The effects of rapid eye movement sleep deprivation and recovery on spatial reference memory of young rats

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It is known that rapid eye movement (REM) sleep plays a crucial role in learning and memory. Previous studies have demonstrated that postlearning REM sleep deprivation (RSD) impairs memory consolidation. Most of these studies observed only the effects of RSD on learning and memory. In the present study, we not only investigated the impacts of 48-h RSD on the spatial reference memory of young rats in a Morris water maze, but also specifically examined whether an REM rebound for 24–48 h after 48-h RSD affected the maintenance of spatial reference memory. RSD was induced by the modified multiple platform method, and spatial reference memory was tested in a Morris water maze. The results demonstrated that, compared with the control groups, posttraining RSD for 48 h produced a significant impairment in the retention of acquired spatial reference memory, and the impairment continuously existed after 24 and 48 h of release from sleep deprivation, which indicates that REM sleep plays a critical role in reference memory maintenance and consolidation. Moreover, postlearning RSD may lead to a long-term impairment in the consolidation of newly acquired memories.

Sleep is composed of two prominent phases—namely, rapid eye movement (REM) sleep and non-rapid eye movement (NREM) sleep—and its function remains elusive. There is increasing evidence that postlearning REM sleep plays a crucial role in the consolidation of newly acquired memories for long-term storage (Backhaus, Hoeckesfeld, Born, Hohagen, & Junghanns, 2008; Born, Rasch, & Gais, 2006; Ishikawa et al., 2006; Marshall & Born, 2007; Stickgold, 2005; Walker & Stickgold, 2004, 2006). A number of studies have demonstrated that REM sleep deprivation (RSD) after learning results in memory deficits and that REM sleep is increased after learning activities in both humans and animals (Fu et al., 2007; Ishikawa et al., 2006; Saxvig et al., 2008; R. H. Silva et al., 2004). In the prevailing model, it is assumed that this con-

solidation relies on a covert reactivation of the novel neuronal memory representations during sleep after learning (Born et al., 2006; Marshall & Born, 2007; Stickgold, 2005; Walker & Stickgold, 2006). One hypothesis accounting for sleep-dependent memory processing is that alterations in synaptic plasticity may be induced during sleep (Benington & Frank, 2003; Born et al., 2006; Marshall & Born, 2007; Stickgold, 2005). The reactivation of the hippocampus in sleep after spatial learning has been observed in humans with positron emission tomography (Maquet et al., 2005; Orban et al., 2006).

Studies on the homeostasis of sleep have indicated that there is an REM sleep augmentation after RSD, called *REM rebound*, which is considered to be a homeostatic compensation for a deficit in REM sleep (Ocampo-

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Garcés, Molina, Rodríguez, & Vivaldi, 2000; Suchecki, Duarte Palma, & Tufik, 2000). More recent research has further demonstrated that REM rebound happened in the first 24 h after 96 h of RSD (Machado, Hipólido, Benedito-Silva, & Tufik, 2004). However, the functional significance of REM rebound after postlearning RSD on the maintenance of memory has not been clarified. To our knowledge, there have been only three studies that have specifically examined whether REM rebound could restore the RSD-induced impairment of long-term potentiation (LTP; Ishikawa et al., 2006; Kim, Mahmoud, & Grover, 2005; Romcy-Pereira & Pavlides, 2004). A study on the effect of RSD for 24–48 h after induction of LTP in the dentate gyrus (DG) on LTP maintenance revealed that REM-deprived rats displayed a deficit in the maintenance of LTP and that the deficit continued to exist after 4 h of release from RSD (Ishikawa et al., 2006). Another study observed the impact of RSD for 4 h after LTP induction in DG and medial prefrontal cortex on the maintenance of LTP; it was shown that the impairment of the maintenance of LTP lasted for 48 h (Romcy-Pereira & Pavlides, 2004). Kim et al. examined LTP in area CA1 of the rat hippocampus, also indicating that 5 days of RSD continuously affected hippocampal function for more than 24 h following the end of deprivation. Since LTP is thought to be the major cellular model for synaptic plasticity and to be associated with memory consolidation (A. J. Silva, 2003), we hypothesize that postlearning REM sleep is critical for newly acquired memory consolidation. Moreover, REM rebound may be ineffective for the restoration of RSD-induced impairment of memory.

