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Original Article

REM sleep deprivation – A Stressor

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A R T I C L E I N F O

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A B S T R A C T

As the world is moving forward with a lot of development in materialistic aspects, health is taking a back seat. The basic need in life – 'sleep' is the one, that is most affected and sleep deprivation is becoming common. Sleep deprivation has been shown to have deleterious effect on the human body, giving rise to many diseases and disorders and especially REM sleep deprivation is shown to affect the HPA axis. This cause an increase in cortisol secretion, which is related to stress. The aim of this study is to assess the effect of REM sleep deprivation on plasma corticosterone. Wistar strain male albino rats were used to study the effect of REM sleep deprivation. They were divided into three groups, control, REM sleep deprived and REM control. Inverted flower pot technique was used to achieve REM sleep deprivation. Heparinised blood sample was taken and assessed for plasma corticosterone level. Food intake and body weight were also monitored to know the effect of REM sleep deprivation. The values obtained were statistically analysed using one way analysis of variance (ANOVA). On significant f test ratio, Tukey's multiple comparison tests were also performed. The level of significance was kept at $P<0.05$. All the results obtained from the study are expressed as mean + standard deviation (SD). The results of this study showed an increase in plasma corticosterone level, 24 hrs (34.725* ± 7.5), 48hrs (43.17* ± 9.2), 72hrs (35.17* ± 5.7) and not much change in 96hrs (10.96 ± 3.62) when compared to the control animals (12.293 ± 3.790). Corticosterone is a hormone related to stress; hence an increase in corticosterone level shows that REM sleep deprivation is a stressor. Sleep is important in life and deprivation of REM sleep can cause stress and affect the quality of life.

1. Introduction

In this world of ours with cost of living increasing, people are only aiming to earn money. They are least bothered about their health. The saying goes 'health is wealth' so it is important to maintain the health of the individual, and literature says sleep is one of the main promoters of health. Unfortunately sleep is the first to be sacrificed. Sleep is one of the important needs of the human body, like oxygen and nutrition, for survival and well being of the individual. Several studies have been conducted on sleep and on the effects of sleep deprivation in human volunteers and animals. Sleep is a periodic reversible physiological state. There are two stages of sleep, non rapid eye movement (NREM) and rapid eye movement (REM) sleep. Rapid eye movement (REM) sleep is important as REM deprivation has shown many deleterious and harmful effects.

REM sleep deprivation study in animals showed there is hyperphagia, increased food competition, aggressiveness, altered sexual behaviour, increased activity levels, increased preference for novelty, and decreased fear [1]. Young animals were more susceptible and dogs die after 14 days of deprivation [2]. Effects of sleep deprivation or prolonged wakefulness in human, kept awake for 60-114 hours showed less physical changes but marked mental changes. There was no change in composition of blood and in body temperature. Slight changes in heart rate, blood pressure and respiration were seen. Ability to maintain equilibrium was grossly impaired. The subjects showed marked inattention, irritability, loss of memory, hallucination and delusions. The symptoms were noticeable after 30-60 hours of sleeplessness. Some subjects also showed symptoms of acute schizophrenia [2].Some disorders stress related diseases like Alzheimers disease, arthritis,

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haemorrhoids, Parkinson's disease, rheumatism, myocardial infarction, AIDS, cataract, retinopathy, cerebral stroke, cancer, stress, jet lag, radiation injury, senility, varicose vein, phlebitis, immune system disorders and a long list of degenerative diseases including ageing. It also seems to contribute to heart diseases [3]. In humans, short periods of sleep loss can result in significance cognitive deficits [4] and long –term disturbance in sleep to be associated with reduced longevity [5]. In rats prolonged sleep deprivation leads to a syndrome characterized by increased food intake, weight loss, increased energy expenditure, progressive decline in body temperature which is invariably fatal [6,7]. It was proposed that the neural activity associated with sleep deprivation for longer durations may damage brain cells and eventually to cell death [8,9]. According to Zepelin and Rechtschaffen, [10], sleep seems to limit metabolic requirements. Therefore sleep deprivation could enhance the metabolic rate and in turn increase oxidative stress. But Almeida et al., [11], showed that there is no oxidative stress following paradoxical sleep deprivation in rats. Cirelli [12], has shown that there is no evidence of brain cell degeneration after a long –term sleep deprivation. Tobler et al [13] has told that stress is not a major factor of effects of sleep deprivation as there was a marked increase in 20min forced locomotion than 24 hrs of sleep deprivation. Here both REM and NREM were deprived. The reports, both favouring and contradicting this hypothesis is seen. Hence an attempt has been made to evaluate this hypothesis. The physiological significance of REM sleep is still unclear. A widely used experimental practice is the selective deprivation of REM sleep[14]. It is said that sleep deprivation activates the hypothalamic-pituitary-adrenal-axis and increases the corticotrophin releasing hormone which impairs sleep and as sleep is deprived, it cause stress. The level of corticosterone may be increased in the blood due to stress. The animals deprived of REM sleep are said to be under stress. So an estimation of the level of plasma corticosterone will provide the necessary information. REM control animals were also study in this to rule out the possible effects of stress caused due to immobilisation. The aim of this study is to estimate the level of corticosterone in plasma in REM sleep deprived animals.

