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Research report

Effects of exercise on depressive behavior and striatal levels of norepinephrine, serotonin and their metabolites in sleep-deprived mice

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ABSTRACT

Exercise is a promising adjunctive therapy for depressive behavior, sleep/wake abnormalities, cognition and motor dysfunction. Conversely, sleep deprivation impairs mood, cognition and functional performance. The objective of this study is to evaluate the effects of exercise on anxiety and depressive behavior and striatal levels of norepinephrine (NE), serotonin and its metabolites in mice submitted to 6 h of total sleep deprivation (6h-TSD) and 72 h of Rapid Eye Movement (REM) sleep deprivation (72h-REMSD). Experimental groups were: (1) mice submitted to 6h-TSD by gentle handling; (2) mice submitted to 72h-REMSD by the flower pot method; (3) exercise (treadmill for 8 weeks); (4) exercise followed by 6h-TSD; (5) exercise followed by 72h-REMSD; (6) control (home cage). Behavioral tests included the Elevated Plus Maze and tail-suspension. NE, serotonin and its metabolites were determined in the striatum using high-performance liquid chromatography (HPLC). Sleep deprivation increased depressive behavior (time of immobilization in the tail-suspension test) and previous exercise hindered it. Sleep deprivation increased striatal NE and previous exercise reduced it. Exercise only was associated with higher levels of serotonin. Furthermore, exercise reduced serotonin turnover associated with sleep deprivation. In brief, previous exercise prevented depressive behavior and reduced striatal high NE levels and serotonin turnover. The present findings confirm the effects of exercise on behavior and neurochemical alterations associated with sleep deprivation. These findings provide new avenues for understanding the mechanisms of exercise.

1. Introduction

Sleep disorders are common and potentially inflict negative effects on conditions of health and disease [1,2]. Sleep is vital for the maintenance of interconnections and neurotransmitters that are essential for emotion and memory [3]. Previously, Xie et al. demonstrated that sleep deprivation impairs the removal of neurotoxic waste products affecting functional performance [3,4]. In view of this, it seems right to assume that sleep is essential for the brain. Experimental procedures of sleep deprivation offer the opportunity to evaluate cerebral changes and behavior in controlled conditions [5].

The neural sequence generating sleep and wake is a complex chain influenced by physiological factors and pathological conditions. Mainly, the sleep/wake cycle regulatory system includes an ascending network extending from the medulla to the forebrain and involving thalamus, basal forebrain, posterior hypothalamus, and brainstem monoaminergic nuclei e.g. noradrenergic neurons of the *locus coeruleus* (LC) and serotonergic neurons of the dorsal raphe [6]. Dopamine neurons of the substantia nigra and ventral tegmental area, glutamate, hypocretin and the histaminergic tuberomammillary nucleus are all involved innervating through the forebrain the cerebral cortex [7]. The striatum, vital for motor control and cognition, has extensive functional interactions with multiple brain structures e.g. the hippocampus, thalamus and the prefrontal cortex. Interactions between hippocampus, striatum and the cerebral cortex generate sleep-related memory consolidation processes [8]. It is proposed that sleep can reorganize the activity within, as well as the functional interactions between these structures [9]. In fact, several studies describe the modulating effects of sleep deprivation on monoaminergic network [10,11]. For instance, sleep is related to the amygdalo-striatal system that is persistent

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throughout evolution and constitutes an essential part of the primordial emotional brain [3,12].

Animal and human experiments have provided evidence for the role of striatum in sleep [13]. In the rotenone experimental model of Parkinson's disease, Rapid Eye Movement (REM) sleep deprivation compromises memory similarly to a nigrostriatal lesion [14]. Durrant et al., testing auditory memory and using polysomnography and magnetic resonance in healthy volunteers, showed that weaker parahippocampal responses and stronger striatal responses occurred after sleep and this was predicted by the amount of slow wave sleep [8]. The latest findings provide further evidence consolidating the relationship of the striatum with sleep [15].

Physical exercise positively modifies mood symptoms and mobility [16,17]. Moreover, exercise improves sleep quality [18], memory [19] and influences anti-inflammatory activity [20]. Importantly, a study evaluating the effects of exercise in Parkinson's disease showed that physical activity improved mobility and general signs of the illness [21]. Sleep disturbances are common in neurodegenerative conditions, e.g. Parkinson's disease, influencing motor performance, cognition, mood and therapeutic response [22]. Given that sleep alterations are common in Parkinson's disease and exercise improves disease related symptoms, it is important to investigate, in conditions of sleep deprivation, how exercise affects behavior and the striatum.

The objective of this study is to evaluate the effects of a treadmill exercise protocol on behavior and neurochemical alterations in the striatum in mice submitted to two protocols of sleep deprivation: 6 h of total sleep deprivation (6h-TSD) and 72 h of REM sleep deprivation (72h-REMSD).

2. Material and methods

2.1. Animals

The experimental protocol involved 60 male adult Swiss mice, weighing 25–30 g housed in standard conditions (light-dark cycle: 12 h/12 h, temperature: 23 ± 1 °C, humidity: 50 ± 10%) with food and water *ad libitum*. The study followed the ethical principles of animal experimentation established by the Brazilian College of Animal Experimentation (COBEA) and was previously approved by the Ethics Committee in Animal Research (CEPA-67-09).

