Sleep Deprivation and its Effects on Male Wistar Rats' Behaviour and Oxidative Stress

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KEYWORDS	ABSTRACT
Flower pot technique, Sleep deprivation, Open Field Maze, Lipid peroxide, Reduced glutathione	**Background:** Sleep is characterized by well-defined changes in the brain electrical activity. It is a fundamental phenomenon with no known molecular function. Insufficient sleep and circadian rhythm disruption are associated with wide range of health problems. But the mechanism involved remains largely unexplored. In this modern life style (24-h society) due to increased recourse to shift and night work, prolonged use of electronic media delays bed time, altering sleep duration and quality. It causes deficits in cognitive functions and synaptic plasticity which is essential for long term adaptive changes in behaviour and antioxidant levels. **Aim:** To study the effect of sleep deprivation on behaviour and its oxidative stress levels in rats. **Methodology:** This study was done in the department of Physiology, MMCH & RI, Enathur. 18 male Wistar rats were divided into 3 groups. Group I: home cage control, Group II: sleep deprivation (flower pot technique – small platform), Group III: sleep deprivation (Large platform). Group II, III were subjected to sleep deprivation for 96 hours. Behavioural assessment was done using open field maze test and antioxidant status was measured for all the groups. **Results & Conclusion:** Sleep deprivation for 96 hours showed behavioural alterations with increase in oxidative stress.

1. Introduction

The sleep-wake cycle requires multiple brain regions and neuronal populations. It has one of the great mysteries of science. It has been proposed that the sleep-wake cycle is regulated by a balance between a sleep and an arousal circuit.[1] Sleep play an integral function in major tasks like learning and memory. Sleep is involved in maintenance of behaviour, acquisition, retention and stabilization of information in memory formation.[2] This concept was postulated based on various experimental evidences showing increase in neuronal activity on prolonged wakefulness at cellular and molecular level.[3] Sleep deprivation is the condition of not having enough sleep. The most common complication of sleep deprivation is depressive illness. This relationship between sleep and depression is the most significant characteristics feature for most of the diseases. Depressive behaviour leads to tissue damage and increased inflammatory cytokines. This study aims to identify the cause of sleep deprived depressive changes and its possible causes.[4]

2. Methodology

The study was done in the Department of Physiology, Meenakshi Medical College Hospital & Research Institute, Enathur, Kanchipuram, Tamil Nadu, India with the Institutional Animal Ethical Clearance. 12 hours light/dark cycle was maintained with free access to food and water. 18 male Wistar albino rats weighing 150-180 grams were divided into three groups. It was categorized as Group I: Home cage control (n=6), Group II: Flower pot technique (small platform) (n=6), Group III: Flower pot technique (large platform) (n=6).

Flower Pot Technique

It is the standard method used to perform sleep deprivation which was 1st proposed by Jouvet et al. It consists of an outer chamber (22´22´35 cm) and an inverted inner chamber of 10 cm high, 6.5 cm

(small platform) and 13 cm (large platform) in diameter (base) which was submerged in water until 1-2 cm below its surface. The animal was placed on top of inverted platform for 96 hours with free access to food and water. 12 hours light and dark cycle was maintained with free access to food and water.[5]

Open Field Maze

Open field maze (OFB) test was widely used to measure general locomotor and explorative activity. The apparatus was made of wood covered with impermeable Formica and has a floor of 100 X 100 cm (divided by white line into 25 squares of 20 X 20 cm) and walls to the height of 40 cm. The 100 watts white bulb was placed 1 m above the apparatus [6]**(Coll Andres et al., 1989)**. Rats used in this study were placed in the centre of the open field maze and allowed to explore for three minutes.

Biochemical Analysis

Animals were anesthetized and dissection immediately after 90 hours of sleep deprivation and lever tissue was collected for the analysis of lipid peroxide (LPO) and reduce glutathione level (GSH).

3. Results

Table: 1: Comparison of behavioural assessment by open field maze test

OFM (Numbers)	CONTROL	SSP	SLP
Rearing	19.83 ± 2.34	49.33 ± 4.89 *	44.00 ± 2.77
Grooming	18.83 ± 3.28	28.5 ± 6.92 *	27.33 ± 2.62
Peripheral ambulation	41.33 ± 1.51	33.33 ± 3.44 *	38.17 ± 1.92
Central ambulation	7.67 ± 1.34	18.00 ± 3.70	21.83 ± 6.39
Immobilization time (sec)	8.83 ± 4.73	12.50 ± 1.9	10.67 ± 2.21

$p < 0.05$ is significant value (*)

In open field maze there was a significant increase in rearing and grooming with significant decrease in peripheral ambulation in single small platform group when compare with control and single large platform group.

Table: 2: Comparison of lipid peroxide (LPO) and reduced glutathione (GSH)

LIVER	CONTROL	SSP	SLP
LPO (µmoles/g of tissue)	2.37 ± 0.46	7.61 ± 0.94 *	7.10 ± 0.81
GSH (mg/g/ptn)	51.66 ± 1.25	23.72 ± 2.31 *	24.68 ± 1.67

$p < 0.05$ is significant value (*)

There was a significant increase in lipid peroxide level with significant decrease reduced glutathione level in single small platform group when compare with control and single large platform group.

4. Discussion

In the present study, rats subjected to sleep deprivation by single small platform showed a significant increase in rearing and grooming which could due to increased level of anxiety induced by sleep deprivation [7]

In open field maze there was a significant decreased in peripheral ambulation in single small platform when compare to control and single large platform this concurs with the findings of Olakunle et al., where significant decrease in ambulatory activity was absorbed in caffeine administered sleep deprived mice groups.[8]

In the present study there was significant increase in the lipid peroxide which was similar to the findings of **Antonio et al** where he explained that increase lipid peroxide was seen in stress due to its high polyunsaturated fatty acid content.[9]

In our study there was significant decrease reduced glutathione in single small platform group then the other groups which concurs with the finding of Janssens BJ et al**.** He states that reduce glutathione have greater participation in detoxifying the peroxide formed during stress condition thus decreases its content in brain and liver due to its metabolic activity.[10]

5. Conclusion

This study concludes that 96 hours of sleep deprivation produced significant changes in number of rearing, grooming and peripheral in open field maze test. In our study there was also a significant increase in the lipid peroxide level with concomitant decrease in reduced glutathione level. This concludes that sleep deprivation results in depressive changes and induces anxiety levels in rats which were confirmed by its increased exploratory behaviour. The present study also confirms that sleep deprivation increases the free radical accumulation in the body and it also decreased the antioxidant scavenging activity of reduced glutathione level in the body which produced oxidative stress.

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