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Original Article

Sex-specific effects of fluoxetine on REM sleep deprivation-induced behavioral impairments and BDNF downregulation in rats

Rojin Ehsan[1], Batool Ghorbani Yekta[2,3,4*], Maryam Eslami[1], Mahdieh Gholami[5], Salar Vaseghi[6]

[1] Department of Genetics, Faculty of Advanced Sciences and Technology, TeMS.C, Islamic Azad University, Tehran, Iran
[2] Department of Physiology, Faculty of Medical Science, TeMS.C, Islamic Azad University, Tehran, Iran [3] Applied Biotechnology Research Center, TeMS.C, Islamic Azad University, Tehran, Iran
[4] Herbal Pharmacology Research Center, TeMS.C, Islamic Azad University, Tehran, Iran
[5] Department of Pharmacy, Alma Mater Studiorum University of Bologna, Bologna, Italy
[6] Cognitive Neuroscience Lab, Medicinal Plants Research Center, Institute of Medicinal Plants, ACECR, Karaj, Iran

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Abstract

Rapid eye movement (REM) sleep is a critical phase in which most dreaming occurs, and its deprivation (REM SD) can lead to disturbances in mood, cognition, synaptic plasticity, and neuroplasticity-related genes such as brain-derived neurotrophic factor (BDNF). Fluoxetine, a selective serotonin reuptake inhibitor (SSRI), is known to modulate BDNF expression, and sex-dependent variations in its effects have been suggested. This study investigated the effects of REM SD, fluoxetine, and their combination on behavioral and molecular outcomes in male and female rats. Results: Following 24 hours of REM SD, animals exhibited impaired memory, increased immobility, reduced rearing, elevated pain threshold, and decreased locomotor activity, along with reduced BDNF levels in the prefrontal cortex. Females were less sensitive to REM SD regarding pain threshold and locomotor activity. Fluoxetine administration (10 mg/kg, i.p.) reversed the effects of REM SD on immobility and rearing exclusively in females, while also mitigating locomotor deficits induced by REM SD. In both sexes, fluoxetine counteracted REM SD-induced changes in pain threshold, but only in females did it restore BDNF downregulation. In conclusion, the findings suggest sex-specific variations in the consequences of REM SD and in the therapeutic response to fluoxetine. While REM SD broadly impairs cognitive, affective, and molecular parameters, females exhibit partial resilience, particularly in pain threshold and locomotor activity. Moreover, fluoxetine demonstrates sex-dependent protective effects, especially in restoring BDNF expression and behavioral outcomes in females.

Keywords: Rapid eye movement (REM), Sleep deprivation (SD), Fluoxetine, Sex differences, Brain-derived neurotrophic factor (BDNF).

1. Introduction

Sleep disturbances have a bidirectional relationship with cognitive dysfunctions and mood disorders. Evidence has shown that sleep deprivation (SD) may be a severe stressor that negatively affects behavioral and cognitive performance [1-4]. Although controversial results have also been reported [5]. Rapid-eye movement (REM) sleep is an important sleep stage that is associated with proper cognitive functioning and maintaining mental health [6]. It has been revealed that REM disturbances or REM sleep deprivation (REM SD) are closely related to cognitive dysfunctions. Research has demonstrated that REM SD in rats causes a variety of behavioral abnormalities as well as cognitive decline.[7-10]. Additionally, neurogenesis, synaptic plasticity, and the expression of genes linked to plasticity, such as brain-derived neurotrophic factor (BDNF), are impacted by REM SD.

The most researched neurotrophin, BDNF, is essential for regulating mood state, cognitive functioning, and neuroplasticity [11, 12]. Previous studies have demonstrated that 14 straight days of chronic REM SD in female rats drastically lowers the level of BDNF expression in the hippocampus, resulting in manic-like behaviors[10]. It has been demonstrated that two months of REM SD increases anxiety and depressive-like behaviors in rats and lowers BDNF levels in the hippocampal regions of mice.[13]. Furthermore, in the hippocampus of the rat neonate, maternal REM SD reduces neurogenesis and lowers BDNF levels.[14]. However, it has been shown that REM SD for 21 consecutive days leads to a greater concentration of BDNF in the dorsal hippocampus of rats [15], suggesting a compensatory mechanism. As mentioned, there are multiple links between REM patterns, mood disturbances, and endogenous depression [16, 17]. Of note, disturbances of serotonin transmission may be involved in REM sleep changes in depression and anxiety disorders [18, 19].

