EFFECT OF SLEEP DEPRIVATION ON LEARNING STRATEGIES IN THE MORRIS WATER MAZE

By

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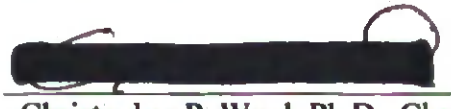

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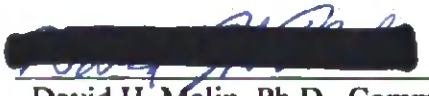

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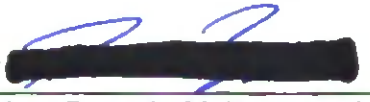

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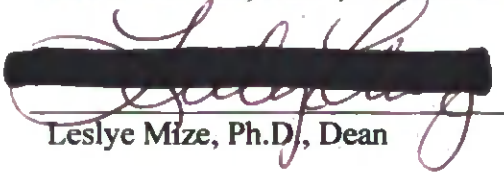

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ABSTRACT

EFFECT OF SLEEP DEPRIVATION ON LEARNING STRATEGIES IN THE MORRIS WATER MAZE

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Sleep deprivation may result in learning and memory impairment. The present study seeks to explore the effect of sleep deprivation on learning and memory in a spatial memory task by utilizing the Morris Water Maze. In the pilot study, Sprague-Dawley rats ($N = 12$) were randomly assigned to either the sleep deprived group or the control group. Through gentle handling, the sleep-deprived group was kept awake for a 6- hour period prior to testing. While the sleep-deprived rats were slower to learn the task and locate the hidden platform, the difference between groups was not robust. In the second experiment, Sprague-Dawley rats ($N = 24$) were randomly assigned to either the sleep deprived group or the motor control group. All rats were housed in a motorized wheel, with the sleep-deprived group being kept awake for a 6-hour period of time by movement for 3 seconds on and 12 seconds off. Motor control animals moved for the same distance but were allowed a rest period of 2 hours 24 minutes between movement episodes. Acquisition of the water maze task was not affected between groups, nor was search

strategy utilized different. In the third experiment, sleep deprivation time was increased to 24 hours, while all other protocols remained the same as in experiment two. No significant differences were noted between the sleep-deprived and control groups.

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CHAPTER I

INTRODUCTION

Changing sleep habits in society along with sleep problems associated with a variety of disorders are important reasons to study the role sleep plays in learning and memory (Van Der Werf et al., 2009). It is estimated that we spend almost one-third of our lives sleeping. Yet, the function of sleep remains relatively unknown. There are, however, several hypotheses regarding the contribution of sleep. These include the idea that sleep contributes to energy conservation (Berger & Phillips, 1995), serves in brain thermoregulation (McGinty & Szymusiak, 1990), aids in brain detoxification (Inoue et al., 1995) and aids tissue restoration (Adams & Oswald, 1977). One hypothesis that is debated rather vigorously is the idea that sleep contributes to learning and memory. There is a large body of research to support the claim that sleep plays a significant role in this area. According to Maquet (2001), various sleep stages partake in the consolidation of memory. Most often memory consolidation refers to the process where memories become more stable and are less susceptible to outside interference from competing factors (McGaugh, 2000). It is thought that the information we acquire when awake is actively altered, restructured and strengthened when we are asleep (Peigneux et al., 2001). There are, however, detractors whose body of research suggest that sleep may not play any role in the consolidation of memory (Vertes & Eastman, 2000; Siegel, 2001; Vertes, 2004; Frank & Benington, 2006).

Sleep & Sleep Stages

Sleep is divided into two types: non-rapid eye movement (NREM) and rapid eye movement (REM). Each of these types of sleep have distinct characteristics. NREM sleep is divided into three stages with the last of these stages also referred to as slow-wave sleep (SWS). During the first stage (N1) of NREM sleep characteristics include the experience of strange noises, lights, or sensations. This is known as hypnogagic hallucinations. Random twitches in the skeletal muscles, also called hypnic jerks, may also be experienced. N2 sleep is characterized by a loss of consciousness where the individual is no longer aware of outside influence. N3 is the last stage of NREM sleep. At this stage, very deep sleep is experienced and it is quite difficult to wake someone during this time. REM sleep is the period where dreams occur. Often referred to as paradoxical sleep, brain activity is heightened while muscle paralysis occurs (Silber et al., 2007).

During the night, humans cycle through non-REM and REM sleep about every 90 to 110 minutes. This cycle remains relatively stable throughout the night, but SWS appears to predominate during the first part of sleep while REM is more abundant during the second portion of the night. With the aid of the electroencephalograph (EEG), researchers have been able to "see" the electrical activity that takes place in the sleeping brain. The large, sharp waves at stage 2 non-REM, as well as the low frequency waves characteristic in stage 3, and the high frequency activity during REM sleep that is very similar to the waking state indicate that sleep is more than a just a static, consistent state. (Llinas & Ribary, 1993).

Memory & Memory Stages

Like sleep, memory is multidimensional. Human memory is divided into declarative and non-declarative types (Tulving, 1985). Declarative memory consists of fact-based information that is consciously accessed. Episodic memory and semantic memory are subcategories of declarative memory. Episodic memories allow us to recall events of personal importance. These memories can be general or very specific. Semantic memories are memories associated with general knowledge that is not associated with a specific event. Non-declarative memory, however, could be considered as our unconscious memory. Also referred to as implicit memory, this occurs when prior experience aids in performing a task without our being consciously aware of the prior experience. This includes procedural memory like learning an action or a skill such as riding a bike or driving a car. Priming is also seen in implicit memory. Here an individual can show improvement on a task for which they have been subconsciously prepared (Schacter, 1987).

Evidence for the Role of Sleep in Memory Consolidation

Evidence for sleep-dependent processing of memories has been found in many species. Early studies investigating sleep and memory focused primarily on declarative memory tasks. In a study by DeKoninck et al. (1989) participants went through very intensive foreign language learning. It was reported that there was an increase in REM sleep after learning. The degree of success at recalling what was learned was correlated with the increase of REM sleep. These findings suggest that REM sleep plays an active role in memory consolidation. In addition, Stickgold & Walker (2005, 2007) suggest that

both REM and non-REM sleep play a role in memory consolidation when memories are of a complex nature or are of emotional importance.

Likewise, there exists a large body of research on the importance of sleep in consolidating procedural memory. For example, Fischer et al. (2002) examined the effect of sleep deprivation on the learning of a procedural task that involved typing a combination of digits. After training, where all participants performed similarly on the task, some participants were allowed to sleep for 8 hours while others were not allowed to sleep. On follow-up, the subjects who were allowed to sleep performed significantly better on the task than did the sleep-deprived participants. In another study, 51 subjects followed a defined route located in an unfamiliar neighborhood. After walking the route, they were tested on a sequence-recognition task. Then subjects were placed in one of three groups: sleep group, sleep deprived group, or day-control group. The sleep group was retested after one-night's sleep, the sleep deprived group was retested after not being allowed to sleep for one night and the day-control group was retested on the same day 8 hours later. It was reported that all group performance speeds increased at retest, but the accuracy in the sequence recognition task was improved only in the sleep group. These findings suggest a role sleep plays in spatial memory consolidation (Ferrara et al., 2006).

