The Effect of Caffeine on Short-Term Spatial Learning in *Periplaneta Americana*

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The American cockroach, *Periplaneta americana*, has been used extensively in recent years to study aspects of the complex vertebrate nervous system as a simple and accessible invertebrate model. This experiment examined the effects of caffeine on short-term spatial learning in cockroaches using a modified Barnes maze. Prior to every trial, the treatment group was injected with a caffeine solution and the control group was injected with a saline solution. The amount of time required for the cockroach to complete the maze during the initial learning trial was recorded. One hour later, the amount of time required for the subjects to complete a second memory trial was also recorded. A one-tailed t-test between the individual trial times for the control group established that learning had occurred. A two-tailed t-test between the individual trial times for the treatment group showed no significant difference. A t-test of means compared the difference in maze completion times between the groups and proved there was a significant difference. Therefore, caffeine did not positively affect short-term spatial learning in cockroaches.

Introduction

Caffeine is likely the most widely used psychoactive stimulant in the world (Dellermalm et al., 2009). While studies have shown that caffeine has nootropic effects, or cognition enhancing abilities, on memory in certain subjects, other studies have found less definitive, or even negative, effects on learning and memory (Han et al., 2007). For example, while humans under the influence of caffeine in one study showed increased brain activity in the frontal lobe and the anterior cingulate cortex, the regions of memory and attention control, another study found that mice that were given low doses of caffeine had reduced hippocampus-dependent learning and impaired memory (Koppelstaetter, 2008; Han et al., 2007). Additionally, another study found that mental performance was increased by low doses of caffeine at the risk of impaired short-term memory and broad-range thinking abilities (Lesk and Womble, 2004). Thus, much ambiguity remains over the benefits of caffeine.

While an experiment performed by Brown and Strausfeld (2009) found that P. americana have the ability to learn tasks through spatial recognition, only the effect of age on memory was tested. No scientific literature exists on the subject of whether caffeine improves neural function in cockroaches. Therefore, exploration into this topic would not only help determine what, if any, effects caffeine has on memory tasks, but also help uncover more information about caffeine's effects on organisms as a whole since prior discoveries were varied.

A maze designed by Barnes (1979) was modified according to the experiment performed by Brown and Strausfeld (2009). Two groups of 20-25 week old, male cockroaches, where one group (n=40) was injected with 25μL of a 3.0μg solution of caffeine, were tested in a series of learning tasks in the mazes. First, the cockroaches were placed in the brightly lit maze for up to our minutes to enable them to evaluate the maze and determine the correct spatial arrangement of the exit. This procedure was repeated 60 minutes after the first injection to determine each cockroach's ability to remember the correct spatial sequence for the exit.

Our hypothesis proposed that cockroaches injected with caffeine would have improved maze completion times due to enhanced short-term spatial learning capabilities.

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Methods

Colonies of P. americana were maintained in a laboratory setting under a 24-hour dark cycle at room temperature. The male adults were individually placed in extra-large petri dishes (numbered randomly) where they remained in darkness at room temperature (~22.5ºC) in between rounds of testing. The cockroaches were provided with a piece of potato or apple wedge for sustenance. Three minutes prior to testing, each cockroach in the treatment group (n=40) was injected with 25μL of a 3.0μg caffeine solution through the joint on the third appendage. The concentration of the dose was determined by using a ratio of an effective dosage by weight in larger organisms reduced proportionally to the average size of a cockroach. The control group (n=40) was injected with 25 microliters of a saline solution.

The modified Barnes maze consisted of a 12-inch circular aluminum platform surrounded by a 10-in aluminum wall. Overhead heat and light was used to motivate the subjects to find the real exit among the 3 false exits.

A Barnes maze was modified according to the experiment performed by Brown, et al. to enable the testing of the short-term spatial learning capabilities of P. americana. The maze, composed of a 12-inch circular poster board, was enclosed by a 10-inch poster board wall. The interior of the maze was covered in aluminum foil to increase the intensity of heat and light during the experimentation and heighten the discomfort for the subjects. Five exits, marked by differing visual patterns, were added equidistant along the circumference of the wall. Four of the doors were false and led to dead-end exits. One of the exits was randomly selected to lead to a dark, safe area for the cockroach. A 1-inch piece of plastic tubing was used to lead from the real exit to a cool, dark box. In addition to typical room lighting, the interior of the maze was warmed by a heat lamp to a temperature of 35 ºC. The maze was monitored by a thermometer and a light intensity probe. This heating, along with the aluminum foil, ensured that the cockroach would attempt to escape to its preferred cool, dark habitat.