* Corresponding author.
E-mail address: bahareh59gh@yahoo.com (B. Ghorbani Yekta).
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Fluoxetine, as the most prescribed selective serotonin reuptake inhibitor (SSRI), is a well-known medication used for the treatment of depression, Obsessive-compulsive disorder (OCD), panic disorder, bulimia, and binge eating disorder, etc.[20]. Interestingly, there is no study investigating the effects of fluoxetine on REM SD-induced cognitive and mood disturbances. Nevertheless, fluoxetine has been proven in a prior work to ameliorate the neuroinflammation and complete SD-induced neuronal deficits in mice.[21]. Of note, preclinical and clinical studies have shown that selective serotonin reuptake inhibitors (SSRIs) significantly affect the level of BDNF [22, 23]. Previous research has shown that fluoxetine may restore memory deficits induced by tamoxifen, along with an increase in BDNF level in the prefrontal cortex and the hippocampus of rats [24]. Furthermore, fluoxetine ameliorates Alzheimer's disease progression, improves behavioral functions, increases brain monoamines, and increases BDNF levels in rats [25]. A recent systematic review has also reported that SSRIs can change BDNF levels in rodents and humans. However, it appears that these medications' effects on depression are not entirely reliant on BDNF levels (at least in human trials)[26]. The effects of fluoxetine may be sex-dependent. Although evidence is limited, Studies have indicated variations in fluoxetine's effects between genders. For instance, it has been demonstrated that early fluoxetine exposure affects rats' baseline anxiety and cognitive functioning in adulthood, with males appearing to be more vulnerable than females.[27]. Additionally, prior studies have demonstrated that fluoxetine increases and decreases the expression of genes associated with myelin in the prefrontal cortex and basolateral amygdala, respectively, with a greater effect in male juvenile rats.[28].

Based on these results, we plan to examine how fluoxetine affects the level of BDNF expression in male and female rats in order to better understand how REM sleep loss causes behavioral and cognitive dysfunctions.

2. Materials and Methods

2.1. Animals

Both sexes of Wistar rats were used in the present study. In order to perform behavioral studies, 204 male and female Wistar rats (102 males and 102 females; 190-210g, 8-9 weeks old) were used. In every 25*50*25cm Plexiglas cage, four rats were housed. The lighting in the lab was set to a 12 hr / 12 hr cycle, with the lights coming on at 7:00 h. Additionally, the standard temperature of 22±1°C was noted. There were 17 male or female rats in each group: 7 rats were used for behavioral tests other than the shuttle box, 7 rats were used for the shuttle box, and 3 rats were used to analyze gene expression. Animals were obtained from the in-house breeding colony of the Cognitive Neuroscience Lab, Medicinal Plants Research Center, ACECR (Karaj, Iran). We followed the National Institutes of Health's Guide for the Care and Use of Laboratory Animals when designing our experimental protocol.[29].

2.2. Fluoxetine administration

Fluoxetine was purchased from Tehrandarou Company, Tehran, Iran. 10 mg/kg of dissolved fluoxetine was delivered intraperitoneally after being diluted in normal saline. The fluoxetine dosage was selected in accordance with earlier reports.[30, 31].

2.3. REM SD apparatus

REM SD apparatus (the multiple-platform apparatus, Tajhiz-Gostar Omid Iranian Co, Tehran, Iran) was employed to bring on REM SD. The multipurpose device consisted of a dark reservoir that held heated water. (90×50×50 cm). Also, in the tank, there were multiple 7 cm-diameter circular platforms. The platforms were 1 centimeter below the water level in the tanks. Each rat was placed on a single platform for the REM SD induction, although it was allowed to move around on it. And all the rats could swim freely. The rat experienced REM SD because it fell into the water during the REM period of sleep and woke up. For the sham-REM SD rats, platforms with a bigger diagonal (15 cm) were available.[32]. Duration of REM SD was 24h.

2.4. Open field test

The open field test (OFT) (Tajhiz-Gostar Omid Iranian Co, Tehran, Iran) was a box made of clear Perspex (30×30×40cm). Locomotion and anxiety-like phenotypes can be evaluated using this apparatus. The OFT arena was divided into 16 squares of the same size. Each rat was free to explore the whole arena for 300 seconds. The squares that were passed by the rat were recorded and considered as locomotor activity [33]. Rearing behavior (while the rat stands on the hindlegs in an upright posture) was also reordered to assess anxiety-like state of the rat [34]. The OFT was done 30 minutes after saline or fluoxetine injection.

2.5. Marble burying test

The marble burying test is a tool used to evaluate behaviors similar to obsessive-compulsive disorder (OCD). Ten ordinary glass toy marbles (15 mm in diameter and 5–6 g in weight) were used in this test, and they were placed on the bedding's surface in each Plexiglas cage. The marbles were arranged in two rows of five each.[35]. Each rat had 30 minutes to freely move throughout the cage. The number of buried marbles was considered an OCD-like behavior. The marble burying test was done 30 minutes after the OFT.

2.6. Hot plate apparatus

A sheet heated by an electric current was the hot plate (Tajhiz-Gostar Omid Iranian Co, Tehran, Iran). Every rat was placed on a heated sheet at 50 degrees Celsius in order to determine the threshold of thermal pain. One hundred seconds was the deadline.[36, 37].

2.7. Forced swim test

The forced swim test (FST) is a useful tool for assessing rodent depression. Water (20–22°C) was poured into the FST, a transparent, cylindrical container, filling it to two-thirds full. The rat was allowed to float inside the container with no way out in order to evaluate depressive-like behavior. Every rat had to swim after floating inside for 300 seconds. It was around this time that the immobility was noted. The length of immobility was regarded as a sign of a melancholy mood.[38].