In rodents, Smith & Rose (1996) suggest a window of 5-8 hours following the learning of a new task whereby sleep is necessary for the consolidation of memories. In this study, three groups of animals were trained in the Morris Water Maze. Group 1 was sleep deprived for four hours immediately following the training trial, Group 2 was sleep deprived for four hours beginning at the fifth hour after training, and Group 3 was sleep deprived for four hours beginning at the ninth hour after training. On the second day of

training, the animals in Group 2 took significantly longer to locate the platform in the water maze when compared to the other two groups. It was reported, however, that the learning deficit appeared to affect only Day 2 training. In a later study (Smith & Rose, 1997) it was reported that the sleep window appeared to be tied to several factors, including the nature of the task, the strain of animals and the number of trials per session. In the follow-up study, a single training session of 12 trials was followed 24 hours later by a 4 trial session. Here it was discovered that animals that were sleep deprived during 1-4 hours post training showed significant deficits in locating the platform. Bjorness et al. (2007) reported similar findings in their study. In this study, the eight-box spatial task was utilized. This is a spatial task that requires animals to learn the locations of food placement in three of eight available box choices. The boxes are located on a rectangular track with cues such as posters or other colored objects surrounding the maze. For this experiment, animals were REM sleep deprived at either the 0-4 hour period or 4-8 hour period after testing. While it was reported that both groups of sleep deprived animals eventually reached the same level of performance, it was noted that the 0-4 hour group were delayed in improvement on the task.

Arguments Against the Role of Sleep in Memory Consolidation

It should be noted that some researchers suggest that findings for the role sleep plays in memory consolidation are weak, faulty and contradictory. Siegel (2001) states that, while sleep is important, any significant role it may play in memory consolidation is unproven. He suggests that, instead, in many of the animal studies that indicate an increased amount of REM sleep after learning a new task it could be stress and frustration that are playing a role in this increase. It is argued that moderate levels of stress can

increase REM sleep and if stress is not accounted for, there is a confounding variable. Because it is difficult to separate stress from learning in animal studies, it also makes it difficult to determine which event causes the increase in REM sleep.

Probably the most compelling evidence against the role of sleep in memory consolidation is that of subjects who take antidepressants. Most antidepressants suppress REM sleep, sometimes for months after beginning the medication, yet these people have no deleterious effects on memory and learning (Vertes & Eastman, 2000; Vertes, 2004).

Frank & Benington (2006) suggest that, despite positive findings on the role sleep plays in memory consolidation, approaches that can combine both the behavioral and neuropsychological aspects of memory may help to resolve existing disputes.

Sleep Deprivation Prior to Learning

While the vast body of work has focused on the effect (or lack of an effect) sleep has on memory consolidation, very little research has been conducted that deals with the effect of sleep deprivation prior to learning. It is reported that being sleepy prior to learning appears to cause a deficit in forming new memories. Yoo, et al. (2007) suggests that sleep prior to learning is critical and functions to help the brain prepare for consolidation of memories. Subjects in this study underwent testing whereby they were shown a series of pictures in the encoding session. Subjects in the sleep-deprived group received a total of 35 hours of sleep deprivation prior to the initial encoding session. All subjects returned two days later and were shown some of the original pictures and some new pictures. On the fourth day, after two nights of recovery sleep, all participants were retested on the original pictures from the encoding session. It was found that, on day 4

retest, subjects in the sleep deprived group performed significantly worse than their counterparts in the sleep control group. These results appear to suggest a deficit in the ability to encode new human memories when sleep deprived. Gibson et al. (2006) found sleepiness in adolescents can be a major obstruction to learning. Through use of a survey and utilization of the Epworth Sleepiness Scale, it was discovered that a significant number of adolescents slept less than 8.5 hours per night during the week. Sleep-deprived students showed decreases in grades because of excess sleepiness reported in the classroom combined with lack of sleep prior to learning. It was also noted that early morning was the most difficult time for students to absorb and recall new information. Another study noted that the quality and quantity of sleep had an impact on the learning capacity and academic performance of students. In addition to learning, behavior control was also impacted (Curcio et al., 2006).

In rats, Guan et al. (2004) found that total sleep deprivation for 6 hours prior to testing on a spatial learning task in the Morris Water Maze produced a significant deficit when retested 24 hours later. This suggests that, while initial spatial learning was not impaired by sleep deprivation, spatial memory was impacted. In a similar study, Youngblood et al. (1997) also found impairment in spatial memory in rats. In both studies, decreases in hippocampal activity suggest a correlation with spatial memory. Sleep deprivation in humans also shows a decrease in activity of the hippocampus (Yoo et al., 2007). In a study on the effect of sleep deprivation in spatial reference memory and spatial working memory, Ward et al. (2009) found that sleep deprivation prior to testing impaired the ability to retain spatial reference memory but did not the impair the initial acquisition of spatial reference memory. According to Tartar et al. (2006), spatial

learning was shown to be impaired after 24 hours of sleep fragmentation. In testing with the Morris Water Maze, it was found that sleep impairment prior to learning trials served to interfere with the acquisition of a spatial learning task. In the study, the sleep-impaired rats increased their swim distance to the target and had poorer performance on the probe trial than did the control animals.

The Hippocampus & Memory

The importance of the hippocampus in the processing of memories is widely recognized. It is thought to be the early storage center for long-term memories and is involved in spatial navigation (Neubauer, 2009). Sleep deprivation can have a significant impact on cell proliferation in the hippocampus, causing the possible disruption of memory formation. To study this, Guzman-Marin et al. (2003) utilized 3 groups of rats (sleep deprived, treadmill control, and cage control) for a period of 4 days. The first two groups were housed in a treadmill apparatus whereby the sleep-deprived group had the treadmill activated for 3 seconds on and 12 seconds off and the treadmill controls for 15 minutes on and 60 minutes off. This was done to equate total movement in these groups, yet afford a sustained rest time for the treadmill control animals. It was found that new cell proliferation in the hippocampus in the sleep deprived group compared with the treadmill control group was reduced by 54%. In comparing the sleep deprived group to the cage control group, the reduction was 68%. Additionally, to address the question of stress related issues, the researchers measured corticosterone levels in all subjects and found no increase in these levels in the sleep-deprived group. This suggests the decrease in new neurons (BrdU positive cells) in the sleep-deprived group was a result of sleep loss and not stress. Ruskin et al. (2006) went further in attempting to dispel the problem

of stress in animal sleep deprivation studies. In this study, a portion of rats underwent an adrenalectomy (ADX) in order to eliminate the adrenal stress response. In these animals, a stable level of corticosterone was maintained by implanting a pellet that was shown to release corticosterone over a 13-day period at normal, non-stress-induced amounts. All animals in the sleep-deprived group, both intact and ADX, were sleep deprived for 72 hours prior to testing. The study utilized the platform-over-water method; if the animal fell asleep it would fall off the platform into water, thus waking the animal and causing it to retreat back to the platform. This method was utilized because of its ability to almost totally deprive the animal of REM sleep, while also reducing the amount of SWS by a significant margin. All animals in the study (cage control intact, cage control ADX, sleep deprived intact, and sleep deprived ADX) received two trials in the Morris Water Maze. On the first trial, latency to locate the platform was similar in all groups. However, on Trial 2 the improvement in the control animals to locate the platform was significant in contrast to no significant improvement in the sleep-deprived group. Because both groups of sleep deprived rats performed similarly, it was suggested that memory impairment to the task was due to sleep deprivation and not to an adrenal stress response. Other research has also found memory that is hippocampal dependent to be very sensitive to sleep disruption. Graves et al. (2003) used a single trial task of fear consolidation to study the effect of sleep on memory consolidation. Here, the animals learned to fear either a new environment or a conditioned stimulus or cue because of association with an aversive unconditioned stimulus. When the animals were exposed to the same environment or cue after training occurred, the animals exhibited various fear responses. The animals in this study were either sleep deprived at the 0-5 hour or 5-10 hour mark

post-training. They found that sleep deprivation from 0-5 hour post-training did impair memory consolidation for contextual fear conditioning, a hippocampal-dependent task but not cue conditioning, which is amygdala dependent. In a similar study, Ruskin et al. (2004) substantiated these findings, noting that sleep deprivation dramatically impaired contextual learning while at the same time having no effect on amygdala dependent cued learning.

