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Research report

The neuroprotective effect of vitamin E on chronic sleep deprivation-induced memory impairment: The role of oxidative stress

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ABSTRACT

Sleep deprivation induces oxidative stress and impairs learning and memory processes. Vitamin E, on the other hand, is a strong antioxidant that has neuroprotective effect on the brain. In this study, we examined the potential protective effect of chronic administration of vitamin E on chronic sleep deprivation-induced cognitive impairment. In addition, possible molecular targets for vitamin E effects on chronic sleep deprivation-induced cognitive impairment were determined. Sleep deprivation was induced in rats using modified multiple platform model. Vitamin E (100 mg/kg) was administered to animals by oral gavage. Behavioral study was conducted to test the spatial learning and memory using the radial arm water maze (RAWM). In addition, the hippocampus was dissected out and antioxidant markers including glutathione (GSH), oxidized glutathione (GSSG) and GSH/GSSG, glutathione peroxidase (GPx), catalase, and superoxide dismutase (SOD) were assessed. The results of this project revealed that chronic sleep deprivation impaired both (short- and long-term) memories ($P < 0.05$), while vitamin E treatment prevented such effect. Additionally, vitamin E normalized chronic sleep deprivation-induced reduction in the hippocampus GSH/GSSG ratio, and activity of catalase, SOD, and GPx. In conclusion, sleep deprivation induces memory impairment, and treatment with vitamin E prevented this impairment probably through its antioxidant action in the hippocampus.

1. Introduction

Humans spend one-third of their life sleeping, which reflects the fact that sleep seems to be vitally important for human being. Sleep consists mainly of two stages non-rapid eye movement, and rapid eye movement (REM) [1]. A central cognitive function of sleep is to consolidate newly acquired memories for long-term storage [2]. Many studies proved the link of learning and memory consolidation to REM sleep through the observation that the duration of REM sleep increases after learning tasks [3,4].

Sleep deprivation has been shown to induce memory deficit in animal studies [5–10]. The mechanism of such impairment is still unknown but in general, sleep deprivation increases oxidative stress in the hippocampus and many regions in the brain [11–14] that is usually detoxified by sleep [15].

Vitamin E is an essential nutrient in humans and well known antioxidant substance. It reduces free radicals and reactive oxygen species activity. Like other antioxidants, vitamin E slows or prevents memory impairments that accompany several conditions such as mental stress [16], diabetes [17], cerebral ischemic injury [18], Alzheimer's disease [19,20], stroke [21] and aging [22]. On the other hand, studies showed a shared link between endogenous plasma concentration of vitamin E and cognition status [23,24].

In this study, we investigated the hypothesis that chronic vitamin E supplementation prevents chronic sleep deprivation-induced impairment of hippocampal learning and memory via its anti-oxidative properties. Both behavioral approach using the radial arm water maze (RAWM) to test learning and memory functions, and molecular enzymatic assays approach were used to test this hypothesis.

2. Methods

2.1. Animals and treatments

Young adult male Wistar rats weighing 150–250 g were used in this study. The animals were housed in metal cages (six rats per cage) under hygienic conditions and maintained at 24 °C and 12 h light/dark cycle (light on at 8 am) with free access to food and water. All experimental procedures were performed during the light cycle. Before starting the experiment, animals were allowed to stay in the same cage for two weeks to establish a social hierarchy within the group. All procedures

