The impact of sleep deprivation on hippocampal-mediated learning and memory in rats

Saygin M1,2, Ozguner MF1,2, Onder O2,3, Doguc DK4, Ilhan I4, Peker Y5,6

Abstract

BACKGROUND: To examine the impact of different types of sleep deprivation on hippocampal-mediated learning and memory in rats.

METHODS: Forty-eight Sprague-Dawley male rats were randomly assigned to 1 of 4 equal-size groups: (1) 12 hours of sleep per day (control), (2) total sleep deprivation (TSD), (3) rapid eye movement (REM) deprivation (RD), and (4) sleep restricted to 4 hours per day (SR). All rats were subjected to swimming training in the Morris water maze (MWM). At the end of the experiments, the rats were decapitated, and hippocampus tissue was analyzed for several neurotransmitters and receptors.

RESULTS: The time spent at the target quadrant increased from 20.2 to 30.0 seconds in the control group on the third day of the experiment, whereas corresponding values increased from 20.2 to 21.8 seconds in the TSD group, 22.1 to 25.4 seconds in the RD group, and 21.2 to 32.0 seconds in the SR group (p = 0.026). On the seventh day of the experiment, the values decreased to 25.0 seconds in controls, 22.5 in the RD group, and 23.6 in the SR group (p = 0.045). The TSD group demonstrated significant decreases in glutamate and serotonin levels compared with the control group. There was a significant increase in 5-HT2α receptor expression in all intervention groups compared with the controls.

CONCLUSIONS: Our results of glutamate levels and 5-HT2α receptor expression in the hippocampus seem to be primarily involved in sleep and memory regulation (Tab. 2, Fig. 4, Ref. 59). Text in PDF www.eils.sk.

Key words: sleep disorders, learning and memory, 5-HT2α, NMDA, a7, glutamate, serotonin, acetylcholine.

Introduction

As scientists have sought to determine the function of sleep, research results have increasingly pointed to the important role that sleep plays in synaptic plasticity, reorganization of the brain, and memory processing (1). Much of this work has taken place in studies that employed sleep deprivation, the results of which have shown that learning that takes place before or after sleep deprivation results in memory deficits in both humans (2) and animals (3, 4). In rodents, the relationship between sleep and memory consolidation has primarily been studied through the testing of spatial memory, which is dependent on the hippocampus (5).

A number of studies that have incorporated sleep deprivation in animals have explored the impact of potential cellular and molecular changes on learning, such as neurotransmitter levels (6), impairment of formation of long-term potentiation (cellular correlate of learning and memory); (7), and effects on levels of extracellular signal-regulated kinases (8). However, the molecular mechanism of changes in neuronal properties induced by sleep deprivation remains poorly understood.

A number of methods can be used to assess the role of sleep in learning and memory processes in animals. One is to record sleep patterns after acquisition of a learning task. Previous studies have shown that rapid eye movement (REM) sleep increases following learning sessions (9, 10). The synaptic activities that occur during learning initiate complex molecular cascades through the activation of different related receptors, intracellular signal messengers, and transcription factors. These receptors, messengers, and transcription factors play essential roles in regulating memory-related gene expression and protein synthesis that are necessary for the acquisition of long-term memory. Sleep deprivation has a strong impact on memory: sleep deprivation that occurs before learning results in reduced learning ability and impaired memory, whereas sleep deprivation that occurs after learning impairs memory formation (11). The question is then, what happens to hippocampal cell pro-

1Department of Physiology, Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey.
2Department of Pulmonary Diseases, Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey.
3Department of Medical Biochemistry, Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey.
4Department of Molecular and Clinical Medicine, Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden.
5Department of Pulmonary Medicine, Marmara University, Istanbul, Turkey.
6Department of Physiology, Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey.

Address for correspondence: M. Saygin, Dr, PhD, Department of Physiology, School of Medicine, Suleyman Demirel University, Morfoloji Binasi, Cunur, TR-32260 Isparta, Turkey.
Phone: +90.246.2113604, Fax: +90.246.2371165

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The activity of the neural networks within the hippocampus can be modulated by the induction of long-term potentiating, (13) as evidenced by Kim et al (2005) (14) who showed that sleep deprivation impairs long-term potentiating area CA1 of the hippocampus. The released glutamate binds to the glutamatergic N-methyl-D-aspartate (NMDA) receptors on the postsynaptic membrane, causing a large influx of calcium ions. The increased intracellular level of calcium ions activates various kinases, including calcium-calmodulin dependent kinase II (CaMKII), which is generally known to be the regulator of short-term memory and long-term potentiation (15).

