

# Effect of REM sleep deprivation on the antioxidant status in the brain of Wistar rats

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## KEY WORDS

REM sleep  
Free radicals  
Hypothalamus  
Midbrain  
Hindbrain

## ABSTRACT

**Background:** Rapid eye movement [REM] sleep deprivation is a stressor. It results in a predictable syndrome of physiological changes in rats. It has been proposed that reactive oxygen species and the resulting oxidative stress may be responsible for some of the effects of sleep deprivation. **Purpose:** The present study was undertaken to investigate the reversible nature of the effects of 96 hours of REM sleep deprivation on lipid peroxidation and total reduced glutathione level in the hypothalamus, midbrain and hindbrain of Wistar strain rats. **Methods:** The rats were deprived of REM sleep using the inverted flowerpot technique. All the animals were maintained in standard animal house condition with 12-h light and 12-h dark cycles. At the end of the stipulated time Jugular venous blood sample of 2 ml was collected under mild ether anesthesia for the assay of stress index, plasma corticosterone. Lipid peroxidation using thiobarbituric acid, total reduced glutathione using DTNB (GSH) were assayed in the brain regions dissected out. **Results:** This study showed that 96 hours of REM sleep deprivation results in increased lipid peroxidation and reduction in total reduced glutathione level in the discrete regions of brain studied. However following restorative sleep for 24 hours all the changes reverts back to base line value. This study shows that oxidative stress produced by 96 hours of REM sleep deprivation is reversible. **Conclusion:** From this study it is clear that, REM sleep deprivation is a potent oxidative stressor. This could probably play a role in the behavioral and performance alteration seen in both experimental animals as well as humans following REM sleep deprivation. Further investigations in this line are needed to highlight the importance of REM sleep.

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doi : 10.5214/ans.0972.7531.190405

## Introduction

REM sleep is an integral part of sleep-wakefulness physiology, though its precise mechanism of generation, function and mechanism of action are unknown. Evidence suggests that it is an essential physiological phenomenon to the extent that prolonged REM sleep deprivation may be fatal. REM deprivation increases basal arousal and enhances drive-related behaviors like hyperphagia.<sup>1-3</sup> It also affects physiological, psychological process as well as neurotransmitter levels.<sup>4</sup> The mechanisms behind these changes are not fully understood. Reimund<sup>5</sup> hypothesized that free radicals or reactive oxygen species produced during wakefulness are removed during sleep, i.e., sleep has an antioxidative function. Maintenance of steady state concentration of free radicals is essential for adequate functioning of aerobic organism.<sup>6</sup> REM sleep deprivation alters membrane bound ATPases, membrane fluidity, calcium ion concentration and gene expression.<sup>7</sup> Alterations in these are potentially capable of inducing changes in cellular physiology including generation of free radicals. Hence, this study was designed to evaluate the oxidative stress in discrete regions of the brain in REM sleep deprived rats and possibility of its reversible nature.

## Methods

### Animals

Adult male Wistar rats weighing 150–180 g were used in this study. The study was conducted following the approval of the institutional animal ethics committee. The rats were divided into 6 groups of 6 animals each; Cage control (CC), REM control (REMC), 96 hours of REM sleep deprived (REMSD), and three groups of restorative sleep of 12 hours, 18 hours and 24 hours following 96 hours of REM sleep deprivation.

### REM deprivation Technique

The rats were deprived of REM sleep using the inverted flowerpot technique.<sup>8</sup> Briefly, rats were placed on a circular platform of diameter 6.5 cm in the center of a small water tub surrounded by water up to 1 cm below the surface of the platform. This setup permits non REM sleep and prevents REM sleep because the decrease in muscle tone during REM sleep makes the animal fall into the water, which wakes up the animal. This procedure is well accepted as depriving rats of REM sleep selectively without the requirement for monitoring the EEG.<sup>9</sup> The REMC group animals were placed on similar setup with diameter of the platform being 15 cm, large enough to permit the animal to go into REM sleep without falling into the water. This group was included to exclude the nonspecific effect of the REM deprivation technique and forms a better control for the REM group.<sup>7</sup> All the animals were maintained in standard animal house condition with 12-h light and 12-h dark cycles. Foods and water were available ad lib.

### Restorative Sleep

Animals of these groups were subjected to 96 hours of REMSD by the flower pot technique described above. This was followed by transferring the animals to their home cage and they were allowed to sleep with food and water *ad lib*. After 12 hours of restorative sleep the animals were sacrificed every 6 hrs till the parameters studied returned back to normal, i.e. 12 hrs, 18 hrs and 24 hrs restorative sleep.