The studies mentioned above on the impact of REM rebound on memory consolidation were focused on electrophysiological analysis, and, to our knowledge, there has been no behavioral research applied to test the hypothesis up to now. To specifically explore this issue, we designed and conducted a behavioral experiment to examine the effects of postlearning RSD, achieved by the modified multiple platform method (MMPM), on newly acquired spatial reference memory in a Morris water maze. Furthermore, we particularly assessed whether REM sleep recovery after RSD influences the maintenance of reference memory.

The MMPM is a technique widely used to deprive subjects of REM sleep. However, the procedure, in addition to completely abolishing REM sleep, also decreases NREM sleep by approximately 30% (Machado et al., 2004). Recent studies have suggested that NREM sleep and REM sleep correlate with memory enhancement (Born et al., 2006; Rasch, Büchel, Gais, & Born, 2007). High-frequency oscillations—known as *sharp-wave/ripple* (SPW-R) complexes and occurring during NREM—have an important role in neuronal communication and synaptic plasticity and promote memory consolidation (Jensen, Kaiser, & Lachaux, 2007). A recent study found that learning resulted in an increased number of SPW-Rs during the first hour of postlearning NREM sleep (Eschenko, Ramadan, Mölle, Born, & Sara, 2008). Therefore, there is the possibility that part of the memory consolidation damage induced by the MMPM may be related to the decrease in the amount of NREM sleep.

METHOD

Subjects

Young, 3-week-old male Sprague Dawley rats with a body weight range of 53.0–68.3 g were used in the present study. All of the animal protocols used in the experiment were reviewed and approved by the Shanghai Jiaotong University School of Medicine Committee of Ethics on Animal Experiments. The rats were housed and maintained under a 12:12-h light:dark cycle with lights on at 7:00 a.m. Room temperature was kept at $20^{\circ}\text{C} \pm 2^{\circ}\text{C}$, and the animals had free access to water and food at all times during the experiment.

Morris Water Maze Test (Spatial Reference Memory Test)

The Morris water maze test was performed as described in Vorhees and Williams (2006). The water maze, consisting of a large circular black pool 120 cm in diameter and 50 cm in height, was divided into four quadrants of equal size on the monitoring screen of a computer. The water, kept at $26^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and colored by a nontoxic black dye, was filled to a depth of 30 cm, 1.0 cm above the surface of a round platform (8 cm in diameter). The water maze was placed in a test room homogeneously brightened at 100 lx and containing various prominent visual cues.

Each rat was given four trials per day. Starting points varied randomly in different quadrants from trial to trial, with each quadrant as the starting point of one trial. The location of the hidden platform remained constant, in the middle of Quadrant 2. Each trial was started by placing the animal into the water with its face toward the wall of the water maze. The animal was allowed to swim until it located the platform or for a maximum duration of 120 sec in each trial. When the rat reached the platform, it was allowed to stay there for 30 sec. The animal was then removed and put into a cage to wait 30–35 sec before the next trial was initiated. If the platform was not located within 120 sec, the animal was placed on it and was allowed to stay for 30 sec. The time elapsed and distance traveled before the animals found the hidden platform were recorded using a video tracking system.

Memory-acquisition trials (training) were performed for 3 consecutive days before sleep intervention, and memory-retention tests were performed immediately after sleep intervention (see Figure 1).

REM Sleep Deprivation (MMPM)

Rats were tested in socially stable groups of 6 in separate water tanks ($88.0 \times 88.0 \times 36.0$ cm) containing either 18 round platforms 4.0 cm in diameter (small platforms) or 18 round platforms 10.5 cm in diameter (large platforms). The platforms stood 8.0 cm above the tank bottom. The tanks were filled with water to a level of 1.0 cm below the surface of the platform. This was so that the animals would be capable of moving inside the tank by jumping from one platform to another. The large-platform group is often used as a control in sleep deprivation experiments. An additional control group was placed in a cage—the *cage control* group.

In developing an adaptation of the MMPM for young rats, we relied on behavioral observations during pilot studies in order to determine the optimal size of the platform. The platform size finally identified as ideal for REM sleep deprivation in this age rat was sufficiently small (4.0-cm diameter) to initially allow the youngest

Figure 1. Experimental protocol of the Morris water maze trials follow-up.

animals (24 days old) to contact the water shortly after losing neck muscle tone (observed as dropping the head toward the water). Behavioral observations also confirmed that, on a large platform (10.5-cm diameter), even the oldest rats (31 days old) did not contact the water with their heads or fall off the platforms when they lost muscle tone after adopting an initial sleep posture.

