Original Article

Effect of Rapid Eye Movement Sleep Deprivation on Memory in Rats: Role of Brain Derived Neurotropic Factor and Oxidative Stress.

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Abstract:

Background: Several studies have shown that sleep deprivation, especially rapid eye movement (REM) sleep enhances learning and consolidation of some forms of memory. In fact it has been shown that REM sleep is increased after learning sessions. On the other hand, sleep deprivation interferes with learning and memory. The exact mechanisms underlying such disturbances are still not fully elucidated. **Aim:** of the present work was to assess the effect of rapid eye movement sleep deprivation (REMSD) on memory consolidation and to investigate the role of brain derived neurotrophic factor (BDNF) in memory impairment of REM sleep deprived rats and the possible effect of oxidative stress as an underlying mechanism. **Material and Methods:** The study was conducted on 24 adult male albino rats with weights ranging from 200-300 g. Rats were divided into 3 groups (8 rats each). Group I (stress-control group): exposed to same experimental conditions for 6 days without sleep deprivation. Group II (REM deprived group): exposed to 6 days REMSD by multiple platforms method. Group III (recovery control group): exposed to 6 days REMSD then allowed to recover from REM sleep loss for 3 days in their normal home cages. All rats were subjected to daily water maze training using modified Morris water maze to reach a hidden platform in the pool. Immediately after daily training, both groups II and III were deprived from REM sleep using multiple platforms method. At the end of the training period, memory retention was tested. Hippocampal BDNF protein level and oxidative stress markers in brain tissues including reduced glutathione (GSH) and melondialdehyde (MDA) were assayed. **Results:** REM sleep deprived rats showed impairment in memory retention testing in modified water maze task as compared to stress control group. In addition, REMSD resulted in a significant decrease of hippocampal BDNF, brain level of reduced GSH and a significant increase of brain level of MDA as compared to stress control group. Recovery from sleep deprivation resulted in a significant improvement in memory retention testing, hippocampal BDNF and oxidative stress parameters as compared to REM sleep deprived rats. Although recovery from sleep deprivation returned hippocampal BDNF back to basal line, there was a significant decrease in results of memory retention testing, level of GSH and increase of MDA as compared to stress control group. In addition a negative correlation was found between the level of hippocampal BDNF and brain tissue MDA. **Conclusions:** The present study supports the involvement of reduced hippocampal BDNF in the memory deficits induced by REMSD and hypothesis that the increased brain oxidative stress markers may be one of possible mechanisms for reduction of BDNF and subsequently impairment of memory. Sleep recovery after REMSD is effective in reduction of oxidative stress and memory deficit but not to base line emphasizing the importance of implementing prompt treatment of sleep deprivation to prevent memory deficit.

Keywords: REM sleep deprivation, memory impairment, oxidative stress, brain derived neurotrophic factor.

Abbreviations:

REM, rapid eye movement; **REMSD,** REM sleep deprivation; **BDNF,** Brain derived neurotrophic factor; **GSH,** reduced glutathione; **MDA,** malondialdehyde; **LTP,** Long term potentiation; **CREB,** cAMP response element-binding protein; **NMDA,** N- methyl-D-aspartate.

Introduction:

Sleep is defined as a natural and reversible state of reduced responsiveness to external stimuli and relative inactivity, which is important for the maintenance of physiological homeostasis and psychological balance. The disturbance or shortening of normal sleep has recently been shown to be harmful for metabolism and endocrine functions, in a way that resembles the effects of premature aging. Lack of sleep may also result in irritability, fatigue, slurred speech, memory lapses, mood changes, depression and even death due to accidents.[1]

Among the multiple functions of sleep, its role in the establishment of memories seems to be particularly important. Sleep has been shown to promote primarily the consolidation of memory.[2] Previous studies in rats and humans aiming to determine whether different sleep stages have different roles in memory consolidation, mainly focused on rapid eye movement (REM) sleep and the consequences of REM sleep deprivation (REMSD).[3,4] REM sleep was shown to increase after learning a certain task during specific time periods dependent on the task. In the Morris water maze task, it started more than 2 h after learning and persisted for 22 h. The increase in REM sleep during the specific time periods predicted later memory recall, whereas selective deprivation of REM sleep during these time periods was shown to impair memory.[5]

Memory is composed of several stages including acquisition, consolidation (retention) and retrieval. Acquisition is the step during which a task is learnt, consolidation is the process during which the memory is stabilized, and retrieval is the bringing back of the learned task.[2] These three stages of memory can be tested in experimental animals using water maze task.[6]

