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Cognitive and hippocampus biochemical changes following sleep deprivation in the adult male rat

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ABSTRACT

Sleep deprivation (SD) influences physiological processes such as cognitive function. The balance of oxidant and antioxidant markers, neurotrophic factors and magnesium are affected by sleep deprivation but there is no difference between pre and post training sleep deprivation. This study was designed to investigate memory retrieval and biochemical factors such as oxidant and antioxidant enzyme, brain-derived neurotrophic factor (BDNF) and magnesium levels in the hippocampus following pre and post-training sleep deprivation.

Male Wistar rats (weighing 200 ± 20 g) in below groups were used: control 1, 24, 48 and 72 h SD before training groups, control2, 24 h SD1 after training (being evaluated 24 h after training) and SD2 24 after training (being evaluated 48 h after training). Memory was evaluated 90 min, 24 h or 48 h after training by step-through passive avoidance apparatus. Multiple platforms method was used to induce SD. Oxidant and antioxidant markers including glutathione (GSH), glutathione reductase (GPx), malonedialdehyde (MDA), Total antioxidant concentration, catalase, superoxide dismutase (SOD), magnesium and BDNF were assessed in the hippocampus or/and brain.

72 h pre-training SD impaired short and long-term memory significantly. There was no significant difference in hippocampus oxidant and antioxidant markers compared to control. Hippocampal BDNF and magnesium did not show any changes in all SD groups.

Lack of correlation between memory impairment and levels of BDNF, magnesium and/or oxidant and antioxidant balance in the hippocampus is likely to be related to animal locomotor activity in the multiple platforms method. More research is needed to clarify the role of neurochemical systems.

1. Introduction

Sleep has an essential role in the normal functioning of the central nervous system. It has been indicated that a vast majority of adults do not get adequate sleep and sleep deprivation (SD) produces serious physiological results [1,2]. Experimental procedures of SD effects on molecular, cellular, physiological as well as cognitive levels are studied to reveal different and unknown aspects of sleep functions [3]. In some studies, the positive effects of SD in the control of diseases such as depression, Parkinson's is considered [4,5]. SD is known to causes learning and memory deficit [6,7] using behavioral animal models, such as active avoidance [8], Morris water maze [9], radial maze [10] and passive avoidance [11]. Some researchers believe the memory impairment caused by SD is due to a stop in-memory processing, and some scholars are of the opinion that it is caused by stress [12,13,1415].

SD can affect cognitive functioning by making neurobiological changes associated with stress as well as oxidative processes. This idea is based on the observed physiological disorders after SD. SD might be the responsible cause of the homeostatic disturbances observed in diverse conditions of prolonged wakefulness [16]. It seems that sleep has a variety of neurophysiological effects that if SD occurs, various neurophysiological mechanisms begin to produce significant changes. Many different theories have been proposed, one hypothesis is that sleep may trigger antioxidative mechanisms [17,18] and SD can promote oxidative stress [2,19] and decreases glutathione levels as an antioxidant marker in the brain region [20]. Singh and et al found that after paradoxical SD(PSD) the oxidative stress increase in the hippocampus in the rat [21]. Benedetta and Gould [22] revealed that the hippocampus regulates functions such as learning and memory, anxiety, and stress [22] and also is reported that the hippocampal oxidative stress in passive avoidance memory deficits induced by SD in mice has an important role [23]. And stress decreases passive avoidance memory and changes the oxidative stress parameters [24]. Sahaya et al.

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showed that oxidative stress induces cognitive dysfunction (passive avoidance) [25]. There is no information about the effect of the SD on the biochemical changes on hippocampus using an avoidance task.

Magnesium is essential for the proper functioning of nervous systems. In the brain, Mg^{2+} has a role in blocking the voltage-dependent *N*-methyl-D-aspartate (NMDA) receptors, and opens them during coincidence detection, and causes synaptic plasticity [26,27]. Magnesium is important in synapse changes control, increasing brain magnesium leads to the enhancement of learning and memory in rats and can compensate for memory impairment in aging [28,29]. And surprisingly some results have indicated that SD decreased intracellular magnesium concentration [30].

Brain-derived neurotrophic factor (BDNF) is a neurotrophin that enhances the growth and differentiation of neurons and synapses [31]. Torabi-Nami et al. [32] demonstrated that total sleep deprivation and chronic partial sleep restriction induce deduction of serum BDNF level [32] Protein and mRNA expression of BDNF significantly decreased after 72 h SD in ovariectomized rats [33]. Alzoubi et al. [35] showed that SD can decrease BDNF levels and impair memories [34,35].

