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The Effect of REM Sleep Deprivation on Spatial Memory in Male and Female Rats

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THE EFFECT OF REM SLEEP DEPRIVATION ON SPATIAL MEMORY IN MALE AND FEMALE RATS

by

Kaitlyn Heywood

A Thesis Submitted In Partial Fulfillment of the Requirements for the Master of Science in Experimental Psychology-Thesis with a Concentration in Cognitive Neuroscience

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APPROVAL FOR SUCCESSFUL DEFENSE

Masters Candidate, Kaitlyn Heywood, has successfully defended and made the required modifications to the text of the master's thesis for the M.S. during this Fall Semester 2018.

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Abstract

It is important for an organism to excel in spatial memory abilities. An organism's survival is dependent on their ability to navigate through their environment to find resources, such as food and water, while being able to navigate home safely. With how important it is to navigate an environment safely, it is important to investigate things that impact spatial memory, such as rapid eye movement (REM) sleep deprivation. Sleep research has primarily utilized male rodents to model sleep disturbances studies which cannot be easily studied in human participants. Research has been divided on whether four hours of REM sleep deprivation impacts spatial memory: some suggesting that there is an impact while others claim that there is no link between the two. Furthermore, with primarily focusing male rats, the literature is missing valuable data on the impact of REM sleep deprivation on spatial memory in female rats. The present study modeled human REM sleep deprivation on spatial memory to further investigate what impact REM sleep deprivation may have on spatial memory while also taking into account the sex differences that may occur. To model this, for 7 consecutive days the rats were REM sleep deprived for four hours after testing in the Radial Arm Maze (RAM). The results showed that REM sleep deprivation did not impact the male or female rats' performance in the RAM. This may suggest that REM sleep deprivation does not impact spatial memory when performing in the RAM.

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Introduction

Sleep in mammals is characterized by a sustained period of inactivity in a species-specific posture, reduced responses to the outside world, the ability to wake up quickly, as well as changes in brain activity (Zepelin, Siegel, & Tobler, 1989). Sleep is further divided into two stages: rapid eye movement (REM) and non-REM sleep, each exhibiting distinct brain activity (Colavito et al., 2013). REM sleep is characterized by an increase in ocular movement, high-frequency EEG wave activity similar to that seen in wakefulness, as well as a decrease in muscular tone, also known as muscle atonia. Muscle atonia occurs naturally at the start of REM sleep in which the muscles relax, resulting in a loss of the muscle tone necessary to keep the body upright (Colavito et al., 2013).

Sleep has been found to be particularly important to cognition, especially the consolidation of new information (Frank, 2006; Sara, 2017). During sleep, the brain allows newly encoded information to be consolidated by moving them from a short-term or working memory to long-term memory (Born & Wilhelm, 2012). Recent research has focused on the distinct role of REM sleep in this process. By selectively depriving male rodents of REM sleep, researchers have examined its role in spatial learning and memory abilities, specifically reference and working memory. However, the literature surrounding whether not REM sleep plays a role in spatial learning and memory is divided: some results show spatial memory deficits as a result of REM deprivation (Smith & Rose, 1996; Smith & Rose, 1997); while other results fail to reach the same conclusion showing, rather, a lack of an effect of REM deprivation on spatial memory (Hunter, submitted; Walsh, Booth, & Poe, 2011).

While sleep research over last 100 years has favored the used males, less than one third of articles published in neuroscience and the biomedical field have included both sexes and of those, only 34% analyzed the data by comparing males and females (Beery & Zucker, 2012).

Female humans were frequently excluded from research until 1993 when the NIH mandated the enrollment of women in clinical trials (Beery & Zucker, 2011). Beginning in October 2014, the NIH launched a program to provide additional resources to encourage researchers to include both male and female subjects in their studies (Clayton & Collins, 2014). Due the historical strong bias towards male subjects in both human and animal studies, it not only compromises our understanding of females but may result in harmful side effects in the treatment of various diseases (Beery & Zucker, 2011; Zucker & Beery, 2010). As the vast majority of studies focus solely on a single sex, it cannot be assumed that those results can extend to females or that no sex differences exist. There is evidence of differences between the sexes in both spatial memory and sleep (Jonasson, 2005), leading to the question of whether there is also a difference in the effects of REM sleep deprivation on spatial memory.

Effect of REM Sleep Deprivation on Spatial Memory

An organism's survival depends on its ability to successfully navigate an environment; that is, it is important for an organism to excel in spatial memory abilities. It is, therefore, also important to investigate the components that are necessary for this ability and that could cause a disruption. Spatial learning is an organism's ability to find its way through a novel environment to locate food, water, suitable mates, and to avoid predators, while safely making his or her way back home (Vorhees & Williams, 2014). Spatial learning often relies on the reliance of reference memory, the ability to remember information that remains constant across time. Search behavior may also improve by making use of working memory, the ability to store and manipulate information to alter decision making and behavior (Kelly & McNamara, 2008; Malenak, Nestler, & Hyman, 2009). Furthermore, there are two types of navigation used to move through an environment: allocentric and egocentric (Vorhees & Williams, 2014). Allocentric navigation is the ability to navigate through a spatial location using distal cues, such as objects located at a distance. Conversely, egocentric navigation is the ability to navigate using internal cues, such as limb movement, speed, and sequence of turns. Because spatial navigation is important for survival, it is important to investigate its mechanisms.

Sleep research tends to utilize rodents to model human sleep disturbances which cannot be easily studied using human participants, including, but not limited to, REM sleep deprivation. The use of REM sleep deprivation allows for a comparison, and dissociation, between the roles of REM and non-REM on a myriad of processes, such as spatial memory (see McCoy & Strecker, 2011, for review). This requires the researcher to selectively deprive the rodent of REM sleep, while allowing the rodent to achieve non-REM sleep (i.e., not exposing the rodent to total sleep deprivation). A common method is the non-invasive flowerpot technique (Mendelson, Guthrie, Frederick, & Wyatt, 1974). The apparatus used in this methodology is simple: an inverted flower pot inside a trashcan. The trashcan is filled with water to just below the rim of the flower pot, leaving a small platform for the rat to rest on. The flower pot technique selectively deprives the rodent of REM sleep by utilizing the muscle atonia that naturally occurs at the onset of REM sleep: the rat experiences a loss of muscle tone that is needed to remain upright and as a consequence awakens, either through falling off the platform and being woken up by the water, or through experiencing the feeling of falling (Colvatio et al., 2013). While the rodents in the experimental group are in this apparatus, some researchers elect to return those rats in the control condition to the home cage (Walsh et al., 2011). Other researchers elect to do a modified version of the flower pot technique, also leaving the rats in a trashcan with water, but allowing them to rest on a wide platform (e.g. a pie plate) instead of a small inverted flower pot, thereby allowing them to sleep and stay asleep during REM (e.g., Silvestri, 2005). The flower pot technique is a simple and effective method for observing the effect of REM sleep deprivation on spatial memory.

Commonly used apparatuses. Research has commonly used apparatuses, such as the Morris Water Maze, to examine the effect of REM sleep deprivation on spatial memory. The Morris Water Maze (MWM), designed by Richard Morris (1981, 1984), is a behavioral technique used to study spatial memory in rodents. In this maze, the rats are placed in an open circular pool filled with opaque water. The rats must escape the water by finding the fixed platform that is hidden below the water. Variables that can be recorded include how long it takes the rat to get to the platform, what direction the rat swims, how many times the rat crosses into a new quadrant, and how much time the rat spends in each quadrant.

Two studies have utilized the MWM to examine the effect of short periods of REM sleep deprivation after training in the MWM on the consolidation of spatial memory. Smith and Rose (1996) deprived male rats of REM sleep after training in the MWM. For the first four days of the study, the rats were trained in the MWM for 12 trials a day. After the training on the fourth day, the rats experienced REM sleep deprivation through the flower pot technique for four hours. The rats were divided into three groups, with each experiencing REM deprivation at a different time: immediately, four hours after training, or eight hours after training. The results indicated that the group that experienced REM sleep deprivation four hours after training took longer to find the position of the hidden platform compared to those that were REM sleep deprived immediately or after an eight-hour delay. In a subsequent study, the male rats were trained in the MWM for 1 day, including 12 trials, and then underwent REM deprivation (Smith & Rose, 1997) As in the previous study, the rats were REM deprived for 4 hours at different intervals: immediately, 4, 8, 12, 16, or 20 hours after training. Only the rats that underwent 4 hours of REM deprivation immediately after training showed longer times to reach the platform, demonstrating a deficit in their ability to find the hidden platform and, thereby demonstrating an impairment in spatial memory.

By contrast, more recent studies have been unable to replicate the findings of Smith and Rose (1996, 1997). Walsh and colleagues (2011) REM sleep deprived male rats via the flower pot technique for six hours while the control group rats were returned to their home cage after training in the MWM. The results concluded that across twelve training trials per day for 6 days, the rats performed similarly regardless of whether they experienced REM sleep deprivation. The researchers concluded that because the rats performed similarly across all trials per day, there was no detectable REM sleep deprivation associated performance deficits.