Memory consolidation involves the strengthening of memory representations through anatomical changes at the synaptic level (synaptic consolidation).[7] Long-term potentiation (LTP) is considered a major mechanism of synaptic consolidation that is induced in the hippocampus during REM sleep. REM sleep has been shown to mediate the expression of plasticity-related immediate early genes (IEGs) like activity regulated cytoskeleton associated protein (Arc), Early growth response protein 1 (Egr1) and brain derived neurotropic factor (BDNF) through hippocampal LTP. Of those, BDNF is especially considered a key protein involved in hippocampal synaptic plasticity. It participates in multiple forms of learning and memory formation[8]. Monfils et al[9] found that consolidation of fear memory increases the binding activity of phosphorylated cAMP response element-binding protein (CREB) to BDNF promoters and upregulates BDNF expression in the amygdala. In addition, hippocampus-dependent contextual learning, or a spatial learning task (water maze task), increases BDNF expression in the hippocampus.[10,11] Upregulation of BDNF has been also observed in the inferior temporal cortex during declarative memory formation[12]. On the other hand, REMSD has been shown to impair the induction of hippocamal LTP and to increase the oxidative stress in the brain resulting in memory impairment.[1,7] However, the exact mechanism underlying memory impairment after REMSD is not fully elucidated.

The aim of the present work was to assess the effect of REMSD on memory consolidation and to investigate the role BDNF in memory impairment of REM sleep deprived rats, and the possible effect of oxidative stress as an underlying mechanism.

Materials and Methods:

The study was conducted on 24 adult male albino rats, with a body weight ranging from 200-300 g. The animals were kept under standard laboratory conditions, maintained on a 12-h light–dark cycle with free access to food and water. Rats were divided into 3 groups. Group I: included 8 rats **(stress-control group),** this group wasn't sleep deprived but rats were exposed to the same experimental conditions by being placed on large platforms in the water pool allowing them to sleep. Group II: included 8 rats that were subjected to REMSD for 6 days **(REM deprivated group)** by multiple platforms method.[13] **Group III:** included 8 rats

(recovery control group) that were subjected to REMSD for 6 days then allowed to recover from REM sleep loss for 3 days in their normal home cages. Behavioral studies were used to assess the effect of REMSD on memory retention follows: Rats were subjected to daily water maze training using modified Morris water maze., immediately after daily training they were deprived from REM sleep using multiple platforms method. At the end of the training period, memory retention was tested.

Water maze task was performed for all rats. The water maze consisted of a dark circular pool, 125 cm in diameter, 55 cm in height, filled with opaque water (about 22° ± 3°C) to a depth of 20 cm., a submerged circular black platform (10 cm in diameter) was placed 20 cm away from the edge in a fixed location and 1 cm below the water surface. The pool was divided into 4 quadrants by 4 starting points marked on its wall: north, south, east, and west (N-S-E-W). The platform provided the only escape from the water. Several cues were placed outside the maze in a fixed position relative to the pool as a window, colored curtain and a red flag; they helped the rat to locate the position of the escape platform hidden below the water surface.[6] The path taken by the animal was recorded by a digital video camera that was mounted above the center of the pool to record the distance traveled and time taken by each rat to reach the platform. The distance was measured by aid of a grid with 10 cm squares placed over the pool (figure 1).[14]

All experimental rats were subjected to daily water maze training before testing of memory retention on last day. During the training session each rat was placed in the water facing the wall of the pool at one of the four designated starting points (north, east, south and west) and allowed to swim and find the hidden platform located in the NW quadrant (target quadrant) of the maze. Each of four starting positions was used once in four training sessions.[15] During each trial, each rat was given 120 seconds (sec) to find the hidden platform. After mounting the platform, the animals were allowed to remain there for 20 sec, and were then placed in a holding cage for 30 sec until the start of the next trial. After completion of training, the animals returned to their home cages. If the rats couldn't find the platform in 120 sec they were placed on it by the examiner and left there for 20 sec. The duration of training varied according to the studied group. It continued for 6 days for groups I and II and 9 days for group III (6 days REMSD + 3 days recovery).

