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The Effects of Caffeine on Spatial Learning and Memory

Romina G. Cupo
Seton Hall University

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The Effects of Caffeine on Spatial Learning and Memory

by

Romina Cupo

Department of Psychology

Seton Hall University

**A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science
in Experimental Psychology with a concentration in Behavioral Neuroscience**

June, 2012

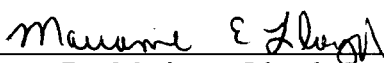
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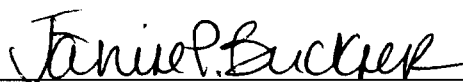
Dr. Amy Hunter, Faculty Mentor



Dr. Michael Vigorito, Committee Member



Dr. Marianne Lloyd, Committee Member



Dr. Janine Buckner, Director of Graduate Studies

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Abstract

Caffeine is the most commonly used psychostimulant. In addition to its widely known peripheral effects, caffeine is also an adenosine antagonist. Adenosine, a neuromodulator, is present in all areas of the brain, making caffeine's effects widespread. These effects differ based on variables such as dose, prior exposure, and timing of administration. The goal of the present study was to examine the effects of acute and chronic caffeine on spatial learning.

A radial arm water maze task was used to assess the behavioral effects of caffeine on caffeine-acclimated and caffeine-naive rats. After an initial caffeine pretreatment (caffeine administration for four weeks), half the rats were given caffeine injections during the learning task. This resulted in four groups: caffeine administration during the pretreatment and during the learning task (caffeine/caffeine), caffeine during the pretreatment and saline during the training task (caffeine/saline), saline during the pretreatment and caffeine during the training task (saline/caffeine), and saline during the pretreatment and during the training task (saline/saline). The differences in latency to reach the platform, reference, and working memory errors were observed between all groups.

The results of the pilot study and the main experiment are consistent with each other, showing that rats given chronic (pretreatment) caffeine make significantly more memory errors than rats given acute caffeine only. These results imply that while acute caffeine may not cause any impairment in learning, chronic caffeine impairs memory over time.

The Effects of Caffeine on Spatial Learning and Memory

As many as 87 percent of people in the United States consume caffeinated products on a daily basis (Myers & Izbicki, 2006). Caffeine, a psychostimulant, is present in coffee, tea, energy drinks, and other food products, such as candy. Caffeine causes increased heart rate, increased respiration rate, lessens fatigue, and causes a disruption of sleep through its actions on the peripheral nervous system. The cognitive effects of caffeine are biphasic and highly dependent on dose, prior exposure, and cognitive and physical state at the time of consumption. In humans, the subjective effects include enhanced mood, increased alertness, and reduced fatigue. Caffeine's effects change dramatically as dose increases and there is still some argument as to whether positive subjective effects are present in all who consume caffeine or only in those who are chronic users (Myers & Izbicki, 2006). This implies that part of caffeine's perceived beneficial effects may come in the lessening of withdrawal symptoms.

Because caffeine can produce tolerance and dependence and has some undesirable withdrawal effects (e.g. headache, fatigue, lethargy, and impaired concentration), use of the drug is commonly maintained because of the lessening of these withdrawal symptoms (Meyer & Quenzer, 2005). While this explains the results of some human studies, animal studies in which there was no prior exposure show that low to moderate doses of caffeine can have beneficial effects on some measures of learning.

Caffeine's Effects on Learning and Memory

Caffeine is a nonselective adenosine (A) antagonist with its primary function in the brain at A₁ and A_{2A} receptors (Rahman, 2009). Adenosine can function as a neurotransmitter and is

present in every cell in the brain (Ribeiro & Sebastiao, 2010). The expression of adenosine receptors suggests that caffeine can potentially affect all areas of the brain. Evidence that caffeine has an effect on learning comes from the fact that adenosine has been shown to produce an inhibitory effect on long-term potentiation (LTP; a strengthening of synapses which facilitates learning) in hippocampal rat slices (De Mendoca & Ribeiro, 1994; Ribeiro & Sebastiao, 2010). In addition, adenosine, through activation of A₁ receptors, interferes with synaptic plasticity in the hippocampus (Alhaider, Aleisa, Tran, Alzoubi, & Alkhadi, 2010). Inactivation of A₁ and A_{2A} receptors has been shown to counteract cognitive deficits related to age (Rahman, 2009). This suggests that caffeine could enhance learning through its antagonism of adenosine, particularly hippocampus-dependent learning where A₁ receptors are densely distributed (Yu, Gupta, Chen, & Yin, 2009).

Despite neurobiological evidence that suggests a mechanism for learning enhancement through adenosine antagonists, the published literature on caffeine shows many inconsistencies. A variety of factors seem to influence whether caffeine enhances or impairs learning. Some of these factors include duration of administration, dose of the drug, and timing of administration relative to the learning task.

One common design is to administer caffeine acutely, with a single administration. Acute caffeine studies show that there is some learning enhancement present depending on timing of administration. In a study done by Angelucci and colleagues (1999), mice were subjected to an inhibitory avoidance task and administered caffeine or saline in doses of 1, 3, 10, 30, or 100 mg/kg, ip, under one of the following schedules: 30 minutes before training, immediately after training, 30 minutes before the test, or both 30 minutes before training and 30 minutes before the test. The inhibitory avoidance task training consisted of two compartments -

an illuminated one and a dark one. Rats were placed in the illuminated compartment and allowed 30 seconds to enter the dark compartment. Once in the dark compartment, a footshock was delivered through the compartment floor and the rat was placed back in the home cage. The test session was similar, but the rats were allowed 600 seconds to enter the dark compartment. The latency to enter the dark compartment during the test session was measured to assess retention. Animals that successfully learned to avoid the dark compartment had higher latency scores, which showed improved retention of the task.

The results of Angelucci et al. (1999) showed that under certain conditions, low doses of caffeine enhanced memory. Specifically, retention was found to be improved when caffeine was administered at doses of 1, 3, 10, or 30 mg/kg and when given immediately after training. Memory retrieval was improved when caffeine was administered at doses of 3 or 10 mg/kg 30 minutes before the test session. However, memory was impaired at doses of 10, 30, or 100 mg/kg when given before training and at 3, 10, 30, or 100 mg/kg when given 30 minutes before training and 30 minutes before testing. Caffeine appeared to impair acquisition, assessed by ambulatory behavior during training, at doses higher than 10 mg/kg. Also, despite pre-training caffeine still being present in the body after training and possibly helping consolidation of new information, it showed no enhancement of memory. This suggests that caffeine can enhance memory, but only when given after training or before tests of retrieval.

In 2002, another study on the acute effects of caffeine was conducted by Angelucci, Cesario, Hiroi, Rosalen, and Da Cunha. In this study, rats were administered caffeine at doses of 0.3, 1, 3, 10, and 30 mg/kg (ip) 30 minutes before training, immediately after training, or 30 minutes before the test session. The learning/behavioral task used was the Morris water maze. Learning was assessed by recording the latency to reach the escape platform. The data they

acquired showed that caffeine administered immediately after training improved retention at the test session at doses of 0.3, 1, 3, and 10 mg/kg. The higher dose of 30 mg/kg had no effect on learning. However, an enhancing effect of caffeine was found only when administered immediately after training, despite the fact that caffeine administered before training would still be present in the body after training due to its half-life of 60-70 minutes in rats and mice (Bonati, Latini, Tognoni, Young, Garatini, 1984). These results suggest that not only does caffeine have differing effects dependent on dose, but it also affects stages of memory differently. Retention seemed to be the most affected stage of memory. It can also be concluded from the study done by Angelucci et al. (2002) that caffeine may impair retention when administered pre-training. A study conducted on mice by Sansone, Battaglia, and Castellano (1994), also suggests that acquisition is not affected by low doses of caffeine. This study included an avoidance task in which mice were placed in a shuttle-box with two compartments. A light would be turned on in one of the compartments, followed by a shock. Mice were then removed from the apparatus. The training for this task consisted of five daily 100-trial sessions. Acute caffeine (2.5, 5, or 10 mg/kg), nicotine (.25 or .5 mg/kg), or a combination of both was administered to each subject 15 minutes before each avoidance task. The results show that no effect of caffeine was found at doses of 2.5 and 5 mg/kg. However, there was a reduced avoidance response in subjects that were given caffeine at 10 mg/kg. This suggests that mice given this higher dose of caffeine failed to learn to avoid or escape from the shocked compartment.

Another stage of memory shown to be affected by caffeine is retrieval. Acute caffeine administration has been shown to improve memory recall in adult mice in an object recognition task, but only when tested right after administration (Costa, Botton, Mioranza, Souza, & Porciuncula, 2008). In this study, mice were given an acute dose of caffeine (10 mg/kg) for four

consecutive days. On the fifth day, training took place, which consisted of a 10 minute session in which mice were presented with two identical objects. Testing occurred either 15 minutes, 90 minutes, or 24 hours after training. During testing, mice were presented with a novel object and a familiar one and recognition of the familiar object was measured to assess memory. Caffeine treated mice recognized familiar objects more efficiently when tested 15 minutes after training. The mice that were tested 90 minutes or 24 hours after training showed no significant differences in recognition when compared to controls. This suggests that acute caffeine administration may enhance short-term retention.

Further evidence of caffeine improving memory recall was found in a study done by Valzelli, Baiguerra, and Giraud (1986). This study consisted of mice being exposed to a shuttle-box avoidance task. In one compartment of the shuttle-box, a light and buzzer would be presented together 5 seconds before a shock was delivered to the cage floor. If mice escaped to the other compartment of the shuttle-box before the shock was delivered, an avoidance response was recorded. After 30 consecutive trials, mice that reached at least 50% of correct-avoidance responses were identified as good learners, and the others were marked as poor learners. After this initial training task, mice were given caffeine (10 mg/kg) and exposed to the same avoidance task 1 hour later. The results showed that caffeine improved recall of the poor learners, but did not affect the performance of the good learners.

While acute caffeine administration may help short term memory recall, the effects of chronic caffeine administration differ. In a study done by Abreu, Silva-Oliveira, Moraes, Pereira, and Moraes-Santos (2011), rats were placed on distinct diets which included different concentrations of either coffee or caffeine. The amount of caffeine consumed daily was approximately 20 mg/kg or 40 mg/kg which was consumed directly or through coffee intake (a

special chow mix was prepared). Rats were placed on these diets starting at post-natal day 21 and continued on them through testing, which began on post-natal day 90. The tasks were an open field test and an object recognition task. In the object recognition task, rats were presented with two identical objects for five minutes (sample phase). Two memory tests were done in which rats were presented with a familiar object and a novel one. One test was done 90 minutes after the sample phase to observe short-term memory and the other was done 24 hours after the sample phase to observe long-term memory. No differences between both caffeine-fed groups and the control group were detected for the sample phase or the short-term memory test. In contrast, in the long-term memory test, caffeine-fed rats performed better than the control group. These results suggest that chronic caffeine consumption may enhance long-term memory. However, not all studies confirm this effect (Han et al., 2007).

Han and colleagues (2007) did a study on the effect of long-term (4 weeks) consumption of a low dose of caffeine (0.3g/L) in drinking water in the Morris water maze (MWM). The learning tasks that Han and colleagues used were the spatial and cue versions of the Morris water maze. While caffeine significantly impaired learning in the spatial version of the MWM, there was no significant difference between the caffeine-fed and control rats in the cue version of the MWM. The spatial version of the MWM is dependent on the hippocampus and the cue version is dependent on the striatum, which may explain the differences in the performances of both groups. The researchers found that long-term consumption of low dose caffeine slowed hippocampus-dependent learning in the Morris water maze task. This study suggests that while caffeine can impair hippocampus-dependent learning, there are no effects of caffeine on striatum-dependent learning.

Although Han and colleagues (2007) showed that chronic caffeine can impair spatial learning, a study done by Alhaider, Aleisa, Tran, Alzoubi, and Alkadhi (2010) showed different results. In this study, rats were given caffeine (0.3 g/L) in drinking water for four weeks. The rats were acutely deprived of sleep for 24 consecutive hours and were later tested for spatial learning and memory in the radial arm water maze (RAWM), a combination of the Morris water maze and the radial arm maze. The results of the test session show that rats treated with caffeine did not differ from controls in the RAWM, but caffeine did prevent the sleep-deprived rats from making as many errors as the rats that were sleep-deprived and not treated with caffeine. The results also show that the rats who had been sleep-deprived and treated with caffeine learned at a rate equivalent to the control group (which was not sleep-deprived nor administered caffeine), whereas rats that had been sleep-deprived but not treated with caffeine performed significantly worse in the later training trials.

These studies (summarized in Table 1), despite their somewhat contradictory findings, agree on several things: first, caffeine seems to improve memory; second, there is a clear dose response curve, with very low doses having little to no effect and very high doses having adverse effects; and third, schedule of administration is just as important as dose, with administration immediately after the task serving as the most beneficial to learning while other schedules of administration may cause an impairment or have no effect at all. It can also be seen that even if caffeine can enhance learning, it cannot be generalized to all learning situations, as caffeine can be helpful to some learning tasks and a hindrance in others.

