Using scorpion activity variation to locate extraocular cuticular light receptors.

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Fall 2017

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Summary

When exposed to ultraviolet (UV) light, scorpions fluoresce at wavelengths in the cyan-green range, but the reason behind this fluorescence remains unknown. There is behavioral and molecular evidence that corroborates the presence of extraocular photoreceptors in the scorpion cuticle; however, little is known as to whether or not scorpion photoreceptors are present throughout the entire body or localized to a specific region. Our research intended to determine whether specific areas of the scorpion cuticle are photosensitive by attaching fiber optic cables to three different segments of the body. We exposed the third dorsal mesosomal segment, the fifth ventral metasomal segment, and the first dorsal metasomal segment of striped bark scorpions to UV, cyan-green, and no light using the fiber optics. We filmed the trials and made qualitative observations. We also used an automated tracking program to calculate the average instantaneous velocities of the scorpions' movements. We expected body segments possessing photoreceptors for a specific wavelength to induce greater average scorpion activity when stimulated; ultimately though, due to certain design flaws and time constraints, our data were inconclusive. However, the question we proposed remains valid and worth further investigation.

Introduction

Scorpions have the unique trait of fluorescing under UV light, but little is known about why this exists or how it works. When exposed to UV light, the cuticle of scorpions glows cyan-green. While the majority of scorpions are capable of fluorescence, certain species, such as those belonging to the Pseudochactidae family, live exclusively in caves and do not fluoresce, most likely because of the absence of light (Lourenço 2012). The function of fluorescence has not yet been identified, but recent research proposes a role in scorpion light detection.

Some information exists on both the chemicals responsible for the fluorescence and behavioral responses to UV and cyan-green light. Mechanistically, the presence of both beta carboline and 4-methyl-7-hydroxycoumarin within the cuticle causes scorpions to fluoresce under wavelengths of UV light (Satchel et al. 1999; Frost et al. 2001). The overall activity of scorpions increases when exposed to UV or cyan-green light, but not with other wavelengths such as infrared (Blass & Gaffin 2008). Also, UV wavelengths with the intensity matching those present one hour before sunset were found to invoke the highest scorpion activity levels (Gaffin & Barker 2014). When scorpions were tested with their eyes either blocked or unblocked under UV and cyan-green light, scorpions with their eyes blocked were far less likely to react to cyan-green light but remained reactive to UV light. This study suggested that the entire cuticle may act as a UV photon collector, transducing UV to green light (Gaffin et al. 2012).

These data indicate that a scorpion's ability to detect variation in light may assist it in detecting shelter. Scorpions that have lost fluorescence as a result of photobleaching are less inclined to seek shelter and demonstrate higher activity levels compared to those with average fluorescence levels; these data suggest that scorpions use their sensitivity to UV light as a way to determine if they need to seek shelter (Kloock et al. 2010). In the field, scorpions are often observed to position themselves under blades of grass or some other cover (Gaffin et al. 2012). Moreover, scorpions with their eyes covered and uncovered found shelter from UV light equally well (Zwicky 1968). These results imply the

presence of photoreceptors in at least some section the scorpion cuticle. A study on the genetic basis for scorpion light sensitivity found Mmopsin3 genes in the tail, proposing it as a primitive light-sensing organ similar to those found in sea urchins and hydra (Cao et al. 2013).

The aim of our project was to gather more evidence regarding the possible localization of scorpion photoreceptors on the cuticle. The ventral nerve cord of the scorpion tail shows a photosensitivity range from long UV to cyan-green light (Zwicky 1968). As such, we tested scorpion behavioral responses to those wavelengths on the ventral side of the fifth metasomal segment. We also tested two other locations: the dorsal mesosoma and the dorsal side of the first metasomal segment on the dorsal side. We selected these areas because they are regularly exposed to overhead light, and they differ in their pigmentation. The darker pigment of the mesosoma segment may be a factor that affects light absorption (Zwicky 1968).

