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Electroacupuncture at Baihui and Shenting Improves Learning and Memory Impairment and Anxiety-like Behavior Induced by Electromagnetic Pulse

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Abstract: Following exposure to electromagnetic pulses (EMP), the brain may experience impaired learning and memory capabilities, as well as anxiety-like behaviors. Microglial activation and inflammatory responses have been shown to be associated with EMP-induced brain damage. Electroacupuncture treatment has demonstrated potential in inhibiting microglial activation and mitigating inflammatory responses in various neurological disorders. This study aims to investigate whether electroacupuncture treatment can alleviate EMP-induced learning and memory impairments and to elucidate its underlying mechanisms. Compared with the non-acupoint group and the EA + LPS, the Y-maze and open field tests indicated that the EA group spent more time in the novel arm and the central area of the open field, the proportion of $iNOS^+/iba\text{-}1^+$ cells in the hippocampus decreased, and the concentrations of pro-inflammatory factors IL-1β, IL-6, and TNF-α decreased. EA improved EMP-induced learning and memory impairment and anxiety-like behavior by inhibiting microglial activation and inflammatory response in the hippocampus.

Keywords: Electroacupuncture; Electromagnetic pulse; Microglia; Activation; Inflammation; Learning and memory; Anxiety

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1. Introduction

An electromagnetic pulse (EMP) is a pulsed high-energy transient electromagnetic field that can induce multi-system damage in organisms exposed to its irradiation range. The brain is particularly vulnerable to EMP irradiation, and exposed animals may experience impairments in learning and memory as well as anxiety-like behaviors [1]. Currently, there is still a lack of effective treatment for EMP-induced brain damage. Microglia appear activated in EMP-related brain injuries, and inflammatory responses are involved in the EMP-induced decline in learning and memory abilities [2]. In various neurological diseases, electro-acupuncture has been shown to effectively suppress microglial activation and mitigate inflammatory responses, promoting tissue repair [3–4]. This study aims to determine whether electroacupuncture can improve EMP-induced impairments in learning and memory and elucidate its underlying mechanisms.

2. Materials and methods

2.1. Experimental animals

Sixty SPF-grade male C57 mice (aged 6-8 weeks) were assigned to five groups: the sham irradiation group (Sham group), the electromagnetic pulse group (EMP group), the electro-acupuncture group (EA group), the non-acupoint control group (EACtl group), and the electro-acupuncture + LPS group (EA+LPS group), with 12 mice in each group. This experiment was approved by the Experimental Animal Ethics Committee of Shaanxi University of Chinese Medicine (Ethics Approval Number: SUCMDL20240306008).

2.2. Main reagents and instruments

Disposable acupuncture needles (0.25 mm×25 mm), electroacupuncture therapy instrument (Huatuo, Suzhou Medical Supplies Factory), microplate reader (Thermo Fisher Scientific), high-speed frozen centrifuge (Eppendorf), microscope (Nikon), fluorescence microscope (Nikon). Iba-1 and iNOS antibodies (Abcam), goat anti-rabbit fluorescent secondary antibody, donkey anti-mouse fluorescent secondary antibody (Zhongshan Golden Bridge), Nissl staining kit (Solarbio), HE staining reagent (Solarbio), and ELISA kit (Boster).

2.3. Modeling and intervention methods

The sham group was placed in a non-electrified EMP irradiator for sham exposure, while the other groups received EMP irradiation at 400 kV/m, 400 pulses per day, repeated for 3 days, to establish an EMP-induced learning and memory impairment model. The EA group received electro-acupuncture treatment at the "Baihui" and "Shenting" acupoints according to "Experimental Acupuncture and Moxibustion" [5]. The needles were inserted 2-3 mm deep using the pinching insertion technique. The waveform of the electroacupuncture therapy instrument was set to a sparse-dense wave at 2 Hz/10 Hz, with a current intensity of 1~2 mA, administered for 20 minutes per session, once a day for 7 consecutive days. The EACtl group was treated at 3 mm above the iliac crest on both sides, with the same electro-acupuncture parameters as the EA group. The EA+LPS group received an intraperitoneal injection of LPS (the microglial activator) at a dose of 3 mg/kg before electroacupuncture intervention, with the remaining treatment the same as the EA group.