Table 1

Summary of Relevant Caffeine-Related Behavioral Research

Authors	Species	Schedule of Administration	Type of Administration	Experimental Task	Results
Angelucci et al. (2002)	Male Wistar Rats	Caffeine at doses of 0.3, 3, 10, 30 mg/kg i.p.--administered 30 min before training, immediately after training, or 30 min before testing.	Acute	Spatial (Morris water maze)	Caffeine at low-moderate doses improved memory retention and retrieval.
Angelucci et al. (1999)	Male Albino Swiss mice	Caffeine at doses of 1, 3, 10, 30 or 100 mg/kg i.p. administered 30 min before training, immediately after training, 30 min before test, or 30 min before testing and 30 min	Acute	Non-Spatial (Inhibitory avoidance task and habituation)	Caffeine at high doses impaired acquisition. Memory consolidation and retrieval was improved at low-moderate doses.
Sansone et al. (1994)	Male CD1 mice	Caffeine at dose of 2.5, 5, or 10 mg/kg was injected intraperitoneally.	Acute	Non-Spatial (Shuttle box avoidance learning)	Caffeine impaired acquisition at the dose of 10 mg/kg.
Valzelli et al. (1986)	Male Albino Swiss CD1 mice	Caffeine at dose of 10mg/kg given orally 1h before each avoidance session of the 5-day retention trial.	Acute	Non-Spatial (Avoidance task)	Caffeine improved memory recall of PL mice.
Costa et al. (2008)	CF1 Albino mice	Caffeine at dose of 10mg/kg i.p. for 4 consecutive days; last dose was 45-60 min before habituation session.	Acute	Non-Spatial (Object recognition)	Caffeine improved recognition memory.
Han et al. (2007)	Male Sprague-Dawley Rats	Caffeine at dose of 0.3 g/L in drinking water for 4 weeks.	Chronic	Spatial (Morris water maze) & Non-Spatial (cue version of the Morris water maze)	Caffeine impaired spatial learning.
Alhaider et al. (2009)	Male Wistar rats	Caffeine 0.3 g/L was administered in drinking water for 4 weeks.	Chronic	Spatial (Radial arm water maze)	Chronic caffeine treatment prevented impairment of hippocampus-dependent learning and short-term memory.
Abreu et al. (2011)	Male Wistar rats	Diets consisted of 3% coffee, 6% coffee, .04 % caffeine, or .08% caffeine from post-natal (PN) 21 through testing which began at PN90.	Chronic	Non-Spatial (Open field & object recognition)	Chronic caffeine administration enhanced long-term memory retrieval.

Radial Arm Water Maze

The radial arm water maze (RAWM) is a combination of the radial arm maze and the Morris water maze (MWM). In 1985, Buresova, Bures, Oitzl, and Zahalka combined the Morris water maze and the radial arm maze in an attempt to control for confounding variables (e.g odor left by previous rats that could unintentionally guide others into the correct arm) found in the radial arm maze. This first radial arm water maze, however, proved to be too complicated. Because of this Hyde, Hoplight, and Denenberg (1998) redesigned the RAWM in to a simpler, more effective apparatus. The RAWM used by Hyde and colleagues consisted of eight arms, but the number of arms can vary to make the task more complex. There are multiple goal arms, each with an escape platform at the end of the arm. The start location varies by day or trial. The goal is for the animal to learn the locations of the escape platforms based on intra- or extramaze cues. The RAWM allows for assessment of reference memory by determining the number of repeated entries into an arm throughout a session and the assessment of working memory by determining the number of repeated entries into an arm throughout a single trial.

Working memory is regulated by the hippocampus, specifically the ventral hippocampus, although studies have shown that the cholinergic pathway from the medial septal area of the basal forebrain to the hippocampus is also important (Givens & Olton, 1994; Seamans, Floresco, & Phillips, 1998). Reference memory, however, is thought to be regulated by the nucleus basalis magnocellularis of the basal forebrain and its cholinergic pathways to the neocortex, as has been shown by lesion studies (Givens & Olton, 1994). The RAWM's design allows for a test of these areas simultaneously, allowing for a broader view of brain functionality.

Combining these two experimental procedures allows for a test of both reference and working memory simultaneously, without having to deprive the animal of food (Hyde et al., 1998) as in the standard radial arm maze. While the water is an aversive stimulus for the rats, it is also an ideal motivator to find a means of escape from the maze.

Rationale

While there have been many studies done to observe the effects of chronic or acute caffeine administration, none have been done that examines both in the same subject. Because of caffeine's ability to cause tolerance and a dependence on the drug, the effect of caffeine on a chronic user would likely be different than the effect on a non-user. Present published studies all greatly differ in the dosage of the drug and the learning task used to examine performance. The inconsistencies present in the current literature may be attributed to the large range in doses that have been used in studies thus far. This study hopes to bridge the gap by using the same dose for both chronic and acute caffeine administration.

The purpose of this study is first to examine whether long-term caffeine impairs or enhances learning and memory of the radial arm water maze; and second, to examine whether the effect on learning differs when small doses of caffeine are given to subjects that have already been exposed to the drug as opposed to when small doses are given to subjects that are naive to caffeine.

Pilot Study

Materials and Methods

Animals. Sixteen adult male Sprague-Dawley rats weighing 450-600 g were housed in a temperature controlled room in the Behavioral Neuroscience Laboratory. Rats were kept on a 12/12 hr light/dark cycle with lights on at 07:00 a.m. Food and water was provided ad libitum. Fourteen of the 16 rats were housed in pairs while the two remaining were housed individually. In a previous, unrelated study, nine of the rats used were housed in an enriched environment. In addition, all 16 rats previously experienced a morphine conditioned place preference, as well as shock-cue drug reinstatement and drug-cue reinstatement.

Radial Arm Water Maze. The radial arm water maze (Figure 1) consisted of a black plastic pool 55 inches in diameter and 23 in. in depth with the water 8 in. deep and 70-74 °F. Six open plexiglass arms extended from a central area of 17 in. in diameter. Each arm was 15 in. long and 6 in. wide, allowing enough room for the rats to turn around easily within them. Two of the arms were designated as goal arms and had escape platforms at the end. The escape platform was submerged approximately 2 cm below the surface of the water. At the start of each arm surrounding the center area, packing peanuts were placed on the surface of the water to obscure the rats' view into the arms and the location of the platforms.

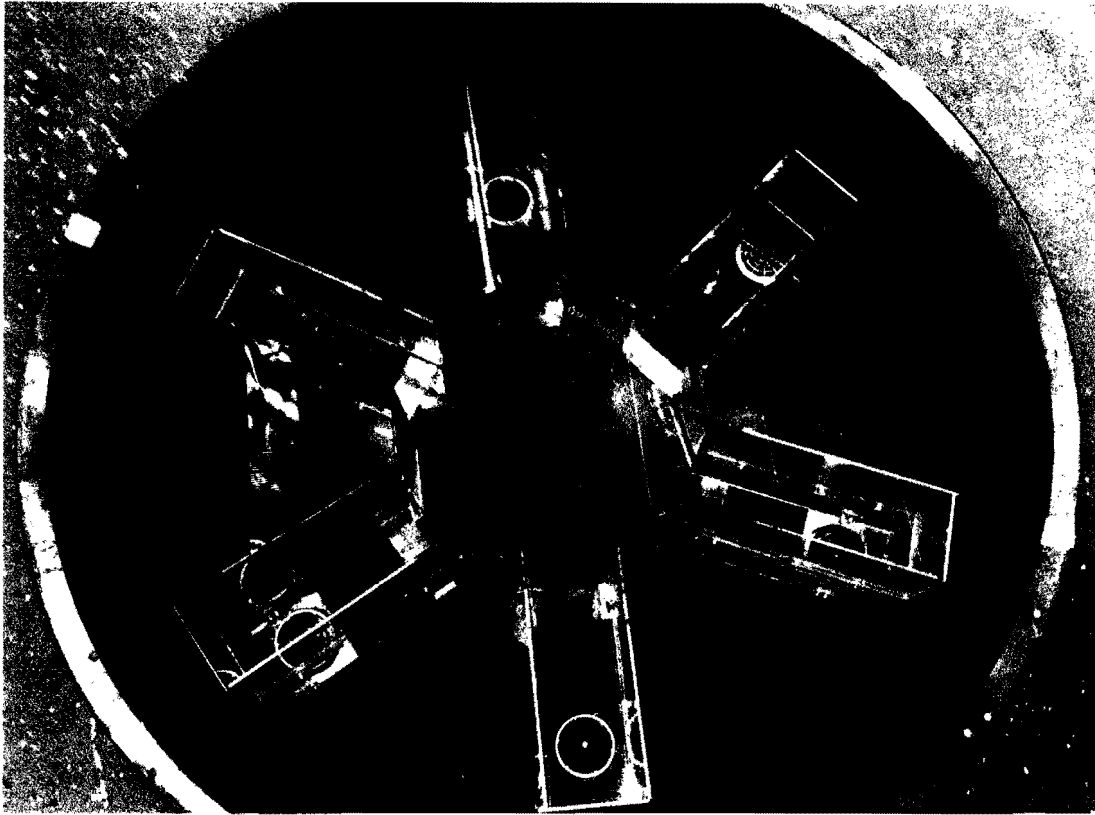


Figure 1. Radial arm water maze.

Drug Pre-Exposure. Rats were divided into two groups, a caffeine-exposure group and a caffeine-naive group. The caffeine exposure group received daily caffeine injections for 9 non-consecutive days over a 13 day period. The dose of the caffeine was 3 mg/kg and was dissolved in 0.9% saline. This dose was chosen based on existing literature and is the equivalent of low to moderate caffeine intake (Angelucci et al., 2002). The caffeine-naive group received saline injections in an equal volume and on the same days to serve as a control. There was a three week delay between drug pre-exposure and behavioral testing (Table 2). A delay was necessary between drug pre-exposure and the learning task to allow the drug to completely exit the rats' systems.

Behavioral Procedures. For the learning task, the rats were divided into four squads, each containing two rats that had been previously exposed to caffeine and two rats that had been exposed to saline. Each squad was taken into the RAWM room, placed in holding cages and run in the RAWM task. There were four spaced trials per rat, therefore, all four rats in a squad would undergo Trial 1 before Trial 2 would begin. The spaced trials were done to ensure that exhaustion would not be a confounding variable. The start locations remained the same throughout the day, but were changed for each subsequent day. This was done to ensure that the rats would learn the location of the goal platform based on extra-maze spatial cues present around the room and not motor responses. Each rat was permitted a maximum of 120 seconds to locate the platform. An entry into an arm was counted only when all four paws were inside the arm. Once a rat reached the platform, it was allowed to remain on it for 10 seconds before being placed back into the holding cage until the next trial. Each rat was scored for latency to locate the platform, reference memory errors, and working memory errors, although no platforms were removed. A reference memory error was any entry to into an arm without an escape platform. A working memory error was any entry into an already visited arm within a trial.

Prior to the training phase, rats were randomly assigned to the caffeine or saline groups. The training period lasted for 6 consecutive days with four trials per day. Once the day's trials were done for a squad, all four rats were given the training injection. Two groups received caffeine dissolved in 0.9% saline at a dose of 3mg/kg and the other two groups received saline in an equal volume.

Following the training period, rats were given three retention tests which took place on days 7, 8, and 15. These tests were done in the same manner as the training days, but no

injections were given. These tests were done to assess long-term retention and to see observe any behavioral differences in the maze when subjects are not being administered caffeine.

PRETREATMENT		3 D E A W E E K	BEHAVIORAL TASK	
Drug Administration			Training	Retention
9 days over a 13 day period			Days 1-6	Days 7, 8, & 15
Group 1	Caffeine		Caffeine	No Drug
Group 2	Saline		Saline	
Group 3	Caffeine		Saline	
Group 4	Saline		Caffeine	

Figure 2. The pilot study experimental design.

Results

Data Analysis

A repeated measures analysis of variance (ANOVA) was conducted to investigate the differences between the drug groups for their training and retention performance. Pretreatment drug, training drug, and housing were each counted as separate independent variables. For the training phase, a 2 (pretreatment drug: caffeine or saline) X 2 (training drug: caffeine or saline) X 2 (housing: enriched or standard) X 6 (training days) analysis was conducted. For each retention test, a 2 (pretreatment drug: caffeine or saline) X 2 (training drug: caffeine or saline) X 2 (housing: enriched or standard) X 4 (trials) analysis was done.

