhalation exposure sources include cosmetic product use such as antiperspirants, sun creams, and toothpaste (Tietz et al., 2019). Upon ingestion and dermal exposure, the aluminum compounds are typically poorly absorbed (Exley & Mold, 2015). Because there are multiple sources of aluminum exposure, even with low absorption rates and low relative toxicity to humans, toxicity can still occur. Symptoms of toxicity include muscle weakness, seizures, slow growth, and aluminum-induced encephalopathy (Exley, 2012). Following absorption, aluminum is distributed throughout the body to several tissues, including bone and muscle, where it has no physiological function (Kim et al., 2021). In bone, for example, it competes with calcium for absorption and can affect skeletal bone mineralization (Tietz et al., 2019).

Aluminum is excreted through urine, sweat, and fingernails (Tietz et al., 2019) with a half-life of one day. But with the frequency of aluminum exposure increasing due to manufacturing and product use, aluminum accumulation occurs at a faster rate than excretion. Thus, it is harmless in small amounts, it simply exits the body; but with higher concentrations, it accumulates and can become toxic (Centers for Disease Control and Prevention, 2015).

Aluminum has a variety of interactions with other ions in the body. These aluminum ion interactions include antagonism, or competition, with different types of cations (Exley & Mold, 2015). For example, iron(II) or iron(III) ions are found at the center structure of heme, which is a precursor to hemoglobin. Hemoglobin binds oxygen, transporting it throughout the body (Jancik & Mashlan, 2002). Aluminum ions can out-compete other cations of +2 charge because of their large magnitude +3 charge (Exley & Mold, 2015).

The present experiments examine the relationship between aluminum ion exposure on planarian behavior, such as their response to different wavelengths of light. Planarian flatworms have been used as models of stem cell biology, regeneration, toxicology, and evolution. They have also been studied extensively because they have a rudimentary nervous system and cerebral eyes with connections to the brain. Their eyes, called ocelli, are located on their dorsal side. A variety of planarian species are commonly used in research. Much previous research has focused on *Schmidtea mediterranea* (*S. mediterranea)* and *Dugesia japonica (D. japonica)*. However, due to their availability in the United States, we will be focusing on the freshwater species *Girardia dorotocephala (G. dorotocephala)*, which is also common in toxicological regeneration, neurobiology, behavior, and reproduction studies (Almanza et al., 2018).

Previous planarian research has found that photophobic reactions to ultraviolet (UV), blue, green, and white light can be modulated by their environment (Birkholz & Beane, 2017).

Photophobia is the reaction planarians have when exposed to a light source. They react by swimming away or around the light source, a response known as negative phototaxis. When exposed to red light, on the other hand, *S. mediterranea* behaves as if the light is not present; they quickly adapt after exposure and swim through it (Paskin et al., 2014). There is a spectrum of red, green, and blue-colored lights within white light, so white light can have an array of planarian reactions because of this mixture of colors of different wavelengths. The planarian reaction to green light is to quickly swim through, showing they will swim through this color of light if they have to (Paskin et al., 2014). When it comes to the visible light spectrum, blue is the shortest wavelength and planarians avoid sources of it. Under normal physiologic conditions, planarians exhibit the most extreme sensitivity to ultraviolet light, escaping it most quickly or choosing to avoid it completely (Paskin et al., 2014).

A possible environmental modulator of photophobic behavior could be aluminum interaction with ion channels. Knockdown studies of *S. mediterranea* suggest ion channels as a potential target of toxicity (Birkholz & Beane, 2017). Aluminum has been shown to interact with such channels causing adverse effects (Exley, 2012). A potential mechanism by which aluminum exposure exerts ion channel-related toxicity is via the transient receptor potential ankyrin 1 (TRPA1) protein. This protein belongs to a larger class of proteins, the transient receptor potential (TRP) cation channel family. This channel can be both voltage-activated and ligand-activated; TRPA1 ion channel activation is triggered by ligands binding to the channel including cinnamaldehyde, N-methylmaleimide, and mustard oil. However, the full range of stimulators by which TRPA1 can be activated is unknown (Ren & Bai, 2019). Importantly, it is responsible for thermotaxis, locomotive response to heat, and pain reception (Arenas et al., 2017). Upon

activation, calcium is transported through TRPA1 either into or out of the cell, depending on its intracellular concentration (Bang & Hwang, 2009).

Typical of ligand-gated ion channels, the activity of TRPA1 is increased by exposure to stimuli or agonists. Alternatively, channel activity is inhibited when exposed to an antagonist (Bang & Hwang, 2009). When TRPA1 expression is prevented, episodic pain syndrome usually develops (Kremeyer et al., 2010). Symptoms of this condition are characterized by pain in the upper body in response to cold, fatigue, or hunger with onset after the individual is born. Given its importance in response to pain, heat, variety of ligands, and its importance to survival, it is valuable to understand how different environmental factors such as aluminum affect TRPA1 activity. Previous research has looked at aluminum interactions and TRPA1, but these types of experiments have not used model organisms such as planaria (Lee et al., 2017). The past aluminum experiments that have been performed on planarians were examining the impact of exposure on regeneration. One such study showed varied adverse reactions at high concentrations of aluminum ions (Kim et al., 2021). Upon exposure, planaria developed reduced motility and seizure-like behavior. Regeneration after amputation was also slowed. Planarian cellular organelle morphology was also disrupted. The mitochondria, Golgi apparatus, and endoplasmic reticulum were irregularly structured and contained empty gap spaces. Depending on concentration, planarians even died after exposure.

Few studies have examined the toxicology of planarian photophobia responses. Furthermore, to our knowledge no studies have looked at aluminum exposure effect on photophobic behaviors, specifically. This study addresses the hypothesis that aluminum ion exposure increases photophobic behavior. The potential mechanism for activation being aluminum binding to the channel, causing subsequent activation. With TRPA1 activation in this

manner, the movement of ions between the cell and its external environment may be adversely affected. Additional studies, such as this, are necessary to fill in the gap on planarian photophobic response and behavioral responses to aluminum ion exposure.

Materials and Methods

Materials

Aluminum sulfate octadecahydrate was purchased from Carolina Biological Supply Company, Instant Ocean Sea Salts were from Instant Ocean, Chamber slides (1-well glass slide) were from Lab-Tek, HC-030031 from APExBIO, dimethyl sulfoxide (DMSO) was from Thermo Scientific, white, red, green, and blue flashlights were from Wayllshine, and the UV flashlight was from uVBeas.

Animal subjects: **Girardia dorotocephala**

Planarians were stored in a dark incubator at 23°C and starved for two or more weeks before the experiment. Worm size ranged from 2 mm to 13 mm. After being exposed to test compounds, worms were not used again.