In line with the findings of Walsh and colleagues (2011) Hunter (submitted) also did not find an effect of REM sleep deprivation on spatial memory, tested with the MWM. Male rats were trained in the MWM for one day, 12 trials, before exposing them to six hours of REM sleep deprivation via the flower pot technique. In Experiment 1, the rats were tested the subsequent day while Experiment 2 added a day of rest between REM deprivation and testing, as to not test the rats while sleep deprived. However, instead of controls being returned to the home cage, they underwent a control condition similar to those in the REM deprivation condition in an effort to reduce potential confounds such as changes in sleep architecture and stress associated with a new sleep environment. As the rats in both conditions, in both Experiment 1 and Experiment 2, showed successful performance in the MWM regardless of REM deprivation, the results of the study also concluded that REM sleep deprivation not affect performance for a spatial memory task.

Examining the effect of REM sleep deprivation on spatial memory has also produced conflicting results when tested with the eight-box task. The eight-box task by Poe and colleagues (2002) is a newer design to measure spatial memory. In this task, a food-deprived rat is required to follow a rectangular track in a single direction. Symmetrically located around this track are eight boxes. Each day the boxes are baited or non-baited in the same pattern, and once the rat reached a baited box, it could eat the food. After the completion of the 10th lap, there was a probe test in which the rat was removed from the track and the track was rotated 180°. Now, different physical boxes were baited, but done so in a way that the new boxes were at the same location to the distal cues as the previously baited boxes. As a result, the probe test indicated what cues that rat was using to complete the maze because the internal cues used while navigating the track were disjointed from those distal cues. If the rat was using distal cues, the rat would find the new baited boxes easily and no increase in the amount of errors would be observed. However, if the rat was using internal cues, the rat would have difficulty, as indicated by an increase in errors.

After the track was rotated, the rats that received REM sleep deprivation immediately after training committed more errors of commission compared to those that were not REM sleep deprived (Bjorness, Riley, Tysor, & Poe, 2005). In addition to showing that the rats that were REM deprived were using internal cues instead of distal cues, it also demonstrates the impact of REM sleep deprivation immediately after training: REM deprived rats solved the maze through egocentric instead of allocentric navigation; however, to our knowledge, this rotation has not been tested in other mazes.

Radial arm maze. The radial arm maze (RAM), developed by Olton and Samuelson (1976), is a widely-used test for spatial memory with rodents. The maze is constructed to have a central hub with a number of arms radiating out from the center. The rat needs to be food deprived to be motivated to navigate the maze, which increases their motivation to explore the arms in attempt to find the food that is placed at the end of some of the arms. During each testing period, some arms are baited while others are not. The arms are baited and non-baited in the same pattern across test days, and once the rat reaches the end of the arm, it is able to eat the food. The RAM also allows for the use of both allocentric and egocentric navigation (Vorhees & Williams, 2014). In the RAM, cues on the wall could be used in allocentric navigation, while distance walked and sequences of turns could be used in egocentric navigation. Olton and Samuelson's (1976) maze had eight arms to measure reference memory errors and working memory errors. By examining the number of times a rat enters an arm that has never been baited with food, the researchers are able to measure reference memory errors. Furthermore, working memory errors can be measured by observing the number of times it reenters a baited arm

Smith, Conway, and Rose (1998) showed that REM sleep deprivation impaired male rats' ability to successfully complete the RAM. In the study, the rats were trained on the RAM for 10 days. After each daily training session, the rats underwent 4 hours of REM sleep deprivation immediately, 4 hours, or 8 hours after training. The results indicated that rats exposed to REM deprivation immediately after training displayed an impairment on the ability to find the correctly baited arms when compared to those that were REM deprived 4 and 8 hours after training. More specifically, REM deprived rats entered more arms that were not previously baited compared to non-REM deprived rats, which indicates an impairment of reference memory as a result of REM sleep deprivation. Similarly, Legault, Delay, and Madore (2010) also found that male rats that were REM sleep deprived for four hours immediately after training in the win-shift RAM had significant impairments in their ability to learn the positions of the baited arms. Although there is no manipulation of reference memory, it does indicate an impairment in working memory. These two studies suggest that there is a window, four hours immediately after training, during which REM sleep deprivation has the most impairment on spatial reference memory compared to REM deprivation four to eight hours after training. The results from these two studies demonstrate that REM sleep deprivation does impact spatial reference memory when measured with the RAM.

To summarize, past literature on the effects of short periods of REM sleep deprivation has shown to have differing effects on spatial memory. According to two studies, four hours of

REM sleep deprivation immediately after training, causes deficit in the consolidation of spatial memory in the MWM by inhibiting their ability to find the hidden platform (Smith & Rose, 1996, 1997). By contrast, other studies suggest that similar periods of six hours of REM deprivation does not have this effect (Hunter, submitted; Walsh et al., 2011). As a result, a question still remains about the role of REM deprivation in spatial memory abilities. For example, there are no published studies to contest the results found for the RAM; however, this does not mean that these studies do not exist and may have not been published yet. In addition, the literature on the MWM shows conflicting outlooks on whether REM deprivation negatively affects spatial memory. The uncertainty surrounding the effect of REM deprivation when tested with the MWM highlights the need to do additional studies that use different spatial memory tasks, such as the RAM.

Sex Differences

Among the aforementioned studies, a trend becomes clear: male rats are consistently used as subjects in studies that look to examine effects of REM sleep deprivation on spatial memory. In focusing solely on male rats, the literature is missing valuable data about the effect REM sleep deprivation might have on spatial memory in female rats, and whether the effect differs between the sexes. Some differences that may affect memory performance for males and females if tested after REM sleep deprivation have been shown. For example, when tested in the MWM, males tend to find the hidden platform faster than females (Monfort et al., 2015; Jonasson, 2005). Additionally, sleep patterns in males and females differ: during the first day of estrus a female rat spent more time awake during the wake period and the increase was compensated for through spending more time in REM sleep that night (Colvin, Whitmoyer, Lisk, Walter, & Sawyer, 1968). Because sex differences exist in spatial memory and sleep, it becomes necessary to include both sexes in studies involving REM sleep deprivation and its effects on spatial memory.

Sleep. With the lack of inclusion of females in the vast majority of studies, it is not surprising that there have only been six studies that sought to observe the sex differences that occur in sleep. These studies have ranged from observing the differences in sleep cycle while the females are in estrus, to removing the ovaries to observe what effect the lack of circulating sex hormones has on the sleep cycle (Colvin et al., 1968; Koehl, Battle, & Meerlo, 2006). The evidence of these studies suggests that the sex differences that are observed in sleep are dependent on sex hormones.

Female rats and mice, with their ovaries intact, have shown differing sleep patterns compared to males. Under baseline conditions, female mice are, on average, awake for 1.5 hours longer than their male counterparts regardless of their estrus cycle, and at the expense of time spent in non-REM sleep (Paul, Dugovic, Turek, & Laposky, 2006). However, during the estrus cycle of female rats, the amount of time spent in REM sleep was impacted. During this time, the hormonal changes that naturally occur during the first day of estrus increase the female's alertness during the day; the increase was compensated for through subsequently spending more time in REM sleep the following night (Colvin et al., 1968). These results suggest that, at baseline, the circulation of hormones cause a shift in time spent awake, causes changes in the time spent in non-REM and REM sleep — thus differentiating their sleep schedules from those of males.

Although sex differences can be seen in intact and ovariectomized (OVX) adult females, manipulation of sex hormones earlier than adolescence can impact sleep. Cusmano, Hadjimarkou, and Mong (2014) provided clear evidence that sex differences in sleep are caused by the early activational and organizational effects of sex hormones. Masculinized female rats, who had female anatomy but a male brain, were injected with either testosterone or estradiol in adulthood and showed similar sleep patterns to those of males regardless of what injection they received. This suggests that there was a lack of an activational effect of estradiol in the masculinized females. However, when the ovaries were removed, estradiol treatment impacted the time spent awake and in REM. As a result, the time spent awake and in REM may be mediated by the brain's organization and once established, may be impacted by sex hormones.

Sex hormones not only affect the time spent sleeping, but the nature of recovery from sleep deprivation, as well. Schwartz and Mong (2013) found that estradiol treatments of ascending dosage caused a change in time spent in REM sleep after experiencing REM deprivation and that change was dependent on what phase rats entered sleep. OVX female rats were injected with ascending estradiol treatments, which mimics the natural preovulatory increase of sex steroids in OVX female rats. The results indicated that, for the OVX females, when sleep recovery from REM sleep deprivation began during the light phase, their normal sleeping hours, there was an increase in time spent in REM sleep and a decrease in the following dark phase. Interestingly, when recovery for the OVX females injected with estradiol began in the dark phase, there was a decrease in time spent in REM. These results suggest that the estradiol treatment promoted wakefulness during the dark phase while also suppressing sleep, regardless of what phase their sleep began.