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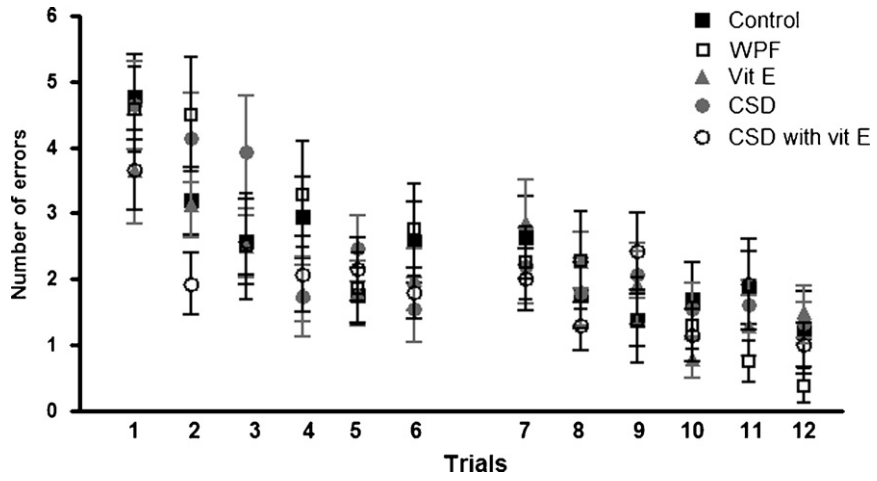

Fig. 1. Animal learning performance in the radial arm water maze. Comparison of control (control), wide platform (WPF), vitamin E (Vit E) 100 mg/kg, chronic sleep deprivation (CSD) and chronic sleep deprivation with vitamin E (CSD with Vit E). *Indicates significant difference from other groups, ($P < 0.05$; $n = 12$–14/group).

were approved by Animal Care and Use Committee (ACUC) at Jordan University of Science and Technology.

Animals were randomly assigned into five groups, each containing 12–14 rats. Control, vitamin E (Vit E), chronic sleep deprivation (CSD), chronic sleep deprivation with vitamin E, and wide platform form (WPF). The Vit E and the CSD with Vit E groups were treated with vitamin E (α-tocopherol, Sigma, St. Louis, MO) at a dose of 100 mg/kg once daily via oral gavage for 6 weeks. Similar dose, route of administration, and duration were previously used by other groups, and were shown to be protective for cognitive functions [17,25,26]. The control, CSD and WPF groups were administered vehicle once daily for 6 weeks by oral gavage. All the treatments were administered between 8:00 and 9:00 am. Right after dosing, both the CSD and CSD with Vit E groups were subjected to REM-SD; 8 h/day for 6 weeks.

2.2. Induction of sleep deprivation

Chronic sleep deprivation was induced using columns-in-water (modified multiple platform) model as described [5–8,27]. Briefly, animals were placed on platforms (20 platforms; 20 cm high and 5 cm diameter, 7 cm apart edge-to-edge) surrounded by water ($24 \pm 1\,^{\circ}C$) in an aquarium where water and food were accessible to animals. The water level in the aquarium was about 4 cm below the edge of the platform. This method has been reported to interfere with total sleep, but it mainly eliminates REM sleep [28]. Loss of muscle tone during REM sleep caused animals to fall into the water and waken. Furthermore, to test the possible effects of stresses of the tank environment, wide platforms (diameter: 12 cm) were used to allow the WPF rats to sleep without falling in the water.

2.3. Behavioral test: radial arm water maze

All animal groups were tested for spatial learning and memory performance on the radial arm water maze [29–33]. This test was carried out only once after six weeks of sleep deprivation and/or vitamin E administration. The radial arm water maze contains six swim paths (stainless steel arms) extending out of black central area tube. With a hidden platform located at the end of one arm (the goal arm). The experiments were done in dimly lit room with two different pictures on the walls which serve as cues for the rats. The animals must find the hidden platform on the goal arm. The goal arm was not changed for a particular rat in a single day. At the morning of the testing day, rats were allowed six consequent trials separated by 5 min rest, then another six consequent trials (acquisition phase), followed by 30 min short-term memory and 5 h and 24 h long-term memory tests. Every trial starts in different arm except the goal arm for a particular rat. In each trial, the rat was allowed to swim freely in the maze for 1 min or until the rat finds the hidden platform. Once the rat is on the platform, the rat was allowed 15 s to observe visual cues before the next trial. When the rat was unable to find the hidden platform the allowed 1 min period, the experimenter guided it toward the platform where it was allowed to remain for 15 s to observe visual cues. Each time the rat enter an arm other than goal arm, an error is counted. Correct entry occurred when the whole body of the rat (not including the tail) is inside the arm.

