The chemistry of sex games: Why do male crickets transfer large amounts of dopamine to females during copulation?

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Abstract

Dopamine is an important biological molecule that plays a critical role in how behaviors are "punished" or "rewarded". And while dopamine has been studied with regard to a wide range of behaviors, including memory, diet and addiction, it has previously not been found to be transferred from one individual to another during copulation. However, the ejaculates of male ground crickets (*Allonemobius socius*) can contain up to 100pg of dopamine, which is roughly 10X the normal physiological dose required to modify behavior in insects. So, the question is, why are male crickets transferring so much dopamine to females during sex? There are several alternative hypotheses, derived from sexual selection and sexual conflict theory, which may explain the function of dopamine as an agent of sexual reward or punishment, respectively. Our preliminary data suggest that dopamine acts as a punishment, whereby the greater the amount of dopamine transferred to the female, the longer it takes for the female to re-mate. As a consequence, females receiving larger doses of dopamine are likely forced to store and utilize more of that male's sperm relative to a female who receives smaller amounts of dopamine. This "punishment" effect is particularly strong when a female re-mates with a different male. While further experiments are needed to clarify the role of dopamine in the chemistry of cricket sex, our data suggest that sexual conflict over mating rates may be driving the evolution of dopamine usage as a sexual punishment in this system.

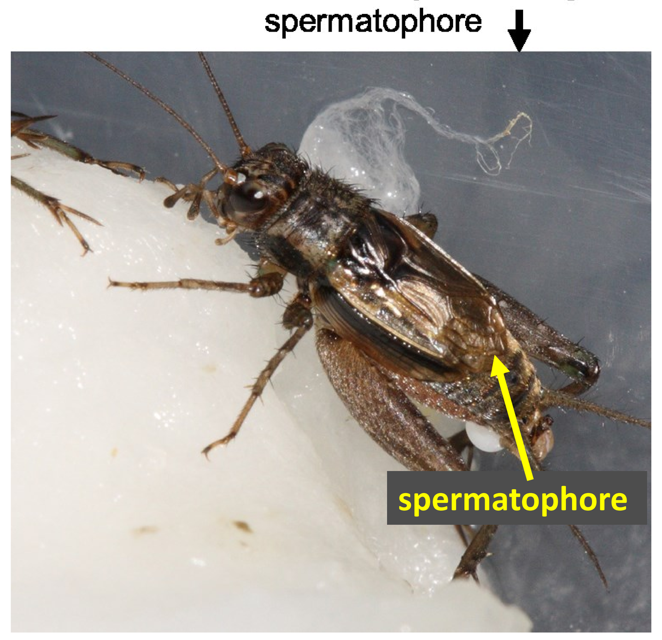
Purpose

The purpose of this research is to determine why high levels of dopamine are found in the spermatophore of *Allonemobius socius.* Previous studies have determined that dopamine plays a crucial role in insect learning and memory. Therefore, the fact that dopamine is present in the spermatophore implies that it influences mating behavior.

Mating

The male ground cricket, *Allonemobius socius,* produces a spermatophore prior to copulation with a female. A spermatophore (Figure 1) is a capsule made out of proteins that contains spermatozoa, as well as various nutrients and seminal fluid proteins. Once the protein coat has sufficiently hardened, the male initiates copulation with the female having control over whether or not to mount the male (Figure 2). After mounting, the male attempts to attach the spermatophore to the female genital opening. After dismounting from the male, the spermatophore stays attached to the female (Figure 3), where the ejaculate continues to be transferred to the female and is stored in her spermatheca (a balloon-like structure attached to the reproductive tract). In addition to receiving the seminal fluid through mating, female crickets can also consume the spermatophore by scraping the spermatophore off and eating it.

