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Short-term REM deprivation does not affect acquisition or reversal of a spatial learning task

Amy Silvestri Hunter

Department of Psychology, Seton Hall University, 400 South Orange Avenue, South Orange, NJ, 07079 USA

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ABSTRACT

Although there is a general belief that rapid eye movement sleep (REM) is essential for spatial memory tasks such as the Morris water maze (MWM), there is conflicting evidence for this assertion. This study investigated the effects of short-term REM deprivation on acquisition and reversal of the MWM by varying the timing of REM deprivation and the degree of task acquisition in three separate experiments. There was no evidence for a detrimental effect of REM deprivation on acquisition, retention, or reversal in the MWM. These data add to a growing body of evidence that although REM is important for certain types of learning and memory, spatial memory, as assessed by the MWM, is not among them.

1. Introduction

One of the most enduring beliefs in the field of sleep research is the impairing effect of rapid eye movement sleep (REM) deprivation on spatial memory. Animal studies have investigated the role of REM in a variety of different spatial learning paradigms, all of which require an animal to remember the location of stimuli in its environment. One of the most commonly used spatial learning tasks is the Morris water maze (MWM; Morris, 1984; Morris et al., 1982), in which an animal is required to use environmental cues to learn and remember the location of a submerged platform in a pool of water. Several studies have demonstrated deficits in acquisition of the MWM as a consequence of short-term – that is, 8 h or less – of post-training REM deprivation (RD). For example, Smith and Rose (1996, 1997) found that 4 hours of RD following daily MWM training produced an increased latency to locate the hidden platform on only the second day of the four day training procedure. This transient impairment suggests that the deleterious effects of RD are limited to the time period during which task knowledge is still being consolidated but is without effect after performance is stable.

Similar results have been found with other spatial learning tasks. Smith et al. (1998) determined that 4 hours of post-training RD impaired reference memory in the radial arm maze, a task in which an animal uses environmental cues to remember which arms of a maze are baited with a food reward. Bjorness and colleagues (2005) utilized the eight-box spatial task, in which animals move along a track in a rectangular box with eight recessed food cups learning which are baited. Four hours of RD after each day of a 15-day training protocol in this task produced impairments in task acquisition. Interestingly, both RD and non-RD animals reached similar levels of performance by the end of the study. This is consistent with the interpretation of the previous study in which RD has no effect after a task has been successfully acquired (Smith et al., 1998). Additional findings supporting a connection between REM and spatial memory come from Smith and Rose (1997), who found a significant increase in time spent in REM following MWM training. This increase began approximately 2 hour into the 24 -hour recording period and continued up to 20 hours after training. There was no change in the sleep patterns of animals exposed to a non-spatial version of the MWM accomplished with a visible platform.

However, not all findings are consistent with the theory that REM is essential for spatial memory and some of the studies previously described have inconsistent findings or confounds that complicate interpretation of the results. For example, Smith and Rose (1996) observed that only RD performed during hours 5–8 after training – but not hours 1–4 or 9–12 – had deleterious effects on subsequent MWM performance. In contrast, in a subsequent paper the same authors found an impairing effect of RD conducted from 1 to 4 hours post-training but not 5–8 hours (Smith and Rose, 1997). Furthermore, Smith and Rose (1996) found that RD animals made more entries into the goal quadrant than other quadrants in the tank, suggesting that RD rats remembered the platform location. Bjorness and colleagues (2005) found the effect of RD to be subtle, with only errors of commission – but not hesitation or omission – to be transiently affected by RD and equivalent performance in both groups by the end of training. Additionally, only RD performed from 0 to 4, but not 4–8, hours after training affected performance. Finally, most studies have compared RD to a condition in which rats are

E-mail address: amy.hunter@shu.edu.

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placed in their home cage as a control. Given that RD is conducted in a unique apparatus in a distinct context, which most frequently consists of placing a rat on a platform above a tank of water, it is likely that placement in the novel apparatus and environment itself causes non-specific sleep disruption. This is supported by the finding that seemingly minor environmental changes such as placing a novel object into a cage (Schiffelholz and Aldenhoff, 2009) or even standard cage change (Tang et al., 2005) affects sleep-wake parameters. Therefore, the sleep architecture of rats exposed to RD and those allowed to sleep in their home cages is likely to be different on a number of variables in addition to the disruption in REM.

In a series of four experiments, Walsh and colleagues (2011) attempted to replicate the findings of Smith and Rose (1996, 1997) but found that latency to locate the platform during MWM acquisition was unaffected by 6 hours of post-training RD. There were also no effects of RD on memory for platform location in subsequent reversal learning, in which the location of the platform was moved to the opposite quadrant of the tank. This lack of effect was apparent whether 12 or 4 daily training trials were used. Wang et al. (2009) demonstrated an impairing effect of 3 days of post-training RD on time spent in the goal quadrant during a probe trial immediately after RD, but no such effect with 18 hours of recovery sleep after RD. This demonstrates a memory impairment only when rats are tested while sleep deprived even after extended REM deprivation and does not permit a distinction between a performance deficit due to extended sleep deprivation at time of testing and one due specifically to post-training RD.

Studies investigating the effects of RD prior to MWM training also cast doubt on the REM-spatial memory connection. Beaulieu and Godbout (2000) exposed rats to 8 hours of RD followed immediately by MWM training in one of two versions of the MWM: a standard version, in which rats were required to use external spatial cues to locate the platform in a fixed location, or an alternation version, in which the location of the platform alternated between two positions. RD rats took longer to find the platform in the alternation version of the task but were unimpaired in the standard version. Similar results were found by LeMarec and colleagues (2001) with only 4 hours of RD prior to training.

In summary, while early literature suggests that REM is important for spatial learning, many contradictory findings exist and confounds limit interpretation of results. In particular, the lack of an appropriate control group exposed to an apparatus similar to the one used for RD but without specific effects on REM leaves open the possibility that the observed effects are due to a nonspecific disruption in sleep. The present study was designed to replicate prior studies with the addition of an appropriate control group and to investigate the influence of short-term RD on both acquisition and reversal of MWM learning. Reversal learning has similarities to extinction, the process by which the relationship between two previously associated stimuli is weakened. While extinction has traditionally been investigated with the use of classical conditioning procedures, it can also be produced in a spatial task such as the MWM (Lattal et al., 2003; Prados et al., 2003). Although RD has been shown to produce deficits in extinction of classically conditioned fear (e.g. Fu et al., 2007; Hunter, 2018; Silvestri, 2005), to date only one study has investigated the effects of short-term RD on reversal learning in the MWM and found no effect (Walsh et al., 2011).

The present study had several goals. The first was to conduct a replication of previous work investigating the effects of post-training RD on MWM acquisition and retention as well as reversal (Smith and Rose, 1996, 1997; Walsh et al., 2011) with the addition of a more appropriate control procedure. A second goal was to extend previous work by manipulating the amount of training provided as a means to manipulate degree of task acquisition, which has been proposed to be specifically affected by RD (Smith and Rose, 1996, 1997). Additionally, the delay between RD and subsequent testing was manipulated to ensure rats were not tested while sleep deprived, which could obscure effects of RD. A clearer understanding of the role of RD in MWM acquisition and retention is necessary to establish an accurate model of the functions of various sleep stages.

2. General materials and methods

2.1. Subjects

Subjects in all experiments were male Sprague-Dawley rats (Envigo, Indianapolis, IN) and were approximately 8 weeks of age at the start of the study. Animals were housed in pairs, given food and water *ad libitum*, and maintained on a 12:12 light-dark cycle (lights on at 8am). All procedures were approved by the Seton Hall University Institutional Animal Care and Use Committee and followed the guidelines for the care and use of laboratory animals from the United States Public Health Service.

