2015

Effects of Sleep Deprivation on Performance in a Water Radial Arm Maze (WRAM) Task

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Effects of Sleep Deprivation on Performance in a Water Radial Arm Maze (WRAM) Task

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science

By

Saline Rose Hughes
B.S., Wright State University, 2013

2015
Wright State University
WRIGHT STATE UNIVERSITY
GRADUATE SCHOOL

December 11, 2015

I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION
BY Saline Rose Hughes ENTITLED Effects of Sleep Deprivation on Performance in a Water
Radial Arm Maze (WRAM) Task BE ACCEPTED AS PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

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Abstract
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Effects of Sleep Deprivation on Performance in a Water Radial Arm Maze (WRAM) Task

Sleep deprivation causes many adverse effects on work performance. Many experiments in both human and rodent models reveal detriments that sleep deprivation has on learning and memory, including performance in a water radial arm maze (WRAM) task. This study utilizes the modified multiple platform method (MMPM) of sleep deprivation; rats were sleep deprived in order to study memory errors they may make during the WRAM task. The findings indicate that 6 hours of sleep deprivation for 2 five-day week periods did not affect performance in the WRAM task except on the initial day compared to the large platform group. The mRNA levels of BDNF and NGF were not changed. These findings are important since they help elucidate the relationship between sleep deprivation and environmental factors as well as supporting previous research conducted.
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Acknowledgements

I would like to thank my parents for listening to me during the time of writing my thesis. They were always trying to support me with encouraging words and taking the time to sit down with me and help me see the bigger picture. You did everything you could to help me finish this, even asking if there was anything you could do to help. Without your continued support I never would have made it through this.

I would like to thank my advisor, Dr. Ryan Jankord, for allowing me to be a part of his lab for this research project. You allowed me to learn the valuable lesson of designing an experimental project, and through that I have learned new ways and aspects to look at each question and the different components that are necessary to understand. You made sure that things were taken care of and supported me with encouragement near the end. Thank you for the opportunity and the skills that you have provided me with.

I would like to thank the following members of Dr. Jankord’s lab who assisted in the completion of this project and were great support when I needed to talk about the experiments: Dr. James Chambers, Mrs. Naomi Bechmann, Ms. Raquel Moore, Ms. Jessica Wagner, Dr. Milene Brownlow, Mr. Mark Jackson, and Mr. Kevin Schmidt. Their contributions and support are what drove this experiment and helped me to complete it. I would especially like to thank Dr. James Chambers for his work in making the platforms for the sleep deprivation tank, Ms. Jessica Wagner for being there to talk to when I needed the push, and Dr. Milene Brownlow and Mr. Kevin Schmidt for helping me see a bigger, more positive picture from my results.

I would like to thank the remaining members of my thesis committee, Dr. Larry Ream and Dr. Kimberly Carhuatanta for assisting me with my project. Dr. Kimberly Carhuatanta supported the idea before the experimentation had begun, and was essential in helping me with certain information related to sleep deprivation.
Introduction

Sleep

Sleep is a necessity for many forms of life including reptiles, insects, and mammals. In humans, it is believed that at least eight hours of nightly sleep is required for optimal function the following day\textsuperscript{30}. Sleep in mammals can be described in two phases comprising of non-rapid eye movement (NREM) and rapid eye movement (REM) sleep\textsuperscript{2}. NREM sleep is further subdivided into three different stages, each containing different waveform activity when measured by electroencephalogram (EEG)\textsuperscript{33}. Stage 1 is represented by alpha (8-13 Hz) and theta activity (3.5-7.5 Hz) and is considered to be the transition stage between wakefulness and sleep\textsuperscript{10}. Stage 2 differs from stage 1 in that the electrical activity is not as consistent and is also considered the point at which an individual has entered sleep. Stage 2 is also the stage where an individual is easily awoken by loud noises\textsuperscript{10}. Stage 3 is characterized by containing large delta activity (less than 3.5 Hz) and categorized as Slow-wave sleep\textsuperscript{10}. Stage 3 is when a person is in their deepest sleep and is where neurons will begin a brief resting period before rapidly firing. REM sleep is characterized by a rapid increase in eye movement, a form of muscle atonia (a paralyzed state of muscle relaxation), and a similar wavelength form of theta activity on the EEG\textsuperscript{13}. Sleep loss has been shown to affect humans in as little as a week; sleep loss causes an increase in reaction time, increased distractibility, forgetting known facts, increased difficulty in memorizing new
information, and increased chances of making a mistake or omitting information\textsuperscript{30}. Decreased reasoning happens not only that night of the sleep loss, but also on the following day. A higher level of stress is displayed as an increase in tiredness, drowsiness, and irritability. Work effectiveness decreases when subjects perform long, difficult, compulsory, monotonous, sitting activities in an unchanging environment with limited lighting, little supply of sound, and low motivation or little interest particularly at low points of the circadian rhythm. Shortened sleep is also correlated with a higher probability of developing diabetes and high blood pressure\textsuperscript{30}.