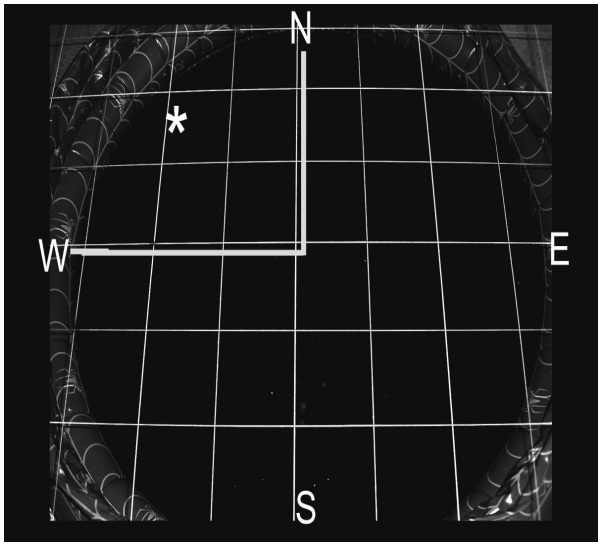

Fig 1: Water maze pool with 4 starting points (N-S-E-W) dividing it into 4 quadrants. The mark points to the location of the hidden platform in the target quadrant of the pool (N-W). A grid with 10 cm squares was placed over the pool to measure the distance swum by the rat.

I- Memory retention testing:

At the end of the training period memory retention testing was performed 24 hours after last day of training for each group, to test for memory consolidation. Retention testing consisted of 60 sec of a free swimming period for each rat with the hidden platform removed from the pool. The better the memory is consolidated the more time will the rat swim around the location of the platform and less escape trials will be attempted from the wall of the pool. The time spent in the target quadrant [expressed as percent of the time spent in the pool (total of 60 sec)], the distance swum in the target quadrant (expressed as percent of the distance swum in the pool) and the number of escape trials from the edges of the pool were calculated.(15)

II- REM sleep deprivation procedure:

REMSD began immediately after each daily training session using the modified multiple platforms method.(1)

Stress control group was submitted to the same procedure, except the platforms were 13 cm in diameter allowing them to sleep on it. Except for the size of the platform, all other conditions remained identical to that of the experimental animals.(16)

Recovery control group included those animals that had been REM sleep deprived and then allowed to live in normal cages for 3 days to recover from lost REM sleep.(16)

The modified multiple platforms method involved placing the rats inside a circular water tank (125×55 cm), containing 12 circular platforms, 6.5 cm in diameter, with water up to 1 cm of their upper surface. The rats could thus move around inside the tank by jumping from one platform to another. When they reached the REM phase of sleep, muscle atonia set in and they fell into the water and woke. Food and water were provided for all groups through placing food on the platforms inserted in the centre of the pool close to the other platforms. The water in the tank was changed daily, throughout REMSD period.(1,3)

III- Biochemical studies:

On the last experimental day for each group; the rats were sacrificed by decapitation immediately after behavioral assessments. The whole brain was removed and washed with ice cold saline. Hippocampus was dissected through the following way: a small curved forceps was placed between the cerebral halves in a closed position, and then the forceps was repeatedly opened and closed. Once an opening was obtained along the midline, the forceps was opened and directed to both sides to separate the cortex from the hippocampus. This movement was repeated on either side until the upper part of the hippocampus was visible (identified by its whitish color). The cortex was gently picked up with the forceps and the hippocampus was freed from the cortex without damaging the cortex. The hippocampus was cut and separated from the fornix. The two halves of the hippocampus were separated and the hippocampus on one side was rolled out of the brain to remove it from the cortex.(17) Hippocampus and the remaining brain tissue from each rat were stored at a temperature of -80 ^{o}C for biochemical analysis.

At the time of analysis the hippocampus and remaining brain tissues for each rat were removed from the freezer. Lysis buffer (137mM NaCl, 20mM Tris–HCl pH 8.0, 1% NP-40, 10% glycerol, 1mM phenylmethylsulphonyl fluoride, 10 μgml^{-1} aprotinin, 1 μgml^{-1} leupeptin, 0.5 mM sodium vanadate) was added to each sample (2 samples for each rat one for hippocampus and the other for remaining brain tissues). After that all the samples were homogenized. The homogenate was centrifuged at 12,000 rpm for 20 min at 4°C to remove tissue debris. The supernatant was aliquoted and frozen at -80°C until analysis.(18)

The protein concentration of each sample was measured in supernatants of hippocampus and the remaining brain tissues using lowry method(19) and was expressed in mg/ml. Supernatant from hippocampus homogenate was used for estimation of BDNF protein level by the enzyme-linked immunosorbent assay (ELISA) kit (Boster Biological Technology., LTD).(18) Its concentration in the hippocampus was divided by the concentration of protein (mg/ml) to be finally expressed as pg/mg protein. The minimum detectable dose of rat BDNF was less than 2 pg/ml. No detectable cross- reactivity with any other cytokine was detected by the manufacture. Supernatants from brain tissues homogenate were used for estimation of:

Reduced glutathione level (GSH) which was estimated by colorimetric method and was finally expressed as ng GSH/mg protein of the remaining brain tissue;[20] as well as malondialdehyde (MDA) level which was estimated by colorimetric method. Lipid peroxidation was finally expressed as nmol MDA/mg protein of the remaining brain tissue.[21]

Statistical analysis:

The values of the measured parameters were expressed as mean±SD. The difference between the studied groups was determined using ANOVA test (F-test) and least significant difference (LSD). Pearson's correlation coefficient was performed for evaluating the behavioral, neurochemical and biochemical variables. P<0.05 values were considered significant. All statistical analyses were processed using SPSS for windows Version 18.

Results:

Comparison between different studied groups regarding memory retention testing:

Water maze memory retention testing after 6 days REMSD in group II caused a significant decrease in the percent of the time spent in the target quadrant of the pool as compared to stress control group where p<0.0001. Recovery from REMSD in group III caused a significant increase in the percent of the time spent in the target quadrant of the pool as compared to group II where p<0.0001. However, the percent of time spent in the target quadrant in group III was still significantly less than in the stress control group where p<0.001.

In addition, 6 days REMSD in group II caused a significant decrease in the percent of the distance swum in the target quadrant of the pool as compared to stress control group where p<0.0001. Recovery from REMSD in group III caused a significant increase in the percent of the distance swum in the target quadrant of the pool as compared to group II where p<0.05. However, the percent of distance swum in the target quadrant in group III was still significantly less than that stress control group where p<0.001 indicating incomplete recovery of memory.

Moreover, REMSD for 6 days in group II caused a significant increase in the number of escape trials from the edges of the pool as compared to stress control group where P<0.05. Recovery from REMSD in group III caused no change in the number of escape trials as compared to group II. However, the number of escape trials in recovery control group was significantly higher than the stress control group where p<0.01. (Table 1)

Hippocampus BDNF (pg/mg protein):

The mean values for hippocampus BDNF in group I, II and III were 5335±942.7 (pg /mg protein), 3377±1781.4 (pg /mg protein) and 5570±1783.6 (pg /mg protein) respectively. There was a significant difference between the groups where F value was 16.98 and p < 0.001.

In group II, 6 days REMSD caused a significant decrease in hippocampus BDNF as compared to stress control (group I) where p<0.05. Recovery from REMSD in group III caused a significant increase in hippocampus BDNF as compared to group II where p<0.05. However, recovery from REMSD caused no change in hippocampus BDNF as compared to stress control group. (Table 2)

Brain tissue GSH (ng/mg protein):

The mean values for brain tissue GSH in group I, II and III were 9.88±1.35 (ng/mg protein), 6.96±1.18 (ng/mg protein) and 8.38±1.48 (ng/mg protein) respectively. There was a significant difference between the groups where F value was 16.85 and p<0.001.

REMSD for 6 days in group II resulted in a significant decrease in GSH as compared to stress control (group I) where p<0.0001. Recovery from REMSD in group III caused a significant increase in GSH as compared to group II where p<0.05. In addition, recovery from REMSD caused a significant decrease in GSH as compared to stress control group where p<0.05 indicating incomplete return of brain tissue GSH to basal line. (Table 3)

Brain tissue MDA (nmol /mg protein):

Brain tissue MDA mean values in Stress-control, REM deprivated and the recovery control groups were 0.49±0.29 (nmol /mg protein), 3.75±1.1 (nmol/mg protein) and 1.29±0.9 (nmol/mg protein) respectively. There was a significant difference between the groups where F value was 16.98 and p<0.001.

It was observed that 6 days REMSD in group II caused a significant increase in brain

tissue MDA as compared to stress control (group I) where p<0.0001. Recovery from REMSD in group III caused a significant decrease in brain tissue MDA as compared to group II where p<0.0001. In addition, Recovery from REMSD caused a significant increase in brain tissue MDA as compared to stress control group where p<0.05 indicating incomplete return of brain tissue MDA to basal line. (Table 4)

Correlation study:

I- Correlation study between level of hippocampus BDNF and performance in memory retention testing:

A significant positive correlation was found between level of hippocampus BDNF and the percent of time spent in the target quadrant of the pool where correlation coefficient r = 0.468 and P<0.05 (figure 2).