2.2. Apparatus

2.2.1. Morris water maze

The Morris water maze consisted of a circular plastic tank (130 cm diameter, 52.5 cm tall) filled with water to a depth of approximately 30 cm. The goal platform was located 2 cm below the surface of the water and 21 cm from the edge of the tank. Styrofoam packing peanuts were placed in a single layer on top of the water, thereby obscuring the location of the submerged platform (Cain et al., 1993). Each wall in the room contained a number of cues that were visible from the tank, including a curtain on one wall and visually distinct posters on 2 other walls. A camera was suspended from the ceiling above the center of the pool to record rat behavior during probe trials. The experimenter stood in the same position during all training sessions.

2.2.2. REM deprivation

REM deprivation was produced with the flowerpot technique, in which each rat was placed onto an inverted flowerpot (10 cm in diameter) inside a large pail (33 cm diameter, 37 cm tall) filled with water up to 1 cm below the level of the flowerpot. This technique has been shown to selectively deprive rats of REM but not non-REM sleep (Mendelson et al., 1974; Smith and Gisquet-Verrier, 1996). Rats in this apparatus can obtain non-REM, but the inhibition of antigravity muscles that accompanies the start of a REM episode (Chase and Morales, 1990) causes them to lose their balance and wake up as they do so. Importantly, rats are typically dry when removed from the apparatus, suggesting they do not fall off the platform into the water during the procedure. The apparatus used for the RD control procedure was similar to that used to produce RD with the exception that the diameter of the platform was larger (21.5 cm). This apparatus controls for nonspecific effects of the RD apparatus on sleep without affecting REM (Mendelson et al., 1974; Smith and Gisquet-Verrier, 1996).

2.3. Procedure

Starting approximately 1 week after their arrival, rats were handled for approximately 5 min per day for 3 days. The first day of MWM acquisition began with a pretraining trial in which rats were placed on the goal platform for 90 s. If at the end of the 90 s period the rat had left the platform, it was guided back to it and left on the platform for another 10 s. For the next acquisition trials, rats were placed in the pool facing the wall of the tank and given 90 s to swim to the platform. The goal platform was in a constant location for all pretraining and acquisition trials, but the location of the rats' entry into the pool varied for each trial on a given day. If a rat did not locate the platform in the allotted time it was guided there. After locating the platform, each rat was left there for 10 s before being removed from the pool, dried off, and put into a holding cage for an intertrial interval of approximately 10 min while other rats in the cohort were run. The packing peanuts were

straightened after each trial to ensure that the entire surface of the pool was covered and the submerged platform remained hidden. Latency to locate the platform was recorded for all trials. For probe trials, the platform was removed from the pool and time spent in each quadrant of the pool (target, adjacent, or opposite to previous platform location) was recorded and later calculated with Any-maze video tracking system (Stoelting Co., Wood Dale, IL).

The timing of RD or the control condition with respect to MWM training and testing varied in each experiment and is detailed below. Rats were placed into the RD or control condition and left undisturbed for 6 h, at which point they were returned to their home cages.

2.4. Data analysis

Statistics were computed with IBM SPSS statistical software. Latency to locate the platform in acquisition and reversal trials of the MWM was analyzed with two-way mixed design ANOVAs (trials x sleep condition) or one-way repeated measures ANOVAs (trials), depending on experimental design (i.e., timing of RD). Time spent in each quadrant of the tank during probe trials was analyzed with one-way repeated measures ANOVAs and *post hoc t* tests with Bonferroni corrections. In cases where Bonferroni corrections were not necessary, p values of .05 or less were considered statistically significant. Effect size was determined through interpretation of η^2_p using standard conventions (small = .01, medium = .06, large = .14; Cohen, 1988). Although data on some trials violated the assumption of normality, the ANOVA is robust to such violations (Field, 2013).

3. Experimental methods, results, and discussion

3.1. Experiment 1

3.1.1. Method

The design of this study was similar to that of Smith and Rose (1996, 1997) with the addition of a control procedure more comparable to the RD condition. Rats were given 12 acquisition trials in the MWM followed immediately by 6 hours of RD or the control condition, in which they were placed on a larger platform so as to produce less sleep disruption (n = 8 per group). The next day, rats were given 4 reminder trials followed by 12 reversal trials, in which the submerged platform was moved to the opposite side of the tank. On the third day rats were given 4 reversal reminder trials. The location of the goal platform was randomly assigned to one of the four tank quadrants for each rat. The design of this and all subsequent experiments is illustrated in Fig. 1.

3.1.2. Results and discussion

As shown in Fig. 2, a repeated measures ANOVA revealed a significant decrease in latency to locate the platform across the 12 acquisition trials on the first day of training [$F(11,165) = 5.67, p < .001$, $\eta^2_p = .27$]. A 2 × 4 (sleep condition x trials) mixed design ANOVA revealed a significant decrease in latency to locate the platform during the reminder acquisition trials the next day [$F(3, 42) = 6.46, p = .001$, $\eta^2_p = .32$]. However, this did not vary by sleep condition [$F(1,14) = 2.4, p = .14, \eta^2_p = .146$] nor was there a trials by sleep condition interaction [$F(3,42) = 0.09, p = .96, \eta^2_p = .01$]. During the reversal learning phase there was also a decrease in latency to locate the platform across trials [$F(11, 154) = 2.76, p = .003, \eta^2_p = .16$], but this did not vary by sleep condition [$F(1,14) = 0.55, p = .47, \eta^2_p = .04$], nor was there a trials by sleep condition interaction [$F(11, 154) = 0.30, p = .99, \eta^2_p = .02$].

A similar pattern of results was found for performance during the 4 reminder reversal trials on the following day. A decrease in latency to locate the platform was observed [$F(3, 42) = 10.59, p < .001$, $\eta^2_p = .43$], but no effect of sleep condition [$F(1,14) = 2.44, p = .14$, $\eta^2_p = .15$] nor a trials by sleep condition interaction [$F(3, 42) = 0.16, p = .93, \eta^2_p = .01$].

Taken together, these results indicate that rats successfully learned the initial location of the platform and its reversed location but were not affected by RD conducted immediately after acquisition. This is in direct contrast to the findings of Smith and Rose (1996, 1997) but consistent with the findings of Walsh and colleagues (2011). However, it is possible that confounding variables obscured an effect of RD. As noted previously, an experimental design with a learning task the day after a sleep manipulation may result in animals being tested in a sleep deprived state, which would likely impair performance. The current study used a control condition that produces nonspecific sleep disturbances as compared to the home cage control utilized in most other studies. With the use of a more similar control condition in the present study, both groups may have been sleep deprived at the time of testing, resulting in a similar response in both groups that obscured a more subtle effect of RD. It is also possible that given the speed with which animals acquired both the acquisition and reversal phases of the task, there may have been a ceiling effect which obscured any effects of RD on task acquisition. Finally, previous research has shown some effects of RD on MWM probe trial performance (Walsh et al., 2011; Wang et al., 2009). As previous work by Smith and Rose (1996, 1997) did not utilize a probe trial and this experiment was a replication of that work, no probe trials were used. However, addition of probe trials provides an additional means of assessing memory for platform location. The next experiment attempted to address these possibilities.

3.2. Experiment 2

This experiment was designed to address several of the possible issues in Experiment 1 that may have obscured an effect of RD on MWM acquisition or reversal. A rest day was added between RD and subsequent testing days to ensure that rats were not tested while sleep deprived. The same number of trials were used for each phase of training, but they were spaced across several days to slow task acquisition and reduce the likelihood of a ceiling effect. A probe trial was added after initial training and after reversal training as an additional assessment of memory for platform location. Finally, given the previously observed impairing effect of RD on extinction of fear conditioning (Fu et al., 2007; Hunter, 2018; Silvestri, 2005), its effects on a version of spatial extinction in the MWM in which the platform location was reversed were investigated in a separate group of animals.

