The Effect of the Social Isolation of Honey Bees on Caffeine Addiction

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Abstract

Social isolation may affect one's vulnerability to become addicted to caffeine, a common stimulant taken daily, and may lead to a caffeine substance use disorder (Meredith et. al., 2013). The honey bee (Apis mellifera) was used as a model system to test for caffeine addiction between isolated and grouped bees because their learning can be quantified. It's hypothesized that socially isolated bees will learn the caffeine reward better than the grouped bees because social isolation may cause an organism to be more prone to caffeine addiction and caffeine enhances a bee's memory (Whitaker, Degoulet & Morikawa, 2013; Wright et al., 2013). Bees were socially isolated or grouped for 0, 1, 2, or 4 days and underwent massed trials with odors paired with a sucrose or caffeine reward. The proboscis extension reflex (PER) responses were recorded based on a binary system. The hypothesis was partially supported. The isolated bees learned better overall than the grouped bees possibly due to stress-induced learning, in which the memory of the odor and reward was enhanced by the stressful isolation (Vogel & Schwabe, 2016). However, no significance was found between learning and the caffeine reward, possibly due to an insufficient amount of caffeine. Future studies can focus on addiction and stressed induced learning.

Introduction

90% of people in the United States consume caffeine regularly and the United States is one of the top 10 countries that consume coffee (Meredith et al. 2013; CBC, 2016). Caffeine is a stimulant like amphetamine and cocaine that increases dopamine in the brain and dependence can form if taken in high concentrations (Meredith et al., 2013). The susceptibility of becoming addicted to a drug and the difficulty to extinguish the established dependence increases when rats are socially isolated during adolescence (Whitaker, Degoulet & Morikawa, 2013). The eusocial honey bee (Apis mellifera) was used as a model system to test for caffeine addiction between isolated and grouped bees because honey bees' learning can be quantified.

Honey bees interact with other honey bees throughout their life cycle. Foragers are female bees that collect pollen and nectar and they undergo associative olfactory learning to remember flowers for maximum honey production. Caffeine may interact in the dopamine pathway in invertebrates, similar to other stimulants in vertebrates (Mustard, 2013). Caffeine may enhance a bee's learning and memory as bees given a caffeine reward were three times more likely to respond to the odor after 24 hours than the sucrose reward; and, bees treated with caffeine responded significantly greater than control bees in a 24-hour olfactory task (Wright et al, 2013; Shao, Zhang & Maleszka, 2005).

Social isolation may affect the learning and memory of organisms. The smaller the group, the more responsive a bee was to a sucrose reward however isolated bees had a lower learning accuracy compared to grouped bees (Tsvetkov, Cook & Zayed, 2019). Also, isolated bees in the presence of dead bees learned more than the number of solely isolated bees possibly due to the dead bees being additional stressors to isolation (Maleszka, Barron, Helliwell & Maleszka, 2009). These studies suggest that isolated bees are more stressed and aware of certain stimuli but have a lower learning capability than grouped bees.

It's hypothesized that if bees are socially isolated or grouped for a duration of time then the isolated bees will learn the caffeine reward better than the grouped bees because social isolation may cause an organism to be more prone to caffeine addiction and caffeine enhances a bee's memory (Whitaker, Degoulet & Morikawa, 2013; Wright et al., 2013).

Methods

The following are the methods for one week (conducted for 5 weeks).

Odor and Reward. Hexanol and octanol were the two odors used (Figure 1) to reinforce either the 0.7 M sucrose or 0.7 M sucrose + 0.0007 g caffeine reward.

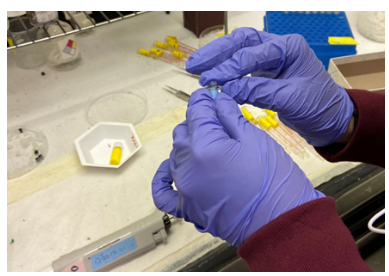

Fig. 1. One of the scientists set up one of the odor tubes by inserting a filter paper with 3 μL of the odor into a tuberculin syringe used in the massed conditioning trials.