Several studies have addressed the biochemical basis for memory deficiencies brought about by sleep deprivation. Guan et al. (2004) reported that extracellular signal-regulated kinase (ERK) is involved in memory consolidation and phosphorylation is needed to activate ERK. This study was designed to determine the effect of sleep deprivation on memory and phosphorylation of ERK in the brain. After 6 hours of sleep deprivation in one group and normal sleep in another group, rats were trained in the Morris Water Maze. While sleep deprivation had no effect on spatial learning, significant impairment was shown on spatial memory when tested 24 hours after training. In the sleep deprived group, ERK levels were decreased after 6 hours of sleep loss but returned to similar levels of the control group after 2 hours of sleep recovery. Given the spatial memory impairment and the ERK phosphorylation, the indications are that the hippocampus is, indeed, vulnerable to sleep deprivation.

Importantly for working memory, glutamate receptors (AMPA) in the hippocampus also appear to be affected by sleep deprivation. Here, it seems that 12 hours of sleep deprivation is sufficient to cause significant impairment of spatial working memory in mice. This period of time correlated to a decrease in hippocampal AMPA receptor phosphorylation. The data in this study (Hagewoud et al., 2009) suggest that

spatial working memory may be affected when the hippocampal AMPA receptors are functioning at reduced levels due to sleep deprivation.

Several studies have explored the effect of REM sleep deprivation on long-term potentiation (LTP) in the rat hippocampus. Davis et al. (2003), by use of the inverted flower pot method, sleep deprived rats for 24, 48 or 72 hour periods. Results suggest a significant decrease in LTP at all levels of sleep deprivation. McDermott et al. (2003) substantiate these findings, suggesting that REM sleep deprivation leads to decreases in membrane excitation, which could impede the ability of rats to utilize fully their ability to perform at certain learning and memory tasks. Ishikawa et al. (2006) agree with the previous studies, but also suggested that REM rebound did not restore the decrease in LTP caused by REM sleep deprivation.

Kalonia et al. (2008) explored the effects of sleep deprivation on various biological and metabolic processes necessary for health. Utilizing a sleep deprivation paradigm whereby rats were suspended over water on a grid for a period of 72 hours, the study investigated mechanisms mediating sleep deprivation-induced dysfunction of behavioral, biochemical and neurochemical parameters. The study found that 72 hours of sleep deprivation significantly altered the sleep-wake cycle by delaying sleep latency, decreasing REM and/or NREM sleep time and increasing time awake. In the water maze, a significant decline in memory and learning was noted in the sleep-deprived rats. Additionally, researchers found a significant increase in acetylcholinesterase activity compared to the control group. This would lead to lower levels of the transmitter acetylcholine, which is important for memory function.

Morris Water Maze

The Morris Water Maze, used in studies dealing with spatial navigation, is a hippocampal dependent task (Morris, 1981, 1984). According to Frick et al. (1995), rodents will swim to find the platform with no other motivation needed. For this reason, food rewards or shock punishment are not a required elements of the task.

The water maze is a large circular pool filled with opaque water where a small escape platform is hidden below the surface of the water. Training trials are utilized to help the rodent learn where the platform is located so they can escape the water. Because the platform is hidden, no visible cues as to its location are available to the animal. External cues, visible to the animal, are normally positioned around the room to aid in place learning. It is suggested that animals can make use of three strategies to locate the platform during trials. The animal can use learned sequences of movement to find the platform, use proximal cues inside the pool, or use distal cues outside the pool (Brandies et al., 1989). After trials are complete, a probe trial is normally used to determine spatial accuracy for location of the platform. During the probe trial the platform is removed and the amount of time spent in the area where the platform used to be is calculated. Well-trained animals are known to spend more than 50% of their swim time in this quadrant (Morris, 1981, 1984).

Unlike maze tasks where rats have only a few directional choices, the water maze requires rats to constantly make directional decisions as they solve the task. Therefore, the escape strategy utilized can indicate the brain regions used. Spatial strategies associated with hippocampal learning are associated with direct paths to the hidden

target. Systematic non-spatial strategies include scanning by searching the interior part of the water maze without having a spatial bias to area, a random search of the water maze with no bias to quadrant, or utilizing a focal target that involves searching a small part of the water maze that did not contain the platform. Repetitive looping non-spatial strategies include swimming patterns around the periphery of the tank. These include chaining which can be described as swimming a distance away from the wall of the tank, peripheral looping which is swimming near the wall of the tank and is similar to thigmotaxic ("wall-hugging") behavior, or by swimming in tight circles around the pool (Brody & Hotlzman, 2006).

Purpose of this Study

Prior evidence suggests that rats perform poorly in the water maze following sleep deprivation (Guan et al., 2004; Tartar et al., 2006; Ward et al., 2009). Additionally, there is strong evidence that the hippocampus is impaired following sleep deprivation (McDermott et al, 2003; Tartar et al, 2006). Even though hippocampal function is impaired, rats following sleep deprivation still produce a learning curve as measured by traditional means (i.e., latency and swim distance to the platform). While the water maze task requires a functioning hippocampus to navigate using distal spatial cues (Morris et al., 1982), rodents can use other strategies to find the hidden platform. Therefore, it is hypothesized that hippocampal impairment due to sleep deprivation will result in rats utilizing alternative strategies to solve the water maze task, rather than rely on spatial cues. The present set of studies will analyze the swim strategy utilized by both sleep deprived and non-sleep deprived rats. It is expected that sleep deprived rats will use

alternative strategies such as systematic searching and looping strategies, while control rats will use more spatial strategies.

CHAPTER II

Experiment 1: Pilot Study

Introduction

In the pilot study a detailed analysis of the strategies utilized in the water maze was performed following total sleep deprivation. It was hypothesized that sleep deprivation would impair hippocampal function and, therefore, rats would choose a non-spatial strategy to escape from the water.

Method

Subjects. Adult male Sprague-Dawley rats (*N=12)* (Harlan Laboratories, Indianapolis, IN) were group housed in a climate controlled animal facility. Food and water was made available *ad libitum*. Rats were under a 12:12 light dark cycle (lights on at 0700 hours). All procedures were approved by the Institutional Care and Use Committee of the University of Houston-Clear Lake before experiments were started.

Sleep Deprivation. Rats were randomly assigned to either the sleep deprivation group (*n=6)* or the control group (*n=6)*. Rats were sleep deprived using the "gentle handling" technique. When rats indicated behavioral signs of sleep, experimenters introduced tactile, olfactory, or visual stimulation to prevent sleep (e.g. lightly touching the rat or introducing a novel object to the cage). For control rats, experimenters attempted to also interact with the rats but only during spontaneous wake periods.

Water Maze. To allow for a single time point to be assessed, a one-day water maze protocol was utilized (Frick et al., 2000). Rats were tested in a 1.5-meter pool containing opaque water concealing a platform that remained in a constant position. Performance was measured with a video tracking system (Videomex, Columbus Instruments, Columbus, OH). Testing consisted of 3 blocks of 4 trials each for a total of 12 trials. There was a 45-minute break between blocks. Each trial contained a 60 second inter-trial interval. Rats were given 60 seconds to locate the hidden platform. If the platform was not found, the experimenter guided the rat to the platform. Rats remained on the platform for approximately 15 seconds before being removed and dried off by the experimenter.

