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Research report

Modafinil ameliorates cognitive deficits induced by maternal separation and sleep deprivation

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HIGHLIGHTS

- Maternal separation induces memory impairment in adult rats.
- Sleep deprivation impairs recognition memory.
- Modafinil ameliorates deficits induced by maternal separation and sleep deprivation.

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ABSTRACT

Animals exposed to an early adverse event may be more susceptible to a second source of stress later in life, and these stressors may have additive deleterious effects. Sleep deprivation is known to be a stressor, affecting multiple body functions such as the cognition. Modafinil enhances working memory and attention in healthy non-sleep deprived subjects and in animal models of sleep deprivation. The first aim of the present study was to investigate the effects of maternal separation (MS) combined with paradoxical sleep deprivation (PSD) in adulthood on recognition memory in rats. Second, we aimed to evaluate whether the administration of modafinil would be able to ameliorate memory deficits induced by MS and PSD. Wistar rat pups were initially distributed into MS and handling (H) groups, with their litters standardized in 4 females and 4 males. In adulthood, the male rats were submitted to PSD or control condition, being redistributed afterwards in modafinil- or vehicle-treatment immediately after the training session of object recognition task. PSD did not potentiate the cognitive deficit due to MS. However, modafinil was able to recover memory impairments associated to PSD and also to MS in the neonatal period. This study demonstrates for the first time that modafinil ameliorates cognitive deficits associated to MS and to PSD in adulthood, independent from MS in the neonatal period.

1. Introduction

Stressful events early in life can have lasting effects on living organisms. Adverse early life experiences might be implicated in an increased susceptibility to psychiatric diseases, such as depressive disorders and schizophrenia [1–3]. In addition, obesity, smoking, drug abuse, and increased use of psychotropic medication in adults have also been associated to adverse childhood experiences [4,5].

Neonatal maternal separation (MS), a widely used model of early life stress in rodents is known to cause permanent cytochemical, morphological and electrophysiological changes in the central nervous system (CNS) [6]. A study conducted by Feng and coworkers [7] has shown that adult rats submitted to MS present disturbed sleep with features of insomnia, including decreased total sleep and increased total wake time during the light period. Additionally, some studies indicate that MS during the critical period of brain development may lead to impairments of cytoarchitecture in various brain regions, such as hippocampus and cortex which are known to be involved in learning and memory [8–10].

We and others have already demonstrated that MS causes persistent memory deficits in rodents [11–14]. In addition, our group also showed that exposure to a second adverse event, such as

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chronic exposure to the psychostimulant D-amphetamine, potentiated the effects of MS on cognition in adult rats [14,15]. According to the "two-hit" hypothesis, animals exposed to an early adverse event may be more susceptible to a second source of stress later in life, and these stressors may have synergistic deleterious effects [16].

Evidence indicated that the effects of acute sleep deprivation (SD) include a temporary activation of the major neuroendocrine stress systems, i.e., the autonomic sympathetic system and the hypothalamic-pituitary-adrenal (HPA) axis [17,18]. Strong correlations between paradoxical sleep deprivation (PSD) and the loss of brain functions in animals and humans have been observed [19–22]. Consistently, studies have shown that SD significantly impairs learning and memory [23–26].

Currently, SD is very frequent among young people [27], who make use of stimulants to prolong wakefulness and to enhance cognitive, emotional and motivational functions [28]. Modafinil [(diphenyl-methyl) sulphinil-2-acetamide] is a medication that has been prescribed to improve excessive daytime sleepiness, alertness, attention, and memory in dementia [29,30]. Although its exact mechanism of action is not completely elucidated, modafinil has been shown to enhance dopaminergic and noradrenergic neurotransmission, increase glutamate release in the thalamus, hypothalamus, hippocampus and striatum, and to dose-dependently decrease GABA levels (for a review see [31]). Moreover, modafinil was shown to enhance spatial learning, working memory and attention in healthy non-sleep deprived animals [30,32] and humans [33,34].

Thus, the present study investigated the effects of MS-induced stress combined with PSD, as a second hit in the adulthood, on recognition memory in rats. We also aimed to evaluate whether the administration of modafinil would be able to recover memory deficits caused by MS and PSD.

2. Materials and methods

2.1. Animals

Pregnant Wistar rats were obtained from Centro de Desenvolvimento de Modelos Experimentais para Medicina e Biologia (CEDEME) – Universidade Federal de São Paulo (UNIFESP), Brazil. After birth, each litter was adjusted within 48 h to contain 8 rat pups with the same proportion of male and female individuals. Pups were maintained together with their respective mother in individually ventilated cages with corn-cob bedding in a room at temperature of 22 ± 1 °C and a 12-h light/dark cycle. At the age of 4 weeks, the pups were weaned and the males were selected and raised in groups of 3 rats. The animals were supplied with standardized pellet food and tap water *ad libitum*. All behavioral experiments took place between 9:00 h and 16:00 h. Rats used in this study were maintained and treated in accordance with the guidelines established by the Ethical and Practical Principles of the Use of Laboratory Animals [35]. Since these experiments may result in a certain amount of stress-related behavior, the number of animals was kept at a minimum but sufficient to allow for statistical significances.

