

Spring 4-17-2013

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Recommended Citation

Pauldurai, Jenn, "The Effects of Selected Drugs on the Memory Recall of Sleep Deprived Mice" (2013). *Senior Research Projects*. 166.
https://knowledge.e.southern.edu/senior_research/166

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The Effects of Selected Drugs on the Memory Recall of Sleep Deprived Mice

Senior Project

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April 17, 2013

The Effects of Selected Drugs on the Memory Recall of Sleep Deprived Mice

Research Report

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Special thanks to the Department of Biology and Allied Health for their provision of laboratory space, laboratory materials, and continuous support throughout this project. I would also like to thank the School of Nursing and, of course, the School of Education and Psychology for making the logistics of this study possible.

Abstract

Humans often make use of the neurotransmission altering effects of drugs such as caffeine, ephedrine, and ethanol. These drugs induce changes in memory and learning ability, specifically when used for sleep deprivation. English white mice (*Mus musculus*) have been shown to exhibit similar cognitive changes when administered drugs while sleep deprived. A sample of 20 mice was divided into three experimental groups (each group receiving a different drug) and one control group. Maze-testing demonstrated that mice performance decreased with sleep deprivation (Treatment-1) but all groups returned to baseline performance when injected (IP) with selected drugs (Treatment-2), regardless of the selected drug. However, in the absence of sleep deprivation, each drug showed less of an effect. Caffeine and ephedrine produced no significant differences between themselves, and alcohol treatment decreased performance. Overall, sleep deprivation decreases memory recall performance in mice. Drug use can counterbalance those effects of fatigue. However, on non-fatigued mice, caffeine and ephedrine demonstrated no positive effect, while alcohol demonstrated a negative effect on performance.

Keywords: house mice, sleep deprivation, caffeine, ephedrine, ethanol, memory recall

The Effect of Selected Drugs on the Memory Recall of Sleep Deprived Mice

Modern society has seen an increased prevalence of cognitive improvement methods and it seems that everyone is intent on maximizing their brain power. Memory recall has been particularly targeted by those under the demands of the academic environment (Pilcher & Huffcutt, 1996). The recall ability of the average college student is influenced by many behavioral and chemical factors. This population has been known to regularly make use of chemicals such as caffeine, alcohol and more recently, ephedrine (Durmer & Dinges, 2005; Pilcher & Huffcutt, 1996). In addition to these drugs, college brains are exposed to chronic sleep deprivation. The dynamics of exposing the brain to all of these circumstances can affect the limits of memory recall.

Previous research has well documented the independent physiological effects of caffeine, ethanol, ephedrine, and sleep deprivation on mice. Research was compiled using academic journals and databases such as *PSYCHBooks*, *SAGE*, and *Web of Knowledge*. Keywords used in this literature review were: *caffeine*, *ethanol*, *ephedrine*, *sleep*, *mice*, *Mus musculus*, *cognition of*, *memory recall of*, *sleep deprivation*, *fatigue*, *stimulants on*, *drug use of*, and *learning ability of*. The following review highlights the results of this literature search and will lay a foundation for the experimental procedure of this research study. Studies will be presented first on sleep deprivation and its effects in both humans and mice. Studies will then be presented more specifically on each of the drugs that will be tested. This information will provide more background details on the specifics behind the behavioral test results.

English white mice (*Mus musculus*) have often been used as the behavioral model for cognition and memory recall experiments simply because of the similarities in learning and brain functioning between this animal model and human behavior. Mice learn quickly and have many

of the same neurotransmitter activities as the human brain. Mice also have been widely used as the model for fatigue and sleep deprivation (Tanaka et al., 2003; Vecsey et al., 2009; Patti et al., 2010). Their light/dark schedules for sleep-wake cycles allow for easy manipulation and control over the amount of sleep, and correspondingly, the amount of fatigue that each mouse is subjected to (Dall'igna et al., 2003). Additionally, administration of drugs into the mouse environment can be controlled for accurate experimentation as well. Mice can be introduced to selected chemicals in various ways. Mice can ingest drugs *ad libitum* through their diet or the drugs can be measured out and injected into the mouse's bloodstream. Use of these methods has shown success in altering the brain chemistry of mice. Aply, mice have often been used in drug studies as their behavior is readily testable and the effect of the drug on the physiology of *Mus musculus* is similar to the behavioral changes expected in the human population (Tanaka et al., 2003).

Sleep Deprivation in Mice

Mice sleep for approximately 85% of their day and sleep deprivation is defined as loss of this regular amount of sleep. The causes of this behavior are usually laboratory induced and not the result of voluntary decisions to stay awake on the part of the mouse. Deprivation can range anywhere from four hours to 10 hours of no sleep or partial sleep in the sleep-wake cycle (Tanaka et al., 2003). Ten hours of total sleep deprivation has shown a change equivalent to that of about a week of no sleep in the human population (Kayir & Uzbay, 2004). The effects of sleep deprivation in mice are similar to those found in humans in that this behavior can induce restlessness, lack of coordination, forgetfulness, distractibility and longer reaction times. Cognitively, sleep deprivation can cause attention deficits and a lack of concentration, with an

increased number of errors and memory disturbances. Long term effects of this behavior include high blood pressure, obesity and diabetes in both mice and humans.