Wavelengths of Light

The wavelengths of the light sources were red 620 nm, green 515 nm, blue 450 nm, and UV 395 nm. White was 400-700 nm.

Planarian reference dosage for aluminum

To find the reference dose, the maximum amount of toxic substance that individuals can be exposed to without adverse effects, planaria were exposed to 0 mg/L, 150 mg/L, 300 mg/L, 450 mg/L and 600 mg/L aluminum sulfate in Instant Ocean Salt (IOS) water. These concentrations are based on past experiments which found that exposure to 500 mg/L and higher caused seizure and C- shape formation (Kim et al, 2021). The solutions used in the experiments

were kept at room temperature. Controls consisted of standard IOS water (negative control) and 2% ethanol (positive control; Hagstrom etc., 2015).

Planarians were exposed for 1, 3, or 5 hours in a darkened room at room temperature, approximately 21°C. Following exposure, they were monitored for their movements in aluminum-free IOS water. Six worms were placed in the center region of a 10 cm petri dish and worm movements were recorded for 2 min. Video was then scored for seizures, formation of C-shapes, and corkscrew movements (Kim et al., 2021) by three blinded observers. Behaviors were defined as follows: Seizure behaviors were denoted when the worms locked into a single position and while being scrunched. C-shape movements were scored when the worm curls both its anterior and posterior towards each other. While corkscrew behavior was denoted when the worms spiral their vertical and dorsal sides completely. No two behaviors were counted to be happening at the same time. Seizure-like behavior ends once the worm is no longer locked into position and starts moving. C-shapes were considered complete once the worm straightened out and stopped curving over to one side. Corkscrew behavior concludes once the worm stops spiraling side to side. The percentage agreement between observers was between 50%-68% for seizure, 50%-60% for C- shape, and 61%-75% for corkscrew movements.

Planarian phototaxis responses to varying wavelengths of light

This experiment consists of a replication of previous work examining planarian differential responses to colored light (Paskin et al., 2014). Briefly, six planarian worms were placed into the first quadrant of a rectangular chamber slide containing IOS water. The slide chamber was split into four equal quadrants. Splitting the slide into quadrants provides a simple system to track the location of the worms after exposure to light over time. The first quadrant was marked with a half-circle indicating the arc with light exposure and the highest intensity

exposure. A plastic gate, that was made specifically for this experiment, was placed on the border separating quadrant 1 and 2 so the worms would not swim out of quadrant 1 before the experiment started. The light source was secured above the dish set to their lowest intensity settings. Worms were exposed to intensity gradients of differently colored lights: white, red, green, blue, or ultraviolet. The control for this experiment consisted of exposing to no light, that is the experiment occurred in the same darkened room without specific light exposure. Once all the worms were in the dish, video recording began and the light was turned on. Worm location was documented at 30 sec intervals over a 2 min time period. After each trial, the worms were given a day of rest and added back to the main population to be used in future experiments.

Effect of aluminum on planarian negative phototaxis to varying light wavelengths

Planarians were exposed to the reference dose of aluminum sulfate as reported above. The worms were exposed for 1, 3, and 5 hr and then moved to quadrant 1 of the chamber slide in fresh IOS water, as previously described. They were then exposed to control (no light), white, or ultraviolet light, and their phototaxis responses were be recorded for a total of 2 min each.

Pharmacological inhibitor HC-030031 effects on* G. dorotocephala *photophobia

This experiment observed planarian *G. dorotocephala* negative phototaxis when TRPA1 is pharmacologically modulated (Lee et al., 2017). Because it was previously used in experiments with different planarian species, HC-030031 was used as the TRPA1 antagonist in this experiment. Six worms were exposed to 100 μM HC-030031 in <1% dimethyl sulfoxide (DMSO) in IOS water, DMSO solvent control, or IOS water alone. After 5 min exposure, they were transferred into a fresh dish and exposed to control (no light), white light, or ultraviolet light for assessment of photophobic responses, as previously described.

Statistical analysis

The experiments in which aluminum reference dosage and effect of aluminum ion on phototaxis were performed in triplicate, with 5-7 worms per treatment. Planarian phototaxis responses to varying wavelengths experiments were repeated 10 times with 6 worms per light treatment. Experiments with the pharmacological modulator HC-030031 were repeated six times per wavelength of light, six worms per treatment.

Reference dose data were analyzed by one-way ANOVA with Tukey multiple comparison of means post hoc analysis. A multinomial test was performed for interpreting the planarian phototaxis responses to varying wavelengths of light. It considers the location of the worms within the dish at all four quadrants concurrently. The multinomial test was chosen since it is able compare experimentally collected distributions to a hypothetical even distribution between quadrants, as would be expected if worms failed to produce a photophobic response. Fisher's exact test was performed for interpreting the planarian responses to varying wavelengths of light while being exposed to aluminum and HC-030031 on quadrant-collapsed data. The decision to choose the multinomial test for the light comparison while treatment comparison used a Fisher exact test was due to gaps in exposure time. In the aluminum photophobia experiment, the light comparisons with just IOS water were done at the same time. Quadrants 1 and 2 collapsed, while quadrants 3 and 4 collapsed together before running the test. These data were collapsed for analysis because of the low numbers of worms per experiment (thus, per quadrant) violating requirements for other tests (i.e., chi-square test) (McHugh, 2013).

Results

Planarian reference dosage for aluminum

This experiment was performed to determine the reference dosage for aluminum, which is the concentration to be used for subsequent experiments. We identified the reference dose for toxicity by exposing planaria to varying concentrations of aluminum sulfate and identifying the concentration at which aluminum exposure elicited the least number of acute toxicity behaviors (i.e., seizures, C-shapes, and corkscrew movements).