The results from the aforementioned studies suggest that the sleep patterns for male and female rats differ simply by the nature of being male or female. The differences in sleep patterns at baseline can be attributed to the naturally circulating sex hormones; as demonstrated by studies which examined the consequences of OVX, which stops this circulation (Koehl et al., 2006). However, the circulation of sex hormones was dependent on the differentiation of the brain that occurs during a selective window from day 18 of embryonic development until PND 10 (Cusmano et al., 2014). Changes at this stage, such as giving a female an injection of testosterone, may cause a female rat to have a male brain thus, becoming insensitive to estradiol and subsequently displaying sleeping patterns similar to their male counterparts. The findings that male and female sleep patterns and the nature of sleep recovery are different at baseline further promote the necessity to include females in sleep deprivation research.

Spatial memory. Given the sex differences in sleep shown in previous research, it is important to look for possible sex differences in spatial memory. Jonasson (2005) conducted a meta-analysis and concluded that male rats have an advantage over female rats when measured on reference and working memory protocols, regardless of a variety of variables, such as inclusion of pretraining, rearing environment, and the interaction between age and number of pretraining trials. The following studies highlight that males outperform females in spatial tasks, such as the MWM and RAM, and it may be due to sex hormones, as was the case with sleep patterns.

The literature shows that males outperform females in the MWM; however, the phase of estrus the female was experiencing may impact their performance. Monfort and colleagues (2015) found that females took longer than males to find the hidden platform on the first few days of training, but when spatial memory was assessed 24 hours later, there was no difference between the two. Healy, Braham, and Braithwaite (1999) compared the performance of males against females in the four different stages of their estrous cycle: estrus (low estrogen), met-estrus, di-estrus, and pro-estrus (high estrogen). The results suggested that the phase of estrous cycle did not affect their performance in the MWM, when compared to males. However, the researchers also found that females took less time to reach the hidden platform in pro-estrus when compared to the other three stages while, over the course of the same time frame, there was no difference in performance in the males. It is suggested that studies which report sex differences may be due to testing on days which females underperform, such as the extra laps and time needed to complete the task in the estrus stage.

Similar to the MWM, evidence that males have an advantage over females has also been found using the RAM. A study conducted by Seymoure, Dou, and Juraska (1996) indicated that males outperformed females in almost all measures of memory. Males made fewer reference and working memory errors, in addition to finishing the maze by visiting fewer arms. Roof (1993), found that the manipulation of sex hormones impacted the females' ability to navigate the RAM. On PND 2, the males and females were injected with either testosterone or sesame oil (which served as a control). They found that the time it took the rats to reach criterion, when they retrieved the food in five out of eight arms in under three minutes, was dependent on the sex. Females that were masculinized with testosterone, so they had a male brain but female anatomy, took less trials to reach criterion compared to the control females. By contrast, the males that were injected with testosterone took more trials to reach criterion compared to the control males. These findings are in line with the idea that there is an optimal level of testosterone needed for spatial memory. With this in mind, injecting the females with testosterone cause them to reach an optimal level, while the levels in the males were pushed outside this range. These results suggest that, at baseline, males outperform females, and this may be due to the levels of circulating testosterone.

The research so far into male versus female performance on spatial memory tasks suggests that males tend to outperform females in both the MWM and RAM, and performance in these spatial tasks is mediated by the circulating sex hormones. It is suggested that this differing performance of males and females on spatial tasks may be due to not taking into account the varying levels of performance that occur over the course of the estrus cycle (Healy et al., 1999). However, when females are injected with testosterone during their sensitive window, they outperform the female controls (Roof, 1993). The injection of testosterone during the sensitive window shows that a female rat, with male brain organization, out performs the female controls, regardless of estrus cycle of the control females. While both groups have the same gonads, it is possible that the sex differences seen in spatial tasks may be due to brain organization and the circulation of testosterone vs estradiol (activational effects). As such, levels of testosterone, especially with the appropriate brain organization, results in an advantage in spatial tasks. These findings reiterate the substantial effect of sex hormones. The literature suggests hormonal levels that naturally differ between males and females affect both spatial memory performance and sleep pattern. Despite this, research in both these domains, to date, has neglected to utilize female rats in addition to male rats. The present study aims to fill this hole in the literature by examining the effect of REM sleep deprivation on spatial memory of both male and female rats.

Interaction of REM Deprivation and Sex Differences on Spatial Memory

To date, only one study has examined sleep and memory performance while considering potential sex differences (Hajali, Sheibani, Esmaeili-Mahani, & Shabani, 2012). The researchers compared three different groups of adult rats: intact males, intact females, and OVX females. The researchers then subjected all rats to either 72 hours of REM sleep deprivation via a modified version of the flower pot technique or a wide platform version. The modified version of the flower pot technique included multiple platforms, so the rats could jump from one to another. The wide platform version served as a non- REM sleep deprived control group and was wide enough that the rats could sleep, including REM sleep as well. Thirty minutes after the end of the REM sleep deprivation they were placed into the MWM during their light on phase. The rats were then recorded on their ability to find, and escape, to the hidden platform as well as the percentage of time spent in the correct quadrant during a probe trial. The researchers concluded that there were no significant differences between the control male and female’s ability to learn where the hidden platform was located. 72 hours of REM deprivation did not have an effect on males; an effect of REM deprivation was not seen in male’s ability to escape to the hidden platform or the percentage of time spent in the correct quadrant, when compared to non-REM deprived males. Conversely, REM deprivation impacted the time spent in the correct quadrant for the intact and OVX female rats; when compared to their controls, those that were REM deprived, on average, spent less time in the correct quadrant. Lastly, after REM deprivation, the females' ability to escape to the hidden platform differed: intact females did not differ from the intact female controls nor the intact males while the OVX took significantly longer to find the hidden platform when compared to the REM deprived intact males and females. These results suggest that 72 hours of REM deprivation impacted the spatial memory of intact and ovariectomized females but not the males.

Although past research on the effect of REM deprivation on spatial memory has been divided, the results from this study provide further evidence that REM sleep deprivation does not affect spatial memory in male rats. Although researchers have claimed that four hours of REM sleep deprivation causes deficits in spatial memory (Smith & Rose, 1996, 1997), others suggest that similar periods of six hours of REM deprivation does not (Hunter, submitted; Walsh et al., 2011). Interestingly, in line with Hunter (submitted) and Walsh and colleagues (2011), Hajali and colleagues (2011) were also unable to find an effect of REM deprivation on spatial memory, even after 72 hours.

Although there was no effect of REM deprivation on males, contradictory findings were found in the females' ability to complete the MWM. Hajali and colleagues (2011) found, on average, the OVX females' ability to escape to the hidden platform was impaired when compared to intact females; however, the intact males and females did not differ from one another. When comparing the percentage of time spent in the correct quadrant on the probe trial, REM deprived intact females and OVX females spent less time, on average, in the correct quadrant when compared to REM deprived males. These results suggest that females' ability to find the hidden platform and percentage of time in the correct quadrant was impaired by REM deprivation, but intact females were only impaired on the percentage of time they spend in the correct quadrant. This is contradictory to past research that states that males have an advantage over females in the MWM (Jonasson, 2005; Monfort et al., 2015). These contradictory results across two separate variables highlight the need for further investigation into the effects of REM sleep deprivation on spatial memory in female rats.

Purpose of the Present Study

Previous research has investigated the spatial memory deficits caused by REM deprivation in male rats. This research has had conflicting results when spatial memory is tested in the MWM (Hunter, submitted; Smith & Rose, 1996, 1997; Walsh et al., 2011). However, the current literature suggests that four hours of REM deprivation does impact spatial memory in the RAM (Legault et al., 2010; Smith et al., 1998). Therefore, the present study replicated the methodology used by Smith and colleagues (1998) by REM depriving the rats for four hours immediately after training in the RAM. Based on these results, we predicted that REM deprivation would impair spatial memory in the RAM.

However, the literature lacks valuable data on how REM deprivation impacts spatial memory in female rats. Therefore, the present study included females as well as males. It has been shown that sex differences exist in both baseline sleep patterns and spatial memory. Males perform better than females in the RAM, which may be attributed to the circulation of sex hormones (Jonasson, 2005; Roof, 1993; Seymoure et al., 1996). We predicted that males, regardless of whether they were REM deprived, would outperform females.

The results of Hajali and colleagues (2012) provided preliminary evidence that REM deprivation affects MWM performance differently in males and females. Some of their findings conflict with past research, and the relative lack of studies conducted with both sexes propose an interesting and somewhat unexplored question of whether the effects of REM deprivation on spatial memory vary by sex in the RAM. For example, Hajali and colleagues (2012) were unable to find an effect of REM deprivation in the intact males. However, for the intact females, they were only able to find an effect in one of the two variables but were able to find an effect on both variables for the OVX females. Thus, they concluded, after REM deprivation, OVX females showed a greater impairment in spatial memory when compare to the intact male and females. Furthermore, after REM deprivation, the intact males only had an advantage over the intact females on their ability to escape to the hidden platform while they had similar performances on percentage of time spent in the correct quadrant. Somewhat similar to their findings, we predicted an interaction between REM deprivation and sex differences on spatial memory; specifically, REM deprivation will impair the spatial memory of females but not non-REM deprived females or REM deprived males.