Procedure

Seventy-two 3-week-old male Sprague Dawley rats were cage housed and handled daily for 1 week to allow adaptation to the environment. At the end of the week, each rat was trained in the Morris water maze for 3 days. The 18 rats that were least successful in training were excluded from the experiment.

The remaining 54 animals were randomly divided into nine groups of 6: Three groups were deprived of REM sleep, which was induced by the MMPM. After 48 h of RSD, two groups were released from sleep deprivation and were put in cages for 24 h (REV1) and 48 h (REV2), respectively. Six groups of time-matched controls were housed either in tanks with large platforms (tank control, TC; three groups) or in cages (cage control, CC; three groups) (see Figure 2).

Statistical Analysis

Statistical descriptions were made by use of the mean and standard deviation. Body weight, swimming speed, distance, and escape latency differences in groups were analyzed using a one-way ANOVA. Changes in weight, distance, and latency with training days were evaluated by a repeated measures ANOVA.

To evaluate the impact of body weight and swimming speed on distance and escape latency (the time taken to locate the hidden platform), multiple linear regression analyses were adopted. Associations of sleep intervention with distance and escape latency were examined using an ANCOVA with body weight as a covariate.

All analyses were performed using SPSS for Windows (Version 12.5). In the presentation of the results, the statistical significance was set at $p < .05$ (two-tailed).

RESULTS

Baseline Analyses of Body Weight, Swimming Speed, Distance, and Escape Latency Before Sleep Intervention

Tables 1–4 summarize the descriptive statistics of body weight, swimming speed, escape latency, and distance, respectively, in the Morris water maze for all groups before sleep intervention. As can be seen in the tables, there were no significant differences in body weight, swimming speed,

escape latency, or distance among the nine groups. There was a significant tendency toward shorter escape latencies and distances with increased training days, which indicated that the performance of the rats in the Morris water maze improved during the memory-acquisition training.

The Impacts of Sleep Intervention on the Retention of Acquired Memory

The effects of body weight and swimming speed on escape latency and distance were analyzed using multiple linear regression analyses. The results demonstrated that rats with heavier body weights and slower swimming speeds tended to have longer escape latencies (standardized regression coefficient, $STDB = -.35$, $t = -2.56$, $p < .05$ for body weight; $STDB = .86$, $t = -32.14$, $p < .01$ for swimming speed) and longer distance ($STDB = -.24$, $t = -2.06$, $p < .05$ for body weight; $STDB = .93$, $t = -48.76$, $p < .01$ for swimming speed) to locate the hidden platform in the Morris water maze, which suggests that the effects of body weight and swimming speed on performances should be considered in analyzing the impact of sleep intervention on learning and memory.

There were significant differences among the nine groups of rats in body weight ($F = 19.95$, $p < .01$) after sleep intervention. Meanwhile, there were no significant differences in swimming speed. Compared with the cage and large platform groups, 48 h of REM sleep deprivation produced a lower weight growth. After 48 h of REM sleep deprivation, the body weights in the RSD, CC1, and TC1 groups were $M = 77.41$ g ($SD = 1.87$), $M = 90.5$ g ($SD = 1.77$), and $M = 86.58$ g ($SD = 1.59$), respectively.

An ANCOVA was used to analyze the effects of sleep intervention on escape latency and distance with body weight as a covariate (Figure 3). Forty-eight hours of RSD produced longer distances and escape latencies than were found in the cage and large-platform groups. After 24 and 48 h of release from sleep deprivation, there was no significant improvement in performance.

Figure 4 shows the paths taken by rats before location of the platform as a function of training and sleep intervention. There was a significant interaction of escape latency

Figure 2. Experimental protocol of the Morris water maze trials follow-up.