Sleep is important in many biological processes; a study dating back to the 1970s have reported that the process of RNA transcription is increased during sleep\textsuperscript{14}. Sleep is thought to promote mRNA translation, while an extended period of wakefulness can negatively impact families of genes that are critical in the protein translation process\textsuperscript{1}.

\textit{Sleep Disturbances}

Acute sleep deprivation is defined as a period during the day that disrupts the normal night of sleep\textsuperscript{30}. Acute sleep deprivation is defined as anywhere from 4-18 hours of wakefulness during a normal sleep cycle and tends to last for a brief period, normally one or two nights. Chronic sleep deprivation is an increased time of sleep deprivation, and has ranged as far as 264 hours in humans\textsuperscript{30}. The longest recorded chronic sleep deprivation in a scientific sleep study was for 205 hours\textsuperscript{30}.

Two of the focused types of sleep deprivation used in animal models are total sleep deprivation and REM sleep deprivation. In total sleep deprivation, the animal is deprived of both phases in their sleep cycle, while in REM sleep deprivation the animal is
allowed to enter NREM sleep, but is awoken when the animals have entered REM. The most frequently used method for REM sleep deprivation is the flower pot method. The flower pot method consists of placing a flower pot (or platform) upside down in a pool of water, leaving enough room for the animal to stand on the ‘platform’ without falling in the water. Once REM sleep has been attained, muscle atonia will cause the animal’s muscles to relax and make the animal unable to sustain posture allowing for an appendage to touch the water, shocking the animal awake. The flower pot method, while abolishing REM sleep, has also been reported to cause considerable loss of NREM sleep. The earliest use of the flower pot method was on cats placed on an upside-down clay pot; this methodology was later adapted for mice and rats. The technique was changed to the multiple platform method, which allowed the subject the opportunity to move around the sleep deprivation tank and reduce stress caused by isolation and immobility. Later, the technique was updated again to be called the modified multiple platform method (MMPM), which included the addition of other subjects from the same litter providing them with an added social component thereby reducing stress caused by social isolation.

In this study we examined the effects that sleep deprivation have on learning and memory. To investigate whether these effects exist, we looked at the neurotrophic factors brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) because of their involvement in the learning and memory process.
Behavioral Paradigm

The water radial arm maze (WRAM) is an updated version of the radial arm maze (RAM), and is meant to avoid the problems that are found with the appetitive RAM\textsuperscript{39}. In one protocol of the WRAM 1 arm is designated as the start and the other 7 are escapes containing submerged platforms at the end of the arms\textsuperscript{9}. When the animal finds a platform the animal and the platform are removed from the maze. The animal is made to wait and then returns to the maze for 6 more times until all platforms have been found. Another WRAM protocol consists of some arms containing platforms while other arms never contain platforms. This protocol allows for further testing of working and reference memory simultaneously and requires a heavier memory load on the animals subjected to the task\textsuperscript{3}.