3.2.1. Method, Experiment 2A

Training was conducted in 3 daily sessions with 4 trials per session. Immediately following each acquisition session rats were placed in either the RD (n = 8) or control apparatus (n = 8). After 6 hours animals were removed and returned to their home cages. To ensure that rats were not being tested while sleep deprived, a minimum of 48 hours elapsed between MWM sessions. Between 48–60 hours after the last acquisition session, rats were given a 90 s probe trial in which they were placed in the tank with no platform and the time spent in each tank quadrant was measured. Reversal training began between 48–60 hours following the first probe trial and was accomplished by moving the platform to the opposite quadrant of the pool. Platform location for training and reversal were the same for all animals. Reversal training and the subsequent probe trial were conducted in the same manner as described above for acquisition except that RD was not performed. Due to technical malfunction, probe trial data were lost for 2 rats each from the RD and control groups, resulting in 6 rats per group for probe trial data analysis.

3.2.2. Results and discussion, Experiment 2A

A repeated measures ANOVA revealed a trend with a large effect size for a decrease in latency to locate the platform across the first day of training [$F(3, 45) = 2.67, p = .059, \eta^2_p = .15$]. A 2 × 4 (sleep condition x trials) mixed design ANOVA on the second day of training showed a decrease in latency to locate the platform [$F(3, 42) = 3.10$,

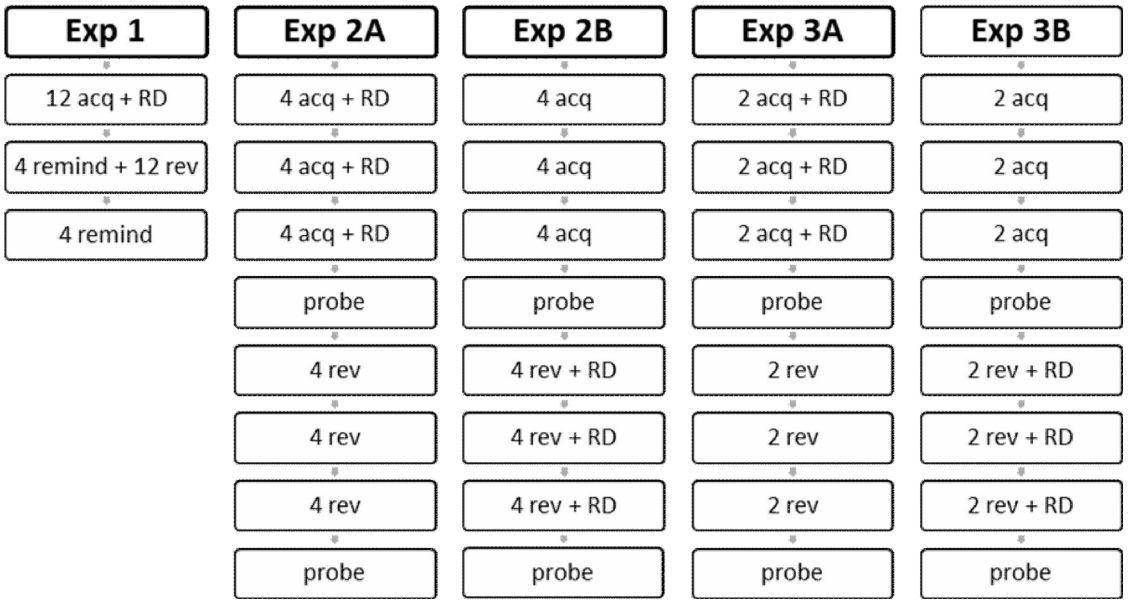

Fig. 1. Schematic of design for all experiments, in which the number of trials per day and timing of REM deprivation was systematically varied. Each cell represents procedures performed on a different day. Acq = acquisition trials; RD = REM deprivation or control condition; probe = probe trial, in which the platform was removed and time spent swimming in each tank quadrant was assessed; remind = reminder trials, in which the submerged platform was in the same location as training; rev = reversal trials, in which the location of the submerged platform was reversed from its location during acquisition.

p = .04, η^2_p = .18] although there was no effect of sleep condition [F(1, 14) = 0.15, p = .70, η^2_p = .01] nor was there a trials by sleep condition interaction [F(3, 42) = 1.61, p = .20, η^2_p = .10]. A similar pattern of results was seen on the third day of acquisition with a decrease in latency across trials [F(3, 42) = 4.77, p = .006, η^2_p = .25] but no effect of sleep condition [F(1, 14) = 0.15, p = .70, η^2_p = .01] or a trials by sleep condition interaction [F(3, 42) = 0.28, p = .84, η^2_p = .02]. These results are shown in Fig. 3.

Fig. 4 (top panel) displays data from the first probe trial, during which rats spent varying amounts of time in the tank quadrants [F(3, 30) = 3.72, p = .022, η^2_p = .27]. This did not vary by sleep condition [F(1, 10) = 0.89, p = .37, η^2_p = .08] nor was there a quadrant by sleep condition interaction [F(3, 30) = 0.58, p = .63, η^2_p = .06]. Surprisingly, rats spent less time in the target quadrant than the opposite quadrant [t(11) = -3.08, p = .01] but time spent in the target quadrant did not differ significantly from time spent in either of the adjacent quadrants (p's > .2). This, in combination with the platform location latency data, suggests that although rats became more efficient at locating the submerged platform during training, they had not yet completely consolidated the memory for platform location as they were not searching in the correct location when it was removed.

As shown in Fig. 3, across the first day of reversal learning there was no change in latency to locate the platform [F(3, 42) = 1.26, p = .30, η^2_p = .08], nor was there an effect of sleep condition [F(1, 14) = 1.787,

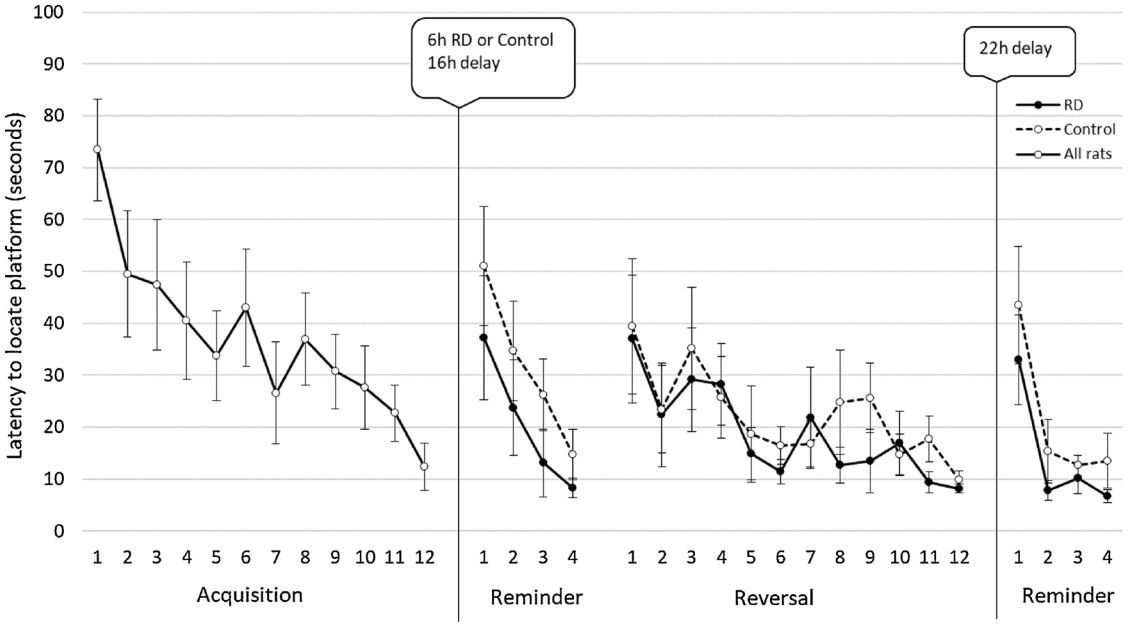

Fig. 2. Latency to locate the platform during Experiment 1 in REM deprived and control rats. Sleep manipulations were implemented immediately after acquisition training. Acquisition data are shown for all rats combined as the sleep manipulation occurred after this phase. RD = REM deprivation. N = 8 per group. Data are presented as M ± SEM.