We used light transmitted by fiber optics that would be directed to a pinpoint location of the body to determine whether extraocular photoreceptors exist. We also explored the effects of different light wavelengths on those specific body areas. Because scorpions are negatively phototactic, we hypothesized that if scorpions possess extraocular photoreceptors in the regions of the body under consideration, and we use fiber optics to direct isolated UV and cyan-green light wavelengths at the selected parts of the scorpion cuticle, then the animals' activity will increase as if their entire body was exposed to the wavelengths. As for our results, most of the data collected was found to be inconclusive and widely varied, due to inconsistencies in testing and lack of sufficient trials.

Methods

Animal care

We used nine male *Centruroides vittatus* Say, 1821 collected on September 20th, 2017 from Lake Thunderbird State Park near Norman, OK (35.2160°N, -97.2483°W). We kept the animals in individual glass jars with 2-3 cm of Eco Earth coconut fiber substrate with a 5 cm^2 terra cotta pot shard. We fed the scorpions one cricket every two weeks and gave them 20-30 mL of water three times per week. We obtained crickets, food, and hydration crystals from Rainbow Mealworms Inc. (Compton, CA). The room temperature remained between 21 & 24 ℃ with room lighting following a natural light cycle.

Behavioral Apparatus

We filmed the scorpions from beneath a Plexiglas sheet mounted on a PVC stage on a vibration minimizing mat using an infrared-sensitive indoor camera (Nest;

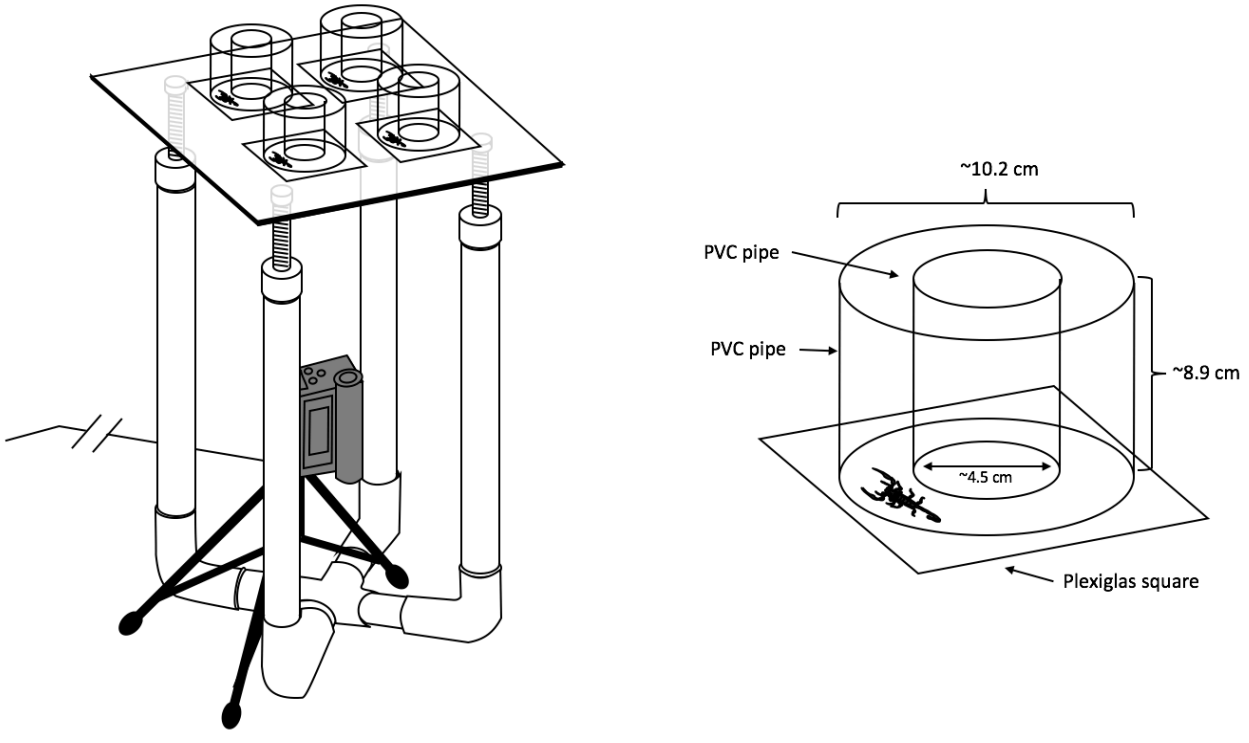

Fig. 1: Experimental design. Concentric PVC pipes form round tracks with open tops where scorpions with fiber optics connected to light sources can move freely. A transparent Plexiglas base provides a platform and enables video recording for later analysis. Entire apparatus was surrounded by a cardboard structure to create total darkness.