Physiology of Sleep Deprivation and Fatigue

In mice, sleep deprivation can cause irreversible changes in hippocampal potentiation activity. Fatigue reduces cortical activity and dendritic spine formation. Fatigue also changes the amounts of tryptophan, indoleamines and catecholamines available for cell signaling. These are all involved in the regulation of melatonin levels (Tanaka et al., 2003; Vecsey et al., 2009). Serotonin levels are also affected by decreased levels of sleep and this in turn affects other hormonal levels including those of dopamine, cortisol, and glucose. Glucose uptake is a primary indicator of brain activity in PET scans and other systemic brain scans. Thus a PET scan run on a sleep deprived mouse brain would exhibit very little overall activity. One study also showed that sleep deprivation interferes with cAMP signaling pathways in the hippocampus of mice. Cyclic AMP (cAMP) is a vital secondary messenger not only in hippocampal signaling but in numerous other intracellular and intercellular pathways (Tanaka et al., 2003).

Sleep Deprivation and Learning

Beyond the effects on memory of sleep deprivation and fatigue, there are also changes in learning, which is intimately connected with recall ability. Some research suggests that sleep deprivation has such a large effect on learning in part because of the state-dependency of learning (Patti et al., 2010). The sleep-wake behavior of initial learning is different than the environment that the mouse is presented to exhibit recall while sleep deprived. Also, because the efficiency of learning is directly linked to memory recall, changing chemical levels with the sleep deprivation alters the memory ability before recall is even initiated. Thus the effects of

sleep deprivation are two-fold: this behavior alters the learning state before memory recall and the ability of the mouse to retrieve information after it has been stored in memory.

Sleep Deprivation Methods

There have been several documented methods of depriving mice of sleep. Total sleep deprivation can be induced by procedures involving water. The disk-over-water method involves a motor that plunges the mice into water if a decrease in electromagnetic activity is detected. Mice can also be inactively submerged in water for a period of time—or forced to keep moving by rising levels of water (Ruiz-Medina, Pinto-Xavier, Rodriguez-Arias, Minarro & Valverde, 2012). This use of water is justified in that mice cannot comfortably sleep while under the stress and fear of drowning, as well as the change in temperature, humidity, and wetness that alter the specific environmental specifications that mice need for sleep (Chen et al., 2009).

Other methods of sleep deprivation include the removal of bedding and the changing of the sleep-wake cycle to include more light hours (Tanaka et al., 2003). *Mus musculus* is sensitive to environmental changes and responds to disturbances by staying alert for any potential dangers. Gentle tapping or gentle handling involves the use of a brush to gently nudge the mouse awake if fatigue and napping is observed, but this method is tedious and time consuming on the part of the experimenter. Newer, more automated methods include the use of chambers that measure brain activity and correspondingly change the environment without the need for the subjectivity of observation.

Pharmacology and Usage of Caffeine

Caffeine is a chemical that has a stimulatory effect on brain activity. This effect is accomplished through two mechanisms. Firstly, caffeine antagonizes the binding of adenosine to receptors thus blocking the inhibitory effect of adenosine and allowing for increased flow of

neurotransmitters such as norepinephrine, cortisol and acetylcholine. With the muted influence of adenosine, the brain increases in energy exchange and is more alert and responsive to stimuli. At higher dosages, caffeine can also block other neurotransmitter reception, such as GABA, and this can cause increases in respiration and heart rate (Ruiz-Medina et al., 2012). Caffeine has also been shown to affect metabolic rate. Caffeine is a xanthine which means it acts as a phosphodiesterase inhibitor allowing for an increase in the levels of free fatty acids in the bloodstream. This decreases the necessity for the breakdown of stored fats and energy is readily available from the increased oxidation of the free fatty acids. This sudden abundance of energy can account for the jittery, hyper-alert, wakefulness associated with caffeine usage (Ruiz-Medina et al., 2012).

The lethal dosage of caffeine (LD_{50}) for mice has been measured to be 168 mg of caffeine per kg of body mass. The stimulant and depressant effects of caffeine on the mouse can be observed in changes in locomotor behavior well before the LD_{50} concentration. This can be observed in the laboratory setting and to a much lesser extent in the open field (Ruiz-Medina et al., 2012).

Pharmacology of Ephedrine

The mechanism of action of ephedrine is an indirect stimulation of the adrenergic receptor system. As a sympathomimetic, ephedrine will bind to alpha and beta receptors of noradrenaline and stimulate the release of noradrenaline and dopamine. Both of these neurotransmitters have stimulating effects on the nervous system and have been known to clear up asthmatic sinuses and induce weight loss (Blizard, 2007). The lethal dosage of ephedrine in the mouse is 55.74 mg/kg of body mass. Similar bronchodilation and weight loss effects are

expected of mice. Ephedrine has also been used to observe energy dynamics using mice models (Malinauskas, Aeby, Overton, Carpenter-Aeby, & Barber-Heidal, 2007; Dullo & Miller, 1986).

