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The effect of electromagnetic fields on the *Arion distinctus* in the presence of various wavelengths of light

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Abstract

Electromagnetic sensing ability found in many different vertebrates and invertebrates is largely mediated through the opioid receptors and internal opioid peptides. This sensing ability has been shown to be light dependent, and therefore also likely dependent on a photoreceptor. Most of the research done in invertebrates has been conducted using *Cepaea nemoralis* (the land snail), but none has been done using shell-less gastropods or slugs. We tested the effect of two different wavelengths of light (red light- 650nm and blue light - 450nm) on the electromagnetic sensing abilities in the Arion *Distinctus* (aka the garden slug). This experiment was performed by injecting the slugs with an enkephalinase inhibitor, followed by a 15min exposure to a ±60µT magnetic field in the presence of either red light, blue light, or white light. This experiment demonstrated for the first time that an electromagnetic sensing ability is present in the *Arion distinctus* and is light sensitive. Blue light was the most effective light setting for slugs to sense an electromagnetic field.

Keywords: electromagnetic Sensing, wavelength, analgesia, enkephalins, pain measurement

1. Introduction

Although humans are the most advanced mammals, they lack a sixth sense that is found in many different vertebrates and invertebrates. This 6th sense is electromagnetic sensing that exists in a wide variety of animals, including but not limited to, sharks, birds, rats, salamanders, flies, fish and snails [1]. Electromagnetic sensing is used for various purposes including migration, hunting, defense, and possibly pain management. For example, homing pigeons use the earth's electromagnetic field (EMF) as their 'compass' to determine their location or estimate how far they are from a specific goal [2].

This electromagnetic sensing ability is mediated through opioid receptors, which also use endogenous opioids to control the feeling of pain [3]. In order to relieve pain, endogenous opioids are released by the nervous system when the body experiences external pain sensations. However, the electromagnetic field negates the effects of an endogenous opioid, a natural painkiller, and makes the subject feel even more pain by distorting the opioid receptors rendering them unable to accept opioids [3, 4]. Thus, in order to test whether an electromagnetic field is affecting an animal, a pain reaction is measured and if they have been exposed to the electromagnetic field the reaction to a pain stimulus will be more extreme.

Previous studies demonstrated a connection between exposure to an EMF and reaction to a pain stimulus. These studies show that 15 minute exposures to ELF (extremely low frequency) magnetic fields increase the amount of pain experienced in snails. Although, in these same studies when injected with an enkephalinase inhibitor, which prevents the breakdown of the opioid enkephalin, the pain is decreased [5]. Enkephalinase inhibitors (e.g. racecadotril) increase the amount of enkephalins, enhancing the analgesic effect, acting like a painkiller [6]. Since an ELF electromagnetic field makes the opioid receptors less able to receive opioids, such as enkephalin, the snails will experience more pain after being placed in the electromagnetic field [5]. The opposing interaction between the EMF and the enkephalinase inhibitor on pain demonstrates that the EMF sensing ability of the snail is opiate mediated.

Researchers have also tested how different conditions during exposure to an electromagnetic field affect the snails' sensitivity to the EMF induced pain. Snails have been shown to react differently to the electromagnetic fields when exposed in lighted conditions versus dark conditions. When snails were placed in a magnetic field with light exposure, the snails reacted faster to the pain stimulus because the electromagnetic field affected more opioid receptors making them less able to receive the enkephalins, which would normally inhibit pain [7]. The magnetic field's orientation in relation to the vertical field affects the degree of the analgesic effects of the enkephalins [8]. Much of the previous research on the sensing ability of electromagnetic fields and their relation to pain has been conducted on snails, but none appears to have been done with slugs. Although slugs are in the same phylum as snails (mollusca), they have both evolved into different species [9]. So, slugs, because of their close taxonomic relationship to snails, are predicted to behave in a similar manner when placed in an electromagnetic field, although this hypothesis has not been previously tested. One goal of this research is to compare the ELF sensing abilities in closely related species to further understand if evolution has affected this sensing ability.