2.8. Shuttle box

With the shuttle box, passive avoidance memory can be assessed.[1, 39]. The shuttle box had two compartments (dark and light) with equal sizes (25x25x25 cm). Both chambers had mesh floors and Plexiglas walls. Elec-

tric current was delivered to the grid floors to induce foot shocks. There was a guillotine door separating the compartments. There were two testing and training phases in this test. The mouse was trained by spending sixty seconds in the light room. The door to the guillotine was opened after this time. The guillotine door was shut as soon as the rat entered the dark chamber, and the grid floor of the chamber received a 3-second electric shock at 0.6 mA. 30 seconds later, the rat was taken out of the apparatus. The exam was taken 24 hours after the instructions. Every rat was put in the light chamber for the duration of the test session. A minute later, the door opened. The memory performance of each rat was determined by measuring its delay to enter the dark chamber. [36].

2.9. Experimental groups

Twelve groups (n = 7 male or female rats) were created from all of the rats:

Group 1 & 2 (saline): male and female rats received saline (0.5 mL/rat).

Group 3 & 4 (fluoxetine): male and female rats received fluoxetine (10 mg/kg).

Group 5 & 6 (sham-REM SD + saline): male and female rats were placed in REM SD apparatus with larger platforms (these rats had normal sleep) for 24h, and immediately after this period, received saline (0.5 mL/rat).

Group 7 & 8 (REM SD + saline): male and female rats were kept out of the REM stage for 24 hours while housed in REM SD apparatuses with smaller platforms, and immediately after this period, received saline (0.5 mL/rat).

Group 9 & 10 (sham-REM SD + fluoxetine): male and female rats were put in a larger platform REM SD equipment (the rats slept normally during this time), and immediately after this period, received fluoxetine (10 mg/kg).

Group 11 & 12 (REM SD + fluoxetine): male and female rats were kept out of the REM stage for 24 hours while housed in REM SD apparatuses with smaller platforms, and immediately after this period, received fluoxetine (10 mg/kg).

For molecular analyses, three rats per cohort (n=3 per group) were humanely euthanized. Animals were deeply anesthetized with isoflurane (5% induction, 2–3% maintenance in oxygen) and euthanized by decapitation; death was confirmed by loss of reflexes and respiration before tissue collection.

2.10. Real-time PCR

Real-time PCR, a cornerstone technique in molecular biology, was employed to precisely quantify gene expression using cDNA synthesized from extracted RNA. The protocol was meticulously designed to ensure specificity and accuracy in measuring gene activity under varied experimental conditions[36, 37, 40-42].

Primer Design and Validation- Prior to the PCR, primer pairs were designed to specifically amplify target cDNA sequences, avoiding amplification of genomic DNA. Each primer spanned exon-exon junctions to enhance specificity. Primer pairs underwent validation for specific binding through in silico analysis using tools like Primer-BLAST against the reference genome to avoid off-target effects. Additionally, the melting temperatures (Tm) of the primers were optimized to range between 58-60 degrees Celsius to facilitate uniform annealing, thereby minimizing the potential for primer-dimer formations. The exact sequences of all primers used in this study are listed in Table 1.

PCR Reaction Setup- for the PCR setup, the reaction mixture was prepared in a sterile environment to prevent contamination. Among the parts were: Master Mix: 5 µL of a 2X concentrated Master Mix containing MgCl2, Taq DNA polymerase, and the required buffers was utilized; cDNA template: 2 µL of cDNA extracted from RNA samples that were subjected to DNase treatment in order to eliminate any excess genomic DNA; Primers: 0.5 µL of each primer (10 µM concentration) ensured efficient and specific initiation of DNA synthesis; Nuclease-Free Water: Added to adjust the final volume to 10 µL, maintaining the reaction's ionic strength and osmolarity.

Thermal Cycling Conditions: The amplification was carried out using a standardized cycling protocol: Initial Denaturation: 95°C for 15 minutes to activate the Taq polymerase; Cycling Phase: 40 cycles, which included: The double-stranded DNA was denatured for 15 seconds at 95°C, and the primers annealed and DNA polymerase stretched the new DNA strands for 30 seconds at 60°C.

Melting Curve Analysis and Specificity Check- Following the amplification cycles, a melting curve analysis was conducted to assess the specificity of the PCR products. This involved slowly increasing the temperature from 60°C to 95°C while continuously measuring the fluorescence emitted by the intercalating dye, which binds to double-stranded DNA. Distinct melting peaks confirmed the specificity of the PCR amplifications, indicating successful amplification of the target genes without primer-dimers or non-specific products.