Pharmacology and Usage of Ethanol in the Mouse

Ethanol is a depressant that binds to certain neurotransmitter receptors and limits their activity. Effected neurotransmitters and hormones include acetylcholine, GABA, serotonin and NMDA (Dullo & Miller, 1986). This antagonistic activity also stimulates an increase of dopamine levels, allowing for the initial euphoric feeling (Hunt, Momjian, & Wong, 2011; Carvalho et al., 2012). The nervous system is suppressed however, which can lead to the uninhibited behavior of the subject administered ethanol. Alcohol can be similarly administered to mice below an LD₅₀ level of 0.0066mg/kg of body mass. Mice are susceptible to overdose but also susceptible to tolerance over time (Carvalho et al., 2012). Mice will choose to voluntarily partake of alcohol after addiction. Similarly to humans, inhibition-free activity is reported in mouse behavior after alcohol usage.

Critique of the Literature

The studies appear to validate the mice model as appropriate for empirical study because mice effectively model both sleep deprivation and selected drug use in a controlled environment while allowing for the easy testing of memory recall. The changes in memory can be behaviorally tested and the effects of these various drugs and fatigue can be behaviorally observed. The relationship between caffeine, ephedrine, and ethanol, and memory recall has been established and explained. Similarly, the relationship between sleep deprivation and cognition has also been modeled and neurobiochemically understood.

Major strengths of these studies include the abundance of information on testing and observing these behavioral changes. The methods have ranged from dissection to observation to

computer monitoring of electromagnetic brain activity. Despite this range of methods, almost all research has agreed on the behavioral effects that can be induced in the mouse by the factors of fatigue and drug usage.

Weaknesses among the research include a lack and variety of drugs tested and administered to mice. Well-documented drugs include ecstasy, dopamine derivatives and medications. More pertinent drug choices could have been the testing of energy drinks such as Red Bull or the commonly used ADHD drug, Adderall. These would provide more relevant information on the human behavior and use of drugs. Another gap in the studies is the lack of information on the synergistic effects of combining any of these drugs. Rarely are any of these stimulants or depressants taken independently. Alcohol consumption is often tied intimately with other drug use in the human population. Modeling these interactions would provide a more realistic simulation of the brain environment.

This current study is a behavioral study using male English white mice (*Mus musculus*) to address the latter gap in that it will combine the effects of sleep deprivation with the effects of drug usage to see what effect this interaction will have on memory recall in mice. This combination of two factors will hopefully model the dynamics of chemical interactions on college campuses.

Definition of Terms

In this study, the following terms will be operationally defined:

1. Sleep deprivation is defined as the partial control of no sleep. This will be induced by the removal of bedding and the gentle tapping method. Deprivation will be observationally determined by the mouse behavior indicative of sleep: closing of eyes, heavy breathing,

curling of the body and limited movement. It is assumed that when not under observation, mice did not rest and were indeed sleep deprived.

2. Maze success is defined as the completion of the maze when the mouse has successfully found the food and begun eating. Merely sniffing the food reward does not deem successful completion of the maze. This is a measurement of time in seconds.
3. Gait is defined as the pace and movement of the mouse through the maze. This will be determined by speed of movement.
4. Decision making is defined as the number of times that the mouse enters a maze arm. The mouse does not need to reach the end of the arm to be considered having turned into the arm. A change in decision making is therefore when a mouse enters an arm and then exits to enter the other arm.
5. A mistake is defined as every time a mouse enters the arm which does not contain the reward.
6. Learning resistance is defined as the inability of the mouse to locate the correct arm with the reward within 120 seconds.
7. Fatigue is defined as tiredness induced by sleep deprivation and is presented and observed in these forms: slower than normal gait, light breathing, barely open eyes, lack of reaction to touch, decreased reaction times and decreased overall movement.

Hypotheses

Seven research hypotheses were addressed in this study. Each of these hypotheses was tested in its null form.

1. There are no pre-existing statistically significant differences among the drug groups at the baseline treatment.
2. There are no pre-existing statistically significant differences within the control group over the course of the three treatments.
3. Sleep deprivation does have a statistically significant effect across all test groups.
4. Drug injection does have a statistically significant effect across all test groups.
5. Drug injection plus sleep deprivation has a statistically significant effect across all test groups.
6. There is a statistically significant difference in effect among the different drug treated groups administered during Treatment-2.
7. There is a statistically significant difference in effect among drug treated groups, independent of Treatment-1.

Method

Subjects

Twenty adult male English Mice (*Mus musculus*) were the main subjects of this study. This was a sample of convenience and was assumed to be representative of the behavior of mice in the general population. Each mouse was randomly assigned to one of four different groups: three test groups (CAFF, ETOH & EPHD) and one control group. These mice were bought from the same litter at Petco Incorporated. (Chattanooga location on Gunbarrel Road). All mice were ethically handled and housed according to the Core Values Statement and Code of Ethics of the

American Association of Laboratory Animal Science (AALAS, 2013) and according to the Responsible Conduct of Research published by the American Psychological Association. (APA, 2010). No human subjects were involved.