### Biochemical Analysis

At the end of the stipulated time Jugular venous blood sample of 2 ml was collected under mild ether anesthesia for the assay of stress index, plasma corticosterone.<sup>10</sup> Time of sacrifice

was kept between 8–9 am to avoid the influence of circadian rhythm on corticosterone level. Following blood collection animals were decapitated and brains were rapidly removed and hypothalamus, midbrain and hindbrain were dissected out on ice following the method of Glowinski and Iverson.<sup>11</sup> They were weighed and homogenized in 0.1 M Tris buffer (pH 7.4) for the analysis of various biochemical parameters. Lipid peroxidation using thiobarbituric acid,<sup>12</sup> total reduced glutathione using DTNB (GSH)<sup>13</sup> were assayed in the brain regions dissected out.

#### Statistical Analysis

Data was analyzed using one way analysis of variance (ANOVA) followed by Tukey's Multiple comparison test. The level of significance was set at  $p < 0.05$ . SPSS version 17 was used for the analysis.

#### Results

REM sleep deprivation for 96 hours resulted in a significant increase in plasma corticosterone level. Lipid peroxidation showed a significant increase in all the three regions was investigated. There was a significant reduction in the level of total reduced glutathione in all the three regions. Following restorative sleep, there was gradual change in all the parameters and it reached the base line value following 24 hours of restorative sleep (Table I).

#### Discussion

Laboratory rats normally sleep for about 12 hrs/day, of which 15–20% of the sleep time corresponds to REM sleep stage.<sup>14</sup> This study has shown that REM sleep deprivation is a stressor, which is evident from the elevated plasma corticosterone levels. This is in correlation with earlier reports.<sup>15,16</sup>

The inverted flowerpot technique is the most widely used method for REM sleep deprivation studies.<sup>17</sup> This causes maximum REM sleep deprivation without significantly affecting non-REM sleep. It causes total loss of REM sleep in rats.<sup>18</sup> Studies on behavioural evaluation of the stress induced by platform method for short term REM sleep deprivation in rats showed that the effect of stress induced by short term confinement to platform do not seem to be a remarkable confounding factor and large platform acts as an adequate stress control for the small platform.<sup>19</sup> Hence large platform was used in the REMC group for all the comparisons. Plasma corticosterone level in the REMC group is almost near the control value for the Wistar strain animals which is in corroboration with earlier report.<sup>15</sup>

Sleep seems to limit metabolic requirements. Therefore sleep deprivation could enhance metabolic rate and in turn increase oxidative stress. Increase in lipid peroxidation in the discrete regions of the brain following REM sleep deprivation in the current study suggests free radical generation and free radical induced neuronal damage. An increase in malonyldialdehyde level is related to an increase in the levels of lipid peroxidation in cell membrane.<sup>6</sup> Mallick *et al*<sup>20</sup> have shown that REM sleep deprivation decreases membrane fluidity in the rat brain. Deep destructive changes in the brain and erythrocyte mitochondria have also been demonstrated following REM sleep deprivation.<sup>21</sup> Increase in lipid peroxidation was accompanied by decrease in total reduced glutathione following REM sleep deprivation. Ramanathan *et al*<sup>22</sup> have shown similar biochemical changes in the hippocampal region of the brain in Wistar rats. D'Almeida *et al*<sup>23</sup> showed that thalamus and hypothalamus are more susceptible to free radical damage following sleep deprivation as evidenced by decrease in GSH levels in these regions.

Table 1: Effect of 96 hrs of REM sleep deprivation on biochemical parameters

	Cage Control	REM control	REMSD (96 hrs)	Rest SI (12 hrs)	Rest sl (18 hrs)	Rest sl (24 hrs)	F test ratio df 5,30
Corticosterone µg/dl	12.292 ± 1.55	13.179 ± 1.67	18.458 ± 1.47 <sup>ab</sup>	26.645 ± 1.91 <sup>abc</sup>	20.058 ± 3.35 <sup>abc</sup>	14.145 ± 3.55	14.397
HYLPO µM/gm tissue	0.2182 ± 0.015	0.2346 ± 0.015	0.4132 ± 0.021 <sup>ab</sup>	0.4933 ± 0.029 <sup>abc</sup>	0.4734 ± 0.013 <sup>ab</sup>	0.1834 ± 0.0152 <sup>c</sup>	55.170
HYGSH mg/gm tissue	5.576 ± 0.68	4.206 ± 0.565	1.746 ± 0.214 <sup>ab</sup>	3.5256 ± 0.282	4.620 ± 0.604 <sup>c</sup>	9.226 ± 0.441 <sup>abc</sup>	20.182
HBLPO µM/gm tissue	0.2078 ± 0.01	0.2088 ± 0.005	0.4268 ± 0.013 <sup>ab</sup>	0.3216 ± 0.014 <sup>ac</sup>	0.3162 ± 0.0214 <sup>ac</sup>	0.2115 ± 0.019 <sup>c</sup>	28.825
HBGSH mg/gm tissue	2.870 ± 0.151	2.749 ± 0.144	1.749 ± 0.144 <sup>ab</sup>	2.733 ± 0.398	2.540 ± 0.219	9.773 ± 0.637 <sup>abc</sup>	83.767
MBLPO µM/gm tissue	0.2082 ± 0.0124	0.258 ± 0.021	0.3960 ± 0.009 <sup>ab</sup>	0.3865 ± 0.014 <sup>ab</sup>	0.3076 ± 0.017 <sup>ac</sup>	0.1958 ± 0.014 <sup>abc</sup>	35.982
MBGSH mg/gm tissue	3.444 ± 0.235	4.519 ± 0.792	1.912 ± 0.156 <sup>ab</sup>	2.767 ± 0.382	3.368 ± 0.306	9.581 ± 0.581 <sup>abc</sup>	35.052