In addition, a significant negative correlation was found between level of hippocampus BDNF and number of escape trials from edges of the pool where correlation coefficient r = -0.401 and p<0.05 (figure 3).

II. Correlation between level of hippocampus BDNF and brain tissue MDA

A negative correlation was found between level of hippocampus BDNF and brain tissue MDH where correlation coefficient r = -0. 523 and p < 0.05 (Figure: 4).

Table (1): Comparison between different studied groups of albino rats regarding memory retention testing

	Group I (Stress control)	Group II (REM deprived)	Group III (Recovery control)	F test (P value)
Percent of time spent in target quadrant	38.13±6.45	7.50 ± 3.98• •p<0.0001	17.71 ± 9.47•# •p < 0.001 #P < 0.0001	F= 26.85 (p<0.0001*)
Percent of distance swum in target quadrant	60.34±18.57	15.32±4.34• •p<0.0001	28.98±15.56•# •p<0.001 #P<0.05	F=25.65 (P< 0.0001*)
Escape trials	1.12 ±1.12	6.50±5.18• •p<0.05	4.50 ± 2.82• •p<0.005	F=14.25 (P <0.01*)

Data are presented as mean±SD *significant • Significant vs group I, #significantvs group II

Table (2): Hippocampus Brain derived neurotrophic factor (BDNF) (pg /mg protein) in the different studied groups of albino rats

BDNF (hippocampus) (pg /mg protein)	Group I (Stress control)	Group II (REM deprived)	Group III (Recovery control)	F test (P value)
Min- Max **Mean ± SD**	3731- 6358 5335 ± 942.7	1261- 6420 3377±1781.4• •p< 0.05	2092- 7983 5570±1783.6# #p< 0.05	16.98 (P < 0.001*)

Data are presented as mean±SD *significant • Significant vs group I, # significant vs group II

Table (3): Brain tissue reduced glutathione (GSH) (ng/mg protein) in the different studied groups of albino rats.

GSH (ng/mg protein)	Group I (Stress control)	Group II (REM deprived)	Group III (Recovery control)	F test (P value)
Min- Max **Mean± SD**	8.20 -11.62 9.88 ±1.35	6.10 - 9.28 6.96±1.18• •p<0.0001	6.37-10.83 8.38±1.48•# •p<0.05 #p<0.05	16.85 (P<0.001*)

Data are presented as mean ± SD *significant p • Significant vs group I, #significant vs group II.

Table (4): Brain tissue melondialdehyde (MDA) (nmol /mg protein) in the different studied groups of albino rats

MDA (nmol/ mg protein)	Group I (Stress control)	Group II (REM deprived)	Group III (Recovery control)	F test (P value)
Min- Max	0.21- 0.90	2.42 - 5.89	0.21- 2.50	16.98
Mean± SD	0.49 ± 0.29	3.75 ± 1.1•	1.29 ± 0.9•#	(P<0.001*)
		•p<0.0001	•p < 0.05	
			#p<0.0001	

Data are presented as mean ± SD * Significant • Significant vs group I, # significant vs group II.

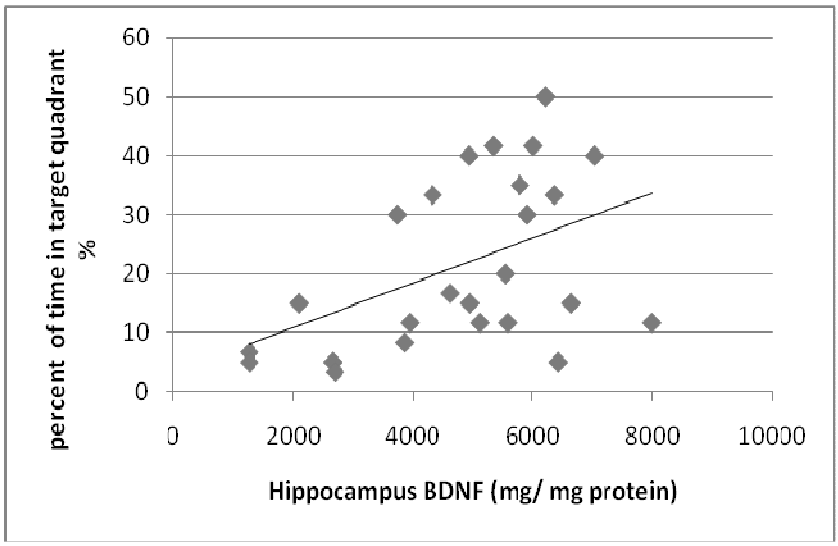

Figure 2: Correlation between level of hippocampus BDNF and performance in memory retention testing as regard the percent of time in the target quadrant of pool in albino rats