2.4. Hippocampus dissection

Animals were killed after 6 weeks of vitamin E and/or CSD. Dissection was carried out as described in Refs. [34,35]. Briefly, the brains were removed immediately from the skull, and placed on a filter paper containing 0.2 M ice-cold sucrose solution, over a glass plate filled with crushed ice. Dissected hippocampus parts were placed in test tubes then, immediately, transferred into liquid nitrogen and stored frozen until time of tissue processing.

2.5. Calorimetric immunoassays

To determine activities or levels of oxidative stress enzymes, hippocampus tissues were homogenized manually using small pestle in lysis buffer (137 mM NaCl, 20 mM Tris–HCl pH 8.0, 1% Nonyl phenyl polyethylene glycol ether with 20 molecules ethylene oxide, 10% glycerol, 0.5 mM sodium vanadate, 1 mM polymethane sulfonyl floride), and protease inhibitor cocktail (Sigma–Aldrich Corp., MI, USA). Homogenates were centrifuged to remove insoluble material ($14{,}000 \times g$ for 5 min, $4\,^{\circ}C$). Thereafter, homogenates were divided into several aliquots; each of them was used for only a single assay. All the work was carried out over crushed ice. Total protein concentration was estimated using commercially available kit (BioRAD, Hercules, CA, USA). To quantify total GSH, tissues homogenates were deproteinized with 5% of 5-sulfosalicylic acid (SSA), centrifuged ($10{,}000 \times g$ for 10 min, $4\,^{\circ}C$) to remove the precipitated protein, and then assayed photometrically for glutathione according to manufacturer's instructions (Glutathione Assay Kit, Sensitivity: 1 nmol/mL, Sigma–Aldrich Corp., MI, USA). Colors were read at 405 nm using an automated plate reader (ELx800, Bio-teak instruments, plate reader, Highland park, Winooski, USA). For GSSG measurement, 10 μL of 1 M 2-vinylpyridine (Glutathione Assay Kit, Sigma–Aldrich Corp., MI, USA) was added per 1 mL of supernatant of the sample, then the procedure was carried as described above for GSH. For all the assays and to ensure minimum variability between experimental runs, each single plate contained samples from every experimental groups. GSH was calculated by subtracting total glutathione species value from GSSG value.

Activity of GPx was determined using Glutathione Peroxidase Cellular Activity Assay Kit according to manufacturer's instruction (CGP1, Sensitivity: 0.005 units/mL, Sigma–Aldrich, MI, USA). In brief, 10 μL of tissue homogenate was added to a 990 μL reaction mixture containing 0.25 mM NADPH, 2.1 mM reduced glutathione, 0.5 units/mL glutathione reductase, and 30 mM tert-butyl hydroperoxide. The change in absorbance of the reaction product was kinetically quantified every 10 s for 1 min at 340 nm using spectrophotometry (UV–VIS spectrophotometer, UV-1800, Shimadzu, Japan).

Catalase activity was measured using commercially available kits according to manufacturer's instructions (Catalase: Sensitivity: 2.5 units/mL, Cell Biolabs, San Diego, USA). In brief, 20 μL of each tissue homogenate was added to 50 μL hydrogen peroxide working solution, which contains 12 mM H_2O_2 in 100 mM potassium phosphate buffer, pH7.0. Then, the reaction was incubated for 1 min, and stopped by adding 50 μL of the catalase quencher provided by the kit. Finally, 200 μL of the catalase chromagen (4-amino-3-hydrazine-5-mercapto-1,2,4-triazole) was added to the mixture. After 40 min of vigorous shaking of the mixture, ELISA plates were read at 540 nm.