The cholinergic system and others, such as the glutamatergic, dopaminergic, and serotonergic systems, are known to be involved in learning and memory. Modulation of 1 or more of these neurochemical systems may affect different stages of learning and memory. NMDA receptors, which are 1 of the subtypes of the glutamate receptor, are known to play a key role in neural physiology, synaptic plasticity, and behavioural learning and memory (16).

The aim of the present study was to determine the effects of total sleep deprivation (TSD), REM deprivation (RD), and sleep restriction (SR) on rats’ ability to function in the Morris water maze (MWM) and on α7, NR2A, NR2B and 5-HT2A hippocampal receptors and glutamate, acetylcholine, and serotonin levels, which are known to be involved in learning and memory.

Materials and methods

Animals

Forty-eight Sprague-Dawley male rats, weighing 320–360 grams, were obtained from the Animal Investigation Laboratory of Suleyman Demirel University. The rats were housed individually in solid-floored cages with wood chips in a temperature-controlled (23 ± 1 °C) room for 1 week. Throughout the training and at the conclusion of each experimental procedure, the rats were given ad libitum access to water and food, with food quantity sufficient to maintain the rats’ body weights at 80 % of their free-feeding levels. On the days on which experiments took place, the food and water were provided after the testing. For 1 week prior to experimental conditions, the rats were adapted to the laboratory under 12/12-hour reverse light/dark conditions (lights off at 08:00). The animals were handled in accordance with the guidelines for animal care and experimentation of the pertinent European Communities Council Directive (86/609/EEC), and all the procedures were approved by the Ethics Committee of Suleyman Demirel University (Number: 22.11.2011/01).

Experimental groups

After the learning period, the rats were randomly assigned to 1 of 4 equal-size groups: (1) TSD, (2) RSD, (3) sleep restricted to 4 hours per day (SR), or (4) 12 hours of sleep per day (control). From Day 6 through Day 8, rats in the TSD Group were completely deprived of sleep. From Day 6 through Day 12, the rats in the other experimental groups were exposed to RD and SR, respectively.

General design

All of the groups were trained and tested in the Morris water maze (MWM) on spatial learning and memory (17). Behavioural testing was performed during the dark phase, which is the animals’ most active period (18). All rats were subjected to swimming training in the MWM float assembly (learning period) 4 times per day for 5 days. Twenty-four hours after the last training test, we implemented the TSD protocols and began conducting probe trials, hidden-platform tests, and visible-platform tests in the MWM each day for each group. Beginning on the sixth day, we implemented the TSD protocol for 3 days and the RD and SR protocols for 7 days in the respective groups. We removed the platform from the assembly for 60-second periods (probe test): for the TSD group, we conducted the visible platform test on the day before the experiment and on Day 3 of the experiment, whereas, for the RD and SR groups, we conducted the visible platform test on the day before the experiment and on Day 7 of the experiment. We conducted a probe trial test on each day of the experiment. During this test, the platform was removed, and the rats were released from the other 3 quadrants that had not previously contained the hidden platform; the rats were allowed to swim for 60 seconds. The time spent in the target quadrant in which the platform had been during the acquisition period was recorded, and these data were used as measures of spatial memory. Thereafter, the visible platform procedure a cued version of the MWM escape task was executed every day for each group except on the final day of the experiment. We included this procedure to study whether the observed effects of the drug were direct or indirect (such as impaired vision or motivation or an increase in anxiety). Each rat was released from the fourth quadrant in each trial; the visible platform was placed in different quadrants (zones 1-3) for each trial, and the escape latency to the visible platform was recorded.

Following the last probe trial, we anesthetized all of the animals by intraperitoneally injecting 2 % xylazine (10 mg/kg) and then 10 % ketamine (80 mg/kg). Rats were sacrificed, and hippocampi were rapidly removed for analysis by Western blot testing.

Sleep deprivation

Control Group

Within the 4 cages of the control group, we placed three 13-cm scale platforms. During the experimental period, the animals were provided free access to food and water. During the experiment, the control group underwent daily probe tests, and at the completion of the experiment, we performed the platform test.