Data Acquisition and Analysis- The real-time PCR instrument captured fluorescence data in real time, which was used to calculate the threshold cycle (Ct) values for each sample. The Ct, indicative of the cycle at which fluorescence reaches a significant threshold above the baseline, reflects the quantity of target nucleic acid in the sample. The comparative Ct method, or 2^(-ΔΔCt) method, was employed to quantify gene expression relative to a housekeeping gene (GAPDH) and normalized against a calibrator or control sample. This relative quantification approach allowed for the comparison of gene expression levels across different samples, correcting for potential variations in cDNA quality and quantity.

The accuracy of the real-time PCR results depended heavily on the precision of each step of the protocol, from primer design and reaction setup to thermal cycling and

Table 1. Primer sequences for BDNF and GAPDH Genes for Polymerase Chain Reaction (PCR).

Gene	Primer Sequence (5' → 3')	Length (bp)
BDNF	Forward: TGCAGGGGCATAGACAAAAGG	21
	Reverse: CTTATGAATCGCCAGCCAATTCTC	24
GAPDH	Forward: TGACATCAAGAAGGTGGTGAA	21
	Reverse: CCCTGTTGCTGTAGGCGTAAT	21

All primers were designed to span exon-exon junctions to prevent amplification of genomic DNA.

data analysis. Each phase was executed with careful consideration to ensure reproducibility and reliability of the gene expression data, crucial for downstream applications such as gene regulation studies, disease modeling, and therapeutic target validation. The comprehensive detailing of each step in the protocol also facilitates replication in other laboratories and consistency in research findings.

The specific sequences of the primers used are listed in Table 1, which can be referred to for detailed sequence information and their respective target genes. (Table 1).

2.11. Statistical analyses

V. 26 of the SPSS program was utilized for data analysis. To analyze the data, post hoc Tukey's, one-way ANOVA, and two-way ANOVA were used. The statistical significance level was set at P<0.05, and all data were presented as mean ± SD. To identify significant differences between the male and female groups, a one-way ANOVA was performed. To find the significant differences between six groups of males and females, a two-way ANOVA was used. A post hoc Tukey test was used to identify any significant group differences.

3. Results

3.1. Locomotor activity

One-way ANOVA revealed significant differences between the male ($F_{5, 36} = 85.41$, $P < 0.001$) and female ($F_{5, 36} = 197.01$, $P < 0.001$) groups. Additionally, REM SD considerably reduced both male and female locomotor activity, according to the post hoc Tukey test ($P < 0.001$). Feline, however, did not affect males; nonetheless, it diminished but did not completely reverse the effect of REM SD in females ($P < 0.001$) (Figure 1).

The two-way ANOVA revealed a significant effect of sex ($F_{1, 72} = 6.42$, $P < 0.05$), interventions ($F_{5, 72} = 241.33$, $P < 0.001$), and sex*interventions ($F_{5, 72} = 10.86$, $P < 0.001$). Additionally, the post hoc Tukey test revealed that only REM SD females had higher locomotor activity after taking fluoxetine ($P < 0.001$), but locomotor activity was lower in REM SD males than in females (Figure 1).

3.2. Rearing

One-way ANOVA revealed significant differences between the male ($F_{5, 36} = 19.37$, $P < 0.001$) and female ($F_{5, 36} = 61.09$, $P < 0.001$) groups. Additionally, REM SD dramatically reduced rearing in both men and females, according to the post hoc Tukey test ($P < 0.001$). Nevertheless, fluoxetine corrected the effect of REM SD in females but not in males ($P < 0.001$) (Figure 2).

The two-way ANOVA revealed a significant effect of sex ($F_{1, 72} = 13.93$, $P < 0.001$), interventions ($F_{5, 72} = 58.17$, $P < 0.001$), and sex*interventions ($F_{5, 72} = 9.09$, $P < 0.001$). Additionally, fluoxetine only corrected rearing in REM SD females, according to the post hoc Tukey test ($P < 0.001$). (Figure 2)

3.3. Pain threshold

One-way ANOVA revealed significant differences between the male ($F_{5, 36} = 10.74$, $P < 0.001$) and female ($F_{5, 36} = 29.03$, $P < 0.001$) groups. Additionally, REM SD significantly reduced pain threshold in both males and females, according to the post hoc Tukey test ($P < 0.001$). But in both genders, fluoxetine counteracted the impact of REM SD ($P < 0.001$) (Figure 3).

The two-way ANOVA revealed a significant influence of sex ($F_{1, 72} = 2.97$, $P < 0.05$), interventions ($F_{5, 72} = 35.86$, $P < 0.001$), but not sex*interventions ($F_{5, 72} = 1.42$, $P > 0.05$). Additionally, the post hoc Tukey test revealed that REM SD females had a lower pain threshold than males ($P < 0.05$) (Figure 3).