Figure 1



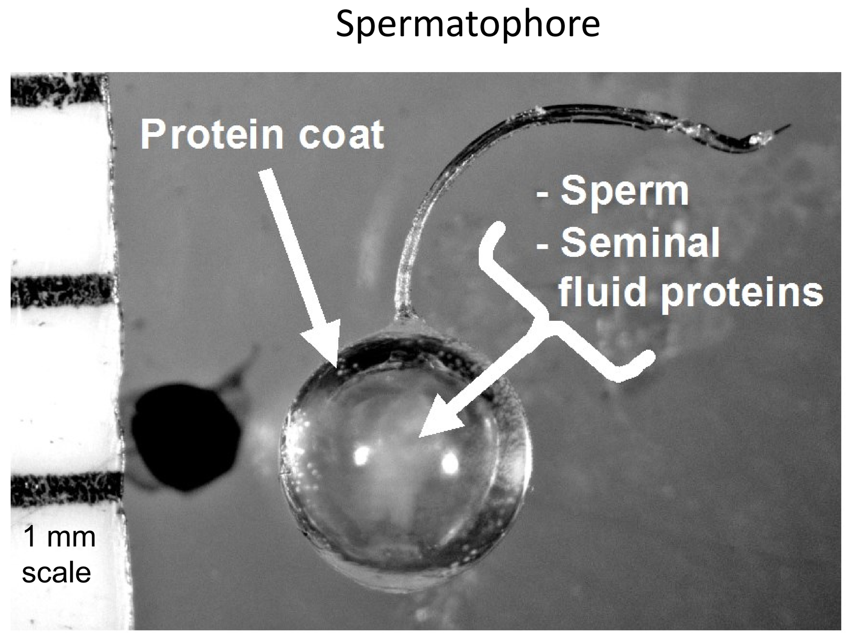


Figure 2

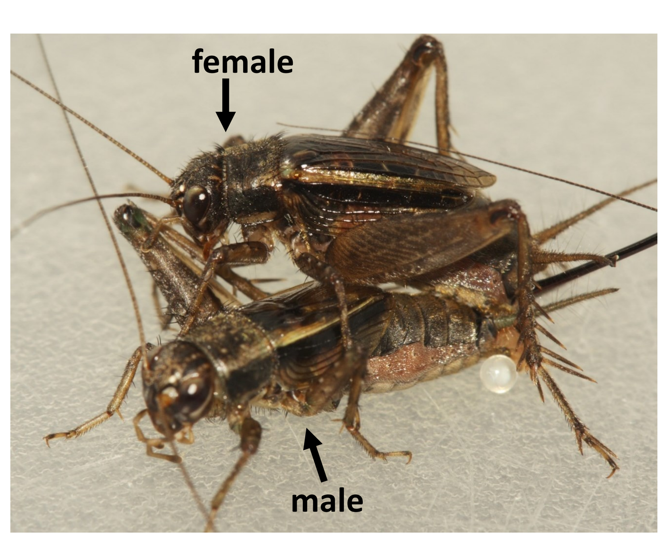


Figure 3

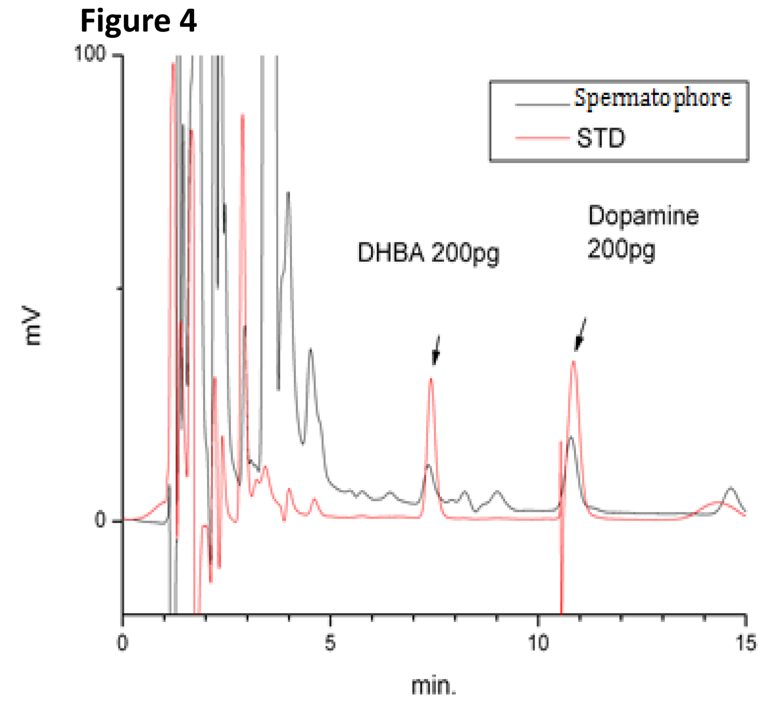
Learning and Behavior in insects

Learning is essential in order for animals to survive in their environment. One way that animals learn is by associating neurotransmitters , usually biogenic amines, in response to stimuli. These neurotransmitters provide feedback on what is going on in the environment by activating neurons, which control motor as well as sensory responses. Depending on the type of stimuli and how it is received, the effect can vary great. These stimuli usually fall into two categories, the reward pathway and the punishment pathway. In the reward pathway, a stimuli occurs after a certain behavior, and increases the probability of that behavior being repeated. Likewise, in the punishment pathway, a stimuli occurs after a certain behavior and decreases the probability of that behavior being repeated.