2.4. Behavioral testing

Y-maze test: This test consisted of two phases. In the first phase (acquisition phase), one arm was closed, and the mouse was placed in the center of the Y-maze, allowed to freely explore the two open arms for 3 minutes. Two hours later, during the second phase (recall phase), all arms were opened, and the mouse was again placed in the center of the Y-maze to freely explore all three arms for 3 minutes. The time spent and distance explored in each arm were recorded. Mice with memory impairment typically exhibit reduced exploration time and distance in the novel arm compared to the familiar arms. The parameters measured included the number of entries into each arm, the time spent, and the distance explored in each arm, which were then statistically analyzed. Open field test: The open field was divided into central and peripheral zones. The experimental animal was placed in the environment of the open field test for 2 hours of adaptation. At the start of the experiment, the mouse was placed in the center of the open field, and its movement trajectory was recorded using a tracking and recording system.

2.5. Histopathological staining

The brain tissue was removed, fixed, dehydrated, embedded, and sectioned. HE staining: After dewaxing and rehydration, the sections were stained with eosin staining solution for 5–10 minutes and hematoxylin staining solution for 2–3 minutes. Nissl staining: After dewaxing and rehydration, the sections were stained with 1% toluidine blue staining solution for 30 minutes.

2.6. Immunofluorescence staining

The brain tissue was removed, fixed, dehydrated, embedded, and sectioned. After antigen retrieval of the sections, goat serum was added and incubated at room temperature for 30 minutes to block non-specific binding. The blocking solution was then removed by washing, and the primary antibody dilution was added for incubation at 4°C overnight. The next day, the unbound primary antibody was washed off, and the sections were incubated with a fluorescent secondary antibody at room temperature for 2 hours. The samples were observed under a fluorescence microscope.

2.7. ELISA detection of rat hippocampus tissue

Remove the brain tissue of the mouse, separate the bilateral hippocampus on ice, add ice-cold PBS solution, homogenize at 4°C, and centrifuge to obtain the supernatant. Following the kit instructions for subsequent detection steps, the absorbance was measured by a microplate reader, and the content of inflammatory factors was calculated.

2.8. Statistical analysis

SPSS 23.0 was used for statistical analysis. Measurement data were expressed as (mean ± standard deviation). For normally distributed and homoscedastic data, one-way ANOVA was conducted, followed by pairwise comparisons between groups using the Least Significant Difference (LSD) method. $P < 0.05$ indicated that the difference was statistically significant.

3. Results

3.1. Comparison of Y-maze test and open field test results among mice in each group

In the Y-maze test, compared with the Sham group, the EMP group mice exhibited shorter stay times in the novel arm and the central area of the open field; compared with the EMP group, the EA group mice exhibited longer stay times in both the novel arm and the central area of the open field; there was no significant difference in the stay times in the novel arm and the central area of the open field between the EACtl group and the EA+LPS group compared with the Sham group; compared with the EA group, the EACtl group and the EA+LPS group exhibited shorter stay times in both the novel arm and the central area of the open field (**Figure 1**).

