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Scopolamine during the paradoxical sleep window impairs radial arm maze learning in rats

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Abstract

It has been proposed that there are paradoxical sleep windows (PSW) during which REM sleep is required for effective learning. Thus, rats deprived of REM sleep during 0–4 (but not 5–8) h after training show impaired learning of a radial maze task. As cholinergic (ACh) systems are active during REM sleep and may be involved in learning, this experiment investigated the effects on learning of pharmacological manipulation of the cholinergic system during the period identified as the PSW. Sprague–Dawley rats were randomly assigned to groups that were physically deprived of REM for 4 h either immediately after training or beginning 4 h after training or treated with the ACh receptor antagonist scopolamine (0–0.4 mg/kg at 0 and 2 h after training or 0.006 mg/kg at 4 and 6 h after training) on each of 9 days of radial maze training. Post-training REM deprivation (0–4 h but not 5–8 h after training) and scopolamine dose-dependently impaired learning. Results suggest that REM sleep and intact ACh neurotransmission are required during the PSW for rats to learn the radial maze task.

Keywords: Acetylcholine; Learning; Paradoxical sleep window; Radial maze; REM sleep; Scopolamine

1. Introduction

Sleep manifests in two distinct phases. Slow wave sleep (SWS) is characterized by slow, rhythmic traces on an electroencephalogram (EEG). In humans it is divided into 4 separate levels of increasing depth as the EEG slows from 7–9 Hz to 0.5–3 Hz. There are no eye movements and autonomic activity is stable. Rapid eye movement (REM) sleep is associated with an EEG similar to that of light non-REM sleep but is accompanied by complete muscle atonia except for REMs and autonomic activity fluctuations (Jouvet, 1967).

The function of either phase of sleep is poorly understood. Without reference to specific sleep phases, Maquet (2001) cited several authors in his review who suggested that sleep acts to conserve energy. Others suggested that sleep is involved in thermoregulatory processes, detoxification of the brain, has a restorative function and that sleep is involved in neural plastic processes (Maquet, 2001). With specific reference to REM sleep, Vertes and Eastman (2000) suggested that it is a mechanism for activating the sleeping brain in SWS for a waking state. In support of this view, persons awoken from SWS were impaired on cognitive tasks relative to those woken from REM (Dinges, 1990). On the other hand, Smith (1985) suggested that REM sleep is associated with learning.

REM sleep is not a singular requirement for effective learning and memory consolidation (see Stickgold et al., 2001), however, alterations in REM patterns have an impact on consolidation processes; consolidation is a time-dependent process that converts recent experience to lasting memory. REM has been associated with the learning of complex logic games (Smith, 1993), foreign language

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acquisition (DeKoninck et al., 1989), and intense studying (Smith and Lapp, 1991). There is evidence suggesting that the amount of REM sleep changes from baseline following training (Hennevin et al., 1995). Using positron emission tomography, Maquet et al. (2000) showed that brain regions that were active during the execution of a task were re-activated during subsequent REM sleep. This was correlated with improved performance on the following day.

Rodent experiments have showed that REM and learning may be related. Thus, REM increased after mice learned a shock-avoidance task (Smith, 1996). REM sleep deprivation blocked cortical mass increases resulting from enriched environments (Smith, 1996) and impaired learning of spatial memories from the Morris water maze (Youngblood et al., 1997) and the radial arm maze (RAM) task (Smith et al., 1998). Results implicate REM sleep in learning and memory.

The paradoxical sleep window (PSW) is defined as a period of paradoxical sleep specifically involved in learning (Smith, 1985). Different PSWs have been identified for several maze-tasks. For example, spatial memories for the water maze were impaired by REM sleep deprivation (REMD) during hr 4–8 following training but not when REMD was applied for four hr intervals begun at times other than hr 4–8 (Smith and Rose, 1996). In a related study, a mass training protocol in the water maze was used. REMD during hr 0–4 following training but not intervals of four hr begun at times outside of hr 0–4 impaired memory (Smith and Rose, 1997). The PSW for the conditioned cue preference task was reported to be between hr 9 and 13 (Vallance et al., 1999). Using the RAM task, Smith et al. (1998) similarly showed that REMD during hr 0–4 following training impaired acquisition. Results support the hypothesis that PSWs exist for several tasks during which REM sleep is important for learning.

