Cantaurus, Vol. 24, 2-5, May 2016 © McPherson College Division of Science and Technology

Crotalus atrox Venom Tolerance through Parental Envenomation in *Mus musculus*

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ABSTRACT

The purpose of this study is to explore possible relationships between parental environmental experience and offspring fitness in a particular environmental setting. The aim is to determine if maternal mice' progeny show an increased fitness if the maternal was envenomated prior to giving birth to her offspring, specifically if the offspring are likely to be envenomated in the environment which they live. In order to explore this possible relationship, a treatment group of female mice were injected with a 25% lethal dose of *Crotalus atrox* (Western Diamondback) venom mixed with 85 % saline solution two separate times. A control group of female mice were injected with only the 85% saline solution. The two groups of female mice were then impregnated with non-treated male mice. The offspring of both groups were then injected with a 50% lethal dose of *Crotalus atrox* venom solution. Survival rates and hours of survival for all offspring were recorded. Results show a slight favorable increase in both survival rate and hours of survival for the treatment group offspring with mean survival rate values at 18.467% and 25.872% for control and treatment groups respectively. Mean hours of survival were 7.303 hours and 9.105 hours respectively, for control and treatment groups. The p-value for both survival rate and hours of survival were 0.545 and 0.610 respectively, indicating no significant difference between treatment and control groups.

Keywords: *Environment, Fitness, Offspring, Venom Tolerance*

INTRODUCTION

Until recently, we thought of our DNA as being completely relatively static and unchanging throughout an individual's entire life, and that this relatively conserved sequence of DNA is passed on to the next generation determining its phenotype, with the exception of mutations to the DNA sequence occurring of course. We now know that our phenotype is affected by not only our genotype, but also the environment in which we live. Some phenotypes can even change depending on the environment in which an organism experiences.

While our DNA may stay the same, epigenetic effects have shown that environmental stressors can cause some of the genes which we pass on to become methylated which results in an inexpression of certain genes. (Weng et al. 2014).

Meanwhile, maternal effect has shown that development of offspring in some species can be determined by the mother's genotype and not determined by the offspring's genotype. This occurs as a result of environmental interaction between the developing offspring and the proteins which the mother produces.

Chimerism is yet another situation in which a phenotype is expressed not on the basis of the true genetic makeup. Chimerism is the state in which an individual has genetically different cells. This is most common of maternal-offspring, but can also be present in siblings who shared a uterus together. As it would turn out, maternal-fetal chimerism can have a negative and positive effect on both the mother and offspring. (Marleau et al. 2003). Having distinctly different genetic cells could have an effect on the individual's fitness in a broad range of environments.

Similar to the afore mentioned mechanisms, Passage of immunoglobulins from mother to offspring through milk in mammals is yet another mechanism in which an individual can affect it's offspring and increase fitness. (Lemke, Coutinho, and Lange, 2004)

While we understand that phenotypes are an outward expression of both genetic factors and environmental interaction, this research is looking into the possibility of an environmental interaction that was experienced by a parental that may benefit the offspring; A "secondary" environmental interaction that directly affects the phenotype and fitness of an offspring if the offspring is interacting in the same environment which the parental experienced.

One scenario which could potentially arise is a mouse (*mus musculus*) found in an environment which is filled with rattlesnakes. If a mouse were to have a couple of close encounters with a rattlesnake and survive envenomation attempts, could this mouse's experience help increase fitness of its' future offspring in some degree?