Materials and Apparatus

A standard Y-arm maze made out of 2 cm thick opaque plastic was used for testing. Each of the three arms of the maze was approximately 0.305 meters in length (1 foot). The maze was set atop a cardboard floor for easy traverse. Certified rodent diet, water, bedding and an exercise wheel was available *ad libitum* to ensure a controlled environment while non-testing. Plastic rodent carrier cages with aerated lids were used to house the mice. The mice cages were stored on a cart with a blanket used to control the sleep-wake cycle.

A timer was used as necessary during testing—this was not in physical contact with the animals. During testing, the same food source was used as a reward in the behavioral maze testing. Sterile 25-gauge hypodermic needles and 1-ml syringes were used for the intraperitoneal injections. Injected chemicals included 200 mg of caffeine in tablet form (Jet-Alert, OTC boxes), 5 ml of ethanol (Smirnoff Vodka, 100 proof), 20 ml natural saline (0.9%), and 200 mg of ephedrine in tablet form (Pseudofed, OTC boxes). The reward used in the maze was the same odorous, certified rodent diet used in housing. Cleaning supplies as necessary were used to keep the mice housed in clean environments.

Design and Procedure

This study was a blind between-subjects experiment. Each of twenty English mice (*Mus musculus*) was randomly assigned to one of four groups: the first group was the control (CTRL, $n = 5$); the second group was the caffeine group (CAFF, $n = 5$); the third group was the ethanol group (ETOH, $n = 5$); and the final group was the ephedrine group (EPHD, $n = 5$). The mice

were caged at the Psychology Resource Area in Herin Hall. During the training and testing phases they were transported to the Biology Department in Hickman Science Center where testing occurred in open laboratory space. All interaction with the mice was limited to these two sites on the campus of Southern Adventist University.

Treatment-0. All 20 mice were allowed to situate and acclimate to the new environment and diet for approximately one week. After this time period, mice were introduced to the Y-maze in the learning phase of this experiment. Each mouse was placed in the Y-maze in a quiet and non-distracting environment. The time that the mouse traverses the maze was recorded until the mouse finds the food that was placed as a reward at the end of one arm. Discovery of the food was marked by the ingestion of food, not just the sniffing of food. Mice were declared learning resistant if they did not find the food within 120 seconds. This procedure was repeated three times for each mouse to ensure learning. This phase was done at the same time of day and after a one meal fast to induce hunger in the mice.

Treatment-1. Testing began 48 hours after all the mice had undergone learning. CTRL mice remained in their housing to be well rested for this time interval and were retested for memory recall of the same Y-maze exactly 48 hours after training. CAFF mice remained in their housing until a 10 hour period before their testing. In this ten hour period, all bedding was removed and lights were kept on. For the last five hours of this deprivation period, intermittent gentle handling of mice added to the partial sleep deprived environment. Testing ensued for each mouse individually immediately after this partial sleep deprivation state. The testing procedure mimicked the training procedure in that mice were given 120 seconds to find the food, three times. The maze was wiped down with sanitizing alcohol to reduce any scent trails. There was ample time for the alcohol to evaporate before mice were reintroduced to the maze. This

procedure was repeated with the ETOH and EPHD groups as well. Mice were returned to a normal sleep-wake and diet environment as soon as testing was complete.

Treatment-2. Testing began 48 hours after all the mice had undergone Treatment-1. CTRL mice remained in their housing to be well rested for this time interval. Immediately after their rest period, the CTRL mice were injected with approximately 0.2 ml of 0.9% natural saline. The mice were then retested for memory recall of the same Y-maze according to the protocol listed in Treatment-1. Similarly, CAFF mice were sleep deprived for the immediate 10 hour period before testing. This sleep deprivation involved the same protocol of removing bedding and gentle handling as listed in Treatment-1. Immediately after sleep deprivation, each mouse was removed from the cage and injected with a caffeine solution. Caffeine solutions were prepared from a mix of natural saline (0.9% NaCl) and dissolved caffeine tablet at a ratio of 0.2mg of caffeine/ 0.2 ml of saline. This solution was an intraperitoneal injection with a volume of 10 ml/kg (approximately 0.2 ml per mouse). This concentration is midway between the effective dose and the lethal dose of caffeine for mice. After the injection of the caffeine solution, mice were returned to their cages for a 30 minute reaction period. After observational recordings during this time, testing followed the same procedure as the previous two treatments.

ETOH mice were sleep deprived for 10 hours as well and then injected with the ethanol solution. The ethanol solution was prepared using 0.2 mL of pure ethanol in 0.8 mL of reverse osmosis water for a 20% ethanol solution. This concentration is midway between the effective dose and the lethal dose of ethanol for mice. This was also an intraperitoneal injection with a volume of 10 ml/kg (approximately 0.2 ml per mouse). EPHD mice were similarly sleep deprived. The ephedrine solution was prepared by mixing 5 mg of synthetic ephedrine in 1 mL of 5% sucrose water. This injection was not weight-dependent and a constant 0.02 mL of

solution was injected into each mouse. A similar procedure for injection and sleep deprivation was maintained for all three experimental groups. A 30 minute wait period was also maintained for all test groups. Injections were all intraperitoneal with each mouse injected with a clean needle. Mice were restrained by hand without the use of anesthetics during the injection and all other transporting involved carefully picking up the mice from the base of their tails. All mice were returned to housing after Treatment-2.