Activity of SOD was measured using commercially available kits according to manufacturer's instructions (SOD kit; Sensitivity: 0.001 units/mL: Sigma–Aldrich Corp., MI, USA). The assay used the Dojindo's tetrazolium salt, WST-1 (2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfo-phenyl)-2H-tetrazolium). In brief, tissue homogenate (20 μL) was mixed with 200 μL of WST-1 solution. Then, 20 μL of SOD enzyme working solution (Sigma–Aldrich Corp., MI, USA) was added to the mixture, and incubated for 20 min at $37\,^{\circ}C$. Thereafter, plates were read at 450 nm using an automated reader (ELx800, Bio-tek instruments, plate reader, Highland Park, Winooski, USA).

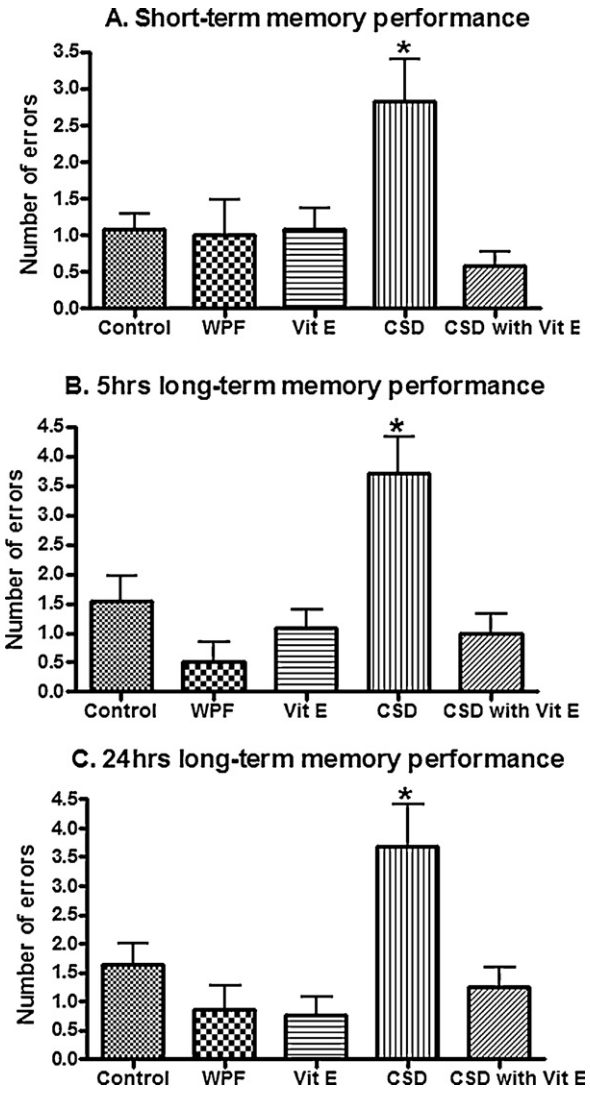

Fig. 2. Memory performance in the RAWM. Memory tests were performed short-term memory (A) 30 min, long-term memory tests (B) 5 h and (C) 24 h, after the last trial of the learning phase. Each point is the average values ±SEM from 12 to 14 rats. *Indicates significant difference from other groups, ($P < 0.05$).

2.6. Statistical analysis

One-way ANOVA followed by Tukey's test was applied to assess differences among groups. All statistical tests were carried out using GraphPad Prism software (version 4.0, GraphPad software, LA jolle, CA) followed by Tukey's post hoc test. Significance levels were considered at $P < 0.05$, and values are presented as mean ± standard error of mean (SEM).

3. Results

3.1. The effect of chronic sleep deprivation and vitamin E on learning and memory

All the animal groups learned the location of the submerged platform, as determined by the marked reduction of errors in the learning phase (trials 1–12; Fig. 1), with no significant difference among these groups in all training/learning trials.

