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Rapid eye movement sleep deprivation disrupts consolidation but not reconsolidation of novel object recognition memory in rats

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HIGHLIGHTS

- Post-sample RSD from 0 to 6 h disrupted object recognition memory consolidation.
- Post-reactivation RSD had no effect on object cognition memory reconsolidation.
- Suggests a dissociation effect of RSD on consolidation and reconsolidation.

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ABSTRACT

There is increasing evidence that sleep plays a critical role in memory consolidation. However, there are comparatively few studies that have assessed the relationship between sleep and memory reconsolidation. In the present study, we explored the effects of rapid eye movement sleep deprivation (RSD) on the consolidation (experiment 1) and reconsolidation (experiment 2) of novel object recognition memory in rats. In experiment 1 behavioral procedure involved two training phases: sample and test. Rats were subjected to 6 h RSD starting either immediately after sample (exposed to 2 objects) or 6 h later. In experiment 2 behavioral procedure involved three training phases: sample, reactivation and test. Rats were subjected to 6 h RSD starting either immediately after reactivation (exposed to the same 2 sample objects to reactivate the memory trace) or 6 h later. Results from experiment 1 showed that post-sample RSD from 0 to 6 h but not 6 to 12 h disrupted novel object recognition memory consolidation. However, we found that post-reactivation RSD whether from 0 to 6 h or 6 to 12 h had no effect on novel object recognition memory reconsolidation in experiment 2. The results indicated that RSD selectively disrupted consolidation of novel object recognition memory, suggesting a dissociation effect of RSD on consolidation and reconsolidation.

1. Introduction

Based on the traditional memory consolidation hypothesis, newly acquired memory is initially present in a transient labile state in which the memory trace can be disrupted by various agents, but becomes resistant to disruption over time [2,26]. This process is called consolidation. However, a well-consolidated memory could be again rendered labile and susceptible to disruption upon its reactivation. For instance, a recall of the memory (reactivation) can return it to a labile state [12,18]. Then a process called reconsolidation may begin by which the original memory becomes resistant again [18,26]. Although the issue of memory reconsolidation is controversially discussed [16], the reconsolidation phenomenon has been indicated in a variety of learning paradigms [18,22,26]. Although consolidation and reconsolidation share brain circuits and molecular processes, the neuronal mechanisms involved respectively do not completely overlap [12,34].

Sleep is composed of two widely known phases: non-rapid eye movement (NREM) sleep and rapid eye movement (REM) sleep. Up to date, however, the function of sleep still remains elusive. Considerable evidence indicates that sleep may be involved in memory processes [37]. Sleep deprivation is a well-known paradigm often used to assess the roles of sleep in memory process. Results from this paradigm suggest that the role of sleep in memory is complex and appears to depend on multiple factors. For example, both pre- and post-training REM sleep deprivation (RSD) caused memory deficits in the plus-maze discriminative avoidance and passive

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avoidance tasks in mice [28]. In rats, post-training RSD produced learning impairment in the hidden platform, but not in the visual platform in the water-maze task [30]. In the eight-arm radial maze task, post-acquisition RSD impaired spatial reference but not working memory in rats [29].

The novel object recognition tests is simple behavioral assay of memory that relies primarily on the spontaneous tendency of rats to explore a novel object more than a familiar one in the absence of externally applied rules or reinforcement [3]. Currently the novel object recognition task has become a widely used paradigm for the investigation on memory alterations, and has also been used to test the effects of various pharmacological treatments and brain damage [9]. For example, microinfusion of the protein synthesis inhibitor anisomycin into the ventromedial prefrontal cortex or the dorsal hippocampus impaired consolidation and reconsolidation of object recognition memory [1,25].

Several studies have shown that total sleep deprivation impaired novel object recognition memory consolidation in mice [10,19,24]. Recent evidence suggests that REM sleep may play a crucial role in learning and memory formation [32,33,37]. RSD may produce differential effects compared with total sleep deprivation. For example, RSD has a profound effect on mood more than total sleep deprivation in humans [20]. Thus it is interesting to explore the effects of RSD on novel object recognition memory consolidation. Furthermore, there remain comparatively few studies that have assessed the relationship between REM sleep and memory reconsolidation. We found previously that RSD did not affect reconsolidation of cued and contextual fear memory [35]. However, the effects of RSD on novel object recognition memory reconsolidation remain to be elucidated. At present study, we designed two experiments to explore the effects of RSD on consolidation (experiment 1) and reconsolidation (experiment 2) of novel object recognition memory in rats.