Brain-Derived Neurotrophic Factor

BDNF is highly expressed in the hippocampus of the brain and is believed to play an important role in neuronal survival and memory and learning in adulthood\textsuperscript{8}. This neurotrophic factor is being examined because the water radial arm maze (WRAM) task is daunting and subjects need to utilize spatial navigational cues to successfully find the platforms. BDNF has been considered to promote growth and maintenance of dendrites and synapses while also enhancing the production and survival of new neurons\textsuperscript{23}. It is known that mice deficient in BDNF have impairments in spatial learning and their ability to recognize objects\textsuperscript{17}. 
**Nerve Growth Factor**

NGF regulates the survival and development of specific neuronal populations in the peripheral and central nervous systems\(^\text{38}\). NGF is thought to be important for long-term potentiation (LTP) mechanisms and is known to be elevated in the hippocampus of rats housed in environmental enriched conditions compared to isolated controls\(^\text{35}\). Modulation in NGF expression is known to influence hippocampal plasticity and behavior and it is believed to modulate neuronal plasticity\(^\text{12}\). It is known that the disruption of a single NGF allele can cause deficiencies in memory acquisition and hippocampal cholinergic innervation, indicating that NGF is involved in the formation and maintenance of nerve signaling\(^\text{32}\).

**Conclusion and Hypothesis**

For these experiments the effects that six hours of sleep deprivation have on cognitive performance in a WRAM task and the mRNA transcript levels of BDNF and NGF were determined. Based on a previous study\(^\text{31}\), four hours of sleep deprivation following the WRAM task would affect the memory consolidation window of learning.

Many experiments previously performed utilizing the flower pot method sleep deprived either rats or mice and showed significant differences in the results when it came to performance in a behavioral task\(^\text{4,22,41}\). During a rapid-eye movement (REM) sleep deprivation (RSD) experiment, rats were connected to a polygraph and computer that read when the subjects would enter REM sleep and then gently stroke their backs with a brush to wake them\(^\text{31}\). In this experiment, NGF was found to decrease in the hippocampus of RSD rats, but not in the cerebellum or brainstem, while BDNF showed a
decrease in the RSD rats in the cerebellum and brainstem, but not in the hippocampus.

We are examining the effects of sleep deprivation on performance in a water radial arm maze (WRAM) behavioral task.
Methods

Animals

Male Sprague-Dawley rats (Charles River, Wilmington, MA) between 300-600 g were used for this study. Animals were quarantined for 10 days upon arrival at an AAALAC (Association for Assessment and Accreditation of Laboratory Animals) accredited animal facility and were paired housed with ad libitum access to food and water. Rats were moved into single housing after quarantine under a 12:12 light/dark cycle. All testing was conducted during the light phase. All procedures were approved by the Wright-Patterson Air Force Base Institutional Animal Care and Use Committee and were performed in accordance with the National Institute of Health standards and the Guide for the Care and Use of Laboratory Animals.

Sleep Deprivation Paradigm

The sleep deprivation paradigm was altered between experiments to address different hypotheses. In both experiments, the sleep deprivation tank (194 cm diameter x 73 cm tall) contained both small platforms (SP; 7 cm diameter) and large platforms (LP; 14 cm diameter, shown in Figure 1). Platforms were spaced 7 cm apart from their bases so the rats were still able to move between platforms. Each platform was 2 cm above water level, allowing the subject to sleep until the onset of REM.
sleep, which lead to muscle atonia and causing the subject to fall into the pool. The sleep deprivation pool was split into two sections by a plexiglass divider, which separated the large platform rats from the small platform rats; the animals were able to see through the divider. Room temperature (22°C ± 1) was generally constant, but a problem occurred with the animal facilities heating and there were three days where the room temperature was much lower (16°C ± 1). This event caused water temperature to be cooler on those days, while it remained at room temperature during normal circumstances.

Figure 1: Representation of the Sleep Deprivation tank set-up

Water Radial Arm Maze (WRAM) Protocol

All animal groups (cage control, large platform control, and small platform experimental) were tested for working and reference memory performance in the WRAM. Each rat was tested for four trials a day with a 30 second interval between each trial before being returned to their home cage (experiment 1) or placed back in the sleep
deprivation pool (experiment 2; LP and SP). Each trial lasted for a maximum duration of 2 minutes; if a rat was unable to find a platform they would be lead to a platform and made to sit for 10 seconds before being removed from the maze arm. Once a platform was located, that platform was removed from the maze for the remainder of the four trials. Platforms were placed in the same arms during the entire experimental procedure (Figure 2) and all rats started their trials in the East arm facing the wall. Working memory errors were defined by a rat entering an arm where a platform was located and then leaving, and when a rat entered a platform arm that they had previously found in one of the previous trials. Reference memory errors were defined as the entrance of the rat into one of the arms that a platform never existed in. The WRAM apparatus that was utilized in both experiments was an octagonal structure with platforms in four of the eight arms (goal arms).