Table 1
Baseline Analysis of Body Weight (in Grams)
Before Sleep Intervention

Group	Body Weight						$F^\dagger (p < .01)$
	Training Day 1		Training Day 2		Training Day 3		
	M	SD	M	SD	M	SD	
CC1	65.13	7.43	70.18	7.10	75.73	8.00	11.93
TC1	64.41	6.22	69.95	5.94	75.27	6.75	17.74
RSD	65.13	6.71	69.55	6.44	75.28	7.39	13.21
CC2	65.15	7.43	71.19	6.09	75.74	7.98	11.92
TC2	64.42	6.21	70.05	6.01	75.27	6.75	16.69
REV1	64.83	8.67	69.95	8.06	75.23	8.40	7.98
CC3	65.14	7.40	70.18	7.23	75.74	7.99	11.96
TC3	64.73	6.52	69.96	6.11	75.33	6.77	17.73
REV2	65.73	5.09	71.99	5.26	75.41	5.93	25.21

Note—CC, cage control group; TC, large platform control; RSD, sleep deprivation group; REV1, sleep recovery for 24-h group; REV2, sleep recovery for 48-h group. One-way ANOVA F values were 0.42, 0.54, and 0.08 (all $ps > .05$) for Training Days 1–3, respectively. † Repeated measures ANOVA.

and training day: The rats tended to more directly locate the platform on the training day before sleep deprivation. The 48-h REM sleep deprivation produced more circuitous swimming paths, and this delay in paths did not show significant improvement during sleep recovery.

DISCUSSION

The present study demonstrated that 48 h of posttraining RSD impaired the retention of acquired spatial reference memory for young rats in a Morris water maze. Furthermore, REM sleep recovery for 24–48 h has no effect on RSD-induced impairment of reference memory. These results first tested our hypothesis that REM rebound may be ineffective for the restoration of RSD-induced impairment of memory through behavioral experiments, indicating that postlearning REM sleep was critical for newly acquired memory consolidation. Moreover, the present study particularly showed an effect of RSD on memory consolida-

tion in the developing brain, which was different from most related research studies. Therefore, this study entailed an extended data set for interpreting the associations between REM sleep and memory consolidation.

The Morris water maze task, a well-established test of spatial navigation, was used to assess spatial reference memory (Morris, 1984; Vorhees & Williams, 2006). Several studies have demonstrated that synaptic plasticity changes in neuronal circuits, including the hippocampus, are involved in spatial memory consolidation, which indicates that spatial reference memory is hippocampal dependent (Burwell, Saddoris, Bucci, & Wiig, 2004; Holup, Kjelstrup, Hoff, Moser, & Moser, 2001; A. J. Silva, 2003). In recent years, evidence has been accumulating that consolidation of hippocampus-dependent memories relies on a dialogue between the neocortex and the hippocampus and that the crucial feature of this dialogue is the neuronal reactivation of newly formed memories during sleep (Born et al., 2006; Fu et al., 2007; Ishikawa

Table 2
Baseline Analysis of Swimming Speed (in Centimeters per Second)
Before Sleep Intervention

Group	Swimming Speed						$F^\dagger$ ($p < .01$)
	Training Day 1		Training Day 2		Training Day 3		
	M	SD	M	SD	M	SD	
CC1	10.81	3.18	6.87	3.52	5.29	2.91	18.75
TC1	11.33	4.37	6.20	3.42	5.94	3.07	16.59
RSD	10.77	3.78	7.42	4.33	5.44	3.04	7.91
CC2	10.77	3.16	6.90	3.54	6.01	2.98	18.43
TC2	11.31	4.34	6.23	3.41	5.88	2.75	16.60
REV1	10.63	4.08	7.51	3.50	5.76	3.10	10.10
CC3	10.81	3.19	6.83	3.46	5.63	3.25	18.37
TC3	11.35	4.35	6.28	3.45	6.24	2.77	16.47
REV2	12.08	3.44	7.35	3.09	6.01	2.83	30.20

Note—CC, cage control group; TC, large platform control; RSD, sleep deprivation group; REV1, sleep recovery for 24-h group; REV2, sleep recovery for 48-h group. One-way ANOVA F values were 0.75, 1.04, and 0.42 (all $ps > .05$) for Training Days 1–3, respectively. † Repeated measures ANOVA.

Table 3
Baseline Analysis of Escape Latency (in Seconds)
Before Sleep Intervention

Group	Escape Latency						$F^{\dagger} (p < .01)$
	Training Day 1		Training Day 2		Training Day 3		
	M	SD	M	SD	M	SD	
CC1	70.32	9.15	38.81	7.94	22.50	3.94	10.93
TC1	71.06	9.06	28.27	6.57	17.91	2.75	17.95
RSD	63.20	9.55	42.73	8.30	21.43	3.41	7.61
CC2	70.45	9.18	38.86	7.94	18.99	3.98	10.94
TC2	71.05	9.06	30.25	6.50	20.05	3.75	17.92
REV1	74.48	9.08	37.99	8.06	22.19	4.40	12.36
CC3	70.32	9.15	38.77	7.90	22.53	3.99	10.94
TC3	70.99	9.04	32.24	6.56	19.11	3.77	17.95
REV2	73.34	8.44	29.98	5.26	17.86	2.99	43.22