It is well-established that cholinergic neurons in the basal forebrain are active during REM (Steriade and McCarley, 1990). Cholinergic neurons in the basal forebrain are implicated in memory (Beninger et al., 2001). Cholinergic neurons in the medial septum may influence learning by modulating plastic processes within the hippocampus or elsewhere (Graves et al., 2001; Woolf and Butcher, 1989). Injections of the muscarinic cholinergic receptor antagonist scopolamine during hr 0–4 following avoidance learning impaired acquisition. As hr 0–4 corresponded to the PSW, results supported the involvement of cholinergic neurons in learning that takes place during the PSW. The related finding that injections of the protein synthesis inhibitor anisomycin during the PSW but not other times following avoidance training impaired learning implicates protein synthesis in the learning processes that appear to occur during the PSW (Smith et al., 1991).

The current experiments evaluated the effects of REM sleep deprivation and the muscarinic cholinergic receptor blocking drug scopolamine given within and outside of the PSW following daily training sessions in the RAM. It was hypothesized that scopolamine and selective REM deprivation would impair learning when they were given within but not outside of the PSW.

2. Methods

2.1. Subjects

Male Sprague–Dawley rats were obtained from Charles Rivers Laboratories (St. Constant, Que.) and allowed to acclimatize to the Trent University Animal Facility for 7 days. Rats were individually housed on soft texture paper chip bedding in opaque plastic cages [45 (l)×25 (w)×20 (d) cm] located in a temperature-controlled (21±1.5 °C) colony room maintained on a 12/12 hr light/dark cycle with lights on at 0700 hr. Prior to any experimental manipulation, animals were handled for 3–5 min each day for 5 consecutive days. Food deprivation (see below) was initiated on the third day of handling. The Trent University Animal Care Committee approved the animal-related procedures a priori.

2.2. Apparatus

2.2.1. Radial Arm Maze

The RAM, elevated 36 cm above the floor, consisted of a central platform (23 cm diam) with eight arms (49×9 cm) radiating from it. Fastened to the floor at the distal end of each arm was a small food dish (4.4 cm diam). The central platform and the arms were painted flat gray. The RAM was situated in a room measuring 2.9×2.1 m divided by a black curtain so that the area containing the maze had the dimensions 1.7×2.1 m. That area had posters on each of the remaining three walls to provide visual cues. A Hitachi video camera was positioned at the junction between the rod holding the curtain, the ceiling, and one of the walls to monitor the animals on the maze. On the other side of the curtain were a chair, desk and video screen. An opaque cylinder (22 cm diam×30 cm high) was placed over the central platform and the experimenter raised this by a pulley system from the monitoring station at the beginning of each trial.

2.2.2. Sleep deprivation

An inverted flowerpot with a bottom diam of 8.5 cm was placed into the bottom of a green plastic can (35 cm bottom diam×57.5 cm high). The can was filled with water to a depth of approximately 26 cm matching that of the platform formed by the inverted flowerpot.

2.3. Drugs

Scopolamine hydrobromide was obtained from Sigma, Oakville, ON and was diluted with 0.9% saline to appropriate concentrations (see below).

2.4. Procedure

2.4.1. Food restriction

Three days before training in the RAM, the animals were restricted to approximately 20 g of rat chow per day. On the first day of exposure to the RAM the animals were further food restricted to approximately 10 g of rat chow per day plus whatever food they obtained from the maze. The animals' masses were monitored frequently to ensure that the animals' health was maintained. An endpoint mass of 250 g was established and one animal was removed from the study when it fell below this value.