Dopamine in Crickets

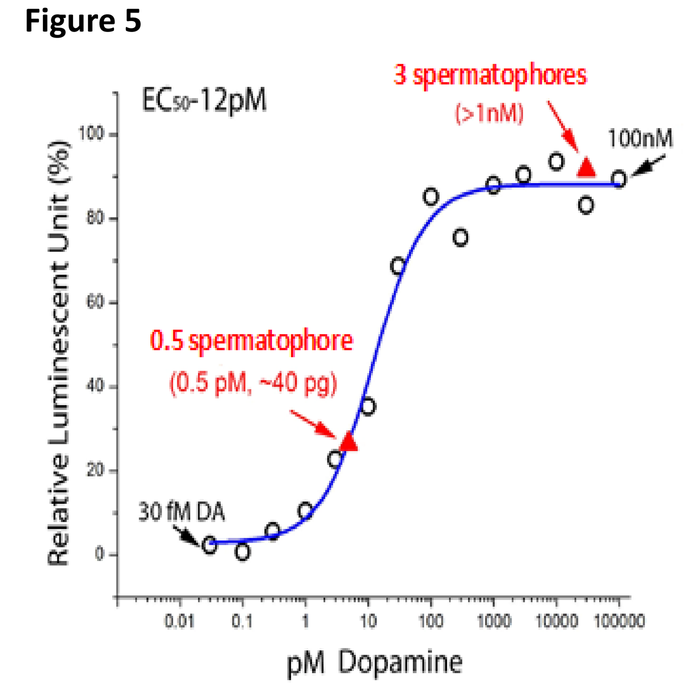
With the help of Dr. Yoonseong Park in the Department of Entomology at Kansas State University, an HPLC-ECD assay was performed (Figure 4), as well as a dopamine D1 receptor assay (Figure 5), on the contents of male spermatophores. To our surprise, we found an average of approximately 100pg of dopamine in each spermatophore – which is roughly 10 times the amount necessary to have a physiological effect. Dopamine has been found to be involved in both the punishment and reward learning systems in insects. The fact that it is present in both systems makes it difficult to elucidate the exact role in plays by being in the spermatophore. For example, in a punishment learning system, dopamine would be released in response to a negative stimuli, and the insect would avoid the stimuli. In a reward system, a stimuli triggers the release of dopamine, which in turn lets the insect know that the stimuli is beneficial. In *Allonemobius socius*, it is unclear whether dopamine is involved in the punishment or reward pathway. From the perspective of males, dopamine acting in a reward pathway could cause the female to determine that the recently copulated with male is a good mate, and thus compel the female to re-mate with him. On the other hand, should dopamine be acting in the punishment pathway, then dopamine could delay female re-mating regardless of the male – which benefits the male as a larger percentage of her eggs will be fertilized by that male.

Figure 4



HPLC-ECD showing presence of ~100pg of dopamine in 1 spermatophore

Figure 5



dopamine receptor assay using the tick D1 dopamine receptor

Hypotheses

(I) If males are utilizing dopamine as a reward, then we hypothesize that male-specific cues are being associated with the large amounts of dopamine. As a consequence, females will re-mate a second time more quickly with the same male than with a different male – a result not predicted by the punishment hypothesis.

(II) If males are utilizing dopamine as punishment, then the greater amount of dopamine transferred to the female (i.e., both during copulation and through the female's post-copulatory eating of the spermatophore) should be positively correlated with the time it takes a female to re-mate regardless of the male partner – a result not predicted by the reward hypothesis.

We have designed a pilot study to determine which of these alternatives, if either, is more likely. The results obtained from this study will dictate the direction that this project takes.

Experimental Design

A series of preliminary mating trials will be performed to determine how dopamine could be influencing mating behavior (Figure 6). There will be two groups of females (i.e., Same vs. Different). In the "Same" treatment, female crickets will mate twice with a single partner, and during each trial, time points will be recorded at the start of the trial, when the spermatophore is produced, when copulation beings, when the female dismounts the male, when she wipes off the spermatophore, and how long it takes her to re-mate. We will also record whether or not the female eats the spermatophore following copulation. In the "Different" treatment, everything will be identical to the "Same" treatment, except the female will be given a different male for the second mating (i.e., a male that successfully mated with another female during the previous trial). During these trials, if a significant difference in time to re-mate is observed (with the "Same" treatment showing a significantly shorter time to re-mate), then we will infer that dopamine is acting as a reward. On the other hand, if such a pattern is not found, but we find that re-mating time in both treatments is positively correlated with the amount of dopamine transferred (measured as the total time the spermatophore was attached and whether or not the spermatophore was eaten), then we will infer that males are utilizing dopamine as punishment.

