THE EFFECTS OF CAFFEINE AND PIRACETAM ON *Mus musculus* IN RELATION TO MAZE COMPLETION TIMES

Katherine Good, Kassy Nikkahl & Trung Dinh. Department of Biological Sciences, Saddleback College, Mission Viejo, Ca 92692.

ABSTRACT

Memory formation and learning involve a combination of highly complex physiological systems and require a delicate balance of homeostasis unique to all organisms. The addition of variables, the substances caffeine and piracetam, disrupts the organism’s homeostasis therefore potentially altering the ability to learn and form new memories. Ten house mice, *Mus musculus*, were tested by being exposed to a maze and timed! After establishing five days of control trials, two groups were formed and introduced to either caffeine or piracetam prior to running the maze for another five days. We hypothesized that there would be a difference in the mean maze completion times between the control group, and the groups exposed to a substance. The control group of *Mus musculus* exhibited a mean maze completion time of 117.8 sec ± 12.19 sec. The caffeine-enhanced *Mus musculus* group had a mean completion time of 63.2 sec ± 9.018 sec, and the piracetam group’s mean completion time was 30.6 sec ± 8.133 sec. These data reflect a significant difference between each of the three groups (ANOVA P < 0.05), and supported our initially proposed hypothesis.

INTRODUCTION

Memory formation, learning, and cognitive thinking are three of the most powerfully interrelated processes composing the very foundation of human life. Memories are often thought of as merely being past events, however, it is memory that allows us to utilize past knowledge and experience to think, reason, and plan for the future. For the past few decades, a variety of factors such as the weakening economy, fierce competition for higher education, longer life expectancies, traumatic brain injuries, and genetic diseases, have all contributed to an amplified interest throughout the scientific community with respect to memory and learning, emphasizing ways in which they can be preserved, prolonged, and most importantly, improved. Two psychoactive substances that are currently of particular interest to researchers are caffeine and piracetam.

Caffeine is a white, crystalline xanthine alkaloid derived from the seeds, leaves, and fruits of various plants (Lancu et al., 2007). When consumed, caffeine acts as both a metabolic and central nervous system stimulant; giving it properties that help temporarily reduce fatigue and restore alertness (Neghli, 2010).

There are three primary mechanisms of action that described the exertion by caffeine on the central nervous system—(Nehlig & Daval, 1992). To begin with, caffeine acts as an antagonist to adenosine receptors in the brain, thereby reducing overall adenosine transmission. Second, caffeine stimulates the mobilization of intracellular calcium and inhibition of specific phosphodiesterases, and third, methylxanthine acts as the antagonist directly at the level of adenosine receptors (Nehlig & Daval, 1992). Studies have suggested that a significant amount of the stimulating effects of caffeine may be related to the action of the methylxanthine on serotonin neurons (Hameleers et al., 2000).

In addition to its desired stimulant properties, side effects of the drug can include sleep disturbances, dependency, muscle twitches, headaches, heart palpitations, pregnancy complications, and dehydration as the result of its diuretic properties (Snel & Lorist, 2011).

In North America, 90% of adults consume caffeine on a daily basis (Lovett, 2005). The sheer availability and growing prevalence of caffeine in a massive variety of popular beverages, and sports drinks significantly contributes to the immense amount that is consumed everyday. With the development of over the counter caffeine pills, the unregulated, ubiquitous and easily accessible
nature of caffeine has made it the world’s most widely consumed legal psychoactive drug (Nehlig & Daval, 1992).

Extensive research has been conducted regarding both the positive and negative effects of caffeine on memory, learning, and cognition, along with an equally vast amount of published literature. According to research performed by Nehlig & Daval (1992), caffeine increases energy metabolism throughout the brain, but at the same time decreases cerebral blood flow, which results in a relative state of brain hypoperfusion. Caffeine also activates noradrenaline neurons and seems to affect the local release of dopamine, which may have a minor stimulatory effect, but not likely enough to stimulate memory and learning.

On the other hand, Angelucci et al., (2002) found that moderate doses of caffeine induce behavioral stimulant effects that suggest an increase in cognitive stimulation, but higher doses can suppress behavioral activity and even performances associated with learning and memory. According to another study performed by Reidel et al., (1995), although caffeine is widely recognized as simply a mild central nervous system stimulant drug, the most important consequence of its adenosine antagonism is cholinergic stimulation, which might lead to improvement of higher cognitive functions, particularly memory.