2.4.2. RAM procedure

Animals were tested between 1000 and 1500 h during the experiments. All animals were exposed to the unbaited RAM for 10 min on each of two consecutive days. On each of the next 10 days, rats were individually placed onto the central platform of the RAM and contained there using the opaque cylinder. A trial began when the experimenter using the pulley system raised the cylinder. For each rat, the same four arms of the maze for each exposure to the RAM were baited each with one half of a piece of the breakfast cereal Froot Loops (approx. 0.05 g). The baiting pattern was different from rat to rat. The animal had a maximum of 6 min to find all of the baits within the RAM. The animal was then removed from the maze and subjected to an experimental manipulation as detailed below. Manipulations were undertaken on each of the first 9 days of training. Dependent variables recorded included latency to the consumption of the last bait or six minutes, whichever occurred first, and the numbers of baited and unbaited arm entries made per trial.

2.4.3. Experiment 1

The REM Deprivation Study replicated the PSW for the RAM task, as shown previously by Smith et al. (1998). Rats were randomly assigned to one of three groups: CONTROLS (n=9), REM deprived using the flowerpot technique during the first four hr following training (REMD 0–4; n=9) and REM deprived using the flowerpot technique for a 4-h interval beginning 4 h after each exposure to the RAM (REMD 4–8; n=10). The flowerpot technique used a platform sufficiently large (8.5 cm diam) that the animal could enter SWS sleep; however, when the animal lost tonicity during REM, it fell from the platform and awoke when it entered the water (Youngblood et al., 1997).

2.4.4. Experiment 2

For the scopolamine study, animals were randomly assigned to each of the following groups:

(i) Saline 1.0 mL kg^{-1} intraperitoneally (IP) 0 and 2 h after training (SAL; n=10).
(ii) Scopolamine 0.4 mg kg^{-1} IP 0 and 2 h after training (SCOP 0.4; n=9).
(iii) Scopolamine 0.1 mg kg^{-1} IP 0 and 2 h after training (SCOP 0.1; n=10).
(iv) Scopolamine 0.025 mg kg^{-1} IP 0 and 2 h after training (SCOP 0.025; n=10).
(v) Scopolamine 0.006 mg kg^{-1} IP 0 and 2 h after training (SCOP 0.006; n=10).
(vi) Scopolamine 0.006 mg kg^{-1} IP 4 and 6 h after training (SCOP OW; n=10). Note that experiment 1 confirmed that the PSW was during hours 0–4 following training; thus, giving scopolamine at the end of the PSW corresponded to giving it outside of the window (OW).

Animals received two injections, spaced 2 h apart, as scopolamine has a half-life of 2.9 h; this ensured muscarinic blockade during the entire PSW (Benet et al., 1996) or for a corresponding period outside of the PSW.

2.5. Analyses

Dependent variables included latency and the numbers of baited and unbaited arm entries per trial. An arm entry was defined as a completed trip from the central platform to any food dish. Arm entry data were used to calculate the ratio of baits consumed to the total number of arm entries per trial (equation 1). This was termed the Performance Index (PI) and as rats learned the task, its value approached 1. A small number (0.001) was added to the denominator of the PI to avoid the possibility of the denominator equaling zero.

$$PI = \frac{\text{total \# of baits consumed in a trial}}{\text{total \# of arm entries in a trial} + 0.001} \quad (1)$$

All statistical analyses were conducted using Statistica '99. The dependent variables described above were subjected to mixed-design analyses of variance (ANOVA). To test for homogeneity of variance, Hartley's Fmax was calculated and the F-ratios for each within-subjects level were computed. When a significant value of Fmax was found for a given day, no post hoc analyses were calculated for that level. Otherwise, significant main effects were evaluated post hoc using Neuman–Keuls pairwise comparisons. Significant between- and within-measure interactions were investigated using ANOVA for simple effects with the planned contrasts interface offered by Statistica '99. For the ANOVA for simple effects calculated on a given day, contrast coefficients were applied to group means such that an omnibus F-ratio was obtained. When those omnibus F-ratios were significant, further post hoc analyses were conducted by applying different contrast coefficients to specific group means within a level to directly compare them. Another F-ratio was obtained in that fashion and when it was significant, it was interpreted that the specific group means being contrasted were different from one another.