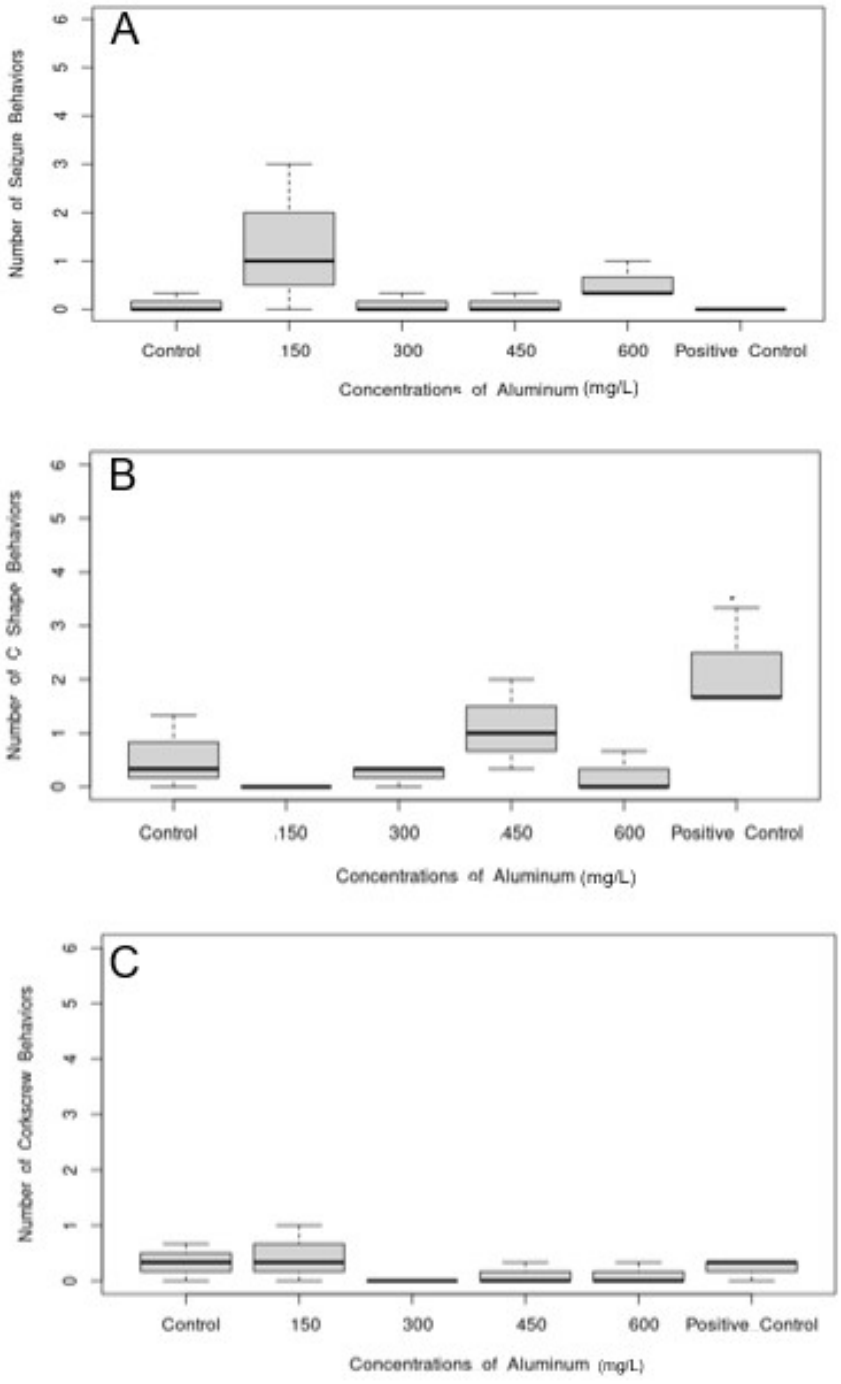
For the 1hr of treatment there was no statistical significance for seizures results ($p<0.01$; Figure 1 A). The 3 hrs and 5 hrs of treatment, seizures steadily increase with exposure to 600 mg/L of aluminum compared to the time-matched no-treatment control ($p<0.001$; Figure 2 A and 3 A).When it comes to C-shape at 5hrs, the positive control of 2% ethanol had the highest number of this behavior occurring with 2 planarians exhibiting C-shapes compared with no-treatment control ($p< 0.001$ Figure 3 A). Aluminum exposure failed to stimulate a significant increase in C-shape behavior at 5 hr exposure for any of the concentrations.

When it comes to corkscrew, the positive control of 2% ethanol had the highest amount exhibiting this behavior with up to 3 worms. These instances occurring for the 3 hrs and 5hrs. ($p< 0.001$ Figure 2 B and 3 B). Taken together, these behaviors suggest that aluminum is having an impact on the worms in both dose- and duration-dependent manners. When observing the different behaviors, for 450 mg/L and 600 mg/L aluminum, C-shape and corkscrew more readily occur with 1-3 hr (Figure 1 B-C and Figure 2 B-C, respectively), while seizures occur after 5 hr (Figure 1 A). Because exposure to both 450 and 600 mg/L aluminum increased seizures and C shapes, and 300 mg/L appears to be the highest concentration with the least number of

significant effects, we determined it to be the reference concentration for subsequent experimentation.

Figure 1:

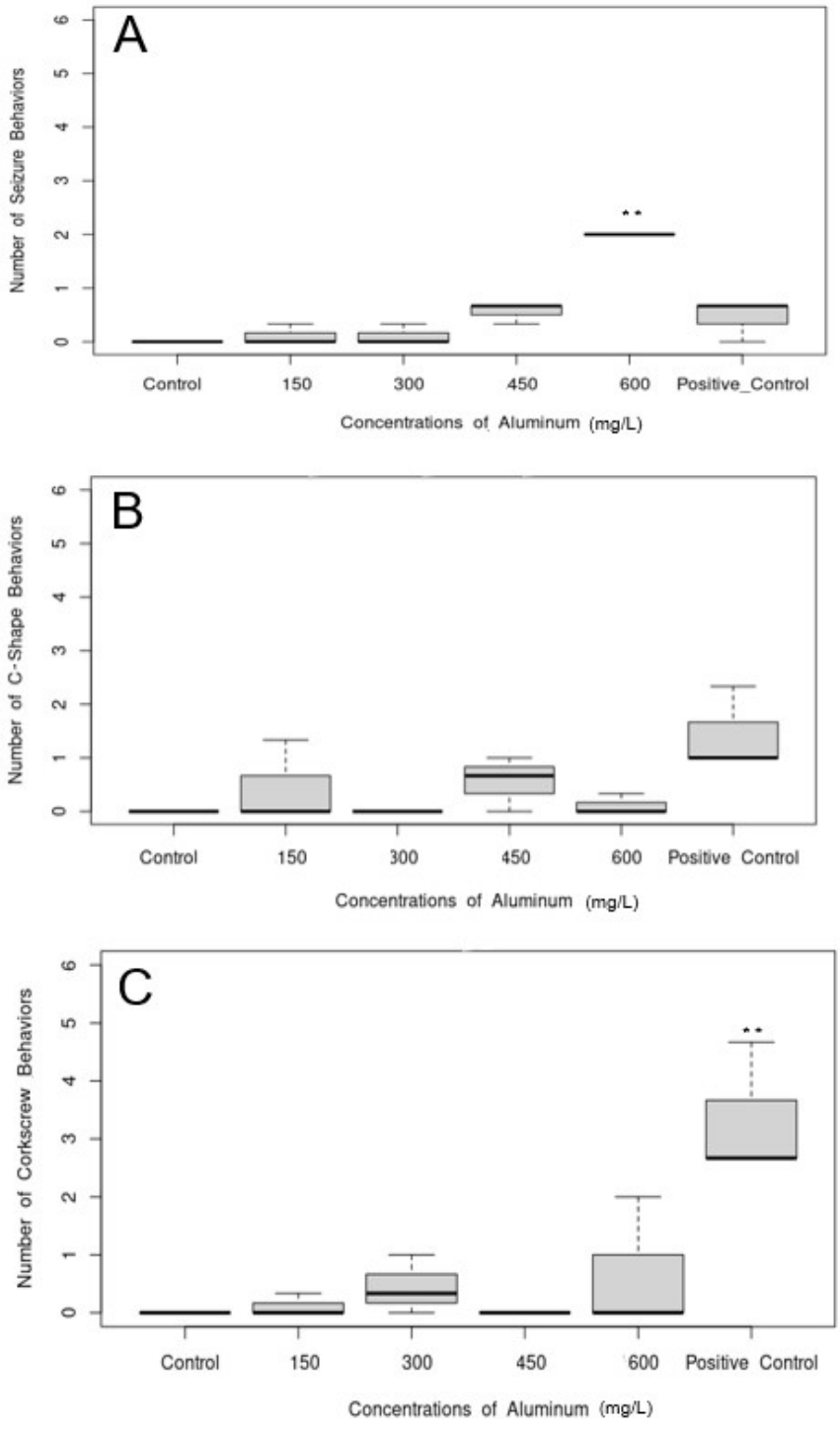
Planarian behavior following exposure to varying concentrations of aluminum for 1 hr