Additional control measures. After 48 hours of recovery, mice performed the final step of testing. This run was a secondary control measurement with the maze testing done after the mice were well-rested, with a one-meal fast. After proper recording and cleanup, mice were returned to housing for recuperation. Testing was then complete and mice were appropriately disposed of.

Data Analysis

After data collection, four dependent sample *t*-tests were conducted relating Treatment-0 and Treatment-1. This procedure was repeated between Treatment-1 and Treatment-2, and between Treatment-0 and Treatment-2. A one-way ANOVA was run on the differences in medians between Treatment-1 and Treatment-0 to determine the effect of each test group. This procedure was repeated for the differences in medians between Treatment-2 and Treatment-1.

Results

The sample of 20 mice had a total of 56 trials instead of 60 trials due to subject sickness. Means and medians were calculated according to each test group and each treatment. Overall, learning took approximately 37.0 seconds for each mouse. After Treatment-1, memory recall of the maze was approximately 37.5 seconds. After Treatment-2, overall average maze completion

was clocked at 35.4 seconds. Changes within each group are further analyzed below and in the Appendix in Tables 2-4, as well as in Figure 1.

Hypotheses

1. There were no statistically significant differences among the drug groups at the baseline treatment ($F = 1.09, p = 0.36$). The one-way ANOVA showed that there was insufficient evidence to reject the null hypothesis. This means that there were no significant pre-existing differences between the mice before treatment began.
2. There were no statistically significant differences within the control group over the course of the three treatments ($F = 0.14, p = 0.87$). The one-way ANOVA showed that there was not sufficient evidence to reject the null hypothesis. This means there were no differences among the control group over the three treatments.
3. Treatment-1 does have a statistically significant effect across test groups ($r_1 = -0.35, k_1 = 45, p_1 = 0.02; r_2 = -0.22, k_2 = 45, p_2 = 0.14; r_3 = 0.40, k_3 = 42, p_3 = 0.01$). Paired-sample t -tests were conducted for each drug group with group-specific number of trials (k). There was sufficient evidence to reject the null hypothesis, suggesting that there is a difference induced by Treatment-1. Sleep deprivation did have a statistically significant effect on the memory recall of all the tested mice (See Figure 1).
4. Treatment-2 did not have a statistically significant difference from Treatment-1 across any of the test groups ($r_1 = 0.25, k_1 = 30, p_1 = 0.19; r_2 = 0.26, k_2 = 30, p_2 = 0.17; r_3 = 27, k_3 = 27, p_3 = 0.08$). This series of paired sample t -tests for each test group did not provide enough evidence to reject the null hypothesis. This suggests that the drug treatment of Treatment-2 did not have a significant effect on the memory recall of the sleep deprived mice (See Figure 1).

5. Treatment-2 did not have a statistically significant difference from baseline treatment across any of the test groups ($r_1 = -0.15$, $k_1 = 45$, $p_1 = 0.34$; $r_2 = 0.12$, $k_2 = 45$, $p_2 = 0.45$; $r_3 = 0.02$, $k_3 = 39$, $p_3 = 0.90$). This next series of paired sample *t*-tests for each test group did not provide enough evidence to reject the null hypothesis. This means that there was no significant difference between the well rested mice and the sleep deprived plus drug treated mice (See Figure 1).
6. There are no statistically significant differences in effect between the different drug treated groups administered after Treatment-2 ($F = 0.62$, $p = 0.61$). The one-way ANOVA did not provide enough evidence to reject the null hypothesis, suggesting that there were no differences among the drug treated groups. Each drug had the same effect regardless of the chemical substance (See Figure 1).
7. There is a statistically significant difference in effect among drug treated groups, independent of Treatment-1 ($F = 3.81$, $p = 0.02$). The one-way ANOVA on the differences between Treatment-1 and Treatment-2 showed that there was enough evidence to reject the null hypothesis. This means there was a difference among one or more of the drugs in the absence of sleep deprivation. The only significant difference in this test was as a result of the ethanol treatment. Ethanol was statistically different from the control, caffeine, and ephedrine treatments (See Figure 2).

Other Interesting Findings

Several mice were resistant to learning and these trials were not taken into consideration when calculating means and medians. Other trials showed evidence of outliers in each test group, where some mice would just find the food incredibly quickly ($M = 4.0$ seconds) and because of these outliers, medians were reported when running ANOVA analysis.

Summary of Results

Baseline treatments were approximately the same across all the groups, ensuring that no one group of mice had a pre-existing advantage. Sleep deprivation in Treatment-1 had a negative effect on the memory recall of the treated mice. Treatment-2 was statistically identical to Treatment-0. This suggests a return of memory recall performance to baseline efficiency after the negative plunge of sleep deprivation. When combined with sleep deprivation, no one drug group was more efficient at restoring performance. However, when administered independently of sleep deprivation there were differences in the effects of the drugs. These results are further summarized in the following Table 1.

