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Mild regular treadmill exercise ameliorated the detrimental effects of acute sleep deprivation on spatial memory

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ABSTRACT

Vulnerable areas like the hippocampus are sensitive to insults such as sleep deprivation (SD); they are also susceptible to environmental enrichment. Much evidence is accumulating that chronic sleep deprivation causes alterations in the hippocampus that responsible for spatial memory. However, there is conflicting about the differences between acute and chronic SD results. The purpose of this study was to determine the protective effects of mild treadmill exercise on acute SD rats.

Four groups were created as control, exercise, sleep deprivation, exercise + sleep deprivation. Multiple platforms method was used to induce REM sleep deprivation (RD) for 48 h. The exercise was applied five days per week for four weeks (5 × 4). For the first and second weeks, the length of the exercise was 15 min in two sessions (5 min interval) followed by 15 min in three, 15 min in four sessions. Morris water maze (MWM) was used as a spatial memory test. Gene level was determined by using the qPCR technique. Malondialdehyde (MDA) content in the hippocampus was measured as an extent of peroxidative damage to lipids by using the ELISA method.

48 h RD impaired long-term spatial memory significantly. Mild, regular treadmill exercise ameliorated the detrimental effects of acute sleep deprivation on memory. There was no significant difference in MDA between groups. Hippocampal gene expression did not show any changes in all groups.

Lack of correlation between memory impairment and levels of genes in the hippocampus is likely to be related to the differences in behavioral and genetic mechanisms.

1. Introduction

Sleep plays a significant role in maintaining body functions and protecting brain health. Sleep loss and sleep disorders are among the most common yet frequently overlooked and common problems in the modern era people who live in a culture that promotes reduced sleep due to the burden of work-life and social pursuits (Patrick et al., 2017). Various factors; including technology using, lifestyle, work-life, and jetlag, cause changes in sleeping patterns, and poor sleep quality is correlated with both neurotic disorders and depression (Wong et al., 2017). For adolescents, extensive television viewing and growing social and academic requests promote sleep loss or sleep problems (Wolfson and Carskadon, 1998, Johnson et al., 2004). An association between lower academic performance and short sleep duration has been indicated (Drake et al., 2003, Shin et al., 2003). Sleep deprivation affects cognitive functions and leads to problems such as coronary heart disease, hormonal deregulation (Kang et al., 2017, Rao et al.,1996), hypertension (Adachi et al., 2011), oxidative stress, and attention deficiency (Um et al., 2017).

Animal studies have demonstrated the positive effects of adequate sleep on the consolidation and storage of memories, learning, and new synapses development (Blissitt, 2001, Gais et al., 2007, Dos Santos et al., 2013, Li et al., 2017). Besides, poor sleep has been associated with deficits in learning and memory (Stickgold et al., 2000, Walker et al.,

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2003). Sleep can be primarily divided into 2 main stages: the non-rapid eye movement (non-REM) followed by a shorter period of rapid eye movement (REM) sleep that especially important in terms of the consolidation and retention of memories (Smith, 1995). However, the two sleep states are regulated by distinct processes. This has been elaborated by selective deprivation experiments (Endo et al., 1997). Non-REM sleep is considered to be oriented towards body restitution, REM sleep provides membrane stabilization in neurons and plays a role in the programming of memory. Notably, rapid eye movement (REM) sleep deprivation decreases the ability of perceptual learning and consolidation of information (Peigneux and Smith, 2011, Süer et al., 2011). Especially for complex tasks (like maze learning), which involve the integration of different information and the development of adaptive behaviours are particularly sensitive to REM sleep deprivation (Rauchs et al., 2005). There are also methodological differences between REM sleep deprivation (fragmented sleep) and total sleep deprivation (absence of sleep) (Trošt Bobić et al., 2016). In fragmented sleep deprivation (like REM or slow-wave-sleep sleep deprivation) subjects getting at least some sleep. From a sociological point of view, REM sleep disorders need to be more thoroughly investigated as people struggle with fragmented sleep disorders in their daily lives more.

Moreover, several theories demonstrated that sleep deprivation causes oxidative damage by reactive oxygen species (ROS) (Vollert et al., 2011). In our previous study, we showed that 21 days of REM sleep deprivation might damage the maintenance of long-term potentiation induced in the dentate gyrus and the balance between oxidant and antioxidant defenses of the hippocampus (Süer et al., 2011). Sleep and exercise have complex interactions that involve physiological and psychological pathways (Chennaoui et al., 2015a, Chennaoui et al., 2015b). Salary et al. demonstrated that forced and regular exercise restored spatial learning and memory impairments in female sleep-deprived rats (72 h) (Salari et al., 2015). Zagaar and colleagues also showed that treadmill exercise prevented sleep deprivation (24 h) caused deficits in late long-term potentiation (L-LTP) (Zagaar et al., 2018). Physical exercise and sleep deprivation produce contradictory effects on cognitive function and synaptic plasticity and there is no enough knowledge available how exactly the combined exercise and sleep deprivation application affects the hippocampus-dependent learning and memory (Zagaar et al., 2013).