Values given are Mean SE of 6 animals in each group. Anova and Tukeys multiple comparison performed with level of significance set at  $p < 0.05$ . a – compared with Cage control, b – compared with REM control, c – compared with REM sleep deprived. Hypothalamus (HY), Midbrain (MB), Hind brain (HB), Lipid peroxidation (LPO) and total reduced glutathione (GSH) level.

Brain antioxidant enzymes provide a mechanism inherent to an organism for removing free radicals. Oxidised glutathione, GSSG, production from GSH occurs as an antioxidant defense when the formation of reactive oxygen species is observed.<sup>6</sup> A decrease in total reduced glutathione following REM sleep deprivation could result in an increase in the level of GSSG. Several studies have experimentally also shown the sleep promoting effect of GSSG.<sup>24,25</sup> If the organism accumulates free radicals during waking period, then GSSG would also accumulate which in turn would induce sleep. Honda *et al*<sup>26</sup> have shown that GSSG has an inhibitory action on the excitatory synaptic membrane of rat brain. They have also speculated that the sleep-enhancing activity of GSSG was caused by its physiological modulation on the glutamatergic neurotransmission in the brain.

Further studies on the histopathological changes in these regions along with the biochemical changes would probably throw more light on the cellular level damages produced by REM sleep deprivation.

Restorative sleep following 96 hours of REM sleep deprivation returns lipid peroxidation and total reduced glutathione back to the base line values gradually by 24 hours of restorative sleep indicating that 96 hours of REM sleep deprivation does not cause permanent damage to the brain of Wistar rats. To emphasise on this fact, further investigation needs to be carried out in same lines along with histochemical and histopathological studies and by increasing durations of REM sleep deprivation.

Increase in plasma corticosterone level in the initial stages of restorative sleep indicates that the body homeostatic mechanisms are impacted by the stress. However, plasma corticosterone level also returns back to base line value by 24 hours of restorative sleep. The decrease in lipid peroxidation following restorative sleep indicates that there is decrease in free radical production. The other possibility is that the free radicals are scavenged by the antioxidant mechanism. This is well correlated by increase in the total reduced glutathione level following restorative sleep. These results are in accordance with the study of Mallik *et al*<sup>20</sup> where they have shown that norepinephrine activity and synaptosomal calcium levels returns to normal by 24 hrs of restorative sleep following 96 hours of REMSD. Datta and Desarnaud<sup>27</sup> have shown that recovery sleep following 3 hours of REMSD is due to activation of intracellular protein kinase A in the pedunculopontine tegmental nucleus. Mendelson and Bergmann<sup>28</sup> have shown that there is age dependent change in the recovery sleep after 48 hrs of sleep deprivation in rats with recovery largely confined to the first 6 hours in the young and middle aged rats but maximum for the old rats occurred in the second six hours.

From this study it is clear that, REM sleep deprivation is a potent oxidative stressor. This could probably play a role in the behavioral and performance alteration seen in both experimental animals as well as humans following REM sleep deprivation.

Importance of REM sleep has been suggested by the study of Ranjan *et al*<sup>29</sup> who have concluded that REMSD could lead to neurodegeneration memory loss and Alzheimer's disease. Sleep deprivation or prolonged wakefulness leads to decrements in cognitive performance, which is recognized as a major hazard to public safety and implicated in vehicular accidents, industrial catastrophes and other incidents involving error in human performance.<sup>30</sup> Though importance is given to the total duration of sleep, due importance is yet to be given to REM

stage of sleep. Further investigations in this field are needed to highlight the importance of REM sleep.

### Acknowledgement

The authors wish to thank the Life Sciences Research Board, Ministry of Defence for the financial support provided to Dr. D. C. Mathangi.

The article complies with International Committee of Medical Journal Editor's uniform requirements for the manuscripts.

Competing interests: None, Source of funding: Life Sciences Research Board, Ministry of Defence

Received Date : 19 March 2012; Revised Date : 30 June 2012

Accepted Date : 05 September 2012

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