Note: Planaria were exposed to 0, 150, 300, 450, or 600 mg/L aluminum for 1 hr. Ethanol (2%) was included as a positive control. Seizures (A), C-shapes (B), corkscrew behaviors (C) were measured for 2 min. Data are presented as box plots with median number of behaviors for n=5-7 worms per treatment from triplicate experiments. Whiskers indicate range of behaviors and gray boxes show interquartile range.

Figure 2:

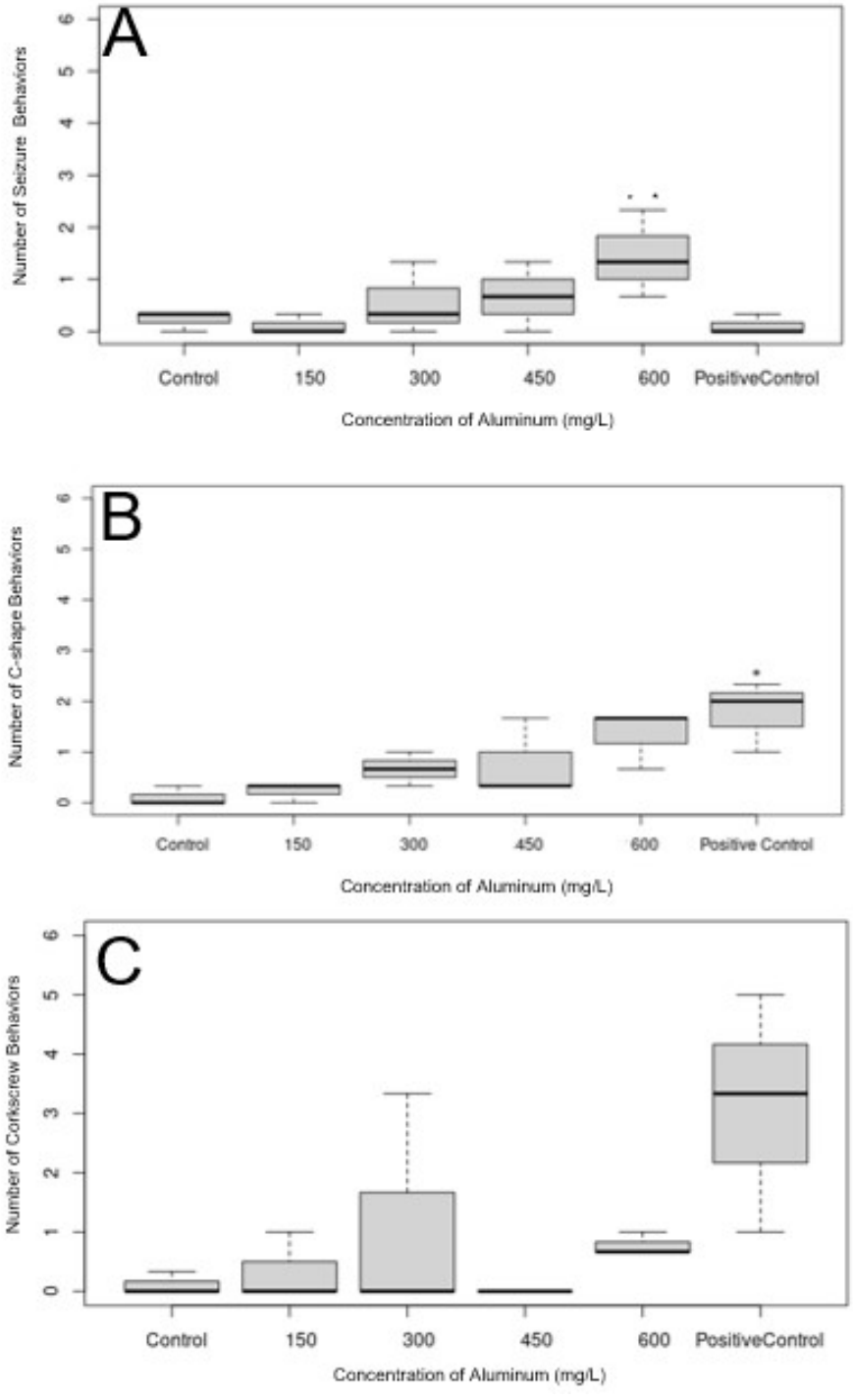
Planarian behavior following exposure to varying concentrations of aluminum for 3 hr



Note: Planaria were exposed to 0, 150, 300, 450, or 600 mg/L aluminum for 3 hr. Ethanol (2%) was included as a positive control. Seizures (A), C-shapes (B), corkscrew behaviors (C) were measured for 2 min. Data are presented as box plots with median number of behaviors for n=5-7 worms per treatment from triplicate experiments. Whiskers indicate range of behaviors and gray boxes show interquartile range. *, $p<0.01$, and **$p<0.001$ compared with time-matched controls by one-way ANOVA with Tukey post hoc analysis.