Table 1

Testing the Research Hypotheses in Summary

Hypothesis	Expected	Actual	Result
1. No differences at baseline.	$p > 0.05$	$p > 0.05$	Hypothesis Supported
2. No differences in CTRL.	$p > 0.05$	$p > 0.05$	Hypothesis Supported
3. Sleep deprivation causes change.	$p < 0.05$	$p < 0.05$	Hypothesis Supported
4. Drug use causes change.	$p < 0.05$	$p > 0.02$	Hypothesis Rejected
5. Drug use + deprivation causes change.	$p < 0.05$	$p > 0.02$	Hypothesis Rejected
6. Each drug group causes different change in sleep deprived mice.	$p < 0.05$	$p > 0.02$	Hypothesis Rejected
7. Each drug group causes different change in well-rested mice.	$p < 0.05$	$p < 0.05$	Hypothesis Supported

Note: The phrase “causes change” indicates a statistically significant difference within or between the results as suggested by a *t*-test or an ANOVA. The presence or lack of “change” does not correlate with the presence or lack of an effect.

Discussion

The purpose of this study was to quantify the effects of drug use on the memory recall of sleep deprived mice. Three different drugs were used: caffeine, ethanol, and ephedrine. These drug treatments were combined with experimental sleep deprivation methods. Over the course of the experiment the effect of this combination on memory recall was measured using a Y-maze. The research hypotheses first established that the changes in memory recall were indeed a result of the treatments. There were no differences among the mice; all changes could be attributed to the methods and no genetic predisposition provided an unfair advantage. The next set of research hypotheses addressed the changes induced over the course of the three treatments. The results show that sleep deprivation does have a negative effect on the memory recall of mice with very little variability. This negative change was counterbalanced by the injection of the selected drugs in Treatment-2. The differences between baseline (Treatment-0) and Treatment -2 were essentially negligible. Upon first glance this could be misinterpreted to mean that the combination has no effect on memory recall. However, looking at Treatment-1 shows that sleep deprivation does in fact have an effect and that effect was just reversed upon the additional drug treatment.

Surprisingly, there were no differences in effect between the drugs used in Treatment-2. It was expected that the two stimulants, caffeine and ephedrine, would show a greater than average memory recall efficiency and performance would be enhanced beyond the baseline. It was also expected that ethanol would have depressing effects and memory recall would be further stunted by the ingestion of alcohol. However this was not the case. All three drugs had the same effect on sleep deprived mice, returning memory recall performance to baseline efficiency.

Upon further statistical analysis examining the differences between sleep deprivation and the combined treatments, it was seen that the drugs had different effects independent of sleep deprivation. This leads to two general conclusions. One, the combined effects induced by sleep deprivation and drug use were not equally split between the two factors. In some test groups (specifically the caffeine and ephedrine groups), the drugs played a large role in counterbalancing the sleep deprivation. However, in the ethanol test group, sleep deprivation had the greater effect of memory recall and the combination of the two factors was more synergistic than either of the factors alone. This is seen in the results of Treatment-2 minus Treatment-1. The drugs had different effects when they were not acting in conjunction with sleep deprivation. Caffeine and ephedrine had very little significant effects on memory recall and alcohol treatment had a significantly negative effect on overall memory recall. This finding suggests that drugs may have varying effects on cognition that is already varied on other factors such as sleep deprivation.

These findings are based on the repeated trials of twenty mice which is a relatively small sample size and may not actually be representative of the mice population, much less human cognition. Additionally, the methods used in this experiment may not be valid with every animal study. Sleep deprivation was induced with the removal of bedding but there was no camera-recording device to ensure that the mice were actually sleep deprived. Along the same lines, there was no drug-only treatment to test the effects of drugs on the well-rested mouse. These results were surmised using the means obtained from the combined treatment and the sleep-deprivation only treatments. The introduction of a drug-only treatment brings to light the need to control for order effects. Drug-only before fatigue-only could be a different effect than the

opposite chronological order. With more time and resources, perhaps this weakness could be addressed in future studies.

Future studies could also address a great range of variable drugs. Energy drinks and sugar are also greatly abused on college campuses and these could have a large influence on memory recall as well as on sleep patterns. Another angle for future research could be the alteration of the concentrations of drugs and the introduction of each drug at different time increments. This study did not address external factors such as addiction and repeated exposure and these could also be points of interest in future studies. This study did however validate the use of the mouse model of sleep deprivation and these findings contribute to the knowledge of stimulant use on cognitive performance. This mice model could provide information for commonly sleep deprived populations, such as college communities and emergency personnel. Results show that if sleep deprivation is absolutely necessary, the consumption of any of the three selected drugs could potentially counterbalance the negative memory recall performance associated with fatigue. This study addresses gaps in previous research that documented the effects of either one or the other but not the combination of sleep deprivation and drug use. Focusing on the combination not only provides empirical information on the science of drug abuse but it also introduces new methods of approaching experimental methodology testing several variables and the overall significance of each variable.