Figure 6

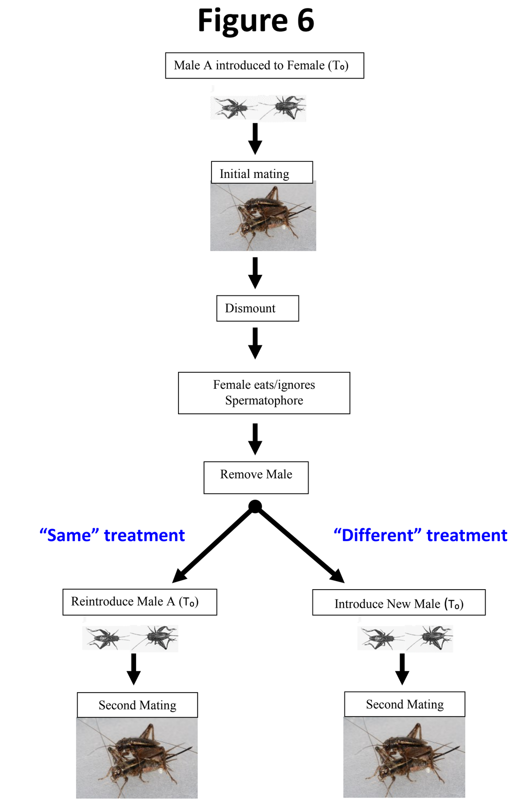
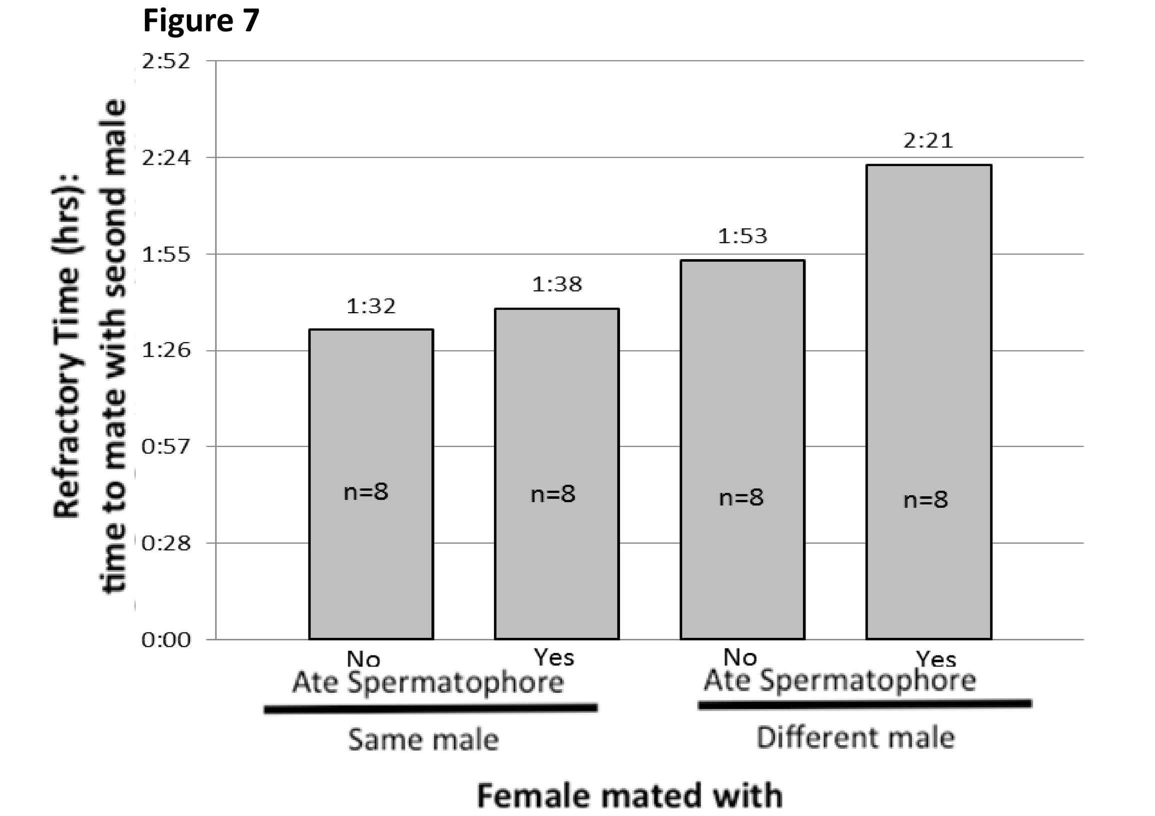


Figure 7



Pilot Study Results - The results from the pilot study (Fig 7) show an effect of male type (i.e., different vs. same treatment) and an effect of females eating the spermatophore (i.e., females receive more dopamine if they consumed the spermatophore). In both trials where a different male is introduced, a sharp increase in refractory time is seen, whether the spermatophore is eaten or not. Upon eating the spermatophore, the refractory time continues to increase, which implies that dopamine is having a "punishment" effect on the female.

