

INCREASED DOPAMINE LEVELS DO NOT INFLUENCE AGGRESSIVE
BEHAVIOR IN BLACK WIDOW SPIDERS (*LATRODECTUS HESPERUS*)

By

MATTHEW SCOTT OLENSKI

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Approved by:

Dr. Anna Dornhaus

Abstract

Discovering the driving forces of individual personality differences in animals is an emerging goal of animal behavior research. Biogenic amines have been known to mediate various aspects of behavior, including aggression, which is one of the most commonly varying individual personality differences among animals. In addition to aggression, a large number of other behaviors across a wide variety of species are influenced by biogenic amines. Arthropods specifically provide an ideal system to manipulate biogenic amines and observe behavioral changes. In this study, we used *Latrodectus hesperus*, the western black widow spider, as our model organism because of their consistent individual behavioral differences, which appear to relate to differing individual life history strategies. For example, higher aggression, as measured by attacks on simulated prey items, relates to higher rates of egg laying in “fast life history strategy” individuals. Here, I investigated the role that dopamine (DA) has on the individual’s aggressive behavior, measured by the number of times they attack a potential prey in their web. I injected spiders with the neurotransmitter, which is a precise way to manipulate the biogenic amine concentration and observed the changes in behavior that may follow. I looked for whether there was a significant difference between aggressiveness of biogenic amine-injected individuals, individuals injected with a saline solution, and non-injected individuals. We discovered that the injection of dopamine had no effect on black widow aggression.

These results indicate that biogenic amines may not play as big a role in individual personality differences as previously thought, and that aggressive behavior is influenced by other factors, such as genetics, metabolic rates, environment, or some other traits.

Introduction

Understanding animal behavior and variation in behavior between individuals is a major goal in understanding the evolution and life history strategies of various animal species. Life history is the way an individual behaves throughout its life, on a broad scope being “Live fast, die young”, expending more energy to have more offspring faster but dying sooner, or “Live slow, die old”, expending more energy over a longer period of time, surviving longer. Studying individual variations in behavior within a single species can also then give insight into various life history strategies and a basis of phenotypic variation. Using the nervous system is a solid mechanism to understand the evolution of phenotypic variation due to its highly conserved nature throughout animal evolution (Arendt, et al. 2008). Life history drives individual variation (Adamo, S., et al., 1995), which is driven in part by the nervous system, which produces, transmits, and receives the signals that determine actions, and thus, the overall behavior of an individual. Other studies suggest other factors that also play a role in aggression such as environmental factors and genetics (Ariyomo, et al. 2013). Due to the potential role of the nervous system in individual personality variances, we are looking to further understand how much an influence the nervous system has on intraspecific life history variations.

Behavior is currently understood to be influenced by a wide variety of factors, including environment, genetics, and an individual’s nervous system (Brembs, 2013). The nervous system uses signals of neurotransmitters, or biogenic amines, to enable nervous system communication. Numerous studies have been done to study the effect of different levels of certain biogenic amines across a wide variety of different species. Dopamine (DA) is one of the most commonly studied biogenic amines. In previous studies, we see that in the ant species *Formica polyctena*, increased dopamine levels lead to increased aggression (Szczyka, A. et al., 2013). We also see that amines alter the individual behaviors of the closely related Redback spider, *Latrodectus hasselti*, with dopamine being correlated with increased aggression (DiRienzo, N., et al., 2017). This was discovered after dissecting the central nervous system of the Redback spider. Seeing that increased levels of dopamine was correlated with increased levels of aggression, the question remains, does increased levels of dopamine *cause* aggressive behavior? In vertebrates, such as the zebrafish (*Danio rerio*), correlations exist between aggressive behavior and dopamine levels, as these zebrafish are used as a model organism to test the effects of certain drugs with these biogenic amines on aggressive tendencies (Jones, L., Norton, W., 2015). In contrast to these however, a study performed with rats have shown that a an increase in dopamine occurs after aggressive interactions, indicating that these aggressive interactions more so influence neurotransmitter levels, rather than the other way around (Annemoon, M. 2000). Individuals naturally produce various amounts of these biogenic amines in response to certain environmental situations, as well as from other effects such as genes and body mass. These varying amounts lead to individual degrees of variation in terms of behavior (Kralj-Fiser, S., Wiebke, S., 2014). In this study we aimed to experimentally test the role of increased biogenic amine level on individual behavior in *Latrodectus hesperus* to see whether the effect of dopamine is consistent among the ant and the spider, two distantly related arthropods. This will give us an idea of whether increased biogenic amines have different effects in different species, indicating different physiological mechanisms between species.

