Neuroscience Letters 463 (2009) 74–77

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Rapid eye movement sleep deprivation does not affect fear memory reconsolidation in rats

Shaowen Tian[a,*,1], Fulian Huang[a,*,1], Peng Li[b], Xinping Ouyang[a], Zengbang Li[a], Haifeng Deng[a], Yufeng Yang[a]

[a] Department of Physiology, College of Medicine, University of South China, Hengyang, Hunan 421001, PR China
[b] Department of Biology, College of Life Science and Technology, University of South China, Hengyang, Hunan 421001, PR China

ARTICLE INFO

Article history:
Received 9 April 2009
Received in revised form 9 July 2009
Accepted 15 July 2009

Keywords:
Sleep
Rapid eye movement sleep deprivation
Fear memory
Reconsolidation
Rats

ABSTRACT

There is increasing evidence that sleep may be involved in memory consolidation. However, there remain comparatively few studies that have explored the relationship between sleep and memory reconsolidation. At present study, we tested the effects of rapid eye movement sleep deprivation (RSD) on the reconsolidation of cued (experiment 1) and contextual (experiment 2) fear memory in rats. Behaviour procedure involved four training phases: habituation, fear conditioning, reactivation and test. Rats were subjected to 6 h RSD starting either immediately after reactivation or 6 h later. The control rats were returned to their home cages immediately after reactivation and left undisturbed. Contrary to those hypotheses speculating a potential role of sleep in reconsolidation, we found that post-reactivation RSD whether from 0 to 6 h or 6 to 12 h had no effect on the reconsolidation of both cued and contextual fear memory. However, our present results did not exclude the potential roles of non-rapid eye movement sleep in the reconsolidation of fear memory or sleep in the reconsolidation of other memory paradigms.

According to the traditional consolidation hypothesis, newly acquired memory is initially present in a transient labile condition in which the memory trace can be disrupted by amnesic treatment, but becomes resistant to disruption over time [1,28]. This process is called consolidation. However, a well-consolidated memory is not permanently resistant to change but may return to a labile state. For instance, a recall of the memory (reactivation) can return them to a labile state [3–5,17,22,23]. Then a process named reconsolidation may begin by which the original memory becomes resistant again [21,23,27,28]. Although there remains controversial for the phenomenon of memory reconsolidation [16,20], the reconsolidation hypothesis has been confirmed using a variety of learning paradigms [2,14,23,25,26,28,36].

Sleep is composed of two widely known phases, namely rapid eye movement (REM) sleep and non-rapid eye movement (NREM) sleep. Up to date, however, the function of sleep still remains elusive. There is increasing evidence that sleep may be involved in learning and memory processes [41]. A well-established paradigm often used to examine this point is to test the effects of sleep deprivation on learning and memory. Results from this paradigm showed that the role of sleep in learning and memory is complex and appears to depend on multiple factors, such as the nature of the task, a specific time window and the stages of sleep. For example, both pre- and post-training REM sleep deprivation (RSD) produced memory deficits in the passive and the plus-maze discriminative avoidance tasks in mice [29]. In rats, post-training RSD resulted in learning impairment in the hidden platform, but not in the visual platform [32]. In the eight-arm radial maze task, post-acquisition RSD resulted in a deficit of spatial reference memory, whereas working memory was intact [31].

In Pavlovian fear conditioning paradigm, subjects are usually trained by pairing a cued or contextual conditioned stimulus (CS) with a footshock unconditioned stimulus (US). After several trials, the CS comes to elicit conditioned fear responses such as freezing, increased startle reflexes and behavioural response suppression. Conditioned fear responses can be extinguished by repeatedly presenting the CS without the US. Similar to other types of memory, fear memory is also a process that has several stages, such as acquisition, consolidation, retrieval and reconsolidation. Previous results showed that pre-training RSD impaired both cued and contextual fear conditioning in rats [7]. Post-acquisition total sleep deprivation impaired contextual but not cued fear conditioning in C57BL/6 mice [13]. Post-fear conditioning RSD impaired the extinction of a cued but not contextual conditioning task [30]. Furthermore, post-extinction RSD impaired recall of cued but not contextual fear extinction [12]. However, the effects of RSD on fear memory reconsolidation remain to be elucidated. At present study, we designed

---

* Corresponding authors. Tel.: +86 0734 8281389; fax: +86 0734 8281785.
E-mail addresses: tsw182276@126.com (S. Tian), hflscience@126.com (F. Huang).

[1] Authors contributed to the paper equally.