Hippocampus is a brain area where spatial memory takes place, and the impacts of physical exercise in this region have a particular interest among the researchers (Rajizadeh et al., 2018). Hippocampus-dependent learning memory is sensitive to sleep deprivation, and cognitive impairments can be corrected by physical exercise. N-methyl D-aspartic acid (NMDA) subunits Grin2a and Grin2b that located in the hippocampus are involved in synaptic plasticity mechanism and any changes in synaptic plasticity are associated with cognitive functions (Chennaoui et al., 2015a, Chennaoui et al., 2015b). Also, moderate-intensity aerobic exercise increases the brain-derived neurotrophic factor (BDNF) that is expressed in the hippocampus and decreases inflammation (Landers et al., 2013).

One of the essential problems about exercise protocols is to define which type, intensity and duration is the most effective on cognitive processes. According to the experimental data, forced and voluntary exercise have different effects on brain neurochemistry and cognitive functions (Landers et al., 2013, Xie et al., 2015). However, in modern lifestyle, people have not a tendency to do exercise voluntarily and need induction for exercise in an obligated way. For this reason, we assumed that forced treadmill exercise is suitable for people in their daily routine exercise. In addition to the advantage of forced exercise in the daily routine, it is also thought that exercise causes more physiological burden on the body (Griesbach et al., 2012, Bilski et al., 2019). In fact, we did not observe a positive effect of forced long term treadmill exercise on learning and memory in our previous study (Cevik et al., 2018). For this reason, exercise should be both forced and turned into a more moderate (mild) type by dividing it into certain intervals like our mild long-term exercise protocol.

However, it is still challenging to understand precisely how exercise influences sleep and vice versa. Due to the differences seen in studies on exercise, a standard should be established in exercise protocols. This study aimed to examine the effects of REM sleep deprivation and mild exercise training on hippocampus-dependent learning and memory and related gene expressions such as Grin2a, Grin2b, c-Fos, and BDNF (Fig. 1).

2. Results

2.1. Spatial learning and memory alterations after chronic sleep deprivation and exercise treatment

2.1.1. Effects on locomotor motor activity

As shown in Fig. 2, during the training session, all groups improved their performance as indicated by shortened distance moved. As a result of repeated measures analysis of variance (timexgroup interaction), timexgroup interaction was not significant ($p = 0.342$, table 1). Accordingly, the effect size was 0.381.

To determine whether groups differed in their distance moved on specific days of the spatial learning task, significant interactions were further analyzed using repeated measures analysis of variance (ANOVA) and one-way analysis Tukey post hoc with Bonferroni correction. A repeated measures analysis of variance indicated that the distance moved by rats to reach the platform significantly differed between trial days in all groups (C, E, RD, RD + E respectively) ($F_{3,18} = 3.343$, $p = 0.042$; $F_{3,18} = 16.559$, $p < 0.001$; $F_{3,18} = 3.532$, $p = 0.036$; $F_{3,18} = 9.659$, $p = 0.015$, Table 1). According to the one way analysis of variance (between-group comparisons), the distance moved was also significantly different between groups ($p = 0.008$, $p = 0.004$, $p < 0.001$, $p < 0.001$ for 1., 2., 3., and 4. trial days). The results from post hoc analysis with Bonferroni correction revealed that RD and E group moved significantly higher distances compared to the C group ($p = 0.002$, $p = 0.001$) on the first day. RD group had longer length moved compared to other groups in 3th and 4th day ($p < 0.01$) when the RD group moved significantly higher from C and RD + E in 2nd day ($p = 0.003$, $p = 0.002$) (Fig. 2A).

2.1.2. Effects on swimming speed

As a result of repeated measures analysis of variance (timexgroup interaction), timexgroup interaction was significant ($p = 0.012$, table 2). Accordingly, the effect size was 0,568.

To determine whether groups differed in their velocity on specific days of the spatial learning task, significant interactions were further analyzed using repeated measures analysis of variance (ANOVA) and one-way analysis of variance Tukey post hoc with Bonferroni correction. A repeated measures analysis of variance indicated that the swimming velocity was significantly differed between trial days in the RD and RD + E group ($F_{3,18} = 18.\ 318$, $p < 0.001$; $F_{3,18} = 5.168$, $p = 0.009$).

According to the one way analysis of variance (between-group comparisons), the velocity was also significantly different between groups ($p < 0.037$, $p < 0.005$ for 1. and 2. trial days). The results from post hoc analysis with Bonferroni correction revealed that, the swimming velocity was higher compared to control in the RD group on the 1st and 2nd days ($p = 0.009$, $p = 0.001$) (Fig. 2B). Also in 4 nd day, the swimming velocity was higher compared to RD group in E group ($p = 0.01$).

2.1.3. Effects on latency

As a result of repeated measures analysis of variance (timexgroup interaction), timexgroup interaction was significant ($p < 0.001$, table 3). Accordingly, the effect size was 0,886.