Figure 2: Water Radial Arm Maze Set-Up. * = platform arm, ^ = starting arm.
**Euthanasia**

For both experiments, means of euthanasia was by rapid decapitation. Tissue dissection followed the rapid decapitation method to ensure the integrity of the samples. Tissue samples were frozen immediately after dissection.

**Transcript level expression**

Following euthanasia, brains were removed from the skull and tissue was sectioned on a rat brain matrix (Zivic Instruments). The hippocampi were split into left and right halves and the cerebellum and brainstem were dissected, placed in an RNase free tube, and immediately moved onto dry ice and stored at -80°C. Tissue samples were homogenized and allowed rapid disruption to fully release molecules using the TissueLyser II (Qiagen). RNA extraction was conducted using the RNeasy Mini Kit (Qiagen) and followed the manufacturer’s protocol. RNA quality was measured using the Qubit 2.0 fluorometer (Life Technologies) and the concentration was used to normalize the samples for cDNA synthesis. The High Capacity RNA to cDNA kit (Applied Biosystems) was utilized for synthesizing 100 ng of RNA into cDNA. The cDNA was taken for quantitative real-time PCR (q-PCR, Figure 3) which was run using Fast SYBR Green Master Mix kit protocol (Applied Biosystems, Figure 4) to assist in the detection of PCR products when run through the StepOne Plus PCR Machine (Applied Biosystems).
Figure 3: Outline of steps involved with qRT-PCR.

The primers (Eurofins MWG Operon) utilized for the reaction were as follows:

Hprt1 forward 5’GACCAGTCAACGGGGGACAT 3’ and reverse
5’GGGGCTGTACTGCTTGACCA 3’, EDA forward
5’AGTAGGCGTGTTCGCCGCAA 3’ and reverse 5’GTCCCTGGGGTCCTGGAGGT 3’, BDNF forward 5’TAAGAGTCTAGACCTTGGGGAC 3’ and reverse
5’TGGTGGAACTTTTTCAGCTACCTTGGGAC 3’, and NGF forward
5’AAGGGGAGCGCATCGAGTTTT 3’ and reverse
5’CCTTTATTGGGCCCAGACACT 3’. Melt curve analysis was used (StepOne Plus, Applied Biosystems) for confirming reaction integrity. All reactions had a single peak in melt curves indicating a pure product. Fold changes were calculated using ΔΔCt Comparative method with endogenous control value created from averaging Ct values of Hprt1 and EDA.
Validation of Primers

Primers were chosen using Primer Blast (NCBI) and were only considered candidates if they met the following criteria: GC pairing below 60%, T_m temperature around 60°C, and the primer needed to extend over two exons. A minimum of 4 primers were tested for performing in the optimization experiments. Initially, primers were tested at varying temperatures (55°C-65°C) to identify maximal performance based on the lowest C_t value. The resulting melt curves were examined to determine if primers yielded a single product. Primers that passed the listed criteria were put through a serial dilution PCR experiment to analyze the efficacy of each reaction. Primers were chosen if they had an efficacy value between 90-100%.

ΔΔC_t Comparative Method

Analysis of the mRNA data utilized the ΔΔC_t method for determining the differences in fold changes. This method compares endogenous control genes and target
genes to analyze the fold change between the groups. Each sample was normalized to their endogenous control threshold value \((C_{t})\). The \(C_{t}\) value is the value specifying when the reaction begins the exponential phase. The endogenous control value was determined as the average of the Hprt1 and EDA \(C_{t}\) values. The normalization equation is as follows:

\[
\Delta C_{t} = C_{t} \text{ target gene} - C_{t} \text{ endogenous control}
\]

