Behavioural Brain Research 321 (2017) 69–78

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Assessment of mouse cognitive and anxiety-like behaviors and hippocampal inflammation following a repeated and intermittent paradoxical sleep deprivation procedure

Mengmei Yin [a], Yali Chen [b], Hui Zheng [b], Tinglin Pu [b], Charles Marshall [c], Ting Wu [a,*], Ming Xiao [b,*]

[a] Department of Neurology, The First Affiliated Hospital of Nanjing Medical University Nanjing, Jiangsu 210029, China
[b] Jiangsu Province, Key Laboratory of Neurodegeneration, Nanjing Medical University, Nanjing, Jiangsu, China
[c] Department of Rehabilitation Sciences, University of Kentucky Center of Excellence in Rural Health, Hazard, KY, USA

HIGHLIGHTS

- A mouse model of intermittent and repetitive sleep deprivation has been established.
- The effects of paradoxical sleep deprivation on various behavioral tasks are examined.
- Neuroinflmmation and neuronal apoptosis endured for even 3 weeks after deprivation.

ARTICLE INFO

Article history:
Received 21 October 2016
Received in revised form
21 December 2016
Accepted 25 December 2016
Available online 30 December 2016

Keywords:
Animal model
Behavioral test
Neuroinflammation
Sleep deprivation

ABSTRACT

It has been reported that more than one fourth of the world's population suffers from sleep problems. However, there is not a stable and reliable animal model to mimic the persistent and periodic features of sleep disorders, and correspondingly, the feasibility and effectiveness of repeated behavioral tests remains to be determined. In the present study, we repetitively, and intermittently, treated mice with 3 days and 7 days of paradoxical sleep deprivation (SD), using the modified multiple small-platforms-over-water method for 3 months. The behavioral results suggested that repeated open field and Y-maze tests are able to successfully detect anxiety-like behaviors and working memory dysfunction of the model mice. The Morris water maze test is not suitable for evaluating spatial learning ability following SD because the long-term utilization of the flower-pot method increases the familiarity of mice with the water environment. Moreover, neuroinflammation, microglial activation and neuronal apoptosis were observed in the hippocampus of model mice even recovery for 3 weeks later. This animal model and corresponding behavioral evaluation method will help to explore the pathogenesis and therapeutic strategies of chronic sleep disorders.

1. Introduction

Sleep is a necessary physiological activity that helps to renew our strength, improve memory restoration, and facilitate waste clearance from brain [1,2]. Multiple factors, such as working stress, environmental change, various anxieties, and physical diseases, can cause sleep deficiency or loss [3,4]. Furthermore, in the modern society, lack of sleep is often long-term and repeated episodes, which not only leads to decline of emotion, learning, memory and immunity, but also is serious enough to reduce the life-span [5,6]. Therefore, it is of great significance to explore the pathophysiological mechanism of chronic sleep restriction and find effective interventions. To achieve this goal, a stable experimental model and evaluation system for chronic sleep deprivation (SD) is essential.

The rodent models of SD are the most commonly used to study sleep disorder [7,8]. Mice or rats after SD show cognitive functional decline and anxiety-like behaviors similar to humans [9–12]. However, most studies utilize one-time SD models, and there is little literature addressing detrimental effects of persistent and intermittent SD on animal memory and mental function [13,14]. Repeated behavioral tests may increase animal behavioral performance due

* Corresponding authors.
E-mail addresses: mingx@njmu.edu.cn (M. Xiao), wuting80000@gmail.com (T. Wu).

http://dx.doi.org/10.1016/j.bbr.2016.12.034
0166-4328/

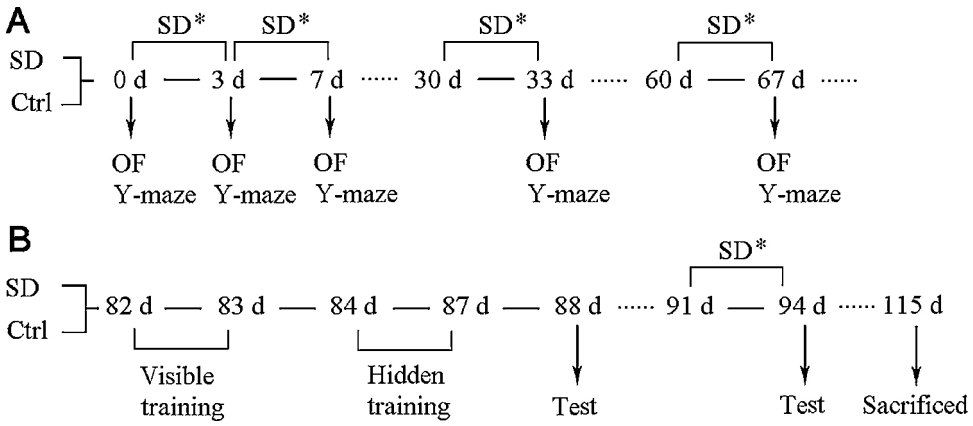

Fig. 1. Experimental procedures. (A) The procedures of repeated and intermittent paradoxical sleep deprivation (SD), and repeated open-field (OF) and Y-maze tests. (B) The procedures of the repeated Morris water maze testing.