To determine whether groups differed in their latency on specific days of the spatial learning task, significant interactions were further analyzed using repeated measures analysis of variance (ANOVA) and Tukey post hoc with Bonferroni correction. A repeated measures within-

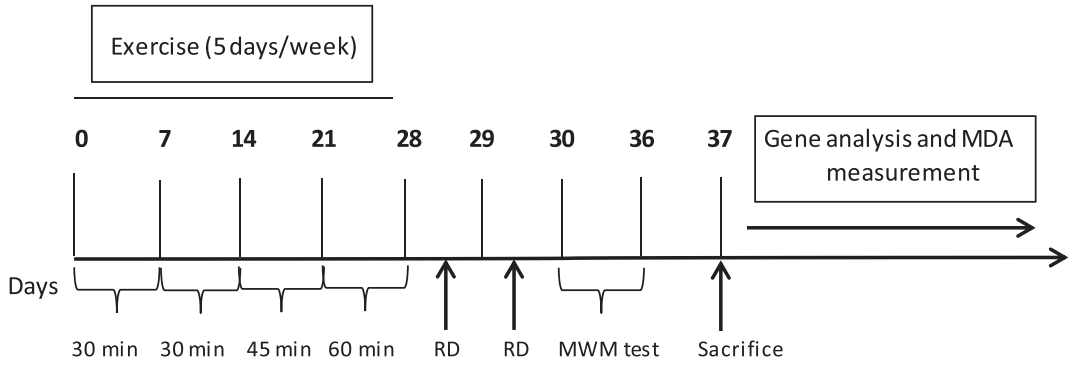

Fig. 1. The timeline of the experimental procedures.

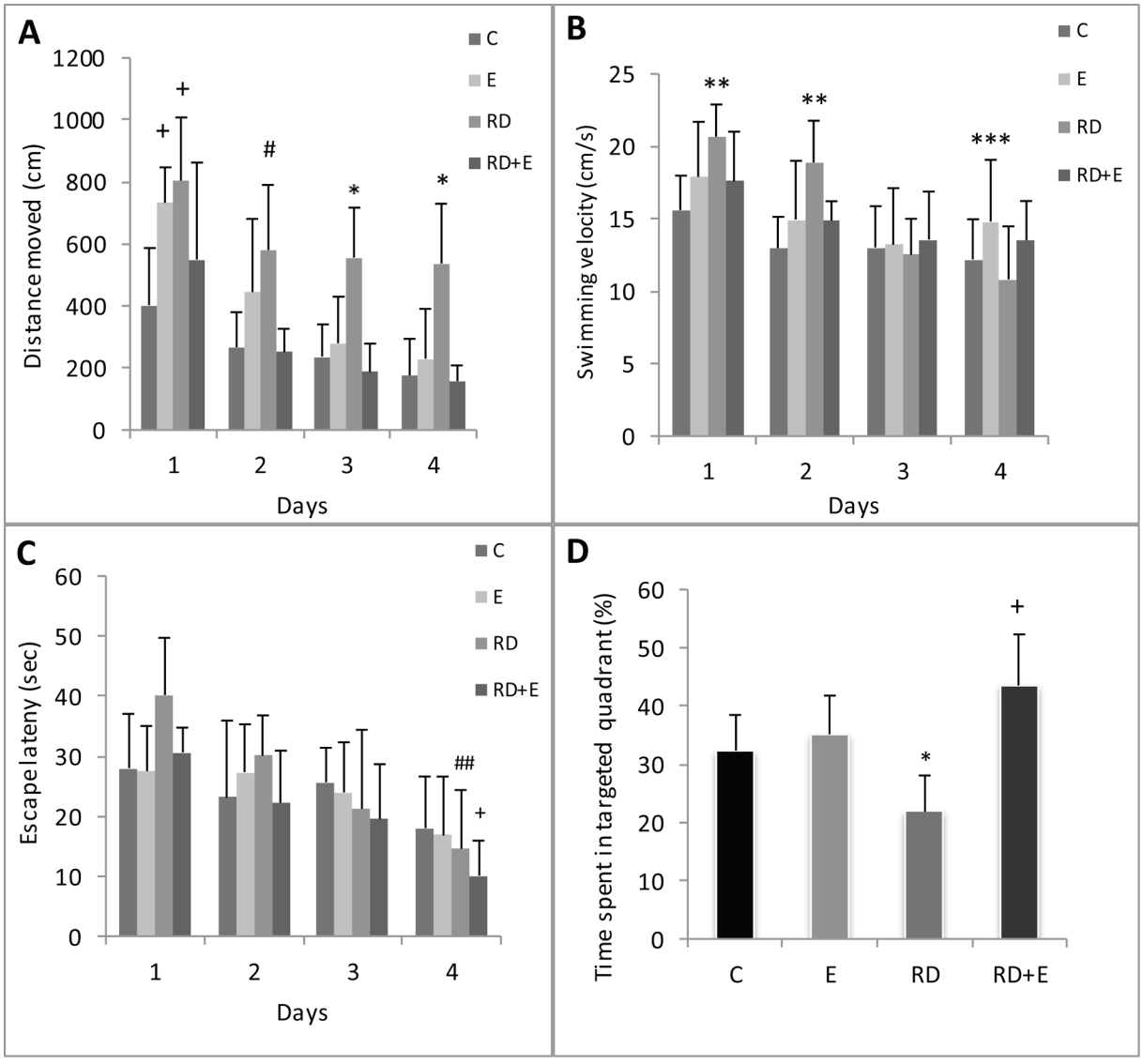

Fig. 2. MWMT performances of groups, the distance moved to reach the platform (cm) (A), swimming velocity (cm/s) (B), escape latency (s) (C), and time spent in targeted quadrant (%) (D). All values represent the mean ± SD from 7 male rats in each group. '*' $p < 0.01$: Significant difference from other groups, '**' $p < 0.05$: Significant difference from C '***' $p < 0.05$: Significant difference from RD, '♯' $p < 0.05$: Significant difference from C and RD + E rats, '♯♯' $p < 0.001$: Significant difference from E and RD + E, '+' $p < 0.05$: Significant difference from C rats.

subjects analysis indicated that the escape latency for rats to reach the platform was significantly differed between trial days in the RD + E and RD groups ($F_{3,18} = 9{,}217$, $p = 0.001$; $F_{3,18} = 7{,}520$, $p = 0.002$).