The result of the above equation is the \(\Delta C_{t}\) value. The next normalization is compared against the average \(\Delta C_{t}\) of the designated control group; in experiment 1 this was the large platform group and in experiment 2 it was the cage control. The equation to calculate this value is:

\[
\Delta\Delta C_{t} = \Delta C_{t} \text{ target gene (treatment group)} - \Delta C_{t} \text{ endogenous control (control group)}
\]

This value represents the difference in \(\Delta C_{t}\) values of the desired gene in both control and treatment groups. When calculating the fold change, the following equation was used:

\[
Fold \ Change = 2^{-\Delta\Delta C_{t}}
\]

Outcomes that were greater than two standard deviations away from the group mean were removed from future analysis. An \(n = 16\) was utilized for the right hippocampus, but an \(n = 15\) was utilized for the brainstem and cerebellum due to inefficient RNA concentration during the isolation process.

*Melting Curve Analysis*
Analysis of the PCR reaction’s integrity was done through a melt curve analysis to verify that there was one pure product. In the final stage of a PCR reaction all copies of transcript are in double strand form, meaning the SYBR green is bound and there is high fluorescence (Figure 4). The melt curve takes a fluorescence measurement every .3°C and increases from 65°C to 95°C. The program indicates a melting temperature when the fluorescence falls suddenly, noting the double stranded DNA has dissociated. This technique is standard protocol when using SYBR Green fluorescent marker for q-PCR reactions.

Experiment 1

Rats in Experiment 1 were assigned into two groups: (1) the large platform controls (Con, n = 8) and (2) the small platforms (SP, n = 8). Initial rat body weights were measured three days before the start of the experiments to later quantify if the 2 hour habituation period was stressful. All animals underwent the 2 hour habituation period to habituate them with the environment. Body weights were measured before each day’s experimental design to determine whether our sleep deprivation protocol would elicit stress-induced effects on body weight (Figure 5). Body weights were converted into percentages as a better way to analyze estimated growth over the experiment. At 0730, rats were removed from their home cages and placed into the sleep deprivation pool either on the large platforms or the small platforms (according to their grouping) where they remained for 6 hours. After 6 hours, the rats were removed one by one (based on a numerical order) and placed into the WRAM test. To evenly distribute sleep deprivation times, the order for the WRAM testing was altered each day to accommodate for the difference in duration animals were kept in the restriction tank and placed back in their
home cages while conducting behavioral testing. Once an animal had undergone all four trials they were returned to their home cages and had the opportunity to sleep for 16-18 hours. This procedure was repeated for 10 days. On the final day, rats were not submitted to WRAM testing and were instead euthanized immediately following 6 hours of sleep deprivation.

Figure 5: Representation of the study design for Experiment 1.

Experiment 2

Rats in Experiment 2 were assigned into three groups: (1) the cage control (CC, n = 8) who remained in their housing room until the time of behavioral testing and were then returned to their housing room following testing, (2) the large platform (LP, n = 8), and (3) the small platforms (SP, n = 8). The number of platforms on the small platform side was increased to 25 and the large platforms were increased to 20 in order to help in keeping a hierarchy from forming among the animals. At 0730 animals from the LP and SP groups were moved into the sleep deprivation tank for an hour in order to prevent them from sleeping. Following this hour, all animals were tested in the WRAM and then CC rats were returned to their housing room while the LP and SP rats were placed back
into the pool to undergo at least 4 hours of sleep deprivation (Figure 6). This procedure was repeated for 10 days. On the final day, rats underwent euthanasia following their 4 hours of sleep deprivation.

![Figure 6: Representation of the study design for Experiment 2.](image)

**Statistical Analysis**

All statistical analysis was completed using SigmaPlot (Version 13.0) and a two-way repeated measures ANOVA was performed to test group differences in body weight percentages, food intake, WRAM working and reference memory errors (Day and Trial). A two-tailed unpaired t-test was run to test group differences between mRNA fold changes. If normality failed, a Mann-Whitney rank sum test was done to analyze the results. Significance was designated as p-value < .05.
Results