TSD Group

The rats in the TSD group spent the entire 3 days of the experiment on a 3.3-cm platform (19). They were removed from the platform only during the test periods. Memory testing was performed each day with the probe test; the platform test was conducted at the conclusion of the 3 days.
RD Group
RD was produced with the well-established “flowerpot” technique, which consisted of placing the rats onto an inverted flowerpot (6.5-cm diameter) placed inside a large pail, which was filled with water up to 1 cm below the top of the flowerpot (20, 21). This technique has been shown to selectively deprive rats of REM but not non-rapid eye movement (NREM) sleep (22). When rats enter REM sleep, their muscle tone diminishes and causes them to touch the water, which arouses them. Because the animals can move from platform to platform within the multiplatform chamber, this device has been reported to produce less immobilization stress, as compared with the widely used single small-platform technique (23). Rats are awakened at this point, thereby terminating the REM episode, although they typically do not fall off the platform.

SR Group
Animals within the SR group were kept in normal cages and allowed ad libitum access to food and water. A typical light-dark cycle of 12 hours was applied. The rats slept less than 75 % of the normal amount of 12 hours of physiological sleep (24) in these experimental rats were allowed to sleep for 4 hours daily. Given that rats have an average sleep onset latency of 20.3 minutes, (25) we applied a stimulus every 15 minutes, for 5 minutes, from 00:00 to 08:00 that kept the rats awake. We continued this SR protocol for 7 days, and at the end of each day, the animals completed the probe test and post-test platform test.

The MWM
Performance in the MWM was tested in a dimly lit, soundproof room with various visual cues. The rats’ task in the MWM was to find a submerged hidden platform in a pool of water. A probe trial test in the MWM was conducted 24 hours after the last training test. This was followed by hidden- and visible-platform tests, as well as a new probe trial on each day of the intervention period. The main outcomes were the time (in seconds), spent in locating the hidden platform, as compared with the widely used single small-platform technique (23). Rats are awakened at this point, thereby terminating the REM episode, although they typically do not fall off the platform.

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Tissue analysis
At the end of the experiments, the rats were euthanized and decapitated; their brains were rapidly removed. The hippocampus was dissected on an ice packet that had been wetted with cold phosphate buffer (50 mmol/L) for further analysis of several neurotransmitters and receptors. The tissue was placed in Eppendorf tubes (Eppendorf AG; Hamburg, Germany) filled with phosphate buffer (50 mmol/L) and frozen at –80 °C until assayed.

Western blotting test
The gel electrophoresis step is included in Western blot analysis to resolve the issue of the cross-reactivity of antibodies. Anti-NR2A, anti-NR2B, anti 5-HT2A, and anti-nAChR were purchased from Millipore (Billerica, MA). Mouse monoclonal antibody to β-actin was purchased from Abcam Company (Cambridge, MA), and prestained molecular-weight marker was purchased from Sigma-Aldrich, Co. (Steinheim, Germany). All other reagents were of analytic grade or the highest grade available. Each hippocampus was weighed. The hippocampi (2 animals per preparation) were homogenized in ice-cold buffer (50 mm Tris-HCl [pH 7.5, 0.15 mol/L NaCl, 1 % Triton X-100, 1 mmol/L ethylenediaminetetraacetic acid [EDTA], 1 mmol/L ethylene glycol tetraacetic acid [EGTA], 25 mg/mL leupeptin, 25 mg/mL aprotinin, 1 mmol/L sodium orthovanadate, 10 mmol/L benzamidine, and 4 mmol/L p-nitrophenyl phosphate) after we obtained the supernatant fractions upon centrifuging the homogenates at 10,000 g for 10 minutes, an aliquot was taken for protein determination. (26) Equal amounts of protein for each sample (50 mg of protein per lane) were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis on 7.5 % minigels, blotted electrophoretically onto an immobilization membrane (Immobilon-P, Millipore), and incubated in tris-buffered saline with polysorbate 20 (Tween 20, Sigma-Aldrich, Co) (TBST; 50 mm Tris-HCl [pH 7.5–8.0], 150 mmol/L NaCl and 0.1 % polysorbate 20 containing 3 % bovine serum albumin (BSA) for 30 minutes. Blots were incubated separately overnight with anti-NR2A (1/500), anti-NR2B (1/500), anti-nAChR 7 (1/200),
anti-5-HT2A (1/200), and anti-β-actin (1/5000) in 1 % BSA. Blots were subjected to 3 additional 10-minute washings in TBST and incubated with alkaline phosphatase-conjugated monoclonal anti-rabbit IgG (1/10 000) in 1 % BSA for 1 hour at room temperature; thereafter, 3 additional washings were performed with TBST for 10 minutes. The membrane was incubated in 20 mL of fresh reagent solution (5-bromo-4-chloro-3-indolyl-phosphate with nitro blue tetrazolium; BCIP/NBT) until color development. Images of immunoblots were analyzed with a computerized image-analysis system (Kodak MD 2000 Image Station; Eastman Kodak Company, Rochester, NY). SDS-PAGE and western blot analyses were carried out on 6 independent hippocampus preparations (2 animals per group; total of 6 preparations per group) (27). Immunoblotting for β-actin was used as an internal standard to confirm equal protein loading and sample transfer. The ratio of such subunit/β-actin of all of the individual samples (control group and 3 other experiment groups simultaneously) was calculated for each blot. The mean of the control-group values for each antibody was set at 100, and each value of the experimental groups was expressed as a percentage of the control-group means (28).