Figure 3:

Planarian behavior following exposure to varying concentrations of aluminum for 5 hr



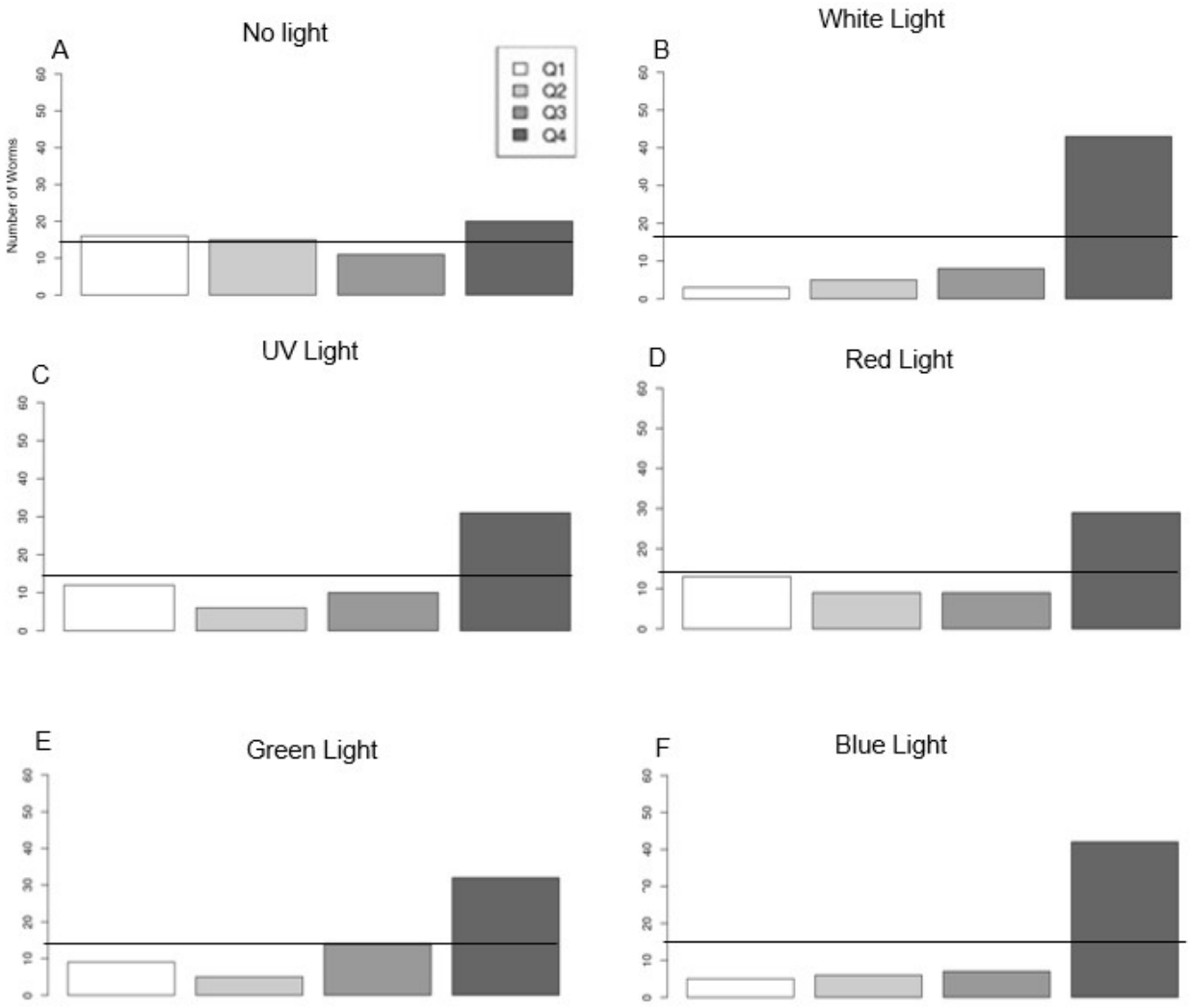
Note: Planaria were exposed to 0, 150, 300, 450, or 600 mg/L aluminum for 5 hr. Ethanol (2%) was included as a positive control. Seizures (A), C-shapes (B), corkscrew behaviors (C) were measured for 2 min. Data are presented as box plots with median number of behaviors for n=5-7 worms per treatment from triplicate experiments. Whiskers indicate range of behaviors and gray boxes show interquartile range. *, $p<0.01$, and **$p<0.001$ compared with time-matched controls by one-way ANOVA with Tukey post hoc analysis.

Planarian phototaxis responses to varying wavelengths of light

Previous research indicates that planarian flatworms have a photophobic reaction to light. Though these studies were performed with *S. mediterranea and D. japonica,* there is a need for data to be gathered on alternative species, such as *G. dorotocephala* (Sabry et al., 2019). Here, we looked at a total of 60 worms per light source and counted their location after 2 min. As expected, worms in the no-light control were evenly distributed across quadrants (Figure 4 A; $p=0.450$). However, light-exposed planaria were not evenly distributed across dishes, with fewer worms in the brightest quadrant (Q1) and more worms in the darkest (Q4). White light accumulated 43 worms in Q4 (Figure 4 B), UV had 32 worms (Figure 4 C), red light 30 worms (Figure 4 D), green had 30 (Figure 4 E), and blue light had 43 (Figure 4 F).

Figure 4:

Planarian quadrant location after 2 min exposure to varying wavelengths of light



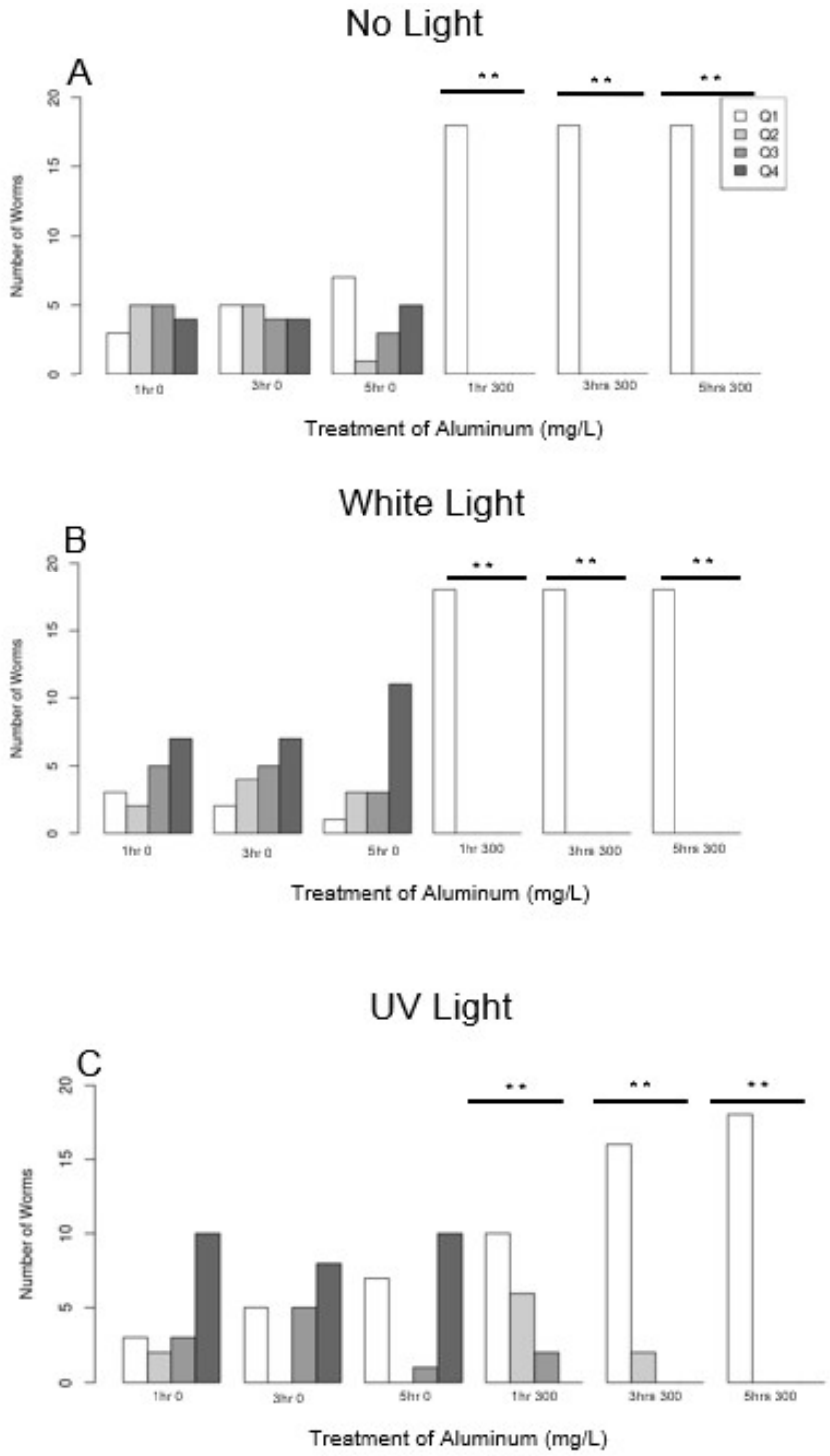
Note: Planaria were exposed to no light control (A), white (B), UV (C), red (D), green (E), and blue light (F) for 2 min. Worm location was then counted across quadrants. Data are presented as total worms for n=60 worms per light. Solid line indicates worm number per quadrant if worms were evenly distributed (15 per quadrant), which would be if a photophobic response was not achieved. White, UV, red, green, and blue light worm distributions all differed from this number ($p<0.001$ for each light) by multinomial test analysis.

Aluminum ion effect on planarian phototaxis reaction to different wavelengths of light

Using the reference dose identified in previous experiments, the worms were then exposed to 300 mg/L of aluminum-IOS water. For this experiment the planaria were exposed to white light, ultraviolet light, and no light, with a control of aluminum-free IOS water. The worms exposed to aluminum without light had all 18 worms remain in quadrant 1 no matter the duration of the exposure see 1 hr ($p<0.001$) and 5 hr ($p<0.001$) (Figure 5 A). Worms exposed to aluminum, then white light, were shown to not behave with photophobic behavior; 18 worms remain in quadrant see 1 hr ($p<0.001$) and 5 hr ($p<0.001$) (Figure 5 B). Among the groups exposed to UV light and 1 hr of aluminum exposure, 9 worms stay in quadrant 1 and no worms were in quadrant 4 amongst the UV exposed worms ($p<0.001$; Figure 5 C). At 3 hr, 2 worms moved to quadrant 2. Lastly, after 5 hours, none of the worms made it out of quadrant 1 for the aluminum and UV exposed planaria ($p<0.001$; Figure 5 C).

Figure 5:

Planarian location in photophobia assay after 2 min exposure to varying wavelengths of light



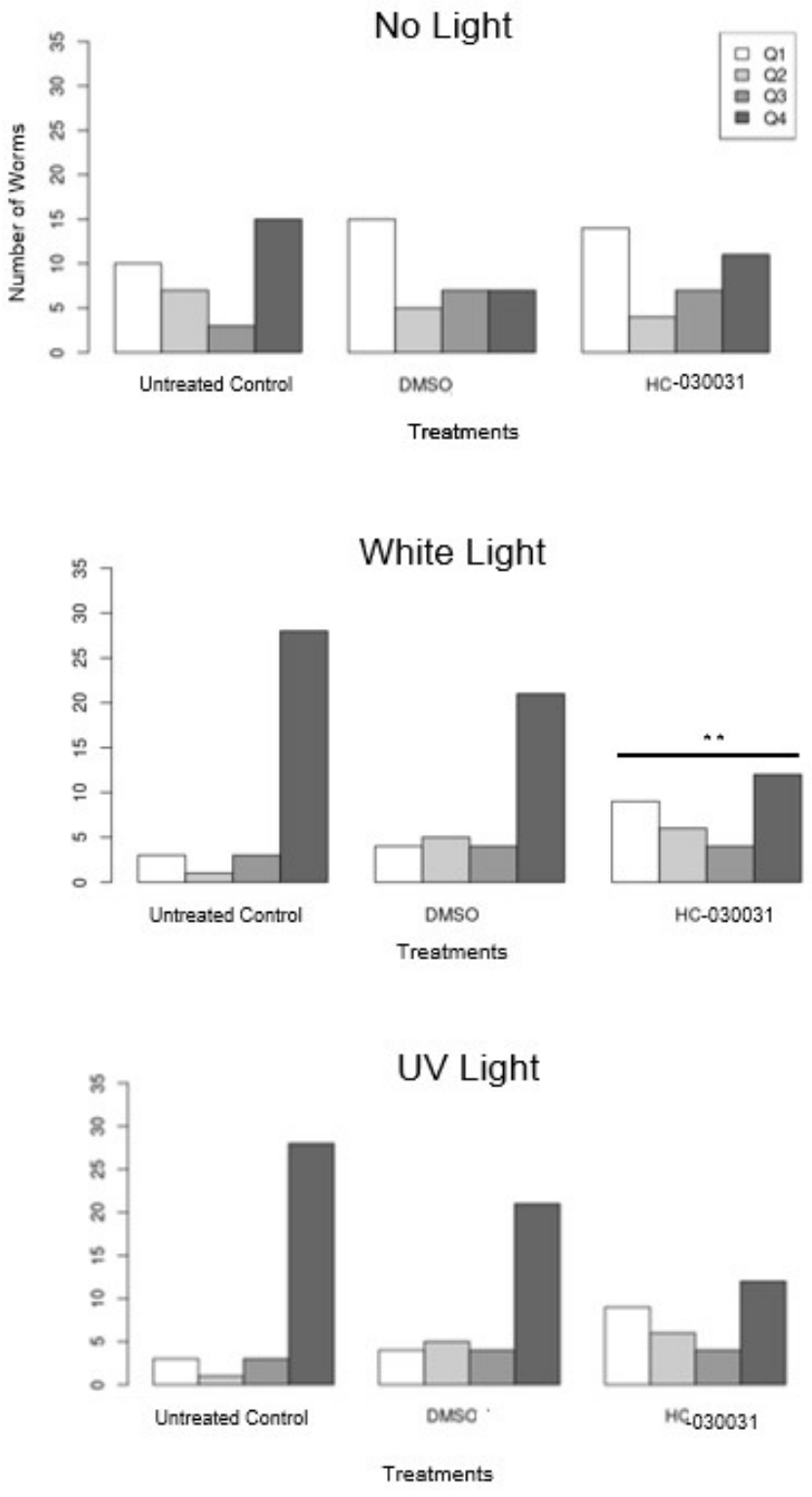
Note: Planaria were exposed to no light control (A), white (B), UV light (C), for 2 min after exposure to 300 mg/L aluminum for 1, 3, or 5 hr. Worm location was then counted across quadrants. Data are presented as total worms for n=5-7 worms per treatment in triplicate. **p<0.001 compared by collapsed Fisher's exact test compared with time-matched no-treatment control.