To our knowledge, there are no studies investigating REM deprivation on allocentric and egocentric navigation in the RAM. Bjorness and colleagues (2005) showed that as little as four hours of REM deprivation was long enough to impact the strategy utilized by the males in the 8-box task: showing a shift from allocentric to egocentric navigation. However, this has yet to be replicated in the RAM. Furthermore, despite effects Bjorness and colleagues (2005) found within males, it cannot be assumed that this finding can be extended to females or that sex differences do not exist in the strategy utilized. Therefore, the present study included a maze rotation. We predicted that both sexes would show an increase in the amount of reference and working memory errors after the maze rotation, indicating a shift from an allocentric to egocentric navigation that occurred due to REM deprivation.

Method

Subjects

A total of 32 Sprague-Dawley male and female rats were used in this study. Male rats were obtained at PND 21 for use as a control group in another study and were approximately 15 weeks of age before beginning the current study. Female rats were obtained from Envigo at 12 weeks of age and were given a minimum of one week to habituate to the lab. All animals were housed in pairs and given food (Envigo Laboratory Environment) and water *ad libitum* until the experimental procedure called for food deprivation. The animals were housed in a controlled environment with a constant temperature (23°) and 12-hour light cycle (lights on at 8:00 am). Approval from IACUC was gained before the experiment began. A power analysis, with a medium effect size, and $\alpha = .05$ indicated that a sample size of 32 was needed to achieve a power above 80%.

Apparatus

Radial Arm Maze. The RAM (38.5 in x 38.5 in) has a circular shape, with 8 arms (11.5 in x 4 in), with short alley ways connecting the receptacle which were locked during the duration of testing, was used to test working and reference memory (Olton & Samuelson, 1976). The walls were made of clear Plexiglas while the floor was made of laminate wood with a white durable surface (see Figure 1). At the beginning of each day of training the rats were placed in the center of the RAM facing north, south, east, or west. Three of the eight arms were baited: a single sucrose pellet, available to be eaten, was placed in a receptacle area (2.75 in x 6 in). Furthermore, a single sucrose pellet was crushed up and a pinch full was sprinkled in each receptacle, including the non-baited arms. However, the powder was able to be consumed but it was recorded that no rats licked this powder up. The receptacle was cleaned often, to not allow a buildup of crushed sucrose pellets. This eliminated any olfactory cues by having every arm smell like the sucrose pellets. The baited arms were positioned near external cues that were placed around the room, such as pictures on the wall. The arms that were baited, and those that were not, remained stable over the course of all the testing days (see Figure 2). On the last day of testing, the maze was rotated 180° so that new arms were baited to match the physical location of the previously baited arms. This allowed for any internal cues that once indicated a baited arm to no longer do so (Bjorness et al., 2005). On all testing days, the rats remained in the maze until all three sucrose pellets had been consumed or 15 minutes had passed, whichever came first. The rats were tested from 11:00 am – 12:00 pm, while the lights were on.

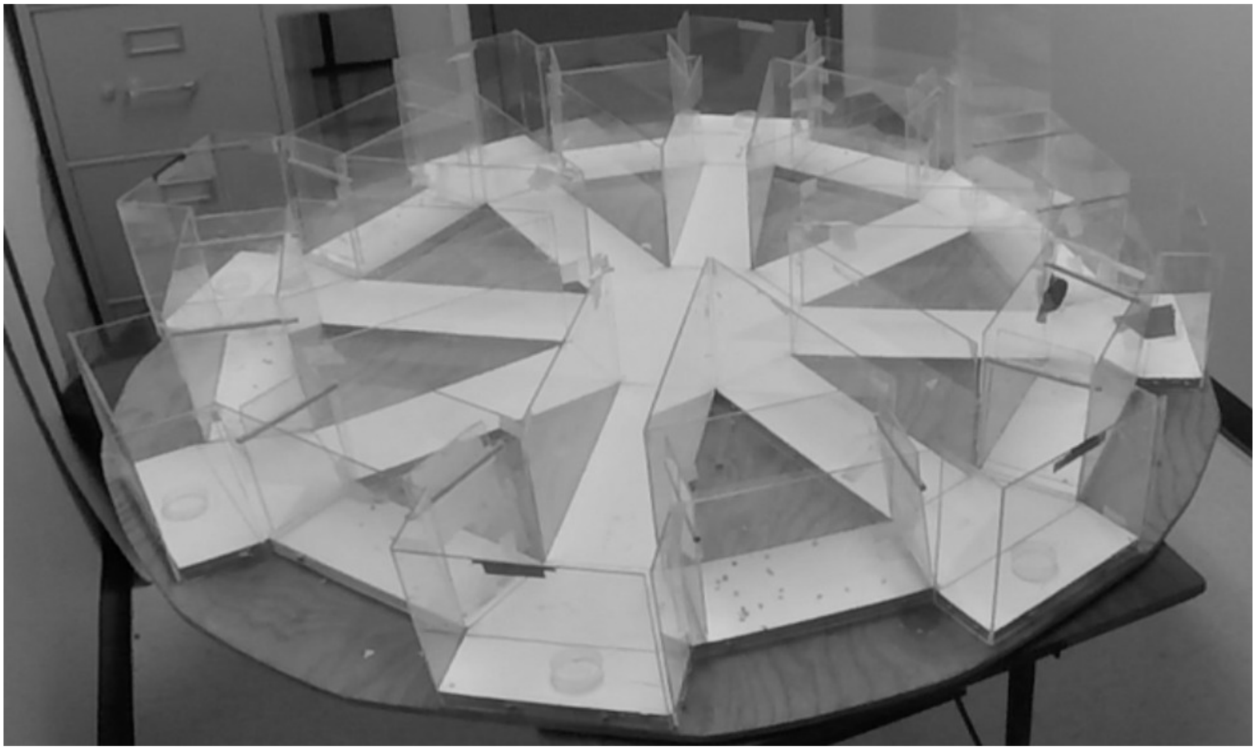

Figure 1. Picture of the RAM used during all 8 days of testing. The alley ways connecting the receptacles were locked as to not allow movement through them. (Heywood, 2018)

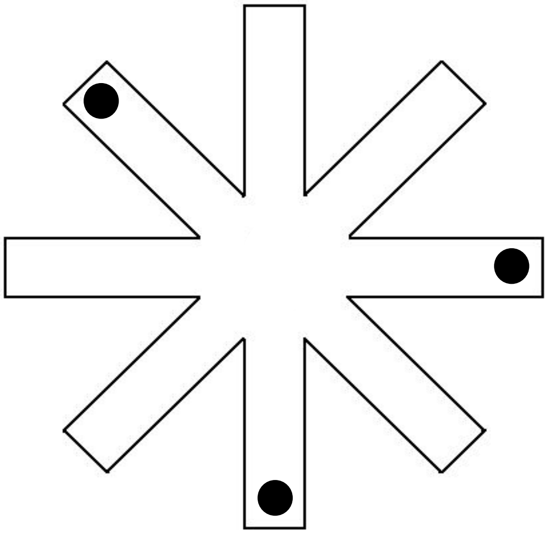

Figure 2. Pattern of baited to non-baited arms used for all 8 testing days in the RAM. Black circle represents a baited arm.

Flower pot technique. The flower pot technique was used to achieve REM sleep deprivation (Mendelson et al., 1974). For four hours, the rat in the REM deprivation condition was placed on an inverted flower pot (5.50 in high x 3.3 in diameter) that was placed inside a trashcan (19 in x 13 in). During non- REM sleep, the rat was able to retain muscle tone to stay on top of the flower pot. On the onset of REM sleep, the muscle tone was lost, causing the rat to lose its balance and to wake up. Those in the control, or non- REM condition, were placed under similar conditions, but instead of being placed on an inverted flower pot, the pot was removed, and they were placed on a wide pie plate (1.5 in high x 8 in diameter)**.** This allowed the rats to enter REM sleep and to better serve as a control group rather than being returned to their home cage. REM sleep deprivation was implemented from 12:00 am – 4:00 pm (four hours) while the lights were on. The rats were placed in a quiet room, with the lights on, and left undisturbed during the four hours. Upon the completion of the four hours, the rats were dried off, if necessary, and returned to their home cages in the colony room. The apparatus was cleaned and refilled after each session. A REM deprived rat and a control rat were housed together during the duration of the study. The sleep manipulation continued over a period of 7 days, immediately after testing in the RAM.

Procedure

Days 1-5. Five days prior to testing, the rats underwent food deprivation and habituation. The rats were given five food pellets per day and weighed daily until the completion of the study to ensure they did not go below 85% of their free feeding body weight. On day three, the rats were exposed to the sucrose pellets. A handful of sucrose pellets was introduced into the home cage and once eaten, five food pellets were made available. On days four and five, the rats were habituated to the RAM. The rats were placed into the maze, with all the arms baited, and were allowed to explore the maze until all the sucrose pellets were consumed or 15 minutes had passed, whichever came first. Once completed, they were returned to their home cages in the colony room and five food pellets were made available. The food deprivation procedure continued until the end of the study.