For each of the above analyses, we were specifically interested in the performance of the groups receiving the same dose during and outside of the PSW. ANOVA was planned a priori to test differences between 0–4 hr and 4–8 hr conditions in each experiment.

Parametric statistical tests were supplemented by survival analyses using the latency data. This allowed use of the results from all 10 trials in the analyses. In survival analysis, when an animal attains a specific criterion, it is said to be censored. The criterion for censoring an animal within any group was the first time that an animal scored less than 360 s in the latency measure. This would require that the animal consumed all four baits.

3. Results

3.1. Experiment 1: REM deprivation study

Changes in the animals' performance day-to-day over the course of training provided indices of learning. These included the latency to consume four baits (up to the criterion of 360 s) (Fig. 1) and the ratio of baits consumed to total number of arm entries (PI) (Fig. 2).

For the latency data, there was little variability among groups during early training and Fmax calculations revealed significant violations of the assumption of homogeneity of variance. Accordingly, survival analyses were used to analyse the latency data.

The survival analysis is a non-parametric statistical test that does not require the assumption of homogeneity of variance. Using the first time the animal scored less than 360 s as the criterion of performance, survival analysis

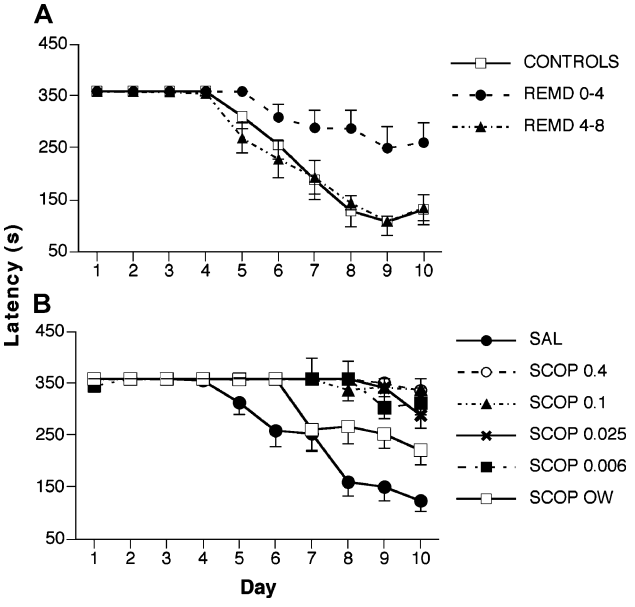

Fig. 1. Latency (s) to completion of the RAM task. (A) REM Deprivation study, (B) Scopolamine study.