Pharmacological inhibitor HC-030031 effects on G. dorotocephala

Previous research indicates that HC-030031 activates planarian TRPA1 ion channel. Though these studies were performed with *S. mediterranea and D. japonica,* there is a need for data to be gathered on alternative species, such as *G. dorotocephala* (Sabry et al., 2019) We looked a total of 36 worms per solution exposed to different wavelengths. For this experiment the planaria were exposed to white light, ultraviolet light, and no light, with a control of aluminum-free IOS water. Amongst the no-light control worms, it appears worms begin to accumulate in quadrant 4 however these results did not reach statistical significance. With white light exposure, photophobic responses were as expected amongst the non-treated controls with 28 worms in quadrant 4. The solvent control (DMSO) had 21, interestingly, treatment with HC-030031 had just 12 in quadrant 4 ($p<0.001$; Figure 6 B). Amongst the UV light exposed, a similar non-significant trend was observed, there were 30 worms in quadrant 4 in the controls, DMSO-solvent control had 20, and HC-030031 had 18 (Figure 6C).

Figure 6:

Planarian location after treatment with TRPA1 inhibitor HC-030031 and exposure to light



Note: Planaria were exposed to no light control (A), white (B), and UV light (C) for 2 min after pre-treatment with 100 μM HC-030031, <1% DMSO solvent control, or no-pretreatment for 5 min. Worm location was then counted across quadrants. Data are presented as total worms for n=36 worms per treatment. **$p<0.001$ compared by collapsed Fisher's exact test compared with no-treatment control.

Discussion

In the present study, we identified a reference dosage to use as a starting concentration for aluminum exposure. Past experiments observed the effect of aluminum exposure on planaria and a significant decrease in motility occurs at 500 mg/L, but not 100 mg/L (Lee et al, 2017). This gave us the range of exposure to narrow concentrations down. We started with 150 mg/L because it was higher than results that stimulated observable behavioral change. We increased by 150 mg/L to keep our increments consistent along the four concentrations used. Our highest exposure concentration stopped at 600 mg/L, because at that concentration the threshold identified in previous studies was exceeded (Kim et al., 2021). Our results showed that the as the duration of exposure to aluminum increased, there was also an increase in acute behavior that denotes toxicity. These atypical behaviors reflect that aluminum was having a toxic effect on them. These behaviors, such as C-shape and corkscrew movement, occur along all concentrations tested, but do not have a clear concentration-dependent pattern. While seizures occurred with a few concentrations such as 450 mg/L and 600 mg/L (Figures 1 A, 2 A, and 3 A).

There are a couple of reasons that can explain the results of such acute atypical behaviors. First, based on our observations it appears corkscrew and C-shape reactions are relatively common behaviors when planarians are exposed to environmental irritants, while seizures occur

less often. It is possible that these two behaviors are a prelude to seizure or are more easily triggered then a seizure in planarians. Secondly, when planarians seize, they are likely inhibited from carrying out other behaviors. For example, planarians could go from a C-shape to a corkscrew, and back to a C-shape. Seizures, however, prevent them from doing other such behavior. There is also the possibility that by 5 hr exposure to aluminum, there is interference with other aspects of planarian physiology, including motility.

While studying photophobic behavior in *G. dorotocephala* results were significant, which aligns with previous research (Paskin et al., 2014). Like past research, the no light control has a nearly even distribution, likely because the planarians swim unabated by light (Figure 4). As expected, worms were not evenly distributed across dishes exposed to white, green, blue, and UV light. Surprisingly, the red-light worms also showed a similar distribution in location, though fewer worms were found in the darkest quadrant. Previous research in *S. mediterranea* had suggested a lack of response to red light in a similar assay (Paskin et al., 2014). This lack of response to red light could be because in their natural environment in streams and ponds, water filters out most light, but red light penetrates further into the streams. Typically, the only wavelengths that can reach them are longer wavelengths such as red light. Shorter wavelengths, like blue light, are filtered and thus their exposure is not common. They evolved this way to adapt to their environment, when they detect other wavelengths like white and UV they swim away. These wavelengths would most likely be found in the presence of predators as planarians stray from the protection of the underside of rocks, so their instinctual response is to swim away. There may be a difference in intensity of the red flashlight that was used in the current study compared to previous research and the magnitude of typical exposure in their environment.

While red light garnered the lowest reaction of the light sources, which reflects previous studies results.