Growing interest in the unique properties of a less common drug, Piracetam, has recently generated a great deal of research and intrigue throughout the scientific community. Piracetam is a cyclic derivative of the neurotransmitter gamma-aminobutyric acid (GABA), yet despite its structural similarity, it has no direct effect on GABA receptors or metabolism of the neurotransmitter in the brain (Winnicka et al., 2005). Physicians will sometimes prescribe Piracetam for a condition called myoclonus, however, the off-label Piracetam supplement available over the counter is used for a much more expansive range of applications (Tacconi & Wurtman, 1996). Piracetam is referred to as a nootropic drug; a group of substances associated with improved mental functions such as memory, intelligence, concentration, and overall cognitive enhancement (Winnicka et al., 2005).

Piracetam has a highly unique mechanism of action, which facilitates influences on neuronal and vascular functions in the brain without operating as a stimulant or sedative (Muller et al., 1999). Though research regarding the complexities of piracetam behavior within the brain is ongoing, numerous studies have been conducted examining specific, isolated reactions, as well as the overall collective mechanism. The research findings thus far provide valuable insight and several pertinent hypotheses about the effects of piracetam on the brain, memory, and learning.

Whereas caffeine acts as a specific competitive inhibitor for adenosine in the brain, Jordaan et al., (1996) performed a study utilizing single photon emission computed tomography to reveal how piracetam acts on ion channels and carriers, which subsequently promotes a non-specific increase in neuron excitability.

Extensive research over the past few years has demonstrated that piracetam can be extremely effective in the treatment of cognitive decline in aging and dementia. Its mechanism of action has been commonly associated with neurochemical deficits, such as acetylcholine, of the aged brain relevant to cognitive dysfunctions. Because many of these neurochemical deficits rely heavily on changes of the membrane properties, such as increased fluidity, it is incredibly significant to note that piracetam is capable of modifying membrane properties by interacting with the polar heads of the phospholipid bilayer (Muller et al., 1999).

Piracetam has also shown to improve the function of the neurotransmitter acetylcholine through muscarinic cholinergic receptors involved in cognitive and memory functions (Winnicka et al., 2005), as well as increase communication between the two hemispheres of the brain, as well as increase neuron firing and activity of the corpus collium (Buresová & Bures, 1976).
Despite the number of existing studies regarding the effects of caffeine and piracetam on memory and learning, cognitive abilities and intelligence are such difficult quantities to measure, making valid, accurate data collection difficult. One of the motivations behind this study was an attempt to make a valid effort to assist researchers attempting to bridge this gap of knowledge. This particular experiment focuses on the hypothesis that house mice, *Mus musculus* will have different maze completion times when they are exposed to the control, piracetam, and caffeine.

**MATERIALS & METHODS**

Ten mice, *Mus musculus*, were used to test the effects of caffeine and piracetam on the ability to complete a maze. The ten mice were purchased at Petco animal supply store in Aliso Viejo, California on Wednesday 26 October 2011. The mice were separated into two groups of five, and housed in controlled environments of cages lined with pine bedding, food pellets purchased from Petco, and plain, distilled water. On 23 October 2011, the experimental maze was constructed using white foam core board purchased from Michael’s Craft Supply Store in Aliso Viejo, California. The dimensions of the maze measured 20 square inches around the edges, and each of the wall structures measured 4 inches high to ensure that the mice would take a path through the maze rather than attempt to climb over the walls. The entrance to the maze had a door-like mechanism that could swing shut when the mouse was placed inside to block the opening, and a reward of cheese and peanut butter were placed at the finish to entice the mouse through the maze.

Data collection began on 27 October 2011 at 7:00pm with the first control group of five mice. Each of the five mice was introduced to the maze, and the average of their completion times was recorded. At 8:00pm, the second control group was tested, and the average maze completion time of each of the other five mice was recorded. This control testing of each group of five mice occurred at the same time everyday and lasted for a total of five days.