Biochemical parameters
All parameters were evaluated by commercial kit. Enzyme-linked immunoassay, colorimetric, and ion-selective electrodes methods were used. Glutamate, acetylcholine, serotonin (Cusabio Wuhan Huamei Biotech, China) and 8-OH-2’-deoxyguanosine (Cayman, Cayman Chemical, Ann Arbor, MI) levels were measured with couples repeated by ELISA method using a commercially available kit. Thiobarbituric acid reactive substances and nitric oxide levels were measured with couples repeatedly using the colorimetric method with commercial kits (Cayman, Cayman Chemical, Ann Arbor, MI). Ca²⁺, Mg²⁺, and Na⁺ levels were measured by the ion-selective electrodes method.

Statistical analysis
Data were analyzed with SPSS® 22.0 for Windows (SPSS Inc, Chicago, IL). The Kolmogorov–Smirnov test was used to test for normal distribution of continuous variables. Data characterized by a normal distribution were expressed as mean ± standard deviation. Parameters of nonnormal distribution were expressed as median and range. The Student t-test (normal distribution) or the Mann–Whitney test (nonnormal distribution) was used for comparing the 2 groups. Continuous variables were reported as mean ± standard deviation, and, categorical variables, as percentages. To compare continuous variables, the Student t-test or Mann–Whitney U test was used where appropriate. Categorical variables were compared with the χ² test. Analysis of variance (normal distribution) or the Kruskal–Wallis (nonnormal distribution) test was used for comparing more than 2 groups. First, we assessed the data for homogeneity of variance using the Levene test. MWM data were analyzed using repeated measures and 1-way analysis of variance followed by the Bonferroni posthoc test. Repeated-measure tests were used for intragroup comparisons. These data are expressed as mean ± standard deviation, and a p value of less than 0.05 was considered as statistically significant.

Because the receptor-expression data were nonhomogeneous, we used the Kruskal–Wallis test for group comparisons. When we encountered significant differences, we used the Bonferroni-corrected Mann–Whitney U test to determine which group was responsible for the difference. These results are expressed as mean ± SEM, and a p value of less than 0.001 was considered to be statistically significant.

Results

MWM: Learning Period
Latency to locate the hidden platform
Day-to-day intragroup comparisons showed that MWM performances were significantly improved by Day 2 and Day 4 when compared with the performance on Day 1 (p = 0.01), indicating that the rats had learned the task. During the training period, comparison of spatial learning times between groups showed no significant differences. Latencies to find the hidden platform in all groups decreased as the experiment progressed, indicating that the rats were learning, but between-groups, the differences were not statistically significant.

Memory Period
Time spent in the target quadrant
Analysis of data from the probe trial day-to-day time spent in the target quadrant using repeated-measure tests showed no significant differences among the control, RD, and SR groups. However, time spent in the target quadrant was increased in the TSD group, as compared with the SR group on the third day (p = 0.001). Time spent in the target quadrant was significantly different when comparing the TSD and SR groups (p = 0.044). Within the SR group, time in the target quadrant decreased from day 3 to day 7 (p = 0.013).