Data Analysis. An experimenter blinded to the group each rat was placed in judged each swim path and determined the rat to have utilized a A) spatial strategy, B) non-spatial systematic strategy, or C) repetitive looping strategy (see Figure 1). The search strategy classifications were based on previously defined schema (Brody & Holtzman, 2006; Janus 2004). Spatial strategies represent swim patterns that indicate the use of spatial cues to navigate to the location of the hidden platform. Non-spatial systematic strategies are swim patterns that utilize a search of the interior of the pool. Circling strategies represent swim patterns in which the rat either remains close to the peripheral wall (thigmotaxic behavior) or loops as to swim over all possible platform positions at a given distance from the wall.

Water maze testing was analyzed by mixed model analysis of variance (ANOVA). Chi Square analysis was performed to determine if particular spatial strategies were preferred by each experimental group. All data analysis was conducted

utilizing SPSS (version 18.0, SPSS Inc., Chicago, Il, USA) with an alpha level of 0.05.

Results

There was a significant interaction between sleep deprivation and latency to find the platform over 12 trials ($F(11,110) = 2.03$, $p = .032$) where sleep deprived rats were slower in learning the task. However, there was not a robust difference between groups. Additionally, there was no difference observed in the frequency of strategy style chosen by rats in the sleep deprivation group as compared to the cage control group ($\chi^2 = 3.73$, $p = .15$). In this study, it was concluded that there were no discernable differences observed in the strategy utilized by the sleep deprived rats in solving a spatial task during the initial testing period (Ward et al., 2009). It was determined that further testing would be necessary to look at increased sleep deprivation time or to change the task with respect to difficulty.

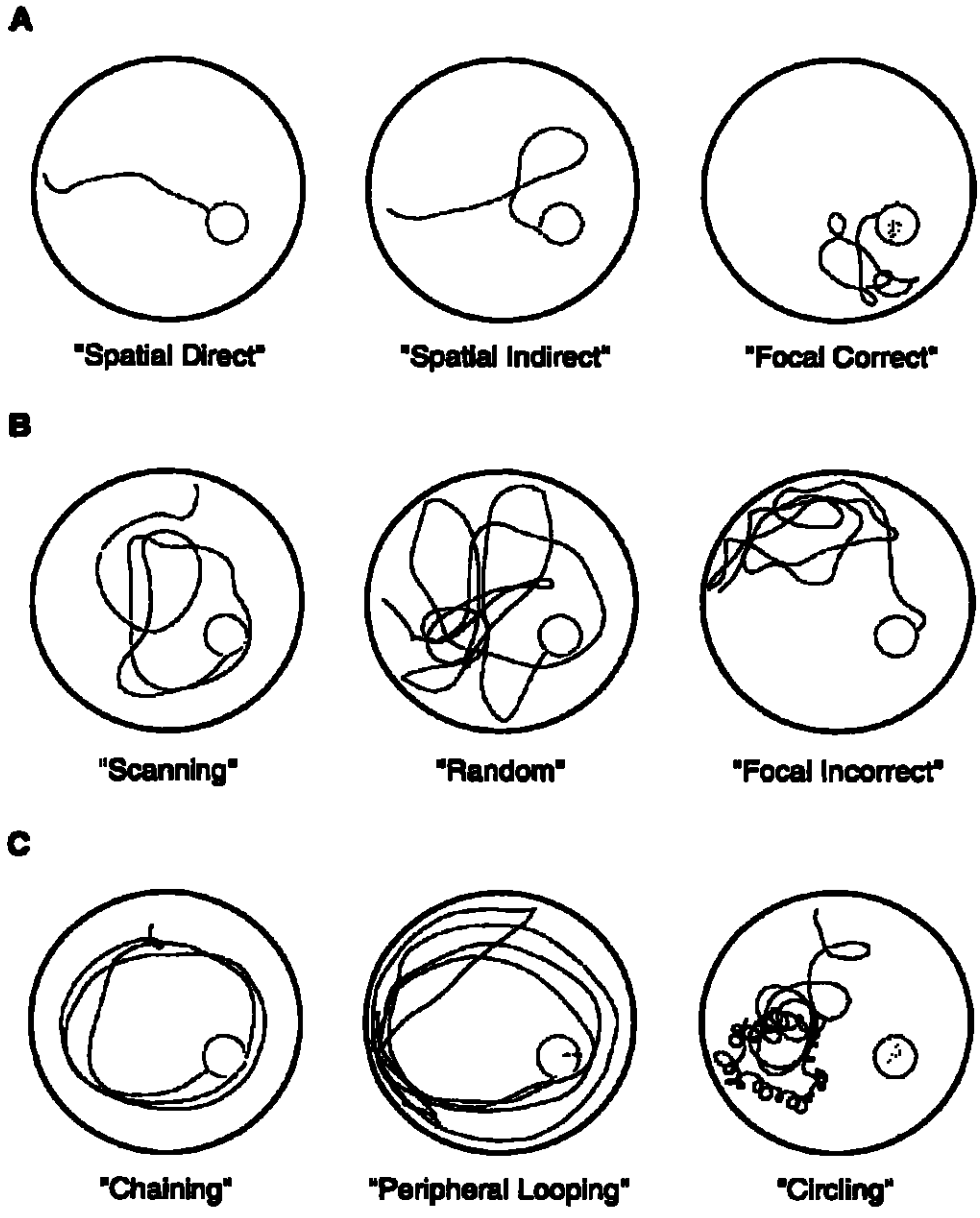

Figure 1. Representative swim patterns of various search strategies in the water maze. (A) Spatial strategies, (B) Nonspatial, systematic strategies. (C) Strategies based on repetitive looping.

CHAPTER III

Experiment 2

Introduction

In this follow-up study to the pilot, a different approach was taken in sleep depriving the animals. Where the pilot study made use of gentle handling, this study utilized a motorized activity wheel. Gentle handling is a resource intensive method of sleep depriving rats. An experimenter must be present at all times while watching the behavioral activity of rats. Additionally, it is difficult to interact with the control group an equal number of times so that all groups have received the same amount of manipulations. An automated activity wheel provides the ability to better control for non-specific stress due to the method of sleep deprivation and allows for a greater number of animals to be tested with fewer experimental personnel. An additional change from the pilot study was that a larger water maze apparatus was used (2 meters compared to 1.5 meters) so as to increase task difficulty.

Method

Subjects. Adult male Sprague-Dawley rats ($N = 24$) (Charles River, Wilmington, MA) were group housed in a climate controlled animal facility. Food and water was made available *ad libitum*. Rats were under a 12:12 light dark cycle (lights on at 0700 hours). All procedures were approved by the Institutional Care and Use Committee of the VA Boston Healthcare System before experiments were started.

Sleep Deprivation. Rats were randomly divided into two groups. Rats in the sleep deprivation group ($n = 12$) were placed in a motorized activity wheel (Lafayette Inst., Lafayette, IN, see Figure 2) that was programmed to rotate for 3 seconds and remain off for 12 seconds. Rats in the activity wheel control group ($n = 12$) were placed in the same motorized wheels but were scheduled to move continuously for 36 minutes and remain off for 2 hours 24 minutes so that they traveled an equal distance as the sleep deprivation group during the same period of time. These procedures have previously been utilized to effectively sleep deprive rats (Christie et al., 2008). Rats were placed in the wheel at lights on (0700 hours) and remained for 6 hours.

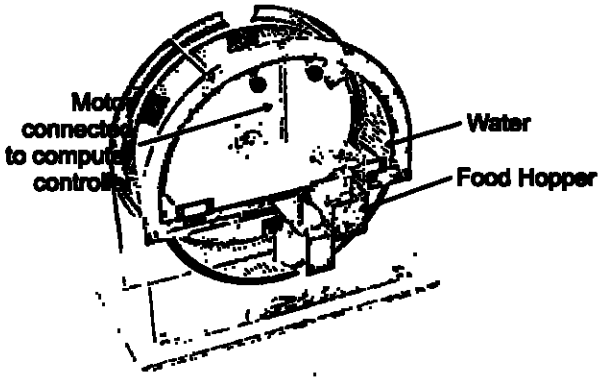

Figure 2. Motorized activity wheels (35.5 cm in diameter) allowed rats free access to food and water while inducing locomotor activity according to a programmed schedule (Figure adapted from Layette Inst.).