2. Materials and method

Male albino rats of Wistar strain weighing between 150 - 180 gms, were used as the experimental animal for this study. The animals were divided into three groups of Control, REM sleep deprived and REM sleep control.

Group I – Control animals (n=6): These animals were used for evaluating the baseline values for the various parameters to be assessed.

Group II – REM sleep deprived animals: These animals were used to study the effect of REM sleep deprivation on plasma corticosterone level in the blood.

Group III – REM sleep control animals: These animals were used to rule out any other effect like isolation, immobilisation involved in the REM sleep deprivation procedure.

The groups II and III were further subdivided into four groups based on the duration of study as (a) 24 hrs. (b) 48 hrs. (c) 72 hrs. (d) 96 hrs. Six animals were used in each group.

The animals were provided with food and water ad libitium, with 12 hrs light and 12 hrs dark cycles. The room temperature was ambient (24 - 28°C).

REM sleep deprivation

Inverted flower technique by of Jovet et al. [15], was used for REM sleep deprivation. The animals were placed on small platform (diameter 6.5 cms) surrounded by a pool of water, where the platform is small enough with reference to the body size of the animal. As the animal goes into REM sleep there is loss of muscle tone which is typical of REM sleep, the animal falls into the water.

Ethical clearance

Ethical clearance was obtained from the Ethical Committee through the institution before the study

Food intake and body weight

The body weight of the animals was estimated before and during the REM sleep deprivation and amount of food intake was also measured. This was done to see whether there was any effect of REM sleep deprivation on body weight and amount of food taken.

Mild ether anaesthesia was administered and heparinised blood samples were collected from the jugular vein at the stipulated time as per the subgrouping (ie.24,48,72 and 96 hours). The heparinised blood was centrifuged at 3000 rpm for half an hour and plasma was obtained, which was used to assess the level of corticosterone.

Estimation of plasma corticosterone [16]

The free and protein bound corticosterone were extracted into dichloromethane. This extract was shaken with a sulphuric ethanol reagent. The fluorescent acid layer was measured in a flourimeter at excitation of 470 nm and emission of 530nm.

Statistical Analysis

The values obtained were statistically analysed using one way analysis of variance (ANOVA). On significant f test ratio, Tukey's multiple comparison tests were also performed. The level of significance was kept at $P<0.05$. All the results obtained from the study are expressed as mean + standard deviation (SD).

I.REM sleep Deprivation
Food Intake (gms) (Table -1, Figure-1)

The animals subjected to REM sleep deprivation showed an increase in food intake in all the subgroups, 24hrs(57 + 19), 48hrs(57 +26) , 72 hrs (73 + 25) and 96hrs (70 + 27), as compared to control(17 +2.4)

Figure 1 Effect of REM sleep deprivation on food intake.

