Research Paper

Intermittent Theta Burst Stimulation Mitigates Depressive-Like behavior in Rats Subjected to Maternal Separation

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Citation Pirzad Jahromi, G., Afshari, M., Roghani, M., Gharibzadeh, Sh, & Ghorbani Alvanegh, A. (2026). Intermittent Theta Burst Stimulation Mitigates Depressive-Like behavior in Rats Subjected to Maternal Separation. *Basic and Clinical Neuroscience*, *17*(1), 123-134. http://dx.doi.org/10.32598/bcn.2026.8409.1

doi http://dx.doi.org/10.32598/bcn.2026.8409.1

Article info:
Received: 26 Oct 2025
First Revision: 14 Nov 2025
Accepted: 16 Dec 2025
Available Online: 01 Jan 2026

Keywords:
Intermittent theta burst stimulation (iTBS), Maternal separation (MS), Depression, Oxidative stress

ABSTRACT

Introduction: Early life stress, such as maternal separation (MS), can lead to serious mental health problems, such as depressive-like behavior in later life. Intermittent theta burst stimulation (iTBS) is a transcranial magnetic stimulation (TMS) protocol. In this study, we sought to determine the effectiveness of the iTBS protocol on depressive-like behavior and cognitive impairment induced by MS in male rats because the therapeutic effect of this protocol on this model remains unknown.

Methods: To induce depression, we used the MS method from postnatal day (PND) 2 to PND 20 for 4 hours each day. At PND 30, the iTBS treatment began and continued for 10 consecutive days. At PND 40, behavioral tests (Barnes maze, open field test (OFT), elevated plus maze (EPM), and forced swim test [FST]) were conducted. The rats were sacrificed on day 50, with brain tissue removed for biochemical analysis (oxidative stress, tumor necrosis factor alpha [TNFα], brain-derived neurotrophic factor [BDNF], and beta-secretase 1 [BACE1]).

Results: Based on the results, iTBS significantly improved spatial memory impaired by MS, as evidenced in the Barnes maze, and significantly reduced anxiety-like behavior shown in the EPM and depressive-like behavior demonstrated in the FST. Biochemical analysis showed that iTBS significantly reduced oxidative stress (superoxide dismutase [SOD], reactive oxygen species [ROS], malondialdehyde [MDA], catalase [CAT], and glutathione [GSH]), inflammation (TNFα), and BACE1, but increased BDNF in the hippocampus.

Conclusion: Taken together, our study suggests that the iTBS protocol may have therapeutic effects on depressive-like behavior and cognitive impairment induced by MS.

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Highlights

- MS caused depression, anxiety, and cognitive deficits.
- iTBS improved behavioral and cognitive outcomes.
- iTBS reduces inflammation, oxidative stress, and BACE1, but increases BDNF.

Plain Language Summary

Early life stress can have a long-lasting effect on the brain and mental health. This early life stress can also be used as a model in animals, using maternal separation, to study the effect of this stress on the brain and to look for new therapeutic options. In our study, we induced depression by separating pups from their mothers for certain periods of time, and then we explored whether a protocol of transcranial magnetic stimulation called intermittent theta burst stimulation (iTBS) could reduce depression and other negative effects on the brain or behavior. It is worth mentioning that this method is already used in humans, but the effect of this method on maternal separation–induced depression is underexplored. Rats who received this kind of stimulation for a period of time showed reduced depressive behavior, less anxiety, and improved memory. We also looked for improvement in the brain. Our observations showed that brain damage was reduced with our treatment compared to untreated rats.

Introduction

Major depressive disorder (MDD) is a prevalent disorder that can greatly affect daily functioning and severely impair quality of life (De Risio et al., 2020; Murgatroyd et al., 2015). It can lead to behavioral disorders that continue into adulthood, such as depression (Wang et al., 2020). Evidence suggests that early life maternal separation (MS) may harm brain development and lead to serious mental problems, such as depression and autistic-like behavior (Bian et al., 2015; Mansouri et al., 2021; Zhou et al., 2020) including MS. Moreover, MS has been linked to cognitive deficits later in life (Hao et al., 2025; Suman et al., 2025). These findings highlight the need to understand how early stress alters specific brain regions, particularly the hippocampus, which is critically involved in mood regulation and memory.

