The effects of magnetic fields on the desert grassland scorpion, *Paruroctonus utahensis*.

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Summary

Recent studies have shown that desert ants, *Cataglyphis noda*, use magnetic fields as directional information to return to their nests. This process, known as magnetoreception, is speculated to be the result of UV-A/blue light photoreceptors called cryptochromes inducing radical pair reactions in the retinas when exposed to light under 420 nm. Although research has been conducted on magnetoreception in the arthropod subphylum Hexapoda, the Chelicerata subphylum had yet to be studied. In this study, we tested for traces of magnetoreception in fifteen female dessert sand scorpions (*Paruroctonus uthahensis*). The scorpions were placed in a segmented arena with a treated zone situated between two Helmholtz coils. We tested the amount of time each scorpion spent and their velocities in each zone when exposed to three magnetic fields: 0, 100, and 500 µT, . We predicted there would be a change in animal behavior in the treated zone when a magnetic field was present compared to the control trials. Our results showed no evidence of the animals occupying the treated zone more frequently than the untreated zone. Under the conditions of these experiments, scorpions did not show signs of magnetoreception. For future tests on scorpion navigation, we suggest researching e-vectors, which are used by bees as an internal compass.

Introduction

While we consider ourselves to be an advanced species, humans cannot detect changes in Earth's magnetic fields. Recent studies have shown that certain animals such as loggerhead sea turtles can detect changes in magnetic fields and use them as a means of navigation (Lohmann et al. 2010). However, this navigation technique is not limited to those found in the phylum Chordata. For example, desert ants (*Cataglyphis noda*; phylum Arthropoda) use magnetic fields as directional information for finding their way back to their nests (Buehlmann et al. 2012). In a laboratory setting, fruit flies (*Drosophila melanogaster*) used magnetic fields to navigate a maze (Gegear et al. 2010). Although this information is beneficial for understanding the navigational habits of arthropods, research has been primarily exclusive to the subphylum Hexapoda.

A popular hypothesis behind the magnetic field sensitivity in insects is the cryptochrome hypothesis. This hypothesis relies on the UV-A/blue light photoreceptor proteins known as cryptochromes to initiate the radical-pair reactions necessary to induce the ability to use magnetic fields as a way of perceiving direction and location, also known as magnetoreception (Maeda et al. 2012). Studies have shown that the cryptochrome found in *D. melanogaster* are most responsive when wavelengths were under 420 nm (Geager et al. 2010). These same cryptochromes are found in all eukaryotic organisms, including scorpions. These insects, which fall into the arthropod subphylum Chelicerata rather than Hexapoda, are nocturnal creatures. Moreover, scorpions have a fluorescent cuticle, which allows the animals to glow under UV lighting thus making it easier to track their movements in a dark environment. Stars emit low enough levels of UV light to initiate magnetoreception, and since scorpions are nocturnal they are often exposed to these low levels of UV light. This suggests that they can experience magnetoreception and makes them ideal candidates for testing magnetoreception in the Chelicerate subphylum.

We investigated whether magnetoreception is present beyond the Hexapoda subphylum of arthropods. Because of its fluorescent cuticle and ease of maintenance in captivity, we have chosen to use the desert grassland scorpion *Paruroctonus utahensis* as our test species. We tested scorpion responses under low UV light levels in the presence or absence of an exogenous magnetic field. If scorpions are capable of magnetoreception, we predicted they would have a noticeable behavioral reaction while in an arena containing a magnetic field compared to when there is no field was present. Our results indicated no changes in scorpion behavior when they were exposed to a magnetic field as compared to trials when no field was present. These results suggest that scorpions are not using magnetoreception as a means of navigation.

Methods

Animals

We used fifteen female *P. utahensis,* also known as desert grassland scorpions, that were collected during the fall of 2015 in sandy regions located thirty kilometers southeast of Monahans, Texas. While in the lab, the scorpions were kept in individual 3.8 L glass jars. The bottoms of the jars were covered with 3cm of sand. The animals were fed one live cricket (*Gryllodes sigillatus*) once every two weeks and were given 20 mL of water three times a week. The scorpions' light/dark cycle was set back 8h to allow for behavioral testing during normal daytime hours. To regulate this cycle, jars were placed in a box and a light was placed across the top to act as daylight during the night hours. A black cloth was placed over the box and light to simulate a nighttime environment during the day. The new day/night cycle was established by setting the timer attached to the light back an hour a day until it reached a nighttime timeframe of 12:00 h to 22:00 h.