Latency to Locate the Platform

Training. There was a significant main effect of days during the training phase [$F(5,40)=10.58, p<.001, \text{partial } \eta^2=.57$], with most of the learning occurring on days 1 and 2 (Figure 3). There was an interaction of pretreatment drug and training drug [$F(1,8)=19.70, p<.01, \text{partial } \eta^2=.71$], showing that caffeine seemed to improve latency to reach the platform the

most when it was administered during one phase only, rather than for both phases. There was also an interaction of training drug and days of training [$F(5, 40)=5.44, p<.001, \text{partial } \eta^2=.41$] with rats that received caffeine during training performing better during the first 2 days of training, while the rats that received saline during training performing better towards the end of training. There was also interactions of days, housing, and pretreatment drug [$F(5,40)=2.60, p<.05, \text{partial } \eta^2=.25$], days, housing, and training drug [$F(5,40)=2.63, p<.05, \text{partial } \eta^2=.25$], housing and training drug [$F(1,8)=6.66, p<.05, \text{partial } \eta^2=.45$], showing that both pretreatment and training caffeine enhanced the performance of the enriched-housed rats and impaired the performance of the standard-housed rats during the first few days of acquisition. Lastly, there was an interaction of days, housing, pretreatment drug, and training drug [$F(5,40)=3.36, p<.05, \text{partial } \eta^2=.30$] in which it appears that training caffeine enhanced the performance of enriched-housed rats more so than pretreatment caffeine or both together and ameliorated the impairment caused by pretreatment caffeine in standard-housed rats.

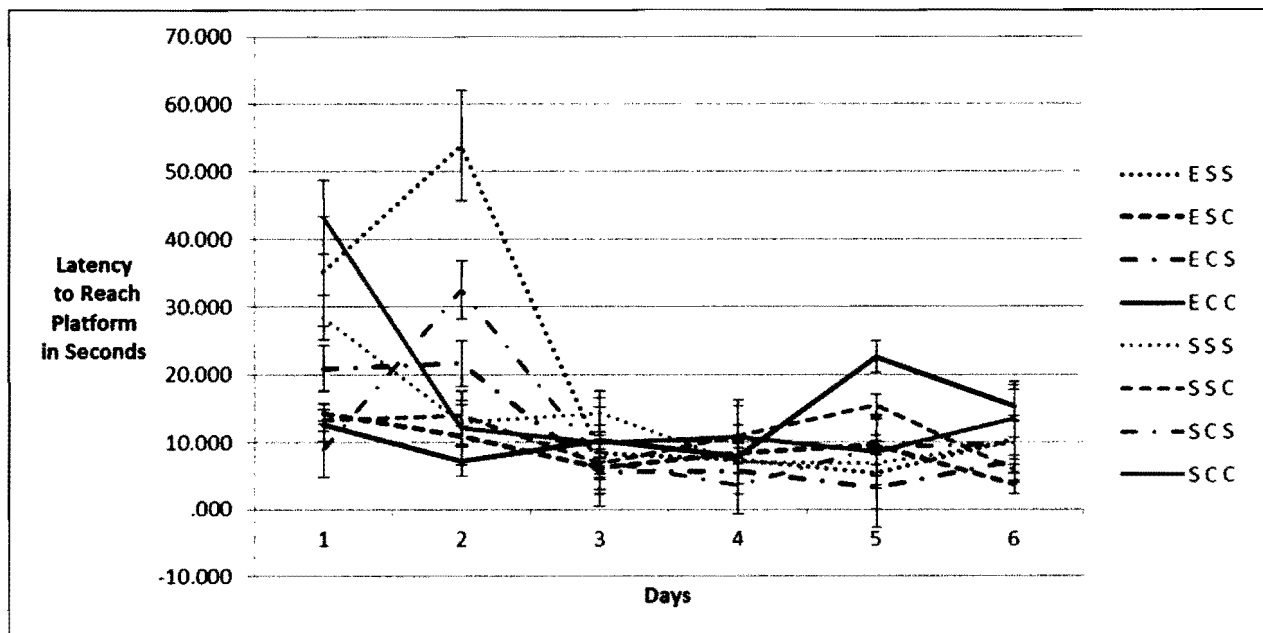


Figure 3. Latency to reach the platform during training. Abbreviations on key stand for housing (enriched or standard), pretreatment drug (saline or caffeine), and training drug (saline or caffeine).

Long-term retention test one. The results of the first long-term retention test show no significant main effects or interactions (Figure 4).

Long-term retention test two. For the second long-term retention test, there was a main effect of training drug [$F(1,8)=5.86$, $p<.05$, partial $\eta^2=.42$], showing that the rats that received saline during training performed better than the rats that received caffeine during training. There was an interaction of trials and pretreatment drug [$F(3,24)=7.90$, $p<.001$, partial $\eta^2=.50$], which shows that rats that were given caffeine for the pretreatment phase did well in later trials. There was also an interaction of trials and housing [$F(3,24)=4.59$, $p<.05$, partial $\eta^2=.37$], showing that enriched-housed rats performed very poorly on trial 3, while standard-housed rats performed very well, but the opposite was observed on trial 4, as was observed by comparing means for both trials (Figure 4).

Long-term retention test three. No significant main effects or interactions were found for the third long-term retention (Figure 4).

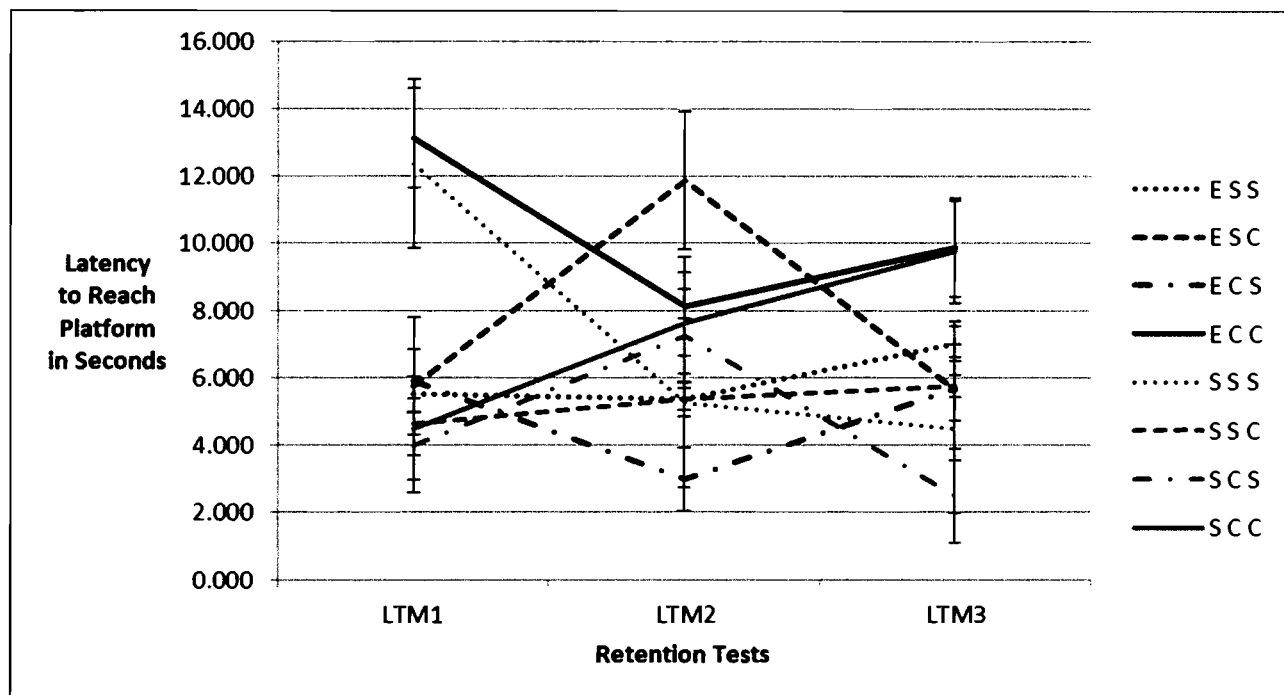


Figure 4. Latency to reach the platform during retention tests. Abbreviations on key stand for housing (enriched or standard), pretreatment drug (saline or caffeine), and training drug (saline or caffeine).

Reference Memory

Training. There was a main effect of pretreatment drug [$F(1,8)=6.37, p<.05, \text{partial } \eta^2=.44$], in which the rats that were given saline during the pretreatment phase had less errors overall during the training phase (Figure 5). There was an interaction of days and training drug [$F(5,40)=5.15, p<.01, \text{partial } \eta^2=.39$], showing that rats that received caffeine after the learning task did better at the beginning of the training phase, but progressively worsened in their performance as days passed. There was also an interaction of days, pretreatment drug, and training drug [$F(5,40)=3.39, p<.05, \text{partial } \eta^2=.30$], showing that generally, rats that received caffeine progressively worsened as days passed, but this effect was less pronounced in rats that received caffeine only during pretreatment or only during training. There was a main effect of housing [$F(1,8)=6.99, p<.05, \text{partial } \eta^2=.50$], showing that enriched-housed rats performed better

throughout the training task than standard-housed rats. There was an interaction of days, housing, and pretreatment drug [$F(5,40)=3.03$, $p<.05$, partial $\eta^2=.28$], in which caffeine appeared to enhance the performance of enriched-housed rats and impair the performance of standard-housed rats during the initial part of the training phase. Lastly, there was an interaction of days, housing, pretreatment drug, and training drug [$F(5,40)=3.26$, $p<.05$, partial $\eta^2=.29$], showing that enriched-housed rats that were given caffeine in both training phases made more errors as days passed, while standard-housed rats, despite initially doing worse with caffeine, improved towards the end of the training phase.

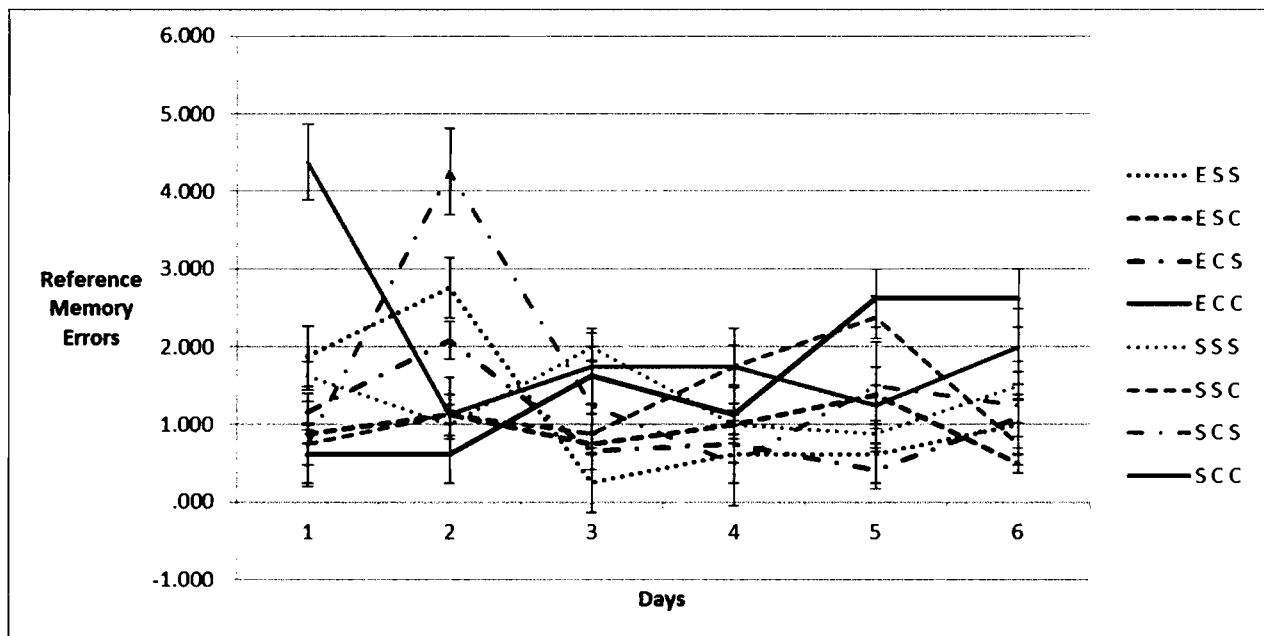


Figure 5. Reference memory errors made during training. Abbreviations on key stand for housing (enriched or standard), pretreatment drug (saline or caffeine), and training drug (saline or caffeine).

Long-term retention test one. For the long-term retention test, there were no main effects but there was an interaction of housing and pretreatment drug [$F(1,8)=6.04$, $p<.04$, partial

$\eta^2=.43$], in which enriched-housed rats made more errors when given caffeine, while the standard-housed rats that were given caffeine made fewer errors (Figure 6).

Long-term retention test two. There was an interaction of pretreatment drug and trials [$F(3,24)=6.30, p<.01, \text{partial } \eta^2=.44$], showing that rats that received caffeine performed poorly on trials 2 and 3, but the opposite was true on trial 4. There was also an interaction of housing and trials [$F(3,24)=5.14, p<.01, \text{partial } \eta^2=.39$], showing that standard-housed rats made more errors than enriched-housed rats on trial 3, but considerably less errors than enriched-housed rats on trial 4 (Figure 6).

Long-term retention test three. No significant main effects or interactions were found (Figure 6).