Note—CC, cage control group; TC, large platform control; RSD, sleep deprivation group; REV1, sleep recovery for 24-h group; REV2, sleep recovery for 48-h group. One-way ANOVA F values were 0.34, 0.86, and 0.38 (all $ps > .05$) for Training Days 1–3, respectively. † Repeated measures ANOVA.

et al., 2006; Marshall & Born, 2007; Saxvig et al., 2008; R. H. Silva et al., 2004; Stickgold, 2005; Walker & Stickgold, 2004, 2006). Our study showed that, after 3 days of training, 48-h RSD produced longer distances and escape latencies in the Morris water maze; it was shown that newly acquired reference memory had been impaired. Youngblood, Zhou, Smagin, Ryan, and Harris (1997) specifically assessed the impact of RSD on spatial reference memory, demonstrating that posttraining RSD for 24 and 96 h resulted in a substantial impairment of reference memory in a Morris water maze. Similarly, Smith, Conway, and Rose (1998) demonstrated that posttraining RSD, especially 4 h after learning, caused an abortive performance in an eight-arm radial maze. Total sleep deprivation for the first 5 h after acquisition has also been shown to impair performance on a hippocampus-dependent contextual fear task (Graves, Heller, Pack, & Abel, 2003), and, in general, retention in the water maze task has been shown to be dependent on posttraining sleep (Graves, Pack, & Abel, 2001). These results were compatible with the present findings that postlearning

REM sleep was critical for newly acquired memory consolidation.

It is well known that RSD could induce compensatory mechanisms and cause rebound of REM sleep during the recovery period (Machado et al., 2004; Ocampo-Garcés et al., 2000; Suchecki et al., 2000). This phenomenon was considered to be a homeostatic compensation for a deficit in REM sleep (Ocampo-Garcés et al., 2000); however, the functional significance for memory is unclear. There is electrophysiological evidence that REM rebound does not restore the RSD-induced impairment of LTP (Ishikawa et al., 2006; Kim et al., 2005; Romcy-Pereira & Pavlides, 2004). For example, Ishikawa et al. specifically examined the effect of RSD after LTP induction and REM rebound on LTP maintenance in DG; it was shown that a 24- to 48-h duration of RSD caused a significant decrease in population spike amplitudes and that REM rebound has no effect on RSD-induced impairment of LTP. Romcy-Pereira and Pavlides focused on the effects of RSD following LTP induction, reporting that a 4-h RSD immediately after a high frequent stimulus (HFS) induced LTP

Table 4
Baseline Analysis of Distance (in Centimeters) Before Sleep Intervention

Group	Distance						$F^\dagger (p < .01)$
	Training Day 1		Training Day 2		Training Day 3		
	M	SD	M	SD	M	SD	
CC1	1,005.47	117.41	545.26	104.54	322.59	56.74	13.03
TC1	1,120.21	140.36	437.27	83.84	314.57	48.83	20.02
RSD	986.18	132.04	608.37	118.56	360.71	56.33	8.19
CC2	1,005.88	113.81	567.31	110.21	325.88	57.98	13.03
TC2	1,125.08	139.56	480.23	98.50	320.89	46.75	19.99
REV1	1,089.22	130.33	562.31	97.66	371.53	68.40	11.49
CC3	1,005.47	117.41	530.79	102.85	318.22	47.99	13.03
TC3	1,119.85	140.25	407.21	93.82	320.55	56.77	20.03
REV2	1,229.85	128.15	437.97	80.36	321.95	48.45	40.65

Note—CC, cage control group; TC, large platform control; RSD, sleep deprivation group; REV1, sleep recovery for 24-h group; REV2, sleep recovery for 48-h group. One-way ANOVA F values were 0.68, 1.01, and 0.15 (all $ps > .05$) for Training Days 1–3, respectively. † Repeated measures ANOVA.