Chronic sleep deprivation markedly impaired short-term memory, which is indicated by the observation that animals in the CSD groups made significantly more errors (2.82 ± 0.57; $P < 0.05$) in finding the hidden platform than those in the control group (1.08 ± 0.26; 1.00 ± 0.49; 1.08 ± 0.30; respectively; Fig. 2A). On the other hand, vitamin E administration prevented CSD-induced impairment of short-term memory in the RAWM. In CSD with Vit E group, animals committed significantly ($P < 0.05$) fewer errors (0.58 ± 0.19) to locate the hidden platform in short-term memory test than the untreated CSD animals (Fig. 2A). Moreover, no significant difference was observed between the control, WPF, Vit E groups, and CSD with Vit E group.

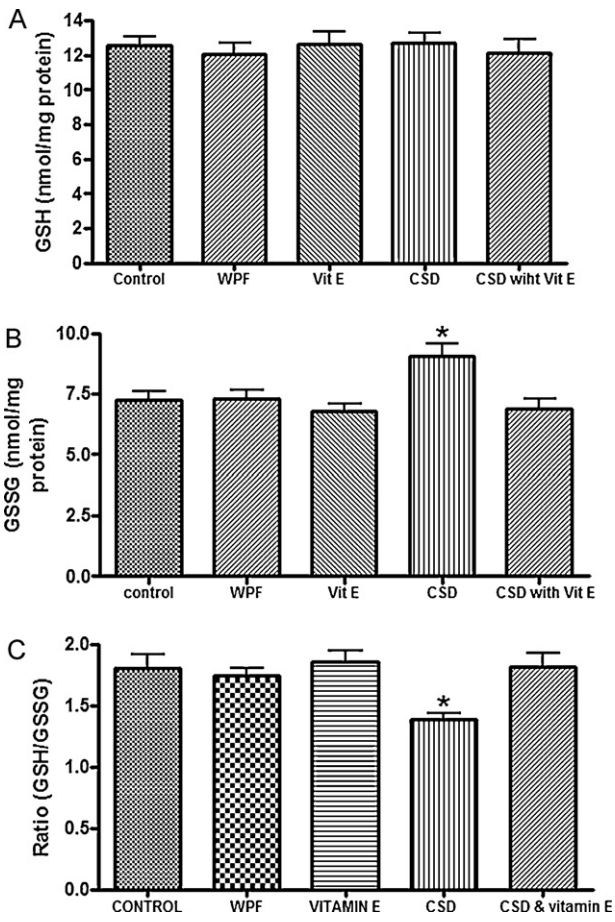

Fig. 3. Changes in glutathione levels in the hippocampus. Comparison of control, wide platform (WPF), vitamin E (Vit E) 100 mg/kg, chronic sleep deprivation (CSD) and chronic sleep deprivation with vitamin E (CSD with Vit E). A: Level of GSH. B: Level of GSSG. C: Level of GSH/GSSG ratio. Each point is the mean ± SEM. *Indicates significant difference from other groups, ($P < 0.05$, $n = 8$–10/group).

In long-term memory tests, animals in CSD group made significantly more errors (5 h: 3.71 ± 0.57; 24 h: 3.69 ± 0.68; $P < 0.05$) compared to other experimental groups. On the other hand, vitamin E administration in chronically sleep-deprived animals, significantly reduced ($P < 0.05$) the number of errors in the long-term memory tests (5 h: 1.00 ± 0.33; and 24 h: 1.25 ± 0.35; Fig. 2B and C). These results show that chronic vitamin E administration prevented sleep deprivation induced long-term memory impairment in the RAWM paradigm.

In summary, chronic sleep deprivation impaired short- and long-term memory and vitamin E administration prevents these effects.