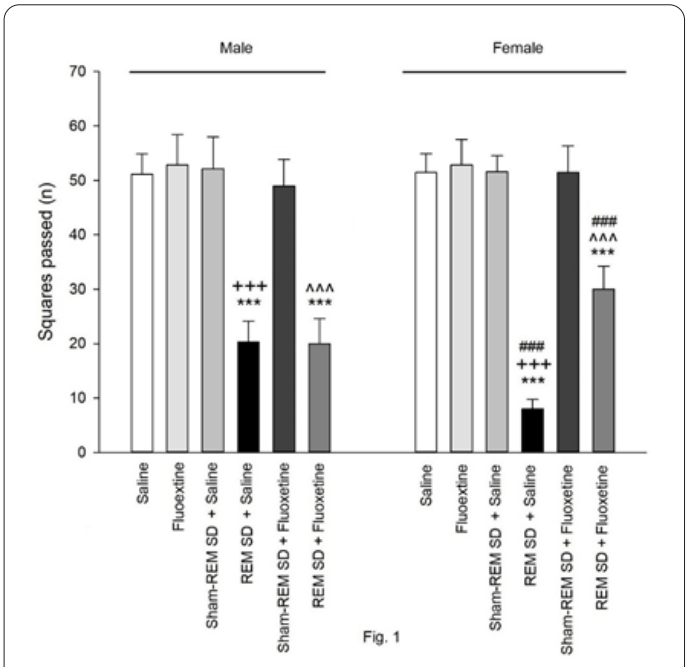


Fig. 1. Locomotor activity in the open field test in all experimental groups in both males and females (n = 7) [***P<0.001 compared to related saline; +++P<0.001 compared to related sham-REM SD; ^^^P<0.001 compared to related sham-REM SD + fluoxetine; ###P<0.001 compared to respective male groups].

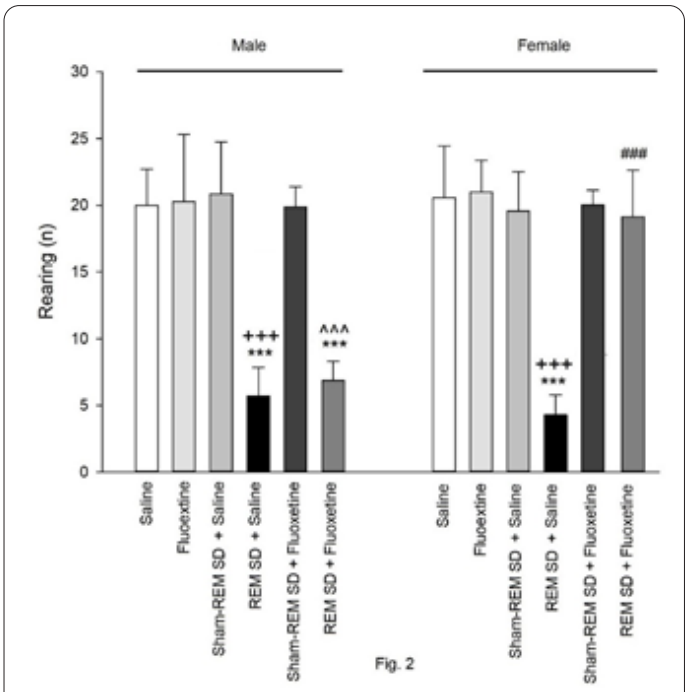


Fig. 2. Rearing in the open field test in all experimental groups in both males and females (n = 7) [***P<0.001 compared to related saline; +++P<0.001 compared to related sham-REM SD; ^^^P<0.001 compared to related sham-REM SD + fluoxetine; ###P<0.001 compared to respective male groups].

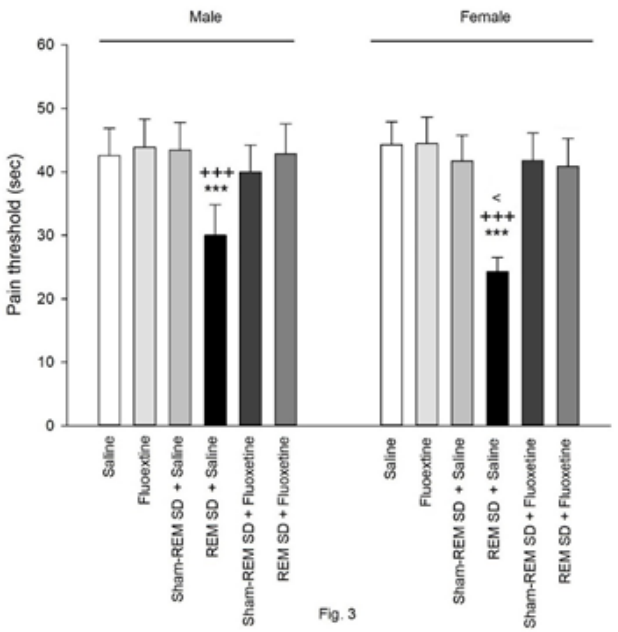


Fig. 3. Pain threshold in the hot plate in all experimental groups in both males and females (n = 7) [***P<0.001 compared to related saline; +++P<0.001 compared to related sham-REM SD; ^^^P<0.001 compared to related sham-REM SD + fluoxetine; #P<0.05 compared to respective male groups].