The hippocampus is an important brain region involved in stress-related depression (Zhou et al., 2020) including MS. Studies report that depression is associated with reduced plasticity and decreased levels of brain-derived neurotrophic factor (BDNF) in areas such as the hippocampus (Ye et al., 2011). Early-life stress, such as MS, can also affect memory performance (Diehl et al., 2012) such as periodic MS, may alter the normal pattern of brain development and subsequently the vulnerability to a variety of mental disorders in adulthood. Patients with a history of early adversities show higher frequency of post-traumatic stress disorder (PTSD). In addition to behavioral changes, MS induces oxidative stress and neuroinflammation in the hippocampus. It can increase anxiety and decrease catalase (CAT) activity in the hippocampus (Malcon et al., 2020). Another study shows that malondialdehyde (MDA) and nitric oxide levels increase, while antioxidant levels decrease, in the hippocampus after MS in mice (Rostami-Faradonbeh et al., 2024). Studies also report increased inflammation in this area with prolonged MS (Zhou et al., 2020) including MS. Tumor necrosis factor (TNF)α expression is reported to increase in the hippocampus in another study (Farzan et al., 2023). These molecular disturbances are relevant to depressive-like mechanisms. For example, beta-secretase 1 (BACE1) is associated with cognitive impairment (Cheng et al., 2014), and oxidative stress is an important factor that can influence BACE1 activity in the hippocampus (Mouton-Liger et al., 2012). Given the combined behavioral, inflammatory, and oxidative alterations, MS provides a suitable model for testing therapeutic interventions with fewer side effects.

TMS is a non-invasive method for modifying brain activity using magnetic pulses (Shirota & Ugawa, 2024). Theta burst stimulation (TBS) is a specific TMS protocol (Lee et al., 2021). There are two commonly used protocols of TBS in research: intermittent TBS (iTBS), which is believed to facilitate neural transmission, and continuous TBS (cTBS), which suppresses brain excitability (Vékony et al., 2018). iTBS, consisting of 3x50 Hz pulses repeated at 5 Hz, has antidepressant effects similar to those of the traditional high-frequency rTMS protocol (Lee et al., 2021). Beyond mood regulation, iTBS has shown cognitive-enhancing effects (Pabst et al., 2022) and has been reported to have antioxidant effects and to increase BDNF in the hippocampus and other regions in a rat model of Alzheimer-like disease (Stanojevic et al., 2023). In another study of autism induced by MS, low-frequency rTMS applied for 14 days attenuated behavioral symptoms by regulating hippocampal gamma-aminobutyric acid transmission (Tan et al., 2018).

While previous studies have explored the effects of low-frequency rTMS in autism induced by MS (Tan et al., 2018) synaptic function and neural circuits. The imbalance of excitatory and inhibitory (E/I, the potential therapeutic role of iTBS in depressive-like behaviors induced by MS remains unclear. Based on this knowledge, we hypothesize that the iTBS protocol may improve depressive-like behaviors induced by MS in rats.

Materials and Methods

Experimental procedure

This study followed ethical guidelines and was approved by the Ethics Committee of Baqiyatallah University of Medical Sciences. Male and female Wistar rats were housed together for mating. Male rat pups were separated from their mother and each other from postnatal day (PND) 2 to PND 20 for 4 hours daily, from 8 AM to 12 PM, and the others were handled daily (Wang et al., 2020). After PND 21, male rats were assigned to 4 groups (8 rats per group): sham, iTBS + H (iTBS + healthy rat), MS, and MS+iTBS. All rats were maintained in standard animal housing conditions (standard nesting materials, 12:12 h light/dark cycle) at 22±1 ˚C and 45±3% humidity, with free access to food and water (Figure 1).

iTBS treatment

iTBS treatment was performed according to reference studies in another neurodevelopmental model (Rittweger et al., 2021). In this study, the Super Rapid2 Magstim device equipped with an air-cooled, figure-eight-shaped coil (D70 air film coil) was used. Rats received 3 blocks of iTBS and 15-minute intervals between blocks (frequency: 50 Hz, number of pulses: 3, number of bursts: 10, cycle time: 10 s, number of cycles: 20, and total number of pulses: 600).