According to the one way analysis of variance (between-group comparisons), the latency was also significantly different between groups just in 4. trial day ($p < 0.001$). These analysis showed that,

Table 1
One-way analysis of variance (p_{group}), repeated measures analysis of variance (p_{time}), and timexgroup interaction ($p_{overall}$) of variable total distance move.

	D1 mean ± SD	D2 mean ± SD	D3 mean ± SD	D4 mean ± SD	p_{time}
C	402.48 ± 184.40	266.62 ± 113.46	236.78 ± 103.81	176.03 ± 118.01	0.042
E	732.84 ± 114.42	444.72 ± 236.17	279.87 ± 150.02	229.80 ± 160.97	<0.001
RD	804.10 ± 204.01	580.43 ± 209.99	556.04 ± 161.19	536.93 ± 192.92	0.036
RD + E	549.90 ± 312.76	251.26 ± 75.11	186.80 ± 91.71	157.34 ± 51.09	0.015
p_{group}	0.008	0.004	<0.001	<0.001	$p_{overall}$ 0.342

Table 2
One-way analysis of variance (p_{group}), repeated measures analysis of variance (p_{time}), and timexgroup interaction ($p_{overall}$) of variable velocity.

	D1 mean ± SD	D2 mean ± SD	D3 mean ± SD	D4 mean ± SD	p_{time}
C	15.60 ± 2.38	12.98 ± 2.16	13.03 ± 2.85	12.20 ± 2.77	0.124
E	17.90 ± 3.80	14.96 ± 4.05	13.21 ± 3.90	14.81 ± 4.26	0.188
RD	20.67 ± 2.23	18.88 ± 2.89	12.51 ± 2.49	10.82 ± 3.55	<0.001
RD + E	17.58 ± 3.44	14.88 ± 1.34	13.57 ± 3.31	13.52 ± 2.71	0.009
p_{group}	0.037	0.005	0.938	0.179	$p_{overall}$:0.012

Table 3
One-way analysis of variance (p_{group}), repeated measures analysis of variance (p_{time}), and timexgroup interaction ($p_{overall}$) of variable latency.

	D1 mean ± SD	D2 mean ± SD	D3 mean ± SD	D4 mean ± SD	p_{time}
C	27.99 ± 8.61	25.64 ± 5.76	23.06 ± 12.86	17.99 ± 9.05	0.169
E	27.50 ± 7.53	27.33 ± 7.98	23.96 ± 8.34	16.78 ± 9.81	0.091
RD	40.18 ± 9.79	30.07 ± 13.18	21.21 ± 9.54	14.56 ± 6.71	0.002
RD + E	19.49 ± 4.14	22.22 ± 8.68	30.61 ± 9.16	10.09 ± 5.83	0.001
p_{group}	0.143	0.107	0.487	<0.001	$p_{overall}$ < 0.001

escape latency was higher in the RD group compare to the E on the 4th day ($p = 0.000$). RD + E rats had short escape latency compared to C and RD group rats on the 4th day ($p = 0.001$, $p = 0.000$) (Fig. 2C).

2.1.4. Retrieval phase

Memory for the previous platform location was assessed in a probe trial without the platform present. The probe test was done 24 h after the acquisition phase to examine long-term spatial memory retention after sleep deprivation. The results included the mean percentage (%) for a time as well as distance and number of crossing in the target quadrant. One-way analysis of variance (ANOVA) was conducted for the percentage time spent in the critical quadrant in the probe trial of the water maze testing. There was a significant difference in the RD group from the other groups in time spent in the targeted quadrant during memory test ($p < 0.01$) (Fig. 2D). The RD + E group showed significant difference from the C.

2.2. Effects of sleep deprivation and exercise on Grin2a, Grin2b, c-Fos, and BDNF expression level

The mRNA expression levels of Grin2a and Grin2b expression levels did not significantly differ between all groups ($p > 0.05$) (Fig. 3A). Similarly, there was no significant difference between all groups in terms of c-Fos and BDNF expression levels (Fig. 3B and 3C) ($p > 0.05$).

2.3. Effects of sleep deprivation and exercise on hippocampal MDA level

Hippocampal MDA had no significant change between groups ($p > 0.05$) (Fig. 4).

3. Discussion

Sleep alters synaptic strength and changes the expression of genes related to synaptic plasticity (Vyazovskiy et al., 2008). It has been known that sufficient sleep is vital for neurocognitive functions, especially for memory consolidation in the hippocampus (Landers et al., 2013, Kim et al., 2005, Dumaine and Ashley, 2015). Hippocampus is not just sensitive to insults; it is also affected by environmental enrichment such as exercise (Salari et al., 2015; Saadati et al., 2015). To our knowledge, no prior study has yet investigated the effects of prolonged-term mild treadmill exercise on acute REM sleep deprivation by demonstrating the hippocampus-dependent learning and memory and related gene expression alterations. Our Morris water maze results suggested that four weeks of mild treadmill exercise ameliorated the detrimental effects of acute REM sleep deprivation on spatial memory. Additionally, we did not find any alterations in gene expression levels such as Grin2a, Grin2b, c-Fos, and BDNF (Fig. 3).