Experiment 1

Animals and their food were weighed immediately before the start of the experimental condition each day (Figure 7), starting with the initial two hour habituation period three days prior to the behavioral paradigm. For this experiment, the body weights were converted into a percentage of initial body weight so that the overall health of the subjects could be observed. Subjects with a body weight ≤ 75% control body weight were intended to be removed from the study, but no subjects needed to be removed. A steady increase in body weight was observed, and food intake remained around a steady 10-20g of food on average per day except for the initial starting day following the weekend. No difference was observed between groups for the body weight percentages $p = 0.75$, or for the food intake $p = 0.76$. 
Animals were placed in the sleep deprivation tank for 6 hours during their light cycle before they were exposed to the WRAM behavioral task where their mistakes in finding the platforms were counted as either a working memory error or a reference memory error. As previously described, a working memory error counted as an entrance, where at least half their body went into an arm that no longer had a platform, or when/if they did not swim down to the platform. A reference memory error counted as an entrance into an arm where a platform never existed. On the initial day of the experiment, the small platform group showed a significant difference (Figure 8A, SP vs. CON $p = 0.03$) when compared to the large platform controls, but overall main group effect showed no significant differences ($p = 0.79$). No significant differences were found for main group effect for average reference memory errors (Figure 8B, $p = 0.28$). To better understand which trials the subjects found most difficult, individual trials for each day...
were analyzed (Figure 8C and 8D). By the behavioral experimental design, the subjects should have been experiencing difficulty during the later trials (3rd and 4th) according to the WRAM task chosen. There were no significant differences in main group effect for the working memory errors overall (Figure 8C, p = 0.79). During specific trials there was significance found between the two groups for working memory errors (Trial 3: SP vs. Con p = 0.001, Trial 8 Con vs. SP p = 0.001, Trial 16: SP vs. Con p = 0.01). The reference memory error trials were also examined for if there were any points during the trials where the subjects were experiencing difficulties. Overall, there were no significant differences in main group effect for reference memory errors (Figure 8D, p = 0.28), but on Trial 11 there was a significant difference found between group performance (SP vs. Con p = 0.02).

A) Mean Daily Working Memory Errors

![A) Mean Daily Working Memory Errors](image-url)
B) Mean Daily Reference Memory Errors

C) WRAM WME
Different regions of the brain were studied for any responses in BDNF or NGF expression from sleep deprivation. The right hippocampus, cerebellum and brainstem were analyzed through q-PCR experiments (Figure 9). The right hippocampus (Figure 9A) and cerebellum (Figure 9B) showed no differences between groups for BDNF (p = 0.78, p = 0.96; respectively) or NGF (p = 0.90, p = 0.68; respectively). The brainstem (Figure 9C) showed trend for decrease for both BDNF and NGF, but there were no significant differences between the groups for either neurotrophic factor (BDNF: p = 0.08, NGF: p = 0.19).

Figure 8: Water Radial Arm Maze results. A) Working Memory Errors by Day, B) Reference Memory Errors by Day, C) Working Memory Errors by Trial, D) Reference Memory Errors by Trial. *= p < .05 vs. Con.
Experiment 2

Body weights were measured at the beginning of each day and converted into percent to evaluate the change in body weights over the course of the experimental procedure. In this format, we were able to assess whether an individual had become unhealthy from a dramatic drop in percentage body weight. If a subject fell to a body weight ≤ 75% of the control group, then they were removed from the experiment. Body weights for main group effect showed no significant differences (Figure 10, p = 0.054);
however, on days 4 and 11, a significant difference (Day 4: CC vs. LP $p = 0.02$, CC vs. SP $p = 0.03$. Day 11: CC vs. LP $p = 0.02$, CC vs. SP $p = 0.03$) was found between the control group and the large platform, as well as the small platform groups.

Figure 10: Body Weight Percentages. * = $p < 0.05$ vs. CC.

Subjects were exposed to the WRAM behavioral test one hour into their sleep deprivation and then placed back into the tank to be sleep deprived for at least 4 hours.

The WRAM test was run for 10 days and subjects were tested on their working memory and reference memory errors (Figure 11A and 11B, respectively). The overall main group effect for working memory errors showed no significant differences ($p = 0.16$), but a significant difference between the cage control and large platform ($p = 0.001$) and the cage control and small platform ($p = 0.002$) groups was found on the third day of testing.