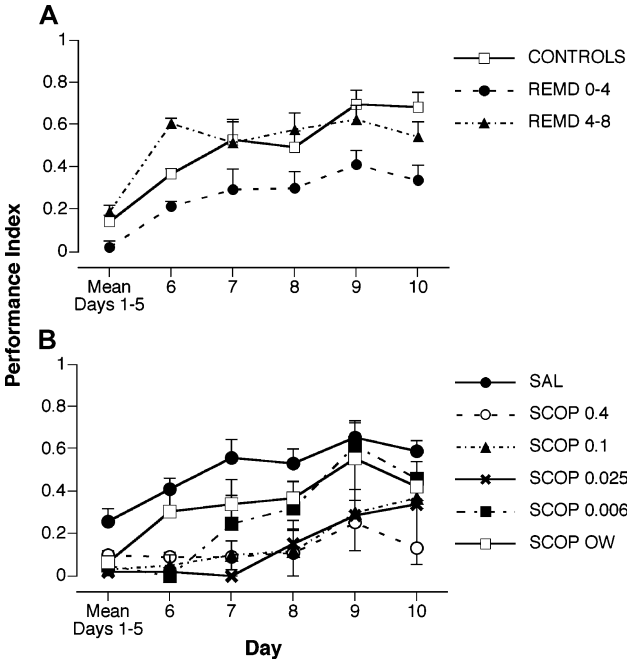

Fig. 2. Ratio of baits eaten to total arm visits (PI). (A) REM Deprivation study, (B) Systemic scopolamine study.

revealed a group difference (χ^2 (2, N=28)=8.63, p=0.013) with animals in the REMD 0–4 group doing less well. Animals in the CONTROL group had a median survival score of 6 days and those in the REMD 4–8 group had a median of 5.5 days. Animals deprived of REM during the PSW had a median of 10 days with half of the group being censored. Thus, REMD during the PSW impaired learning.

The groups showed little change on the PI over the first 5 days; therefore, the PIs were averaged for each group over these days (Fig. 2A). The PI improved over days but the REMD 0–4 group did less well. ANOVA revealed a significant main effect of group ($F_{(2,25)}$=7.87, p<0.01) and day ($F_{(5,125)}$=12.1, p<0.001) but there was no significant interaction. Pair-wise comparisons (Newman–Keuls) confirmed that the CONTROL and REMD 4–8 groups performed better than the REMD 0–4 group and did not differ from each other. Results supported the conclusion from the survival analysis that REMD during the PSW impaired learning.

3.2. Experiment 2: scopolamine study

Latencies for groups are shown in Fig. 1B. All doses of scopolamine given within the PSW led to an impairment compared to the SAL group with performance of the group receiving scopolamine outside of the PSW being intermediate. Fmax analyses revealed that homogeneity of variance was violated, thus, survival analysis was employed for the latency data.

Survival analysis (Fig. 3B) confirmed that groups differed (χ^2 (5, N=59)=31.1, p<0.001). The median number of days for the SAL group to acquire the RAM