According to the studies, most of the researchers prefer classical single-platform and multi-platform for the sleep deprivation model (Nabaee et al., 2018). In the current study, we used the flowerpot technique (multi-platform) that disrupts just the REM sleep period (Süer et al., 2011). Being relatively free in social isolation makes this model advantageous for evaluating cognitive outcomes (Zagaar et al., 2013). Much evidence exists that REM sleep has a more significant role for the LTP rather than non-REM sleep (Shikawa et al., 2006). Also, it is known that REM sleep deprivation diminishes LTP (Ishikawa et al., 2006) and oppositely enhances long-term depression (LTD) (Tadavarty et al., 2011). In our MWM test, during the learning phase, rats in all groups learned to find a hidden platform, as noted by the decline in their escape latency and swimming distance throughout for four days (Fig. 2A and 2C). Almost equal swimming speed and latency to reach the visible platform in the exercise and exercise + sleep deprivation groups showed that the changes were not related to locomotor activity and visual impairments of rats (Fig. 2B and 2C). In line with this, Rashid et al. reported that REM sleep deprivation affected considerably learning and memory performance without changing locomotor functions (Abd Rashid et al., 2017). In the probe trial day, sleep-deprived rats spent less time in the targeted quadrant compare to the other groups, and these findings showed that 48 h acute RD alone impaired the spatial memory of rats. Ebrahim et al. also demonstrated that 72 h acute RD disrupted short and long-term memory significantly (Nabaee et al., 2018). As revealed in our previous study, we indicated that 21 days of sleep deprivation of rats did not show any significant differences for memory performances (Sahin et al., 2019). By these results, we assumed that chronic deprivation models might not affect learning and memory functions compared to acute ones. Hence, we could suppose that post-RD spatial memory impairment was sufficient at both 48 h and 72 h RD.

According to our MWM results, exercise improved the detrimental effects of acute sleep deprivation. Interestingly, exercise alone did not change the spatial memory because exercised rats spent nearly the same time with the control group in the targeted quadrant. Furthermore, our data was consistent with the idea that the exercise was not a stress factor by itself. Sleep deprivation application with continuous exercise or

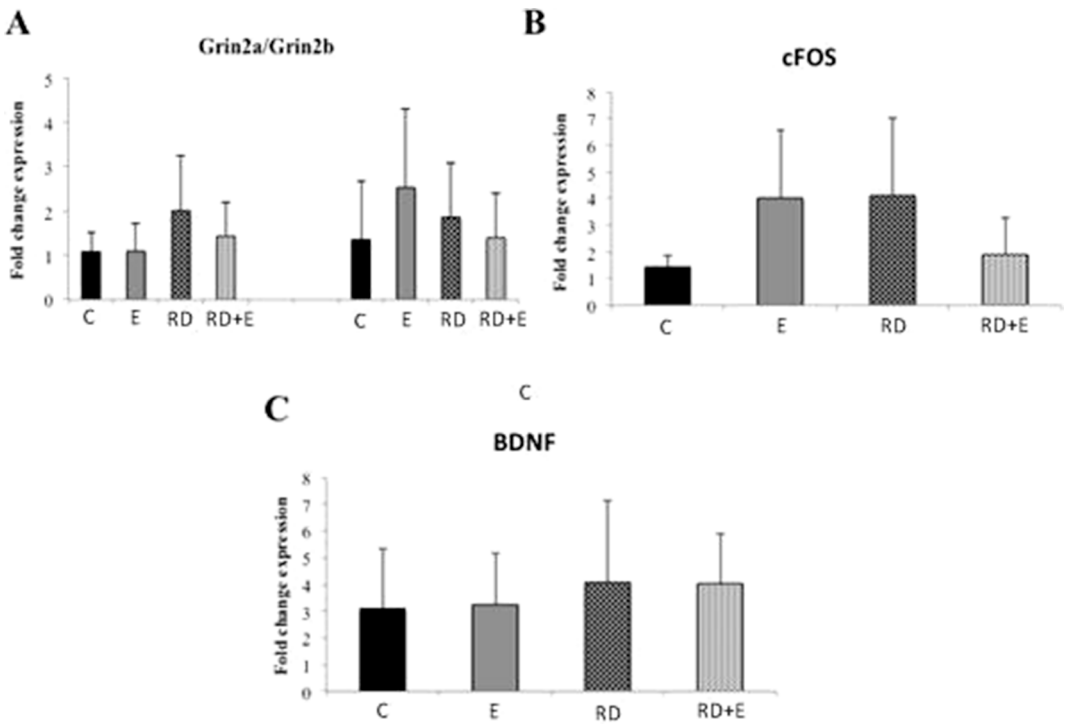

Fig. 3. Expression levels of Grin2a, Grin2b, c-Fos, and BDNF. Graphs showing the levels of fold change expression of Grin2a and Grin2b (A), c-Fos (B), BDNF (C) genes in hippocampal regions of 4 groups. All values represent the mean ± SD from 4 male rats in each group.

