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Low-frequency repetitive transcranial magnetic stimulation mitigates working memory deficit and cortical malondialdehyde besides preservation of dendritic spines in valproic acid-induced model of autism spectrum disorder

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Abstract

Background and Objective: Autism spectrum disorder (ASD) is characterized by behavioral dysfunctions, including repetitive behaviors and impaired social interactions. Previous research has identified working memory deficits in individuals with ASD, often associated with prefrontal cortex abnormalities. Valproic acid (VPA), a well-known antiepileptic drug, has been linked to negative effects on brain development and an increased risk of ASD. This study explored the potential therapeutic impact of low-frequency repetitive transcranial magnetic stimulation (LF-rTMS) in mitigating oxidative stress and preserving neural structures in the context of ASD induced by prenatal exposure to VPA.

Materials and Methods: Our investigation examined the role of LF-rTMS, a non-invasive brain stimulation technique, in modulating working memory (Y-maze), oxidative stress, and protecting neural structures. Oxidative stress, measured by malondialdehyde (MDA) in the prefrontal cortex, serves as a critical marker for evaluating cellular damage. Dendritic spine density was assessed using the Golgi impregnation method as a marker for neural structure protection.

Results: Our data indicated that LF-rTMS treatment significantly improves working memory function, reduces MDA level, and increases dendritic spine density in the prefrontal cortex.

Conclusion: In conclusion, our findings suggest that LF-rTMS holds promise as a neuroprotective intervention, showing potential in reducing oxidative stress and preserving neural structures in a VPA-induced ASD model.

Keywords: Autism spectrum disorder, Low-frequency repetitive transcranial magnetic stimulation, Valproic acid, Working memory

1. Introduction

Environmental factors such as air pollution, adverse early childhood experiences, and exposure to certain drugs during pregnancy may contribute to the development of autism spectrum disorder (ASD) (1). ASD is characterized by behavioral dysfunctions, including repetitive and restricted behaviors and impaired social interactions (2). Valproic acid (VPA), a well-known antiepileptic drug, has been shown to negatively affect brain development when consumed by pregnant women, potentially leading to ASD later in life (3).

Previous research suggests that working memory deficits are commonly observed in individuals with ASD and rodent models (4,5). Working memory is closely associated with the prefrontal cortex (6). One study reports morphological and electrophysiological changes in a rodent model of ASD, possibly linked to

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increased oxidative stress in this brain region (7). Malondialdehyde (MDA) is a crucial marker for evaluating oxidative stress in the brain (8). Elevated MDA levels indicate increased lipid peroxidation in cell membranes, serving as a marker of cellular damage (9). Research has shown that oxidative stress is common in VPA-induced ASD models (10), and it may contribute to damage to neural cells, particularly dendrites and spines (11). Dendrites are critical for healthy neural activity (12).

Repetitive transcranial magnetic stimulation (rTMS) is a well-established non-invasive brain stimulation technique that uses magnetic pulses to modulate cortical activity. The effects of rTMS on the brain vary depending on the frequency of stimulation (13). Studies have shown that lower frequencies tend to have inhibitory effects on neural cells, while higher frequencies tend to be excitatory (14). It has also been suggested that rTMS possesses neuroprotective and antioxidant properties (15).

In summary, the aim of this study was to investigate the effects of low-frequency rTMS (LF-rTMS) on MDA as an oxidative stress marker, as well as its protective effects on dendrites and spines.

2. Materials and Methods

2.1. Experimental procedure

In this study, we followed NIH guidelines for animal experimentation, and the study was approved by the Shahid Beheshti University Ethics Committee (IR.SBU.REC.1401.108). We used male Wistar rats throughout the experimental period, maintaining them under standard animal facility conditions with free access to standard rat food and clean water.

To induce ASD, we administered VPA at a dose of 600 mg/kg via intraperitoneal injection to pregnant rats on gestational day 12.5. Successful mating was confirmed by the presence of a white plug in the vagina or cages (16). Twenty-one days after birth, male offspring rats were separated and randomly assigned to one of four groups: Control, LFC, Autism, and LF+Autism, each comprising eight rats. All experiments were conducted between 8 a.m. and 12 p.m.