to be familiar with of test environment and course, thereby interfering with, or even conceal, cognitive dysfunction caused by repeated SD itself. Therefore, it is necessary to determine the feasibility and effectiveness of repeated behavioral tests after repetitive and intermittent SD.

In the present study, in order to mimic chronic human sleep disturbance, we treated mice with 3 days or 7 days of paradoxical SD, repetitively and intermittently, for 3 months. The open field, Y-maze, and Morris water maze (MWM) test, three classic rodent behavioral tests, were utilized to analyze the behavioral performance of SD mice. The feasibility and availability of these repeated behavioral tests has been systemically evaluated. The long-term detrimental effects of repetitive and intermittent paradoxical SD on neuronal apoptosis and neuroinflammation in the hippocampus were also investigated.

2. Methods

2.1. Animals and experimental design

Ten-month-old male CD1 mice were used in the experiments. All animals were kept in a room of controlled illumination (12:12 h light/dark cycle), humidity (30–50%), and temperature (18–22 °C). All experiments were conducted in accordance with the National Institutes of Health guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978).

The open field and Y-maze tests were conducted 1 h prior to paradoxical SD to obtain the baseline data, and repeatedly performed 3 days, and 7 days after SD, respectively. One month later, these behavioral tests were performed again after another 3-day period of SD. Then, following another month interval, the mice were given 7 days of SD, followed by the above behavioral tests (Fig. 1A). On the 82th day, the MWM training was carried out. Swimming training was conducted for 6 consecutive days, 2 days with a visible platform and 4 days with a hidden platform. A probe test was conducted on the 88th day with the hidden platform removed. Following 48 h of rest, the mice received another 3 days of SD, followed by the probe test again on the 94th day (Fig. 1B). Some intact mice served as control group that was given the same procedure of behavioral tests mentioned above.

2.2. Paradoxical SD

The modified multiple platform method (MMPM) was utilized to establish a paradoxical SD model, as previously described [15]. Briefly, nine small platforms (8.5 cm in height and 2.5 cm in diameter) were placed (8–10 cm apart) inside a water tank made of sheet iron. The tank was filled with 24 °C water that reached 1–2 cm below the platform surface. The platforms were small, which permitted the mice to sit, but not lie down, on the platform. Furthermore, mice in the same cage could easily move between the platforms, but could not stretch across any two platforms to sleep. Thus, the animals were awoken when they experienced paradoxical sleep-induced atonia by touching the water. The water in the tank was changed daily. All mice had free access to food and water and were weighed daily. The control mice were placed on large platforms (8.5 cm in height and 20 cm in diameter). Immediately after each SD period, mice returned to their home cages allowing 3 h of *ad libitum* sleep opportunity before receiving the open field test and Y-maze test in sequence.

2.3. Open field test

The open field test was performed to evaluate anxiety-like behaviors [16]. The field consisted of a square black Plexiglas box (60 cm × 60 cm × 25 cm), with an outlined center area (30 cm × 30 cm). Each animal was placed in the middle of box, which served as a starting point, then allowed to move freely for 5 min within the box. The percentages of time spent and distance traveled in the center area and total distance traveled were measured. At the conclusion of the experiment, defecation number was also counted.

2.4. Y-maze test

The Y-maze test was conducted to evaluate mouse short-term spatial working memory as described previously [17]. The Y-maze included 3 arms: novel arm (NA), starting arm (SA), and other arm (OA). The test contains two stages: training stage and testing stage. During the first stage, the NA was blocked by a black baffle, allowing the mice to move freely only between the SA and OA for 8 min. During the second stage, the NA was opened and mice could freely move throughout all 3 arms for 5 min. The percentages of time spent and distance traveled in the NA, total distance traveled during the test and the percentages of entering number into the NA were calculated.

2.5. MWM test

The MWM task was conducted to measure mouse long-term learning and memory function as described previously [18]. Briefly, a black plastic pool with a diameter of 100 cm, and a height of 50 cm, was filled with water (22 ± 2 °C). From day 82, training was conducted for 6 consecutive days, with 4 trials per day. Mice were