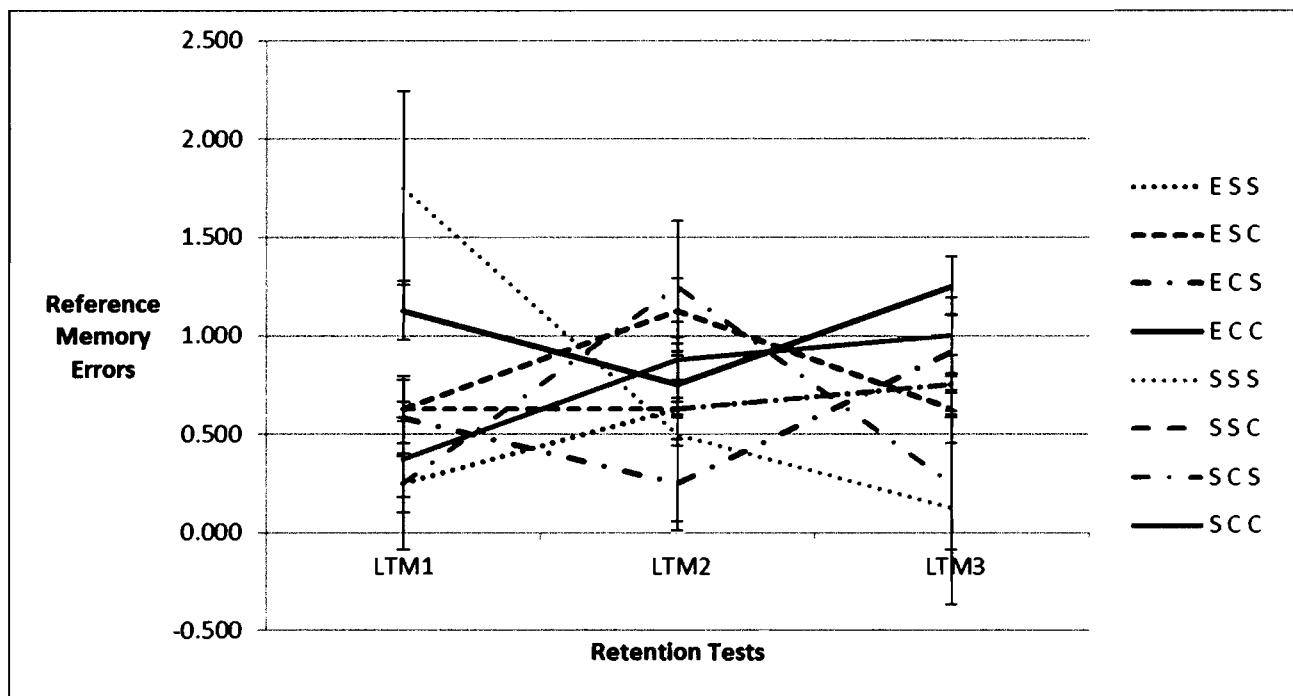


Figure 6. Reference memory errors during retention tests. Abbreviations on key stand for housing (enriched or standard), pretreatment drug (saline or caffeine), and training drug (saline or caffeine).

Working Memory

Training. No significant main effects were found for working memory (Figure 7). There was an interaction of days and training drug [$F(5, 40)=3.45, p<.05, \text{partial } \eta^2=.30$], showing that the rats that were given saline during training improved in their performance as days passed, while caffeine-treated rats made more errors toward the end of training. There was also an interaction of days, pretreatment drug, and training drug [$F(5, 40)=2.74, p<.05, \text{partial } \eta^2=.26$], showing that rats that were given caffeine in both phases did well on the first few days of training, but made more errors toward the end, while rats that received caffeine during the training phase initially did well in comparison to other groups, but made a great number of errors on day 5. Lastly, there was an interaction of days, housing, and pretreatment drug [$F(5, 40)=3.38, p<.05, \text{partial } \eta^2=.30$], with pretreatment caffeine appearing to impair standard-housed rats in the first few days of training, but not impair enriched-housed rats.

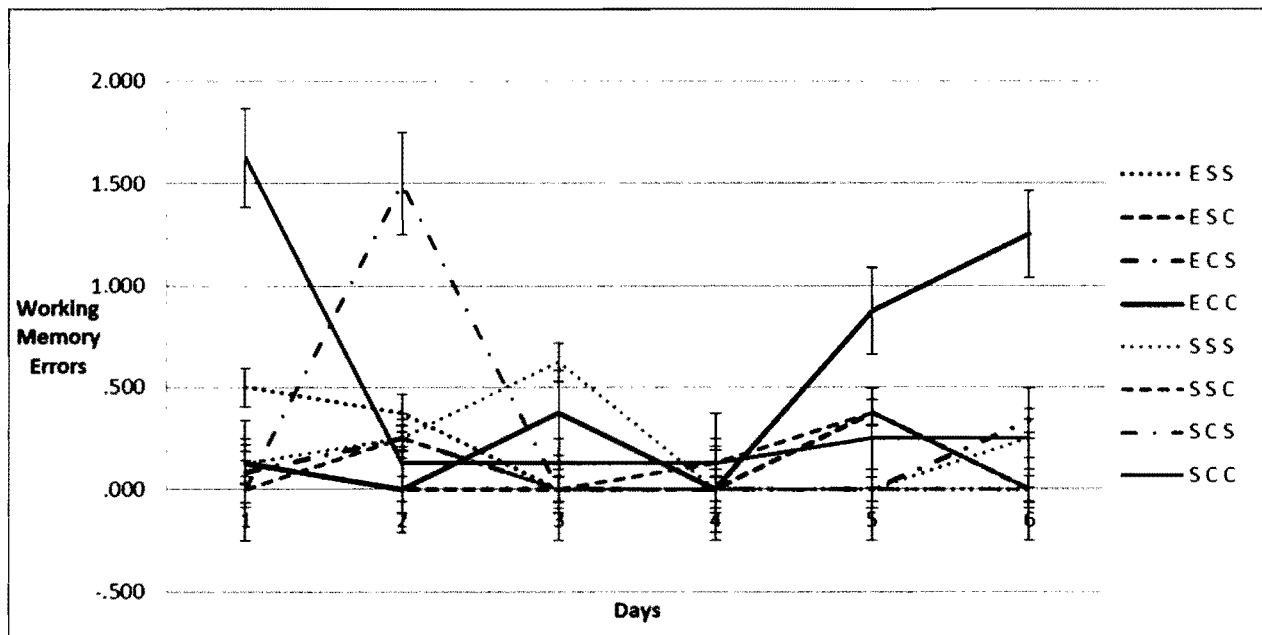


Figure 7. Working memory errors during training. Abbreviations on key stand for housing (enriched or standard), pretreatment drug (saline or caffeine), and training drug (saline or caffeine).

Long-term retention test one. No significant main effects or interactions were found (Figure 8).

Long-term retention test two. For the second long-term retention test, only two rats made errors and these two rats were in the same housing and drug group: enriched, saline (pretreatment drug), caffeine (training drug). This caused significant results in all main effects and interactions, which are described below.

There was significant main effects of trials [$F(3,24)=3.39$, $p<.05$, partial $\eta^2=.30$], housing [$F(1,8)=8.31$, $p<.05$, partial $\eta^2=.51$], with errors occurring only during trials 2 and 4. There was also a main effect of pretreatment drug [$F(1,8)=8.31$, $p<.05$, partial $\eta^2=.51$] and training drug [$F(1,8)=8.31$, $p<.05$, partial $\eta^2=.51$], in which pretreatment caffeine enhanced working memory, and training caffeine impaired it (Figure 8). There was also an interaction of pretreatment drug and training drug [$F(1,8)=8.31$, $p<.05$, partial $\eta^2=.51$], showing that pretreatment saline and training caffeine impaired memory. There was also an effect of pretreatment drug on trials [$F(3,24)=3.39$, $p<.05$, partial $\eta^2=.30$], with pretreatment saline impairing memory, and an effect of training drug on trials [$F(3,24)=3.39$, $p<.05$, partial $\eta^2=.30$], with training caffeine impairing memory. Also, there was an interaction of trials, pretreatment drug, and training drug [$F(3,24)=3.39$, $p<.05$, partial $\eta^2=.30$], showing that pretreatment saline and training caffeine impaired performance on trials 2 and 4.

With housing, there was an interaction of housing and trials [$F(3,24)=3.39$, $p<.05$, partial $\eta^2=.30$], showing that enriched-housed rats did worse. There was an interaction of housing and pretreatment drug [$F(1,8)=8.31$, $p<.05$, partial $\eta^2=.51$], with enriched-housed, saline pretreated rats performing the worst. There was also an interaction of housing and training drug [$F(1,8)=8.31$, $p<.05$, partial $\eta^2=.51$], showing that enriched-housed rats given caffeine during

training performed poorly. There was an interaction of housing, pretreatment drug, and training drug [F(1,8)=8.31, $p < .05$, partial $\eta^2 = .51$], showing that enriched-housed, saline pretreated rats given caffeine during training did poorly. Additionally, there was also an interaction of trials, housing, and pretreatment drug [F(3,24)=3.39, $p < .05$, partial $\eta^2 = .30$] and an interaction of trials, housing, and training drug [F(3,24)=3.39, $p < .05$, partial $\eta^2 = .30$], and an interaction of trials, housing, pretreatment drug, and training drug [F(3,24)=3.39, $p < .05$, partial $\eta^2 = .30$], with all three interactions showing an impairment of learning in enriched-housed rats given saline during pretreatment and caffeine during training performing very poorly on trials 2 and 4.

Long-term retention test three. No significant main effects or interactions were found (Figure 8).

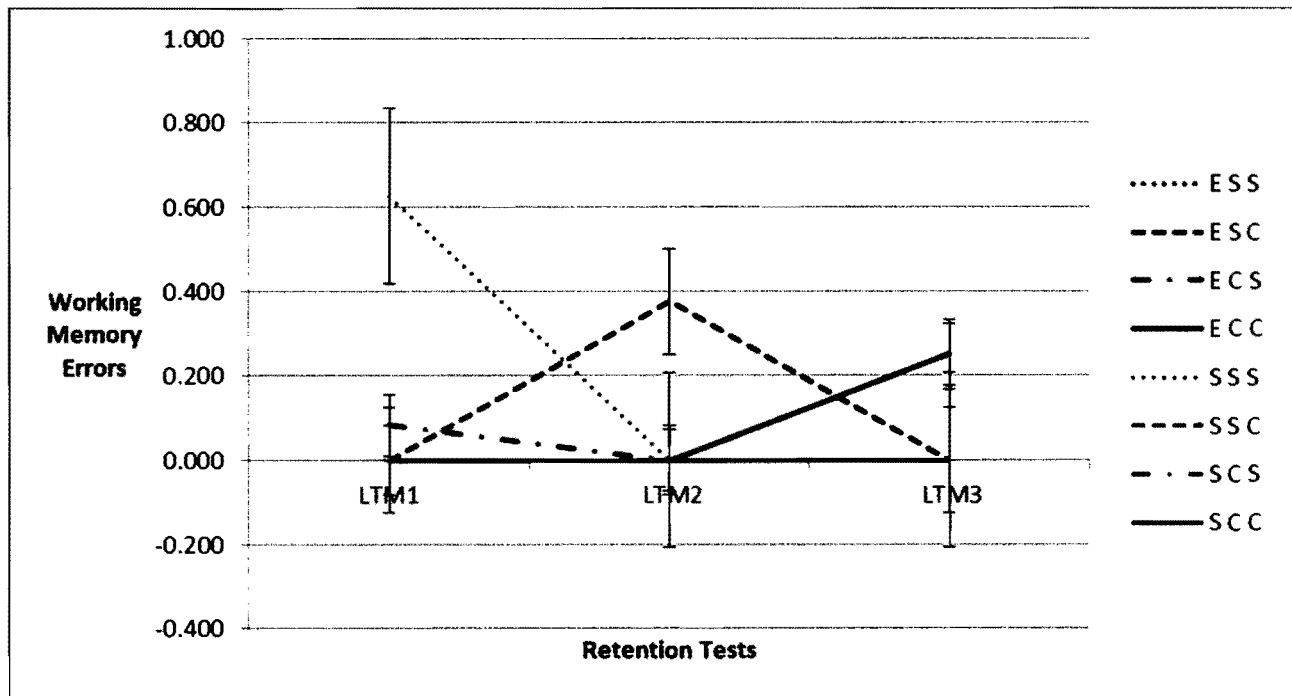


Figure 8. Working memory errors during retention tests. Abbreviations on key stand for housing (enriched or standard), pretreatment drug (saline or caffeine), and training drug (saline or caffeine).

The effects of caffeine on latency, reference memory, and working memory during as assessed by the pilot study behavioral task are summarized in Figure 9.

Timing of Caffeine	Training			Retention		
	<i>Latency to Escape</i>	<i>Reference Memory</i>	<i>Working Memory</i>	<i>Latency to Escape</i>	<i>Reference Memory</i>	<i>Working Memory</i>
Pretreatment	Enhanced	Impaired	Impaired	Impaired	Impaired	Mixed
Training	Mixed	Impaired	Impaired	Enhanced	No Effect	Mixed

Figure 9. Effects of caffeine in pilot study.

Discussion

The results of this study indicate that caffeine administered during training appeared to enhance spatial learning during the initial part of the training phase.