3.2. The effect of chronic sleep deprivation and/or vitamin E on hippocampus oxidative stress markers

3.2.1. Levels of the GSH, GSSG and GSH/GSSG ratio

No change was observed in the levels of GSH among the experimental groups (Fig. 3A). The CSD group exhibited a significant increase in GSSG levels (Fig. 3B), and a significant decrease in GSH/GSSG ratio compared to other groups (Fig. 3C). On the other

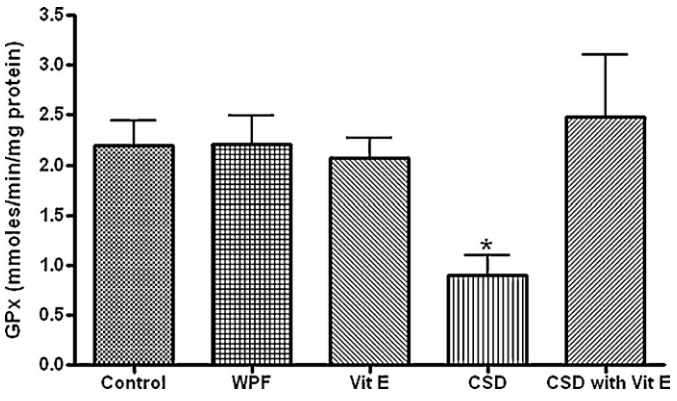

Fig. 4. Level of GPx activity in the hippocampus. Chronic sleep deprivation decreases GPx activity in the hippocampus and the decrease was normalized by Vitamin E (SD/Vit E). Vitamine E alone did not affect basal activity of GPx. Each point is the mean ± SEM. *Indicates significant difference from other groups, ($P < 0.05$, $n = 8–10$/group).

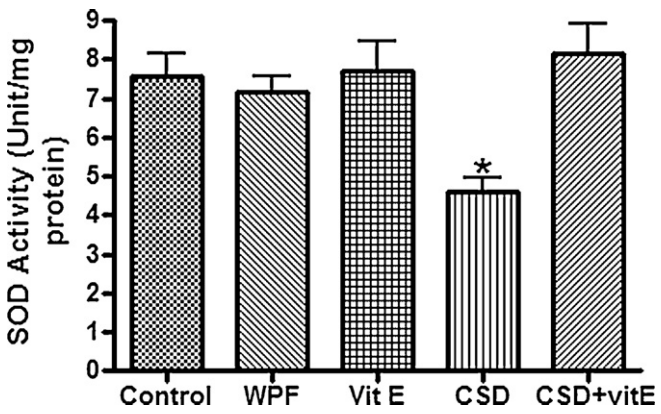

Fig. 6. SOD enzymatic activity in the hippocampus. Chronic sleep deprivation is associated with reduction in the hippocampus activity of SOD, which was normalized by vitamin E administration. Each point is the mean ± SEM. *Indicates significant difference from other groups, ($P < 0.05$, $n = 8–10$/group).

hand, CSD with Vit E group showed comparable level of GSSG (Fig. 3B) and GSH/GSSG (Fig. 3C) to those of control, WPF, Vit E groups.

3.2.2. GPx Activity

In the hippocampus, chronic sleep deprivation significantly decreased GPx levels compared to control ($P < 0.05$; Fig. 4). On the other hand, no difference was detected among the other groups (Vit E, CSD and CSD with Vit E) compared to the control group (Fig. 4), indicating that vitamin E normalizes reduced GPx level during chronic sleep deprivation.

3.2.3. Catalase activity

In the chronic sleep deprivation group, the activity of the catalase enzyme was significantly reduced compared to control group ($P < 0.05$; Fig. 5). On the other hand, no difference was observed among any of the other groups compared to the control group (Fig. 5), indicating that vitamin E normalized hippocampal catalase activity reduced by chronic sleep deprivation.