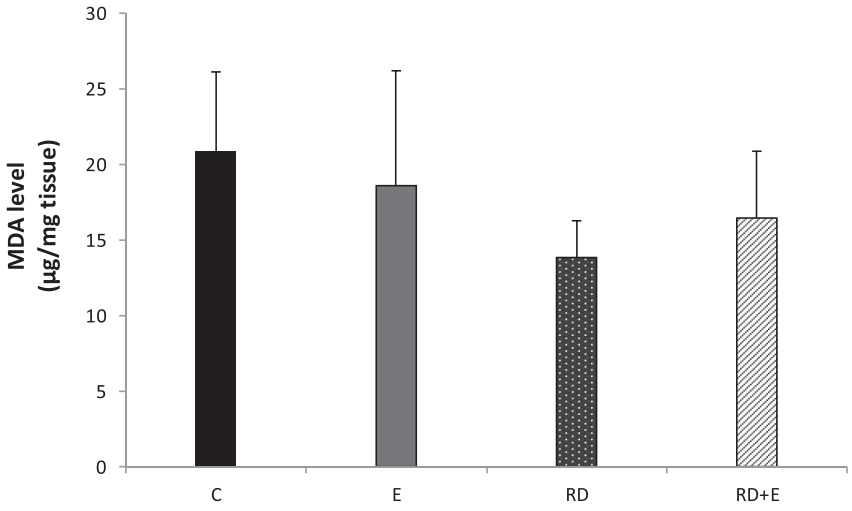

Fig. 4. Effects of long-term mild treadmill exercise on hippocampal MDA concentration in 48 h REM sleep deprivation. Graphs showing the levels of MDA. All values represent the mean ± SD from 4 male rats in each group.

multiple bouts of acute exercise has destructive effects on spatial memory (Slutsky et al., 2017). Besides, several studies speculated that strenuous forced exercise is lacking beneficial effects of exercise on learning and memory by causing additional stress factor by itself (Albeck et al., 2006). As revealed in our previous study, forced continuous 4-week treadmill exercise routine did not have any beneficial effects on learning and memory in socially isolated rats (Cevik et al., 2018). In line with this, we assumed that pairing sleep deprivation with strenuous exercise resulted in deleterious effects on learning and memory because of the total physiological load on body and mind. Some study reports indicated that mild type exercise like wheel running improves cognitive functions. Rajizadeh et al. showed that voluntary running wheel exercise enhanced learning and memory impairments in sleep-deprived rats (Rajizadeh et al., 2018). Our new exercise protocol is known as mild type due to the intervals for resting between the running section, and the duration of exercise is adaptable for rats. In a previous human study, the dose–response effect of exercise also has been confirmed on the sleep quality of women (Kline et al., 2012). Not all exercise protocols have beneficial effects. The effects of exercise vary according to the duration, type, age, gender etc. For instance, older animals showed a significantly higher effect of exercise on BDNF mRNA levels compared to younger animals (Hatchard et al., 2014). Moreover, Hayes et al. (2008) suggested that exercise with a stressful component, rather than voluntary exercise or stress alone, is better able to reduce infarct volume after stroke in rats.

We think that while our protocol clearly showed its effect on stressed young rats, it was insufficient to show a biological effect on non-stressed young rats. According to all these results, we can hypothesize that the type and duration of exercise have remarkable effects on cognitive functions.

To figure out the link between sleep loss and neuronal plasticity, determining of the alterations in NMDA receptor subunits after sleep deprivation is essential. According to our results, Grin2a, Grin2b, c-Fos, and BDNF expression levels were not changed significantly between groups. Our molecular data indicated that mild type of exercise was insufficient to alternate the gene expression level. In some cases, exercise increases the BDNF expression level depending on the neural activity (Griffin et al., 2009, O'Callaghan et al., 2009). In line with the result of some exercise studies, we could not find an increase in BDNF expression level (Lou et al., 2008, Marais et al., 2009). We assumed that in the absence of any cognitive training, exercise alone did not change the level of the neurotropic factor. In some cases, when stress protocols are applied alone, they may not be able to change growth factors. Alzoubi et al. showed that sleep deprivation alone did not alter BDNF expression level significantly, but when combined with dietary restriction, the BDNF level was reduced (Alzoubi et al., 2013).

The immediate early gene (IEG) c-fos encodes a transcription factor. RNA levels of c-fos are rapidly and transiently increased in the dorsal hippocampus by spatial water task training. For instance, Guzowski found that levels of c-Fos was significantly higher than caged controls at 0.5 hr, but not 2 or 6 hr, after training (Guzowski et al., 2001). Moreover, in our previous study, we also indicated that the expression level of c-Fos gene was not changed after MWM training (Keloglan et al., 2019). We supposed that the suppression of c-Fos expression, subsequent to the increase of duration after the maze learning appears to be due to cellular adaptation in the brain. Moreover, multiple previous studies suggested that adult neurogenesis in the hippocampus was decreased by sleep deprivation when duration was for more than two-days (Mueller et al., 2008, Sportiche et al., 2010, Novati et al., 2011). Our 48 h deprivation period may not be sufficient to cause alterations on the c-Fos expression level.