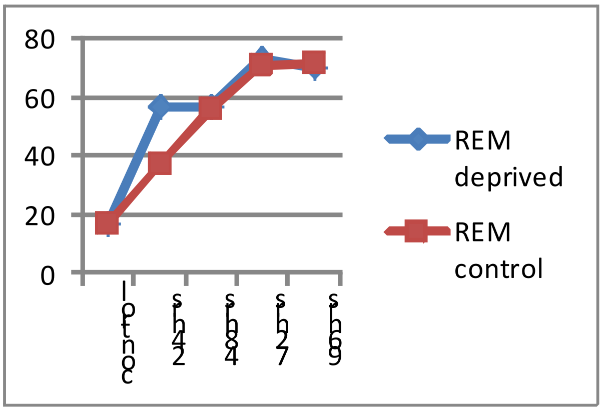

Table -1 Effect of REM sleep deprivation on food intake & body weight changes

Parameters		Control	24hrs	48hrs	72hrs	96hrs	'F' test ratio
Food intake (gms)	REM deprived	17±2.4	57*±19	57*±26	73*±25	70*±25	6.531
	REM control	17±3	37*±9	56*±11	71*±7	72*±6	54.252
Body weight (gms)	REM deprived	180±18	166±19	160±18	157±19	147*±17	2.25
	REM deprived	168±8	159±12	153± 13	153±12	150±12	2.02

n=6 in each group, values given as mean ±SD, *P<0.05

Body weight (gms) (Table -1, Figure-2)

Figure 2 Effect of REM sleep deprivation on body weight

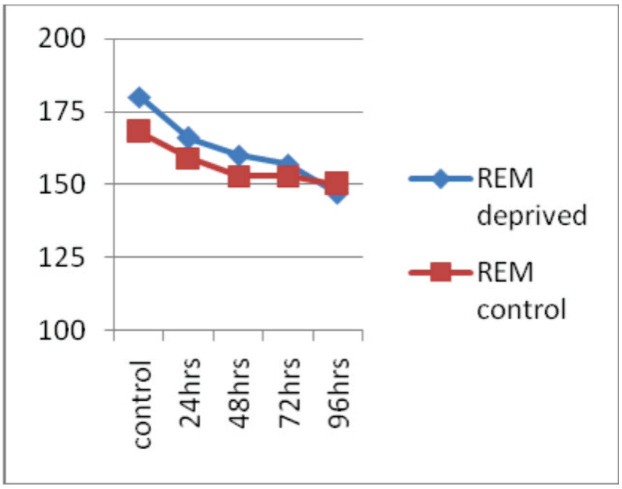

Table -2 Effect of REM sleep deprivation on plasma corticosterone

Parameters	Control	24hrs	48hrs	72hrs	96hrs	'F' test ratio
Corticosterone (µg/dl)	12.293 ±3.790	34.725* ±7.5	43.17* ±9.2	35.17* ±5.7	10.96 ±3.62	32.184

n=6 in each group, values given as mean ±SD, *P<0.05

Plasma corticosterone (µg/dl) (Table-2, Figure-3)

The plasma corticosterone level estimated showed a significant increase in 24 hrs (34.725 + 7.5), 48 hrs (43.17 + 9.20 and 72 hrs (935.17 + 5.7) of REM sleep deprivation in comparison to the control group (12.293 + 3.790). 96 hrs of REM sleep deprivation (10.96 + 3.620) did not show any significant change. To rule out the possible non-specific effects attributed to the technique of REM sleep deprivation like isolation, immobility, all the results were compared with REM sleep control animals.

Figure 3 Effect of REM sleep deprivation on plasma corticosterone

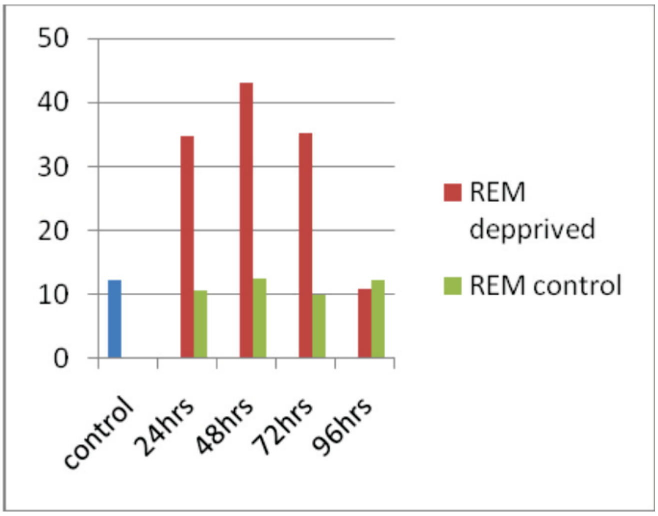

Table -3

24 hrs	Control	REM deprived	REM control	'F' test ratio
Corticosterone (µg/dl)	12.293 ±3.790	34.725* ±7.5	10.7 + 2.7	42.664

n=6 in each group, values given as mean ±SD, *P<0.05

The above table shows comparison between control, REM sleep deprived and REM sleep control, on plasma corticosterone values of 24 hrs duration.