Experimental Apparatus

For the construction of the experimental environment, which is shown in Figure 1, our team set up a 60.69 cm long by 20.32 cm wide plastic planter box; we rounded the corners with four strips of clear vinyl. The arena was filled with 6 cm of washed sand and was divided into three sections. One side was placed in between the two coils of a Helmholtz magnetic field generator so that the magnetic field ran parallel to the dividing lines. The field generator consisted of two 200-turn Pasco brand coils mounted to a 20-cm base. The 20-cm base placed the coils twice the optimal distance apart, which means that the field that they generated was not quite uniform between the coils. The coils were powered by a 5-amp power supply. A small zone in the center, marked with fluorescent paint on the sides of the arena, was designated

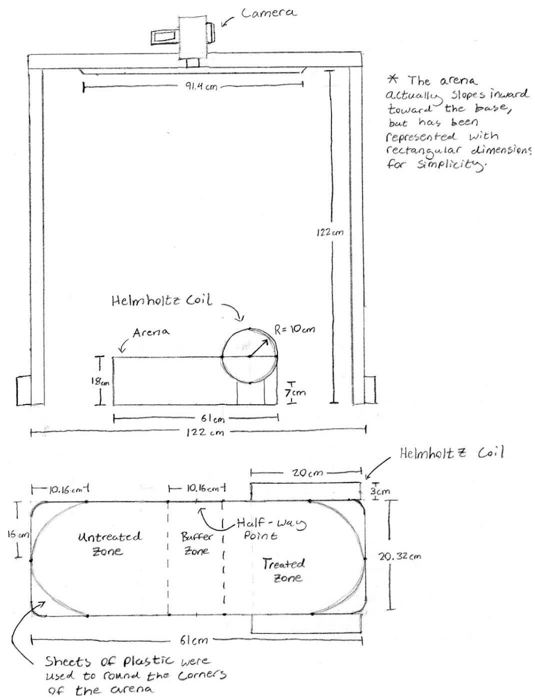

Fig. 1: Set-up figure. The picture depicts how our experimental apparatus was set up.

as a buffer zone to avoid any overlap between the affected and unaffected zones. The side of the arena opposite the coils was not magnetically polarized.

All our tests were conducted under 420 nm wavelength UV-A light since it has been hypothesized by Geager et. al (2010) that, when exposed to such light, cryptochromes develop sensitivity to magnetic fields. To support the UV lights, we constructed a wooden frame using two 243.84 cm long by 5.08 cm by 10.16 cm pieces of wood. It consisted of a 121.92 cm beam suspended 121.92 cm off the ground by two posts. The frame was stabilized by two 60.96 cm beams at the bottom of each post. Once the structure was completed, we removed the sticker attached to the adhesive backing of the UV light strip and mounted it to the bottom side of the bridge piece. Both ends of the light strip were further secured with duct tape.

Procedure

On day one of the experiment we assembled the experimental apparatus as outlined above. Trials were conducted starting at 14:00 h and ended after all fifteen animals had been tested. This process was repeated twice a week for five weeks. Trials lasted 10 min each and began at a constant starting point within the buffer zone of the arena. For each trial, we released an animal at the starting point and allowed it to roam freely. To account for animals that did not start to move right away, a buffer period of 10 min was allotted for the scorpion to begin its motion and leave the buffer zone. If the scorpion did not move during this buffer period, the test was declared a non-trial and a new trial was started. Once it was confirmed that the scorpion had become active, we timed the trial for 10 min. The set of fifteen specimens were tested at two strengths of the magnetic field, plus a set of trials in which the Helmholtz coil was switched off. The low magnetic field measured close to the Earth's field at 100 µT (microtesla); the high magnetic field was 500µT

To eliminate any bias, the order of exposure was randomized. Three groups of five scorpions were rotated through each level of field strength so that no group was exposed to the fields in the same order. After each trial, we cleaned the sand of the enclosure and replaced it so no specimen followed a path laid out by its predecessor. Each trial was recorded using a Sony video 8 XR digital camera. The recordings were analyzed using a Matlab motion tracking program to record the time spent and average velocities in each section of the arena.

Analysis

Our experiment followed a repeated measures design, which required all subjects to be tested under each condition. We looked at the percentage of time the scorpion spent in each area of the arena and its average velocity in each area. To process the footage from our trials, we decreased the framerate to 0.8 frames per second before running the video through the Matlab analysis program. The program changed all color in the video to grayscale and pixelated the images into a 100 by 100 matrix. It then ran frame by frame and measured differences in the brightness of each pixel and compared the differences to a threshold value. Any values that exceeded the threshold were stored and plotted as a "blob" that represented the motion of the scorpion. The values were broken down to find the center of motion in each frame, exported to Excel for plotting and for calculating both the time and the average velocity in each zone. We looked for consistent behavioral changes between our treated and untreated zones. If the scorpion spent substantially more time in the portion without the magnetic field, it could indicate an ability to detect a magnetic field and a tendency to stay away. If they spent more time in the half of the arena with a magnetic field, then it could indicate that they can sense magnetic fields and possibly use them during navigation to orient themselves or determine their direction. Our expected results were that the scorpions would spend a majority of the time in the area containing the magnetic field.