It is important to test various wavelengths of light given the wide range of animals that have EMF sensing ability, from birds to sharks and fish. Birds and sharks have very different environments and are exposed to different wavelengths of light. Therefore, it is important to test different light environments. The red wavelength chosen for this experiment was chosen because in previous research by (Garrick, S. 2015, unpublished preliminary data) red was shown to be the least effective wavelength for enhancing pain in the slugs. The blue wavelength was chosen because sharks and other ocean creatures experience blue wavelengths more frequently than other wavelengths as the water filters out other colors. This experiment tests the differing effects of red light (730nm), blue light (455nm), and broad spectrum white light on the slug's response to pain under each light condition.

This research is significant because very little is known about the relationship of electromagnetic sensing in invertebrates and how it is affected by light [10]. This information could give us more insight into a sixth sense that many animals possess and this natural interaction of light and electromagnetic fields can be affected by human intervention, either deliberate or accidental.

2. Methods

Helmholtz Coil Construction

The electromagnetic field was created using a Helmholtz Coil, built and designed based on the previous research by Kirschvink [11]. A Helmholtz Coil consists of two to four solenoid magnets of the same size and placed on the same axis, creating a uniform electromagnetic field. Kirschvink [11], evaluated various coils and their corresponding field areas. The Merritt *et al.* coil was chosen because it had the largest area of constant magnetic field strength in the center of its four coils. The coils were built using a laser cutter to shape the acrylic support structure and then each was wrapped in magnet wire once the structures were finalized. The coils generate a constant ±60μT electromagnetic field, which is comparable to the Earth's natural magnetic field. The Helmholtz coil had modifications in order to accommodate both snails and slugs. Two trays were designed for holding the slugs and snails: one that was able to keep the snails contained while also accommodating for the size of their shells and one with a smaller cavity for the slugs (Figure 1).

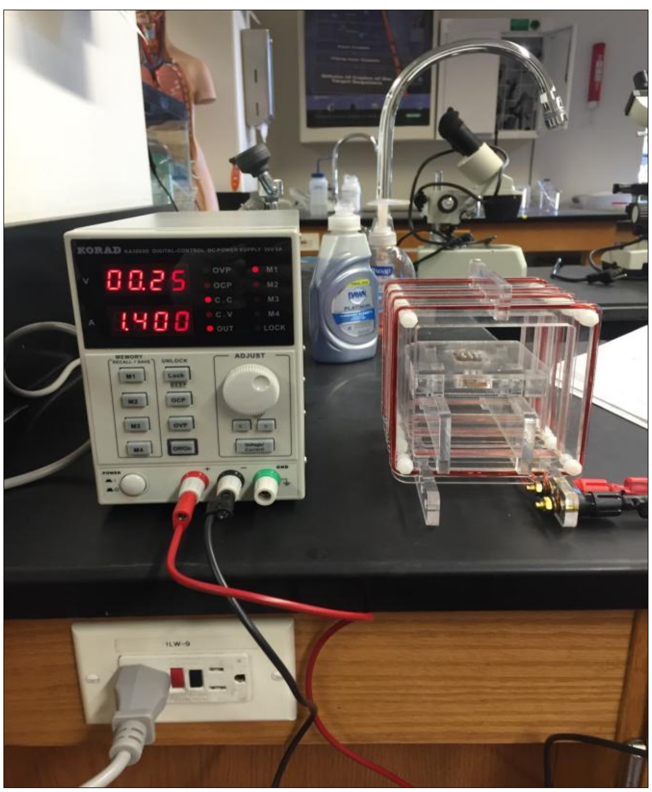

Fig 1: Power source and Helmholtz Coil generating ±60μT electromagnetic field.

Light Box Construction

Two light boxes were constructed for experimental trials. Holes were cut into the top and sides of the box where 10 blue or red LEDs were evenly placed. The LEDs were soldered together on top of the box and connected to a power source to create a uniform intensity of light (LED World 2.5V 3W red high power LED 620-630nm 20mm star base 700mA; 460-470nm 3.5V 3W, blue). The Helmholtz coil was then placed on the platform with the box fitting over it. Experiments with slugs or snails not exposed to single wavelength light as a control were performed in standard fluorescent lighting.