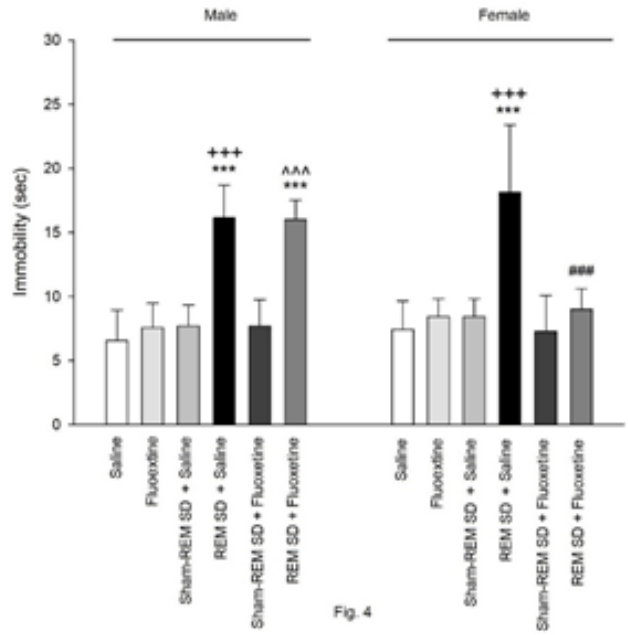


Fig. 4. Immobility in the forced swim test in all experimental groups in both males and females (n = 7) [***P<0.001 compared to related saline; +++P<0.001 compared to related sham-REM SD; ^^^P<0.001 compared to related sham-REM SD + fluoxetine; ###P<0.001 compared to respective male groups].

3.4. Immobility

One-way ANOVA revealed significant differences between the male ($F_{5, 36} = 39.15$, $P < 0.001$) and female ($F_{5,36} = 16.40$, $P < 0.001$) groups. Additionally, REM SD considerably enhanced immobility in both males and females, according to the post hoc Tukey test ($P < 0.001$). Nevertheless, fluoxetine corrected the effect of REM SD in females but not in males ($P < 0.001$). (Figure 4).

The two-way ANOVA revealed that the effect of interventions ($F_{5, 72} = 41.31$, $P < 0.001$) and sex*interventions ($F_{5, 72} = 7.77$, $P < 0.001$) was significant, while the effect of sex ($F_{1, 72} = 0.28$, $P > 0.05$) was not significant. Additionally, the post hoc Tukey test revealed that only in females did fluoxetine restore immobility ($P < 0.001$) (Figure 4).

3.5. Passive avoidance memory

One-way ANOVA revealed significant differences between the male ($F_{5, 36} = 16.39$, $P < 0.001$) and female ($F_{5, 36} = 50.38$, $P < 0.001$) groups. Additionally, REM SD significantly reduced passive avoidance memory in both males and females, according to the post hoc Turkey test ($P < 0.001$). But in both genders, fluoxetine showed no impact (Fig. 5).

The two-way ANOVA revealed that the effect of interventions ($F_{5, 72} = 54.43$, $P < 0.001$) was significant, while the effect of sex ($F_{1, 72} = 0.85$, $P > 0.05$) and sex*interventions ($F_{5, 72} = 2.25$, $P > 0.05$) was not. Therefore, there was no sex difference (Fig.5).

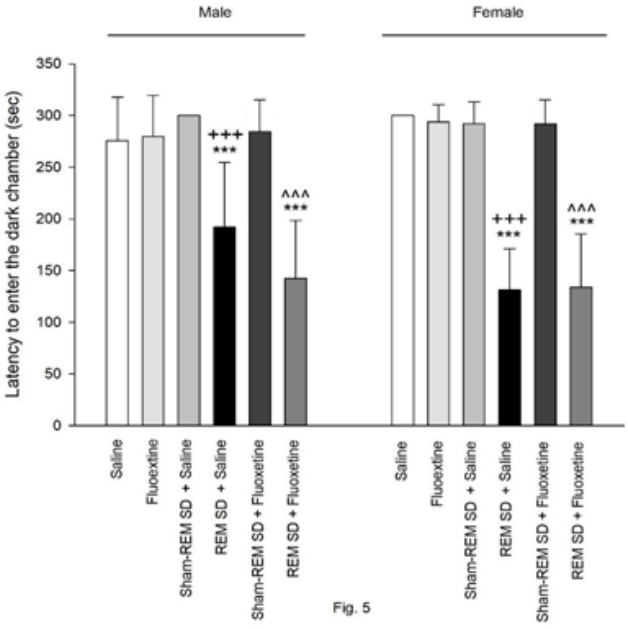


Fig. 5. Latency to enter the dark chamber in the shuttle box in all experimental groups in both males and females (n = 7) [***P<0.001 compared to related saline; +++P<0.001 compared to related sham-REM SD; ^^^P<0.001 compared to related sham-REM SD + fluoxetine].