For treatment, we employed LF-rTMS using a MagStim device equipped with a 70 mm figure-of-eight coil (UK). The stimulation parameters were set at a frequency of 1 Hz, with 20 trials in each session and a 2-second inter-train interval. This protocol was repeated for 14 days, starting from postnatal day 30 to day 43 (17). The stimulation intensity was set to 100% of the resting motor threshold, determined based on preliminary data. The resting motor threshold was established by observing the hand movements of four to five healthy awake rats under single-pulse stimulation. Based on this data, the stimulation intensity was set to 50% of the device's maximum output. Stimulation was applied to the area between the eye and ear in the center of the rat's head. To minimize movement during stimulation, the rats were restrained using a method from a previous study (18). To acclimatize the rats to the restrainer and the stimulation sound, they were exposed to these conditions for one week before the experiment.

2.2. Working memory

The Y-maze used in this study consisted of three arms, each forming a 120-degree angle with the others, designated as A, B, and C. Each arm has a length of 60 cm, a height of 30 cm, and a width of 15 cm. The arms are connected by a triangular plate. To conduct memory tests in the Y-maze, a quiet and dark room with minimal noise and traffic is necessary. The test is performed only once for each rat.

The procedure begins with rats that have no prior exposure to the maze. Each rat is placed in the first segment of one arm, and the guillotine door is closed. The experiment officially commences by opening the guillotine door. Over the course of 8 minutes, the rat's entries into the arms are recorded as they occur sequentially. At the end of the 8-minute period, the rat exits the maze and is returned to its cage.

An alternation is defined as an entry by the rat into all three arms without repeating any of them. The alternation percentage is calculated as the ratio of actual alternations to the maximum possible alternations, subtracted by 2. This metric provides a measure of the rat's working memory and cognitive flexibility during the Y-maze test.

2.3. Malondialdehyde (MDA) assay

MDA concentration was determined utilizing the TBARS assay (19). The procedure entailed mixing the supernatant with trichloroacetic acid and TBARS reagent, followed by an 80-minute incubation at 90°C. After cooling, samples underwent centrifugation at 1000 g for 10 minutes, and the absorbance of the supernatant was measured at 532 nm. MDA levels were then quantified as ng/mg of protein.

2.4. Protein assessment

The quantification of protein content in the samples was achieved through the implementation of the bicinchoninic acid (BCA) method, following a procedure detailed in prior documentation (20).

2.5. Golgi impregnation method

The Golgi impregnation method utilized in this study was adapted from a previously documented procedure (21). In brief, this method consists of two steps: 1) A two-week immersion in a solution containing 1% mercury chloride, 0.8% potassium chromate, 0.5% potassium tungstate, and 1% potassium dichromate; 2) Two days in another solution containing 15% potassium nitrate and 1% lithium hydroxide. The density of spines in the prefrontal cortex was then computed using ImageJ (ver. 1.54h) software.

2.6. Statistical analysis

The Shapiro-Wilk test was employed to assess normality. Statistical analysis was conducted using one-way ANOVA, followed by post-hoc testing using Tukey's method. All data are presented as mean ± SEM, and significance was determined for p-values below 0.05.

3. Results

Y-Maze

One-way ANOVA analysis of the data between groups revealed significant differences ($F(3, 28) = 5.952$, $p < 0.01$; Fig. 1). Post-hoc assessments showed that the autism group exhibited significant differences compared to the control group ($p < 0.01$). LF-rTMS treatment demonstrated a significant effect on working memory disruption in rats prenatally exposed to VPA ($p < 0.05$).