2.2. Maternal separation (MS)

MS was performed based on previous reports [14,15,36]. Rat pups were exposed to one of the following maternal rearing conditions from post-natal days 1–14 inclusive: (1) handling (H), animals were exposed to a daily 15-min period in which the dam was removed and the litter was weighed, (2) maternal separation (MS), animals were exposed to a daily 180-min period in which the dam was removed and the litter was weighed. During the separation period, rat pups of each litter were maintained together in a plastic cage with standard bedding material in an adjacent room to their dams on an incubator at the temperature of 35 °C to avoid hypothermia. After the separation period, pups were returned to the nest and rolled in home cage bedding material, and the dam was returned. In rats, the mother is routinely off the litter for periods of 20–25 min [37]. Thus, only the group exposed to an 180 min period of MS, but not the group exposed to a 15-min period of separation (handling), were exposed to a deprivation of maternal care.

2.3. Paradoxical sleep deprivation (PSD) procedure

The experimental groups were submitted to PSD for 24 (PSD-24 h), 48 (PSD-48 h) or 72 (PSD-72 h) h using the modified multiple platform method [38]. We used these periods because it has been reported that PSD impairs the acquisition of a variety of behavioral tasks. Modified multiple platform methods consist in placing the rats inside a tiled water tank (123 cm × 44 cm × 44 cm), containing 14 circular platforms, 6.5 cm in diameter, with water up to 1 cm of their upper surface. The rats could thus move around inside the tank by jumping from one platform to another. When they reached the paradoxical phase of sleep, muscle atonia set in and they fell into the water and woke. Throughout the study, the experimental room was maintained under controlled temperature (23 ± 1 °C) and a light–dark cycle (lights on at 07:00 h and off at 19:00 h). Food and water were provided *ad libitum* for all groups by placing chow pellets and water bottles on a grid located on top of the tank. Rats kept in their home cages were placed in the same room as the platform apparatus and were used as control in all the experiments. PSD method has proven to be effective in suppressing paradoxical sleep. Indeed, we have demonstrated that, in the same experimental conditions described here in rodents, the multiple platform technique resulted in significant decreases in slow wave sleep and completely abolished paradoxical sleep during the SD period.

2.4. Acute treatment with modafinil

For the experiments investigating the effects of modafinil on memory deficits induced by MS and PSD in adult animals (4 months), rats were trained and tested in the object recognition task. Vehicle (Tween 80-saline solution 1:16, v/v) or modafinil (Stavigile®, Libbs, USA) at the dose of 75 mg/kg (n = 9–13 per group) was administered intraperitoneally immediately after the training session of the object recognition task. This dose was selected based on pilot experiments performed in our laboratory and previous published studies [30].

2.5. Object recognition task

The object recognition task was performed as previously described [14,15]. Briefly, the object recognition task took place in a Plexiglas cylinder (45 cm diameter) arena with corn-cob covering its floor. On the first day, rats underwent a habituation session during which they were placed in the empty open field for 5 min. On the following day, rats were given a 5-min training trial in which they were exposed to two identical objects (A1 and A2). On the long-term memory (LTM) testing trial, performed 24 h after the training session, rats were allowed to explore the open field for 5 min in the presence of two objects: the familiar object (A) and a novel object (B). These were placed in the same locations as in the training session. In long-term retention test trial, the novel object was placed in 50% trials on the right side and 50% trials on the left side of the open field. All objects were made of plastic Duplo Lego Toys and had a height of about 10 cm. Objects presented similar textures, colors, and sizes, but distinctive shapes. Between trials the objects were washed with 10% ethanol solution. Object exploration was measured by an experimenter blind to group treatment assignments; using two stopwatches to record the time spent exploring the objects during the experimental sessions. Exploration was defined as follows: sniffing or touching the object with the nose. Sitting on the object was not considered as exploration. A recognition index calculated for each animal was expressed by the ratio TN/(TF + TN) [TF = time spent exploring the familiar object (A); TN = time spent exploring the novel object (B)].

2.6. Experimental design

2.6.1. Effect of PSD on recognition memory in three different time intervals between training and testing

Adult naïve Wistar rats not subjected to MS were trained in the object recognition task. After the training session, animals were submitted to PSD for 24 h (PSD-24 h group), 48 h (PSD-48 h group), or 72 h (PSD-72 group). For control groups, right after training, animals were returned to their home cages, and kept there for 24, 48 or 72 h (CTRL-24 h, CTRL-48 h, and CTRL-72 h groups). Immediately after the end of the PSD period, all animals, including the control groups were submitted to the test session in the object recognition task.

2.6.2. Effects of MS, PSD and acute modafinil on object recognition memory retention

A separate set of animals, submitted to H or MS during the neonatal period, as described above, when adults, were trained in the object recognition task and subsequently distributed into the following groups: animals that received intraperitoneal (i.p.) injections of modafinil (M) 75 mg/kg, or vehicle (V) immediately after the training session. Those animals were further redistributed and either submitted to PSD for 24 h or returned to their home cages (control group, not submitted to paradoxical sleep deprivation, NPSD). After 24 h of PSD or NPSD, the animals were tested in the object recognition task. Experimental design for this experiment is shown in Fig. 1.

2.7. Statistical analysis

In the experiment designed to evaluate the effects of PSD on object recognition memory retention, data was analyzed by one-way analysis of variance (ANOVA), followed by Tukey's post hoc tests.

The effects of early MS and subsequent administration of modafinil and PSD were analyzed using 3-way ANOVA. The model includes three fixed factors, each