Table -4

48 hrs	Control	REM deprived	REM control	'F' test ratio
Corticosterone (µg/dl)	12.293 ±3.790	43.17* ±9.2	12.61 +4.2	49.25

n=6 in each group, values given as mean ± SD, *P<0.05

The above table shows comparison between control, REM sleep deprived and REM sleep control, on plasma corticosterone values of 48 hrs duration.

Table -5

72 hrs	Control	REM deprived	REM control	'F' test ratio
Corticosterone (µg/dl)	12.293 ±3.790	35.17* ±5.7	10.05 ±2.7	64.906

n=6 in each group, values given as mean ± SD, *P<0.05

The above table shows comparison between control, REM sleep deprived and REM sleep control, on plasma corticosterone values of 72 hrs duration.

Table- 6

96 hrs	Control	REM deprived	REM control	'F' test ratio
Corticosterone (µg/dl)	12.293 ±3.790	10.96 ±3.62	12.167 ±3.19	0.259

n=6 in each group, values given as mean ± SD, *P<0.05

The above table shows comparison between control, REM sleep deprived and REM sleep control, on plasma corticosterone values of 96 hrs duration.

II- REM sleep control

a) Food intake (gms) table-1, Figure-1)

The food intake estimated showed an increase in all the subgroups of 24hrs (37 ± 9), 48hrs (56 ± 11), 72hrs (71 ± 7) and 96 hrs (72 ± 6) compared to control animals.

b) Body weight (gms) (table-1. Figure-2)

Body weight monitored showed only minimal changes in all the subgroups of 24hrs (159 ± 12), 48hrs (153 ±13) , 72hrs (153 ± 12), and 96 hrs (150 ± 12) compared to control animals (168 +8)

c) Plasma corticosterone (µg/dl) (table – 3.4, 5.6, Figure – 3)

Plasma corticosterone level did not show much change in all the REM sleep control animals of 24hrs (10.7 ± 2.7) ,48 hrs (12.61 ±4.2), 72 hrs (10.05 ± 2.7) and 96hrs (12.167 ± 3.19) when compared to the corticosterone level of control animals (12.293 ± 3.790).

7. Discussion

Hypothalamic Pituitary Axis (HPA) is said to play a role in sleep. Axiel [17] showed that the HPA hormones affect sleep- EEG changes. It is well documented that corticotrophin releasing hormone impairs sleep and enhance vigilance. Most studies agree that the circadian pattern of cortisol is relatively independent from sleep and environmental influence. Some data suggest a major effect of light on cortisol secretion. Sleeping is widely associated with blunting and awakenings are linked with increase of HPA hormones.HPA axis plays an important role in maintaining alertness and modulating sleep axis hyperactivity can have many negative effects on sleep. It can lead to sleep fragmentation, decreased SWS, shortened sleep time and sleep disturbances can exacerbate HPA axis and worsen the cycle [18]. Dysfunction of the axis at any level can disrupt sleep and cause an increase in corticosterone. The technique - inverted flowerpot, developed by Jouvet et al.[15], was followed for REM sleep deprivation, where the animals are placed on a small platform surrounded by water. The advantages of this technique are that several animals can be deprived simultaneously, without laborious monitoring of electrophysiological characteristics of sleep. The various REM sleep deprivation protocols like classical platform, pendulum, multiple platform have shown that there was not much difference [19]. Both stress index, (Selyes classical indices, adrenal hypertrophy, thymus atrophy, body weight loss and stomach ulceration [20]) as well as EEG recording did not show significant differences between the various REM sleep deprivation procedures [21]. But this technique was heavily criticised for its several stress factors like restriction of movement, isolation, recurrent wetting, emotional distress which are inherent to the procedure [22]. This technique is at present accepted as a valid method if comparisons were made with animals on large platform allowing the relaxed position of REM sleep and without danger of falling into water [14,23]. Hence a REM sleep control was also included in this study.
The body weight and food intake were monitored in this study and it was found that there was a steady decline in the body weight as the duration of REM sleep deprivation increases, though there was a steady increase in food intake. This loss of weight despite hyperphagia was also shown by other works [19,24,25,26,27].Study by Kushida et al.[26], also showed that REM sleep deprivation caused weight loss in spite of increased food intake, which they attributed to increased energy expenditure with mean levels reaching more than twice base line values. Bradeley Youngblood et al.[28] also showed weight loss. Though increased food intake was also observed in REM sleep control animals it was not accompanied by decreased body weight which is peculiar to REM sleep deprivation as shown by Brock et al.,[27].