Our study finding also suggested that Grin2a and Grin2b expression levels were not changed significantly because of 48 h RD-induced alterations were insufficient. In a previous study, it was shown that 8 h SD resulted in dramatically over-activated NMDAR function that could contribute to the hippocampal plasticity deficits (Xie et al., 2015). Given previous literature, it might be reasonable to think the cause of invariant gene expression levels may be related to the molecular adaptation of the body due to the extended sleep deprivation duration. Because we also observed the same situation in the constant MDA levels which indicate peroxidative damage. Similar to our results, Gopalakrishan et al., and Nabaee et al., had not observed any evidence of oxidative damage at the lipid and/or at the protein level in sleep-deprived animals in comparison to their controls (Gopalakrishnan et al., 2004, Nabaee et al., 2018). Some conflicting studies reports that included oxidative damage examinations (Silva et al., 2004). It seems the difference between the studies could be related to several factors such as; species of animal, strain differences, SD methods, and brain and body regions. For instance, some researches indicate that classical platform (single platform) isolates and limits the animaĺs movement, generates immobilization stress, and produce oxidative stress because of methodological issues (Villafuerte et al., 2015). Mirescu et al. indicated that sleep deprivation ''platform on the water'' method, suppresses adult neurogenesis and causes an increase in plasma levels of corticosterone simultaneously (Mirescu et al., 2006). However, it was shown that multi-platform deprivation causes less stress than the classical platform technique, due to a decline of movement limitation (Coenen and Van Luijtelaar, 1985). Hence, we should consider about discrepancies between deprivation and exercise protocol.

At first, it might seem that our molecular data contradictory with our behavioral finding, but we have to take into other neuronal mechanisms in future studies. For instance, peripheral processes that intermediate positive effects of exercise on the brain remain sparsely explored. Moon and colleagues demonstrated that a muscle secretory factor, cathepsin B protein, is important for the cognitive advantages of running (Moon et al, 2016). We did not investigate whether exercise of individuals influenced dendrite structure and growth, especially in hippocampus neurons. Another point of view, without any neurobiological effects alterations in memory functions by exercise may dependent on neurons morphological changes. In recent years, it was found that serotonin was a key regulator responsible for exercise-dependent neurogenesis (Yuan et al., 2015). Future studies are required to elucidate the role of these factors on hippocampal dependent memory.

3.1. Conclusion

Our results indicated that 48 h RD alone impaired the spatial memory of rats, and mild exercise application ameliorated the detrimental effects of RD. Furthermore, according to the molecular data, we could not find any significant differences in the expression of gene levels and oxidant parameters. Hence, determining the role of sleep at different gene mechanisms is essential to clarify the exact relations between molecular and behavioral underpinnings.

4. Experimental procedure

4.1. Ethics statement

Experimental procedures were performed following the Mersin University Health Guide for the Care and Use of Laboratory Animals. They were approved by the University of Mersin Institutional Animal Care and Use Committee (Approval No: 15/01/2018/02-02).

4.2. Experimental animals

The experiments were carried out on 28 male *Wistar Albino Rats* (postnatal 28 days) at the Physiology Laboratory of the Mersin University Faculty of Medicine. The rats were fed tap water and rodent chow ad-lib. Four experimental groups were designated ($n = 7$ per group): control (C), exercise (E), REM sleep deprivation (RD) and exercise + REM sleep deprivation (RD + E).

4.3. Exercise protocol

For physical exercise procedures, the treadmill was used during the light cycle between 9:00 am and 2:30 pm for four weeks. For running motivation, a grid apparatus was used in a treadmill that applies a direct current shock average of 0.1–0.15 mA. Firstly, to decrease environmental stress, rats were familiarized with the treadmill for 5 min before exercise protocol. According to the exercise protocol, for the first and second weeks, the duration of exercise was 15 min in two sessions (5 min interval) to avoid muscle fatigue. The length of the exercise was 15 min in three sessions for the third week (5 min interval), and four weeks was 15 min in four sessions (5 min interval). The exercise protocol was executed randomly five days a week (Zagaar et al., 2013). When exercise protocols in the literature are examined, various types of mild exercise can be found (Albeck et al., 2006, Inoue et al., 2015, Yook et al., 2019). However all these mild exercise protocols have in common is that they all have beneficial neurobiological effects.

4.4. REM sleep deprivation procedure

The best and most common model for REM sleep deprivation is the 'flowerpot' technique. In this technique, the experimental animals were deprived of sleep by placing them into a plexiglass tank with numerous small platforms surrounded by water (24 °C ± 1 °C) and the platforms approximately 1 cm above the water surface and had a diameter of 6 cm.

The tank contained 11 small platforms where rats could move freely from one platform to another. The low platforms prevented the animals from sleeping because when they fell asleep, they consequently fell into the water and woke up. Therefore, the flowerpot method is selective for eliminating REM sleep. The rats could reach to feed and water above their head. The animals in the RD and RD + E groups remained on the tank for 48 h. The control and exercise group normally slept in their cages day (Süer et al., 2011).

4.5. Assessment of spatial learning and spatial memory in the Morris water maze

The Morris water maze (MWM) is a widely used apparatus for testing spatial learning and memory in rodents. Rats were trained after REM sleep deprivation following a protocol reported previously. MWM were performed during the light period (8:00–11:00 a.m). The MWM is a black circular pool (150 cm diameter and 60 cm height) filled with water at 22 ± 1 °C. The pool was divided into four quadrants of equal size, in the tracking system (southeast, southwest, northeast, and northwest) without physical boundaries. A circular platform (10 cm diameter) was hidden right below (1.5 cm) the surface of the water in the center of the northeast quadrant. On each trial, the animals were left in the pool with the face facing the wall. Performances were recorded by a smart video tracking system (Noldus Ethovision® system, version 7, Wageningen, NL) (Cevik et al., 2018).