0304-3940/$ – see front matter © 2009 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2009.07.044

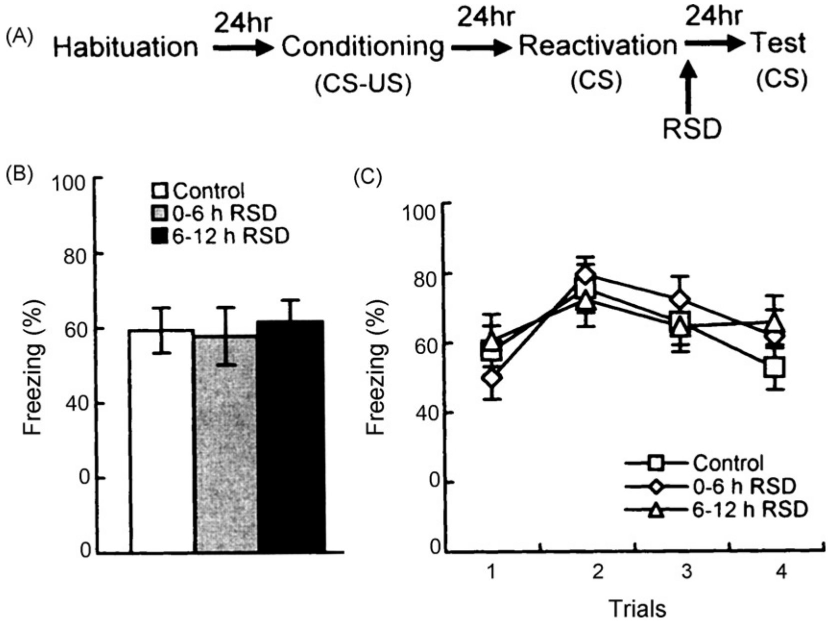

Fig. 1. Post-reactivation RSD does not affect the reconsolidation of cued fear memory. (A) Schematic of the behavioural procedure used. Behaviour procedure involved four training phases: habituation, fear conditioning, reactivation and test. Rats were subjected to 6 h RSD starting either immediately after reactivation (0–6 h RSD) or 6 h later (6–12 h RSD). (B) Percent freezing to the tone was shown for 0–6 h RSD rats, 6–12 h RSD rats and control rats during reactivation phase. (C) Percent freezing to the tone was shown across trials for 0–6 h RSD rats, 6–12 h RSD rats and control rats during test phase. RSD, rapid eye movement sleep deprivation. All data are represented as mean ± SEM.

two experiments to explore the effects of post-reconsolidation RSD on a cued (experiment 1) and contextual (experiment 2) fear memory reconsolidation task in rats.

The subjects were 60 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 environment 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.

RSD was accomplished with the well-established “flowerpot” technique as our previous descriptions [12], 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. 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 for fear memory reconsolidation. This technique has been shown to selectively deprive rats of REM, but not NREM, sleep [19,33].

Experiment 1 was designed to assess the effects of post-reactivation RSD on cued fear memory reconsolidation. Fear conditioning, reactivation and test were conducted in two different observation chambers as same as that previously described [15]: context A and context B. Behaviour procedure was carried out on four consecutive days. On day 0 (habituation phase), rats were taken from their home cages and transported to context A and context B, respectively, for 30 min each with no stimuli presented to habituate them to both contexts. The order of the context exposure was counterbalanced. The following day (day 1, fear conditioning phase), rats received five tone-footshock trials (tone: 4 kHz, 80 dB, 20 s duration; shock: 0.5 mA, 0.5 s duration) beginning 3 min after being placed in context A. The footshock coterminated with the tone. The average intertrial interval was 90 s (range, 60–120 s). Thirty seconds after the final shock, the rats were returned to their home cages. Twenty-four hours after fear conditioning (day 2, reactivation phase), rats received 1 tone alone presentations (4 kHz, 80 dB, 20 s duration) 3 min after placement in context B. Rats were subjected to 6 h RSD starting either immediately after reactivation (0–6 h RSD, $n = 10$) or 6 h later (6–12 h RSD, $n = 10$). The control group rats ($n = 10$) were returned to their home cages immediately after reactivation and left undisturbed. RSD was conducted at about 9:00 a.m. for 0–6 h RSD rats or 3:00 p.m. for 6–12 h RSD rats. Twenty-four hours after reactivation (day 3, test phase), rats received four tone alone presentations in context B. Percent of time spent freezing was used to measure conditioned fear response during reactivation and test phases. Freezing is the absence of all movements except those related to respiration. The total time spent freezing during each tone presentation was scored with a digital stopwatch from digital videos. Observers scoring freezing were blind with respect to the experimental groups.

Experiment 2 was designed to assess the effects of post-reactivation RSD on contextual fear memory reconsolidation. Behaviour trainings were conducted in context A. On day 0 (habituation phase), rats were habituated to context A for 30 min with no stimuli presented. On day 1 (fear conditioning phase), rats received five alone footshock trials (0.5 mA, 0.5 s duration) beginning 3 min after being placed in context A. The average intertrial interval was 90 s (range, 60–120 s). On day 2 (reactivation phase) and day 3 (test phase), rats were reexposed to context A for 90 and 300 s, respectively, without receiving a footshock. As described in experiment 1, rats were subjected to 6 h RSD starting either immediately after reactivation (0–6 h RSD, $n = 10$) or 6 h later (6–12 h RSD, $n = 10$). The control group rats ($n = 10$) were returned to their home cages immediately after reactivation and left undisturbed. RSD was conducted at about 9:00 a.m. for 0–6 h RSD rats or 3:00 p.m. for 6–12 h RSD rats. The total time spent freezing during the 90 s reactivation period and every 60 s during the 300 s test period were scored.

Percent freezing values during reactivation and test phases were analyzed using one-way ANOVA or two-way repeated-measures ANOVA (group × trial or group × time block), respectively (SigmaS-