Dopamine-matrix Study - A new experiment to directly test the effects of dopamine was designed, using the knowledge that females feed on the males' hind tibial spur during mating (Fig 8), which is known as nuptial feeding. Previous studies have utilized liquid Band-Aid to cover the spur (Fig 8); so, in this experiment, a dopamine-liquid band aid mix of known concentration is placed on the spur in order to control the amount of dopamine the female receives during mating (~200pg or 5X that found in a single spermatophore). This was done because only a small portion of the liquid Band-aid will be consumed by the female (~1/20 or 10-20pg) . Our control was a liquid Band-Aid only solution. In both treatments, the females were not allowed to consume the spermatophore, which ensures that their only source of dopamine was from our treatment effect. In the control matings (Fig 9), the refractory time stayed consistent, while a significant increase in refractory time was observed in the dopamine trials ($t=3.72$, $df=18$, $P=0.0016$). This result rejects the reward hypothesis, which predicts increased dopamine levels should result in shorter refractory times.

Figure 8

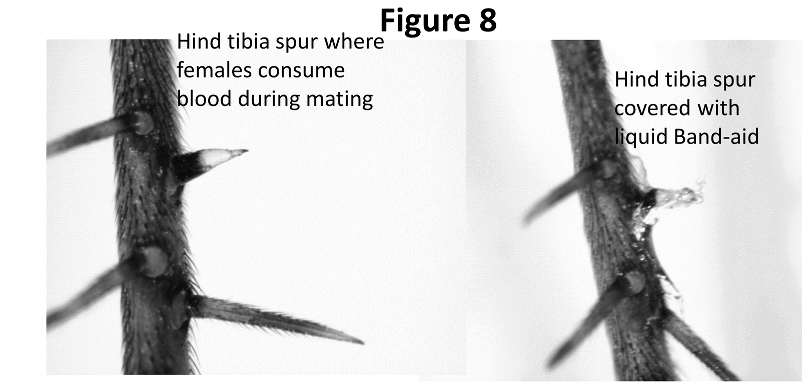
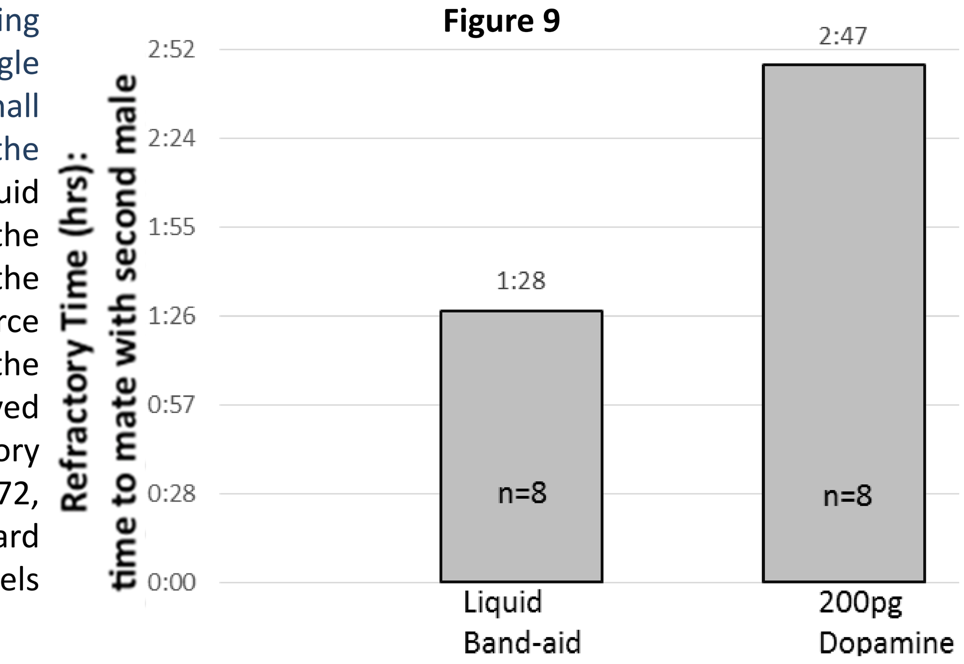


Figure 9



Acknowledgements

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