Results

Although each trial was different, there were some noticeable patterns in scorpion behavior throughout the trials. For example, the animals would generally run away

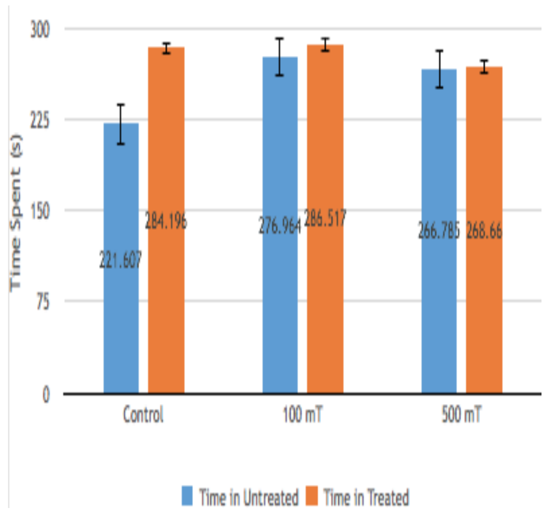

Fig. 2: Average time spent in treated vs. untreated zones under three magnetic conditions. The plots show average time of occupancy in seconds with standard error bars

from the starting position when we released them into the arena. This could have been a behavioral response to the forceps used to carry them or a desire to explore their new surroundings. The animals tended to be more active in exploring the arena during control trials with no field present than when a field was present. On average, the scorpions would move around the arena for 50 ± 6.1 s before stopping for a period of more than 10 s. As they explored the arena, the animals tended to walk close to

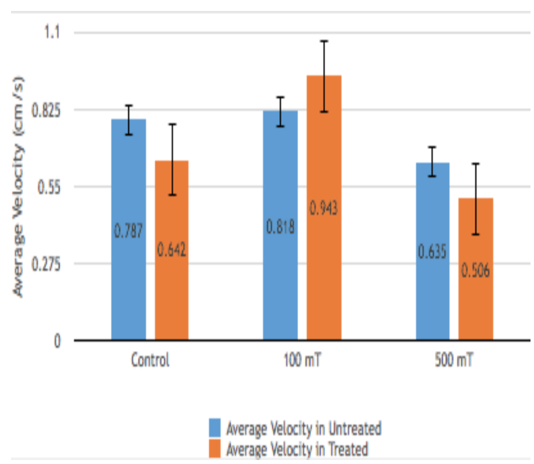

Fig. 3: Average velocity in treated vs. untreated zones under three magnetic conditions. The plots show average velocity in cm/s with standard error bars.

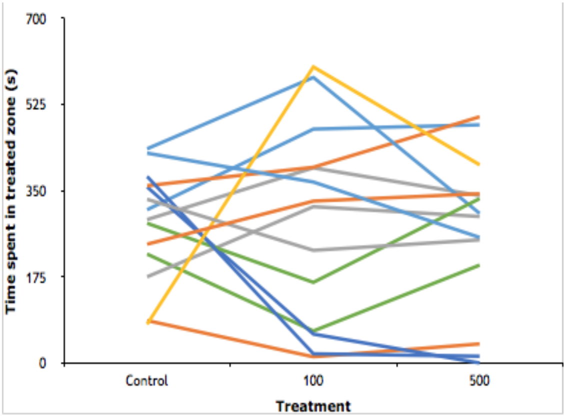

Fig. 4: Time spent in the treated zone under three magnetic conditions for each specimen. Plot shows each animal's time in the corresponding magnetic condition in seconds.