Slug and Snail Core Conditions

The research subjects are brown slugs and garden snails (Niles Biological, Sacramento, CA) Each slug varied in weight from 0.08 grams to 0.8 grams. Slugs act as the experimental group and snails as the control group since previous research using snails has been published. The slugs and snails all were kept in similar conditions: small terrarium, dirt covered base, plenty of vegetables to eat, and a wet paper towel covering the bottom with an automatic mister fogging up the terrariums to keep the environments damp and cool.

Core Assays

For the core assays, a group of twelve snails and fifteen slugs were placed in white light with four different conditions: 1) slugs without Racecadotril solution and with sham EMF, 2) slugs and snails injected with Racecadotril (R; 0.1μg racecadotril/2μl DMSO) solution (Sigma Aldrich, dissolved with DMSO) followed by sham EMF; 3) slugs and snails not injected with Racecadotril solution and placed in a ±60μT EMF; and 4) slugs and snails injected with Racecadotril solution (0.1μg racecadotril/2μl DMSO) and placed in a ±60μT EMF. After each trial the slugs and snails were placed on a hotplate set at 40+/- 0ºC and their nociceptive response was measured. This response was measured based on the amount of time (measured by a stopwatch) they endure on the hotplate before lifting their "foot" reflecting pain. They were then immediately removed from the hot plate and placed back in their terrarium [8]. The outcome measure was time to lift their foot once placed on the 40+/- 0 C hot plate.

Wavelength Groups

There were two different experimental trial groups of slugs: observed and tested at red - 650nm and blue - 455nm. Each slug was injected with a Racecadotril solution and then placed in an electromagnetic field set at ±60μT for 15 minutes exposed to their assigned wavelength color. Once they were removed from the electromagnetic field, they were placed on a hotplate set at 40+/- 0º C and their nociceptive response was measured.

Statistics

All of the statistical testing was done using the "R" statistics application, through the "R Studio" (RStudio, Boston, Massachusetts) interface. For each set of groups, individual data vectors were created. These vectors were then used with the *boxplot* () function to create boxplots for the purpose of identifying the presence of any outliers. Each vector was then run through the *qqnorm* () function to produce a normal probability plot to visually check that the data appeared to be normally distributed. As an additional check, each data vector was also run through the *shapiro test* () function to perform a Shapiro-Wilk test of normality. The data vectors for each group were then combined into a data frame. The function *Levene Test* () was then used to perform Levene's test for homogeneity on the groups in the set.

These tests were all performed for the purpose of ensuring that the data met the underlying assumptions for an analysis of variance test. In the case of both sets of slug data, the group variances were found to be non-homogeneous. As a result, an ANOVA test would not be appropriate. Instead, Welch's test is used to compare the group means. This was done in *R* using the function *oneway. Test* (). Finally, pairwise post-hoc testing was completed for the groups in each set by using the *oneway. Test* () function in conjunction with the *apply* () function to produce a table of p-values for each comparison.

3. Results

Slugs injected with racecadotril (R) and not exposed to EMF took longer to react (13.08 +/- 1.18s) to a pain stimulus than slugs injected with racecadotril and exposed to EMF (6.8 +/- 0.43s) (Figure 2). The slugs exposed only to EMF, without injections of racecadotril, had the shortest reaction time (5.2 +/- 0.73s) compared to the slugs tested for their natural reaction to the pain stimulus (10.67 +/- 1/19s). Each of the control trials were conducted in white/fluorescent light (Figure 2).

Welch's test on the 4 groups are: One-way analysis of means, not assuming equal variances(F=41.7333, Df 3, Denominator df = 23.6672) p<0.05. Pair-wise post-hoc testing revealed all 4 groups were distinct from each of the other 3 (p< 0.05).