Black Widows are a common model organism to study variations in behavior due to their known consistencies in variations among individuals (DiRienzo, 2013), meaning that individuals

maintain a consistency in personality throughout their life. We chose to use the western black widow as a model organism also because one, the local area is a natural habitat leading to easy collection of wild type individuals, and two, the black widow is already a well established model organism for behavioral research (Pruitt 2010; Pruitt & Husak 2010; Pruitt et al. 2011a; Johnson et al. 2012; DiRienzo et al. 2013). Black Widows express variable aggressive behavior based on certain environmental pressures (Blackledge, T., 2007), as they are sit-and-wait ambush predators against small insects that get close enough to their established home, which means that it was easy for us to maintain a small environment for them. Females vary in individual behavior in such that some are more likely to attack a prey cue than others (DiRienzo, Aonuma 2017). The work that does show this variation is correlative, as in the study performed by Dr. DiRienzo and Dr. Aonuma (2017), they dissected the spiders' central nervous systems and found increased dopamine levels in the more aggressive individuals. This shows individual differences may be mediated by biogenic amine levels.

In this study, we sought to answer the question of how increased dopamine levels affect aggression in Black Widow spiders (*Latrodectus hesperus*), and what insights into individual life history strategy we can gain from this variation. We hypothesize that behavioral trait variation is either driven primarily by changes in certain neurotransmitter levels or by some other factor, such as environment, genetics, body size, or some other influence. Based on previous studies, we expect to see that increased dopamine levels would increase aggression (DiRienzo, N. et al. 2015). We did this by manipulating amines via micro-injections and looking at influences on aggression based on attack numbers against a controlled vibratory stimulus.

Methods

Web Environment

Sixty lab-reared mature female black widow spiders total were randomly chosen for each respective trial. The mothers of these females originated from Davis, California. The spiders chosen for the experiment were raised in our lab and were between 2-3 months old. They were maintained in our lab and were not used for any prior experiments. Due to the random selection for trials, some spiders present in both trials may have received different treatments in each trial. Their body weights varied from 232mg to 600mg. Sixty Sterilite containers (L= 40.7 x W=28.5 x H=18.5 cm) were cleaned using ethanol and allowed time to dry overnight. One inch of Quikrete brown play sand (comes washed, screened, and dried) was added to the container along with a skeletonized cardboard box (L=24.5 x W=19 x H=10 cm) (photos a, b, and c) The cardboard box had three walls and all but 3 cm of the top removed, thus leaving a rectangular cardboard frame with the back, bottom, and a 3cm portion of the top walls remaining. During the week of behavioral assays, each spider was fed two Brown House crickets, (*Acheta domestica*) all of similar body size, on Monday in their small, circular containers (radius = 9 cm, height = 7.5 cm). On Tuesday they were removed from their containers and weighed using an Ohaus Analytical Plus scale and transported to the Sterilite container where they were allowed to build webs until Thursday. The spiders were exposed to a 12:12 light/dark ratio and the room was set at 27 degrees Celsius.

a.)



b.)



c.)

Figure 1. Various views of skeletonized cardboard boxes used to house spiders. Photo a.) shows a side view. Photo b.) is top view before black construction paper is added to bottom and back side. Photo c.) is a top/side view of the box without black construction paper added to the bottom and back on the inside.

Injection schedule

We used a pre-made Ringer saline solution consisting of 233mM NaCl, 6.8mM KCl, 8mM CaCl₂, 5.1mM MgCl₂, 10mM HEPES, and maintained at a pH of 7.8 to use with a 3mM dopamine solution. The dopamine was dissolved in the Ringer solution (Bicker, 1995; Barron, et al. 2007). During the weekend before each injection week, we let each individual spider build a web in their containers/boxes to familiarize themselves with the environment, to control against an unfamiliar environment. On the Monday of injection week, we removed the spiders from their webs and fed them two medium sized Brown House crickets. On Tuesday, the spiders were then weighed and placed back on their webs. On Thursday morning, the spiders were removed from their webs and placed into labelled 50mL vials. On Thursday, around noon, we injected each spider using a Hamilton 700 Series 50uL microsyringe with whichever treatment they were randomly selected to receive. Following all injections, the spiders were then placed back onto each of their respective webs. We then did our first behavioral assay at around 15:00 that same day, followed by repetitive assays 24 hours after injection (Friday), and 48 hours after injection (Saturday).

Injection method

Sixty spiders were randomly selected and placed into four treatment groups consisting of fifteen individuals each for the injections. To do the injections we used a 50uL Hamilton syringe to inject the dorsal side of the abdomen of the spider, just off to the side of the heart to avoid damaging any major organs. Before injection, we placed the individuals into a 50mL tube and inserted a hose dispensing CO₂ gas to cause the spiders to become momentarily incapacitated. During this time, we were able to easily inject the spiders with their respective solution. Per spider, one group received 5ul of saline solution, the second received 5uL of dopamine (DA) solution, the third received 5ul of serotonin (5-HT) solution, whose effects were looked at in a separate project, and the fourth and final group received no injection, but still was exposed to the CO₂ gas as to control against the injection process. We then placed the spiders back into their labelled 50mL vials to be transported back into their respective Sterilite containers that contained their webs on the cardboard boxes placed on top of sand.