3.6. BDNF

One-way ANOVA revealed significant differences between the male ($F_{2, 6} = 29.90$, $P < 0.001$) and female ($F_{2, 6} = 15.38$, $P < 0.001$) groups. Additionally, the post hoc Tukey test revealed that in both males and females, REM SD dramatically reduced the level of BDNF expression ($P < 0.01$) and ($P < 0.001$). Nevertheless, the effect of REM SD was reversed by fluoxetine exclusively in females ($P < 0.001$). Feline fluoxetine did, however, raise BDNF levels, though this effect was not statistically significant (Fig. 6).

The two-way ANOVA revealed that the effect of sex ($F_{1, 12} = 7.13$, $P < 0.05$) and interventions ($F_{2, 12} = 34.94$, $P < 0.001$) was significant, while the effect of sex*interventions ($F_{2, 12} = 2.96$, $P > 0.05$) was not. The results of the post hoc Tukey test showed that females ex-

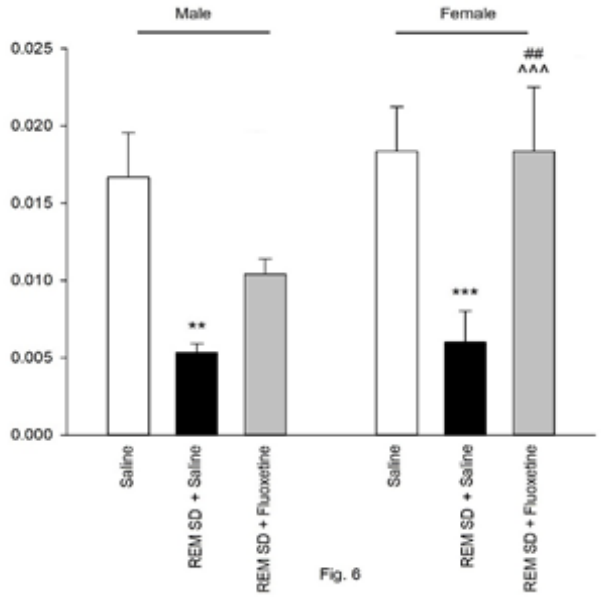


Fig. 6. BDNF expression level in the prefrontal cortex in experimental groups with significant changes in behavior in both males and females (n = 7) [***P<0.001 and **P<0.01 compared to related saline; ^^^P<0.001 compared to related REM SD; ##P<0.01 compared to respective male groups].

posed to REM SD + fluoxetine had higher levels of BDNF than males (P < 0.01) (Figure 6).

4. Discussion

In summary, we showed that REM SD decreased locomotor activity, rearing, pain threshold, and BDNF levels, impaired memory, and increased immobility. The effects of REM SD on pain threshold and locomotor activity were higher in females; fluoxetine attenuated the effects of REM SD on locomotor activity and reversed the effects of REM SD on rearing and immobility only in females, while it had no effect in males; fluoxetine reversed the effects of REM SD on pain threshold in both sexes, while it did not affect memory impairment induced by REM SD; additionally, it restored BDNF downregulation in female but not male REM SD rats; consequently, we observed sex differences in the effects of REM SD on pain threshold and BDNF.

Stressors like SD can have a negative impact on a variety of cognitive functions and mental health. Both SD and REM SD potently attenuate synaptic plasticity, synaptic transmissions, and neurogenesis [14, 43]. SD and REM SD also impair cognitive functions and lead to mood disturbances such as depression, mania, and anxiety [32, 44, 45]. It has been demonstrated that 72 hours of REM SD in rats enhances immobility (as depressive-like behavior) in the forced swim test [46]. Additional research has demonstrated that in the elevated plus maze, rats exposed to REM SD display anxiety-like behaviors.[15]. Additionally, neonatal REM SD leads to an adult depressive-like state in rats [9]. However, the duration of REM SD is important. It has been reported that duration of SD and REM SD determines the final impact of SD on the mood state [5]. For example, chronic short-term REM SD (6h/d for 14d) in female rats can lead to manic-like behaviors, hyperlocomotion, and decreased anxiety level. Another study has shown that 24h hours total SD and REM SD have no effect on locomotor activity in rats [47]. It has also been reported that 72 hours of REM SD restores depressive-like behaviors induced by olfactory bulbectomy in rats [48]. Another study has shown that 4 days of REM SD may induce antidepressant effect in clomipramine model of depression [49]. Furthermore, 24 hours REM SD may restore cognitive deficits induced by intensive exercise in rats [50]. 24 hours REM SD has been shown to leave pain threshold unaffected [51]. However, it has been shown that 24 hours of REM SD in rats leads to an increase in pain perception [52]. Chronic REM SD may decrease pain threshold in female rats [32]. It has also been shown that 72 hours of REM SD increases pain behaviors in Sprague-Dawley rats [53]. Therefore, REM SD can induce inconsistent changes that depend on the duration and the physiological/pathological condition of the animal. Our data revealed that 24 hours of REM SD increased anxiety- and depressive-like behaviors, impaired memory, and decreased pain threshold and locomotor activity.