3.2.4. SOD activity

Sleep deprived animals showed significantly reduced hippocampus activity of SOD as compared to the control and Vit E groups. In the CSD with Vit E group, the activity of SOD was similar to that of the control group, indicating that vitamin E treatment normalized the activity of SOD during CSD (Fig. 6)

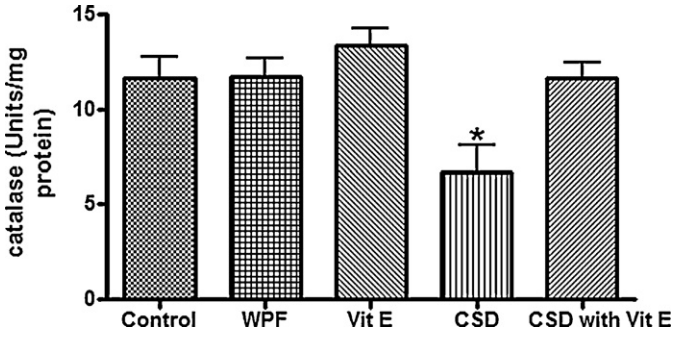

Fig. 5. Catalase enzymatic activity in the hippocampus. Comparison of control, wide platform (WPF), vitamin E (Vit E) 100 mg/kg, chronic sleep deprivation (CSD) and chronic sleep deprivation with vitamin E (CSD with Vit E). Each point is the mean ± SEM of 12–14 rats. *Indicates significant difference from other groups, ($P < 0.05$, $n = 8–10$/group).

4. Discussion

The current study shows that chronic vitamin E administration prevents (short- and long-term) memory impairment induced by chronic sleep deprivation. In addition, vitamin E normalized levels of oxidative stress markers such as GSH/GSSG ratio, and enzymatic systems including GPx, catalase and SOD that are reduced during chronic sleep deprivation.

Several lines of evidence indicate that SD interferes with memory in the hippocampus. Sleep loss was previously associated with memory impairment in both human [36–38] and animal studies [5–8,10,39–41]. Previous studies have shown that acute sleep deprivation for 24–72 h impair hippocampus-dependent short and long term memory [5–8,13]. The present results reveal that chronic sleep deprivation (8 h for 6 weeks) also impairs spatial (short- and long-term) memory. In parallel, a recent study showed that 3 h/day for 14 days of sleep deprivation resulted in memory impairment [42].

The effect of vitamin E on learning and memory impairment has been widely investigated. In one study, vitamin E-supplementation to aged rats resulted in marked retention of their memory function [43]. Furthermore, when vitamin E was given to moderately severe Alzheimer disease patients, those patients showed delayed onset of severe dementia [19]. A combination of vitamin E and C improved learning and memory in controls and reversed learning and memory deficits in diabetic rats [26]. In another study, co-administration of vitamin E with melatonin significantly improved learning and memory performance in diabetic rats compared to control rats [17]. Results of the current study are consistent with these studies in showing that vitamin E prevents chronic sleep deprivation induced short- and long-term memory impairment.

Oxidative stress has been linked to cognitive impairments in several health conditions such as aging [44], traumatic brain injury [45], and Alzheimer's disease [46–50]. In fact, majority of reports underling cognitive impairment in Alzheimer disease relate impairment to oxidative stress via decreased levels of antioxidant enzymes [46–50]. Current results indicate that chronic sleep deprivation decrease the antioxidant defense mechanism, namely, GSH/GSSG ratio, GPx, catalase, and SOD, and perhaps contribute toward impairment of both short- and long-term memory. These studies together with our findings provide initial support for the concept that oxidative stress contributes to the cellular damage and as result cognitive impairments.

Vitamin E is a powerful antioxidant that inhibits propagation of free radical reactions and prevents oxidative stress [51–56]. Results of the current study show that vitamin E normalizes reductions

in antioxidant enzyme systems (GPx, catalase, and SOD) induced by chronic sleep deprivation. Additionally, vitamin E prevents the reduction in ratio of GSH to GSSG during chronic sleep deprivation. Thus, Vitamin E might prevent memory impairment during chronic sleep deprivation via its antioxidative property. This conclusion is consistent with other studies, for example, vitamin E prevented memory impairment and normalized the level of SOD in traumatic brain injury rat model [45]. In another study, it was shown that GSH and catalase levels were decreased in association with restraint stress, while pretreatment with vitamin E normalize these effects [57]. Finally, when vitamin E was administered to an animal model of diabetes, GSH levels, and catalase and SOD activities were normalized [58].