Massed Conditioning Protocol. Olfactory learning in honey bees was tested using massed conditioning protocols.10 isolated and 10 grouped bees were each strapped into harnesses (Smith & Burden, 2014). A 0.5 M sucrose solution was used to test for sucrose responsiveness. At least 5 isolated and 5 grouped bees were used for each massed conditioning. In each trial, an odor is blown at the bee for 4 seconds. On the 3rd second the bee is fed either the sucrose or caffeine reward. Each trial is separated by 30 seconds (Wright et al., 2013) (Figures 2 & 3). The order of which odor was blown at the bee and the corresponding reward was conducted in a pseudorandom order for a total of 8 trials. A binary system was used to record the proboscis extension reflex (PER) responses (Figure 4): 0 = PER with reward (bee has not associated odor with the reward); 1 = PER without reward (bee has associated the odor with the reward thus learning); and NA = no PER.

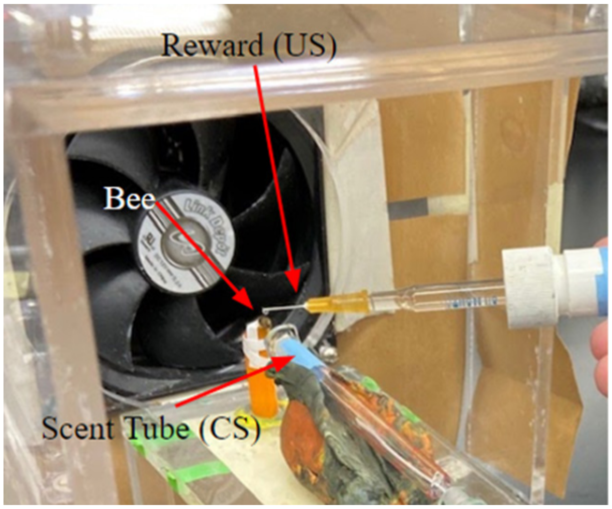

Fig. 2. One of the scientists is testing one of the bees in the massed conditioning trials. The bee is fed one of the rewards on the 3rd second after the odor begins blowing at the bee. US= Unconditioned stimulus. CS= Conditioned stimulus.

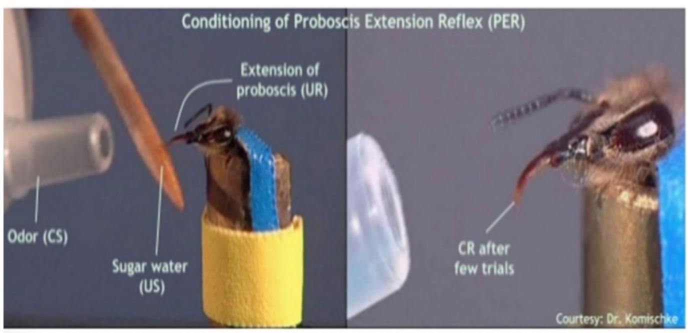

Fig. 3. Proboscis Extension Reflex (PER) (Komischke, 2015). CS= conditioned stimulus, US= unconditioned stimulus; UR= unconditioned response, CR= conditioned response.

Weekly Protocol. On Mondays (Day 0) all bees for one week were collected from the nuclear hive. 25 bees each were placed in two cages for the grouped social group (Figures 4 & 5). 50 bees were placed in individual queen cages for the isolated social group (Figures 6 & 7). Then, the massed conditioning protocol was conducted on 10 bees collected from the nuclear hive for baseline (Day 0) testing. Then on Tuesdays (Day 1), Wednesdays (Day 2), and Fridays (Day 4) the aforementioned behavioral assay was done.

Results

Learning curves were graphed from average PER responses for the bees (Figures 8, 9, & 11).

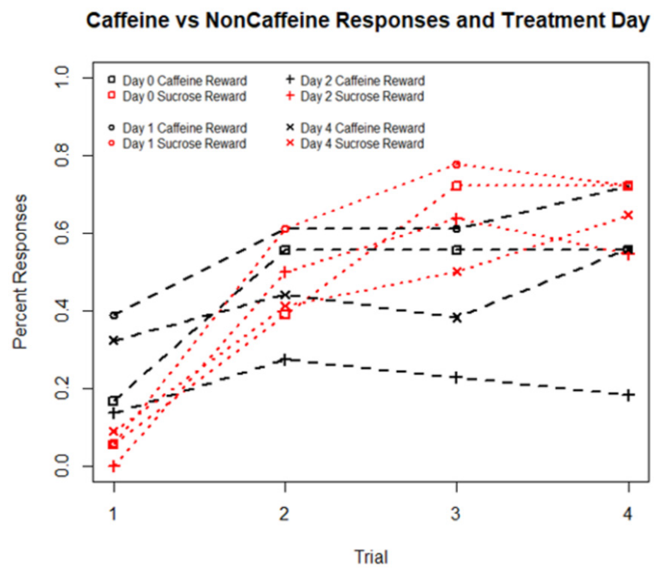

Fig. 8. The graph depicts the learning curves for bees conditioned with the sucrose or caffeine reward for each trial on each Day of social isolation or groups. Overall the bees conditioned with the sucrose reward learned better than the caffeine reward. Each Day is represented by a different icon.