Fig. 2: Fiber optic attachment locations. From left to right: ventral side of the fifth metasomal segment, dorsal side of the first metasomal segment, and dorsal side of third mesosomal segment.

NC1104US) that uploaded the footage to an internet server. We created four individual chambers by mounting PVC pipes (9 cm tall x 10 cm diameter) directly onto clear Plexiglas squares. To create a circular running track, we glued a smaller PVC pipe (10 cm tall x 4.5 cm diameter) to the center of the chamber (Fig. 1). We surrounded our apparatus with a cardboard shelter to block out any ambient light during the tests.

Trial preparation

One at a time, we anesthetized scorpions in the freezer in a pre-chilled coplin jar for about three minutes. Upon removing the scorpion from the freezer, we placed it on a glass lid so that it would remain cold and immobilized, and we used forceps to secure and position it further. We then used Gorilla Super Glue Gel (MN: 7700103) to attach a 48 cm fiber optic (0.25 mm) that we had sheathed in black paint (Apple Barrel Acrylic Paint, MN: 20504) to the scorpions' cuticle at the predetermined locations. Three scorpions were assigned to be attached at each of the three locations: third dorsal mesosomal segment, the fifth ventral metasomal segment, and the first dorsal metasomal segment (Fig. 2). We held the fiber in place above the scorpion by securing it against a dowel in an adjustable ring stand, applied superglue to the tip of the fiber, maneuvered the stand so that its tip rested in the desired location on the scorpion, and added an additional drop of superglue. After ten minutes of drying time, we returned the scorpions to their jars, removed the clay shard to avoid dislodgement, and gave them at least a day to acclimate.

Light calibration

We used an Arduino connected to a breadboard to precisely control and match LED intensities. We circuited two 5 mm LEDs for both light sources: ultraviolet (BIVAR; 15 mA; 15°; 3.4 V; 395 nm) and cyan-green (Broadcom; 20 mA; 30°; 3.2 V; 505 nm), with no light as a control. We inserted the free end of each scorpions' fiber optic lighting filament into a 3D printed connector that held the fiber at the proper angle and distance from the light and that could be placed on and removed from the LEDs easily (Fig. 3). During no light trials, we covered the open end of the connector with electrical tape and set it next to the LEDs. We mounted the breadboard approximately 10 cm above the arenas and used cardstock and electrical tape to shield excess light emanating from below the connectors. Using a light spectrometer (Ocean Optics; USB4000-UV-VIS-ES), we calibrated the LEDs to a standard irradiance of 0.01 W/m^2. Because the area of light is only 0.049 mm^2 (due to the 0.25 mm diameter of our fiber optics), we chose this value because it was the strongest intensity our equipment could deliver.

Trial execution

We used a random-number generator to determine the order in which the scorpions ran by assigning them to two groups of four. (Scorpion #10 was out of schedule with the other scorpions and was run separately). We also randomly assigned the order in which the scorpions would receive each light treatment. Trials began around 21:00 on November 16th, 2017. We cleaned the arenas with a paper towel and 70% isopropyl alcohol, allowed five minutes for drying, and then moved the animals to the chambers. We positioned the connectors on the predetermined light source, turned off the room lights, replaced the lid on the cardboard box, and left the scorpions undisturbed for five minutes to acclimate to the chambers and darkness. We marked the beginning of our footage for analysis later. We filmed the scorpions for 10 minutes, following a light schedule of 2.5 minutes off, 2.5 on, 2.5 off, and 2.5 on. After recording, we returned them to their jars, where we allowed them to rest for at least 30 minutes while the other group ran. In the same night, we