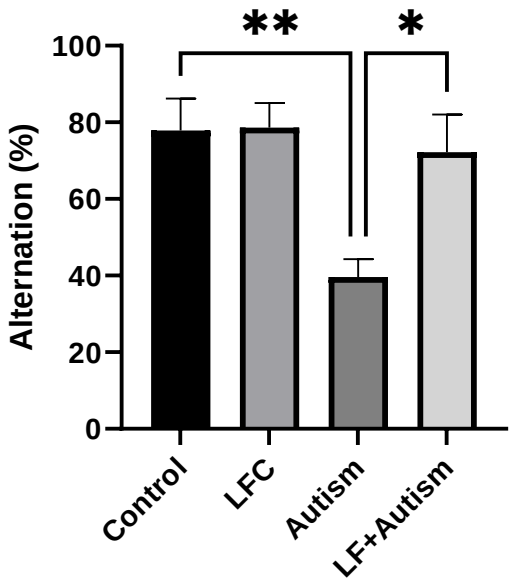

Fig. 1. Evaluation of working memory using the Y-maze test in different experimental groups. Data are presented as mean ± SEM (n = 8). *p < 0.05, **p < 0.01.

Malondialdehyde (MDA) level

MDA serves as a marker for lipid peroxidation in cells and oxidative stress. Analysis of the MDA results indicated significant differences among experimental groups ($F(3, 16) = 5.951$, $p < 0.01$; Fig. 2). Further analysis demonstrated that although autism increased MDA levels in the prefrontal cortex of rats ($p < 0.05$), LF-rTMS treatment significantly reversed this increase ($p < 0.01$).

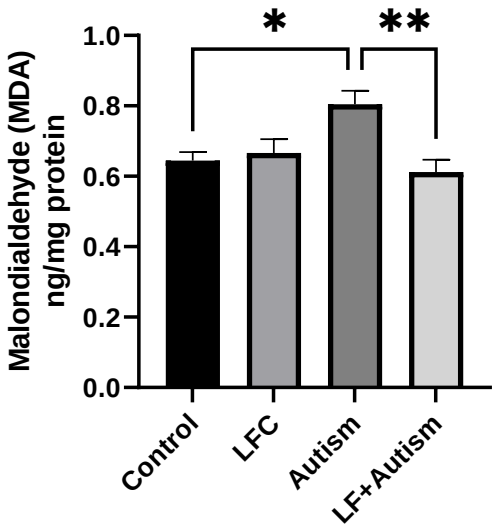

Fig. 2. Comparison of MDA levels, a marker for oxidative stress, across the experimental groups. Data are presented as mean ± SEM (n = 5). *p < 0.05, **p < 0.01.

Dendritic Spine Density

Dendritic spines are essential structures in neurons for the integration of neural signals. Our results showed significant differences among groups ($F(3, 16) = 15.43$, $p<0.001$; Fig. 3). Prenatal VPA exposure reduced spine density in the prefrontal cortex ($p<0.001$), and LF-rTMS treatment was able to reverse these changes ($p < 0.05$) ($P > 0.05$).