2. Material and methods

2.1. Subject

The subjects were 80 adult male Sprague Dawley rats (250–300 g) obtained from the Laboratory Animal Center of University of South China, Hengyang, China. After arrival, the rats were housed individually in a temperature- and humidity-controlled home room with *ad libitum* access to food and water. Animals were maintained on a 12 h light/dark schedule, with lights on at 7 a.m. After being housed, the rats were handled (3–5 min per rat per day) for 1 week to habituate them to the experimenter. Experiments were conducted according to the *National Institutes of Health Guide for the Care and Use of Laboratory Animals*, and experimental protocols were approved by the University of South China animal care and use committee.

2.2. Rapid eye movement sleep deprivation procedure

Rapid eye movement sleep deprivation (RSD) was accomplished with the well-established "flowerpot" technique as our previous descriptions [8], which consisted of placing rats onto an inverted flowerpot (10 cm diameter) placed inside a large pail, which was filled with water up to 1 cm below the level of the flowerpot. In this situation, the rats are unable to completely relax the large muscle groups (a necessary condition for REM sleep to occur) without falling from the platform, getting wet, and waking. After the period of RSD, the animals were dried with a towel, if necessary, and were returned to their home cages until the next day's test. This technique has been shown to selectively deprive rats of REM, but not NREM, sleep [15,31]. The pails were placed in one of two identical rooms (room A and B). Before behavioral tests, rats were habituate to each room for 5 h per day for 1 week, respectively. RSD began about 8:00 a.m. (0–6 h RSD) or 2:00 p.m. (6–12 h RSD).

2.3. Behavioral apparatus

Novel object recognition memory is the ability to discriminate the familiarity of previously encountered objects. The training apparatus consisted of two same black Plexiglas boxes (60 cm × 60 cm × 60 cm) which were used to test 2 animals at the same time. Each box was placed in a sound-attenuating cabinet which was located in a brightly lit and isolated room. Illumination was provided by a 15 W white house light mounted on the ceiling of cabinet, and a 65 dB background noise was supplied by a ventilation fan in the cabinet. The floor of the box was covered with sawdust. The objects used in the task were made of water-repellant materials such as glass and plastic with differences in shape and color. The sizes of the objects were about 6 cm × 6 cm × 8 cm. Objects were fixed to the floor of the training apparatus, 10 cm from the walls. The location and objects were counterbalanced to control for any preferences that the rats might have had for one of the corners or of the objects. The sawdust was stirred and the box and the objects were cleaned with 40% ethanol solution between trials. Exploration of an object was defined as pointing the nose to the object at a distance of <1 cm and/or touching it with the nose.

2.4. Experiment design and procedure

Experiment 1 was designed to assess the effects of post-sample RSD on novel object recognition memory consolidation. Behavioral procedure was carried out on 2 consecutive days. On Day 1 (sample phase), rats were exposed to 2 objects (A and B) for 10 min as described above. The total time spent exploring both objects was recorded. Rats were subjected to 6 h RSD starting either immediately after sample or 6 h later. For 0–6 h RSD, the RSD group rats (0–6 h RSD group, $n = 10$) were transported to room A for RSD immediately after sample. The control group rats (0–6 h control, $n = 10$) were transported to room B and left undisturbed. After 6 h, all rats were returned to their home room. For 6–12 h RSD, the control (6–12 h control, $n = 10$) and RSD (6–12 h RSD group, $n = 10$) groups rats were returned to their home room immediately after sample. After 6 h, the RSD group rats were transported to room A for RSD. The control group rats were transported to room B and left undisturbed. All rats were returned to their home room again after 6 h. On Day 2 (test phase), rats were exposed to a duplicate of an object from the sample trial and a novel object (A and C) for 3 min. The time spent exploring each object and the total time spent exploring both objects was recorded. The discrimination index (DI) used to assess memory was calculated as the difference in time exploring the novel and familiar object [7], expressed as the ratio of the total time spent exploring both objects.

Experiment 2 was designed to assess the effects of post-reactivation RSD on novel object recognition memory reconsolidation. Behavioral procedure was carried out on 3 consecutive days. On Day 1 (sample phase), rats were exposed to 2 objects (A and B) for 10 min as described above. The total time spent exploring both objects was recorded. On Day 2 (reactivation phase), rats were exposed to the same 2 sample objects (A and B) for a 3-min period to reactivate the memory trace. The total time spent exploring both objects was recorded. Rats were subjected to 6 h RSD starting either immediately after reactivation or 6 h later. For 0–6 h RSD, the RSD group rats (0–6 h RSD group, $n = 10$) were transported to room A for RSD immediately after reactivation. The control group rats (0–6 h control, $n = 10$) were transported to room B and left undisturbed. After 6 h, all rats were returned to their home room. For 6–12 h RSD, the control (6–12 h control, $n = 10$) and RSD (6–12 h RSD group,