Putative mechanisms that link oxidative stress with impaired cognitive functions during sleep deprivation are not well understood. However, it could be related to oxidative stress responsive signaling molecules such as CREB and CAMKIV. A recent study has shown that oxidative stress was accompanied with reduced levels of CAMKIV and phosphorylated CREB [59]. Both CREB and CaMKIV are essential signaling molecules for memory functions [e.g., 60, 61]. Thus, it is possible that increased oxidative stress during sleep deprivation leads to suppression of signaling molecules important for memory functions such as CREB and CaMKIV, leading to memory impairment. Vitamin E, which is an antioxidant, could be capable of preventing this sequence of events, thus, preserving memory functions during sleep deprivation.

Result of the current study show that administration of vitamin E did not affect memory functions in normal animals. However, it preserved memory during sleep-deprivation. This is in accordance with results of previous studies [e.g., 13]. Thus, vitamin E, in the dose tested; seem to function as a memory protective, but not enhancing agent, indicating that vitamin E might work only if there is an impairment in memory functions.

Our results show that chronic sleep deprivation induces reduction in antioxidant defense mechanisms. A recent study by Vollert et al. [11] has shown that acute sleep deprivation result in elevation of oxidative stress markers, and antioxidant enzyme systems in the hippocampus and certain other brain areas. Results of Vollet et al. are very important as they work hand-in-hand with current result to explain the possible reaction of the body against oxidative stress. Oxidative stress seem to be acutely associated with a compensatory increase in antioxidant defense mechanism, however, with chronic exposure to oxidative stress, these compensatory mechanisms start to fade, and reductions in antioxidant defense mechanisms is usually detected [current results, 45, 58].

The current study is unique in that in it is the first to examine the interactive effects of chronic sleep deprivation, and chronic administration of vitamin E (for 6 weeks). However, this study also has some limitations. Although the sleep deprivation paradigm – the modified multiple platform – used in this study can be affected by environmental confounds such as stress and anxiety, it has several advantages over other methods. For example, it eliminates immobilization and isolation stress seen in the single platform technique [62]. Additionally, rats in the WPF group who had the chance to sleep on wide columns in the same aquarium, did not show impairment of spatial learning and memory [current results, 5, 7, 8]. Thus, it is unlikely that factors other than direct effects of SD had major contribution to the observed deficits in the RAWM performance. In support of that, eliminating adrenal stress response to SD by the corticosterone inhibitor, metyrapone [63] or by adrenalectomy [64] does not prevent the effect of SD on learning and memory suggesting that the impairment of learning and memory during SD is not due to release of stress hormones. Moreover, stress influences the hippocampus in a substantially different way than SD. For example, 6 weeks of psychosocial stress does not affect long-term memory [65] and does not impair dentate gyrus related memory function [66]. Concerning the duration of SD, a previous study have evaluated sleep homeostasis during chronic 3 weeks exposure of animals to the modified multiple platform, and concluded that the paradigm is effective and consistent in producing chronic sleep deprivation [67]. Although this former study did not extent for longer time durations, we believe that the chronic sleep deprivation pattern is unlikely to change with the longer duration used in the current study. This is because if any adaptation or change in the sleep deprivation effect would have to occur, it will be likely to happen in the initial 3 weeks of the SD induction. Therefore, the sleep deprivation paradigm used in the current study appears to be effective for chronic sleep deprivation.

In conclusion, vitamin E prevents the deleterious effect of chronic sleep deprivation on short- and long-term memory; probably through normalizing the antioxidant defense mechanisms including GPx, catalase and SOD, and the ratio GSH/GSSG that are impaired during chronic sleep deprivation.

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