Figure 3. The comparison of escape latency (sec) and distance (cm) in the Morris water maze among each sleep intervention group and the time-matched control groups. (A) The comparisons among the RSD group and the time-matched control groups. (B) The comparisons among the REV1 group and the time-matched control groups. (C) The comparisons among the REV2 group and the time-matched control groups. The error bars indicate standard deviations. * $p < .05$.

impairment at 48 h but not at 24 h after HFS. The present study, to our knowledge, is the first to observe the impact of REM sleep recovery on the maintenance of newly acquired memory through behavioral experiments. In agreement with the findings of previous electrophysiological studies, the present results show that REM sleep recovery for 24–48 h has no effect on RSD-induced impairment in

a Morris water maze, which supports the hypothesis that REM rebound may be ineffective for the restoration of RSD-induced impairment of memory.

The mechanisms responsible for memory consolidation during REM sleep remain to be elucidated. REM sleep is characterized by hippocampal theta rhythmic activity, which is strongly related to an increase of acetylcholine in

Figure 4. Representative tracings of young rats during training and sleep intervention in the Morris water maze. Train 1, training on the first day; Train 2, training on the second day; Train 3, training on the third day; SD48h, 48-h REM sleep deprivation; REC24h, 24 h released from sleep deprivation; REC48h, 48 h released from sleep deprivation.

the hippocampus (Monmaur, Collet, Puma, Frankel-Kohn, & Sharif, 1997; Yoder & Pang, 2005). Previous studies have shown that acetylcholine during REM sleep following a learning task plays an important role in memory consolidation (Matsuyama, Matsumoto, Enomoto, & Nishizaki, 2000; Smith, Tenn, & Annett, 1991). In addition, a more recent study by Davis et al. (2006) indicated that RSD severely hampers cytoskeletal reorganization in the hippocampus, a process that is essential for synaptic plasticity and memory consolidation. Another study observed the NMDA receptor properties in dendrites of CA1 pyramidal neurons during sleep deprivation; it was shown that the NMDA–AMPA ratio and the amplitude of NMDA currents at Schaffer collateral synapses were reduced after 72 h of sleep deprivation, indicating an impairment in NMDAR-mediated synaptic transmission, which is considered to be a key mechanism in sleep-deprivation-induced memory impairment (McDermott, Hardy, Bazan, & Magee, 2006).

As mentioned in the introduction, the MMPM technique, adopted in our study to induce RSD, decreases NREM sleep by approximately 30% when it completely abolishes REM sleep (Machado et al., 2004). Therefore, there is the possibility that part of the results described in our study may be related to this decrease in the amount of NREM sleep. However, there is evidence that NREM sleep rebound is not found after the use of the MMPM (Alvarenga et al., 2008). Suchecki et al. (2000) specifically assessed the sleep rebound of rats exposed to the MMPM for a period of 90 h; it was found that the exposure induced an exclusive REM sleep rebound (184.7%) and a reduction of NREM sleep (–12%). These results suggest that the NREM reduction during the deprivation period may not be severe enough to induce compensatory rebound or that NREM rebound could have been offset by the large increase in REM sleep. Therefore, our study has significant value in understanding the associations between REM sleep and memory consolidation.

In addition to being deprived of sleep by the MMPM, the rats were physically stressed because of movement restriction, wetness, and muscle fatigue (Suchecki, Lobo, Hipólido, & Tufik, 1998; Suchecki & Tufik, 2000), and the stress experienced by rats during sleep intervention may have implications for conclusions on the associations between posttraining RSD and spatial reference memory. However, it has been confirmed that, compared with the original single-platform technique, the MMPM significantly reduces movement restriction, social isolation, and social instability, and, as a result, the use of the MMPM exhibits attenuated stress indices (Suchecki & Tufik, 2000). A more recent research study by Ruskin, Dunn, Billiot, Bazan, and LaHoste (2006) assessed the impact of 72 h of sleep deprivation on spatial memory in adrenalectomized rats, which provided support for the impairment in memory's being due specifically to the lack of sleep and not to an adrenal stress response.

In conclusion, the findings in the present study are an extended data set for the hypothesis that postlearning REM sleep plays a crucial role in the consolidation of newly acquired memories for long-term storage, although the limitations must be taken into consideration. Further investigation is needed in order to determine the mechanisms underlying the associations between REM sleep and memory consolidation. Moreover, the role of NREM sleep in memory maintenance should receive more attention, since more and more evidence suggests that both NREM sleep and REM sleep correlate with memory enhancement (Born et al., 2006; Rasch et al., 2007; Stickgold, 2005; Walker & Stickgold, 2004, 2006).

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