This research will not be attempting to identify which a specific pathway that could potentially lead to improved offspring fitness, even though several pathways as mentioned could be available, (i.e. epigenetic effect, chimeric effect, maternal effect, or even the possibility of immunoglobulins being passed from mother to offspring) The concept of offspring

being influenced in some positive form from a stressful experience that the parental incurred in a certain environment will be looked in to. More specifically, the purpose of this research project will aim to determine if venom tolerance can be acquired from a parental (mother) that resorted to producing an immunoglobulin as a result of envenomation from the species *crotalus atrox*. Currently it is known that in humans, horses, and sheep, the immunoglobulin (antibody) IgG is responsible for targeting and disrupting the otherwise potentially deadly effects of snake venom. (Vázquez et al. 2013) Since mice are easily attainable and common prey items for rattlesnakes, the subjects for this study will be of the species *Mus musculus. Crotalus atrox* venom is primarily hemotoxic and contains hemorrhagic components called zinc metalloproteinase. Zinc metalloproteinase causes blood hemorrhaging. It is this feature of the venom that will be used to provide measurement for how tolerant an individual is in this research. (Nikai et al. 1985) The question trying to be answered is "Can the envenomation and stress caused by envenomation in a maternal female mouse increase offspring fitness in the same environment? If an epigenetic effect, maternal effect, chimeric effect, or passing of immunoglobulins occurs as a result of the envenomation, the prediction is that survival rates and survival time will differ between control and treatment group offspring, with those differences being favorable to the treatment group offspring. The null hypothesis states that envenomation of maternal mice will have no effect on survival rate or survival time between the treatment and control group. The alternative hypothesis states that envenomation of maternal mice will produce an increased fitness in the treatment group offspring.

MATERIALS AND METHODS

To carry out this research, rattlesnake venom from the species *Crotalus atrox* was acquired from Sigma Aldrich. Due to the extremely potent nature of the venom, only 0.1 grams was needed needed. 10 female mice were acquired from Petco in Wichita, Ks. In order to control for the possibility of skewed results, females were randomly assigned into treatment or control groups and will be mated with the one of two male mice that were also acquired from the same Petco.

All mice are to be kept in the same room at all times, with exception to times of venom injection and blood sampling. The accommodations of the living area is a .3 meter x .2 meter x .15 meter plastic enclosure which consisted of an ever-present supply of water and food. Each mouse is kept alone in its enclosure except for the times of breeding where a male will be introduced into the enclosure for a twelve hour period of time. Before each injection, each mouse was weighed in order to give an accurate dosage based on the LD50 value. (LD50 for various Snakes, 2015) Injections were done using 1 cc syringes. The treatment group was injected with a solution of venom in 85% saline solution consistent with that of half of the LD50 value, (which resulted in .2775 mg/mL for every gram the mouse weighed) for the species *Crotalus atrox*. (This was to induce stress on the parental females without killing them. The control group was injected with only 85% saline solution with the same volume of solution that would have been given considering half the LD50 value. Two injections took place with 12 days in between for the two groups to recover. The males were then introduced 3 days after the 2nd injection date to induce pregnancy of the females. After birth and growth of the offspring, both treatment group and control group offspring were injected with a solution of *Crotalus atrox* venom and 85% saline at the true value of the LD50 (.555 mg/mL for every gram the mouse weighed.) Time of injection and time of death were recorded in order to compare survival rates as well as hours of survival for those that died between the control group offspring and the treatment group offspring. After all survival rates and hours of survival were collected, all mice were terminated via cervical dislocation.

RESULTS

Once all the data was collected, analysis of the data was done using an independent samples t-test. Treatment of mother mice with 50% concentration of LD50 (.2775 mg/ mL) showed no significant difference in either survival time or survival percentage between the control and treatment groups' offspring when the offspring were subjected a venom solution injection at the standard LD50 rate. (.555 mg/mL). An independent samples t-test was the method for analyzing this data. The p-value for survival percentage was 0.545. Mean values for survival were 18.467% and 25.862% for control groups and treatment groups respectively. (see Figure 1.) Mean values for hours of survival were 7.303 and 9.105 hours for control and treatment groups respectively. (see Figure 2.) The p-value for time of survival for all mice that died was 0.610. The null hypothesis is accepted; alternative hypothesis is rejected.