REM SD also changes the expression level of BDNF. Of note, this effect also seems to be inconsistent [5]. Previous studies have demonstrated that rats' hippocampal BDNF levels are unaffected by 48 hours of REM SD.[54]. Neonatal rats from a mother exposed to 72 hours REM SD exhibit increased BDNF levels in the hippocampus [14]. Also, 24 hours REM SD restores BDNF downregulation induced by exercise in rats [50]. It has been shown that REM SD for 21 days can raise BDNF levels in rats' dorsal hippocampus.[15]. Nonetheless, 48 hours of SD dramatically lowers the amounts of BDNF in the rat hippocampal tissues.[55]. It has been discovered that giving mice 24 hours of REM SD causes the prefrontal cortex to express less BDNF.[56]. Constant REM SD in female rats also diminishes BDNF levels within the hippocampus [10]. Our information, moreover, appeared that REM SD for 24 hours decreased BDNF expression level within the prefrontal cortex.

On the other hand, fluoxetine, as one of the most prescribed SSRIs, may affect BDNF levels. Of note, BDNF changes induced by fluoxetine have been shown to depend on the length of drug administration [57]. It has been shown that 3 weeks of fluoxetine treatment leads to increased BDNF levels in the hippocampus of rats [58]. Fluoxetine can raise the amounts of BDNF in mice's hypothalamus.[59]. In a rat model of posttraumatic stress disorder (PTSD) induced by single prolonged stress, fluoxetine increases BDNF levels in the hippocampus [60]. Significantly, it has been determined that whereas chronic fluoxetine administration raises BDNF levels in the hippocampus and prefrontal cortex but not in the rat dentate gyrus, acute fluoxetine treatment has no effect on BDNF [61]. Additionally, fluoxetine does not raise BDNF levels in rats subjected to unexpected mild stress (CUMS), according to a prior study.[62]. Of note, there is no evidence on the interaction of fluoxetine and REM SD on BDNF levels. For the first time, we showed that fluoxetine can restore BDNF downregulation induced by REM SD only in females, suggesting a sex difference. Sex differences were also seen in the effects of fluoxetine on locomotor activity, rearing, and immobility.

Evidence has shown that fluoxetine effects may be sex dependent, although there are inconsistent reports. In both male and female rats, fluoxetine has been demonstrated to impair short-term new object recognition memory, while the expression levels of BDNF are higher in males than in females, and anxiety-like behavior changes differently

in males and females [63]. According to a prior study, in the elevated plus maze, only males who received prenatal fluoxetine exhibited higher anxiety-like behaviors.[64]. Fluoxetine dose-dependently attenuates the performance of male rats, while females receiving fluoxetine show an improved function [65]. Prenatal fluoxetine in prenatally stressed rats leads to an antinociceptive effect, with a greater effect in males [66]. Another study has shown that prenatal fluoxetine affects hippocampal neurogenesis and social investigation in male rats [67]. However, previous study has stated that fluoxetine increases BDNF levels in the hippocampus of both sexes [68]. Furthermore, chronic fluoxetine treatment increases adult hippocampal neurogenesis in both male and female rats exposed to social instability stress [69]. It should be noted that evidence on possible sex differences in the effects of fluoxetine is limited. Also, possible sex differences in the effects of REM SD have not been investigated.

The results of this study indicate that fluoxetine may have sex-dependent effects on changes in locomotor activity, rearing, depressive-like behavior, and BDNF levels brought on by REM SD. It appears that the effects of fluoxetine are more pronounced in females than in males, most likely because of the higher levels of BDNF in females.

List of abbreviations

REM: Rapid Eye Movement; SD: Sleep Deprivation; BDNF: Brain-Derived Neurotrophic Factor; SSRI: Selective Serotonin Reuptake Inhibitor; OCD: Obsessive-Compulsive Disorder; OFT: Open Field Test; FST: Forced Swim Test; PCR: Polymerase Chain Reaction; cDNA: complementary DNA; RNA: Ribonucleic Acid; DNA: Deoxyribonucleic Acid; Tm: Melting Temperature; Ct: Threshold Cycle

Declaration

Ethical Approval: All experimental procedures were conducted in accordance with the guidelines set by the National Institutes of Health for the Care and Use of Laboratory Animals (2011). The study protocol was approved by the Ethical Committee of Tehran Medical Sciences Islamic Azad University. We have conducted all aspects of our research in accordance with the highest ethical standards. The study was evaluated and approved by the Research Ethics Committees of Tehran Islamic Azad University of Medical Sciences under the approval ID IR.IAU.PS.REC.1403.207.

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Not applicable.

Consent to publish

Not applicable.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Availability of data and materials

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

R. E. and B. G. Y. conceptualized and designed the study. R. E. performed the experiments, data collection, statistical analysis, and drafted the manuscript. M. G. contributed to data interpretation and manuscript editing. M. E. and S. V. provided scientific supervision during the experimental procedures and contributed with expert opinions throughout the study. B. G. Y. supervised the entire research process, critically reviewed the manuscript, and approved the final version for publication. All authors reviewed and approved the final manuscript.

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