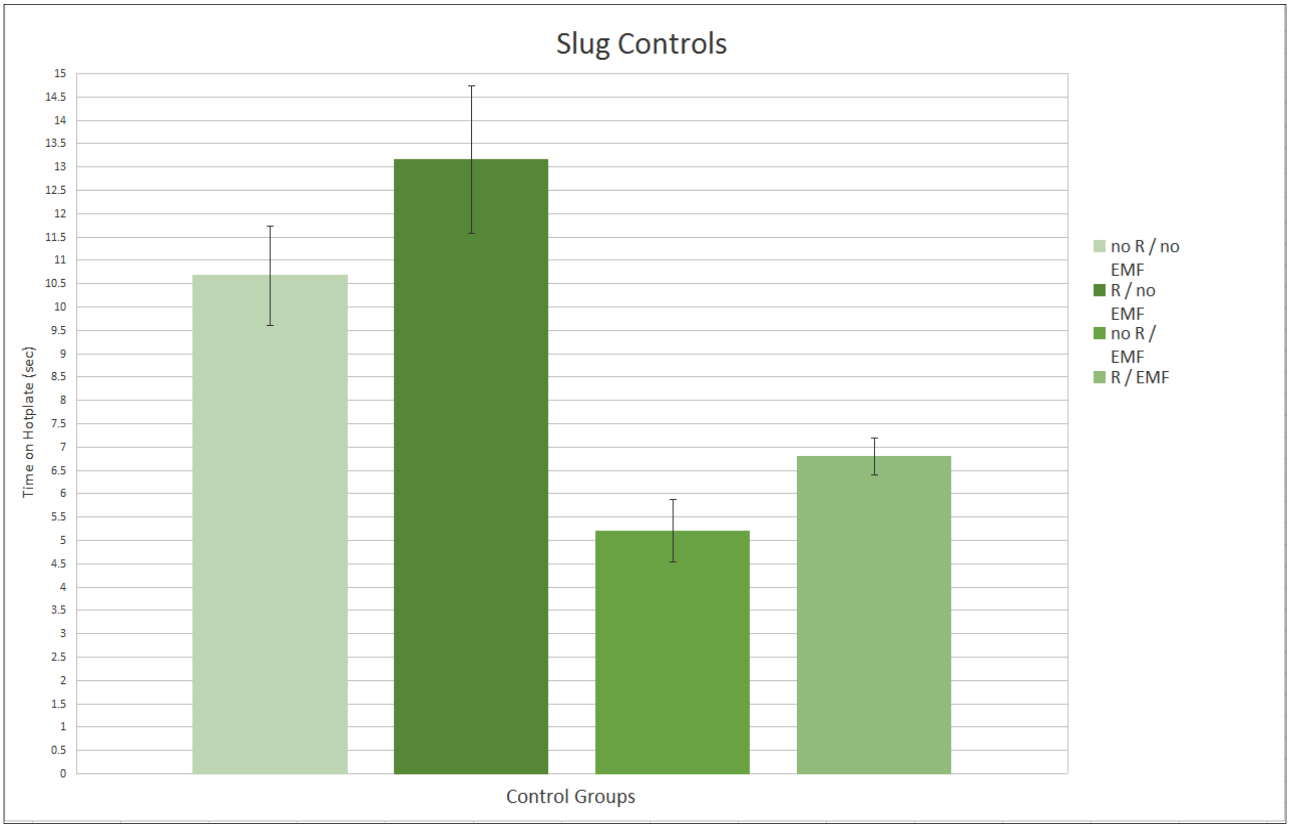

Fig 2: The mean amount of time (sec) for each slug group to respond to the pain stimulus under different conditions +/- 95% Confidence. R = Racecadotril preinjection. EMF = Electromagnetic Field. Compared to no EMF and no R (Group 1), the enkephalin inhibitor increased amount of time to pain response. EMF increased the response to pain, ie. shorten the time to pain response (Group 3 vs 4, and Group 1 vs 3). EMF without R had the shortest time to pain response.