Days 6-12. Immediately following the first five days of food deprivation and habituation, the rats began the sleep manipulation and testing period. During days 6 through 12 (days one through seven of RAM testing), each rat was placed in the RAM daily, facing a randomized direction with three arms baited, each day. The rats were allowed to freely explore the RAM until they had eaten all three of the sucrose pellets. However, if they failed to find all three sucrose pellets within 15 minutes, the session ended, and their time was recorded as 15 minutes.

Immediately after they completed the task, eight male and eight female rats were subjected to four hours of REM deprivation via the flower pot technique. The other eight male and eight female rats were immediately put into the control condition. After the four hours, they were returned to their home cage in the colony room and given five food pellets.

Day 13. On day eight of testing, a probe trial occurred: the maze was rotated 180° so that new arms were baited to match the physical location of the previously baited arms. Once rotated, the arms were baited in the same patterns as days one through seven of testing. The rats were allowed to freely roam the RAM until they had consumed the three sucrose pellets. If they were unable to the find the pellets in 15 minutes, the session ended with their time being recorded as 15 minutes. Once the probe test was completed, the rats were returned to their home cages in the colony room and given food and water *ad libitum*.

Data Analysis

While completing the task, the rat was measured on three different variables: reference memory errors, working memory errors, and time to complete the maze. Reference memory errors were defined as entering an arm that had not been baited previously (Kelly & McNamara, 2008). For example, if the rat went down an arm that has never been baited, this was counted as one reference memory error. Working memory errors were defined as re-entering a baited arm that they had previously visited (Malenak et al., 2009). For example, if the rat was to enter a baited arm, and eat the sucrose, but later returned to the same arm, this was counted as one working memory error. Re-entries to arms that were never been baited were not considered working memory errors. To be recorded as an entry into an arm, the hind legs had to pass over the line where the center hub and the arm connected. Lastly, the rats were timed on how long it took to find and eat the sucrose in the baited arms. If the rats were unable to find all the baited arms within the 15-minute time slot, the trial ended, and their time recorded as 15 minutes.

The results from testing on day 1 were used as a baseline and subsequently used to ensure the groups did not differ significantly before continuing with analyses where the sleep manipulation was present. To do this, an independent samples t-test was conducted. If the results showed that the groups did not significantly differ on day 1, the following analyses were conducted using the raw scores. However, if the groups were significantly different, the raw scores would be converted into percent change from baseline.

The primary focus of this experiment was to determine if there was an interaction between REM deprivation and sex differences on spatial memory. For main effects and interactions, a 2 (male vs female) x 2 (REM vs non-REM) x 6 (testing days 2 through 7) mixed design repeated measures ANOVA was used. A 2 (male vs female) x 2 (REM vs controls) x 2 (testing day 7 vs 8) mixed design repeated measures ANOVA was used to determine if there were any main effects or interactions, in order to determine the consequences of the maze rotation. For repeated measures ANOVAs, a Mauchly's Test of Sphericity was conducted to ensure that variances were equal and, if sphericity was violated, a Greenhouse-Geisser correction was used.

For significance, values of $p < .05$ were considered statistically significant. For measures of effect sizes, a partial eta squared was used for the ANOVAs (small = .01, medium = .06, large = .14) and Cohen's *d* for the *t*-tests (small = .2, medium = .5, large = .8,) (Cohen, 1988).

Results

Reference Memory Errors

Day 1. An independent samples t-test was conducted on the number of reference memory errors made on the first day of training to confirm that there were no pre-existing differences between the rats destined to be REM deprived and those destined for the control condition. There was no significant difference between to-be-REM deprived ($M = 8.13$, $SD = 5.54$) and to-be-control ($M = 6.81$, $SD = 3.53$) conditions; $t(29) = -.80$, $p = .43$, Cohen's $d = 0.28$. These results suggest that there was no difference between the two groups before the sleep manipulation occurred thus, the raw scores were used for all subsequent analyses. Additionally, there was no significant difference between males ($M = 7.73$, $SD = 6.03$) and females ($M = 7.19$, $SD = 2.81$); $t(29) = -.33$, $p = .75$, Cohen's $d = .11$ on the first day of testing.

Days 2-7. There was no statistically significant change in reference memory errors across testing days, $F(5,135) = .96$, $p = .44$, $\eta^2_p = .03$. This suggests that, regardless of sex and sleep condition, the average number of errors made did not vary across the 6 days of testing. This suggests that no learning occurred from day 2 to day 7 of testing. There was no statistically significant difference between the REM deprived and controls on reference memory errors made, $F(1,27) = .50$, $p = .48$, $\eta^2_p = .02$. There was a statistically significant difference between the males ($M = 4.91$, $SD = 1.38$) and females ($M = 6.60$, $SD = 1.84$), $F(1,27) = 7.88$, $p < .01$, $\eta^2_p = .23$ (see Figure 3). This indicates that males made fewer reference memory errors when compared to the females, regardless of what sleep condition they were in.

There was no interaction between sleep condition and day, $F(5,135) = 1.25$, $p = .29$, $\eta^2_p = .04$. There was no interaction between sex and day, $F(5,135) = .63$, $p = .68$, $\eta^2_p = .02$. There was no interaction between sex and sleep condition, $F(1,27) = .05$, $p = .82$, $\eta^2_p = .002$. Lastly, there was no 3-way interaction for sex, sleep condition, and day, $F(5,135) = .70$, $p = .62$, $\eta^2_p = .03$ (see Figure 4).

Days 7-8. On day 8, the maze was rotated 180°. There was no statistically significant change in reference memory errors between testing days 7 and 8, $F(1,27) = .64$, $p = .43$, $\eta^2_p = .02$. There was no statistically significant difference between the REM deprived and controls on reference memory errors made, $F(1,27) = 1.63$, $p = .21$, $\eta^2_p = .06$. There was no statistically significant difference between the males and females, $F(1,27) = .07$, $p = .80$, $\eta^2_p = .002$.

There was no interaction between the sleep condition and day, $F(1,27) = .18$, $p = .66$, $\eta^2_p = .01$. There was no interaction between sex and day, $F(1,27) = .49$, $p = .49$, $\eta^2_p = .02$. There was no interaction between sex and sleep condition, $F(1,27) = 2.14$, $p = .15$, $\eta^2_p = .07$. Lastly, there was no 3-way interaction between sex, sleep condition, and day, $F(1,27) = .03$, $p = .86$, $\eta^2_p = .01$ (see Figure 4).

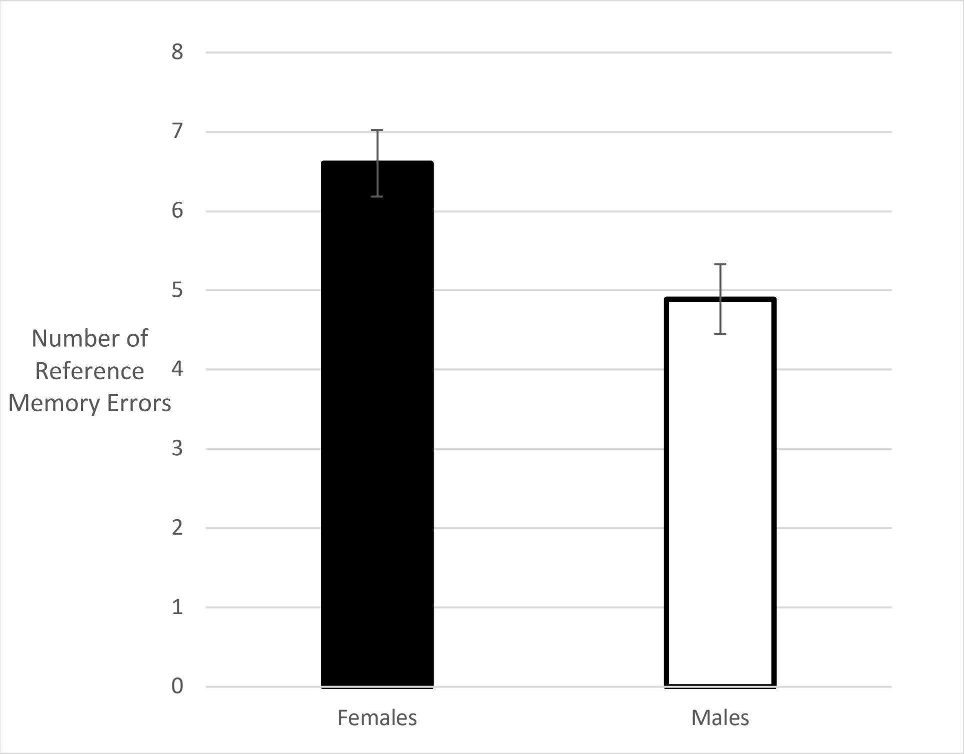

Figure 3. Average number of reference memory errors made by the males and females from day 2 to 7. Data are presented as mean ± standard error. N = 15 for males, N = 16 for females.