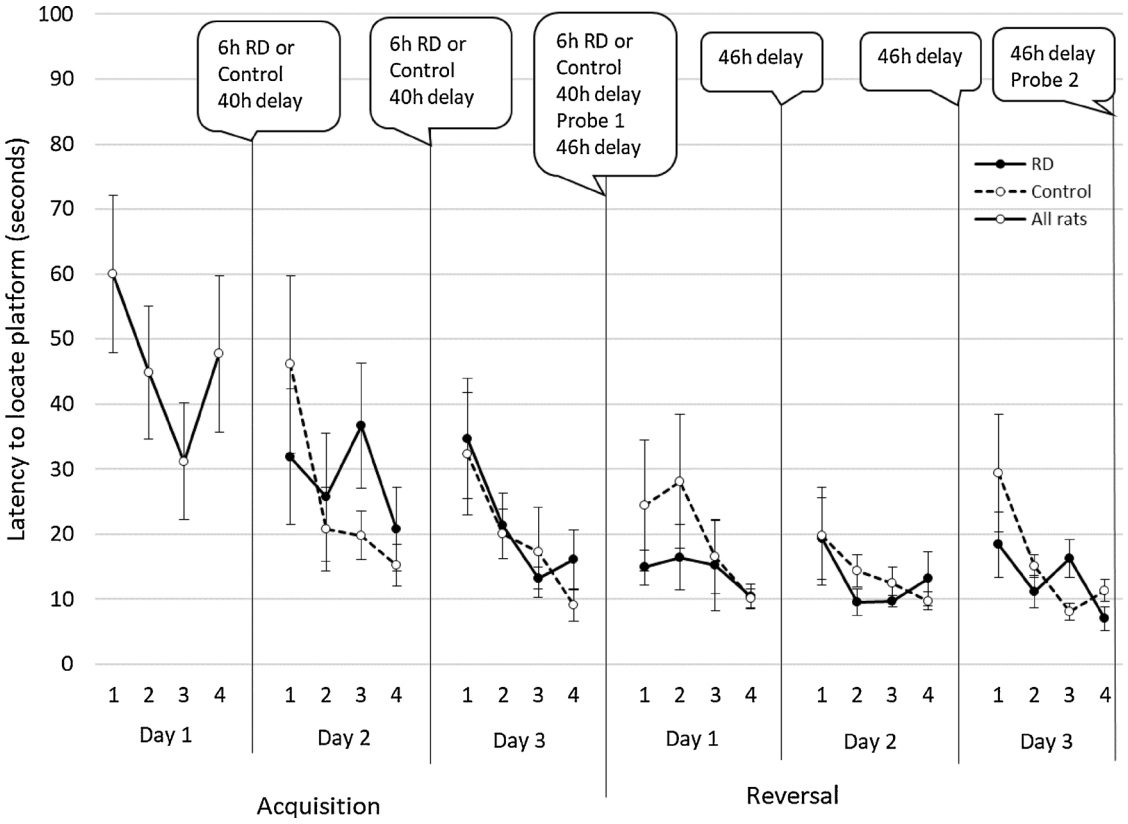

Fig. 3. Latency to locate the platform during Experiment 2A in REM deprived and control rats. Sleep manipulations were implemented immediately after each day of acquisition training. Acquisition day 1 data are shown for all rats combined as the sleep manipulation occurred after this phase. RD = REM deprivation. N = 8 per group. Data are presented as M ± SEM.

p = .203, η^2_p = .113] or a trials by sleep condition interaction [F(3, 42) = 0.42, p = .74, η^2_p = .03]. The pattern of results was similar on the second day of reversal learning, with no change in latency across trials [F(3, 42) = 2.13, p = .11, η^2_p = .13], no effect of sleep condition [F(1, 14) = 0.12, p = .73, η^2_p = .01], and no trials by sleep condition interaction [F(3, 42) = 0.41, p = .75, η^2_p = .03]. The lack of effect of trials on the first two days of reversal training is likely due to a ceiling effect in which rats typically located the platform in under 20 s, even on the first trial. Latency to locate the platform decreased across the third day of reversal training [F(3, 42) = 4.81, p = .006, η^2_p = .26] but there was no effect of sleep condition [F(1, 14) = 1.05, p = .32, η^2_p = .07] and no trials by sleep condition interaction [F(3, 42) = 1.84, p = .15, η^2_p = .12].

During the second probe trial, shown in Fig. 4 (lower panel), rats spent varied amounts of time in the tank quadrants [F(3, 30) = 15.95, p < .001, η^2_p = .62]. This did not vary by sleep condition [F(1, 10) = 0.017, p = .9, η^2_p = .002] nor was there a quadrant by sleep condition interaction [F(3, 30) = 0.86, p = .47, η^2_p = .08]. Rats spent significantly more time in the target quadrant than the opposite quadrant [t(11) = 3.88, p = .003] and one of the adjacent quadrants [t(11) = 8.33, p < .001], with no difference in time spent in the target quadrant as compared to the other adjacent quadrant [t(11) = 2.09, p = .061].

Taken together, these findings indicate that RD conducted immediately after acquisition training did not affect memory for initial platform location, nor did it affect subsequent reversal learning. This pattern of results is similar to Experiment 1 and demonstrates that any nonspecific sleep deprivation due to the RD or control procedures did not affect retention of the MWM task or reversal learning as measured by latency to locate the platform or time spent in each tank quadrant during probe trials. Additionally, it fails to demonstrate any effect of RD on retention of initial learning in the MWM or acquisition of reversal learning.

3.2.3. Method, Experiment 2B

The experimental design was identical to Experiment 2A with the exception that RD or the control condition (n = 8 per group) was performed after the first day of reversal training, similar to Walsh and colleagues (2011). Due to technical malfunction, data from the second probe trial were lost for 2 rats each from the RD and control groups, resulting in 6 rats per group for probe trial 2 data analysis.

3.2.4. Results and discussion, Experiment 2B

As shown in Fig. 5, a repeated measures ANOVA demonstrated a reduction in latency to locate the platform across the first day of training [F(3, 45) = 4.17, p = .011, η^2_p = .22]. A similar effect was seen on the second day of training [F(3, 45) = 9.29, p < .001, η^2_p = .82] and third day [F(3, 45) = 3.51, p = .023, η^2_p = .19].

As shown in Fig. 6 (top panel), during the first probe trial rats spent varying amounts of time in the tank quadrants [F(3, 45) = 7.33, p < .001, η^2_p = .33]. Rats spent less time in the target quadrant than an adjacent quadrant [t(15) = -3.15, p = .007] but there was no difference in time spent in the target vs opposite quadrants [t(15) = -1.6, p = .13] or the target vs the other adjacent quadrant [t(15) = 1.74, p = .1]. Similar to the first probe trial from Experiment 2A, this suggests that rats became more efficient at locating the platform during training but had not completely consolidated the memory for platform location at the time of the probe trial.