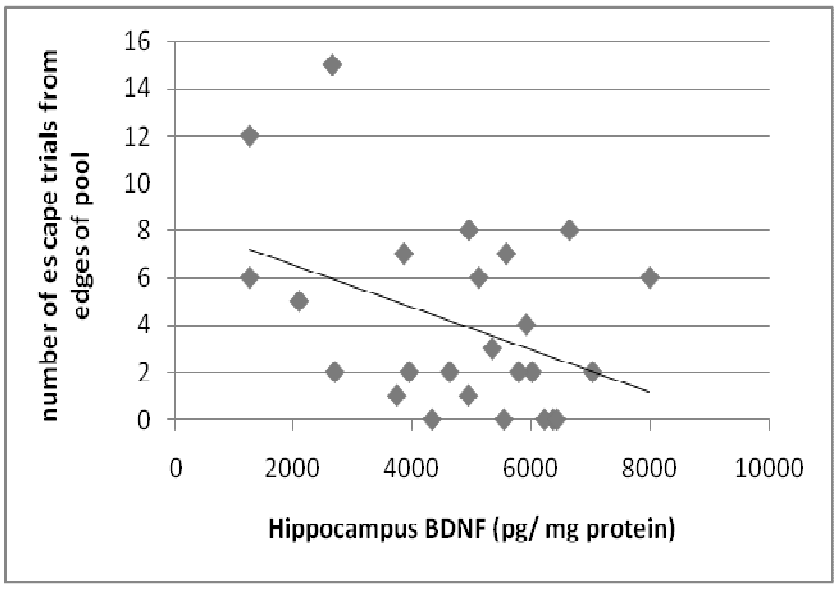

Figure 3: Correlation between level of hippocampus BDNF and performance in memory retention testing as regard the number of escape trials in albino rats.

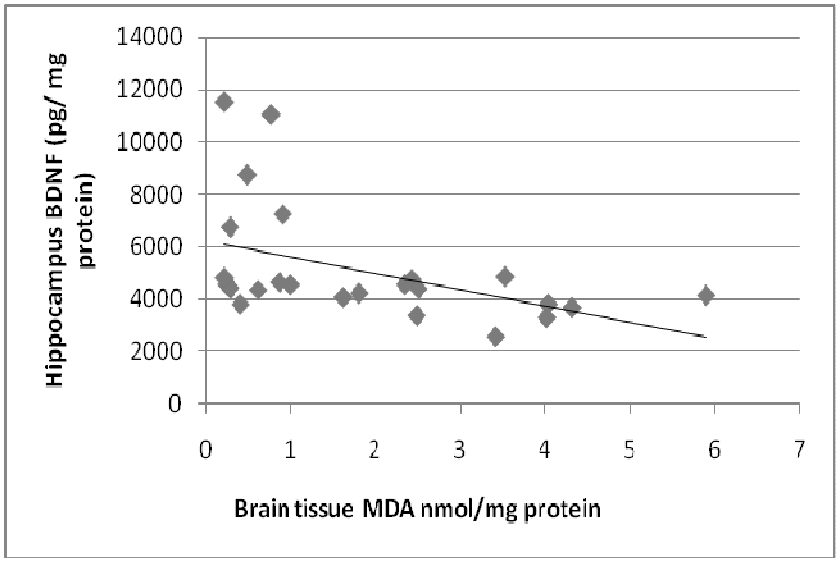

Figure 4: Correlation between level of hippocampus BDNF and brain tissue MDA in albino rats

Discussion:

Sleep loss is a common feature of many sleep disorders in humans, and for this reason analyses of behavioral and neurochemical effects seen in animal models of sleep deprivation are of considerable interest. Several studies (2,22) have shown that sleep (especially REM sleep) enhances learning and consolidation of some forms of memory. In fact it has been shown that REM sleep is increased after learning sessions.[23] On the other hand, sleep deprivation interferes with learning and memory,[24,25,26] however the exact mechanisms underlying such disturbances are still not fully elucidated.