Fig. 4. Front view of grouped cages. About 25 bees in each cage. The two bottles inserted into the top of the cage contain either water or a 1.0 M sucrose solution.

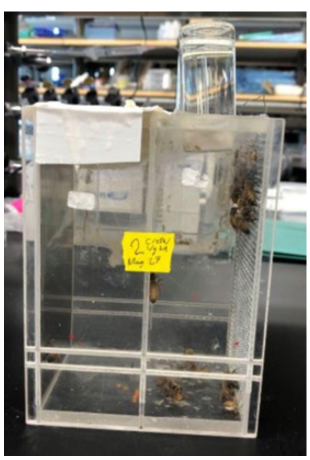

Fig. 5. Side view of grouped cages. About 25 bees in each cage.

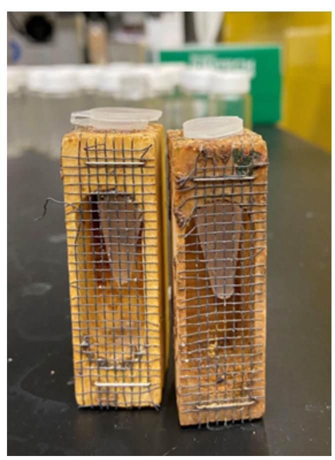

Fig. 6. Set up of the queen cages for the isolated bees. The Eppendorf tube inserted into the opening of each queen cage is filled with 3 mL of a 1.0 M sucrose solution.

Fig. 7. The set-up of all the isolated bees in the queen cages. A paper divider separates each queen cage.

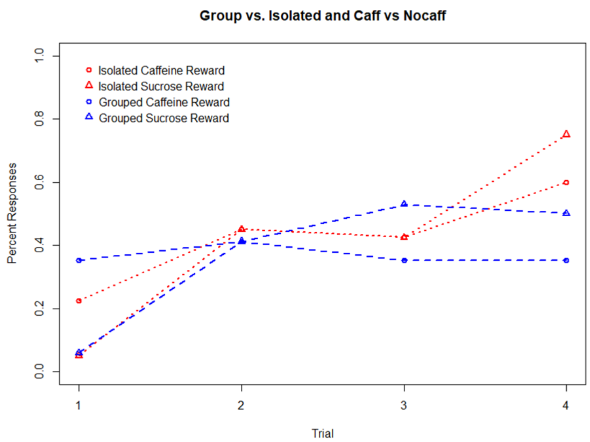

Fig. 9. The graph depicts the learning curves for grouped and isolated bees rewarded with caffeine or sucrose. As the number of trials increased, the socially isolated bees given either reward had higher average PER responses (Trial 4) compared to the socially grouped bees.

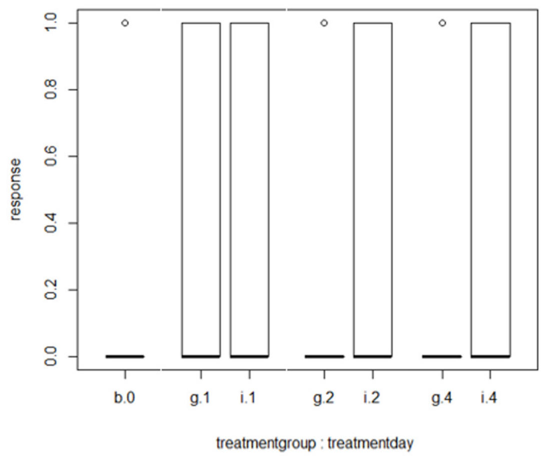

Fig. 10. Treatment Group : Treatment Day Box Plot. The boxplot shows the relationship between the treatment group and treatment day. Grouped bees only learned on Day 1 while isolated bees continued to learn throughout Days 1, 2, and 4. The box plot represents the median (bolded line) and 3rd quartiles for the PER responses of the bees in a certain social group treatment (b= baseline; g= grouped; i= isolated) and day treatment, which is the number of days the bees were in groups or isolation (0-4). The open circles represent outliers.