The results obtained from the present study also showed a significant increase in plasma corticosterone level in 24hrs, 48hrs and 72hrs of REM sleep deprivation in comparison to the control animals. The decrease in the plasma corticosterone level in the 96 hrs REM sleep deprived animals can be attributed to adaptation.

Meerlo et al.,[29] showed that there was an increase in ACTH and corticosterone level in rats deprived of sleep but returned to baseline within 4 hrs of recovery. Also showed 48 hrs deprivation showed increase in corticosterone level. This showed that sleep loss is a mild activator of HPA axis and affects subsequent response to stress. Sleep deprivation elevates plasma corticosterone in rats after short periods of sleep deprivation and sleep deprivation is a stressor. [30]. Increase in weight loss and increase in serum corticosterone was shown by Bradeley Youngblood et al.,[28]. Similar results were also shown by other studies [13], where corticosterone level was markedly elevated in 48hrs REM sleep deprived animals in comparison to controls and 72 hrs of REM sleep deprivation. In the present study control and large platform REM sleep control animals showed little difference in the plasma corticosterone level which was shown by Heiskanen et al.,[31]. This clearly shows that the increase in plasma corticosterone level in the REM sleep deprived animals cannot be attributed to the stress involved in the REM sleep deprivation technique followed and it is purely due to the stress that the animal has undergone due to REM sleep deprivation perse, which has altered the HPA axis and caused an increase in corticosterone level which is an indicator of stress.

8. Conclusion

From the results obtained in this study and on comparing with the results obtained from other studies, in conclusion it can be stated that REM sleep deprivation activates the HPA axis and cause an increase in corticosterone level , which is stress related. This shows that REM sleep deprivation is a stressor. In this fast moving life, with expensive basic requirements people are least concerned about their health and sleep is the first to be sacrificed. Sleep deprivation can lead to impairment of health and performance decrement. Hence sleep deprivation must be avoided to lead a healthy life.

9. References

[1] Coris MC, Poncedeleon M. Juarez J, Ramos J. Effects of Paradoxical sleep deprivation and stress on the waking EEG in the rat. Physiol. Behav.1994;55 (6): 1021 – 1027.

[2] Sarada Subramanyam, Madhavankutty K.,2001. Sleep., Text book of Human Physiology., 6thed: 789 – 793.

[3] Chitra K Pillai, Pillai K S., 2002. Guest Editorial Antioxidants in health., Indian J of Physiol. Pharmacol. 46 (1): 1 – 5

[4] Horne JA. 1988. Why we sleep. The function of sleep in humans and other mammals. Oxford University press Oxford.

[5] Kripke DF, Simons RN, Garfinkel, Hammond EC., 1979. Short and long sleep and sleeping pills – is increased mortality associated? Arch Gen Psych., 36: 103 – 116.

[6] Rechtschaffen A, Gilliland MA, Bergman BM, Winter JB.,1983. Physiological correlates of prolonged sleep deprivation in rats., Science., 221: 182 – 184

[7] Rechtschaffen, Bergmann BM, 1995. Sleep deprivation in the rat by the disk over water method., Behav., Brain REs., 69: 55 – 63.

[8] Inoue S. Honda K, Komoda Y. 1995. Sleep as neuronal detoxification and restitution. Behav.Brain Res. 69: 491 – 496.

[9] Mamelak M, 1997. Neurodegeneration sleep, cerebral energy metabolism a testable hypothesis., J.Gen Psych. Neuro., 10: 29 – 32.

[10] Zeppelin H, Rechtschaffen A,1974. Mammalian sleep, longevity and energy metabolism. Brain Behav. Evol.10: 425 – 470.