In the present work results of water maze memory retention testing revealed that REMSD caused a significant decrease in the percent of the time spent and percent of the distance swum in the target quadrant of the pool as compared to the stress control group. It also caused a significant increase in the number of escape trials from the edges of the pool as compared to the stress control group signifying an impairment of memory retention and retrieval. In agreement with these results are the findings of Yang et al[27] who found that rats subjected to 4 hours of daily sleep deprivation for 7 days traveled a longer distance to find the hidden platform during the acquisition training and had fewer numbers of platform crossings in the memory retention testing than those in the control group during water maze training. Similarly, Kalonia et al[1] found that rats deprived of REM sleep for 72 h using a grid suspended over water, showed impairment in memory retention testing by using elevated plus maze, passive avoidance and Morris water maze tests. In addition, Alvarenga et al[3] studied the effects of REMSD for 96 h on the learning/memory processes in rats submitted to the plus-maze discriminative avoidance task, which simultaneously evaluates learning, memory, anxiety and motor function and concluded that REMSD impaired acquisition, consolidation, and recall of a discriminative avoidance task in rats.

In the present work, when rats were allowed to recover from sleep deprivation, memory retention testing revealed an improvement in memory consolidation in recovery control group as compared to REM sleep deprived group, however memory performance was less than stress control group, indicating that the improvement in memory after recovery from REMSD didn't return to baseline. Similarly, Li et al[28] found that REMSD for 48 h produced a significant impairment in retention of acquired spatial reference memory of young rats in a Morris water maze, and impairment continuously existed after 24h and 48h of recovery from sleep deprivation which indicates that REM sleep play a critical role in memory maintenance and consolidation and REMSD may play a role in long-term impairment in the

consolidation of newly acquired memories which are hippocampus dependant. The role of REM sleep in hippocampus dependant memory consolidation has been further investigated by smith et al[23] who found that REMSD disrupts memory consolidation in hidden platform version which is hippocampus dependant, while the visible platform version was not affected by REMSD as it is hippocampus independent. Furthermore other studies indicate that the firing patterns of neurons in the hippocampus during prior spatial learning tasks were found to replay during subsequent REM sleep.[29,30] In addition, Yang et al[31] demonstrated that REMSD disturbed the rhythmic activity of theta band in the hippocampus, which may be involved in the deficit of the spatial learning and memory retention testing in rats. The above studies suggest that memory consolidation in the hippocampus is affected by REMSD. Such findings prompted us to investigate the effect of REMSD on hippocamal BDNF and its relation to learning and memory deficit. BDNF is a neurotrophin that enhances the growth and differentiation of neurons and synapses.[32] In the CNS, BDNF is functionally active in the hippocampus, cortex, and forebrain, the areas that have a vital role in learning and memory.[33] The present work demonstrated that 6 days REMSD caused a significant decrease in hippocampus BDNF as compared to stress control. Recovery from REMSD caused a significant increase in hippocampus BDNF as compared to REM sleep deprived group; however, the change was insignificant when compared to the stress control group. Our findings are in agreement with Guzman-Marin et al[18] who compared the effects of short- term (8 h) and intermediate-term (48 h) sleep deprivation on the expression of BDNF in the neocortex and the hippocampus. They showed a significant decrease of mRNA of BDNF and its protein level in the hippocampus. However, neither 8 h nor 48 h sleep deprivation or control treatments had significant effects on BDNF in the neocortex. The reduction in the levels of BDNF in the hippocampus after REMSD is consistent with a finding showing that sleep deprivation reduces the protein levels of the phosphorylated form of the extracellular signal-regulated kinase 2 (phospho-ERK2), which is one of the sequences of the BDNF receptor activation.[24] Furthermore, in the present study there was a significant positive correlation between BDNF and percent of time spent in the target quadrant; in addition, there was a negative correlation between BDNF and the number of escape trials from the edges of the pool in all studied groups. These findings demonstrate the relation between BDNF and memory retention testing, where rats spent less time in the target quadrant and had more tendencies to escape from the pool as the level of BDNF decreased.