Water Maze. To allow for a single time point to be assessed, a one-day water maze protocol was utilized (Frick et al., 2000). Rats were tested in a 2-meter pool containing opaque water concealing a platform that remained in a constant position. Performance was measured with a video tracking system (EZVideo, AccuScan Instruments, Columbus, OH). The testing protocol was the same as utilized in the pilot

study. Testing consisted of 3 blocks of 4 trials each for a total of 12 trials. There was a 45-minute break between blocks. Each trial contained a 60 second inter-trial interval. Rats were given 60 seconds to locate the hidden platform. If the platform was not found, the experimenter guided the rat to the platform. Rats remained on the platform for approximately 15 seconds before being removed and dried off by the experimenter. At the 45-minute interval after the last trial, the hidden platform was removed and rats were given a 30 second probe trial.

Data Analysis. As in the pilot study, an experimenter blinded to the group each rat was placed in judged each path and determined the rat to have utilized a A) spatial strategy, B) non-spatial systematic strategy, or C) repetitive looping strategy (see Figure 1). Water maze testing was analyzed by mixed model analysis of variance (ANOVA). Chi Square analysis was performed to determine if particular spatial strategies were preferred by each experimental group. All data analysis was conducted utilizing SPSS (version 18.0, SPSS Inc., Chicago, Il, USA) with an alpha level of 0.05.

Results

As shown in the top panels of Figure 3, no significant differences were observed between the sleep deprived group and the control group in latency to locate the hidden platform ($F(1,22) = 0.23, p = .636$). Also, there were no significant differences between groups in the path length to the target ($F(1,22) = 0.18, p = .673$). Rats were given a probe trial 45 min following the last acquisition trial. No significant differences in percent time ($t(22) = 0.43, p = .673$) or percent swim distance ($t(22) = .075, p = .464$) in the target quadrant was observed between the two groups (see bottom panels, Figure 3).

Table 1 shows the number of swim trials classified as either spatial strategy, search strategy or circling strategy (see Table 1). There were no significant differences between the sleep deprived and the activity control groups ($\chi^2 = 1.67, p = .434$). Additionally, there were no significant differences between the sleep deprived and activity groups ($\chi^2 = 3.16, p = .206$) when classifying the number of swims that utilized a spatial strategy across the 3 blocks of training (see Table 2).

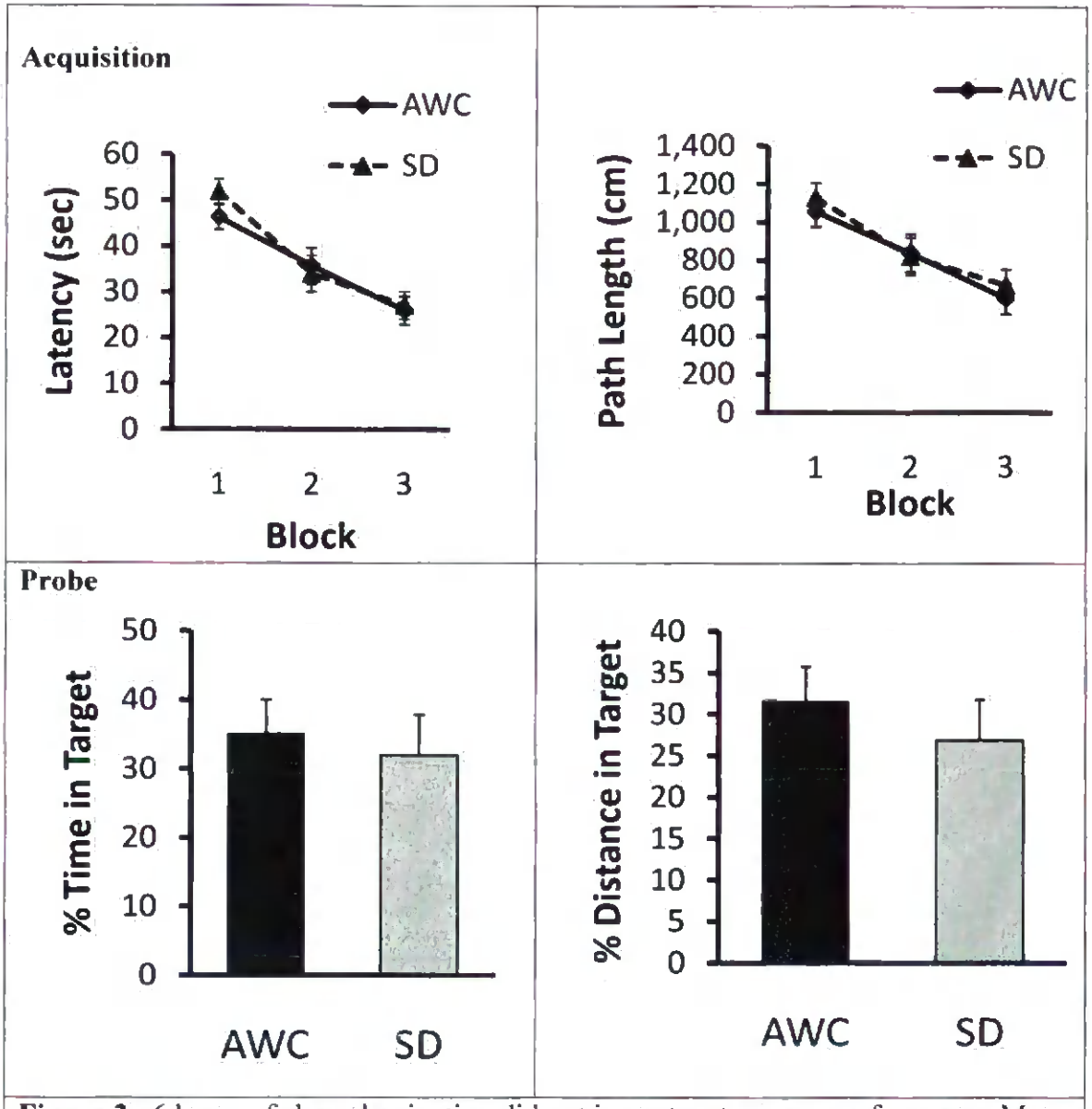

Figure 3. 6 hours of sleep deprivation did not impact water maze performance. Mean (±SEM) latency (top left) and path length (top right) for rats to find the hidden platform for motor control (MC) versus sleep deprived (SD) rats. No significant differences were observed between groups. Probe trials indicated no difference between groups as measured by percent time in target quadrant (bottom left) or percent swim distance in target (bottom right).

Table 1. ***6 hours of sleep deprivation did not affect type of search strategy used by rats.***

	Spatial	Search	Circling
AWC	57 (39.6%)	61 (42.2%)	26 (18.1%)
SD	49 (34.0%)	61 (42.2%)	34 (23.6%)

Table 2. ***6 hours of sleep deprivation did not affect the usage of spatial strategy across acquisition trials.***

	Block 1 (Trials 1-4)	Block 2 (Trials 5-8)	Block 3 (Trials 9-12)
AWC	9 (18.8%)	16 (33.3%)	32 (66.7%)
SD	4 (8.3%)	21 (43.8%)	24 (50.0%)

CHAPTER IV

Experiment 3

Introduction

This study extended the period of sleep deprivation from 6 hours to 24 hours. As in Experiment 2, sleep deprivation by use of the motorized wheel (see Figure 2) was utilized. Since 6 hours of sleep did not produce a significant difference between groups, I hypothesized that extending the period of sleep deprivation to 24 hours would increase experimental power by increasing treatment effect. Once again, it was hypothesized that the strategies for locating the hidden platform in the water maze would be different for the sleep-deprived group as compared to activity wheel controls.