The stimulation intensity was set at 100% of the motor threshold, based on previous experiments with 5 healthy rats for forelimb movement (Tan et al., 2018), and set to 50% of the maximum device output. For restraint, in line with a previous study (Afshari et al., 2024), the rats were introduced to the restraint method and device sound for 1 week before the actual treatment began (they were assigned to groups at PND 21). The rats were immobilized using medical gloves and a self-adhesive elastic wrap to minimize their movement during TMS coil application, with the coil handle aligned perpendicularly to the rat's body (the coil center was positioned at the midpoint between the eye and the ear). All experiments were conducted under environmentally controlled conditions to minimize stress. In the sham group, the coil was placed upside down and positioned at a sufficient distance from the rats' heads to prevent actual stimulation while replicating the coil's auditory output. The treatment protocol started on PND 30 (Tan et al., 2018) and continued for 10 consecutive days.

Barnes maze test

The Barnes maze test was conducted to measure spatial memory, following previously published methods (Zappa Villar et al., 2018). The test includes 3 stages: habituation, training, and probe. During the habituation stage, the rats were given enough time to explore the maze. During training, they learned to find the escape box and were expected to memorize its location. In the probe stage, the escape box was removed, and the rats' spatial memory was assessed. Several parameters were measured during the test. Latency is the overall time the rats needed to find the previous escape hole location for the first time during the probe test. The number of errors is the number of explorations of the holes throughout the maze until the escape hole is found. Strategy is the rats' strategy for finding the escape hole during the probe test. The result is scored as follows: score 1, spatial (the rats directly went toward the escape hole); score 2, serial (the rats explored a series of holes next to each other until they found the escape hole); score 3, random (the rats had no specific pattern for finding the escape hole).

Open field test (OFT)

In accordance with a previously published method (Mansouri et al., 2021) the unknown pathophysiology of autism as well as number of conflicting results, urge for further examination of the therapeutic potential of EE in autism. Therefore, the aim of this study was to examine the effects of environmental enrichment on autism-related behaviors which were induced in the MS, the time spent in the center of the apparatus over 15 minutes was assessed (40×40×40 cm).

Elevated plus maze (EPM) test

In accordance with a previous study, we used the EPM task to assess the anxiety levels of the rats (Servadio et al., 2016). One behavior was recorded: total time spent in the open arms of the EPM over 5 min.

Forced swim test (FST)

To assess depression, the FST was employed. A cylindrical container was half-filled with water (25±1 ˚C). The test included a 2-minute acclimation period followed by a 5-min test period (Hu et al., 2017). The duration of immobility in the water was recorded.

Biochemical analysis

After the behavioral tests, on PND 50, the rats were sacrificed following anesthesia (ketamine/xylazine). The hippocampal tissue was homogenized and stored at -80 ˚C for further analysis. For oxidative stress analysis, the following method was used: reactive oxygen species (ROS) were measured using the dichlorofluorescein diacetate (DCFDA) assay. According to the protocol, 100 µL of prepared supernatant and 10 µL of DCFDA were combined and incubated at 37 ˚C for 30 min (excitation: 488 nm, emission: 525 nm) (Nazari-Serenjeh et al., 2024). MDA was determined using the TBARS (thiobarbituric acid reactive substances) assay. After mixing with supernatant and incubating for 80 min at 90 ˚C, following cooling and centrifugation for 10 min at 1000 g (absorbance: 532 nm) (Alizadeh Makvandi et al., 2021). CAT activity was measured by its ability to decompose hydrogen peroxide into water. For the CAT assay, 20 µL supernatant was added to the buffer (100 µL) and diluted substrate (20 µL), incubated for 20 min, then potassium hydroxide (30 µL) was added to stop the reaction; after 5 min, potassium periodate was added (absorbance: 540 nm) (Nazari-Serenjeh et al., 2024). Glutathione (GSH) was assessed following the method detailed in previous literature by adding supernatant to DTNB (absorbance: 412 nm) (Alizadeh Makvandi et al., 2021). Superoxide dismutase (SOD) activity was measured by incubating the supernatant with xanthine and xanthine oxidase in potassium phosphate buffer (pH 7.8) at 37˚C for 40 min, followed by the addition of nitro blue tetrazolium and reading at 550 nm (Alizadeh Makvandi et al., 2021).