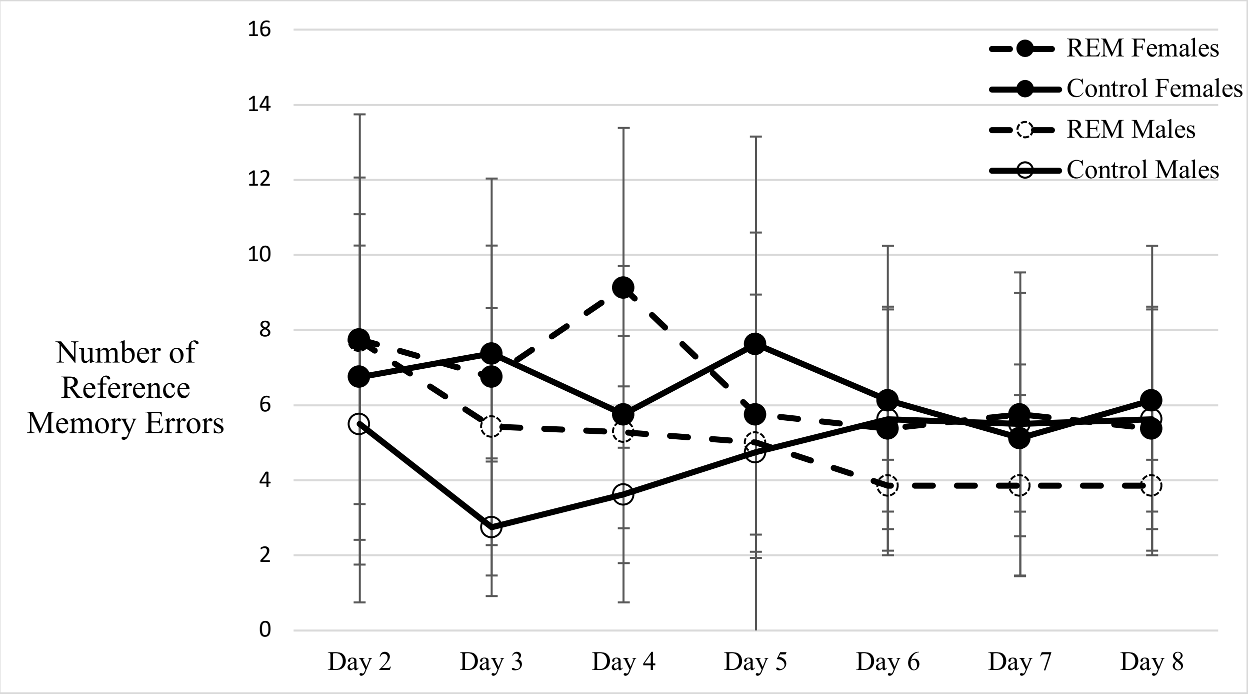

Figure 4. Number of reference memory errors made per experimental group on days 2 through 8. REM= REM deprived (dashed line). Data are presented as mean ± standard error. N = 8 in the control groups and REM females, N= 7 for the REM males

Working Memory Errors

Day 1. The amount of working memory errors was compared to ensure that the to-be-REM deprived and to-be-control groups were not different before the sleep manipulation occurred. For working memory errors, there was no significant difference between to-be-REM deprived (*M* = 2.40, *SD* = 3.36) and to-be-control (*M* = 1.75, *SD* = 1.77) conditions; *t* (29) = -.68, *p* = .50, Cohen's *d* = 0.24. There was also no significant difference between males (*M* = 2.87, *SD* = 3.54) and females (*M* = 1.31, *SD* = .95); *t* (29) = -1.69, *p* = .10, Cohen's *d*= .60. Because there were no significant differences between the groups, the following analyses were completed using the raw data.

Days 2-7. Mauchly's test indicated that the assumption of sphericity had been violated ($\chi^2(14) = 44.99$, $p < .001$), therefore degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity ($\varepsilon = 0.53$). There was no statistically significant change in working memory errors across testing, $F(2.68,72.48) = 1.05$, $p = .39$, $\eta^2_p = .03$. This suggests that, regardless of sex and sleep condition, the average number of errors made did not vary across the 6 days of testing. This suggests that no learning occurred from day 2 to day 7 of testing. There was no statistically significant difference between the REM deprived and controls on number of working memory errors made, $F(1,27) = .70$, $p = .41$, $\eta^2_p = .02$. There was also no statistically significant difference between the males and females, $F(1,27) = 1.10$, $p = .30$, $\eta^2_p = .04$.

There was no interaction between sleep condition and day, $F(2.68,72.48) = 1.24$, $p = .30$, $\eta^2_p = .04$. There was no interaction between sex and day, $F(2.68,72.48) = 1.24$, $p = .30$, $\eta^2_p = .04$. There was no interaction between sex and sleep condition, $F(1,27) = .001$, $p = .99$, $\eta^2_p = .001$. Lastly, there was no 3 way interaction between sex, sleep condition, and days, $F(2.68,72.48) = 1.55$, $p = .21$, $\eta^2_p = .05$ (see Figure 5).

Days 7-8. On day 8, the maze was rotated 180°. There was no statistically significant difference between testing days 7 and 8, $F(1,27) = 1.09$, $p = .31$, $\eta^2_p = .04$. There was no statistically significant difference between the REM deprived and controls on number of working memory errors made, $F(1,27) = .05$, $p = .82$, $\eta^2_p = .000$. There was no statistically significant difference between the males and females, $F(1,27) = .20$, $p = .66$, $\eta^2_p = .01$.

There was no interaction between sleep condition and day, $F(1,27) = .000$, $p = .98$, $\eta^2_p = .00$. There was no interaction between sex and day, $F(1,27) = 2.92$, $p = .10$, $\eta^2_p = .10$. There was no interaction between sex and sleep condition, $F(1,27) = 1.16$, $p = .29$, $\eta^2_p = .04$. Lastly, there was no 3 way interaction between sex, sleep condition, and day, *F*(1,27) = .04, *p* = .85, η^2_p = .001 (see Figure *5*).

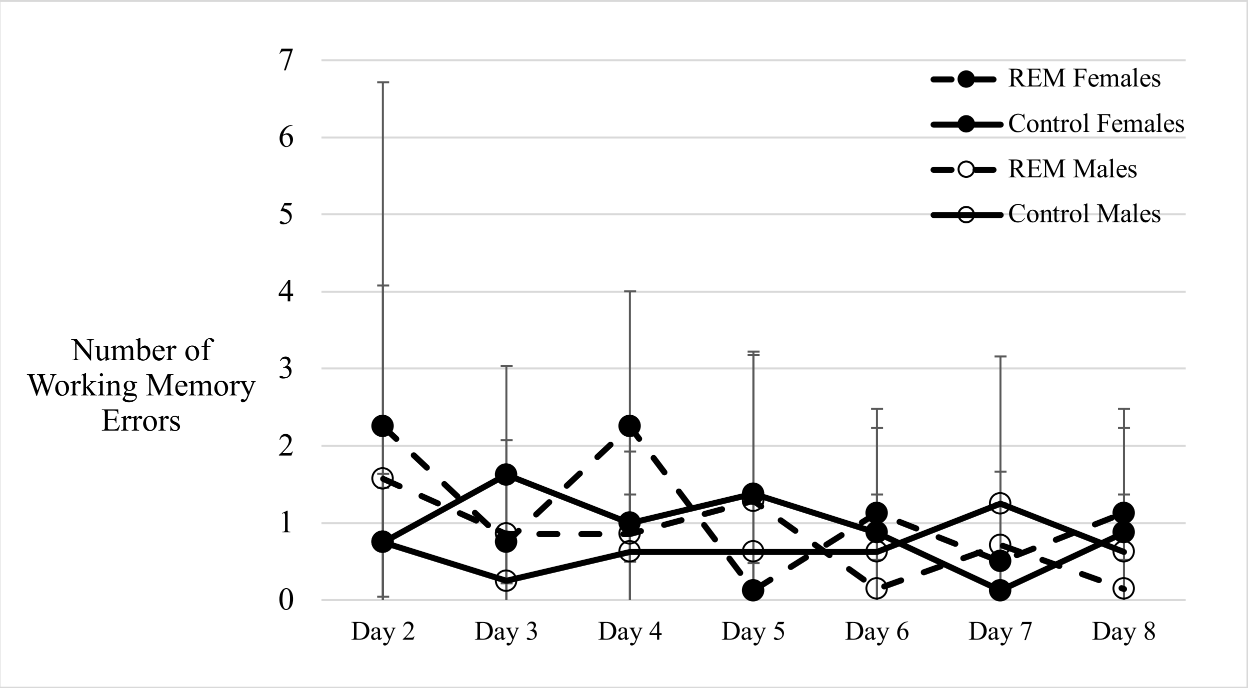

Figure 5. Number of working memory errors made per experimental group on days 2 through 8. REM= REM deprived (dashed line). Data are presented as mean ± standard error. N = 8 in the control groups and REM females, N= 7 for the REM males

Time to Complete the Maze

Day 1. The time until completion was compared to ensure that the groups were not different prior to the sleep manipulation. There was no significant difference between to-be-REM deprived (*M* = 141.13, *SD* = 130.67) and to-be-control (*M* = 101.50, *SD* = 56.20) conditions in time to complete the maze; *t*(29) = -1.11, *p* = .28, Cohen's *d* = 0.39. There was also no significant difference between males (*M* = 138.80, *SD* = 134.74) and females (*M* = 103.69, *SD* = 48.21); *t*(29) = -.98, *p* = .34, Cohen's *d*= .35. Because there are no significant differences between the groups, the following analyses were completed using the raw data.