Animals were grouped (4–5 per cage) Experiment was in accordance with the guidelines established by the Ethical and Practical Principles for the Use of Laboratory Animals [23].

2.2. Experimental procedures

Fig. 1 shows an overview of the experiments. Six groups of animals (N = 10 each) were distributed, as described. Group 1 was control kept in their home cages. Group 2 was submitted to 6h-TSD and group 3 to 72h-REMSD. Group 4 performed up to one-hour of treadmill exercise for 8 weeks. Group 5 included mice that previously exercised (1-h treadmill exercise for 8 weeks) and were right away submitted to 6h-TSD; group 6 included mice that previously exercised (1-h treadmill exercise for 8 weeks) and were immediately REM sleep deprived for 72 h (72h-REMSD).

Time to initiation of sleep deprivation was around Zeitberg Time (ZT) 6 and exercise was also conducted at daytime, under conditions of darkness, from 11:00 AM to 01:00 PM (ZT 6–7). Behavioral tests, firstly, the Elevated Plus Maze (5 min) and secondly, Tail Suspension (6 min), were performed, immediately at the end of exercise or sleep deprivation period. Behavioral tests were scored manually by a blinded examiner.

Animals were decapitated immediately after behavioral tests (11 min) and the striatum (dorsal and ventral) was dissected and frozen for analysis.

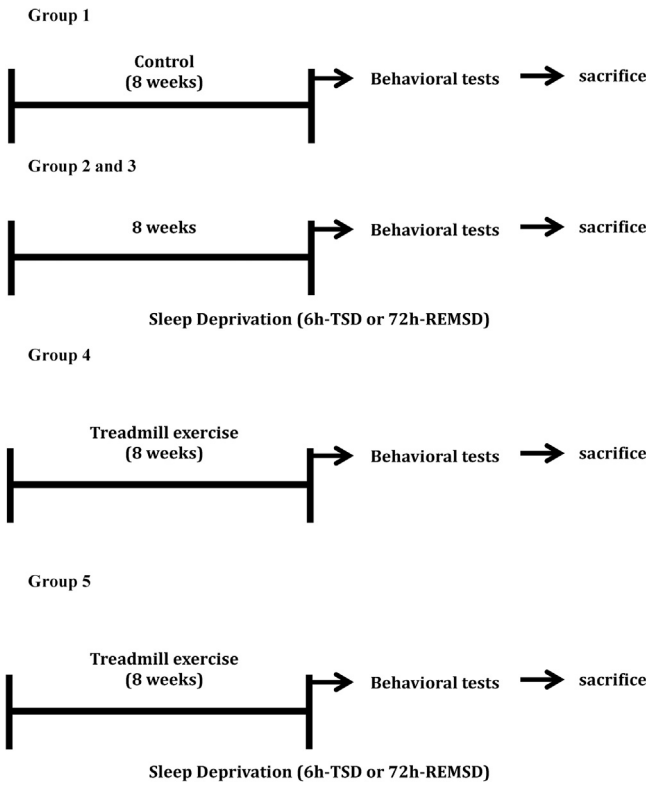

Fig. 1. Overview of experiments. Group 1 was control kept in their home cages. Groups 2 and 3 were subjected to sleep deprivation (6h-TSD or 72h-REMSD). Group 4 performed up to one-hour of treadmill exercise only. Groups 5 and 6 were previously exercised and then subjected to sleep deprivation (6h-TSD or 72h-REMSD). Time to initiation of sleep deprivation was Zeitberg Time (ZT) 6 and exercise was conducted at daytime from 11:00 AM to 01:00 PM (ZT 6–7).

2.3. Sleep deprivation

2.3.1. Total sleep deprivation (6h-TSD)

Mice were sleep deprived during 6 h of the light phase (ZT6 to ZT12) by gentle handling (cage tapping and delicate touching). Gentle handling method (6 h) is described by Fenzl et al. [24] and Franken et al. [25]. Food and water were available *ad libitum*. Mice were acclimatized for similar conditions and housed for at least 2 weeks prior to experimental use.

2.3.2. REM sleep deprivation (72h-REMSD)

Sleep deprivation (72h-REMSD) was initiated at ZT6 using the multiple platform method adapted for mice [26]. Groups of 5 mice were placed on platforms in tanks (32cm × 42cm × 18 cm) for 72 h. Each tank contained 14 platforms (3 cm in diameter) surrounded by water up to one cm beneath the surface of the platforms. Food and water were available through a grid placed on top of the water tank through all experiments. In this model, animals can move inside the tank by jumping from one platform to another and restriction of movement or social isolation are not imposed. The "flower pot" method is the best method to selectively deprive animals of rapid eye movement (REM) sleep for one or multiple days with only intermittent monitoring by the researcher [27]. When the characteristic muscle atonia occurs, the animal contact the water surrounding the platform and immediate awakening occur.

2.4. Exercise protocol

All groups were habituated on an eight-channel motor-drive treadmill (Model Insight®-Equipment, Research and Education-Co., Brazil). Treadmill exercise was also conducted during daytime, under condi-