The results of this experiment do not support the hypothesis that aluminum exposure increases photophobic behavior. With co-exposure to aluminum and white light, the worms either stayed in place contracting and wiggling their bodies in place in quadrant 1 (Figure 5 B). While in UV light, nine worms leave quadrant 1 with 1-3 hr of exposure, but by 5 hrs the worms did not leave quadrant 1 either (Figure 4 C). Importantly, aluminum-exposed no-light controls also remained in quadrant 1. Given the normal exploratory behavior of planarians in dark (Figure 2 A), these data suggest aluminum inhibition of planarian motility. Planarian motility or movement is characterized by gliding motion with the cilia that line the ventral side of their body and to perform muscular undulation. Like other animals, planarian motility is linked to ion channel-mediated transport of ions within muscle cells.

Aluminum may disrupt cellular function by interfering with ion channel function, resulting in a lack of planarian motility. Magnesium, for example, serves as an important regulator of ion channel activity, such as voltage gated channels, and ion binding proteins, like those that bind calcium. Because magnesium mediates calcium ion transport in sarcoplasmic reticulum storage centers, aluminum interreference with magnesium may prevent the muscle contraction required for motility (Exley, 2012). An additional potential mechanism of magnesium disruption includes direct ion competition. In nucleotide synthetic enzymes, for example, magnesium lies at the active site. Competition for aluminum at the active site could thus lead to inhibition of synthesis of ATP. ATP is the essential usable energy form in the cell, with processes such as muscle contraction, nerve impulse propagation, and many others

dependent on its hydrolysis. Disruption in each process may lead to adverse effects like reduced motility.

Aluminum is also a competitor for calcium, which is important for motility (Exley, 2012). Calcium ions play a key role in signal transduction pathways, where they serve as a second messenger. They interact with ion channels that are released by ligand-binding or voltage changes in the cell, which triggers calcium second messenger movement across membranes. Aluminum can lead to improper flow of calcium ions, causing pathways dysfunction, and a subsequent decrease in motility. In addition, several enzymes require calcium as cofactors for reactions. Aluminum ions could bind proteins such as calretinin and calmodulin, causing a conformational change leading to loss of function. Thus, because of its similar chemistry to cations like calcium, aluminum exposure may cause toxic responses, such as interference with muscle contraction, energy requirements for movement, and the signals required to propagate movement.

We speculate another potential target for aluminum disruption of photophobic responses is the TRPA1 ion channel. This channel is expressed throughout the planarian body, not just the eyes (Birkholz & Beane, 2017). TRPA1 ion channel activation has been linked to exposure to light sources on the epidermal membrane (Birkholz & Beane, 2017). Furthermore, previous studies have observed a lack of photophobic response in epidermal TRPA1 planarian knock down experiments, indicating the dependency of photophobic responses on TRPA1 activation. Linking with aluminum, previous research suggests aluminum activates TRPA1, at least in mice (Lee et al., 2017). With TRPA1 activation in this manner, there is flux in ions across the cell membrane. Based on these data we had hypothesized that aluminum may increase TRPA1 activity, thereby increasing photosensitivity. However, with TRPA1 activity also dependent on

the concentration of aluminum ions, a disruption in the flow of ions can affect cellular function including motility responses.

We proposed that TRPA1 activation would impact the planarian behavior through an increase in photophobic behavior, which was based on previous studies (Birkholz & Beane, 2017). It was found in mice that an activation of TRPA1 occurs with aluminum exposure (Lee et al, 2017). The results of our study suggest inhibition of the ability to carry out photophobic behavior. For motility to be carried out, transport of ions is acquired, and aluminum 3+ would interact with the flow of ions. The aluminum ions would interfere with the flow of calcium ions, because of them having a more positive charge. TRPA1 is a candidate for interference of ion flow, leading to inhibition of motility, and the inability to carry out their photophobic behavior.

The present experiment showed the worm location after 2 min of light exposure, but an interesting behavior was observed during this time. Under UV and blue light, the planarians initially swam away from the light source towards or into quadrant 4. But after 2 min they begin to circle back into previous quadrants (Figure 4 C and 4 F). A possible explanation for this behavior is that the worms could be searching for a dark location in the chamber slide. So, they might explore the slide within the first minutes then back track looking for a darker area. To our knowledge, previous experiments did not observe such behavior. It is possible that previous researchers did not report this behavior, or more interestingly, that it did not occur in other species. This finding raises new questions about potential species differences in photophobic responses. Another potential explanation for this behavior is that planaria are over-stimulated by the blue and UV light sources, and they do not stay in the dark end of the chamber because of it. In our assay, the planarians could be unable to escape the light are thus continuously swimming, since such stimulation does not allow them to rest.

The results from pharmacologic disruption of TRPA1 with HC-030031 were not as expected. We predicted that the worms would have an increased photophobic response when compared to their controls. However, based on the observable behaviors there was a decline in their photophobic behavior with HC-03001 treatment. These results can be due to fatigue caused by TRPA1 activation by the modulator causing improper flow of ions. Another possibility comes from the nature of modulator HC-030031 interactions with the planarian TRPA1. In mice and humans, HC-030031 is known to inhibit TRPA1 channels, while in planarian species *S. mediterranea* and *D. japonica,* the agent acts as a TRPA1 activator (Sabry et al., 2019). Thus, a major limitation of using HC-030031 is that we cannot directly test the type of interaction it is having with TRPA1. The TRPA1 ion channel has remained in humans and other species despite millions of years of evolution. Its function in reacting to noxious stimuli has been conserved, but the full array of TRPA1 activators is not known and may vary across species, such as with HC-030031. Thus, future experiments should compare HC-030031 interaction with inhibitors, agonists, or use a genetic approach in *G. dorotocephala* to address the question more fully regarding the role of TRPA1 in planarian photophobic responses.

A further limitation of this study is the accuracy of the light source placement in relation to the dish. Though the light beam was adjusted in this experiment, there was a limit to the extent the adjustment. As a result, a disparity could develop in the alignment of the dish, quadrants marker sheet underneath the dish, and light source which then could have affected their behavior. In addition, the uniformity of light passage through the dish may have been affected by the volume of water in the dish. A dish that could be more precisely aligned with the gradient of light might yield more accurate photophobic behavior.

In summary, this experiment does not support the hypothesis that aluminum ion exposure increases photophobic behavior. Though our observations suggest that aluminum inhibits their photophobic behaviors in white light and UV light at low exposure durations. Our results raise additional questions about the nature of the interaction between aluminum exposure and planarian photophobic responses. This differential response to white and UV light in the aluminum-exposed planarian indicates that they are detecting different wavelengths of light, with distinctive responses despite exposure. The mechanism responsible for wavelength detection and its sensitivity to environmental insult begs further investigation. Another future direction includes exposure to aluminum in a calcium ion-deprived environment or to an antagonist that interferes with calcium-ion transport, based on our speculation about the role of calcium signaling in motility. It is evident that the mechanism by which aluminum exposure inhibits planarian photophobic behavior and motility warrants further exploration.

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