On 2 November 2011, the experimental testing began. Caffeine and piracetam supplements were purchased at Rite Aid Pharmacy in Laguna Niguel, California, and were crushed and measured in the chemistry laboratory at Saddleback College in Mission Viejo, California. The recommended adult dosage for over the counter caffeine pills as well as over the counter piracetam supplements is a single 200 mg pill. The average adult has a body mass of 68 kg, and the average body mass for a single mouse is roughly 25 g. By comparing the recommended 200 mg dosage of caffeine and piracetam for an adult to the average body mass of a mouse, the appropriate amount of substance that was administered calculated out to 0.06 mg of substance per mouse. A total of 25 doses of 0.06 mg of both the caffeine and piracetam were crushed and measured into separate bags. A total of 50 sterile medical supply syringes used for oral dosage administration were donated from the Ronald Regan Emergency Room at the University of California, Los Angeles hospital.

One the first day of experimental testing, the five mice in control group #1 were orally administered a dosage of 0.06 mg of caffeine dissolved in 1 ml of distilled water along with a small amount of table sugar. The mice were returned to their cages for thirty minutes to allow substance absorption, and at 7:30 pm, the mice were again individually introduced to the maze and the average of their completion times was recorded. At 8:00pm, the same process took place with control group #2, except piracetam was the administered variable rather than caffeine. At 8:30 pm, each of the five mice was individually put through the maze and their average completion time was recorded. During the five days of experimental testing, all environmental conditions remained the same, with the only change in variable being the introduction of either caffeine or piracetam. On Sunday 6 November 2011, experimental testing and data collection was completed, and the ten mice were returned to Petco in Aliso Viejo, California. During the collection period, the data were entered into the program Microsoft Excel. Using this program, the appropriate statistical analysis
tests were performed. A two-tailed, paired t-test along with an ANOVA test were conducted, and the p-values were appropriately evaluated to establish results.

**RESULTS**

The mean maze completion time for the group of mice receiving caffeine-enhanced water was significantly greater than the mean completion time of the control group of mice (two-tailed, paired t-test, \( p = 0.00179 \)) (Figure 1). The mean maze completion time for the group of mice receiving caffeine-enhanced water was significantly shorter than the control group (two-tailed, paired t-test, \( p = 0.00179 \)). The mean maze completion time between the control group, the caffeine group, and the piracetam group were all significantly different. The mean maze completion time for the group of caffeine-enhanced mice was significantly greater than the mean maze completion time for the piracetam-enhanced group of mice. An ANOVA test was conducted with \( p = 0.00431 \) and a Post-Hoc test was then conducted to establish a difference between the caffeine and piracetam groups, \( p = 0.005 \).

![Figure 1](image1.png)

Figure 1. The mean maze completion time for the group of mice receiving caffeine-enhanced water was significantly shorter than the control group. A two-tailed, paired t-test was conducted with \( p = 0.00179 \); error bars are mean ± SEM.

![Figure 2](image2.png)

Figure 2. The mean maze completion time for the group of mice receiving piracetam-enhanced water was significantly shorter than the control group receiving regular, plain distilled water. A two-tailed, paired t-test was conducted with \( p = 0.000738 \); error bars are mean ± SEM.
Figure 3. The mean maze completion time between the control group, the caffeine group, and the piracetam group were all significantly different. The mean maze completion time for the group of caffeine-enhanced mice was significantly greater than the mean maze completion time for the piracetam-enhanced group of mice. An ANOVA test was conducted with $p = 0.00431$. A Post-Hoc test was then conducted to establish a difference between the caffeine and piracetam groups $p = 0.005$.

DISCUSSION

The results gathered from the study not only prominently supported the initially proposed hypothesis, the data that were collected provided valuable results with the statistical significance to establish even further specific conclusions regarding the differences between experimental groups.

Similar results to those obtained throughout the study supporting the hypothesis were obtained in a 2002 study performed by Angelucci et al., on the effects of caffeine on learning and memory in rats tested in the Morris water maze. This particular research was extremely thorough and examined the differences between pre-training caffeine administration and post-training caffeine administration in rats, in correlation to maze completion time. The data obtained by the researchers provided evidence that caffeine improves memory retention, but not memory acquisition. These results coincide with those obtained during our study, and also provide a reasonable explanation for some of the discrepancies and contradictions found in other literature.

In a study performed by Diamond and Brouwers (2005), the researchers conducted a double-blind placebo-controlled study on the effects of Piracetam on verbal memory in healthy college students. The subjects were given 3x4 capsules at 400 mg per day for two weeks. Each of the subjects learned series of words presented as stimuli flashed on a memory card. Although no significant effects were observed after 7 days, it was found that after a two-week regimen of the drug, the student’s verbal learning and memory had increased significantly, nearly doubling from the results of the first 7 days of the trial. These data provide support for the results obtained through our study.