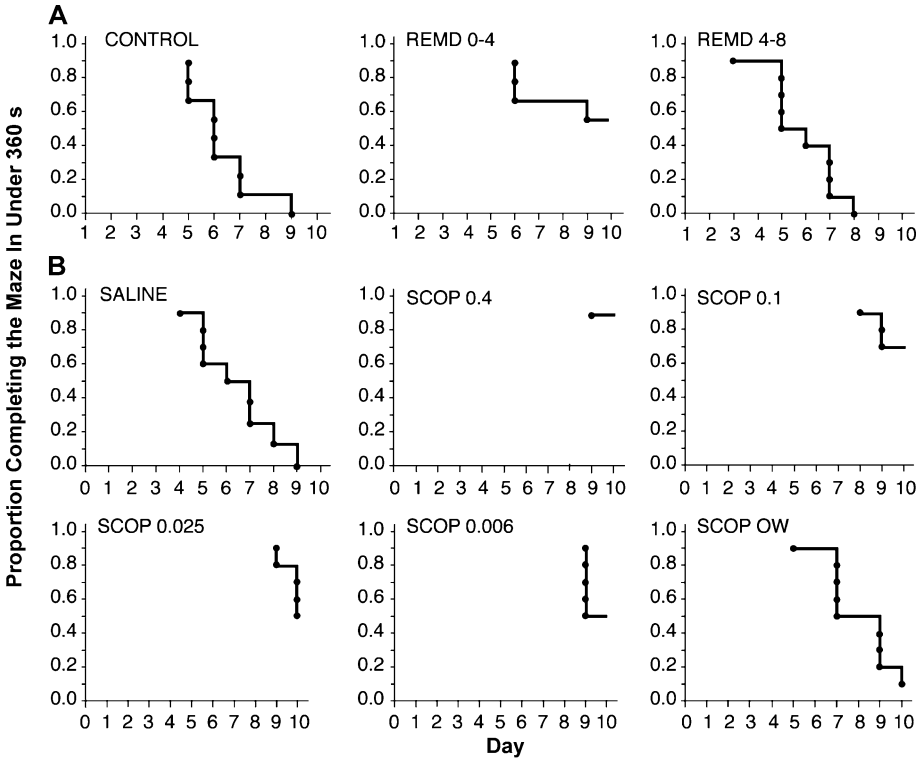

Fig. 3. Kaplan-Meier survival plots of latency data. (A) REM deprivation study, (B) Scopolamine study.

task was 6. Animals given scopolamine outside of the PSW had a median of 8 days. For the groups SCOP 0.4, SCOP 0.1, and SCOP 0.025, the median was 10 days with few animals reaching the criterion for censure. The SCOP 0.006 group had a median of 9.5 days. A separate survival analysis compared groups receiving the same dose of scopolamine either in (SCOP 0.006) or outside of the PSW (SCOP OW). Results showed a significant difference (z=2.39, p<0.05) with the SCOP 0.006 group being impaired. Thus, scopolamine giving within the PSW impaired learning.

PI results (Fig. 2B) revealed the best performance in the SAL group and poor performance in the SCOP 0.4, 0.1 and 0.025 groups with groups receiving 0.006 mg kg^{-1} of scopolamine within or outside of the PSW at an intermediate level of impairment. ANOVA revealed a significant main effect of group ($F_{(5,53)}$=7.28, p<0.001), day ($F_{(5,265)}$=26.9, p<0.001) and a significant interaction ($F_{(25,\ 265)}$=1.67, p<0.05). Fmax tests indicated that the homogeneity of variance assumption of ANOVA was violated for simple effects ANOVA on the means for days 1–5, 6 and 7 and therefore, pairwise comparisons were not carried out on those days. ANOVA for simple effects revealed differences among groups on Days 8, 9 and 10 (Day 8: $F_{(1,53)}$=48.1, p<0.001; Day 9: $F_{(1,53)}$=121, p<0.001; Day 10: $F_{(1,53)}$=145, p<0.001). On Day 8, SAL was different from SCOP 0.025, SCOP 0.1 and SCOP 0.4 but not SCOP OW or SCOP 0.006. The groups that were given scopolamine within the PSW did not differ from each other. With the exception of the SCOP 0.006 group, the SCOP OW group was different from each of the other groups that received scopolamine. On Day 9, SAL was different from the SCOP 0.4, SCOP 0.1 and SCOP 0.025 groups but not different from SCOP 0.006 and SCOP OW groups. With the exception of SCOP 0.006, no other group that received scopolamine during the PSW was different from another. On Day 10, the SCOP 0.025 group had started to perform better although with respect to the SCOP 0.4 group, the differences only approached significance ($F_{(1,53)}$=3.34, p=0.073). The difference in performance between SCOP 0.4 and SCOP 0.1 was significant. The SAL group differed from the SCOP 0.1 group but not the SCOP OW or the SCOP 0.006 groups. The planned ANOVA comparing SCOP 0.006 and SCOP OW yielded a significant main effect of day ($F_{(5,90)}$=12.1, p<0.001) but a non-significant group effect ($F_{(1,18)}$=0.569, p>0.05) and interaction ($F_{(5,90)}$=1.44, p>0.05). In summary, the PI was impaired in a dose-dependent manner by scopolamine injections within the PSW but the 0.006 mg kg^{-1} dose did not produce a different effect when given inside vs. outside of the PSW.

4. Discussion

Results showed that animals deprived of REM sleep during 0–4 h post training (the PSW for the RAM task) or given post-training injections of the muscarinic cholinergic

receptor blocker scopolamine during the PSW were impaired in their latency to find all of the baits and in their accuracy of arm choices. These observations replicated the PSW for the RAM and implicated cholinergic neurotransmission during the PSW in learning.