As shown in Fig. 5, there was no change in latency to locate the platform across the first day of reversal training [F(3, 45) = 0.41, p = .75, η^2_p = .03]. A 2 × 4 (sleep condition x trials) mixed design ANOVA demonstrated a decrease in latency to locate the platform on the second day of reversal training [F(3, 42) = 4.56, p = .007, η^2_p = .25]. This did not vary by sleep condition [F(1, 14) = 0.33,

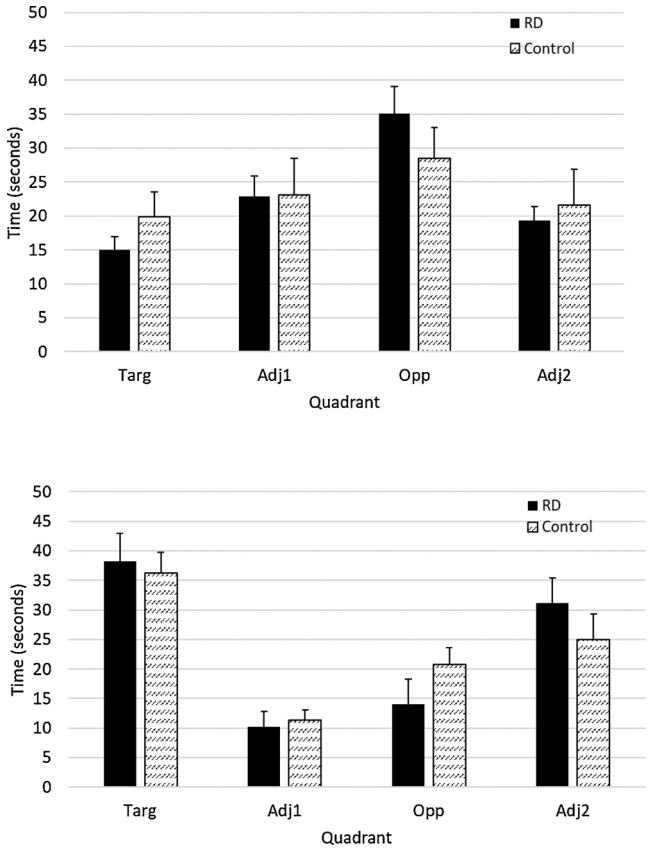

Fig. 4. Time spent in each tank quadrant in REM deprived and control rats for probe trial 1 (top), conducted after acquisition training, and 2 (bottom), conducted after reversal training, in Experiment 2A. Targ = target quadrant; Adj1 and Adj2 = adjacent quadrants; Opp = opposite quadrant. RD = REM deprivation. N = 6 per group. Data are presented as M ± SEM.

p = .58, η^2_p = .02] nor was there a trial by sleep condition interaction [F(3, 42) = 0.83, p = .48, η^2_p = .02].

On the third day of reversal training there was no change in latency across trials [F(3, 42) = 1.18, p = .33, η^2_p = .08] and no effect of sleep condition [F(1, 14) = 0.15, p = .7, η^2_p = .01], nor was there a trials by sleep condition interaction [F(3, 42) = 1.96, p = .14, η^2_p = .12].

Fig. 6 (lower panel) shows data from the second probe trial, during which rats spent varying amounts of time in the tank quadrants [F(3, 30) = 4.95, p = .007, η^2_p = .33] but this did not vary by sleep condition [F(1, 10) = 2.4, p = .15, η^2_p = .19] nor was there a quadrant by sleep condition interaction [F(3, 30) = 0.21, p = .89, η^2_p = .02]. Rats spent significantly more time in the target quadrant as compared to the opposite quadrant [t(11) = 3.37, p = .006] and more time in the target quadrant as compared to one of the adjacent quadrants [t(11) = 3.88, p = .003] but there was no difference in time spent in the target quadrant as compared to the other adjacent quadrant [t(11) = 1.34, p = .2].

These findings demonstrate that post-reversal RD did not affect the retention of reversal learning in the MWM. Data from the first probe trial, which did not show a clear preference for the tank quadrant where the platform was previously located, may be interpreted as evidence that animals were uncertain regarding the platform location. Based on prior research suggesting that RD has its effects only during task acquisition, as may have been occurring during the probe trials, this should have provided an opportunity to see impairing effects of RD; however, RD did not affect time spent in each quadrant during the probe test or the retention of reversal learning.

Similar to the results of experiment 1, animals quickly acquired both the acquisition and reversal phases of the task as assessed by latency to locate the platform. This may be the result of a ceiling effect which obscured any effects of RD while the task was being consolidated, which has previously been demonstrated to be sensitive to RD (Smith and Rose, 1996, 1997). The next experiment utilized fewer trials spaced across several days to slow task acquisition and test this possibility.

3.3. Experiment 3

This experiment attempted to reduce the likelihood of asymptotic learning by using spaced training trials and fewer training trials overall. In a second experiment with a separate group of animals, the timing of RD was varied between acquisition and reversal learning.

3.3.1. Method, Experiment 3A

Rats were given 2 MWM trials per day for 3 days with 6 hours of RD or the control condition (n = 8 per group) immediately after each acquisition session and one rest day between each testing session. Between 48–60 hours after the last acquisition session, rats were given a 90 s probe trial in which they were placed in the tank with no platform and the time spent in each tank quadrant was measured. After another 48–60 hour delay, reversal training was conducted. Reversal training and the subsequent probe trial were conducted in the same manner as described for acquisition except that RD was not performed. Platform location for training and reversal were the same for all animals. Due to technical malfunction, data from the second probe trial were lost for 2 rats in each of the RD and control groups, leaving a total of 6 rats in each group for probe trial data analysis.

3.3.2. Results and discussion, Experiment 3A

As shown in Fig. 7, a repeated measures ANOVA demonstrated no change in time taken to locate the platform across the first training day [F(1, 15) = 0.000, p = .99, η^2_p < .000]. However, a 2 × 2 (trials x sleep condition) mixed design ANOVA showed a significant decrease in latency during the second day [F(1, 14) = 8.99, p = .01, η^2_p = .39] but this did not vary by sleep condition [F(1, 14) = 0.074, p = .79, η^2_p = .005], nor was there a trials by sleep condition interaction [F(1, 14) = 0.48, p = .50, η^2_p = .033]. Similarly, on the third day of training there was a decrease in latency across trials [F(1, 14) = 7.14, p = .02, η^2_p = 33]. There was no effect of sleep condition [F(1, 14) = 0.44, p = .52, η^2_p = .03] but there was a trials by sleep condition interaction [F(1, 14) = 5.19, p = .04, η^2_p = .27]. This was the result of a sharper decrease in latency across both trials in the RD group as compared to the control group. However, both groups had similar latencies on the last trial of the day.

Fig. 8 (top panel) displays the data from the first probe trial, which revealed a difference in time spent in each quadrant [F(3, 42) = 16.75, p < .001, η^2_p = .55] and a quadrant by sleep condition interaction [F(3, 42) = 5.13, p = .004, η^2_p = .27]. The interaction was the result of a significant difference in time spent in each quadrant in control rats [F(3, 21) = 22.66, p < .001, η^2_p = .76] but not RD rats [F(3, 21) = 2.01, p = .14, η^2_p = .22]. However, the effect in control rats was due to spending less time in the target quadrant as compared to the opposite quadrant [t(7) = -5.7, p = .001]. After application of the Bonferroni correction, there was no difference in time spent in the target quadrant and either of the adjacent quadrants [t(7) = -2.87, p = .024 and t(7) = 0.12, p = .91]. Taken together, the probe trial results are similar to those of previous experiments, in which there was uncertainty in memory for platform location in both groups as opposed to RD impairing performance.