The control trials with the snails using the same groups as the slugs, show very similar results (Figure 3). From this collection, the group with the shortest response to the pain stimulus was was the group with only EMF (5.63 +/- 0.77s) compared to no EMF and no racecadotril (11.25 +/- 1.24s). The second shortest was with racecadotril and EMF, with neither EMF or racecadotril following closely behind (11.25 +/- 1.24s). Finally, the group with only racecadotril (17.13 +/- 1.7s) had the longest delay before the subjects showed signs of pain.

The results of Welch's test on the 4 groups one-way analysis of means, not assuming equal variances (F = 75.548, Df = 3, Denominator DF=15.022) p<0.05. Pair-wise post-hoc testing revealed all groups are seen as being different from the others, since the *p*-values are all less than 0.05.

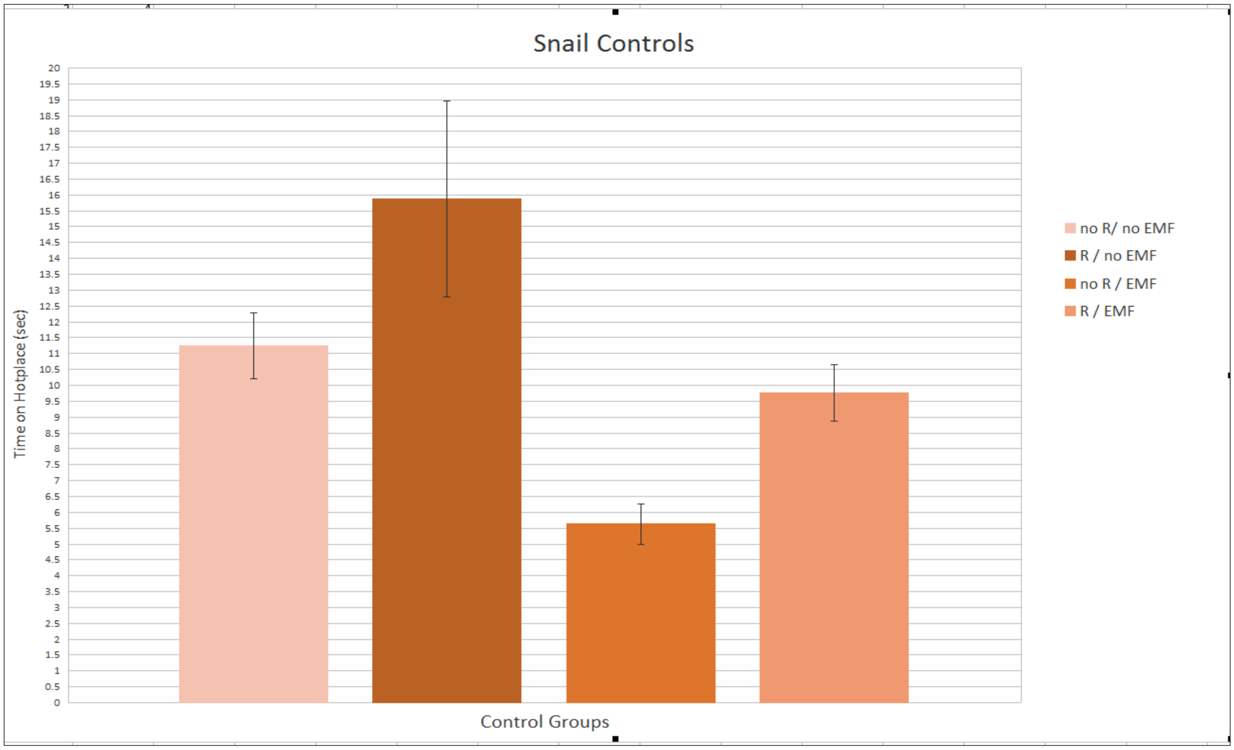

Fig 3: the amount of time (sec) it took for each snail group to respond to the pain stimulus under different conditions +/- 95% Confidence. R = Racecadotril preinjection. EMF = Electromagnetic Field. Compared to no EMF and no R (Group 1), the enkephalin inhibitor, increased the amount of time to pain response (Group 4). EMF increased the response to pain, i.e. Shortened the time to pain response (Group 2 vs 4, and Group 1 vs 3). EMF without R had the shortest time to pain response (Group 3).