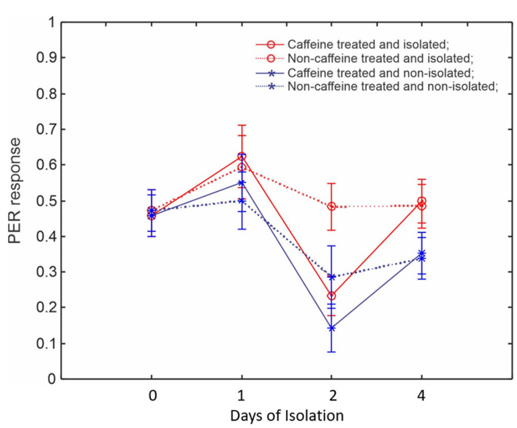

Fig. 11. Grouped vs. Isolated Bees and Caff vs No Caff Treatments on each Day of Isolation. From both social groups, non-caffeine treated bees show equal rates of learning on Day 2 and 4, but the caffeine treated bees show a definite decrease in learning on Day 2 and a definite increase in learning on Day 4. Graph made by Professor Hong Lei with MATLAB.

An ANOVA test was conducted to measure the observed effects and statistical significance. At a 5% significance level, significance was found in the number of trials conducted per bee ($p=0.0430$), in the relationship between isolated bees and the number of trials ($p=0.0471$), and in the relationship between the testing day and grouped bees ($p=0.0552$). The significance in the relationship between isolated bees and the number of trials supports the trend that isolated bees learned better as the number of trials increased (Figure 9). The significance in the relationship between testing day and grouped bees approaches significance, however, it still supports how the grouped bees learned less as the days progressed (Figure 10). A post-hoc pairwise test was conducted to test for significance between the comparisons of the caffeine (caff vs. nocaff) and social group (group-g vs. isolated-i) treatments within each Day. At a 5% significance level the comparisons between caff g-nocaff i on Day 2 ($p=0.0683$) and nocaff g-caff i on Day 4 (0.0636) approached significance. The approaching significance supports that isolated bees may have learned better than the grouped bees (Figure 10). Also, the longer the isolation the greater the difference between the grouped and isolated bees' learning as the two comparisons that approached significance were on Day 2 and Day 4 (Figure 11).

Discussion

The purpose of the experiment was to see the effects of caffeine and social isolation on honey bees' learning, and whether social isolation increases the likelihood of becoming addicted to caffeine. The hypothesis that isolated bees would learn caffeine better was partially supported, because the isolated bees learned better overall than the grouped bees (Figures 9 & 10), but there was no relationship between learning and the caffeine treatment ($p=0.3276$) (Figure 8). There was a significance between the grouped bees and testing day (Figure 10), and the isolated bees and number of trials (Figure 9). In addition, isolation enhancing the bees' learning is apparent when the bees are isolated for a longer duration of time (post-hoc and Figure 10), and supports the hypothesis of stress-induced learning enhancing memory, since the isolated bees become more stressed from the isolation.

Furthermore, the isolated bees may have learned better over time because they were put into a stressed environment, being separate from the hive, hence stress-induced learning. Possibly the isolated bees were more stressed than the grouped bees due to grouped bees still having a social stimulus. The memory of the odor stimulus with the specific reward may be enhanced by the stressful situation therefore improving learning (Vogel & Schwabe, 2016). A future study can focus on the levels of certain hormones that have a role in learning or stress released by the bee during conditioning trials (Even, Devaud & Barron, 2012). Further studies can focus on addiction and/or stress-induced learning.

A possible explanation for why there was no significance with the caffeine treatment is that the concentration of caffeine used in the experiment was not detected by the bee. Previous studies that resulted in positive effects of caffeine on honey bee acquisition used 0.1-100 µM of caffeine (Mustard, 2013). 0.0007 g of caffeine was used in this experiment (0.003604 mM≈3.604 µM). Massed conditioning trials can be done to find the caffeine concentration bees are able to learn the best from and respond to.

Interestingly, the grouped bees learned less each day as previous studies showed that grouped bees learned better than isolated bees; however, the studies did not conduct conditioning trials over a period of days like this experiment (Tsvetkov, Cook & Zayed, 2019; Maleszka, Barron, Helliwell & Maleszka, 2009). In addition, dead bees were in the group cages as time passed which may have added a stressor to the bees (Ichikawa & Sasaki, 2002).

In conclusion, isolated bees learned better than the grouped bees. No conclusion can be made about caffeine's effect on bees as the caffeine concentration used was not significant. Further tests should be done to find a caffeine concentration that affects a bee's learning and whether stress-induced learning is present in isolated bees.

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