Method

Subjects. Adult male Sprague-Dawley rats (N=24) (Charles River, Wilmington, MA) were group housed in a climate controlled animal facility. Food and water was made available *ad libitum*. Rats were under a 12:12 light dark cycle (lights on at 0700 hours). All procedures were approved by the Institutional Care and Use Committee of the VA Boston Healthcare System before experiments were started.

Sleep Deprivation. As in Experiment 2, rats were randomly divided into two groups. Rats in the sleep deprivation group (n = 12) were placed in a motorized activity wheel (see Figure 2) that was programmed to rotate for 3 seconds and remain off for 12

seconds. Rats in the activity wheel control group (n = 12) were placed in the same motorized wheels but were scheduled to move continuously for 36 minutes and remain off for 2 hours 24 minutes so that they traveled an equal distance as the sleep deprivation group during the same 24-hour period. Rats were placed in the wheel at lights on (0700 hours) and remained for 24 hours.

Water Maze. As in the previous experiments, a one-day water maze protocol was utilized (Frick et al., 2000). Rats were tested in a 2-meter pool containing opaque water concealing a platform that remained in a constant position. Performance was measured with a video tracking system (EZVideo, AccuScan Instruments, Columbus, OH). Testing consisted of 3 blocks of 4 trials each for a total of 12 trials. There was a 45-minute break between blocks. Each trial contained a 60-second inter-trial interval. Rats were given 60 seconds to locate the hidden platform. If the platform was not found, the experimenter guided the rat to the platform. Rats remained on the platform for approximately 15 seconds before being removed and dried off by the experimenter.

Data Analysis. Two experimenters blinded to the experimental groups rated the swim path of each trial. Inter-rater reliability (Cohen's kappa) was calculated between the two scorers of swim strategy classifications. Chi Square analysis was performed to determine if particular spatial strategies were preferred by each experimental group. Water maze testing was analyzed by mixed model analysis of variance (ANOVA). Due to a computer error, path length data was not recorded for any trials. All data analysis was conducted utilizing SPSS (version 18.0, SPSS Inc., Chicago, Il, USA) with an alpha level of 0.05.

Results

Twenty-four hours of sleep deprivation prior to testing did not alter acquisition of platform location in massed trial learning. In acquisition trials, no significant differences in learning were observed between the two different groups of rats as indicated by latency ($F(11,242) = 21.604, p = <.001$) to reach the platform. There was not a significant trial x group interaction for latency ($F(11, 242) = 1.283, p = .234$). There was a significant main effect across trials ($F(1,22) = .172, p = .683$) indicating the animals learned the location of the platform (see Figure 4). Rats in both the control and sleep deprived groups performed similarly in the water maze task. No significant difference in frequency of search strategy style was noted ($\chi^2 = .59$, p = .745) (see Table 3). Inter-rater reliability for search strategies utilized was satisfactory (Cohen's kappa = .745). Additionally, across acquisition trials, no discernable difference in search strategy style was seen ($\chi^2 = 1.07$, p = .586) (see Table 4).

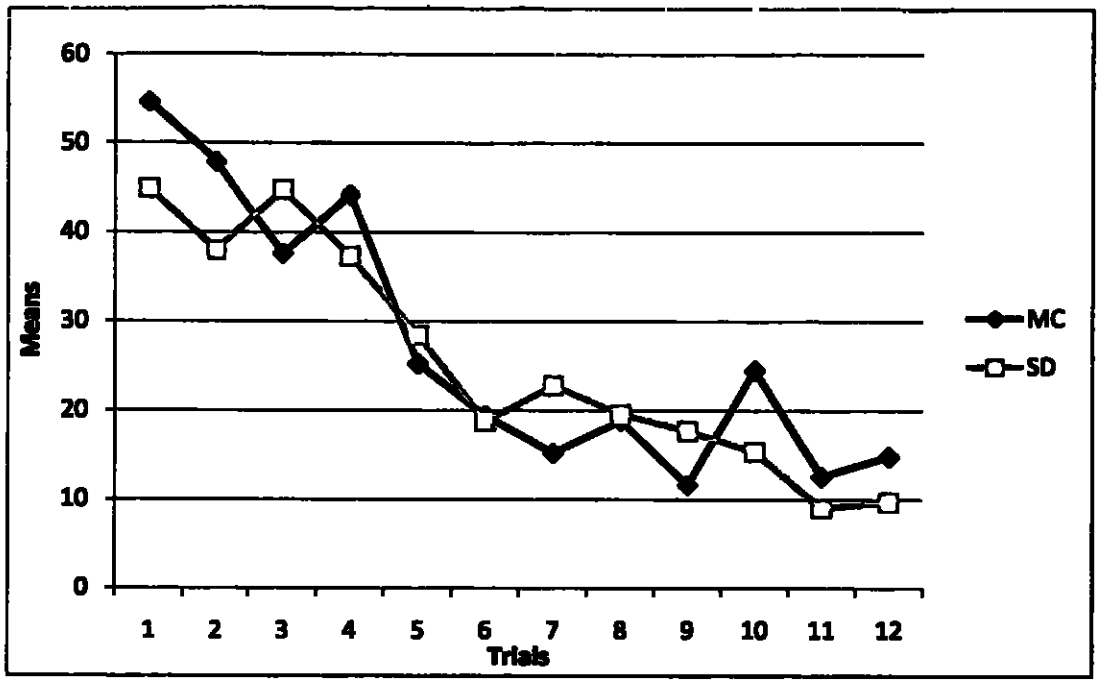

Figure 4. 24 hours of sleep deprivation prior to testing did not impact performance on the spatial memory task. Mean (±SEM) latency for rats to find the hidden platform for motor control (MC) versus sleep deprived (SD) rats. No significant differences were observed between groups.

Table 3. *24 hours of sleep deprivation did not affect type of search strategy used by rats.*

	Spatial	Search	Circling
MC	72 (50.0%)	58 (40.28%)	13 (9.03%)
SD	79 (54.86%)	106 (73.61%)	12 (8.33%)

Table 4. *24 hours of sleep deprivation did not affect the usage of spatial strategy across acquisition trials.*

	Block 1 (Trials 1-4)	Block 2 (Trials 5-8)	Block 3 (Trials 9-12)
MC	6 (12.5%)	30 (62.5%)	36 (75.0%)
SD	10 (20.83%)	28 (58.33%)	41 (85.42%)

CHAPTER V

DISCUSSION

Summary of Results

The initial study indicated that there was a difference, although not robust, in between sleep deprivation and controls to find the hidden platform over 12 trials. It was also noted that were there no differences in observed frequency of strategies utilized to find the platform between sleep deprived and control groups. In experiment two, the method of sleep deprivation was altered. By utilizing the method of motorized wheels, it was thought that the animals would perform differently. However, no differences were observed between sleep deprived and control animals in either their learning of the water maze task or the search strategies used. Experiment three extended the time of sleep deprivation from 6 hours to 24 hours and continued to utilize the motorized wheel as the method of sleep deprivation. Again, no significant differences in strategies to find the hidden platform were observed.