When the slugs were placed in the electromagnetic field in the presence of blue light, their reaction to the heat was faster than the control (4.24 +/- 0.54 vs 6.8 +/- 0.43) (Figure 4). When the slugs were placed in the electromagnetic field in the presence of red light, their nociceptive response was delayed significantly (11.12 +/- 1.02s vs 6.8 +/- 0.43s).

The results of Welch's test on the 3 groups are one-way analysis of means, not assuming equal variances (F=84.672, Df=2, Denominator df=29.072), p< 0.05. Pair-wise post-hoc testing was performed revealing distinct groups (p<0.05).

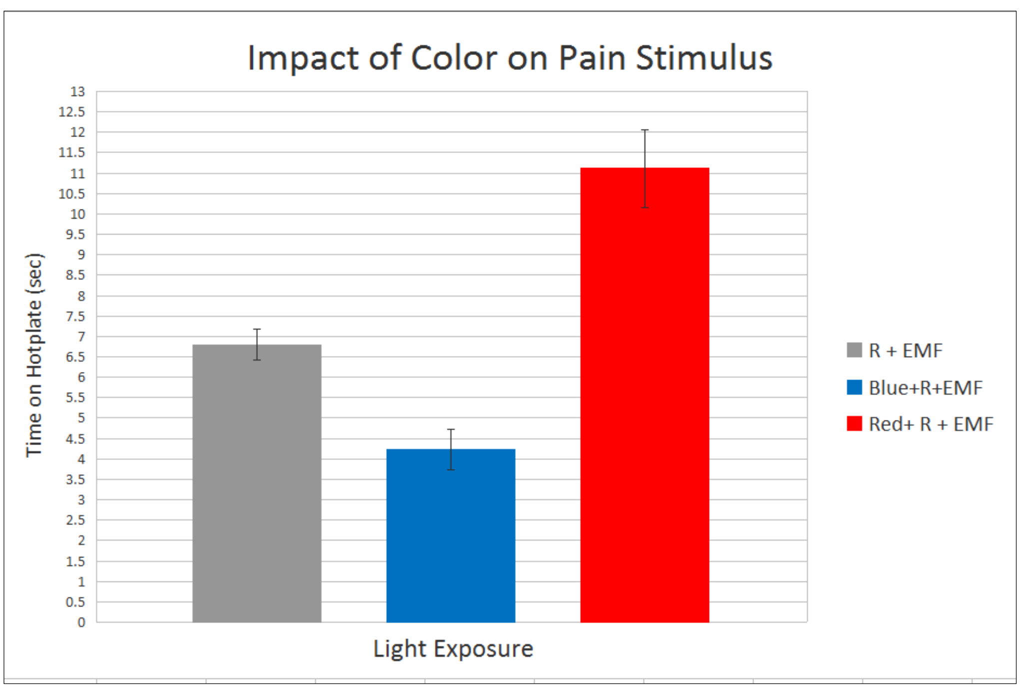

Fig 4: shows the time recorded (+/- 95% confidence) for each trial's slugs' nociceptive responses after being injected with racecadetril and then placed in the electromagnetic field exposed to different wavelengths of light. In addition, the control most similar to the experimental trial is added as white+R+EMF.

4. Discussion

These results demonstrate for the first time that the species, *Arion distinctus*, has an EMF sensing ability. In previous studies e.g. [3, 5, 7, 8] the electromagnetic sensing ability has been evaluated in a closely related species of snails similar to those evaluated in the current study. Since slugs and snails have a similar evolutionary ancestry, these findings suggest that this sensing ability is also found in an evolutionarily more recent species, garden slugs.

Comparisons of the four slug control groups demonstrate that each group is significantly different from the others. These results demonstrate strong support for an EMF sensing ability in slugs that is largely opiate mediated. This work confirms - and repeats - prior research in snails that they, too, have opiate mediated EMF sensing ability [3, 5, 7, 8].