For the first learning trial, each cockroach was placed in the maze 3 minutes after the injection for up to 4 minutes and was observed to determine if the subject could find the correct exit from the maze. If the cockroach's back legs entered the exit tubing, the trial was considered to successful. If the cockroach failed to locate the exit and completely enter the plastic tubing/box by the end of the 4 minute time period, the trial was considered to be unsuccessful. In the results, an unsuccessful trial was marked as having a time of 4 minutes. The exact amount of time required for each cockroach to reach the desired exit was recorded. The second learning trial occurred one hour after the first trial, and the same procedure was followed with an injection preceding the maze by 3 minutes. All sixty cockroaches in both the control group and the treatment group were subjected to this treatment. Each cockroach was subjected to the maze one time per trial for a total of 2 times by the end of the experiment. The order of the subjects that were exposed to the trial remained the same throughout the experiment so that approximately the same amount of time passed between the trials for each cockroach.

A repeated measures analysis of variance (ANOVA) test was performed to compare the time required for the subjects in the control group to complete the maze to the time required for cockroaches under the influence of caffeine to complete the maze. In this statistical test the cockroaches are matched by group (control or treatment) and individually by trial.

Results

There was a tendency for the difference in maze completion times (MCT) between Trial 1 and Trial 2 in the control group to be positive (Fig. 1). A positive change in MCT denoted a faster maze completion. With an average improvement time of 37.2 seconds, the control subjects injected with saline solution were found to have improved MCT from their first to second trial (Table 1). Only 9 out of 40 cockroaches were found to have a negative change in MCT from trial 1 to trial 2 (Fig. 1). Previous literature has shown that cockroaches have spatial learning capabilities (Brown et al., 2009), so this a priori knowledge dictated that a one-tailed t-test be performed. The t-test revealed that the control group had significantly improved MCT with a p-value of 0.0008 (Table 1).Furthermore, there was a tendency for the difference between MCT of the two trials in the treatment group to be negative (Fig. 2). With an average change in trial times of -0.05 seconds, the caffeine subjects were not found to have improved MCT from their first to second trial (Table 1).

Table 1. Summary of results from t-tests on individual groups		
Group	**Control**	**Caffeine**
# of Subjects	40	40
Average ΔT (sec)	37.2	-0.05
P-Value	0.0008	0.5016
DF	39	39
T-Value	3.3727	0.0041

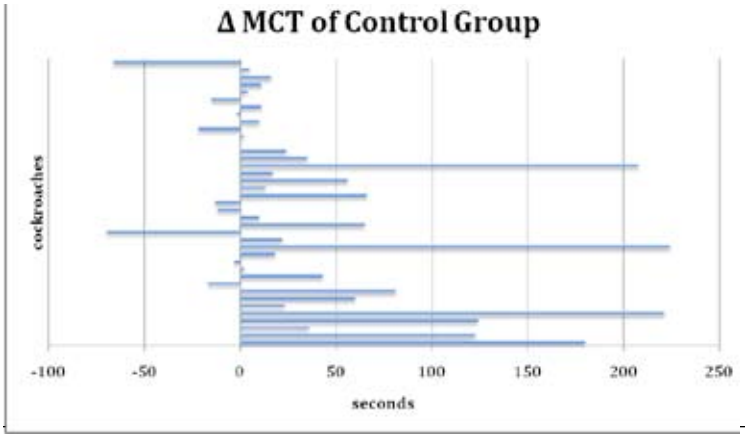
Because no a priori knowledge of the effects of caffeine on learning in P. americana exists, a two-tailed t-test was performed and showed that the caffeine-injected subjects did not have significant improvement in their MCT from trial 1 to trial 2 with a p-value of 0.5016 (Table 1). A total of 13 out of 40 caffeine-injected cockroaches were found to have a negative MCT from trial 1 to trial 2 (Fig. 2). Additionally, a t-test of means determined that the change in MCT of the caffeine group was significantly different from the change in MCT of the control group with a p value of 0.0255 (Table 2). Therefore, caffeine was not found to have a significant effect on the improvement of MCT and, subsequently, spatial learning capabilities in the subjects.