As shown in Fig. 7, after the platform location was reversed, a 2 × 2 (trials x sleep condition) mixed design ANOVA indicated that the time taken to locate the platform did not vary across the first day of reversal training [F(1, 14) = 1.78, p = .20, η^2_p = .11], nor was there an effect of sleep condition [F(1, 14) = 0.11, p = .75, η^2_p = .008] or a trials by sleep condition interaction [F(1, 14) = 1.62, p = .22, η^2_p = .10]. A similar pattern of results was seen on the second day of reversal training,

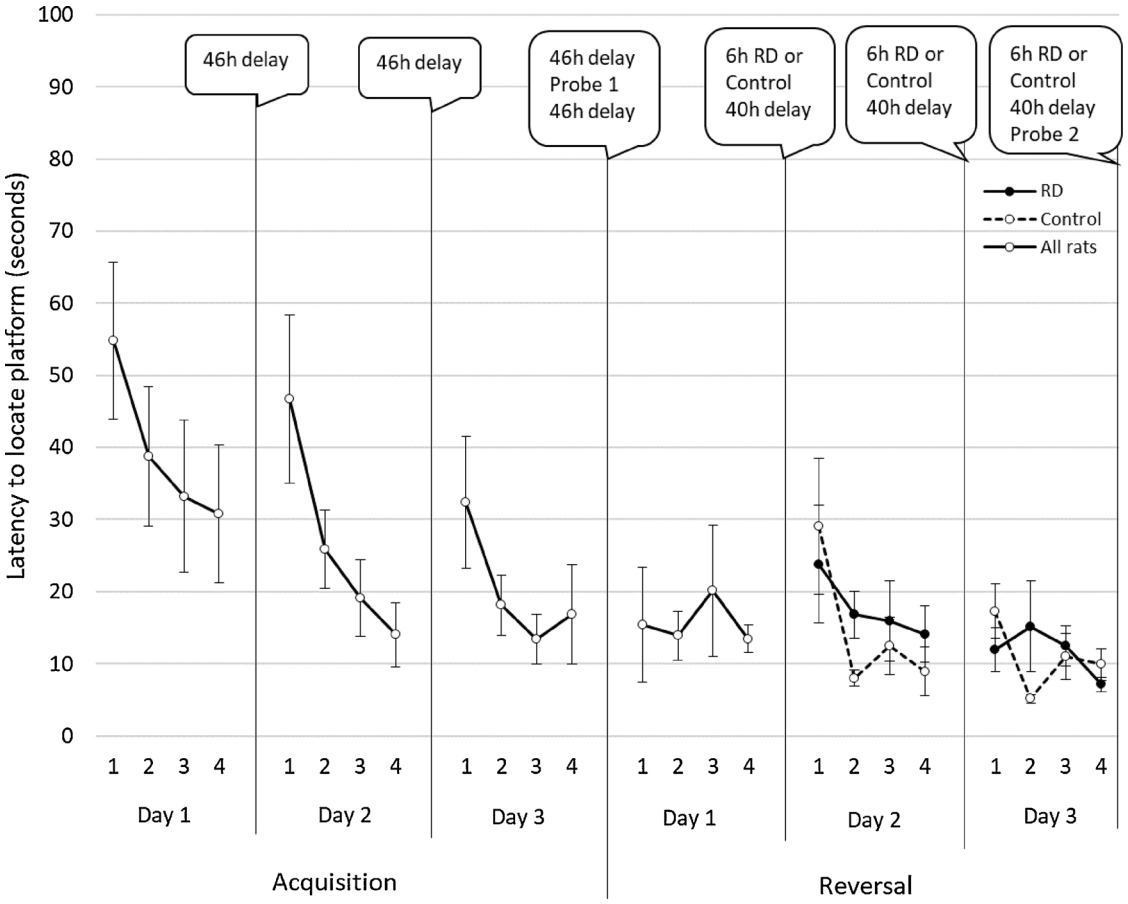

Fig. 5. Latency to locate the platform during experiment 2B in REM deprived and control rats. Sleep manipulations were implemented immediately after each day of reversal training. Acquisition days 1, 2, and 3 and reversal day 1 data are shown for all rats combined as the sleep manipulation occurred after this phase. RD = REM deprivation. N = 8 per group. Data are presented as M ± SEM.

with no significant effect of trials [$F(1, 14) = 1.16$, $p = .30$, $\eta^2_p = .08$], sleep condition [$F(1, 14) = 1.44$, $p = .30$, $\eta^2_p = .08$] nor a trials by sleep condition interaction [$F(1, 14) = 0.3$, $p = .6$, $\eta^2_p = .02$]. The third day of reversal training produced similar results, with no effect of trials [$F(1, 14) = 0.41$, $p = .53$, $\eta^2_p = .03$], sleep condition [$F(1, 14) = 0.005$, $p = .94$. $\eta^2_p < .000$] or a trials by sleep condition interaction [$F(1, 14) = 0.31$, $p = .58$, $\eta^2_p = .02$]. The lack of effect of trials during this phase is likely the result of both intra-rat variability and a ceiling effect, with rats locating the platform in an average of under 30 s for each trial.

Fig. 8 (lower panel) shows the data from the second probe trial, which revealed an effect of quadrant [$F(3, 30) = 17.14$, $p < .001$, $\eta^2_p = .63$], with rats spending more time in the target quadrant as compared to the opposite quadrant [$t(11) = 4.68$, $p = .001$] as well as adjacent quadrants [$t(11) = 6.5$, $p < .001$ and $t(11) = 4.35$, $p = .001$]. There was no effect of sleep condition [$F(1, 10) = 0.12$, $p = .74$, $\eta^2_p = .012$] nor was there a quadrant by sleep condition interaction [$F(3, 30) = 0.35$, $p = .79$, $\eta^2_p = .034$]. These results suggest that regardless of prior sleep condition, rats were able to recall the location of the reversed platform.

3.3.3. Method, Experiment 3B

The experimental design was identical to Experiment 3A with the exception that RD or the control condition (n = 6 per group) was performed during reversal learning instead of acquisition. The location of the goal platform was randomly assigned for each rat.

3.3.4. Results and discussion, Experiment 3B

As shown in Fig. 9, a repeated measures ANOVA showed no significant change in time taken to locate the platform across the first day of acquisition training [$F(1, 11) = 0.73$, $p = .41$, $\eta^2_p = .06$]. There was a significant decrease on the second day of acquisition training [$F(1, 11) = 4.98$, $p = .05$, $\eta^2_p = .31$] but this difference was not significant on the third day [$F(1, 11) = 1.52$, $p = .24$, $\eta^2_p = .12$].

As shown in Fig. 10 (top panel) there was a significant difference in time spent in each tank quadrant during the first probe trial [$F(3, 33) = 8.88$, $p < .001$, $\eta^2_p = .45$]. However, rats spent less time in the target quadrant as compared to the opposite quadrant [$t(11) = -2.9$, $p = .014$] and there was no difference in time spent in the target quadrant as compared to either adjacent quadrant [$t(11) = -0.77$, $p = .46$; $t(11) = 1,27$, $p = .23$].

As shown in Fig. 9, when platform location was reversed there was no significant change in time to locate the platform across the first day of reversal training [$F(1, 11) = 1.55$, $p = .24$, $\eta^2_p = .12$]. A 2 × 2 (trials x sleep condition) mixed design ANOVA revealed no effect of trials on the second day of reversal training [$F(1, 10) = 0.46$, $p = .51$, $\eta^2_p = .044$], sleep condition [$F(1, 10) = 0.02$, $p = .89$, $\eta^2_p = .002$], nor a trials by sleep condition interaction [$F(1, 10) = 2.88$, $p = .12$, $\eta^2_p = .224$]. A similar pattern of results was seen on the third day of reversal training with a trend with a large effect size toward an effect of trials [$F(1, 10) = 4.11$, $p = .07$, $\eta^2_p = .29$] but no effect of sleep condition [$F(1, 10) = 0.11$, $p = .74$. $\eta^2_p = .01$] and no trials by sleep condition interaction [$F(1, 10) = 1.42$. $p = .26$, $\eta^2_p = .12$]. The lack of effect of trials was likely due to the wide-ranging performance of rats across daily trials.