A possible suggestion for the lack of difference in strategies utilized to locate the hidden platform may be explained by the protocol used to sleep deprive the animals. Previous research (Tartar, et al., 2006; Ward et al., 2009) used a treadmill system to fragment the sleep of rats. Findings in those studies did indicate impairments in acquisition of the water maze task. This indicates that the utilization of an automated system to induce locomotor activity is sufficient to sleep deprive rats. A variety of

techniques have shown that sleep disruption impairs water maze performance. The flower pot method (Youngblood, et al., 1997) or a similar method whereby animals were placed on a grid floor that was suspended over water (Kalonia et al., 2008) found significant impairment on the water maze task. Additionally, similar to the pilot study, Guan et al. (2004) introduced novel objects to the sleep-deprived group. This served to stimulate the animals' exploratory behavior and prevented the animals from sleeping. This study also produced findings of impairment on the water maze task.

Another possibility for a lack of impairment could be the time of sleep deprivation utilized. The pilot study and the follow-up experiment utilized a 6-hour time period with no significant findings, so the subsequent experiment made use of a 24-hour sleep deprivation paradigm. It was thought that increased sleep deprivation would produce impairment to acquisition of the water maze task. Studies that utilize 72-hour sleep deprivation (Kalonia, et al., 2008) have been successful in showing impairment to the learning task. However, other studies have been successful in producing impairment with 6-hour (Guan et al., 2004) and 24-hour (Tartar et al., 2006; Christie, et al., 2008) sleep deprivation periods.

Another contrast in this study is the choice of water maze protocol utilized. In the three experiments a one-day water maze protocol was used (Frick, et al., 2000). During the testing, rats underwent 3 blocks of 4 trials each for a total of 12 trials. There was a 45 min break between blocks. Each trial contained a 60 seconds inter-trial interval. Rats were given 60 seconds to locate the hidden platform. If the platform was not found, the experimenter guided the rat to the platform. Rats remained on the platform for approximately 15 seconds before being removed and dried off by the experimenter. Guan

et al. (2004) used a protocol where the rats were not given a break between trials. Sleep deprived rats learned the task similarly to controls. It was not until a 24-hour probe trial that the study found significant impairment on the spatial memory test. Youngblood et al. (1997) utilized a protocol whereby the rats were subject to breaks of 25 minutes between trials with findings that sleep deprivation did affect learning. The time between trials may play a role, but the room itself could also play a role in acquisition to the task. It is recommended that the room have a sufficient amount of external cues that are visible to the animal. According to D'Hooge & De Deyn (2001) it is rather difficult to control the external cues that play a determining factor in the learning process. Therefore, from study to study, the placement of cues may play significantly in this process.

It should also be noted that strain of the animal has been questioned by several researchers. In this study, Sprague-Dawley rats were used. It has been suggested that they perform quite well in the water maze (Diana, et al., 1994). Other studies have used the same strain (Guan et al., 2004; Tartar et al., 2006) with significant results. However, others (Christie et al., 2008; Ward, et al., 2009) utilized Fischer-Norway rats and had mixed results. Youngblood et al. (1997) studied both Wistar and Sprague-Dawley rats in their study and had similar results with both strains. Additionally, Wistar rats were utilized by Kalonia et al. (2008) with successful results. These findings appear to indicate that strain of rat may not play a significant role in learning impairment within the water maze paradigm. Ward et al. (2009) suggested there was an interaction between strain and protocol used, where strains that are better performers in the water maze need harder to learn protocols in order to see deficits due to sleep deprivation.

Future Studies

Future studies in the area of sleep deprivation and its effect on strategies utilized in the water maze should take into consideration the strain of rat used. Also, increasing the time of sleep deprivation to greater than 24 hours may cause an effect. Previous research indicates that 72 hours has an effect on the ability of the animal to solve the maze on initial trials and the ability to retain information in subsequent probe trials (Kalonia, et al., 2008). It is suggested that an initial study with a time of 48 hours sleep deprivation that also considers the water maze protocol used and the visual cue placement in the room be utilized. The method of sleep deprivation should also be considered given that a variety of methods have been utilized, some with mixed results.

Importance of These Studies

It has been noted that sleep habits are changing in society. In addition, a variety of disorders work to impair sleep (Van Der Werf et al., 2009). Yoo et al. (2007) suggest that sleep is critical for consolidation of memory in humans. The study also states that, because humans often deprive themselves of sleep intentionally, it is essential that we understand all of the ramifications of this action. Gibson et al. (2006) and Curcio et al. (2006) indicate that sleep deprivation plays a significant role in the ability to learn. Children and adolescents who are sleepy have more problems learning in a classroom and often fall behind their peers. These young people often experience other problems such as behavioral difficulty that can impact learning.

Harrison & Horne (2000) also suggest that sleep deprivation impacts the ability to make decisions. In executive-type decision making after sleep deprivation, impairment

in communication, innovation, inflexibility in thought processes, excess distraction, unwillingness to attempt a new strategy, and change in mood to include lack of empathy were all noted. These impairments in a variety of situations could have serious implications. Therefore, by studying the effect of sleep deprivation there can be a better understanding about physical and biological changes caused by lack of sleep.

REFERENCES

Adam, K. & Oswald, I. (1977). Sleep is for tissue restoration. *Journal of the Royal College of Physicians of London.* 11(4): 376-388.

Berger, R. & Phillips, N. (1995). Energy conservation and sleep. *Behavioral Brain Research,* 69(1-2): 65-73.

Bjorness, T., Riley, B., Tysor, M. & Poe. G. (2007). REM restriction persistently alters strategy used to solve a spatial task. *Learning & Memory,* June: 352-359.

Brandeis, R., Brandys, Y. & Yehuda, S. (1989). The use of the Morris water maze in the study of memory and learning. *International Journal of Neuroscience,* 48(102): 29-69.

Brody, D. & Holtzman, D. (2006). Morris water maze search strategy analysis in PDAPP mice before and after experimental traumatic brain injury. *Experimental Neurology,* 197(2): 330-340.

Christie M., McKenna J., Connolly N., McCarley R. & Strecker R. (2008). 24 hours of sleep deprivation in the rat increases sleepiness and decreases vigilance: Introduction of the rat psycho-motor vigilance task. *Journal of Sleep Research,* 17(4): 376-384.

Curcio, G., Ferrara, M. & De Gennaro, L. (2006). Sleep loss, learning capacity and academic performance. *Sleep Medicine,* 10: 323-337.

D'Hooge, R. & De Deyn, P. (2001). Applications of the Morris water maze in the study of learning and memory. *Brain Research Reviews,* 36: 60-90.

Davis, C., Harding, J. & Wright, J. (2003). REM sleep deprivation-induced deficits in the latency-to-peak induction and maintenance of long-term potentiation within

the CA1 region of the hippocampus. *Brain Research*, 973: 293-297.

De Koninck, J., Lorrain, D., Christ, G., Proulx, G. & Coulombe, D. (1989). Intensive language learning and increases in rapid eye movement sleep: Evidence of a performance factor. *International Journal of Psychophysiology*, 8(1): 43-47.

Diana, G., Domenico, M., Loizzo, A., Scotti de Carolis, A., & Sagratella, S. (1994). Age and strain differences in rat place learning and hippocampal dentate gyrus frequency-potential. *Neuroscience Letters*, 171(1-2): 113-116.

Ferrara, M., Iaria, G., De Gennaro, L., Guariglia, C., Curcio, G., Tempesta, D. & Bertini, M. (2006). The role of sleep in the consolidation of route learning in humans: A behavioral study. *Brain Research Bulletin*, 71: 4-9.

Fischer, S., Hallschmid, M. Elsner, A. & Born, J. (2002). Sleep forms memory for finger skills. *PNAS*, 99(18): 11987-11991.

Frank, M. & Benington, J. (2006). The role of sleep in memory consolidation and brain plasticity: Dream or reality? *Neuroscientist*, 12(6): 1-12.

