Progress in Neuro-Psychopharmacology & Biological Psychiatry 38 (2012) 216–222

Contents lists available at SciVerse ScienceDirect

Progress in Neuro-Psychopharmacology & Biological Psychiatry

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

Sleep deprivation impairs emotional memory retrieval in mice: Influence of sex

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ARTICLE INFO

Article history:
Received 12 March 2012
Received in revised form 28 March 2012
Accepted 29 March 2012
Available online 13 April 2012

Keywords:
Conditioning fear context
Gender
Memory retrieval
Passive avoidance task
Plus-maze discriminative avoidance task
Sleep deprivation

ABSTRACT

The deleterious effects of paradoxical sleep deprivation on memory processes are well documented. However, non-selective sleep deprivation occurs more commonly in modern society and thus represents a better translational model. We have recently reported that acute total sleep deprivation (TSD) for 6 h immediately before testing impaired performance of male mice in the plus-maze discriminative avoidance task (PM-DAT) and in the passive avoidance task (PAT). In order to extend these findings to females, we examined the effect of (pre-test) TSD on the retrieval of different memory tasks in both male and female mice. Animals were tested using 3 distinct memory models: 1) conditioning fear context (CFC), 2) PAT and 3) PM-DAT. In all experiments, animals were totally sleep-deprived by the gentle interference method for 6 h immediately before being tested. In the CFC task and the PAT, TSD induced memory impairment regardless of sex. In PM-DAT, the memory impairing effects of TSD were greater in females. Collectively, our results confirm the impairing effect of TSD on emotional memory retrieval and demonstrate that it can be higher in female mice depending on the memory task evaluated.

1. Introduction

Several studies have demonstrated the close relationship between sleep and memory in both humans and in animals (Fu et al., 2007; Lim and Dinges, 2010; Palchykova et al., 2006; Patti et al., 2010; Sterpenich et al., 2007; Stickgold, 2005; Stickgold and Walker, 2007). Memory is typically defined as the ability to retain and manipulate previously acquired information by means of neuronal plasticity (de Oliveira Álvares et al., 2010; Thompson et al., 2002). It has been well-established that sleep plays a critical role in learning and memory formation. For instance, many studies have reported that paradoxical sleep deprivation in animals leads to memory deficits in several behavioral models such as avoidance (Bueno et al., 1994; Harris et al., 1982; Skinner et al., 1976), the Morris water maze (Youngblood et al., 1999, 1997), the radial maze (Smith et al., 1998) and the plus-maze discriminative avoidance tasks (PM-DAT) (Alvarenga et al., 2008; Patti et al., 2006, 2010; Silva et al., 2004a, 2004b).

Despite the well-documented consequences of paradoxical sleep deprivation in animal models, the effects of total sleep deprivation (TSD) on learning and memory as well as anxiety and locomotor activity in animal models have been overlooked. In this sense, TSD is a non-selective sleep deprivation and occurs more commonly in modern society, thus representing a better translational model. Specifically, according to Fenzl et al. (2007), one has to differentiate between TSD, when the animal is prevented to from falling asleep at all, and selective sleep deprivation, where only particular sleep stages are eliminated. The later achievement is frequently used to deprive paradoxical sleep in rodents, usually by placing the animals in small platforms surrounded by water (Alvarenga et al., 2008; Patti et al., 2010; Silva et al., 2004a, 2004b). On the other hand, TSD (paradoxical and slow-wave sleep) is less complex, simply achieved by removing or introducing objects within the cages or by gently handling animals, keeping them awake.

Importantly, quite recently we have demonstrated that memory deficits induced by 72 h of acute paradoxical sleep deprivation, but not those induced by 6 h of TSD, are related to state-dependency phenomenon (Patti et al., 2010). In this study, we have also reported that TSD-induced memory deficits had a greater magnitude than those induced by the paradoxical sleep deprivation in the PM-DAT model. In addition, mice subjected to TSD immediately before training and/or testing in the PM-DAT presented memory impairments without alterations in anxiety or locomotor activity. However, this study only examined male mice (Patti et al., 2010).

Sex differences in behavior have been extensively described in the literature. With respect to learning and memory, studies usually show better spatial learning in males (Astur et al., 2004; Blokland et al.,

Abbreviations: TSD, total sleep deprivation for 6 h; PM-DAT, plus-maze discriminative avoidance task; PAT, passive avoidance task; CFC, context fear conditioning; M, male mice; F, female mice; CTRL, control (home cage) condition.

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0278-5846
doi:10.1016/j.pnpbp.2012.03.014

2006; Jonasson, 2005; Rilea et al., 2004) and stronger emotional memory in females (Canli et al., 2002; Hamann, 2005). Beyond these behavioral differences between the sexes, sex may also play a remarkable role in sleep. Indeed, many studies indicate that sleep is differentially regulated between sexes both in humans (Paul et al., 2008) and in animals (Fang and Fishbein, 1996; Yamaoka, 1980), suggesting that distinct effects may modulate their sleep patterns and thus, different behaviors could be expected (Antunes et al., 2006). Indeed, sex influences sleep–wake amount, the daily timing of the sleep–wake cycle, and the ability to restore sleep after extended wakefulness. Several lines of evidence suggest that in mammals, reproductive hormones are responsible for the effects of sex on sleep and may have organizational and activational influences on sleep regulatory mechanisms (Paul et al., 2008)

The goal of the present study was to evaluate the sex differences in mice subjected to TSD using two classic memory models (the passive avoidance task — PAT, and context fear conditioning — CFC) as well as the PM-DAT, which is used to evaluate learning, memory, anxiety, and motor activity, and their respective interactions.