Additionally, TNFα levels were measured using a sandwich enzyme-linked immunosorbent assay (ELISA) with commercial kits from Bio-Techne, USA. BDNF content in the hippocampus was assessed using a BDNF ELISA kit (RAB1138, Sigma, USA). BACE1 activity was evaluated by measuring the hydrolysis of DL-BAPNA per hour (ΔA/h) (Ogunsuyi et al., 2020), and protein content in the hippocampal supernatant was measured using the BCA (bicinchoninic acid) method (Smith et al., 1985).

Statistical analysis

Data analysis was conducted using the software GraphPad Prism software, version 9.5.1. Group comparisons were performed using 2-way ANOVA followed by Tukey's test for all data except for the search strategy and number of errors acquired from the Barnes maze, which were analyzed using the Kruskal-Wallis test followed by Dunn's test. To examine the relationship between depressive-like behavior and biochemical markers, we performed the Pearson correlation analyses between FST immobility and MDA, TNFα, and BDNF levels. Because the distribution of BACE1 did not meet parametric assumptions, its association with FST was assessed using Spearman's rank correlation. All P values obtained from these correlation analyses were corrected for multiple testing using the original Benjamini–Hochberg false discovery rate method. Normality was assessed using Q-Q plots. Results were expressed as Mean±SEM, with P<0.05 considered statistically significant.

Results

Effect of iTBS on spatial memory

The two-way ANOVA indicated significant variations among groups in terms of the latency to find the specific target hole (Figure 2A), which the rats had previously learned to locate ($F_{1, 28}$=44.36, P<0.001) with significant interaction differences ($F_{1, 28}$=50.75, P<0.001). The analysis revealed that MS significantly increased the time to locate the escape hole compared to the sham group (P<0.001). In contrast, iTBS significantly reduced latency compared to the MS group (P<0.001).

Additionally, the analysis indicated significant differences in errors made until finding the target hole (the Kruskal-Wallis test; H=19.05, df=3, P<0.001) (Figure 2B). The MS group made significantly more errors than the sham group (P<0.001). However, iTBS treatment significantly reduced the number of errors compared with the MS group (P<0.05).

Strategy scores (Figure 2C), based on maze performance, were significantly shifted from spatial to random patterns in the MS group (the Kruskal-Wallis test; H=11.91, df=3, P=0.008). Magnetic stimulation signifi-

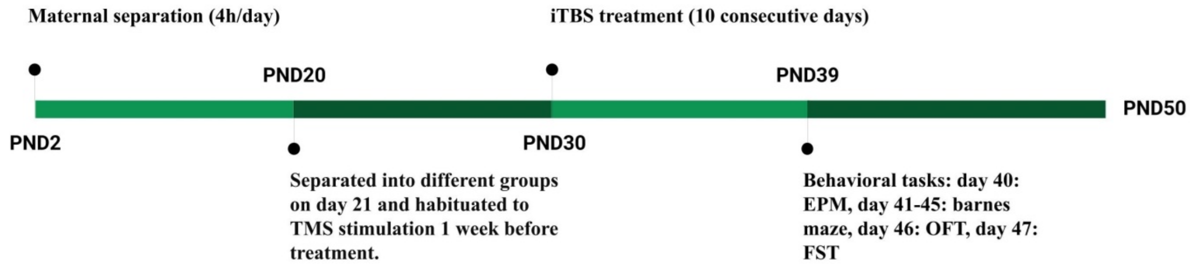

Figure 1. Timeline of this study

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Note: PND 2 to PND 20: Rats undergo MS to induce depressive-like disorder. Day 21: Male rats are randomly divided into groups. PND 30: iTBS treatment starts and continues for 10 consecutive days. PND 40: Behavioral evaluations begin. PND 50: Rats are sacrificed, and brain tissue is collected for further analysis.

cantly shifted the strategy scores toward spatial patterns compared to MS rats (P<0.05).