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Appendix

Table 1

Testing the Research Hypotheses in Summary

Hypothesis	Expected	Actual	Result
1. No differences at baseline.	$p > 0.05$	$p > 0.05$	Hypothesis Supported
2. No differences in CTRL.	$p > 0.05$	$p > 0.05$	Hypothesis Supported
3. Sleep deprivation causes change.	$p < 0.05$	$p < 0.05$	Hypothesis Supported
4. Drug use causes change.	$p < 0.05$	$p > 0.02$	Hypothesis Rejected
5. Drug use + deprivation causes change.	$p < 0.05$	$p > 0.02$	Hypothesis Rejected
6. Each drug group causes different change in sleep deprived mice.	$p < 0.05$	$p > 0.02$	Hypothesis Rejected
7. Each drug group causes different change in well-rested mice.	$p < 0.05$	$p < 0.05$	Hypothesis Supported

Note: The phrase “causes change” indicates a statistically significant difference within or between the results as suggested by a *t*-test or an ANOVA. The presence or lack of “change” does not correlate with the presence or lack of an effect.

Table 2

Mean Completion Times in Seconds for Each Test Group

Test Group	Trial 1	Trial 2	Trial 3	Average
Control	31.0	17.9	27.0	25.3
Caffeine	41.2	33.2	43.6	39.3
Alcohol	30.9	41.9	38.3	37.0
Ephedrine	31.8	22.4	33.6	29.3

Note: Testing Hypothesis #1 suggests that the differences between these averages are not statistically significant. In effect, there are no differences between any of the groups at baseline.

Table 3

Mean Differences in Completion Times in Seconds for Each Test Group Between Treatment-0 and Treatment-1

Test Group	Trial 1	Trial 2	Trial 3	Average
Control	-11.2	14.1	-4.6	-0.6
Caffeine	-23.6	-23	-19.4	-22.0
Alcohol	1.1	11.1	-20.1	-2.6
Ephedrine	-3.6	-5.4	-17.6	-8.9

Note: Testing Hypothesis #2 suggests that the average change seen in the Control group is not statistically significant. Testing Hypothesis #3 suggests that the average changes seen in the three test groups are significant differences from Treatment-0.

Table 4

Mean Differences in Completion Times in Seconds for Each Test Group Between Treatment-0 and Treatment-2

Test Group	Trial 1	Trial 2	Trial 3	Average
Control	-16.8	-1.9	13.8	-1.6
Caffeine	-13.8	-13.0	-3.4	-10.1
Alcohol	-15.6	-23.3	-29.9	-22.9
Ephedrine	23.8	21.8	-8.8	12.3

Note: Testing Hypothesis #5 suggests that these average changes are not statistically different from Treatment-0. In effect, these results are equivalent to the averages seen at baseline.

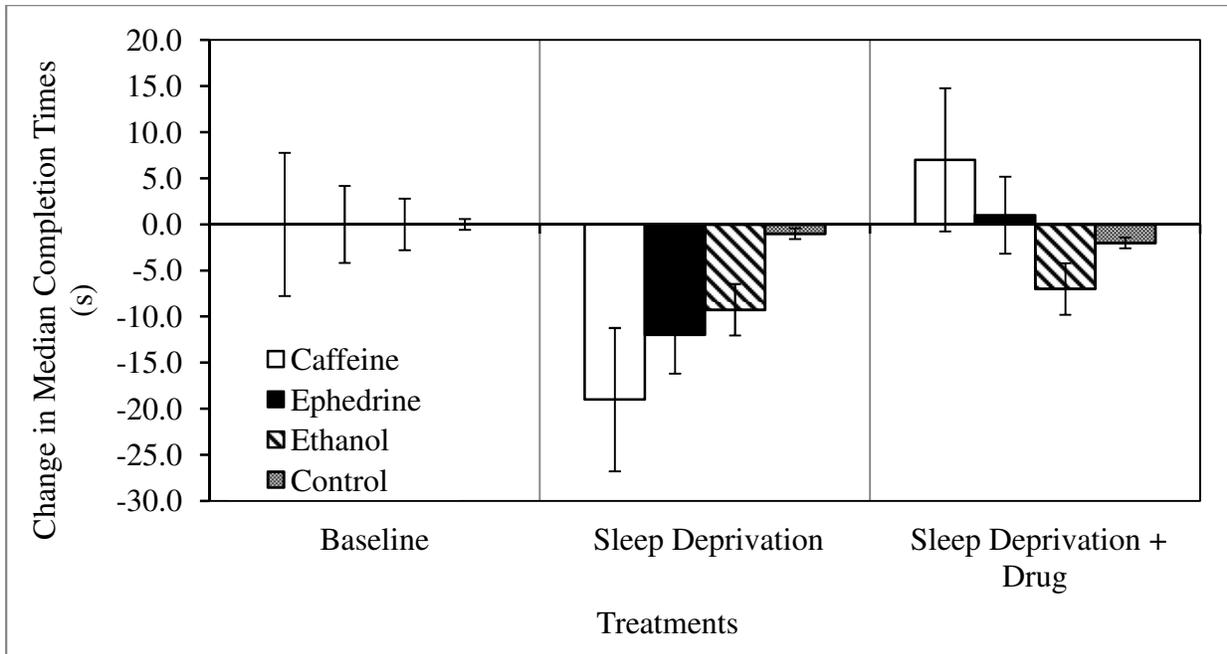


Figure 1. Mice memory recall measured by maze completion time in seconds.