2. Material and methods

2.1. Subjects

Seventy-four three-month-old male and seventy-five 3-month-old female Swiss mice (raised at CEDEME, Universidade Federal de São Paulo) were used in the experiments. Animals weighing 30–35 g were housed under controlled temperature (22–23 °C) and lighting (12 h light, 12 h dark; lights on at 6:45 a.m.) conditions. Food and water were available ad libitum throughout the entire study.

All experimental procedures were approved by the Ethics Committee under protocol (#1203/2009). The mice were allowed at least 2 weeks of adaptation to the housing facilities before the start of the experiments.

2.2. TSD

Mice were subjected to TSD through the gentle handling method, which consists of keeping the animal awake by tapping on or moving the cage and, if necessary, by gently touching them with a soft brush, whenever behavioral signs of sleep, such as closed eyes or sleep posture, were (see Patti et al., 2010). The animals were sleep-deprived for 6 h immediately before testing in the memory paradigms.

Although this method may allow micro-sleeps, the use of the gentle handling method is widely accepted in sleep research and other related fields (Fenzl et al., 2007). In fact, this procedure suppresses 97.8% of paradoxical and slow-wave sleeps across 6 h (Fenzl et al., 2007) and induced memory impairment in previous studies (Palchykova et al., 2006; Patti et al., 2010).

2.3. Animal models for memory evaluation

2.3.1. Contextual fear conditioning (CFC)

On the first day (training session), mice were individually placed in a dark chamber with a grid floor (22 × 22 × 22 cm). After 150 s, 0.4 mA foot shocks lasting 1 s were applied every 30 s for the subsequent 150 s. Thirty seconds after the last foot shock, the animal was removed from the apparatus.

The CFC test was performed 24 h after the training. Each animal was placed in the same dark chamber, without receiving foot shocks. The freezing duration (defined as complete immobility of the animal, with the absence of vibrissae movements and sniffing) was quantified by observers blind to experimental conditions during a 5 min period.

2.3.2. Passive avoidance task (PAT)

PAT was conducted using methods described by Denti and Epstein (1972). A 2-way shuttle box with a guillotine door placed between the 2 modular testing chambers served as the testing apparatus. One chamber was illuminated by a 40-W light, while the other remained dark. During the conditioning session, the animals were individually placed in the illuminated chamber facing away from the guillotine door. When the mouse entered the dark chamber, the door was quietly lowered and a 0.4 mA foot shock was applied for 1 s through the grid floor. A test session was performed 10 days after conditioning using the same procedures, expect for the foot shock. In both sessions (training and testing), the latency to enter the dark chamber was recorded, with a cut-off duration of 300 s.

2.3.3. Plus-maze discriminative avoidance task (PM-DAT)

A modified elevated plus-maze was used to conduct PM-DAT. The maze was made of wood and consisted of two enclosed arms, with sidewalls and no top (28.5 × 7 × 18.5 cm), and 2 open arms with no sidewalls (28.5 × 7 cm), on opposite sides. A non-illuminated 100-W lamp was placed over the exact center of one of the enclosed arms (aversive enclosed arm). In the training session, each mouse was placed at the center of the apparatus, and, during a 10-min period, every time the animal entered the enclosed arm containing the lamp, an aversive stimulus consisting of the illumination of the 100-W light and a cold wind produced by a hair dryer was administered. The aversive stimulus continued until the animal left the arm. A test session was performed in the same room 10 days after the training session. During the test session, mice were again placed in the center of the apparatus and were observed for 3 min; however, the mice did not receive the aversive stimuli when they entered the aversive enclosed arm (although the non-illuminated lamp and the hair dryer were still placed in the middle of this arm to help distinguish between the aversive and non-aversive arms).

Total number of entries, defined as the entry of all 4 paws into the arm, in any of the arms, percent time spent in the aversive enclosed arm (time spent in aversive enclosed arm/time spent in both enclosed arms × 100), and percent time spent in open arms (time spent in open arms/time spent in both open and enclosed arms × 100) were calculated. Learning was evaluated by the decrease in percent time spent in the aversive enclosed arm throughout the training session. Memory was evaluated by the time spent in the aversive vs. non-aversive enclosed arms in the test session. Percent time spent in the aversive enclosed arm among the groups that showed retention of the task in the test session was compared in order to reveal quantitative differences. Anxiety-like behavior was evaluated by the percent time spent in the open arms of the apparatus. Total number of entries in any of the arms was used to evaluate motor activity. All the measures taken during the PM-DAT were obtained manually by observers blind to experimental conditions.

2.4. Experimental design

2.4.1. Experiments I, II and III: effects of TSD on memory retrieval in mice: influence of sex

Male (M) or female (F) mice were randomly assigned to one of the following groups: control (CTRL) or total sleep deprivation (TSD) for 6 h. Animals were trained in the CFC task (Experiment I), in the PAT (Experiment II) or in the PM-DAT (Experiment III). Twenty-four h (Experiment I) or 10 days (Experiments II and III) after training, mice were kept in their home cages (CTRL condition) or were deprived of sleep for 6 h (TSD condition). Immediately after the TSD or home cage time (CTRL), the animals were tested in order to evaluate memory retrieval.