Table 2. Summary of Results from t-test on mean ΔMCT between Control and Treatment Groups

Source	DF	Sum of Sqs	Mean Sq	F Ratio	Prob > F
Group	1	27751.25	27751.3	5.1887	0.0255
Error	78	417172.30	5348.4		
C. Total	79	444923.55			

Fig. 1. Change in MCT (Trial 1 – Trial 2) for Control Group

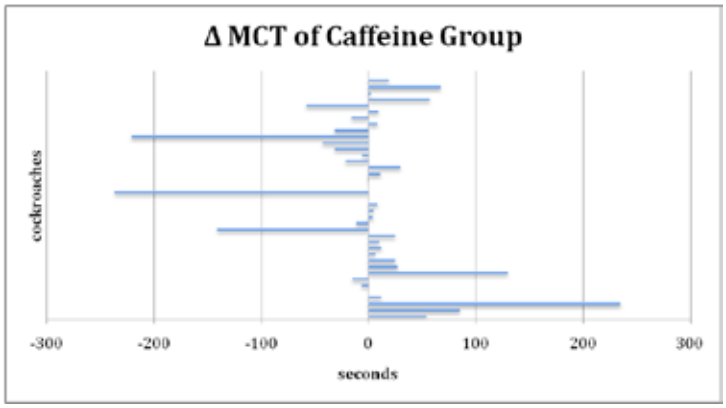
With a mean change of MCT between trial 1 and trial 2 of 37.2 seconds, the control group was found to have significant improvement between trials. The subjects were able to spatially recognize the exit of the maze.



The control group was found to have a significant improvement in MCT between trials (p = 0.0008), while the caffeine-injected group did not have any improvement in MCT between trials (p = 0.5016).

Fig. 2. Change in MCT (Trial 1 – Trial 2) for Caffeine Group

With a mean change of MCT between trial 1 and trial 2 of -0.05 seconds, the treatment group was not found to have any improvement between trials. On average, the subjects were unable to spatially recognize the exit of the maze.



With p = 0.0255, the control group MCT were significantly different from the MCT of the treatment group. Thus, the caffeine did not have beneficial effect on spatial learning in the subjects.

Discussion

Spatial learning has been proven to occur in cockroaches (Brown and Strausfeld 2009). The experiment performed in this paper replicated this hypothesis of the ability of cockroaches to learn, exemplified by the difference in MCT of the control group cockroaches. The one-tailed t-test of the difference in times between the learning trial and the memory trial revealed highly significant results, as shown by the p-value of 0.0008.

The original hypothesis of this experiment proposed that caffeine would enhance short-term spatial learning in the cockroach. Caffeine operates as a competitive inhibitor, binding to adenosine receptors which typically keep the blood flow to the brain regulated (Fison et. al. 2004). Once bound with caffeine, the adenosine receptors no longer keep the blood flow down (Daly et. al. 1987). It was hypothesized that the presence of caffeine would heighten nervous system function in P. Americana thus leading to an increased ability to learn the correct exit from the maze.

However, the analysis of data from the experiment suggested that caffeine does not enhance spatial learning in the cockroach. The control group performed significantly better on the second memory trial when compared to the treat-

ment group, as shown by the t-test of means. Furthermore, when the difference in trial times only for the treatment group was compared, a two-tailed t-test suggested that the cockroaches injected with caffeine did not learn from one trial to the next. Therefore, the original hypothesis must be rejected, and it must be concluded that the treatment of caffeine does not enhance the short-term spatial learning of cockroaches.

Because adenosine receptors are common to many other organisms, including humans, this study acts as a model for the potential effects of caffeine on learning capabilities of those organisms. Thus, the observations recorded can potentially be applied to systems outside those formally tested. Other studies have yielded mixed results about the role of caffeine in learning and memory of different organisms. For example, caffeine has been shown to improve memory retention but not memory acquisition in rats (Angelucci, et al., 2002). The complex effects of caffeine on neurological performance have been studied more extensively in humans, with mixed results. Caffeine has been found to decrease memory retention in humans, as well as increase brain activity in the regions of the brain responsible for memory and attention control (Terry and Phifer, 2006; Koppelstaetter, 2008). The absence of any definitive conclusions about the effect of caffeine on humans suggests the need for more in-depth research of this drug, which can effectively be done using animal models like the cockroach. This same experiment may be modified to examine the full spectrum of effects of caffeine on cockroaches. Altering the dosage of caffeine may allow researchers to examine how the performance of cockroaches varies in response to different concentrations of the drug. Additional learning and memory tasks may also be implement in conjunction with the treatment of caffeine to further examine the specific functions of the brain that may be affected by this drug.

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