Data analysis revealed a significant difference in main group effect for their reference
memory errors (p < 0.001). Surprisingly though, it was only during day three that data showed significant differences between the cage controls and large platforms (p < 0.001) and the cage controls and small platforms (p = 0.003). Data for the individual trials was also analyzed to examine the exact trials during a day (4 trials per day) that subjects may be experiencing significant errors in both working memory and reference memory errors (Figure 11C and 11D, respectively). The working memory errors by trial showed no significant difference in main group effect (p = 0.16), but during Trial 8 (CC vs. SP p = 0.003, LP vs. SP p = 0.004), Trial 16 (CC vs. SP p < 0.001, CC vs. LP p < 0.001), and Trial 20 (CC vs. SP p < 0.001, CC vs. LP p < 0.001) significant differences were found between platform groups and the cage controls. The reference memory errors by trial revealed a significant difference in main group effect (p < 0.001). Closer analysis also revealed significant differences between the platform groups and the cage controls during Trial 2 (LP vs. CC p < 0.001, LP vs. SP p < 0.001), Trial 3 (CC vs. LP p < 0.001, CC vs. SP p = 0.003), Trial 8 (LP vs. CC p < 0.001, LP vs. SP p = 0.003), and Trial 16 (CC vs. SP p = 0.002, CC vs. LP p = 0.01).
Figure 11: Water Radial Arm Maze results. A) Working Memory Errors by Day, B) Reference Memory Errors by Day, C) Working Memory Errors by Trial, D) Reference Memory Errors by Trial. * = p < .05 vs. Con, ^ = p < .001 vs. Con.
Discussion

The aim of this study was to determine whether sleep deprivation had adverse effects on performance in a behavioral task. From Experiment 1, we noted that there were no significant differences in mRNA transcript for BDNF and NGF for the right hippocampus, cerebellum, and brainstem; however, there was a trend for a decrease in both neurotrophic factors in the brainstem. Also there was an adverse effect on working memory errors in the small platform group with the initial day of the WRAM task, but no significant difference for the remainder of the experiment. Following this, in Experiment 2 a method was devised that would test for a memory consolidation window found during a previous study. While it appeared that the six hour sleep deprivation did not cause any significant differences on performance, the first experiment showed the possibility that an acute exposure to sleep deprivation may be enough to cause an initial acquisition difficulty in a spatial memory task. Experiment 2 also showed a decrease in percent body weight, which is to be expected when using the MMPM procedure. This decrease in weight could also be attributed to the age of the rats in Experiment 2 and the possibility of their weight reaching a plateau.

Environmental Enrichment

Sleep deprivation is thought to have negative effects on learning and memory in subjects. The above experiments showed that in most cases, the small platform experimental group performed significantly better than the cage control group. This could
be due to many different aspects of environmental enrichment (EE) that occurred throughout the experimental procedure. The CC subjects remained in their home cages until the behavioral testing, while the LP and SP subjects were introduced to a new, open environment that contained unfamiliar objects (the platforms).

An aspect of EE that may have benefited the small platform subjects was that there were multiple subjects of the same species and same litter in the sleep deprivation tank leading to social buffering. Kiyokawa et al. showed that rats raised together and placed together in an environment buffered the conditioned fear responses expected during an auditory conditioned stimulus. This effect could explain the small platform group, which contained 8 subjects in the same area, performed significantly better than the control group, which was single housed, on occasions throughout the second experiment.

By providing the small platform group with a social environment and the ability to move on multiple platforms, the stress levels experienced during normal sleep deprivation could have been reduced. The ability to move around could be viewed as a form of exercise, which has been shown to have beneficial effects on cognition in sleep deprivation.

Memory Consolidation

In Experiment 1, a significant difference was found between groups during the initial day of sleep deprivation and the behavioral test. This difference disappeared during the following days and did not return for the remainder of either experimental procedure. Since animal subjects have the capability to be sleep deprived around the clock it makes
some of the studies different from human studies. Humans generally are sleep deprived
during the day and are then subject to social constraints that make them unable to
compensate for the sleep that was lost from the previous night\(^\text{25}\). The difference in ages
between the rats in each experiment does not affect their cognition as they are both still young adults.