Swim speeds
Swim speeds were calculated by sampling path lengths (cm) and measuring the time (sec) that each rat spent locating the hidden platform. To evaluate the swim speeds, we used data from the probe trial (Days 1–7) and visible-platform procedure (Days 0, 3, and 7). When comparing the data, distance travelled showed statistically significant differences between the TSD and SR groups on the third day (p = 0.023), and swim speeds increased for the TSD group. Our analysis showed a significant difference (a decrease) between Days 0 and 7 in the control (p = 0.006) and SR (p = 0.001) groups. Differences were also found between the control and RD groups (p = 0.011), with swim speed increased by the seventh day in the RD group and decreased in the control group. Our analysis also revealed significant differences between the RD and SR groups (p = 0.05), with swim speed increased on the seventh day for the RD group and decreased for SR group.

Latency to locate the visible platform
Analysis of data obtained after the learning period revealed no significant differences among groups with respect to latency to visible platform, average swimming speed, and total swimming
time; however, within each group, average swim speed increased from day 0 to day 3 \( (p = 0.05) \), and latency to visible platform \( (p = 0.001) \), average swim speeds \( (p = 0.000) \), and total swimming time \( (p = 0.032) \) differed within groups as well between days 0 and 7.

Western blot

Using the Western blot method, we found no between-group differences in the optical densities of the glutamatergic NR2A and NR2B or a7 nicotinic acetylcholine (nAChR) receptors (all \( p \) values > 0.05); however, 5-HT2A protein expression was significantly increased in all treatment groups, as compared with in the control group (Tab. 1, Figs 1 and 2).

Biochemical results

Neurotransmitters

As compared with the control group, no statistically significant differences were found in acetylcholine levels in any of the experimental groups. However, differences were found between experimental groups with regard to glutamate levels (TSD vs RD and TSD vs SR; \( p = 0.021 \)) (Fig. 3). Compared with the control
group, the TSD group had a statistically significant decrease in serotonin levels (p = 0.029) while the levels were increased in the RD (p = 0.043) and SR groups (p = 0.021) (Fig. 4).

Nitric oxide, 8-OH-2’-deoxyguanosine, TBARS
Ca²⁺ and Na⁺² levels compared were not significantly different when comparing experimental and control groups (p > 0.05); however, when comparing the control and SR, we found statistically significant differences in Mg²⁺ levels (ie, decreased in the SR group; p = 0.021). All biochemical parameters are shown in Table 2.

Discussion

Previous neuroanatomical and electrophysiological studies have clearly established the role of hippocampal formation on memory (29) with spatial memory having been shown to be strongly dependent on hippocampal activity in rats (30). The results of the current study show that sleep deprivation and sleep restriction have obvious effects on the serotonergic system of hippocampus-dependent memory (29) with spatial memory having been shown to be strongly dependent on hippocampal activity in rats (30). The results of the current study show that sleep deprivation and sleep restriction have obvious effects on the serotonergic system of hippocampus-dependent memory (29, 30). The results of the current study show that sleep deprivation and sleep restriction have obvious effects on the serotonergic system of hippocampus-dependent memory (29).

The relationship between sleep deprivation and oxidative stress has been investigated in many studies, but the results are conflicting (42, 43). Serum levels of nitric oxide (NO), 8-hydroxy-2’-deoxyguanosine, and thiobarbituric acid reactive substances (TBARS) have not been found to differ significantly between animals that

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control</th>
<th>TSD</th>
<th>RD</th>
<th>SR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamate, nmol/mg protein</td>
<td>223.18±35.91</td>
<td>176.02±31.34</td>
<td>268.93±46.01</td>
<td>278.51±68.01</td>
</tr>
<tr>
<td>Acetylcholine, ng/mg protein</td>
<td>7.65±1.05</td>
<td>6.48±1.42</td>
<td>7.94±1.31</td>
<td>6.76±1.20</td>
</tr>
<tr>
<td>Serotonin, ng/mg protein</td>
<td>6.26±1.03</td>
<td>4.64±0.30</td>
<td>6.41±1.79</td>
<td>6.45±0.71</td>
</tr>
<tr>
<td>NO µmol/mg protein</td>
<td>3.49±1.14</td>
<td>3.97±1.16</td>
<td>2.63±1.01</td>
<td>3.25±1.32</td>
</tr>
<tr>
<td>8-OH-2’-deoxyguanosine pg/mg protein</td>
<td>2002.76±413.41</td>
<td>2108.04±387.49</td>
<td>2025.25±473.91</td>
<td>2245.34±195.54</td>
</tr>
<tr>
<td>TBARS nmol/mg protein</td>
<td>0.44±0.19</td>
<td>0.48±0.21</td>
<td>0.38±0.12</td>
<td>0.48±0.16</td>
</tr>
<tr>
<td>Ca²⁺ µg/mg protein</td>
<td>0.21±0.06</td>
<td>0.17±0.02</td>
<td>0.17±0.03</td>
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<tr>
<td>Mg²⁺ µg/mg protein</td>
<td>1.26±0.05</td>
<td>1.14±0.08</td>
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</tr>
<tr>
<td>Na⁺² mEq/mg</td>
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<td>0.016±0.00</td>
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</tr>
</tbody>
</table>