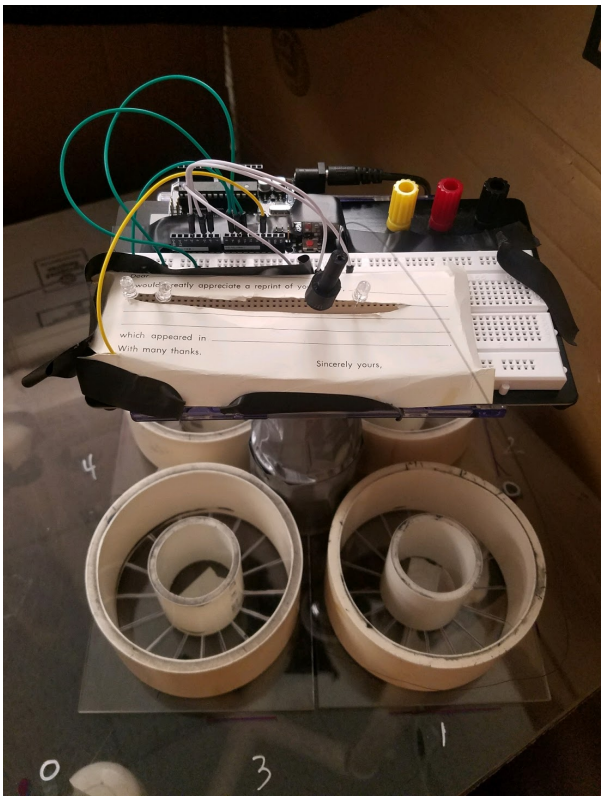

Fig. 3: LED fiber optic connection. A breadboard with two UV (395 nm) LEDs and two cyan-green (505 nm) LEDs connected were controlled using an Arduino computer to standardize irradiance. The breadboard sat above the testing chambers on a platform, allowing the attached fiber optic cables to fall down with minimal tangling and tension. A paper cover was constructed to shield as much light seeping from beneath the LEDs as possible from the arenas below. Fiber optics attached to the scorpions were inserted into 3D printed LED caps shown on the second to farthest right LED to standardize the amount of light entering each fiber optic and to minimize light spread in the dark environment. You should annotate your figure with initials and call them out as you come to them in the legend.

repeated running the groups until every scorpion had experienced all three light sources.

Data collection & analysis

To determine if the animals were affected by the light stimulation, we processed the videos through a specially written MATLAB program. The program used frame-by-frame subtraction to produce an x-y coordinate of the scorpion every two seconds. These data points were sectioned off into groups based on the light condition and attachment location, and the average instantaneous velocities between points were calculated and plotted in Microsoft Excel. We used instantaneous velocities rather than a metric such as total distance travelled to make a quantitative distinction between the slow, constant motion of non-light stimulated scorpions and the fast, sporadic activity of scorpions under UV and green light we expected to find (Gaffin et. al. 2012).

Results

Overall reactions

Animals exhibited a similar variety of behaviors across all trials despite experiencing different wavelengths of light in different locations on their cuticle. The average behavior exhibited consisted mostly of sporadic, frantic scratching at the arena walls rather than lateral motion. Scorpions exposed to the same wavelength of light in the same location exhibited radically different movement patterns from each other. In addition, sometimes the scorpions seemed unable to gain traction on the arena surface due in some part to the fiber optics becoming caught on the arena's walls and hindering their lateral movement.

Data analysis

There was a wide variance in our data, which undermined its reliability (Fig. 4). We attributed some of this variance to spurious data points due to the data analysis software's occasional misinterpretation of animal movement. We inspected average instantaneous velocity during each light/dark period to see if there was a distinction in their activity prior to and after turning on the light. We saw no differences with light type during lights on or lights off periods. The scorpions with fiber optics attached to their backs appeared to have lower average velocities than those that had fiber optics attached to their tails. Once again, however, the data were too inconsistent to draw any conclusions.