A study using the flower pot method where the rats were sleep deprived for 18 hours for 21 days and allowed to sleep for 6 hours each day and found that during the 6 hour sleep window subjects showed an increase in percentage of sleep time, which was reflected by an augmentation in high amplitude slow wave sleep and in paradoxical sleep\(^\text{24}\). This study also showed that the subjects tended to awaken fewer times and stayed awake for fewer minutes, and the animals exhibited a monophasic-type sleep pattern. Thus, it may be possible that the rats in our experiments exhibited a similar change from a polyphasic sleep pattern to a monophasic sleep pattern during their 18-hour recovery sleep period. It is during this monophasic sleep pattern that the subjects may have been consolidating their memory from the WRAM task.

*Behavioral Test*

In the behavioral experiments, the WRAM task was utilized as the behavioral task for testing the rats learning and memory capabilities. As shown, there were no apparent detriments found from the sleep deprivation group for these experiments. An explanation for this observation could be that the WRAM task in the specific set-up utilized may have been too easy for the subjects. A different WRAM protocol that was paired with the MMPM for sleep deprivation has shown significant differences in their sleep deprivation
group’s performance under both 8 and 24 hour sleep deprivation times\textsuperscript{4,5}. In these experiments the WRAM protocol consisted of platforms in 7 of 8 arms and 12 training trials before being subjected to the sleep deprivation protocol. An experiment was conducted that used the radial arm maze (RAM) behavioral test that was followed by blocks of 4 hour sleep deprivation for the next 24 hours and showed that there might be a memory consolidation window for learning a trained task\textsuperscript{21}.

The use of sleep deprivation and fear conditioning has shown mixed results. In one study, rats deprived of sleep for 6 hours impaired extinction of conditioned fear\textsuperscript{37}. Another experiment showed that 6 hours of sleep deprivation had no effects on the reconsolidation of both cued and contextual fear memory\textsuperscript{34}. In a similar fashion, sleep deprivation was paired with the Morris Water Maze (MWM) with mixed results. In a study where the subjects were deprived of sleep for 6 hours, neither spatial or reversal learning are resistant to the sleep deprivation\textsuperscript{40}. Another study subjected male and female rats to 72 hours of sleep deprivation and the MWM test\textsuperscript{15}. This study showed that the 72 hours of sleep deprivation did not affect spatial learning and short-term memory in male rats, but significantly impaired the performance of intact and ovariectomized female rats.

\textit{Moving Forward}

In this study the effects of sleep deprivation on learning and memory performance in a WRAM task were investigated. This study was limited by the amount of sleep deprivation that was experienced and the single behavioral task. Other variables such as light cycle in the animal facility and the start time of experiments and the addition of social interaction for cage control animals should be considered in future experiments.
Another aspect to consider for future experiments is to implant EEG compatible electrodes into the rats to record the stages of sleep experienced during the sleep deprivation and how long the rats remain in each stage.

Following the results collected during these experiments and based on previous studies, the next step would be either to try a full 24-hour sleep deprivation or for a prolonged period of 8-hour sleep deprivation. Another possibility would be to study the effects of acute sleep deprivation lasting for a single night to better understand the results seen in Experiment 1 where on the initial day of the experiment the a significant difference was found between groups. This finding could possibly elucidate whether acute sleep deprivation acts on acquisitions mechanism involved in learning and memory.
Conclusion

Taken together, the results of this study shows that under acute sleep deprivation for 6 hours, no memory deficits appeared. After 6 hours of sleep deprivation for 2 weeks, there appeared to be no changes in BDNF and NGF mRNA levels in the hippocampus, cerebellum, or brainstem. The results indicate supportive evidence in the theory of social buffering, and that the MMPM is still a stressful paradigm of sleep deprivation. Both experiments also indicate supportive evidence in the theory of memory consolidation and the rats transitioning from polyphasic sleep to monophasic sleep patterns. Based on the evidence provided through both experiments, we can speculate that the sleep deprivation paradigm used may have inserted beneficial stress into the rat’s lives. Further studies need to be done to assess the acute effects of sleep deprivation on BDNF and NGF levels in the brain, or whether sleep deprivation affects learning and memory.
References