With respect to latency to locate the platform, caffeine appeared to facilitate performance for enriched-housed animals when administered either during pretreatment or during training, but not both. As far as reference memory, caffeine-treated rats did worse over time, but the impairment was less pronounced when the received caffeine only during training. In this case, it appears that for subjects that have been previously exposed to caffeine and were accustomed to its effects, more injections of caffeine did not enhance their learning. For working memory, caffeine-treated rats did well initially, but made progressively more errors as time went on. Rats that received caffeine during training only did well on all days except day 5. This further suggests that caffeine may be beneficial when administered during training.

No significant effects were observed for the first and third retention tests, however, during the second long-term retention test for latency, rats that received caffeine during training

did well in later trials, while rats that received caffeine during pretreatment did poorly. For long-term retention of reference memory, rats given caffeine during pretreatment did well, whereas, during training the opposite was true. For long-term retention of working memory, caffeine-treated rats did poorly. Despite the results of the retention tests being inconsistent across all three dependent variables it appears that caffeine during pretreatment may impair acquisition of new information.

Regarding housing, enriched-housed rats appeared to better initially, but not much difference was found in housing conditions toward the end of training phase. Retention tests showed that standard-housed rats did better in the first long-term retention test, while enriched-housed rats performed better during the second long-term retention test. Because rats were removed from enriched housing 4 months prior to RAWM task, the benefits from the enriched environment were minimal.

These data suggest that caffeine administration may initially enhance learning under some circumstances, but can impair learning over time. A possible reason for this could be that as rats become accustomed to caffeine's effects, more of the drug is required to keep them on par with controls. Another possibility is that caffeine is causing a sensitization of its motor effects in enriched rats, making them faster rather than enhancing the learning of the task. Swimming speed was not measured, but there is a possibility that caffeine may have been increasing rats' speed but not their accuracy. This may explain why the latency results are not consistent with the reference and working memory results.

Experiment

Overall, the results of the pilot study indicate that caffeine may have an effect on latency to reach the platform, reference memory, and working memory. However, due to the low number of rats used in the study, more research is necessary to determine caffeine's effects on learning and spatial memory. Additionally, the number of pretreatment days and the duration of the delay between the pretreatment and behavioral task may have been inadequate. A more suitable way of assessing working memory is also necessary to observe caffeine's effects on this type of memory. Since the goal platforms were not removed for the pilot study, rats were never properly trained to be assessed for working memory errors.

Materials and Methods

Animals. Thirty-two adult male Sprague Dawley rats weighing 250–400 g were housed in pairs of two in clear plexiglass cages in a temperature controlled room in the Behavioral Neuroscience Laboratory. Rats were kept on a 12/12 hr light/dark cycle with lights on at 08:30 a.m. Food and water were provided ad libitum. Rats were handled by the experimenter prior to beginning pretreatment, but were kept naïve to the radial arm water maze until the first day of the learning task. Due to attrition throughout the course of the study, 5 rats were dropped from the experiment and data analyses.

Radial Arm Water Maze. The radial arm water maze was the same as in the pilot study.

Drug Pretreatment. The drug pretreatment phase consisted of caffeine administration at a dose of 0.3 mg/kg, ip, for 14 days and 3 mg/kg, ip for the following 14 days. The smaller dose was due to experimenter error. There was a one week delay between the pretreatment phase and the training task. The longer pretreatment phase compared to the pilot experiment allows for a more chronic administration of the drug (Han et al., 2007) and the shorter delay is long enough that the drug will no longer be biologically active but increases the likelihood that rats will still be tolerant to its effects upon subsequent administration.

Behavioral Procedures. Rats were trained in the radial arm water maze for a total of 13 days. Water was kept at 70 °F and packing peanuts were placed at the entrance of each arm to obscure the rats' view into the arms. Rats were run in squads of no more than four per session with four spaced trials per session. After each squad completed its last trial, half of the rats were

given caffeine injections, ip, at the same dose that was used for the pretreatment and placed back in their home cage.

During the first phase of the training (8 days), there were two goal arms. Rats were given a total of 120 s to locate a platform. Once on the platform, they were removed and placed back into their holding cage to await the next trial. This first part of the training served to allow the rats to acclimate to the maze and learn the locations of the platforms.

The second phase of training lasted 5 days with only two trials per each session. During trial 1, each rat would be allowed 120 s to locate one platform. Once the first platform had been located, rats would be placed back in their holding cage to await the next trial. That first platform would then be removed, leaving only one platform remaining for the second trial. After each squad completed its last trial, half of the rats were given caffeine injections at the same dose that was used for the pretreatment. The last part of training allows for an accurate test of working memory, as the rats had to not only remember the locations of the platforms but also remember which goal arm had already been visited.

Two test sessions were done to test for long-term memory. Each session consisted of 4 trials. One session was done 48 hours after the last day of training (Test 1), and the second was done one week after the last day of training (Test 2). Figure 10 shows a schematic of the experimental design.

PRETREATMENT		8 D E L A Y Y	BEHAVIORAL TASK		
Drug Administration	Training		Training	Retention	
28 Consecutive Days	<i>PHASE 1</i>		<i>PHASE 2</i>		
	Days 1-8		Days 9-13	Days 15 & 20	
	4 Trials		2 Trials	2 Trials	
Caf/Caf	Caffeine	Caffeine		No Drug	
Sal/Sal	Saline	Saline			
Cal/Sal	Caffeine	Saline			
Sal/Caf	Saline	Caffeine			

Figure 10. Experimental design used for experiment.

Results

Data Analysis

A repeated measures analysis of variance was conducted to evaluate the differences between the groups. Pretreatment drug and training drug were analyzed as independent variables. For the first part of training (days 1-8), a 2 (pretreatment drug: caffeine or saline) X 2 (training drug: caffeine or saline) X 8 (days) X 4 (trials) analysis was done. For the second part of training (days 9-13), a 2 (pretreatment drug: caffeine or saline) X 2 (training drug: caffeine or saline) X 5 (days) X 2 (trials) analysis was done. For the retention phase (days 15 and 20), a 2 (pretreatment drug: caffeine or saline) X 2 (training drug: caffeine or saline) X 2 (days) X 2 (trials) analysis was done. Additional ANOVAs were conducted to observe any relevant interactions.

Latency to Locate the Platform

Training. During training, learning occurred as evidenced by the decreases in latency to reach the platform, both for phase 1 [main effect of days, $F(7,161)=19.23$, $p<.01$, partial $\eta^2=.46$] (Figure 11) and phase 2 [$F(4,92)=3.08$, $p<.05$, partial $\eta^2=.12$] of training (Figure 12). There was

also a main effect of trials for phase 1 [$F(3,69)=6.44$, $p<.01$, partial $\eta^2=.22$] (Figure 11) showing that latency to reach the platform decreased across trials within each day. An interaction of days and trials was observed for phase 1 of training [$F(21,483)=1.74$, $p<.05$, partial $\eta^2=.07$] (Figure 11)/ Additional analyses showed that this interaction was due to a significant effect of trials only on day 2 [$F(3,78)=6.04$, $p<.01$, partial $\eta^2=.19$], day 4 [$F(3,78)=4.21$, $p<.01$, partial $\eta^2=.14$], day 6 [$F(3,78)=3.21$, $p<.05$, partial $\eta^2=.11$], and day 8 [$F(3,78)=4.41$, $p<.01$, partial $\eta^2=.15$]. During phase 2, a main effect of trials was also observed [$F(1,23)=30.89$, $p<.01$, partial $\eta^2=.58$] (Figure 12) which showed that latency to reach the platform increased on trial 2. This is expected because during phase 2 of training, the second trial in each session contained only one goal arm, thus making the platform more difficult to locate.

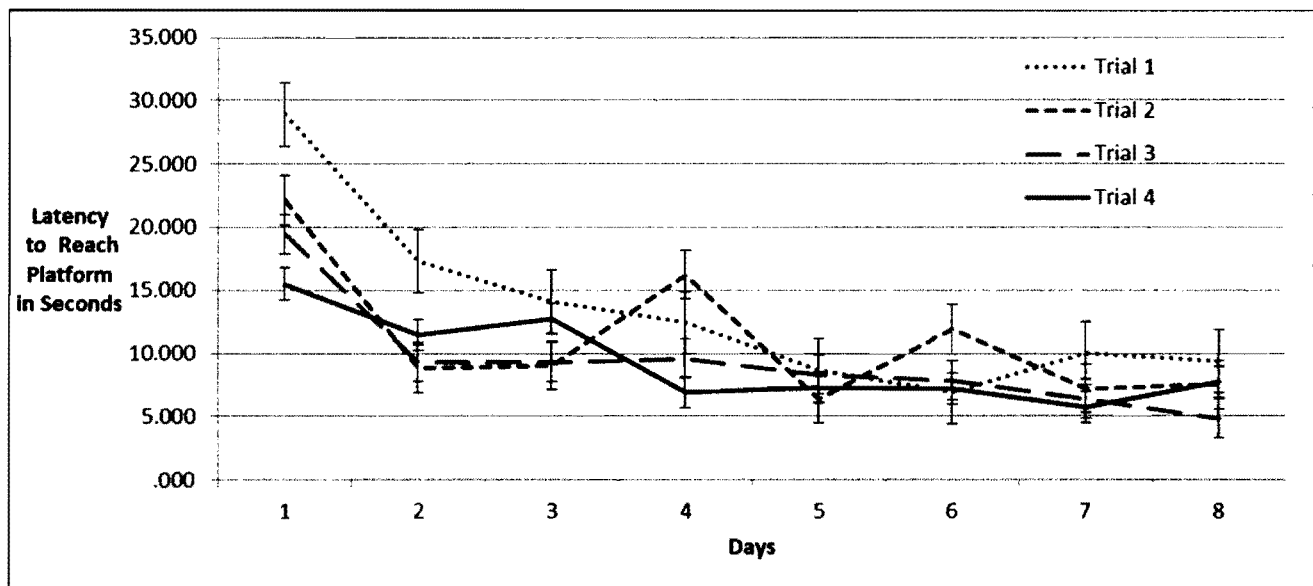


Figure 11. Latency to reach the platform across days during phase 1 of training.

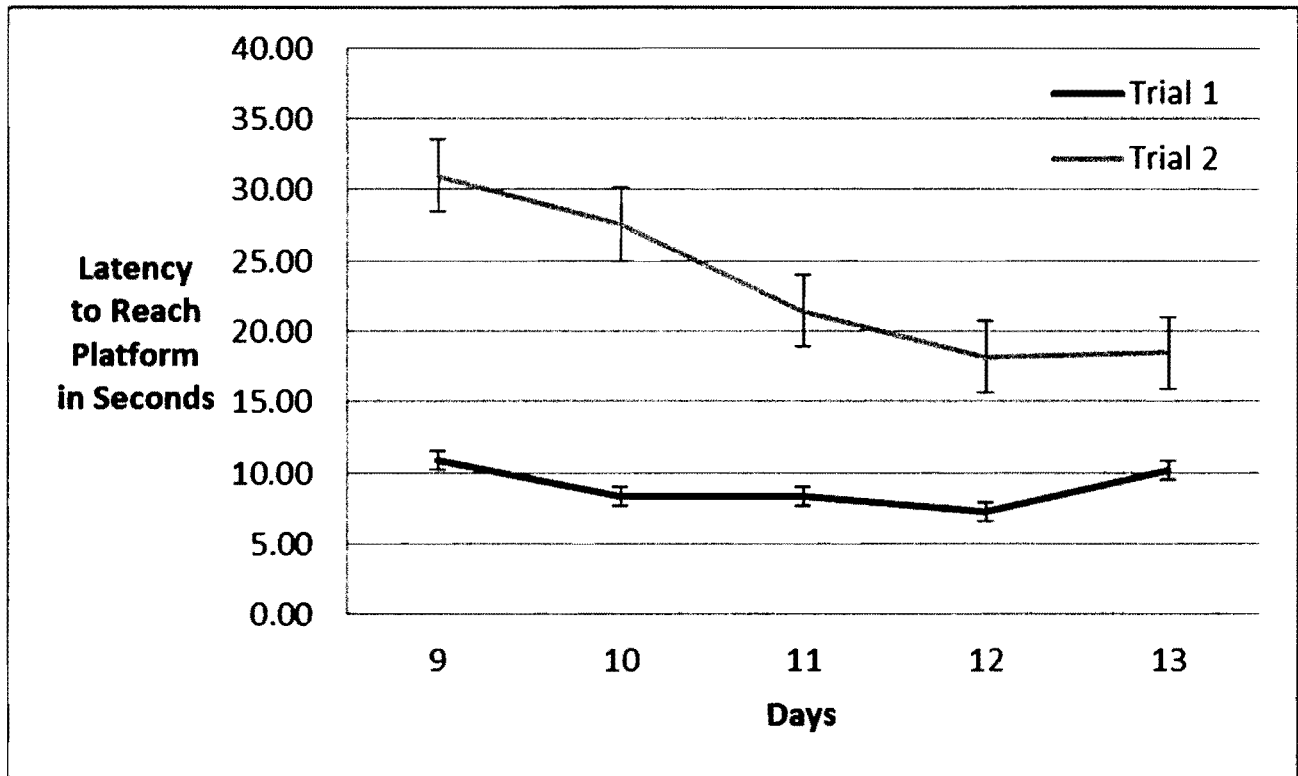


Figure 12. Latency to reach the platform across days during phase 2 of training.