Effect of iTBS on the OFT

In the OFT, significant differences were observed across groups ($F_{1, 28}$=18.32, P<0.001) (Figure 3A), with a significant interaction ($F_{1, 28}$=4.795, P<0.05). Further analysis showed that MS significantly reduced the time spent in the center compared to the sham group (P<0.001), while iTBS significantly increased center time compared to the MS group (P<0.05).

Effect of iTBS on the EPM test

Spending less time in the open arms indicates higher anxiety levels (Figure 3B). Analysis showed significant differences in EPM between groups ($F_{1, 28}$=39.58, P<0.001) and a significant interaction ($F_{1, 28}$=9.823, P<0.01). Further analysis indicated that the MS rats spent significantly less time on the open arms than the sham group (P<0.001). iTBS significantly increased open-arm time relative to MS rats (P<0.01).

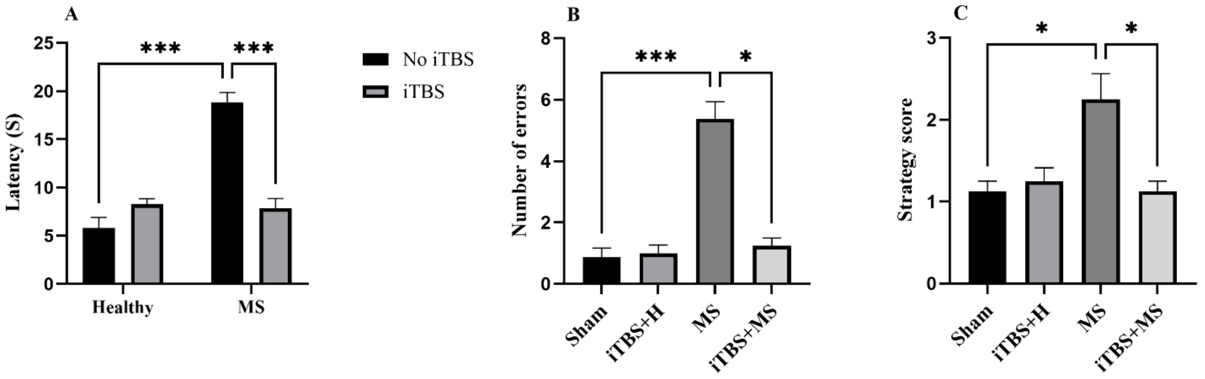

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Figure 2. The effect of intermittent theta burst stimulation treatment on spatial memory with Barnes maze in rat model of depression-like behavior induced by MS

A) Time spent to find the target hole in the probe test, B) Number of errors made to find the target hole in the probe test, C) Strategy score based on how the rats managed to find the target hole in the probe test.

*P<0.05, **P<0.01, ***P<0.001.

Note: All data are presented as Mean±SEM (n=8).

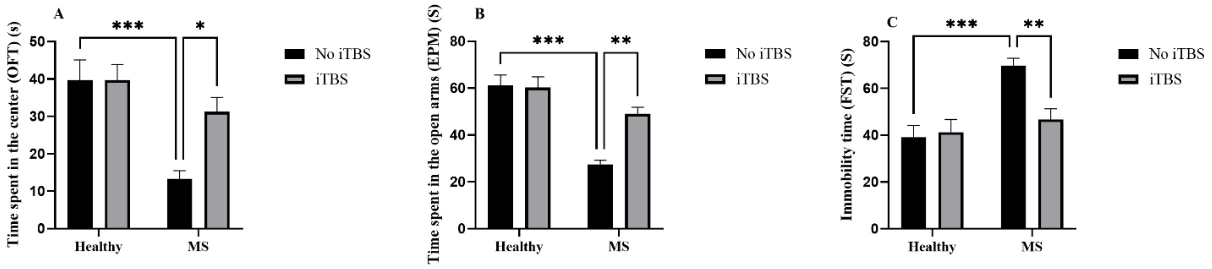

Figure 3. The effect of intermittent theta burst stimulation treatment on OFT, EPM and FST in rat model of depression-like behavior induced by MS

A) Time spend in the center showing the anxiety level in the open field test, B) Time spent in the open arms of the EPM, C) Immobility time in water during the FST.