While the majority of data compiled in preparation for this study serves as support for the hypothesis, it is always critically important to seek out conflicting research, and in doing so, it improved overall conceptual understanding of our topic and helped build an appreciation for the opposing hypothesis. Most importantly, however, including non-supportive data forces to challenge and confront the research, which helps maintain drive, energy, and motivation to strive for better and more accurate results.

DISCUSSION:

- DISCUSS DATA
- DISCUSS CONCLUSIONS (IN MORE THAN ONE SENTENCE)
- RELATE INTRO...
A study conducted by Moran et al., (2002) at the Johns Hopkins University School of Medicine examined the effects of piracetam on cognitive performance in mice. This particular study took place over the course of eight weeks, and utilized the Morris water maze and correlation of maze completion times. The first four weeks of testing involved low dosages of piracetam administered to the mice, which were then put through the visible platform component of the maze. During the second four weeks of testing, the amount of piracetam administered to the mice remained the same, but the maze now consisted of various hidden platform components. These data obtained do not provide support for the hypothesis that treatment with piracetam will decrease maze completion time. These conflicting results, however, could very potentially be explained by differences in experimental procedures. Moran et al., (2002) administered a very low dosage of piracetam, and allowed the study to progress for a total of eight weeks, and included an extra set of variables by testing the maze uncovered and then covered, whereas in this study, an appropriate mass ratio of piracetam was administered to the mice, and the dry maze was tested unchanged for a total of ten days. These differences in experimental methods could very reasonably account for the conflicting results.

A promising study for patients suffering from Alzheimer’s Disease was conducted by Arendash et al., (2009) with the use of treating transgenic mice with moderate levels of caffeine. The study revealed that Alzheimer’s disease transgenic mice given a moderate level of caffeine intake (the human equivalent of 5 cups of coffee per day) were protected from development of otherwise certain cognitive impairment, and had decreased hippocampal amyloid-beta levels. At 4-5 weeks into the caffeine treatment, those impaired transgenic mice that were given caffeine exhibited vastly superior working memory compared to the continuing impairment of control transgenic mice. Even with pre-existing and substantial amyloid-beta burden, aged mice exhibited memory restoration and reversal of Alzheimer’s disease pathology, suggesting a treatment potential of caffeine in cases of established Alzheimer’s disease. These results obtained from Arendash et al., (2009) provide support for the initial hypothesis as well as the results obtained during the study. While this particular study did differ significantly with the use of Alzheimer’s transgenic mice, it helps to emphasize the significance of continued academic research, and provides a positive, hopeful reminder that effective scientific and medical advances are possible.

REFERENCES CITED


Review Form
Department of Biological Sciences
Saddleback College, Mission Viejo, CA 92692

Author(s): Good Nikuh, 3 Dinh

Title: The Effects of Caffeine and Piracetam on Mus Musculus in Relation to Maze Completion Times

Summary
Summarize the paper succinctly and dispassionately. Do not criticize here, just show that you understood the paper.

Caffeine and Piracetam are known to cause variable effects on cognition. The hypothesis stated that there would be a difference in maze completion time of mice exposed to Caffeine/Piracetam compared to their times without any variable substance. The experimental results illustrated that ingesting Caffeine/Piracetam lowered maze completion times. The difference in completion times could be linked to memory formation as discussed by the researchers.

General Comments
Generally explain the paper's strengths and weaknesses and whether they are serious, or important to our current state of knowledge.

Strengths: • Great research and introduction. Showed thorough knowledge of the variables in question.
• Accurate figures
• Literature cited properly

Weaknesses: • Data presentation and explanation
• Discussion only discussed this experiment in two or three sentences.
• No explanation of why the results are important.
• Only used an experimental population of
Technical Criticism
Review technical issues, organization and clarity. Provide a table of typographical errors, grammatical errors, and minor textual problems. It's not the reviewer's job to copy Edit the paper, mark the manuscript.

☐ This paper was a final version  ☐ This paper was a rough draft

- Some long run-on sentences could be pruned to two or more easier to read sentences
- Use the correct header and abstract formatting
- Not all figures are referenced in the text
- How does research relate to problem?
- The conclusion is not explained in the discussion.
- The discussion does not explain how the cited papers relate to this experiment