No main effects or interactions of pretreatment or training drug were observed for latency during either phase of training ($p's > .05$)

Retention. A main effect of trials was observed during the retention phase [$F(1,23)=15.73, p<.001, \text{partial } \eta^2=.41$] showing that latency to locate the platform increased during trial 2 (Figure 13). Again, this is expected because of the increasing difficulty in locating the goal arm when only one platform is present. There was also an interaction of days, trials, pretreatment drug, and training drug [$F(1,23)=4.76, p<.05, \text{partial } \eta^2=.17$] (Figure 14). The interaction shows that during trial 2 on the second retention test, rats that were administered caffeine in both phases and rats that were administered saline in both phases performed significantly worse compared to the other groups and compared to their own performance on the

first retention test. An interaction of days, pretreatment drug, and training drug was also observed [$F(1, 23)=5.69, p<.05, \text{partial } \eta^2=.20$] showing that the performance of rats that received the same compound during pretreatment and training deteriorated from day 1 to day 2, while the performance of rats that received differing compounds during pretreatment and training improved from day 1 to day 2. Additional analyses showed that latency increased when rats had been given the same substance (either caffeine or saline) for both phases. This effect was significant on the second retention test [$F(1,23)=7.67, p<.05, \text{partial } \eta^2=.25$].

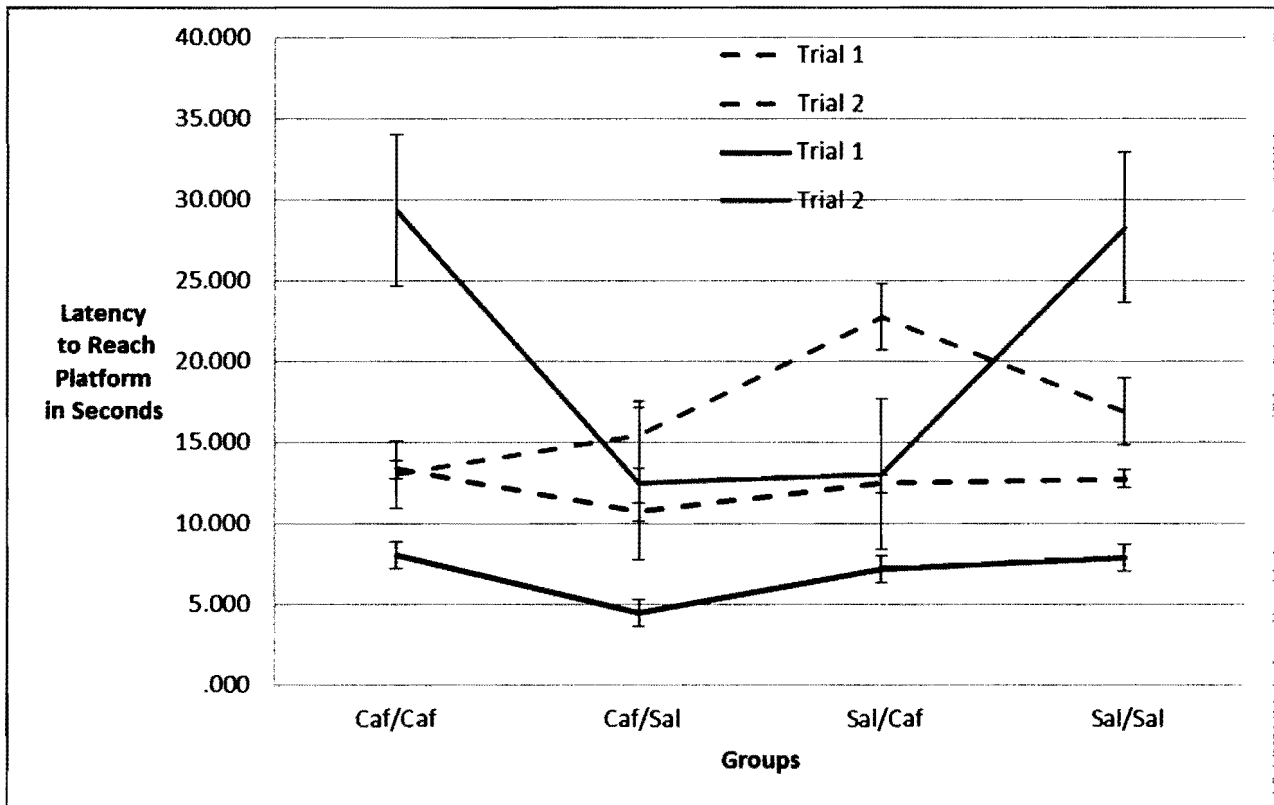


Figure 13. Latency to reach the platform (across groups) during retention tests. The dotted lines represent retention test 1 and the solid lines represent retention test 2.

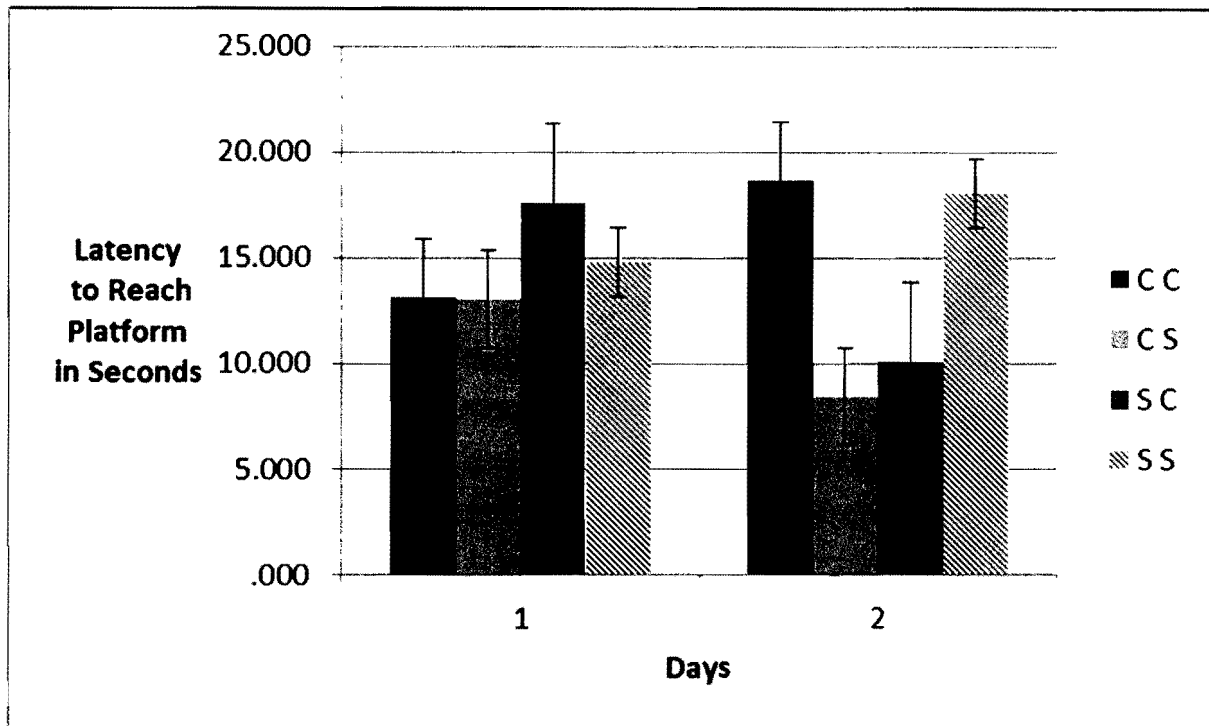


Figure 14. Latency to reach the platform across days for retention tests. Abbreviations on key stand for caffeine (C) or saline (S). The first letter indicates the drug received during pretreatment and the second letter indicates the drug received during training.

Reference Memory

Training. A decrease in reference memory errors across days during phase 1 [F(7,161)=9.45, $p < .01$, partial $\eta^2 = .29$] (Figure 15) and phase 2 [F(4,92)=7.59, $p < .01$, partial $\eta^2 = .25$] of training shows that learning occurred. There was also a main effect of trials during both phases. During phase 1, reference memory errors decreased across trials [F(3,69)=7.61, $p < .05$, partial $\eta^2 = .12$] (Figure 15). During phase 2, reference memory errors show a marked increase on trial 2 as compared to trial 1 [F(1,23)=39.14, $p < .01$, partial $\eta^2 = .63$] (Figure 16), which is expected due to the increased difficulty of the second trial. There was also an interaction of days and trials during phase 1 [F(21,483)=2.93, $p < .01$, partial $\eta^2 = .11$], reflecting

greater variability during trial 2 as compared to other trials. A interaction of days and trials was also seen during phase 2 [$F(4,92)=3.25$, $p<.05$, partial $\eta^2=.12$] (Figure 16), showing that while trial 1 performance remained the same across all 5 days, trial 2 performance dramatically improved as days passed. This reflects rats' improved performance on the more difficult second trial throughout the course of training.

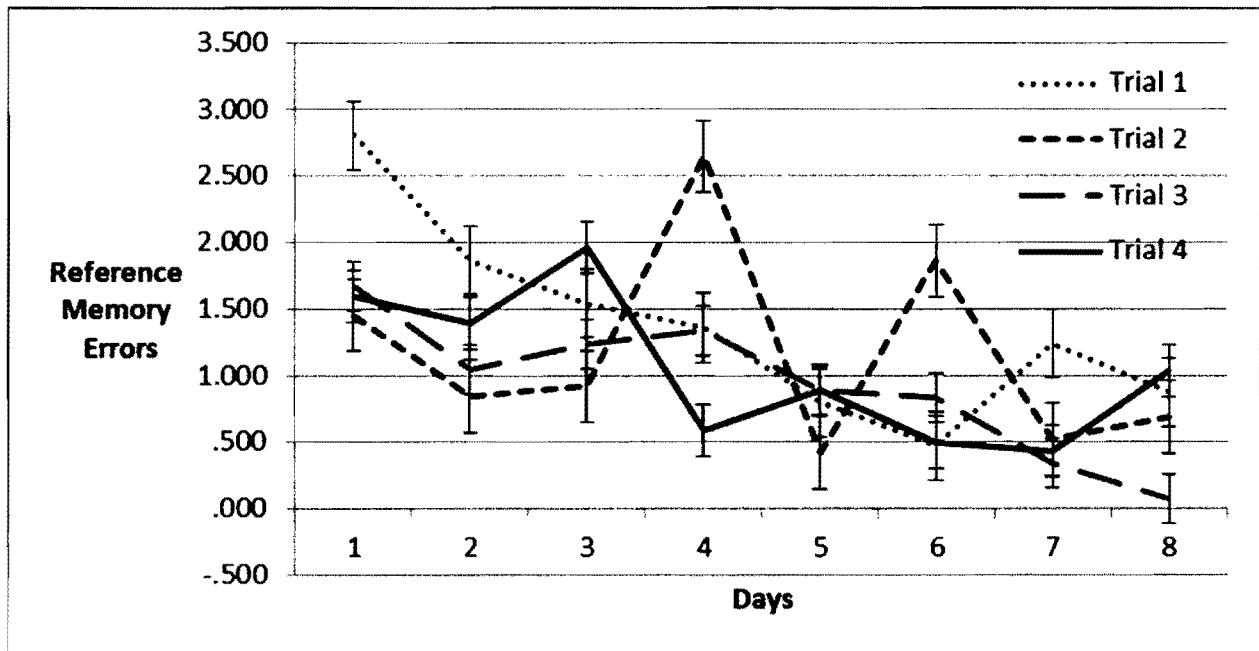


Figure 15. Reference memory errors across days during phase 1 of training.