4.6. Long-Term spatial learning

MWM protocol consists of 6 days. In the first day, a single platform is placed in 4 different quadrants 1.5 cm above the water surface, and spatial clues and quadrants are introduced to the rat, and this day is known as the habituation day (Since the 1st day is a habituation day, it is not included in the analysis). Each rat was trained five times (120 s for each, interval = 5 min). Rats were semi-randomly released into the water from east, south, west, and north directions, respectively. If rats did not found the platform in 120 s, the experimenter forced it to the platform by wooden stick and let it rest for 10 s. The remaining 2, 3, 4 and 5. days are known as training days (learning phase) and for this time same platform was fixed just in a single quadrant. In 2nd 5th training days, each rat was trained five times (60 s for each, interval = 5 min) per day for four successive days. During training days (2–5), the platform was placed southwestern quadrant; rats were allowed to swim 60 s and rest for 5 s. When rats found the platform, we assumed that the platform location had been remembered (Sahin et al., 2019).

4.7. Long-term spatial memory testing

The last day, which is the 6th day, is known as the probe trial and the memory test is performed by removing the platform from the dial (60 s probe trial). The platform was removed from target quadrant 24 h after the 4. day of learning trials. The animal was allowed to explore the tank for 60 s. The time spent in the target quadrant that previously contained the platform, as well as the frequency of crossing to the target quadrant, were recorded to evaluate the spatial memory of platform location (Sahin et al., 2019).

4.8. The collection of hippocampus samples

All animals were decapitated under deep anesthesia at the end of the long-term spatial memory test (09.00 a.m. ± 30 min), and brain regions were separated in phosphate buffer solution (PBS buffer). Both right and left hippocampus tissues were taken for analysis of gene expression and TBARS (Sahu et al., 2019).

4.9. RNA isolation and cDNA synthesis

Total RNA isolation from the tissues was performed by a manual method using Ribozol (Invitrogen). Hippocampal specimens were sectioned manually in Eppendorf tubes containing 1 ml Ribozol (for 5–10 mg sample, 1 ml Ribozol). The tissues were allowed to incubate for 10 min. Then, 200 μl of chloroform was added and mixed. The mixture was centrifuged at 12,000 RCF for 15 min. Next, 500 ml of isopropanol was added to the supernatant. Samples were incubated at room temperature for 10 min and again centrifuged at 12,000 RCF for 10 min. The supernatant was removed and 1 ml of ethanol was added to the pellet portion (for 1 ml RiboZol, 1 ml ethanol). After mixing, it was centrifuged at 7500 RCF for 5 min. For cDNA synthesis, ethanol was removed from the pellet. The obtained RNA was dissolved in 50 μl RNase-free solution.

4.10. Gene expression by qPCR and analysis

qPCR was run on a Fluidigm Biomark Real-Time PCR using Taqman GE Master Mix. The suitability of the cDNAs was determined by RT-PCR. β-actin was used as a housekeeping gene that generally preferred in neurological research. The reaction was carried out using 40 amplification cycles of 50 °C for 2 min, 70 °C for 30 min, 25 °C for 10 min, 96 °C for 15 s and 60 °C for 1 min. The expression level of Grin2a, Grin2b, c-Fos and BDNF were calculated using the cycle threshold (Ct) normalization method to the β-actin (Sahin et al., 2019).

4.11. Hippocampal biochemical measurement

As processing tissue, dissected hippocampal tissue was homogenized in and the

homogenates were centrifuged to remove the insoluble materials (14,000×*g* for 15 min at 4 °C). Hippocampal MDA levels were measured by using enzyme-linked immunosorbent assay using rat TBARS ELISA Kit method (CK-10009055 produce by Cayman). Briefly, 100 ul sample or standart was added and swirt to mixed. Each sample was added 4 ml color reagent and boiled for one hour. After bolied, the vials were incubated on ice for ten minutes, then centrifuged for ten minutes at 1600×*g* at 4 °C. 150 ul of each sample was added clear plate and read at 530 nm. MDA levels was calculated using as a formula: MDA (μM): [Corrected absorbance- (y-intercept)] / Slope.

4.12. Statistical analysis

The results were analyzed by SPSS 11.5 statistic software. The Shapiro Wilk ($p > 0.05$) test examined conformity for normal distribution in each group. The data are expressed as mean values ± SD. For normally distributed data, one-way analysis of variance (ANOVA) was conducted for the percentage time spent in the target quadrant in the probe trial of the water maze testing, and for gene expression and MDA levels followed by Tukey post hoc comparisons assess significant differences between groups (significance deter mined as $P < 0.05$). Bonferroni correction for multiple comparisons were performed where appropriate. Within-subject measurements, such as escape latencies, distance move and velocity across trials (1., 2., 3., 4., days) in the water maze, were analyzed using repeated-measures ANOVA (also timexgroup interactions). For each day in water maze testing, to test difference between groups for variables (distance move, latency and velocity), one-way analysis of variance (ANOVA) was conducted followed by Tukey post hoc comparisons (significance determined as $P < 0.05$).

CRediT authorship contribution statement

Leyla Sahin: Conceptualization, Methodology, Writing - original draft, Supervision. **Ozge Selin Cevik:** Investigation, Writing - original draft. **Kenan Cevik:** Investigation. **Celal Guven:** Data curation. **Eylem Taskin:** Formal analysis. **Sayad Kocahan:** Project administration,

Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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