DISCUSSION

The question this research seeks to answer is "Can the envenomation and stress caused by envenomation in a maternal female mouse increase offspring fitness in the same environment?" Mean values for survival percentage between the control and treatment groups were 18.467 percent and 25.872 percent, respectively. Standard error values

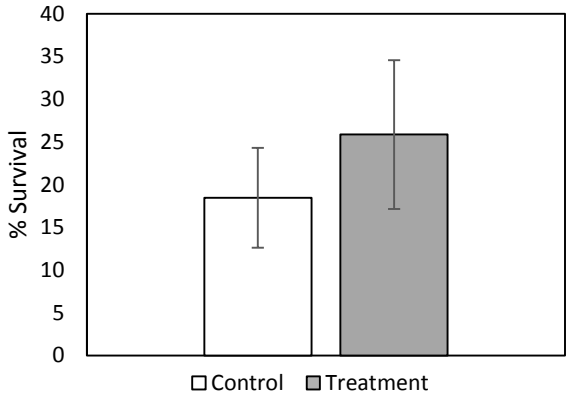

Figure 1. Survival percentages for both the control and treatment groups' offspring.

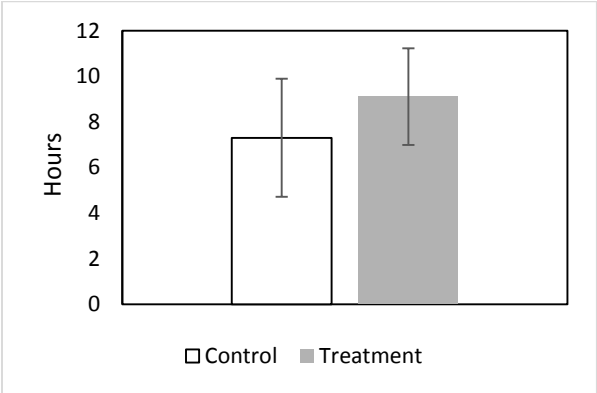

Figure 2. Hours before mortality after LD50 dose injection in both control and treatment groups' offspring.

for the control and treatment groups were 5.839 and 8.724, respectively. Average time of survival after injection was 7.303 hours for the control group and 9.105 hours for the treatment group. Standard error values were 2.593 for the control group and 2.116 for the treatment group. The survival rate and hours of survival after injection both show a minor favorability to the treatment group. The difference we see between the two groups Is not significantly different however but could be looked into further in future experiments.

First and foremost, the sample sizes and number of groups should have been much larger. To do this experiment, 10 female maternal mice were intended to be used. Due to unforeseen circumstances, two of the female mothers died before being able to impregnate them. Along with the loss of any offspring from those two female mothers, one of the females in the control group was found to have eaten all of her offspring. These circumstances resulted in the loss of potentially 30 or so offspring samples from being utilized in this research. Altogether there were 77 offspring that were able to be utilized; 44 treatment group offspring and 33 control group offspring. There were only 3 broods of the control group and 4 broods in the treatment group. Each brood was considered a single sample. In order to have had a significant difference based on the mean values and standard deviation for both survival percentage and hours of survival, 81 broods would be necessary for both the treatment and control groups.

In order to offset the small sample size, a blood viscosity test was going to be performed to look at clotting times when the control and treatment groups' offsprings' blood encountered the *Crotalus atrox* venom. Due to time constraints and a fear of potentially losing offspring before the LD50 injection in this process, the blood viscosity test was not performed.

As I had alluded to in the introduction, a mechanism by which this process could have occurred was not specified. However, one particular mechanism, if any, could be the the passing of immunoglobulins from mother to offspring via the mother's milk. Immunoglobulins resulting from the mother's envenomations could be produced and passed to the offspring which could in turn account for improved survivorship of the offspring if said offspring were to experience a similar environment which the mother endured. Further experiments should include a larger sampling size and looking at the offspring's overall fecundity and survival rates if the F2 generation were subjected to the same venom injection.

ACKNOWLEDGEMENTS

I want to thank the entire faculty of the natural science department for all of their guidance and suggestions. A big thank you to Dr. Wilgers my research advisor for his continued support and help with this project. Also, I'd like to thank my fiancée Elizabeth Stein and friend Evan Hiebert for their help with overall maintenance and well-being of the mice.

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