The effect of sleep deprivation induced decrease in BDNF on memory retention and consolidation could involve hippocampal synaptic plasticity. One prominent form of hippocampal synaptic plasticity is LTP, a long-lasting change in the strength of synaptic connections that is a frequently used model to study the mechan isms underlying learning and memory.[34,35] In rats, the use of forced locomotion to induce total sleep deprivation for 12 h or sleep fragmentation for 24 h, was found to impair hippocampal LTP recorded from Schaffer collateral CA1 synapses. Similar LTP deficits were also produced by REMSD using the platform-over-water technique for 24 to 75h in area CA1 both in vitro and in vivo[36,37] In addition, Marks and Wayner[38] showed that a single sleep disruption of REM sleep for 3-h reduced dentate granule cells LTP in anesthetized rats. These studies indicate that REMSD impaired LTP in CA1 and dentate granule cells of hippocampus. N- methyl –D-aspartate (NMDA) receptors are mediators of LTP induction and spatial memory in pyramidal neurons in CA1 region of the hippocampus. Numerous studies blocking NMDA receptor activity or knocking out genes for selected subunits have been carried out and have lead to reduced LTP induction and impaired spatial memory[39,40]. On the other hand, overexpression of the NR2B subunit of the NMDA receptor leads to an enhancement of learning and memory in mice[41]. McDermott et al[34] found that 72h of REMSD results in a lower density of surface NMDA receptors; whereas modifications that enhance NMDA receptor activity restore the ability of these synapses to undergo potentiation. These observations may explain the reduced ability to induce LTP due to reduction of BDNF which is considered a key protein in hippocampal synaptic plasticity. This may be considered as one of mechanisms that explain sleep deprivation-induced memory impairments.

Several explanations have been proposed for the decrease of BDNF during REMSD, it

was suggested that the decrease in BDNF could be attributed to decrease of hippocmpal theta wave activity that was shown to occur in REMSD. Theta wave activity in REM sleep is thought to enhance hippocampus LTP, BDNF gene expression and its release from stored vesicles.[42] It has also been suggested that sleep deprivation-induced memory deficits might possibly be due to the combined effect of oxidative damage and alterations in several neurotransmitter levels.[1] Therefore in the present study we measured oxidative stress markers to investigate their role as a possible mechanism for reduction of BDNF and subsequently impairment of memory. We found that REMSD for 6 days caused a significant decrease of GSH and an increase of MDA as compared to the stress control group. In addition, Recovery from REMSD caused a significant increase in GSH and decreased MDA as compared to REM sleep deprived group. However, the recovery caused a significant decrease in GSH and an increase in MDA as compared to stress control group, indicating that recovery from sleep deprivation didn't return measured oxidative measures to basal line. Similarly, Khadrawy et al[43] suggested that the hippocampus is more vulnerable to oxidative stress resulting from REMSD. Their finding was supported by the significant increase in nitric oxide in the hippocampus after 72 h of sleep deprivation. Kalonia et al[1] found that 72 h REM deprivation caused a significant decrease in the levels of endogenous antioxidants such as reduced glutathione and catalase, and an increase in the levels of lipid peroxidation and nitrite, resulting in the generation of free radicals, leading to oxidative stress in the brain.

In the current work, a negative correlation was found between level of hippocampus BDNF and brain tissue MDA. Increase oxidative stress may lead to reduction of BDNF. Similarly, Kapczinski et al,[44] found that alterations in oxidative status may be mechanistically associated with abnormal low levels of BDNF observed in individuals with bipolar disorder. The above findings agree with previous studies that showed that increase oxidative stress may lead to reduction of BDNF.[44,45] Several mechanisms by which oxidative stress could decrease BDNF have been suggested, including decreased CREB[46] which is associated with reduction of BDNF gene expression. In addition, increase oxidative stress results in energy depletion and then impairs NMDA channel function. The impairment of NMDA channel function leads to reduction of BDNF gene expression.[44]

Sleep normally involves a process of detoxification at the cellular level. Reimund[47], concluded that free radicals accumulate during wakefulness and are removed during sleep. Their removal during sleep is accomplished by the efficiency of endogenous antioxidant mechanism. Thus sleep essentially has antioxidative role. In addition, the brain has been shown to be more sensitive to oxidative damage as a result of the abundance of polyunsaturated fatty acids, deficient antioxidant defense systems, and a high rate of energy use resulting from the higher metabolic rate.[48] Sleep deprivation on the other hand has been shown to cause oxidative stress resulting in the formation of reactive oxygen species and eventually leading to neuronal and cellular damage, and reduces endogenous antioxidant defense in areas of the brain responsible for memory, particularly the hippocampus, which may be partially responsible for learning and memory deficits.[49,50] Increased brain oxidative stress seems to have an important role in cognitive impairment caused by normal aging and neurodegenerative diseases such as Alzheimer's disease, treatment of chronic sleep deprivation may be of value in preventing such disease.

Conclusions: The present study supports the involvement of reduced hippocampal BDNF in the memory deficits induced by REMSD, and hypothesis that the increased brain oxidative stress markers may be one of possible mechanisms for reduction of BDNF and subsequently impairment of memory. Sleep recovery after REMSD is effective in reduction of oxidative stress and memory deficit but not to base line emphasizing the importance of implementing prompt treatment of sleep deprivation to prevent memory deficit.

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