Behavioral analysis

Behavioral assays were performed every day for three consecutive days, starting on the Thursday of injection. After the third tests were given, each spider was transported into their original circular containers and fed the following Monday. Injections were not given again for exactly 3 weeks. To measure aggression, a 10 cm cable tie was attached to the tip of a vibrating device (Classical Silicone Vibrator, Liler, Shenzhen, China) and was applied to three different areas of the web. The cable tie allowed for a specific portion of the web to be tested while decreasing the risk of damage to the web. The end of the cable tie was placed near the spider's retreat (within 2 cm), in the middle of the box, and again towards the far end of the cardboard box, each for ten cycles of vibrations. If the spider did not build a web in the specified area, no aggression test was conducted. The order in which the cable tie was placed into the box was randomly predetermined. All assays were performed blindly; The person who administered the

injections wrote the ID number and treatment given to the spider on a separate spreadsheet so that the person giving the behavioral assays was unaware of which spider had which treatment. The vibrator was set to vibrate with low intensity consistently, with 1 spike in intensity every 3 seconds. We then let the vibrator go through 10 cycles of vibration on each of the 3 areas of the web (i.e. for a total of 30 seconds), with a 10 second break in between each area, and we observed whether or not the spider attacked. We did these injections a total of two times.

Statistical analysis

To analyze the data, we used an R version 3.6.3 with the tidyverse, lme4, multcomp, and MuMIn packages. We performed a generalized linear model with a binomial distribution on whether each spider attacked the stimulus (coded as 1) or did not attack the stimulus (coded as 0). We also used a Tukey Post Hoc Analysis test to compare the averages of each treatment with the controls to determine whether there was a significant effect of neurotransmitter injection or of any injections, compared to the CO2 anesthetized controls. In addition, we used a poisson distribution to compare the dopamine to the saline control group. An example of our code used for our Poisson distribution is as follows.

R-script used for poisson distribution and r-squared values-

```
m1_pois <- glmer(attack_number ~ (1|ID) +  
  treatment + scale(weight.x) + day + web,  
  data = amine_slim, family = 'poisson',  
  control= glmerControl(optCtrl=list(maxfun = 100000), optimizer="bobyqa"))  
r.squaredGLMM(m1_pois)
```

Results

We found that in dopamine injected spiders, there was no significant increase or decrease in aggression level throughout all days (p-value = .567, n = 30, Tukey Post Hoc Test (Table 1.)) or when each separate day was taken into account (p-value = .567, n=30, Poisson distribution).]

For the dopamine-injected treatment, this supports the hypothesis that dopamine does not have an effect on black widow aggression, which is different than the effects of dopamine seen in similar studies of other arthropods (Szcuka, A., et al. 2013). While aggression may not be affected by dopamine levels, dopamine may affect some other aspect of life history strategy not covered in this study.

Using a Poisson distribution, we see that compared to the saline control group, dopamine had a very high p-value (p= .567). Based on this value, we can say that the increased amines

aren't the key players in aggressive differences between individuals. Furthermore, when using a Poisson model, the r-squared values seen indicate an interesting bit of information. We can see that the conditional r-squared is high ($r^2 = .367$), while the marginal r-squared value is far lower ($r^2 = .059$). This combined with the p-value differences indicates that there is a lot of individual variation and that the amines present actually don't account for a large effect on aggressive behavior.

Discussion

Here, we find that dopamine does not seem to have an effect on aggression in Black Widows. In contrast, dopamine has been shown to influence increased aggression-based behavioral changes in the closely related Redback spider (DiRienzo, N., et al., 2017). A question that comes from this study is why are these same molecules seen to have different effects among arthropods, as even other spiders?

The dopamine treatments which showed no effect on aggression indicate that variations in individual behavior and life history strategy may be primarily affected by other factors, such as body mass, environment, or genetics, and may be a topic looked at in a future study. Considering the results seen in the closely related Redback spider (DiRienzo, 2017), this further suggests that dopamine may be involved in a different physiological process with no influence on aggressive behavior, with a much larger focus coming from other factors. A future study may look to see which other factors have a significant influence on aggression.

Due to the highly conserved nature of the animal nervous system, the results of this study can potentially be applied to a wide array of species within the animal kingdom. The different effects seen in different species shows how neurotransmitters may not play as big a role in certain life history characteristics, such as aggression previously thought. Given as well, that arthropods make up a vast majority of animal species and the conservation of the nervous system throughout animal phyla, these results can be applied to further research in potential management or control mechanisms and is further information into the understanding and study of animal behavior.

Conclusion

Understanding the underlying role of individual variation and their role in various life history strategies is currently a highly studied area of behavioral ecology. Using the Black Widow spider as a model system due to its ease of access and its conserved variations among individuals leads us to a result that has future implications for understanding the behavior of a wide variety of spiders (Sweeny et al. 2013; Royaute et al. 2014; DiRienzo et al. 2015). The highly conserved nature of the animal nervous system could lead to the application of this data to a far greater variety of species. In our study we found that increased concentrations of dopamine within the spiders' nervous systems do not account for a large effect for individual personality variations. We can hypothesize then, that aggressive behavior is influenced more by any number of other factors, such as genetics, environment, body mass, or metabolism. Further studies should be done in order to help us further understand various factors and their roles in life history strategies.

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