Data from the second probe trial, illustrated in Fig. 10 (lower panel), showed a significant effect of quadrant [$F(3, 30) = 14.21$, $p < .001$, $\eta^2_p = .59$] reflecting more time spent in the target quadrant as

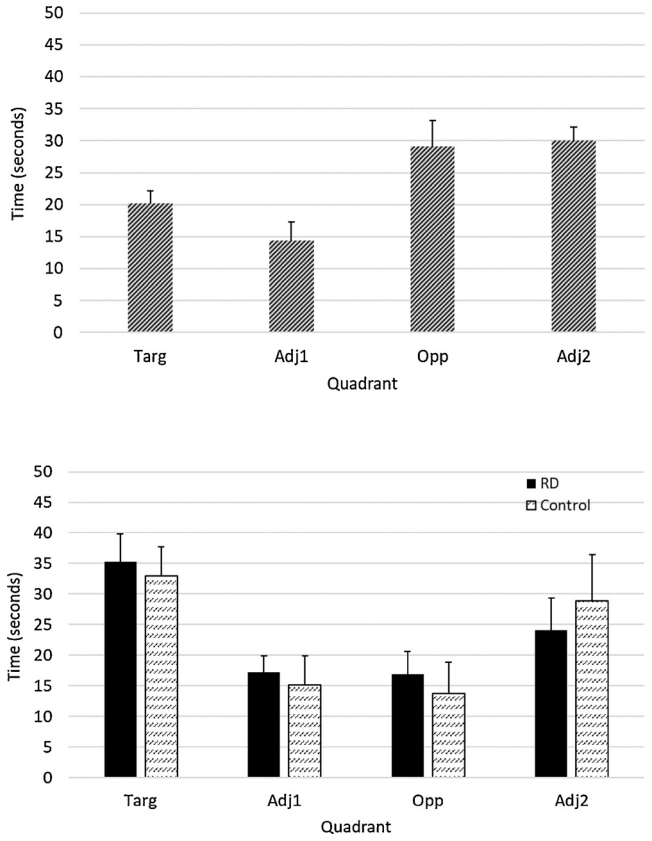

Fig. 6. Time spent in each tank quadrant in REM deprived and control rats for probe trials 1 (top), conducted after acquisition training, and 2 (bottom), conducted after reversal training, in Experiment 2B. Targ = target quadrant; Adj1 and Adj2 = adjacent quadrants; Opp = opposite quadrant. RD = REM deprivation. N = 16 (8 per group) for probe 1 and 12 (6 per group) for probe 2. Data are presented as M ± SEM.

compared to the opposite quadrant [$t(11) = 6.00$, $p < .001$] and both adjacent quadrants [$t(11) = 4.23$, $p = .001$; $t(11) = 3.59$, $p = .004$]. This did not vary by sleep condition [$F(1, 10) = 0.032$, $p = .86$, $\eta^2_p = .003$] nor was there a quadrant by sleep condition interaction [$F(3, 30) = 0.78$, $p = .51$, $\eta^2_p = .073$].

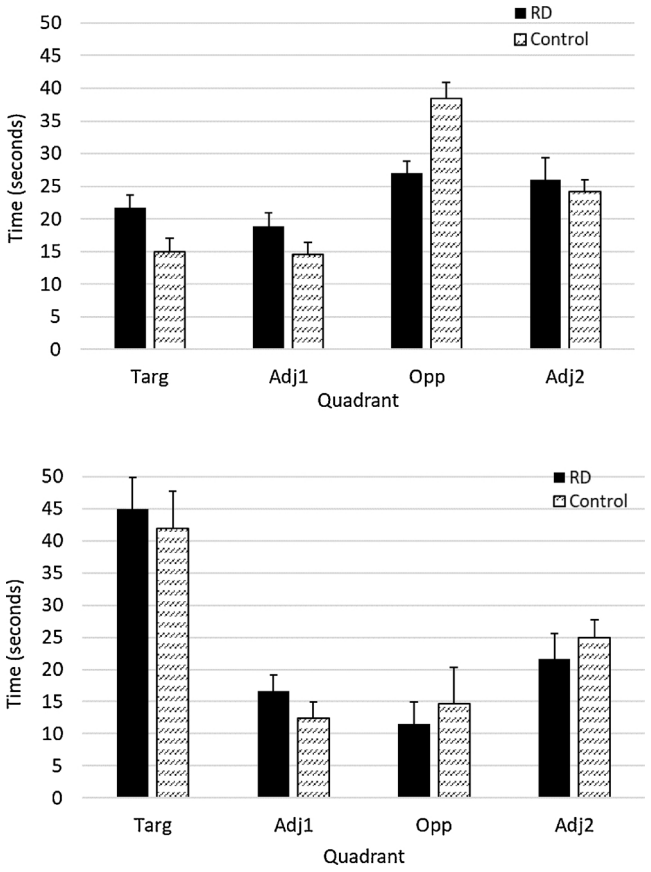

Fig. 8. Time spent in each tank quadrant in REM deprived and control rats for probe trials 1 (top), conducted after acquisition training, and 2 (bottom), conducted after reversal training, during Experiment 3A. Targ = target quadrant; Adj1 and Adj2 = adjacent quadrants; Opp = opposite quadrant. RD = REM deprivation. N = 6 per group. Data are presented as M ± SEM.

4. General discussion

The results of this study indicate that post-training REM is not essential for consolidation of a spatial memory task. This is true for both initial acquisition and the more difficult reversal learning and is also true regardless of the distribution of training (massed or spaced trials)

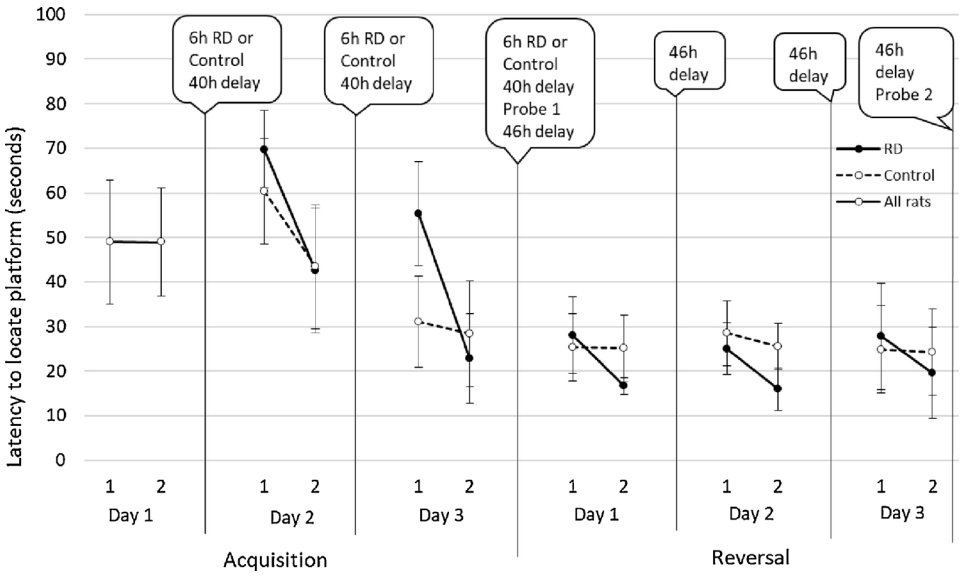

Fig. 7. Latency to locate the platform during Experiment 3A in REM deprived and control rats. Sleep manipulations were implemented immediately after each day of acquisition training. Acquisition day 1 data are shown for all rats combined as the sleep manipulation occurred after this phase. RD = REM deprivation. N = 8 per group. Data are presented as M ± SEM.