Tab. 2. Levels of biochemical parameters in the hippocampus tissuea.

aData are presented as mean ± SD, with 12 rats in each group. In the total sleep-deprived (TSD) and REM-deprived (RD) groups, weight decreased significantly from Day 0 to Day 7 (p = 0.001 in both groups). SR – sleep restricted; NO – nitric oxide; TBARS – thiobarbituric acid reactive substances.

The studies conducted to date have indicated that the neuronal circuits and activity patterns involved in the original learning process are reactivated when sleep takes place after the learning period and that this process may play important roles in memory consolidation (34).

When analysing the effect of sleep disorders on memory parameters, Guan et al (8) and McCoy et al (35) showed that, compared with the control group, those in the sleep-deprived group had decreased time in the target quadrant. Aricanli (36) found that rats that were deprived of REM sleep for 13 days spent less time in the target quadrant, compared with those in the control group, but the results were not statistically significant. Our data were similar to those of McCoy and Guan but different from Aricanli’s results. Aleisa et al (37) performed 24-hour REM deprivation in 2 groups of rats with and without nicotine and showed that the nicotine group found a secret platform with significantly fewer mistakes, leading the researchers to conclude that REM deprivation impaired long-term memory and that nicotine decreased this effect. Wang et al (32) and Hajali et al (33) found that rats in our TSD, RD, and SR groups had increased levels of anxiety, indicated by their faster swimming and time to find the platform.

The increase in swimming speed could be explained by the increased activity of the REM-deprived animals, resulting from the increased levels of sympathetic activity, irritability, and hyper excitability. These studies show that selective RD impairs the reinforcement of hippocampus-dependent memory. (41).

Youngblood et al (39, 40) found a significant difference in the swimming speed in rats after 4 days of RD compared with the control group. When Yang et al (38) deprived rats of REM sleep for 5 days, the rats swam faster, compared with rats in the control group, but the difference was not significant. The increase in swimming speed could be explained by the increased activity of the REM-deprived animals, resulting from the increased levels of sympathetic activity, irritability, and hyper excitability. These studies show that selective RD impairs the reinforcement of hippocampus-dependent memory. (41).

The relationship between sleep deprivation and oxidative stress has been investigated in many studies, but the results are conflicting (42, 43). Serum levels of nitric oxide (NO), 8-hydroxy-2’-deoxyguanosine, and thiobarbituric acid reactive substances (TBARS) have not been found to differ significantly between animals that
were and were not sleep deprived. Although the mechanism of NO being an important neuromodulator has not been elucidated, NO has been shown to regulate the sleep-wake cycle (44, 45). In our study, NO levels increased in the TSD group, results that are comparable with those of other studies. Likewise, our levels of 8-hydroxy-2-deoxyguanosine and TBARS were comparable with those of D’Almeida et al (46). Our study did not find increased measures of oxidative stress parameters, which can be explained by the lack of exotoxicity in hippocampal cells. The changes in biochemical parameters identified by us might be related to the impaired memory functions as a consequence of sleep deprivation. In the SR group, Mg2+ levels dropped after sleep deprivation. NMDA receptor channels connected to the cation of Mg2+ binding site throughout the membrane that block by magnesium ions this connection is agonist and voltage-dependent. In this case, increased glutamate levels and NMDA-channel activation caused intracellular Ca2+ entrance and excitation. This excitation resulted in protein synthesis related to memory. Further studies are needed to understand how to modulate the ion currents of neurons to prevent the effects of sleep deprivation (47).