[11] Almeida VD, Hipolide DC, Azzalis LA, Lobo L, Junqueira VBC, Tufik S.,1997. Absence of oxidative stress following paradoxical sleep deprivation in rats., Neuroscilett., 1235:25-28

[12] Cirelli C, Shaw P J, Rechtschaffen A, Tononi G.,1999. No evidence of brain cell degeneration after long term sleep deprivation in rats., 840: 184 – 193

[13] Tobler L.Murison R, Urisin R, Hursin, Burbely AA,1983. The effect of sleep deprivation and recovery sleep on plasma corticosterone in the rat, Neurosci.Lett., 35: 297 – 300

[14] Vogel GV.,1975. A review of REM sleep deprivation., Arch, Gen Psychia., 32:749 – 761

[15] Jouvet D.P. Vimont F, Delorme, Jouvet M. 1964. Etude de la privation selective de la phase paradoxale de summeil chezle chat. C.R.Seanc Soc Biol. 158: 756 – 759.

[16] Mattingly D. 1969. Determination of plasma 11 hydraxy corticosteroids. Fluorimeteric method. In varely H.Ed. Practical clinical Biochemistry London. Gulab Vazirarl for Arnold Heinman publishers., 669 – 670

[17] Axel Steiger, 2002. Sleep and the hypothalamic pituitary adrenocortical system. Sleep Med.,6 (2) :125 – 138

[18] Theresa M. Buckley and Alan F. Schatzberg, 2005. REVIEW: On the Interactions of the Hypothalamic- Pituitary-Adrenal (HPA) Axis and Sleep: Normal HPA Axis Activity and Circadian Rhythm, Exemplary Sleep Disorders. The Journal of Clinical Endocrinology & Metabolism, 90(5):3106–3114

[19] Coenen AML, Van Luijtelaar ELJM., 1985. Stress induced by three procedures of deprivation of paradoxical sleep., Physiol and Behav., 55) 501 – 504

[20] Selye H, 1950.The physiology and pathology of exposure to stress ., Medical Publisher, Montreal, Canada., ACTA Inc.,

[21] Van Luijtelaar ELJM, Coenen AML, 1986. Electrophysiological evaluation of three paradoxical sleep deprivation techniques in rats. Physiol, and Behav. 36: 603 – 609

[22] Albert IB., 1975.REM sleep deprivation., Biol,phsychia., 33:341-351

[23] Ellman S J, Spielman A J, Luck D, Steinerss, Halperin R., REM deprivation.1978. A review., In the mind in sleep psychology and psychophysiology Eds: An Arkin J S Aritrobus, S J Ellman, Lawrence Erbaum Associates, New Jersey., 419 – 457

[24] Adam K .1977a, “Body weight correlates with REM sleep”, Br.Med.J.,1 : 813-814

[25] Bregmann BM, Everson CA, Kushida CA.,1989, Sleep deprivation in rat: V Energy use and mediation., Sleep.,12 (1): 31 – 41

[26] Kushida CA, Bergmann BM, Rechtschaffen A. 1989. Sleep deprivation in rats – IV, paradoxical sleep deprivation. Sleep. 12 (1): 22 – 31.

[27] Brock JW, Farooque SM, Ross KD, Payne S, Chandan Prasad. 1994. Stress related behavious and central norepinephrine concentrations in the REM sleep deprived rat. Physiol. And Behav. 55 (6): 997 – 1003.

[28] Bradely D Youngblood, Jun Zhou, Gennady N Smagin, Donna H Ryan, Ruth BS Haris, 1997. Sleep deprivation by flower pot technique and spatial reference memory. Physiol, and Behav., (2) :249-256

[29] Meerlo, P., Koehl, M., Van Der Borght, K. and Turek, F. W, 2002. Sleep Restriction Alters the Hypothalamic-Pituitary-Adrenal Response to Stress. Journal of Neuroendocrinology, 14: 397–402. doi: 10.1046/j.0007-1331.2002.00790.x

[30] Illana S Harison, Norman F Ruby, Shiela Brooke, Christelle Peyron, Daniel P Denning, H Craig Heller, Rober M Sapolsky, 2001. Sleep deprivation elevates plasma corticosterone levels in neonatal rats. NeurosciLett., 315 (1-2) :29 -32

[31] Heiskanen TP, Smith S E, Taira T, Urban J H, Levine J E, Turekstenberg D., 1995. Nor adrenergic activity in rat brain during rapid eye movement sleep deprivation and rebound sleep., Physiol Society., r 1456 – 1463