Note: The change in median maze times per test group is indicated by different colors. The horizontal axis shows the progression of treatments in each repeated measures design. The differences among the three test groups after Treatment-1 and after Treatment-2 were not statistically significant at $\alpha = 0.05$ ($k = 57$, $F = 0.62$, $p = 0.61$)

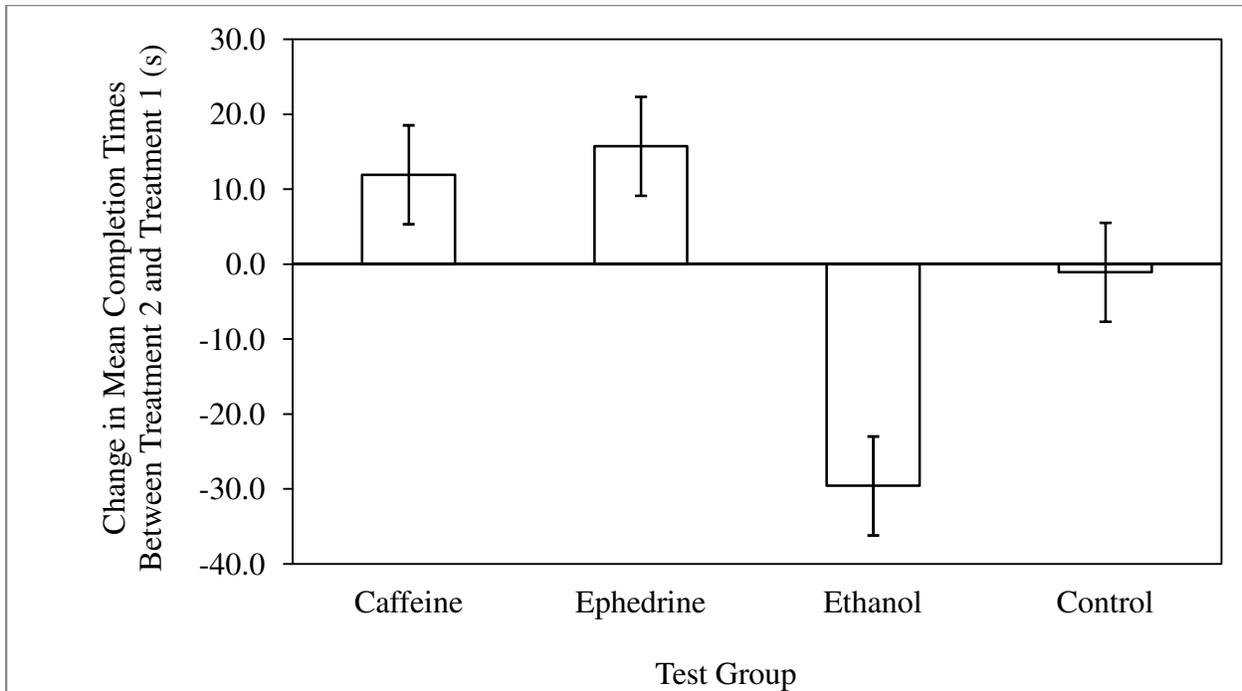


Figure 2. Comparison of the differences in mean maze completion times between Treatment -1 and Treatment-2.

Note: This compares the effect of the drug treatments, independent of the sleep deprivation. The only significant result among the three test drugs was due to the ethanol treatment ($\alpha = 0.05$, $k = 57$, $p = 0.02$, $F = 3.81$)

Figure Captions

Table 1 *Testing the Research Hypotheses in Summary*

Note: The phrase “causes change” indicates a statistically significant difference within or between the results as suggested by a *t*-test or an ANOVA. The presence or lack of “change” does not correlate with the presence or lack of an effect.

Table 2 *Mean Completion Times in Seconds for Each Mouse in Each Test Group*

Note: Testing Hypothesis #1 suggests that the differences between these averages are not statistically significant. In effect, there are no differences between any of the groups at baseline.

Table 3 *Mean Differences in Completion Times in Seconds for Each Test Group Between Treatment-0 and Treatment-1*

Note: Testing Hypothesis #2 suggests that the average change seen in the Control group is not statistically significant. Testing Hypothesis #3 suggests that the average changes seen in the three test groups are significant differences from Treatment-0.

Table 4 *Mean Differences in Completion Times in Seconds for Each Test Group Between Treatment-0 and Treatment-2*

Note: Testing Hypothesis #5 suggests that these average changes are not statistically different from Treatment-0. In effect, these results are equivalent to the averages seen at baseline.

Figure 1. Mice memory recall measured by maze completion time in seconds.

Note: The change in median maze times per test group is indicated by different colors. The horizontal axis shows the progression of treatments in each repeated measures design. The differences among the three test groups after Treatment-1 and after Treatment-2 were not statistically significant at $\alpha = 0.05$ ($k = 57$, $F = 0.62$, $p = 0.61$)

Figure 2. Comparison of the differences in mean maze completion times between Treatment -1 and Treatment-2.

Note: This compares the effect of the drug treatments, independent of the sleep deprivation. The only significant result among the three test drugs was due to the ethanol treatment ($\alpha = 0.05$, $k = 57$, $p = 0.02$, $F = 3.81$)