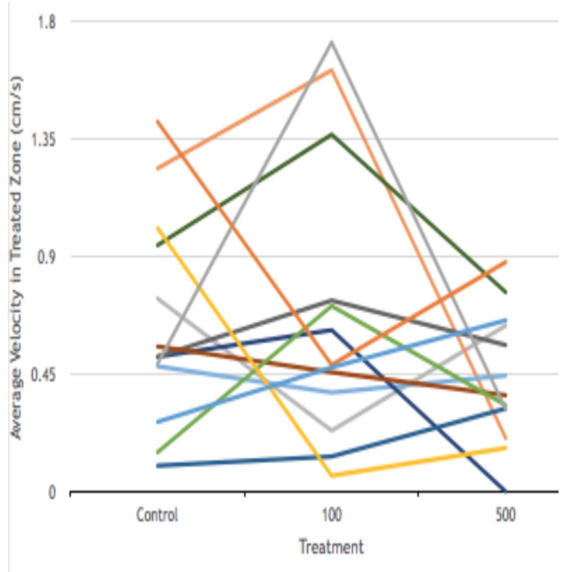

Fig. 5: Average velocity in the treated zone under three magnetic conditions for each specimen. Plot shows each animal's average velocity while in the corresponding magnetic condition in cm/s.

the walls. After their initial explorations, many of the scorpions rested in the rounded edges or attempted to climb the walls of the arena.

The quantitative results show no distinct correlation between the presence of a magnetic field and the behavior of the scorpions. Our analysis of the time spent in the treated zone, which is shown in Figures 2 and 4, showed that the scorpions did not spend significantly more or less time in the treated zone when a magnetic field was applied. In agreement with our analysis of the time spent, the average velocities of the specimens in the treated zone also showed no significant differences under the effects of a field. The velocity analyses can be viewed in Figures 3 and 5. Although the averages from the entire group of specimens in Figures 2 and 3 suggest slight changes in behavior when magnetic fields are present, the sheer variation between individuals (Figs. 4 and 5) completely invalidates any such trends. This observation is reinforced by the Repeated Measures One-way ANOVA Test, which returned a p-value of 0.4681 for the velocities in the treated zone and a value of 0.5685 for the time spent in the treated zone.

Discussion

Under the conditions of our experiment, the scorpions did not show signs of magnetoreception. The relatively even distribution of time spent in each zone depicted in Figure 2 indicates that the animals did not have a specific preference in zones. This would suggest that if the scorpions did exhibit magnetoreception, the fields were probably too weak to have a substantial impact on their movement patterns. Another possibility is that the cryptochromes present in scorpion retinas do not undergo the radical pair reactions necessary for magnetoreception. Moreover, our findings failed to mimic other studies, such as Geager et. al's study on *D. melanogaster*, which found that members of the subphyla Hexapoda use magnetoreception for navigation (2010).

Our experiment showed no evidence that scorpions use magnetoreception. This could mean that the cryptochromes inside of scorpions are not functional, which begs the question as to whether that is true for all chelicerate species. This opens the possibility for experimentation on other chelicerates to determine whether the lack of response to intense magnetic stimulus is unique to scorpions or is observable in all chelicerates. Since the scorpions did not display any clear orientation bias while in the magnetic field, it suggests that they may use other cues. These other possibilities, such as e-vectors, would be great bases for further experimentation.

In Figure 2, the average values from the control group indicated that the scorpions spent much of their time in the treated side of the arena. This raises questions about the nature of the arena, since we expected approximately equal time spent in each zone for the controls. The difference between the two zones is likely due to the wide variation in behavior among the specimens. Figure 4 shows that there was no uniform trend in the motion of each specimen, and that individual values for time in the treated zone spanned a large range. Since the averages were taken from a population with such extreme variability, it is difficult to draw a conclusion from the mean alone. Further investigation may be necessary to determine whether the setup of the arena has any effects on the control trials. If more trials were conducted with a larger group and proved a significant affinity for the treated zone in the absence of a field, that affinity might be attributed to shadows cast by the Helmholtz coils, perhaps resembling shelter. This could be tested by running the control trials without the Helmholtz coils in place.

Because the apparatus and analysis tools used in this experiment were custom made, this research had several areas that could be improved upon in future tests. Most notably, the experiment lacked a way to measure the magnitude of the magnetic field between the Helmholtz coils. Because of the size of the arena, the Helmholtz coils used in the experiment were spaced at a distance that was twice the optimal separation. Our measurements for the magnetic field strength were calculated based on the point located equal distances between the centers of the two coils, where the field should have reached its maximum strength. This measurement, however, is not representative of the entire field because the spacing of the coils resulted in a non-uniform field distribution. Given that this experiment measures the response to a change in magnetic field, this irregularity should have had little effect, but it should still be considered when taking into account possible sources of error. More room for improvement is present in the motion tracking elements that were used to generate the raw coordinate data for our analysis. The program used was a Matlab script had lapses in sensitivity and periodically produced outlier values as a result. Additionally, the camera that we used to film trials was not well suited for dark environments, which produced lower video quality and occasionally caused the tracking software to lose track of the scorpion for two to three frames. These are factors that undoubtedly produced a small degree of error in the results but are ultimately reparable by upgrading to a professional grade tracking apparatus.

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