Paradoxical sleep fosters the consolidation of emotional memories and is assumed to reduce the emotional tone of the memory [36], but is not clear what does different time and duration of it on memory? Here, we investigated the effect of selective PSD on the avoidance task because having an emotional component. According to these studies, our hypothesis is that changing in the timing of PSD can influence in a different way on memory and biochemical and molecular factors in the hippocampus.

Therefore, this study was designed to show the effect of changes in time and period of SD on memory, the oxidative and antioxidant markers, the level of magnesium, and BDNF in the hippocampus (as the memory adjustment center).

2. Materials and methods

2.1. Animals

Male Wistar rats (weighing 200 ± 20 g) were used in this study. Animals were caged in groups of 4–5 with access to food and water ad libitum. The rats were housed under a 12–12 h light–dark schedule (lights on 07:00–19:00 h) and standard conditions of temperature (23 ± 1 °C). Seven groups of rats were randomly selected and classified as shown in Table 1. Each group for behavioral section contained 6–7 rats and for biochemical section 4 to 6 samples.

2.2. Study design and grouping

In order to investigate the effect of time and period of sleep deprivation on memory and neuro-biochemical factors, 7 rat groups were randomly selected. The experimental protocol was designed based on a pilot study. As seen in Table 1, in control group 1, sleep deprivation was not applied but was measured short term (90 min after training), long term memory (24 h after training) and locomotor activity. After that animals were immediately sacrificed and the brain and hippocampus were removed to measure various neuro-biochemical factors (oxidative and antioxidant markers, magnesium, and BDNF) in the hippocampus (as the memory adjustment center).

In three experimental groups (2, 3 and 4), sleep deprivation was performed for 24, 48 and 72 h before training, and the short term (90 min after training) and long term memory (24 h after training), locomotor activity and the neurobiochemical factors were measured just like the control group 1.

In experimental group 5 (24 h post SD1), 24-h sleep deprivation was applied after training, and the short-term memory (90 min after training) assessment was discarded due to interruption of it with sleep deprivation period but long-term memory was evaluated 24 h after training. After that all of behavioral and neuro-biochemical evaluation was done just like the control group 1 so this group was compared with control 1.

In group 6 or control 2, sleep deprivation was not performed. Long-term memory and locomotor activity were evaluated 48 h after training. All stages of extraction of the brain and hippocampus and measurement of neuro-biochemical factors such as control group 1 was performed.

In experimental group 7 (24 h post SD2), sleep deprivation was applied for 24 h after training. 48 h after training (simultaneous with 24 h after sleep deprivation), memory and motor activity were evaluated, after which the brain and hippocampus were extracted and just like control 1 and 2, the neuro-biochemical factors were measured. This group was compared to control 2. Considering the interference of the memory test in sleep deprivation after training, the continuation of the experiment with 48 and 72 h deprivation periods was discarded.

All procedures were performed in compliance with the National Research Council's Guide for the Care and Use of Laboratory Animals and approved by the Ethics Committee of the Shahid Chamran University of Ahvaz, Iran (Code: EE/96.24.3.88375/scu.ac.ir).

2.3. Passive avoidance learning protocol

The task was performed in a two-compartment shuttle box, with a white compartment connected to a black one by a sliding door.

Table 1
Groups and experimental protocol.

No	Groups	SD before training	Training	SD after training	Short-term memory (90 min after training)	Long-term memory test (24 h after training)	Long-term memory test (48 h after training)	open-field test	Brain and hippocampus extraction time
1 N = 6	Control 1	–	+	–	+	+	–	+	After open-field test (24 h after training)
2 N = 7	24 h pre SD	24 h	+	_	+	+	–	+	After open-field test (24 h after training)
3 N = 6	48 h pre SD	48 h	+	_	+	+	–	+	After open-field test (24 h after training)
4 N = 6	72 h pre SD	72 h	+	_	+	+	–	+	After open-field test (24 h after training)
5 N = 6	24 h post SD1	–	+	24 h	– Due to Interference with SD	+	–	+	After open-field test (24 h after training)
6 N = 6	Control 2	–	+	–	–	–	+	+	After open-field test (48 h after training)
7 N = 6	24 h post SD2	–	+	24 h	– Due to Interference with SD	– Due to Interference with SD	+	+	After open-field test (48 h after training)