Discussion

There are several factors that most likely contributed to the irregular behavior of our scorpions. Scorpions with fiber optics attached to their tails showed more erratic and active movement than those attached at the torso, possibly suggesting that attaching the fiber optic to the tail created more stress on the animal. If this experiment were to be repeated, this could be a confounding variable that may have to be accounted for or otherwise avoided.

The cables were not intended to be attached to the cuticle for any longer than approximately 24 hours. However, as we modified the design of our experiment, the scorpions stayed attached longer. Instead of the scorpions becoming acclimated to the cables like we expected, it may have induced high levels of stress for the animals. With the cable restricting movement of the tail, feeding was much more difficult. This and prolonged exposure to the chemicals in the glue may have contributed to the high number of scorpion deaths we

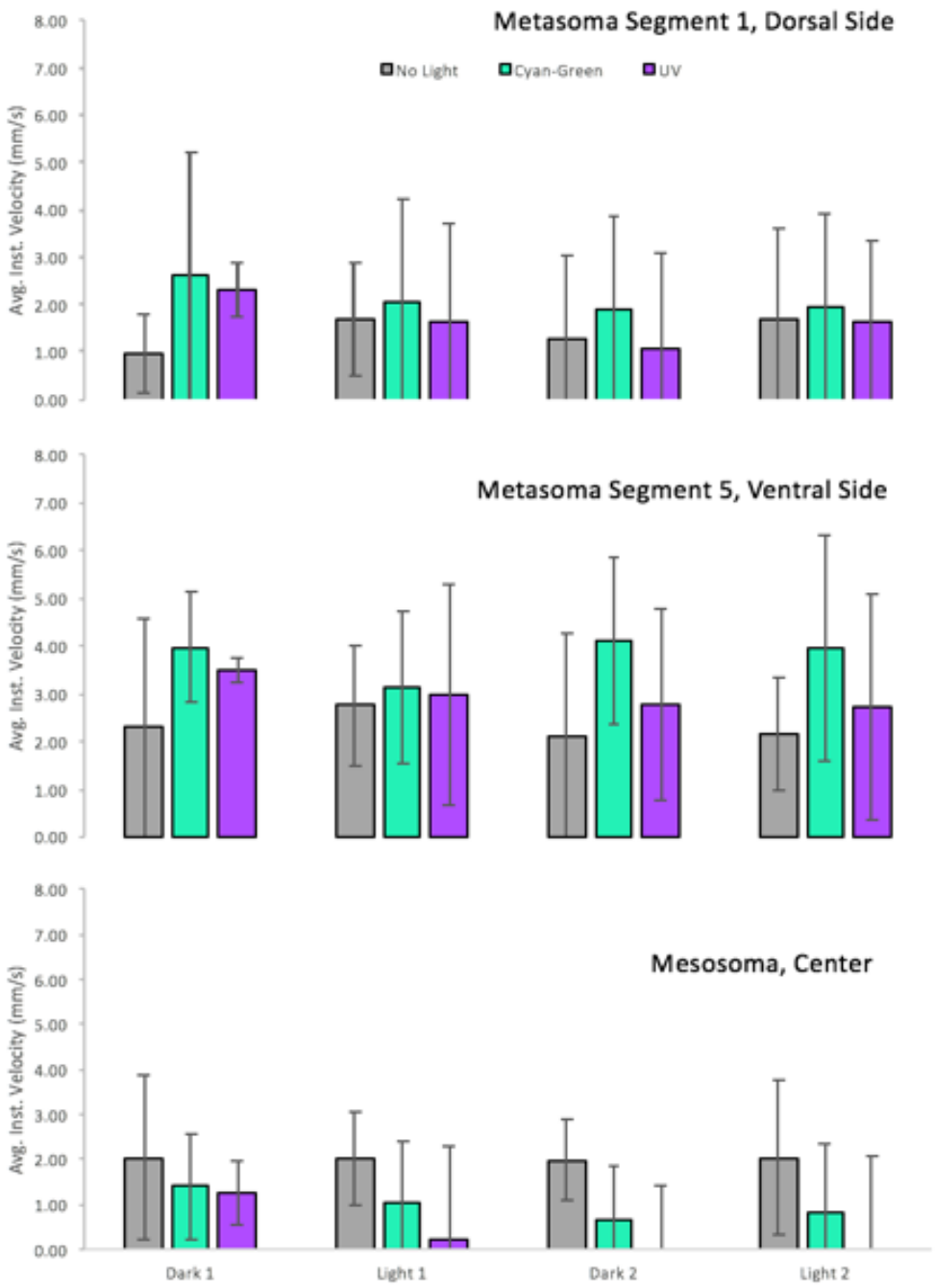

Fig. 4: Average instantaneous velocities under different light conditions. Animals had a fiber optic attached to either the ventral side of the fifth metasomal segment, the dorsal side of the first metasomal segment, or on the center of their mesosoma. Each animal was observed reacting to cyan-green (505 nm), UV (395 nm), and a no light control. The computer software MATLAB used infrared videos of the trials to plot x-y coordinates of the animals' movement using frame subtraction, and we plotted the average instantaneous velocities (+/- SE) in millimeters per second during the dark and light periods.

encountered. We intended to do a repeated measures test, but because many specimens died after our first round of trials, we only collected data for one location on each scorpion.

Due to time constraints and numerous changes, as we approached our deadline, we still had some unresolved problems. Unfortunately, we needed to proceed, attempting to control for errors as much as possible. For example, originally intent on quantifying activity by counting crossing intervals, we designed circular arenas, but this design caused the fibers to twist and catch around the center tube leading to serious scorpion movement impairment. This forced us to use a MATLAB program to track movement instead, but it could only measure

lateral movement rather than the stationary yet frantic motions we sometimes observed.

In addition, some no light trials showed the opposite trend from what was expected. Enough light may have escaped from under the LED caps to affect the animals' behavior. We tried to block as much light as possible, but with time constraints and the fibers requiring us to have the lights close to the animals, we were unable to eliminate all the seeping light. In addition, we developed our connectors to standardize how the fibers were attached to the light sources, but since we could not be certain all the fibers were oriented the same way within the connectors and were unable to measure the intensity from the ends attached to the scorpions, we did not know if all the scorpions were receiving the same light intensity.