Frick, K., Baxter, M., Markowska, A., Olton, D. & Price, D. (1995). Age-related spatial reference and working memory deficits assessed in the water maze. *Neurobiology of Aging*. 16(2): 149-160.

Frick, K., Stillner, E. & Berger-Sweeney, J. (2000). Mice are not little rats: Species differences in a one-day water maze task. *Learning and Memory*, 11(16): 3461-3465.

Gibson, E., Powles, A., Thabane, L., O'Brien, S., Molnar, D., Trajanovic, N., Ogilvie, R., Shapiro, C., Yan, M. & Chilcott-Tanser, L. (2006). "Sleepiness" is serious in adolescence: Two surveys of 3235 Canadian students. *BMC Public Health*,

6:116.

Graves, L., Heller, E., Pack, A. & Abel, T. (2003). Sleep deprivation selectively impairs memory consolidation for contextual fear conditioning. *Learning & Memory,* 10: 168-176.

Guan, Z., Peng, X., & Fang, J. (2004). Sleep deprivation impairs spatial memory and decreases extracellular signal-regulated kinase phosphorylation in the hippocampus. *Brain Research*, 1018: 38-47.

Guzman-Marin, R., Suntsova, N., Stewart, D., Gong, J., Szymusiak, R. & McGinty, D. (2003). Sleep deprivation reduces proliferation of cells in the dentate gyrus of the hippocampus in rats. *Journal of Physiology*, 549.2: 563-571.

Hagewoud, R., Havekes, R., Novati, A., Keijser, J., Van Der Zee, E. & Merlo, P. (2009). Sleep deprivation impairs spatial working memory and reduces hippocampal AMPA receptor phosphorylation. *Journal of Sleep Research,* 19: 280-288.

Harrison, Y. & Horne, J. (2000). The impact of sleep deprivation on decision making: A review. *Journal of Experimental Psychology: Applied,* 6(3): 236-249.

Inoue, S., Honda, K. & Komoda, Y. (1995). Sleep as neuronal detoxification and restitution. *Behavioral Brain Research,* 69(1-2): 91-96.

Ishikawa, A., Kanayama, Y., Matsumura, H., Tsuchimochi, H., Ishida, Y. & Nakamura, S. (2006). Selective rapid eye movement sleep deprivation impairs the maintenance of long-term potentiation in the rat hippocampus. *European Journal of Neuroscience*, 24: 243-248.

Kalonia, H., Bishnoi, M. & Kumar, A. (2008). Possible mechanism involved in sleep deprivation-induced memory dysfunction. *Methods and Findings in*

Experimental Clinical Pharmacology, 30(7): 529-535.

Janus, C. (2004). Search strategies using APP transgenic mice during navigation in the Morris water maze. *Learning & Memory,* 11(3): 337-346.

Llinas, R. & Ribary, U. (1993). Coherent 40-hz oscillation characterizes dream states in humans. *PNAS,* 90(5): 2078-2081.

Maquet, P. (2001). The role of sleep in learning and memory. *Science* 294: 1048-1051.

McDermott, C., LaHoste, G., Chen, C. Musto, A., Bazan, N. & Magee, J. (2003). Sleep deprivation causes behavioral, synaptic, and membrane excitability alterations in hippocampal neurons. *Journal of Neuroscience,* 23(29): 9687-9695.

McGaugh, J. (2000). Memory - A century of consolidation. *Science,* 287: 248-251.

McGinty, D. & Szymusiak, R. (1990). Keeping cool: A hypothesis mechanisms and functions of slow-wave sleep. *Trends in Neuroscience,* 13(12): 480-487.

Morris, R. (1981). Spatial localization does not require the presence of local cues. *Learning and Motivation,* 12: 239-260.

Morris, R. (1984). Developments of a water-maze procedural for studying spatial learning in the rat. *Journal of Neuroscience Methods,* 11: 47-60.

Morris, R., Garrud, P., Rawlins J. & O'Keefe, J. (1982). Place navigation impaired in rats with hippocampal lesions. *Nature,* 297: 681-683.

Neubauer, D. (2009). Sleep and memory. *Primary Psychiatry,* 16(8): 19-21.

Peigneux, P., Laureys, S., Delbeuck, X. & Macquet, P. (2001). Sleeping brain, learning brain: The role of sleep for memory systems. *NeuroReport,* 12(18): A111-A124.

Ruskin, D., Liu, C., Dunn, K. Bazan, N. & LaHoste, G. (2004). Sleep deprivation impairs hippocampus-mediated contextual learning but not amygdala-mediated

cued learning in rats. *European Journal of Neuroscience,* 19: 3121-3124.

Ruskin, D., Dunn, K., Billiot, I., Bazan, N. & LaHoste, G. (2006). Eliminating the adrenal stress response does not affect sleep deprivation-Induced acquisition deficits in the water maze. *Life Sciences,* 78: 2833-2838.

Schacter, D. (1987). Implicit memory: History and current status. *Journal of Experimental Psychology: Learning, Memory & Cognition,* 13: 501-518.

Siegel, J. (2001). The REM sleep-memory consolidation hypothesis. *Science,* 294: 1058-1063.

Silber, M., Ancoli-Israel, S., Bonnet, M., Chokroverty, S., Grigg-Damberger, M., Hirschkowitz, M., Kapen, S., Keenan, S., Kryger, M., Penzel, T., Pressman, M. & Iber, C. (2007). The visual scoring of sleep in adults. *Journal of Clinical Sleep Medicine,* 3(2): 121-131.

Smith, C. & Rose, G. (1996). Evidence for a paradoxical sleep window for place learning in the Morris water maze. *Physiology & Behavior,* 59(1): 93-97.

Smith, C. & Rose, G. (1997). Posttraining paradoxical sleep in rats is increased after spatial learning in the Morris Water Maze. *Behavioral Neuroscience,* 111(6): 1197-1204.

Stickgold, R. & Walker, M. (2005). Memory consolidation and reconsolidation: what is the role of sleep? *TRENDS in Neurosciences,* 28(8): 408-415.

Stickgold, R. & Walker, M. (2007). Sleep-dependent memory consolidation and reconsolidation. *Sleep Medicine,* 8(4): 331-343.

Tartar, J., Ward, C., McKenna, J., Thakkar, M., Arrigoni, E., McCarley, R., Brown, R. & Strecker, R. (2006). Hippocampal synaptic plasticity and spatial learning are

impaired in a rat model of sleep fragmentation. *European Journal of Neuroscience*, 23: 2739-2748.

Tulving, E. (1985). Memory and consciousness. *Canadian Psychology*, 26(1): 1-12.

Van Der Werf, Y., Altena, E., Schonheim, M., Sanz-Arigita, E., Vis, J., De Rijke, W. & Van Someren, E. (2009). Sleep benefits subsequent hippocampal functioning. *Nature*, 12(2): 122-123.

Vertes, R. (2004). Memory consolidation in sleep: Dream or reality. *Neuron*, 44: 135-148.

Vertes, R. & Eastman, K. (2000). The case against memory consolidation in REM sleep. *Behavioral and Brain Sciences*, 23: 793-1121.

Ward, C., McCarley, R. & Strecker, R. (2009). Experimental sleep fragmentation impairs spatial reference but not working memory in Fischer/Brown Norway rats. *Journal of Sleep Research*, 18(2): 238-244.

Yoo, S., Hu, P., Gujar, N., Jolesz, F., & Walker, M. (2007). A deficit in the ability to form new human memories without sleep. *Nature Neuroscience*, 10(3): 385-392.

Youngblood, B., Zhou, Z., Smagin, G., Ryan, D., & Harris, R. (1997). Sleep deprivation by the "flower pot" technique and spatial reference memory. *Physiology & Behavior*, 61(2): 249-256.