*P<0.05, **P<0.01, ***P<0.001.

Note: All data are presented as Mean±SEM (n=8).

Effect of iTBS on FST

FST was used to measure depression levels in our study (Figure 3C). Two-way ANOVA analysis showed significant differences among groups in immobility time during the FST ($F_{1, 28}$=15.30, P<0.001) with a significant interaction ($F_{1, 28}$=7.453, P<0.05). Immobility time during the FST was significantly increased in MS rats (P<0.001), while iTBS significantly decreased immobility time compared to the MS group (P<0.01).

Effect of iTBS on biochemical parameters

Biochemical factors were analyzed for differences between groups using 2-way ANOVA. Significant differences were found in oxidative stress markers: ROS ($F_{1, 16}$=5.498, P<0.05), MDA ($F_{1, 16}$=6.943, P<0.05), GSH ($F_{1, 16}$=52.88, P<0.001), CAT ($F_{1, 16}$=65.59, P<0.001), and SOD ($F_{1, 16}$=17.88, P<0.001) with significant interaction differences ROS ($F_{1, 16}$=4.920, P<0.05), MDA ($F_{1, 16}$=9.783, P<0.01), GSH ($F_{1, 16}$=24.47, P<0.001), CAT($F_{1, 16}$=70.32, P<0.001) and SOD ($F_{1, 16}$=10.21, P<0.01) (Figure 4). Further analysis showed that ROS and MDA levels were significantly increased in the MS group (P<0.05 and P<0.01, respectively), while GSH, CAT, and SOD levels were significantly reduced (P<0.001). iTBS treatment partially reversed these changes, reducing ROS and MDA levels (P<0.05 and P<0.01, respectively) and significantly increasing GSH, CAT, and SOD levels (P<0.001).

In terms of inflammation (Figure 5A), the level of TNFα in the hippocampus showed significant differences between groups ($F_{1, 16}$=10.88, P<0.01) and a significant interaction ($F_{1, 16}$=5.654, P<0.05). TNFα levels were significantly increased in the MS group (P<0.01), and iTBS treatment significantly reduced TNFα levels to levels not significantly different from those in the sham group (P<0.05).

The level of BDNF (Figure 5B) also showed significant changes between groups ($F_{1, 16}$=6.122, P<0.05) with interaction differences ($F_{1, 16}$=4.563, P=0.05). In the MS group, hippocampal BDNF levels were significantly reduced (P<0.05), whereas iTBS treatment significantly elevated them relative to the MS group (P<0.05).

Analysis of BACE1 ($F_{1, 16}$=13.52, P<0.01) activity (Figure 5C) revealed a significant increase in the MS group compared to the sham group (P<0.01). However, our treatment reduced BACE1 activity toward sham group levels (P<0.01). Significant interaction differences ($F_{1, 16}$=6.480, P<0.05).

Correlation analysis for FST and biochemical parameters

To assess the relationship between depressive-like behavior (FST immobility) and key biochemical parameters, we performed the Pearson correlation analyses of MDA (a key oxidative stress marker), TNFα, and BDNF. These analyses revealed significant correlations between FST and MDA (r=0.43, P=0.029), TNFα (r=0.48, P=0.017), and BDNF (r=–0.39, P=0.045). The correlation between BACE1 and FST was evaluated using Spearman's rank correlation, which showed a positive association (r=0.72, P<0.001).