Day 2-7. Mauchly's test indicated that the assumption of sphericity had been violated ($\chi^2(14) = 34.79$, $p < .001$), therefore degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity ($\varepsilon = 0.71$). There was no statistically significant change in latency to complete the maze across testing days, $F(3.56,96.23) = .91$, $p = .45$, $\eta^2_p = .03$. This suggests that, regardless of sex and sleep condition, the average time to locate all rewards did not vary across the 6 days of testing. There was no statistically significant difference between the REM deprived and controls, $F(1,27) = .89$, $p = .35$, $\eta^2_p = .03$. There was also no statistically significant difference between the males and females, $F(1,27) = 3.76$, $p = .06$, $\eta^2_p = .12$.

There was no interaction between sleep condition and day, $F(3.56,96.23) = 1.12$, $p = .35$, $\eta^2_p = .04$. There was no interaction between sex and day, $F(3.56,96.23) = .50$, $p = .72$, $\eta^2_p = .02$. There was no interaction between sex and sleep condition, $F(1,27) = .69$, $p = .41$, $\eta^2_p = .03$. Lastly, there was no 3-way interaction between sex, sleep condition, and day, $F(3.56,96.23) = .71$, $p = .57$, $\eta^2_p = .03$ (see Figure 6).

Days 7-8. On day 8, the maze was rotated 180°. There was no statistically significant difference between testing days 7 and 8, $F(1,27) = .48$, $p = 31$, $\eta^2_p = .02$. There was no statistically significant difference between the REM deprived and controls on number of working memory errors made, $F(1,27) = .04$, $p = .85$, $\eta^2_p = .00$. There was no statistically significant difference between the males and females, $F(1,27) = .40$, $p = .53$, $\eta^2_p = .02$.

There was no interaction between sleep condition and day, $F(1,27) = .29$, $p = .59$, $\eta^2_p = .01$. There was no interaction between sex and day, $F(1,27) = .00$, $p = .99$, $\eta^2_p = .00$. There was a trend towards significant interaction between sex and sleep condition with a large effect size, $F(1,27) = 4.18$, $p = .051$, $\eta^2_p = .13$. On average, REM deprived females took longer to complete the maze when compared to the REM deprived males and the control males and females (see

Figure 6). Lastly, there was no 3-way interaction between sex, sleep condition, and day, *F*(1,27) = .08, $p = .78$, $\eta^2_p = .00$ (see Figure 7).

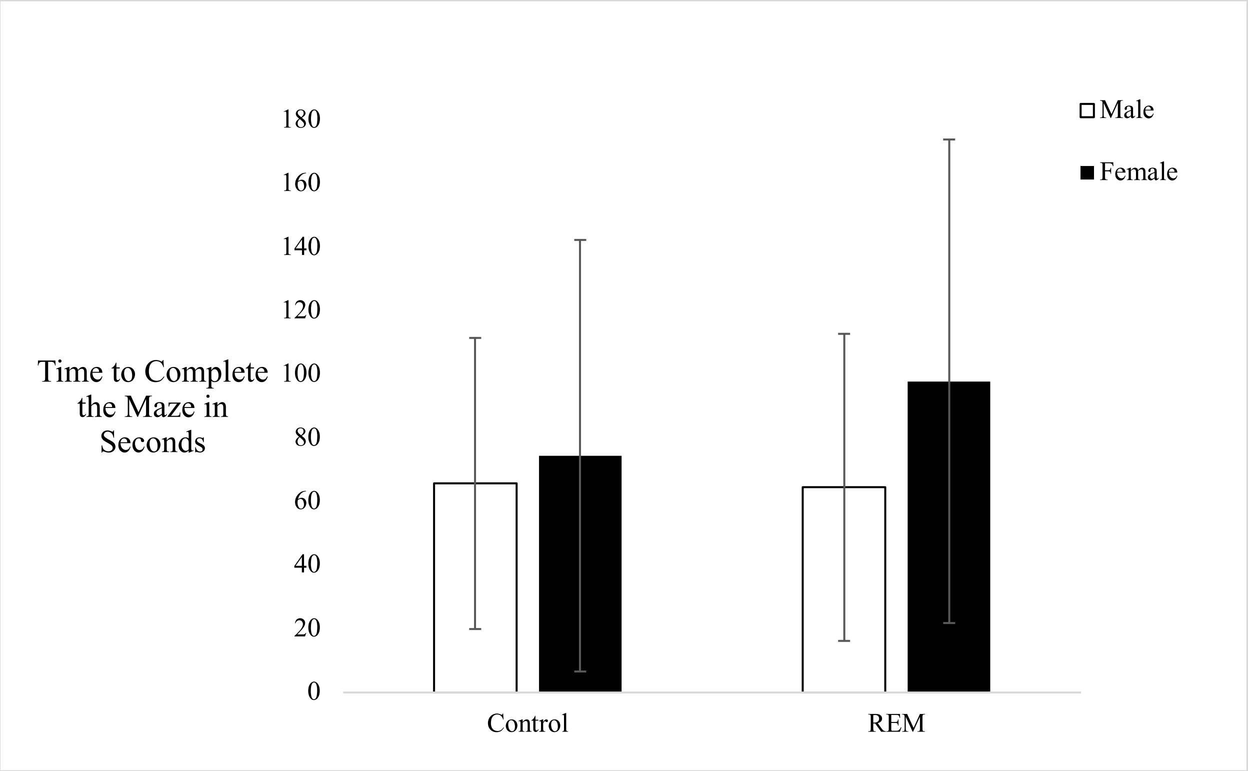

Figure 6. Average time it took the males and females to complete the maze. Data are presented as mean ± standard error. N = 15 for males, N = 16 for females.

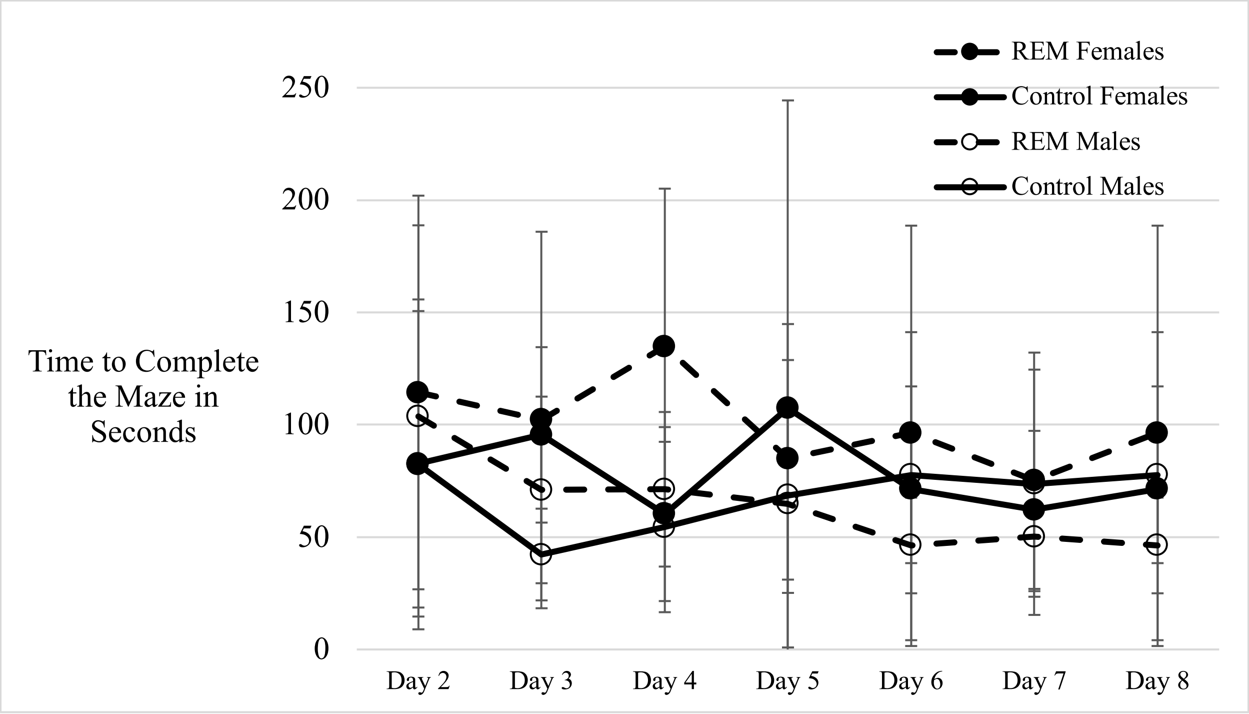

Figure 7. *Time to complete the maze, in seconds, per experimental group on days 2 through 8. REM= REM deprived (dashed line). Data are presented as mean ± standard error. N = 8 in the control groups and REM females, N= 7 for the REM males.*

Discussion

The results indicate that REM deprivation that occurs after training in the RAM does not impact performance in the maze and, more specifically, reference and working memory. These findings are in line with those who were unable to find an effect of post-training REM deprivation on spatial memory in the MWM (Hunter, submitted; Bjorness et al., 2005). However, the present study was able to show that males and females performed differently when comparing reference memory errors, but not working memory errors.