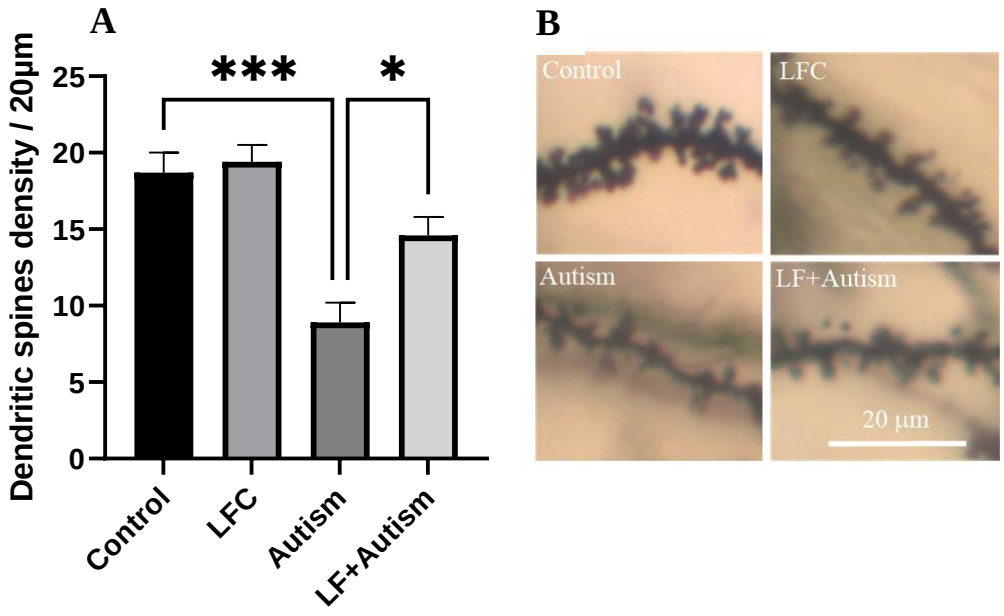

Fig. 3. A) Analysis of dendritic spine density in the prefrontal cortex among different experimental groups. Data are presented as mean ± SEM (n = 5). *p < 0.05, ***p < 0.001. B) Visualization of dendritic spines in representative samples from the different groups.

4. Discussion

In this research, we applied LF-rTMS therapy to address autism-like behaviors induced by VPA, focusing on working memory and its underlying mechanisms. In the Y-maze test, we observed a decrease in working memory capacity in rats prenatally exposed to VPA. However, LF-rTMS therapy successfully ameliorated these working memory dysfunctions. MDA assessment of the prefrontal cortex showed an increase due to VPA exposure, which significantly decreased with LF-rTMS treatment. Additionally, histological analysis indicated that LF-rTMS increased spine density in the prefrontal cortex of VPA-exposed rats.

Research studies have suggested a possible link between prenatal exposure to valproic acid and an increased risk of autism in offspring. Prenatal exposure refers to the period during pregnancy when the developing fetus may be exposed to the medication if the mother is taking it (22). Some studies have reported an increased risk of autism in children exposed to valproic acid during pregnancy (23). However, the exact mechanisms by which valproic acid might contribute to autism are not fully understood.

Extended use of HF-rTMS raises concerns regarding seizures due to its cortical excitatory effects. In contrast, LF-rTMS provides an inhibitory influence on brain cells, thereby mitigating this risk (17).

An accumulating body of evidence underscores the potential for cognitive and memory deficits resulting from prenatal exposure to VPA (24). Our findings on LF-rTMS treatment highlight its effectiveness in enhancing working memory function, as evidenced by the Y-maze test. These results align with previous research that emphasizes LF-rTMS's potential to alleviate cognitive deficits (25,26).

Valproic acid has been shown to affect mitochondrial function, potentially leading to an increase in reactive oxygen species (ROS) production while also reducing certain antioxidants in the body (27). MDA is a key biomarker associated with oxidative stress (8). This molecule serves as a well-established indicator of lipid peroxidation, a process that can result in cellular damage and dysfunction (28). Elevated MDA levels often indicate increased oxidative damage (9). In the VPA model of autism, elevated MDA levels are frequently observed in various tissues, including the brain, potentially contributing to the neurodevelopmental abnormalities seen in this model (29). Oxidative stress can significantly impact the structure and function of dendritic spines, which are small protrusions on neuronal dendrites that play a crucial role in synaptic transmission and plasticity (30). Oxidative stress can lead to the oxidation of proteins involved in spine structure and function, resulting in alterations in spine morphology and impairments in synaptic plasticity (31). Dendritic spines are essential for the neural circuits underlying working memory, a cognitive function responsible for the temporary storage and manipulation of information during mental tasks (32). Therefore, any damage to these structures may cause working memory dysfunction, as seen in our study.

Studies have shown that transcranial magnetic stimulation (TMS) triggers biological effects, including the release of neurotransmitters, heightened neurotrophic factors, and antioxidant effects (33). Research suggests that magnetic stimulation increases the activity of antioxidant enzymes while concurrently reducing oxidant levels (33). LF-rTMS has also been shown to have anti-inflammatory activity (34). In our study, LF-rTMS was found to decrease MDA levels in the prefrontal cortex. Interventions aimed at reducing oxidative stress may positively impact dendritic spine density, as observed in our study .

Conclusion

In conclusion, our research emphasizes the potential therapeutic role of LF-rTMS in addressing autism induced by VPA, specifically by modulating the oxidative stress pathway. LF-rTMS emerges as a promising intervention, offering a non-invasive and effective approach to treatment.

Conflicts of interest

The authors declare that they have no competing interests.

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