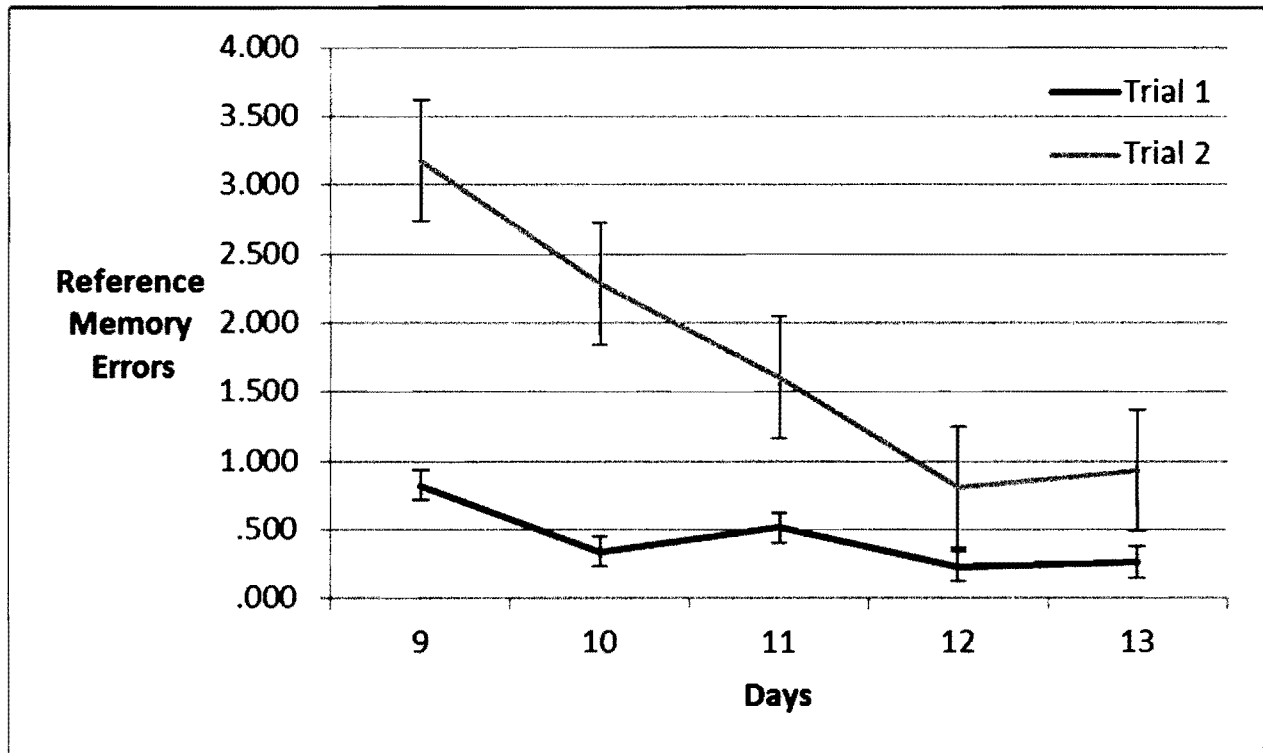


Figure 16. Reference memory errors across days during phase 2 of training.

No main effects of pretreatment or training drug were observed for reference memory during training ($p's > .05$)

Retention. A main effect of trials was observed during both retention tests [$F(1,23)=14.78, p<.001, \text{partial } \eta^2=.39$] showing that significantly more errors were made during trial 2 (Figure 17). Once again, this is expected because of the increased difficulty of the second trial.

No main effects of pretreatment or training drug were observed for reference memory during retention ($p > .05$).

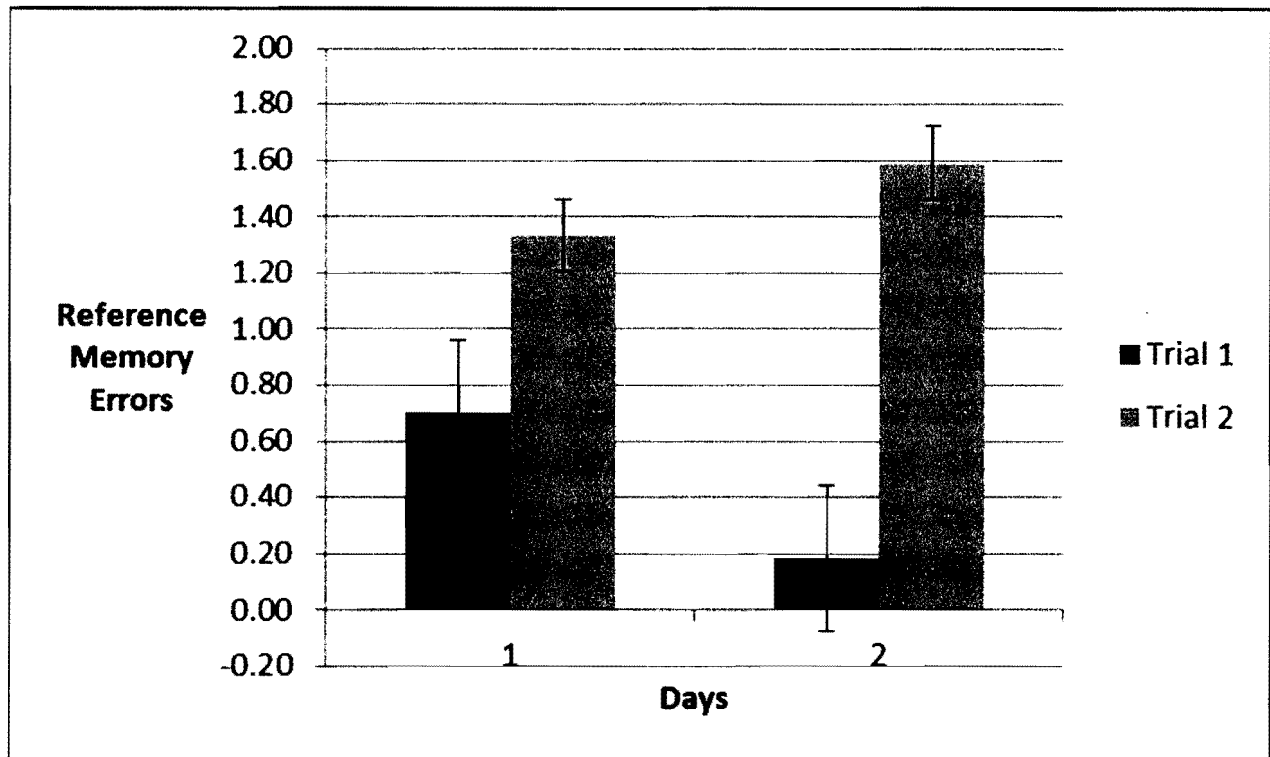


Figure 17. Reference memory errors across days for retention tests.

Working Memory

Training. A main effect of days was observed [$F(4,92)=3.44, p<.05, \text{partial } \eta^2=.13$] during phase 2 of training showing a significant decrease in working memory errors as days passed (Figure 18). A main effect of trials was also observed [$F(1,23)=19.60, p<.01, \text{partial } \eta^2=.46$] showing significantly more errors were made on trial 2. A main effect of pretreatment drug was also observed [$F(1,23)=4.82, p<.05, \text{partial } \eta^2=.17$] showing that pretreatment (chronic) caffeine administration significantly impaired the performance of rats during phase 2 of training (Figure 19). There was an interaction of days and trials [$F(4,92)=3.18, p<.05, \text{partial } \eta^2=.12$], showing that while trial 1 performance across days remained unchanged, trial 2 performance improved dramatically over the first four days (Figure 18). An interaction of trials and pretreatment drug was found [$F(1,23)=5.03, p<.05, \text{partial } \eta^2=.18$], showing that while trial

1 performance was not affected by drug, pretreatment (chronic) caffeine significantly impaired performance on trial 2 (Figure 20).

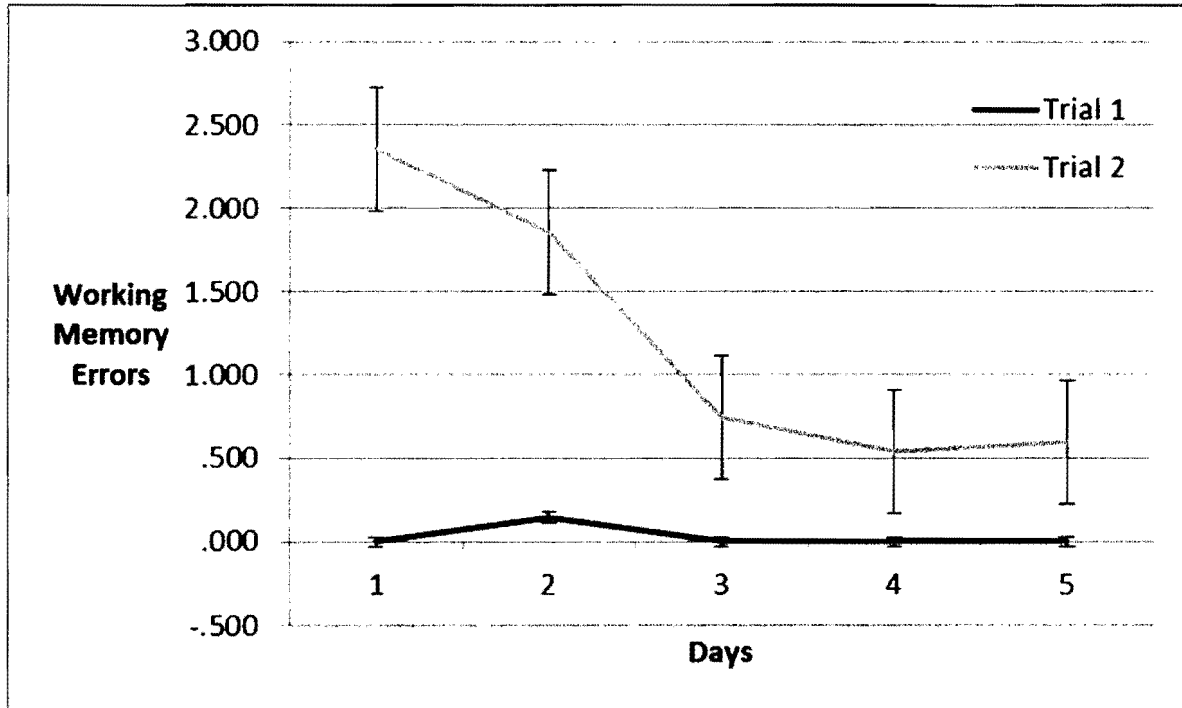


Figure 18. Working memory errors across days during phase 2 of training.

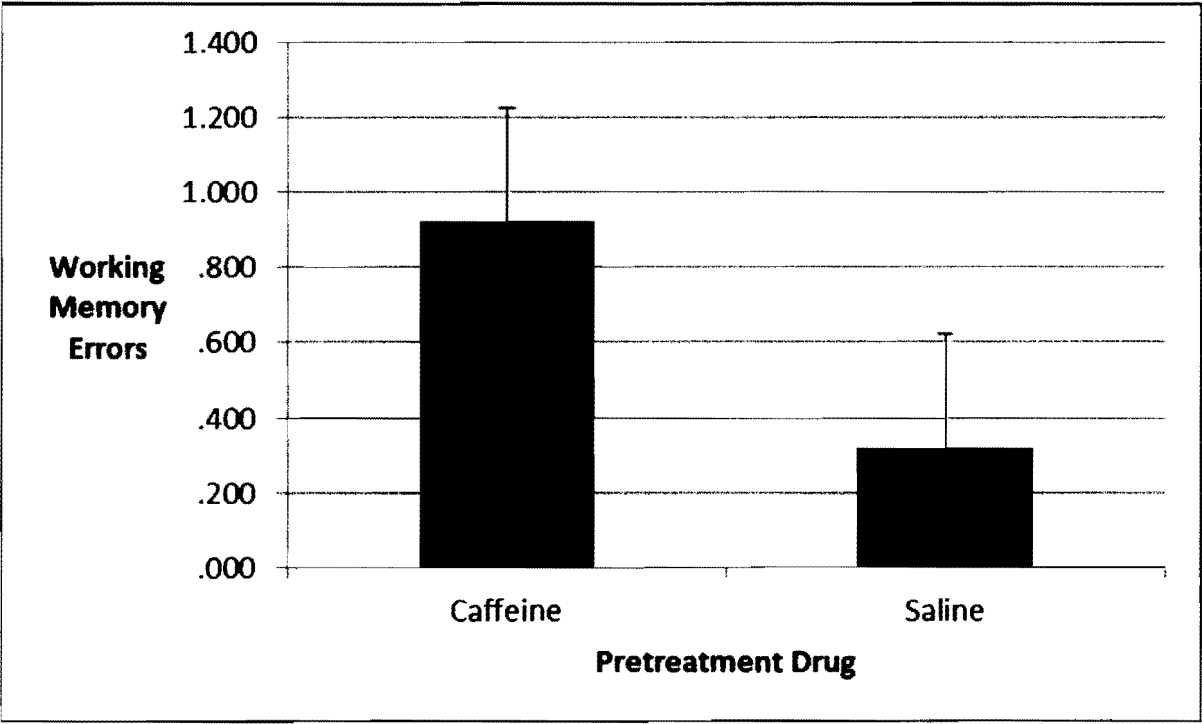


Figure 19. Effect of pretreatment drug on working memory errors during phase 2 of training.