Although the results demonstrated that REM deprivation did not impact spatial memory, it did show that sex differences occurred when looking at the number of reference memory errors made. Regardless of sleep condition or day of testing, it was apparent that, on average, the males made less reference memory errors than females. This is in line with previous work showing that males have an advantage over females in spatial memory tasks (Jonasson, 2005). Additionally, Seymoure and colleagues (1996) also found that males made less reference memory errors compared to females. It is possible that the males in the present study had the advantage over females due to experience in a previous study assessing object recognition and object location memory. However, Seymoure and colleagues (1996) found that males made less working memory errors compared to females, a finding we were unable to replicate. If males had an advantage due to participating in a previous study, it would have been expected that they would also outperform females in regard to working memory errors as well.

Although males made more reference memory errors than females on days 2 through 7, there was no improvement across the days. Since there was no improvement across the days, it is possible that the rats were not using reference memory. Furthermore, this difference cannot be attributed to the amount of time the different sexes spent in the maze since there was no significant difference between the time it took the males and females to finish the maze.

However, during this time, the females could have walked faster and traveled farther in the maze thus resulting in more arm choices, and consequently, reference memory errors than the males.

Reference memory errors were further analyzed using an error fraction. The reference memory error fraction (Smith et al., 1998) of the total choices made, those that were reentries into a baited arm. Smith and colleagues were able to successfully use this error fraction in displaying their results. However, we were unable to find anything statistically significant. Furthermore, the number of arm choices that were made before a correct entry into a baited arm was analyzed per individual rat. This data did not convey any new or insightful information.

The inability to find an effect for working memory errors may be due to a floor effect. All rats, regardless of sex and sleep condition, made very little working memory errors across the eight days of testing. With all rats across all conditions and days only making a few working memory errors per day (*M* = 0.94, *Mdn* = 0), it is not surprising that no effect was found with such high levels of accuracy. However, it is also possible that REM deprivation does not affect working memory, consistent with previous research. Previously, Smith and Rose (1998) found that working memory was not affected by four-hours of REM sleep deprivation immediately after training; however, reference memory was. The results of the present study may suggest that only counting the number of re-entries to a baited arm may not be an adequate measurement of working memory in the RAM. Future studies should look to examine if just counting the number of re-entries into a baited arm is more appropriate than, for example, re-entries into any arm.

The inability to find an effect across days for both the reference and working memory errors may suggest that no learning occurred during these 6 days of testing. If learning was to occur over the period of 6 days, there would have been a significant decrease in the amount of reference memory errors. However, there was no significant decrease in errors over the course of the six days but an apparent stagnation of the average amount of errors thus, suggesting that no learning occurred for any of the groups. With the possibility of a floor effect within the working memory errors, it is possible that the task was too easy.

Although the task may have been too easy to measure working memory, it is possible that the maze was not easy, in terms to measuring reference memory and time to complete the maze. On average, across all conditions and sex, the rats made, on average, 6 reference memory errors per day. Furthermore, the rats took, on average across all conditions and sex, 76 seconds to complete the maze. Due to having a higher rate of reference memory errors and time to complete the maze it indicates there is room for improvement thus, the maze was not easy in terms of both of these measures.

Another possibility for the low number of working memory errors may be due to the strategy used to solve the maze. With no difference in performance between day 7 and 8 on any variable, this lends to the idea that the rats may have used allocentric navigation over egocentric because there were no statistically significant difference in the amount of errors made between the two days. However, it is possible that the rats had utilized a strategy outside of these two options: chaining (i.e., entering each arm in a systematic order), which does not rely on spatial memory (Vorhees & Williams, 2014). It is possible that the rats had entered the arms in adjacent turns which would, in turn, decrease the likelihood of committing an error. Although it was observed that, on a few occasions, a rat would enter each arm sequentially, it is possible that a less obvious pattern was used and went undetected. In the current study, the pattern of baited to non-baited arms was done so that visiting every other, or every third, would not completely solve the maze. However, it is possible that a less obvious chaining method was used that was successful in solving the maze thus circumventing the need to rely on spatial memory.

In addition to using less obvious chaining methods, it is possible that their performance in the maze does not reflect the use of spatial memory during the task. Although reference and working memory errors were recorded in hopes of reflecting spatial memory, it is possible that it does not accurately reflect spatial memory thus, there is no correlation between maze performance and spatial memory. The RAM has been frequently used in two different versions: the first being between-choice confinement where movement is restricted and the second, in which movement is not restricted. In between-choice confinement, once that rat has chosen an arm and exited back into the central hub the doors to the arms shut thus not allowing entrance into another arm for up to 15 seconds. In other versions movement is not restricted and rats are allowed to freely roam the apparatus. Taking these two different versions into consideration, Dubreuil, Tixier, Dutrieux, and Edeline (2003) sought to compare the behavioral performances of rats under these two different testing conditions: confinement and no confinement with all arms being baited. They found that, on the last day of 12 days of training sessions, rats in both groups displayed similar levels of performance. However, this does not mean that similar methods of solving the maze was used or the same information was learned in the maze. This indicated the possibility that performance may not be directly linked to spatial memory measures involving learning and memory.

The inability to find an effect of REM deprivation on spatial memory may be due to differences in methodology. It is possible that the order of testing may matter. There are two primary ways in which the effect of REM sleep deprivation on spatial memory has been studied: pre- and post- training. With post-training REM sleep deprivation, the results vary from observing an effect (Smith et al., 1998; Smith & Rose, 1996; Smith & Rose, 1997) to not being able to replicate the findings (Hunter, submitted; Walsh et al., 2011). However, the study that employed the use of post- training may have only observed results due to having a sustained period of REM sleep deprivation (Hajali et al., 2012).

The majority of research that concludes that REM deprivation does impair spatial memory, in both the RAM and MWM, has arisen from the same laboratory (Smith et al., 1998; Smith & Rose, 1996; Smith & Rose, 1997). Due to this, it is possible that these results are only being found under precise conditions that may be exclusive to that laboratory. Conversely, it is possible that the lack of results may be due to methodology within our laboratory (Hunter, submitted). However, only two other laboratories have sought to replicate these findings, and only one with success (Legault et al., Walsh et al., 2011). This highlights the need for more research outside of these laboratories and a closer examination of the methodology used.

The male rats who were used in this study served as controls in a study which examined if chronic sleep restriction (CSR) in adolescences impacts memory in adulthood (Howard, 2018). Although the rats in the control condition slept undisturbed, those that experienced CSR displayed impairments on hippocampal dependent memory tasks. By having experience in this experiment, the male rats had previous exposure to being tested in hippocampal dependent and non-hippocampal dependent memory tasks, but no previous exposure to sleep deprivation. Meanwhile, the female rats had no previous pre-exposure to any behavioral tasks before starting training in the RAM. By nature of being in a previous experiment, the males also had much more handling compared to the females. Due to these reasons, future studies should compare males and females who are naïve to any type of testing.

The lack of findings may not be attributed to flaws in the flower pot technique. Within the current study, it was noted that a majority of the rats tried to escape the flower pot apparatus, both in the control and REM sleep deprivation conditions. However, this was only observed in the first few minutes of being in the apparatus and, at the end of the four hours, all rats were dry, suggesting that they had not fallen into the water. Past research has suggested that the flower pot technique is effective in reducing REM sleep. Smith and Gisquet-Verrier (1996) found a significant drop in the total amount of REM sleep deprivation while in the flower pot compared to their baseline. Although there was a difference in the amount of REM sleep, the non-REM sleep did not differ from baseline. This suggests that the lack of findings may not be due to the flower pot technique being ineffective.

Despite not observing any effect of REM sleep deprivation on spatial memory, the results show a need to explore the discrepancy of whether or not REM sleep deprivation is necessary for spatial memory through replication of past studies. Smith and colleagues (1998) were able to demonstrate that four hours of REM sleep deprivation following training in the RAM was adequate enough to display an impairment in male rat's spatial memory. The results of the current study, with similar methodology, were unable to replicate these findings. However, under a different spatial task, this is not the first time these findings have been challenged and unable to come to similar conclusions (Hunter, submitted; Walsh et al., 2011). Because these findings could not be replicated with the same spatial memory task, nor a similar one, it is possible that the lack of effect of REM sleep deprivation on spatial memory seen in the present study may be due to there being no relationship between these variables.

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