Although our experiment did not produce conclusive results, we believe that there is still merit in testing for isolated photosensitivity. Our experimental design was forced to change several times, resulting in a somewhat sloppy testing build. Our sample size was also drastically small, only allowing us to conduct qualitative observations rather than the statistical analysis we originally wanted. We did visually observe some isolated instances of scorpion agitation without lateral motion immediately after turning the lights on which suggests that our hypothesis warrants further investigation.

The question of whether or not a scorpion's photosensitivity is isolated to a certain area of the body remains unanswered. Finding out whether or not scorpion photosensitivity is localized to regions of the body would contribute to understanding the scorpion cuticle and fluorescence as a whole. First, however, the experiment must be redone with appropriate modifications and more data points to generate more valid information. Understanding if photosensitivity is localized to a certain part of the body is significant, for it may indicate which part(s) of the scorpion should be investigated further for a deeper understanding of the fluorescence phenomenon.

Acknowledgments

We would like to extend our sincerest thanks to Dr. Doug Gaffin, Brad Brayfield, and Mallory McMahon for their tremendous support over the course of this project and for answering all of our many, many questions. This project would be absolutely nowhere without them. We would also like to acknowledge the contributions of all the unfortunate scorpions of this project, especially #19, also known as "Larry", for undergoing the tortuous process of having fiber optics attached to them. We also greatly appreciate the contributions of George Martin for building our arenas. We want to thank Dr. Katie Marshall for tolerating our, at times, rather disturbing presence as we used her freezer. We will be out of your way from now on. Additional thanks to Liz Cooley for providing supplies and ordering necessary items for our experiment. The OU Biology Department was responsible for our transportation to collect our specimens and supporting us financially throughout the process. Finally, we would like to thank the University of Oklahoma Honors College for offering this Scorpion Navigation class which had such a tremendous impact on all of us.

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