REM deprivation during hours 0–4 but not hours 4–8 after training impaired acquisition of the RAM task, in good agreement with the similar findings of Smith et al. (1998). These results support the hypothesis that REM sleep is required during a specific interval after training (the PSW) for effective learning of the RAM task (Smith, 1985).

We used the version of the RAM task that involves consistently baiting four of the eight arms because it was used previously by Smith et al. (1998) and we set out to replicate the PSW that they had reported. One of the advantages of this version of the RAM task is that is allows independent assessment of working and reference memory errors (Olton and Papas, 1979). Working memory errors are defined as revisits to already visited arms and reference memory errors are defined as visits to never-baited arms. However, these types of errors can only be assessed meaningfully once the animal has learned that a subset of four of the eight arms is baited. Indeed, most studies that have evaluated working and reference memory in this task use well-trained rats (e.g., Wirsching et al., 1984). Because the present study evaluated *acquisition* of this task, meaningful assessment of different types of memory errors could not be made.

Scopolamine administered after training each day dose-dependently impaired acquisition of the RAM task. It is unlikely that the impairment can be attributed to a cumulative effect of the drug with repeated dosing. Thus, drug-treated groups gradually learned the task but their rate of learning was significantly slower than that of the SAL group. If the effect of the drug was cumulative, it might be expected to increase over days, not decrease as was observed. Furthermore, the group that received scopolamine outside of the PSW learned significantly faster than the comparable group receiving the drug inside of the PSW. If the impairment in performance observed in the group receiving scopolamine within the PSW could be attributed to drug accumulation, a similar impairment would have been expected in the group receiving scopolamine outside of the PSW. Thus, the effects of scopolamine do not appear to be attributable to a cumulative drug effect.

The finding that treatment with scopolamine during the PSW impaired acquisition of the RAM task is in agreement with Smith et al. (1991) who showed that acquisition of an avoidance task was similarly impaired by one dose of scopolamine injected during the PSW. The present study is the first to show that the effect of scopolamine during the PSW on the acquisition of a task was dose-dependent. That the effect of scopolamine was greatest during the PSW was confirmed by the finding that an impairing dose (0.006 mg kg^{-1}) given outside of the PSW produced significantly less impairment. A significant difference between 0.006 mg kg^{-1} given within vs. outside of the PSW was seen in the survival analysis of latencies but not in the PI. Results implicate ACh acting at muscarinic receptors in memory consolidation that takes place during the PSW.

Flood and coworkers have shown that both dopaminergic and muscarinic receptor antagonists following training impair acquisition of a discriminated shock-avoidance task with some similarities to the RAM task that required discrimination of eight arms. Using central infusions into the septum, cholinergic receptor antagonists impaired acquisition of the task (Flood et al., 1998). Doses of the drugs used immediately following training were also applied to mice 24 h after training with no significant effects being observed. Results suggest that the interval immediately following training was important for learning. These same researchers found that M1 and M2 muscarinic receptor antagonists injected into the hippocampus following training also impaired learning of the discriminated shock avoidance task (Farr et al., 2000a). Similarly, cholinergic antagonists given into the cingulate gyrus following training impaired learning (Farr et al., 2000b). It may be that cholinergic neurons active immediately following training in each of these structures are recruited during REM sleep. If this was the case, the impairments in learning seen by Flood and his coworkers may be related to a disruption of REM patterns.

The present results showed that learning of the RAM task was sensitive to manipulations of the REM sleep cycle and cholinergic systems. The effects of cholinergic manipulations were time-sensitive and were related to the PSW. The impairments may have been due to a drug-induced delay in the transfer of information from short-term memory to long-term memory, a function of REM proposed by Smith et al. (1998). These results supported the hypothesis that REM sleep and intact cholinergic systems are required during the first 4 hr following training for effective learning of the RAM task.

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