This study also demonstrates that blue light appears to heighten the slugs' response to the electromagnetic field (i.e. faster time to demonstrate pain) compared to any other group. The slugs tested in the red light, on the other hand, had a delayed reaction to the hot plate when compared to tests done under control white lighting. The electromagnetic field in white light was associated with the slugs reacting faster to the pain stimulus compared to slower response in red light. These findings extend prior research suggesting the sensing-transduction mechanism of reaction to EMF involves both light and opiate-mediated mechanisms [7].

The slug core assay group using racecadotril and exposed to EMF is the most comparable to the experimental trials exposed to different wavelengths of light since all of those trials contained slugs injected with racecadotril in addition to the electromagnetic field. Each of the experimental trials included racecadotril because the EMF is theorized to nullify the pain-suppressing effects of the racecadotril. For the control group with racecadotril and with the EMF in white light, the average time for the slugs to respond to pain was 6.8 seconds (+/- 0.43 s), whereas for the red light plus EMF it was 11.12 seconds (+/- 1.02s) (Figure 4). The red light could have negated the effects of electromagnetic exposure because the response time to the pain stimulus is close to the response time for the control trial of slugs just injected with racecadotril (11.2 +/- 1.02s vs 13.08 +/- 1.18s) (Figure 2). Since the blue light demonstrates the slugs' shortest reaction to the hot plate of any group at 4.24 seconds (+/- 0.54s), the blue light was found to be the most effective environment for the slugs in order to sense an electromagnetic field.

A limitation in this study is comparing a control group (white light), which was performed somewhat earlier, with later experimental groups (red and blue light), although the standard errors were low and the groups highly significantly different from each other. Certain other factors could have contributed to potential bias in this study. The most important would be the measurement of each slug's or snail's response to the hot plate test following experimental procedures did not have a blinded rater. This could be minimized in future studies using a researcher blinded to the solutions injected or the EMF exposures previously used in each experimental subject.

Several other variables may have affected the results in this study. In previous studies testing the electromagnetic sensing abilities of snails, the Enkephalinase inhibitor used was SCH 34826. In this study racecadotril was used as the Enkephalinase inhibitor SCH 34826 is no longer manufactured. These substances are both Enkephalinase inhibitors and, in these studies, accomplish the same biological effects. However, since racecadotril is insoluble in water and saline solution it was instead dissolved in DMSO. The DMSO could have had some effect on the slugs' response to the electromagnetic field and/or the hot plate. On the other hand, the core trials with snails reacted similarly to previous studies [3, 5, 7, 8]. Another factor that may have affected variation in response to the pain stimulus is the difference in the weight of each slug. Slugs varied in weight and some slugs appeared to have higher pain tolerances than others, so their responses to the hotplates could vary. For example, the heaviest slug weighed 0.8 grams while the lightest slug weighted 0.08 grams. The amount of racecadotril absorbed could differ by weight. This potentially increased the variability of the results and could have affected the data collected for certain wavelengths depending on which randomly selected slugs were used for each trial. Future studies could more carefully track the weight of each slug per trial and how this may have affected their response to pain. Another factor previously noted, is the exact angle of snails to the direction of the EMF which was only approximated in this study [8].

Future research addressing the effects of different wavelengths of light on the ability to sense an electromagnetic field could consider more wavelengths of light and dark [7] in order to fully understand the photoreceptive qualities of this sensing ability. Furthermore, based on the results of this study, it will be useful to investigate how blue light relates to the electromagnetic abilities of marine animals since many marine animals (such as sharks) have electromagnetic sensing abilities and blue light is the most penetrant in water. The slugs responded best to blue light. Finally, in order to apply this research to supplement environmental change, it would be beneficial to see how light pollution, as a whole and of different wavelengths of light, affect animal's electromagnetic sensing abilities in different regions of the world. The effects of artificial light for the benefit of human use could also have unknown and unintended effects.

5. Acknowledgement

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