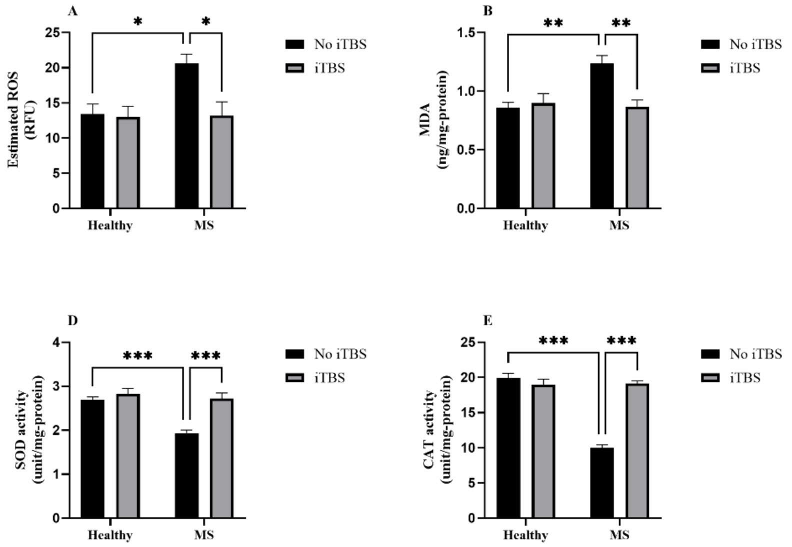

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Figure 4. The effect of intermittent theta burst stimulation treatment on oxidative stress in rat model of depression-like behavior induced by MS

A) ROS, B) MDA, C) GSH, D) SOD, E) CAT

*P<0.05, **P<0.01, ***P<0.001.

Note: All data are presented as Mean±SEM (n=5).

Discussion

Our research showed that 10 days of iTBS had a therapeutic effect on early-life stress induced by MS to some extent. In this study, we showed that MS led to memory impairment and increased anxiety and depression later in this model. These behavioral symptoms were accompanied by oxidative stress, inflammation, and reduced BDNF levels in the hippocampus of MS rats. iTBS attenuated these MS-induced symptoms.

Early-life stress can affect the hypothalamic-pituitary-adrenal (HPA) axis and, in turn, influence behavior and brain structures, such as the hippocampus (Huang, 2014). Research suggests that the hippocampus is vulnerable to oxidative stress (Salim, 2017). MS can cause long-lasting damage to the hippocampus through mechanisms such as inflammation, eventually leading to serious mental problems (Aisa et al., 2007; Diehl et al., 2012; Wang et al., 2020). Consistent with other studies, our experiment showed that MS caused several behav-

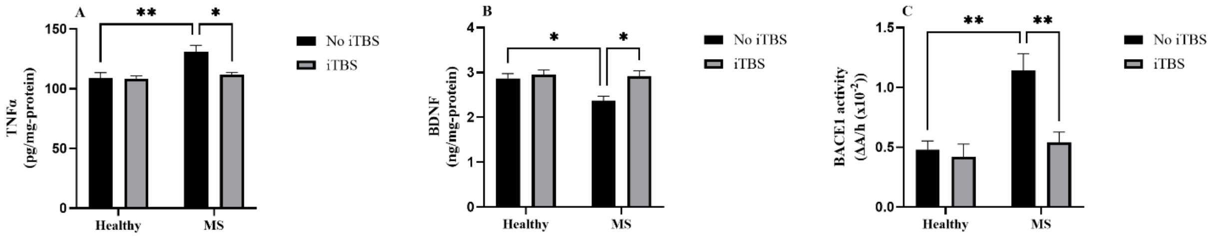

Figure 5. The effect of intermittent theta burst stimulation treatment on other biochemical parameters in rat model of depression-like behavior induced by MS

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A) TNFα, B) BDNF, C) BACE1

*P<0.05, **P<0.01, ***P<0.001.

Note: All data are presented as Mean±SEM (n=5).

ioral problems, including increased latency to find the target hole in the Barnes maze, reduced time spent in the open arms in the EPM, and increased immobility time in the FST.