In our study, we found that rats in the TSD and RD groups lost weight after the experiments. Kumar and Singh (45) observed a similar weight loss sleep in their animals after 3 days of sleep deprivation, which could be explained by a stress-induced increase in energy expenditure. Yang et al (38) and Mohammed et al (48) five- and three-day studies of RD, respectively, likewise identified weight loss in rats. They attributed this weight loss to impaired physiologic mechanisms related to stress, which may induce excessive energy consumption.

The glutamate levels of rats in Mohammed et al (48) study were increased after the aforementioned 3 days of RD. When comparing control animals and those that were sleep-deprived for 24 hours, Chen et al (49) were unable to demonstrate a significant difference in NR2A or NR2B receptors. In addition, they found a significant decrease in NMDA receptor-mediated excitatory postsynaptic potentials in hippocampal slices and hippocampus-mediated memory related to synaptic plasticity in the hippocampal NMDA receptors; their results were similar to ours.

The primary observation in our present study is that TSD, RD, and SR significantly increased 5-HT2A receptor expression in the hippocampus. 5-HT2A receptors were upregulated throughout the period of sleep deprivation. Serotonin is known to play an important role in regulating sleep and wakefulness, and the 5-HT2A receptor is involved in modulating slow-wave sleep (50, 51). In turn, higher serotonin levels result in downregulation of the 5-HT2A receptor, which is a G protein-coupled receptor. In our study, we observed an increase in 5-HT2A receptor expression in the hippocampus tissue of alternate groups, which was reflected in a change in inositol-triphosphate (IP3) and Ca2+ levels, and, in turn, resulted from the increase in G protein and stimulated protein kinase C.

Basal serotonin concentrations in the rat brain are approximately 0.5 Nm (51) and rise several-fold with sleep deprivation (52). Several studies that measured hippocampal serotonin levels by microdialysis methods showed that the maximum release of serotonin occurs during wakefulness and is decreased in NREM sleep, with further reductions occurring during REM sleep (53). Short-term sleep deprivation increases levels of 5-HT in the hippocampal cycle, with levels quickly returning to normal during sleep rebound (53, 54). When Senthilvelan et al deprived rats of REM sleep for 24 hours, the 5-HT cycle, measured after resting for 3 h and 12 h, was increased (55).

Increases in 5-HT2A receptors reinforce learning and memory; conversely, receptor antagonists have a negative impact on learning (56). The 5-HT2A receptor antagonist volinanserin MDL 100907 (MDL) increases sleep and decreases wakefulness in rats. In addition, Morairty et al have shown that 3 different doses of MDL increase sleep in general, as well as NREM sleep and delta sleep (57). The 5-HT2A receptor indirectly controls glutamatergic signaling. Furthermore, slow wave sleep and REM sleep are generally associated with decreased glutamate levels (58), and antagonism of NMDA receptors increases delta power in NREM sleep in rats (59). With regard to the functional consequences of these regulatory processes, we found that sleep deprivation led to an increase in the 5-HT2A receptors.

As did Lopez-Rodriguez et al (52) we found significantly decreased levels of serotonin in the hippocampus of rat brains in our TSD group, a factor that was related to increased expression of 5-HT2A receptors. However, serotonin levels were not significantly decreased in the RD group, which was explained by a decrease in serotonin secretion in NREM sleep, a reduction that continues in REM sleep (53). When Youngblood et al (39) studied the hippocampal serotonergic system in detail in rats that were sleep-deprived for 4 days and given dietary valine, they did not find the concentration of serotonin to be significantly different in the sleep-deprived versus control rats; however, an indicator of serotonin metabolism, 5-hydroxyindoleacetic acid, was significantly increased. Similarly, we found increased 5-HT2A receptors related to sleep deprivation in our rats and showed that a serotonin-related mechanism of spatial memory may be regulated by 5-HT2A receptors in the rat hippocampus.

Conclusion

We conclude that TSD as well as RD have significant adverse effects on memory, whereas short-term improvements seem to occur in rats undergoing SR. Glutamate levels and 5-HT2A receptor expression in the hippocampus seem to be primarily involved in sleep and memory regulation. These receptors mediate long-term memory affected by TSD and RD. Circadian sleep disorders causing long-term memory impairment by LTD regulate 5-HT receptor. These phenomena might target receptor of 5-HT for long-term memory impairment related to circadian sleep disorders.

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