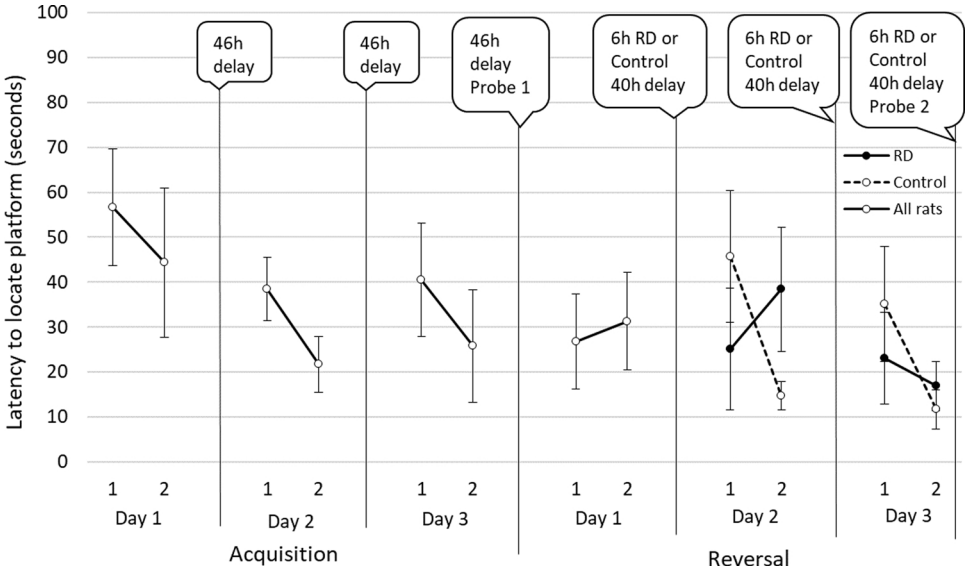

Fig. 9. Latency to locate the platform during Experiment 3B in REM deprived and control rats. Sleep manipulations were implemented immediately after each day of reversal training. Acquisition days 1, 2, and 3 and reversal day 1 data are shown for all rats combined as the sleep manipulation occurred after this phase. RD = REM deprivation. N = 6 per group. Data are presented as M ± SEM.

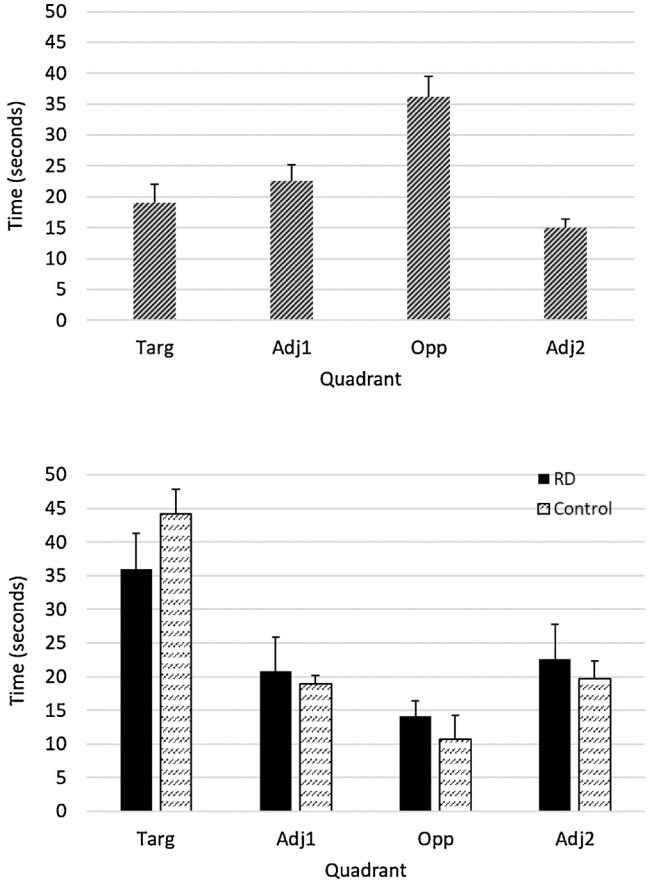

Fig. 10. Time spent in each tank quadrant in REM deprived and control rats for probe trials 1 (top), conducted after acquisition training, and 2 (bottom), conducted after reversal training, in Experiment 3B. Targ = target quadrant; Adj1 and Adj2 = adjacent quadrants; Opp = opposite quadrant. RD = REM deprivation. N = 6 per group. Data are presented as M ± SEM.

and resultant degree of task acquisition. These findings are consistent with those of Walsh and colleagues (2011) and extend their findings through manipulation of the rate of task acquisition as well as controlling for possible nonspecific effects of sleep deprivation at the time of testing.

Despite a lack of effect of REM deprivation on spatial memory, there is substantial research demonstrating the important role of sleep in other types of learning and memory; specifically, sleep patterns can be altered following learning, and certain types of learning are impaired by sleep deprivation (see Vorster & Born, 2015 for review). While nonREM is responsible for memory replay (Feld & Diekelmann, 2015), REM is critical in the stabilization of memories with emotional content (Tempesta et al., 2017; Walker, 2010). Although the specific neurobiological substrates of REM that support learning and memory have not been definitively determined, REM deprivation impairs hippocampal long-term potentiation (Ishikawa, Kanayama, Matsumura, Tsuchimochi, Ishida, and Nakamura, 2006), which is presumed to provide the neurobiological basis for memory. Synaptic plasticity in brain regions essential to learning and memory such as the hippocampus and amygdala are strongly tied to the occurrence of pontogeniculo-occipital waves that occur exclusively during REM (for review, see Hutchison and Rathore, 2015). Additionally, neurotransmitters such as acetylcholine and noradrenaline play important roles in both REM and memory and may provide a point of integration (Hutchison and Rathore, 2015). However, the delineation of the specific neurobiological underpinnings of the REM-memory connection remains a topic for additional investigation.

The probe trial data produced some anomalous results; specifically, there was increased time spent in the opposite quadrant of the tank than the quadrant where the platform had been located during the first probe trial in experiments 2A and 3B, while during the first probe trial in experiment 2B rats did not show a preference for any tank quadrant. The preference for the opposite tank quadrant is not easily explained. While one possibility is a preference or aversion for a particular area of the testing room, this effect was seen even when platform location was randomized. This effect may limit the value of the probe trial data as it is suggestive that some process other than memory for platform location may have influenced time spent swimming in a particular area of the apparatus. While the duration of the probe trials was relatively long at 90 s, analysis of the first 15 s or the first 30 s did not produce differential results (data not shown). However, the lack of any quadrant preference is most parsimoniously explained as rats displaying uncertainty of the exact location of the platform. As previous studies have shown that RD has its most impairing effects on memory when it is still being consolidated (Smith and Rose, 1996, 1997), this should have increased the likelihood of finding an effect of RD. Importantly, the lack of tank quadrant preference did not vary by RD condition; in no case was memory impaired in RD rats as compared to control. In addition, probe trials provide just one measure of memory for platform location. The other memory assessment, latency to locate the platform, showed

no effect of RD on the initial acquisition, retention, or reversal of the task in any of the present experiments. Finally, the number of animals in each group was not optimal, with only 6 animals in some conditions. However, the effect size statistics did not suggest that a lack of power resulted in an inability to see significant effects of RD.

The lack of consistency in the methodology used to induce RD in prior studies, including the use of an appropriate control condition, produces a challenge in summarizing findings across studies. The lack of mobility that is a consequence of the single platform technique used in this and other studies may constitute a confounding variable, as might non-specific stress resulting from exposure to the RD apparatus. An additional complication is that previous studies exposed animals to varying durations of RD ranging from 4 to 72 h. As the present study was an attempt to replicate the work of Smith and Rose (1996, 1997) with the addition of a more comparable control condition, the RD parameters in those studies were replicated to the extent possible. However, without polysomnographic recording the specific nature of the sleep deficit during the 6 hours of the sleep manipulation or the characteristics of rebound sleep cannot be determined. It is possible that the novelty of the apparatus produces changes in sleep architecture that are not limited to REM. However, the finding of differential effects between the RD and control conditions in previous studies (Silvestri, 2005; Hunter, 2018) indicate that animals have differential responses to the two procedures.

5. Conclusions

This study found no evidence for an impairment in MWM acquisition or reversal as a consequence of RD, regardless of the timing of RD or distribution of training. While the effects of RD on other spatial learning tasks have not been as well studied, these findings are consistent with a growing body of evidence that REM is not necessary for spatial learning and memory.

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