As mentioned, MS can affect oxidative stress and inflammation, as shown in our study. An increase in ROS production activates the cellular antioxidant defense system, and when this system fails, oxidative stress increases (de Almeida et al., 2022; Sharma et al., 2023). Our study showed that ROS and MDA levels increased, and the antioxidant system was disrupted. Another study reported decreased total brain antioxidants and increased levels of MDA and nitrite in MS (Rostami-Faradonbeh et al., 2024). Our study showed that CAT, SOD, and GSH levels also decreased later in life after MS. BACE1 activity can be affected by elevated oxidative stress (Mouton-Liger et al., 2012). Our results showed increased BACE1 activity and cognitive impairments. Another study also showed MS involvement in hippocampus inflammation (Farzan et al., 2023). TNFα levels increased in our experiment as a marker of hippocampal inflammation in MS rats.

BDNF is an important factor that influences neural connectivity and shapes neurons (Deinhardt & Chao, 2014). BDNF is also important in depression (Yu & Chen, 2011). In our study, MS was associated with reduced BDNF levels, consistent with previous reports (Ohta et al., 2017).

TBS is increasingly gaining attention because it requires less stimulation time (Stoby et al., 2022). There is evidence that iTBS has an antidepressant effect in rats, mediated by increased dendritic spine density and modulation of BDNF (Lee et al., 2021). Previous studies have also reported that iTBS can enhance cognitive function in a rat model of Alzheimer disease (Stanojevic et al., 2022). Our study showed that iTBS treatment after MS reduced depression-like behavior and partially improved memory.

Several studies report that rTMS has antioxidant effects. It has been reported that TMS stimulation enhanced the antioxidant system in cells and reduced oxidative stress (Medina-Fernández et al., 2018). It was also reported that magnetic stimulation has anti-inflammatory properties (Tian et al., 2020). Both effects can reduce MS-induced early-life stress damage, as observed in our study.

iTBS can also modulate BDNF levels, as observed in our study. Similarly, another study reported that long-term iTBS treatment in an ischemic-reperfusion model can improve recovery via the miR-551b-5p/BDNF/TrkB pathway (Wang et al., 2022). This finding aligns with other related studies showing that rTMS increases BDNF levels and enhances cognition (Shang et al., 2016).

This study has several limitations; the most important is the coil size, which was relatively large for rat brains at this age and may have influenced other brain structures and networks, potentially affecting results. Another limitation is that this study lacks immunohistochemical or histological analyses; future studies could benefit from these analyses to demonstrate the effects of this technique on brain structures. Another limitation is that training data from the Barnes maze were not included; as only probe test data were used, conclusions about spatial learning are not fully supported. Males and females respond differently to acute and chronic stress regarding hormone release and cognitive functions. In this respect, females often display greater resilience to chronic stress (Luine et al., 2017). Also, sex differences were not the main objective of our study; future studies are recommended to consider this aspect as well. Another limitation is that we tested the therapeutic effect of iTBS using tests that may yield different outcomes, and this important issue should be considered in future studies.

Conclusion

Taken together, our data suggest that iTBS has therapeutic effects on depressive-like behavior induced by MS in male rats. Ten days of iTBS improved spatial memory, reduced oxidative stress and inflammation, and increased BDNF levels in the hippocampus.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Research Ethics Committee of Baqiyatallah University of Medical Sciences, Tehran, Iran (Code: IR.BMSU.AEC.1402.030).

Data availability

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

Declaration of generative AI and AI-assisted technologies in the writing process

The authors used Grammarly and generative AI to improve clarity. All authors then reviewed and edited the manuscript and take full responsibility for its content.

Funding

This research did not receive any grant from funding agencies in the public, commercial, or non-profit sectors.

Authors' contributions

Conceptualization: Mehrdad Roghani and Masoud Afshari; Methodology: Gila Pirzad Jahromi, Masoud Afshari, and Mehrdad Roghani; Investigation: Gila Pirzad Jahromi, Masoud Afshari, Mehrdad Roghani, and Akbar Ghorbani; Resources: Mehrdad Roghani, Masoud Afshari, and Shahriar Gharibzadeh; Supervision: Mehrdad Roghani and Gila Pirzad Jahromi; Writing the original draft: Gila Pirzad Jahromi; Review and editing: All authors.

Conflict of interest

The authors declared no conflict of interest.

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