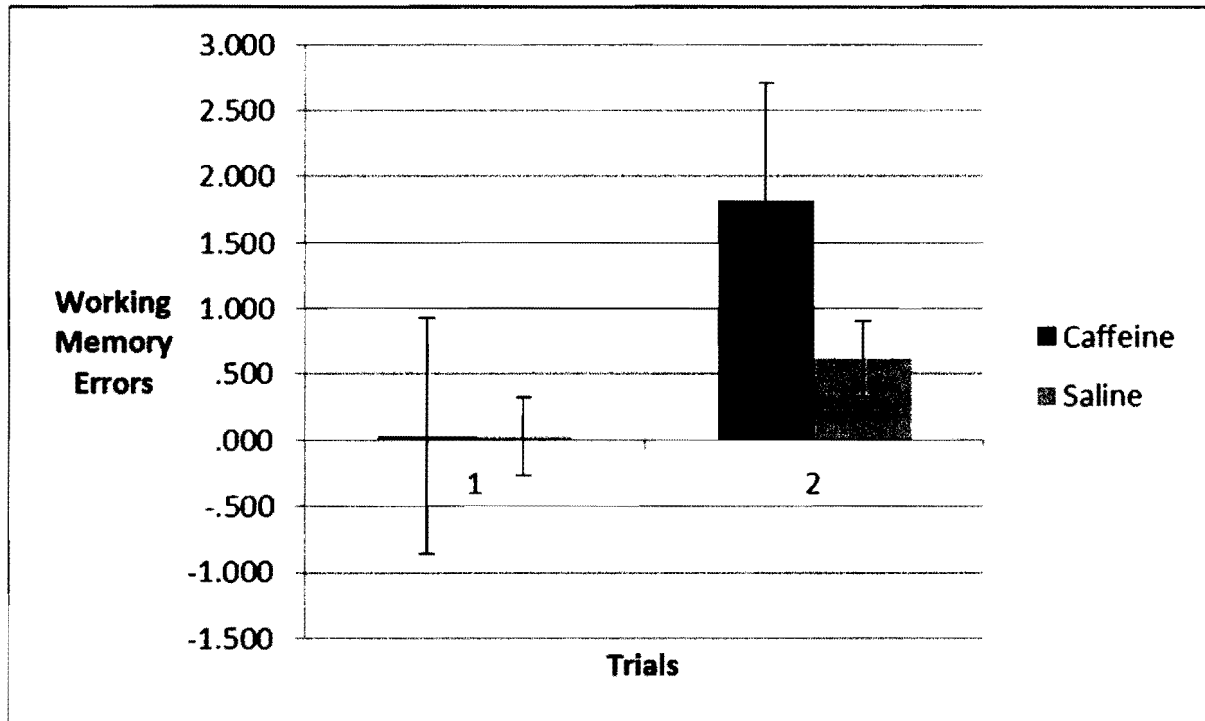


Figure 20. Working memory errors across trials during phase 2 of training.

Retention. A main effect of trials was found [$F(1,23)=9.40, p<.01, \text{partial } \eta^2=.29$] indicating that rats performed significantly worse on trial 2 (Figure 21). No main effects of drug were found for working memory during retention ($p>.05$).

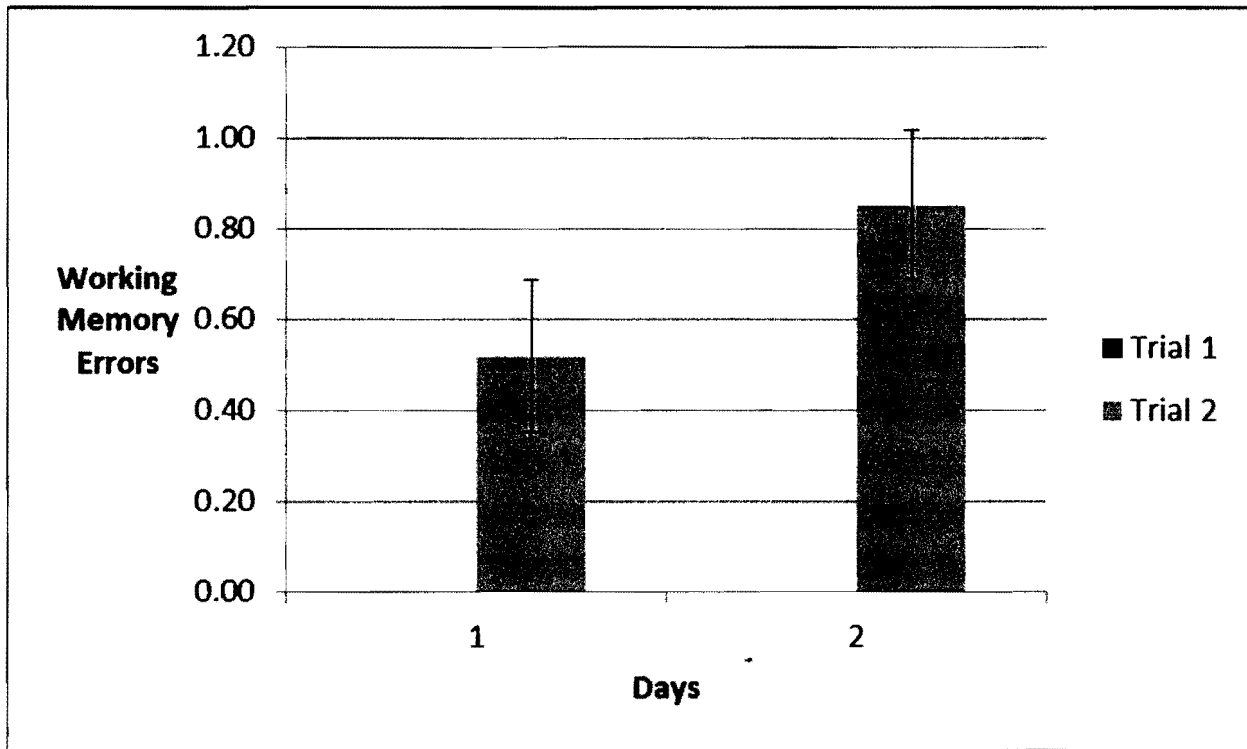


Figure 21. Working memory errors across days for retention tests.

The effects of caffeine on latency to reach the platform, reference memory, and working memory are summarized in Figure 22.

Timing of Caffeine	Training Phase 1		Training Phase 2			Retention		
	Latency to Escape	Reference Memory	Latency to Escape	Reference Memory	Working Memory	Latency to Escape	Reference Memory	Working Memory
<i>Pretreatment</i>	No Effect	No Effect	No Effect	No Effect	Impaired	Mixed	No Effect	No Effect
<i>Training</i>	No Effect	No Effect	No Effect	No Effect	No Effect	Mixed	No Effect	No Effect

Figure 22. The effects of caffeine in the experiment.

Discussion

The results of the study indicate that rats were able to successfully learn the RAWM, as demonstrated by decreased latency to locate the platform as well as a decrease in working and reference memory errors throughout the course of training. As predicted, rats showed impaired performance on the second trial of the retention test (training phase 2) due to the increased difficulty of the task as a result of the removal of one of the hidden platforms.

The effect of caffeine varied by timing of drug administration and by the memory task assessed. Specifically, animals pretreated with caffeine made more working memory errors than rats in other conditions. Additionally, there were varying effects of caffeine on latency to locate the platform during the two trials of the retention test. Rats that received caffeine during pretreatment and during training showed a decrement in performance from trial 1 to trial 2, while rats that received caffeine during only one of the two phases had improved performance across the two trials. However, it is important to note that the rats receiving saline during pretreatment and during training also showed a decrement in performance from trial 1 to trial 2. This may indicate that the impairment is due to a general effect of state dependency and is not an effect specific to drug administration. The finding of improved performance in the rats that received caffeine treatment during a single phase implies that acute caffeine may enhance learning, perhaps by preventing forgetting of previously learned information.

Reference memory appeared to be completely unaffected by drug or drug interactions, however, working memory was clearly impaired by pretreatment caffeine administration. While all the rats had some difficulty with trial 2 during retention and phase 2 of learning, rats that were administered caffeine during pretreatment made significantly more working memory errors

trying to locate the platform, although they did improve in the working memory task. A main effect of pretreatment drug showed that rats given caffeine made significantly more working memory errors overall when compared to the other groups. These results imply that chronic caffeine may impair working memory during training, but this impairment can be overcome with additional training as evidenced by the lack of impairment in this group on retention tests.

When compared to the pilot study results, these results show more consistency. One of the reasons for this could be the use of experimentally naïve rats. The rats used for the pilot study had been previously used for another experiment and were older so there could have been other factors influencing their performance, which could account for the fact that reference memory was affected by the drug in the pilot study, but not in the main experiment. Also, because less rats were used for the pilot study, the analyses could have been affected by a lack of power and many of the significant differences that were found could be due to individual differences in the performance of certain rats. A similar finding in both results is the fact that chronic caffeine may impair learning over time, especially when in regards to latency to reach the platform. This agrees with previous findings by Han et al. (2007) which showed that chronic caffeine impaired spatial learning. The dose used by Han and colleagues is lower than the dose used in the present study which implies that even small amounts of caffeine, when administered chronically, can cause impairment. A study by Alhaider et al. (2009) showed that chronic caffeine prevents impairment of spatial learning, showing that caffeine helps to reverse deficits caused by sleep-deprivation. The present study, however, does not focus on impairment of learning. No efforts were made to impair learning and the only means of forgetting the behavioral task would be through the passage of time while the study conducted by Alhaider used sleep deprivation as a way to test for impairment.

Findings by Abreu et al. (2011) showed that chronic caffeine may improve long-term memory retrieval. The study done by Abreu and colleagues, however, did not involve spatial learning and the memory being observed was object recognition memory, while the present study shows impairment in working memory as a result of chronic caffeine consumption. This discrepancy implies that caffeine affects different types of memory differently, as was seen by the lack of drug effect in reference memory. Part of the reason for this could be because of the different structures that mediate reference and working memory. The hippocampus and the dentate gyrus are heavily implicated in both working and reference memory (Niewoehner et al., 2007). However, working memory was found to be impaired by lacking NMDA receptors in the dentate gyrus, while reference memory was not (Niewoehner et al., 2007). This implies a difference in associations and pathways between these two types of memory. Both reference and working memory are also heavily influenced by the cholinergic system (Hodges, 1996; Wolff, Benhassine, Costet, Segu, & Buhot, 2003), however, the relationship between caffeine and acetylcholine has not been studied enough to draw any significant conclusions that can be applied to memory paradigms.

Although chronic caffeine appears to impair some measures of learning, acute caffeine appears to have either beneficial or protective effects on learning, at least in lower doses. These findings agree with previous studies conducted by Angelucci et al. (2002) and Valzelli et al. (1986). Angelucci's (2002) findings showed that low to moderate doses (0.3-3 mg/kg) of acute caffeine improved acquisition of spatial information while Valzelli's (1986) findings show that acute caffeine administration enhanced the recall of poor-learning mice.

Acute caffeine administration was also shown to improve objective recognition (Costa et al., 2008) and acquisition of an inhibitory avoidance task (Angelucci et al., 1999), showing,

again, that caffeine has differential effects on different types of memory and different types of tasks. Therefore, one finding on the subject of caffeine cannot be applied to multiple spectrums of learning. A study by Sansone et al. (1994) showed that higher doses of caffeine impaired learning of an avoidance task, implicating that while low to moderate doses of acute caffeine may be helpful for learning, higher acute doses are not.

While these results show some degree of consistency on the effects of caffeine administration, there are many questions that have yet to be answered in the literature. Different tasks, types and stages of memory are affected differently by caffeine. The present study only observes caffeine's effects across one task so these findings may not be able to be applied to differing behavioral learning tasks, although they do remain consistent with the current literature. It is important to remember that the present study only observes chronic versus acute caffeine in spatial learning. The behavioral task was also limited by its duration. A longer duration of the testing may shed light on different, longer term effects of caffeine.

Furthermore, in addition to the external validity of this study being compromised because of the specificity of the task, no measures were taken to observe whether tolerance and dependence of caffeine had occurred in the rats that had chronic administration of the drug. Because of the nature of caffeine to cause a tolerance and a dependence on the drug in humans, it can be assumed that the same may have occurred for the rats used in the present study. Additionally, due to the widespread location of adenosine receptors in the brain and caffeine's antagonism of the A₁ and A_{2A} receptors, the effects of caffeine can be more widespread in the brain than initially thought. The density of A₁ receptors in the hippocampus, especially in area CA1, which is essential for spatial learning (Yu et al., 2009), poses some very serious repercussions for caffeine and learning.

As previously stated, the effects of caffeine are far from understood. More research needs to be done focusing on different types of learning and memory and how caffeine may affect them. The effects of chronic caffeine, especially at higher doses, can be far more damaging than what is currently known. While there are many more highly damaging drugs (e.g. methamphetamine, cocaine, MDMA), caffeine is legal, easily accessible, present in many things that are consumed regularly, and widely consumed both by children and adults so its effects should be well understood before it is so readily consumed. These results have some serious implications for humans because of the amount of caffeine that is consumed worldwide. While some studies done on humans insist that chronic caffeine has beneficial effects (Nehlig, 2010) none are controlled enough to truly link the consumption of caffeine to the enhancement of learning. While the present study and many of the others presented focus on caffeine in animal research, future studies should focus more on human consumption. Caffeine's erratic effects should be studied on the population which they are directly affecting, particularly because of how inconsistent the effects can be across differing behavioral tasks, doses, timing and schedule of administration.

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