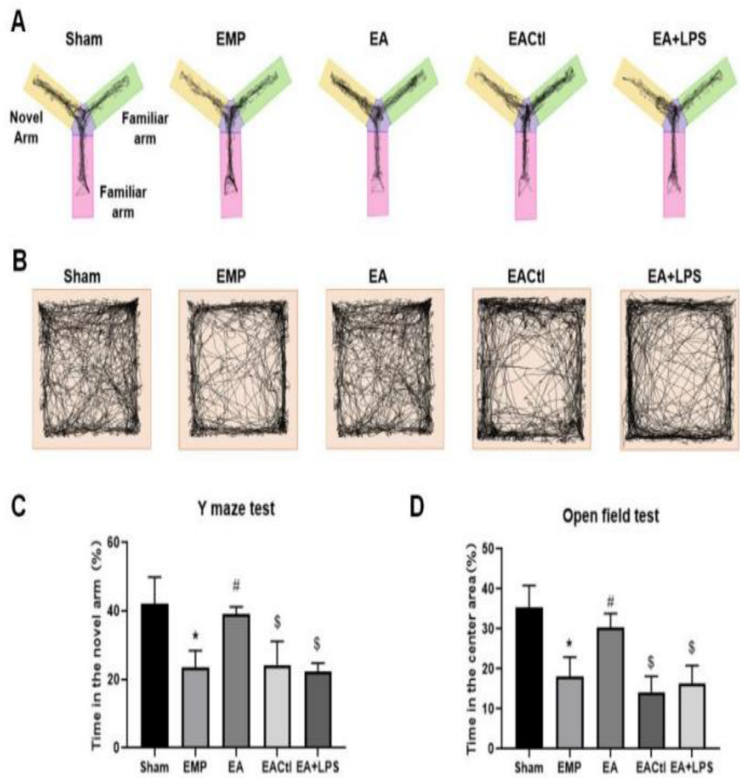

Figure 1. Y-maze test and open field test results among different groups of mice. (A) Movement trajectory in the Y-maze, (B) Movement trajectory in the open field, (C) Percentage of time spent in the novel arm of the Y-maze, (D) Percentage of time spent in the central area of the open field. * represents $P < 0.05$ compared to the Sham group; # represents $P < 0.05$ compared to the EMP group; $ represents $P < 0.05$ compared to the EA group

3.2. Comparison of pathological staining results in the hippocampal CA1 region of mice from different groups

Compared to the Sham group, the EMP-irradiated hippocampal cells showed irregular arrangement, cytoplasmic shrinkage, blurred contours, fragmented nuclei, and a significant reduction in Nissl bodies within the cells. However, in the EA group, the hippocampal cells had clear boundaries, prominent nucleoli, and abundant Nissl bodies. The hippocampal cells in EACtl and EA+LPS groups were similar to those in the EMP group, characterized by blurred or shrunken cell morphology, abnormal nuclear fragments, uneven distribution of Nissl bodies, lighter staining intensity, and signs of disappearance and dissolution (**Figure 2**).

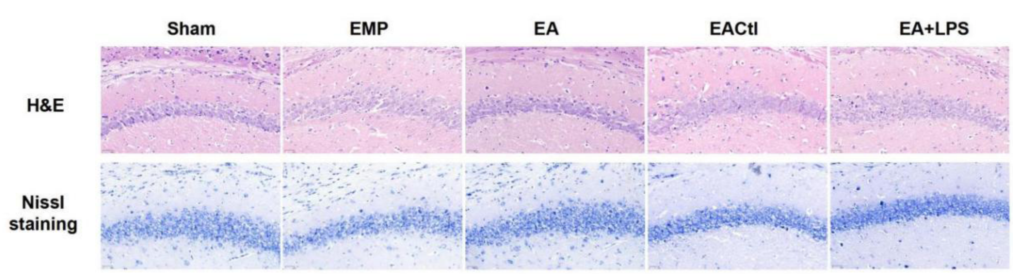

Figure 2. Comparison of pathological staining results among different groups of mice

3.3. Comparison of activated microglia in the hippocampal region of mice from different groups

Compared to the Sham group, EMP irradiation induced the activation of microglia, with an increase in the proportion of iba-1$^+$/iNOS$^+$ cells in the hippocampal region. Compared to the EMP group, the EA group had a lower proportion of iba-1$^+$/iNOS$^+$ cells in the hippocampal region, while there was no statistically significant difference between the EACtl and EA+LPS groups. Compared to the EA group, the EACtl and EA+LPS groups demonstrated a higher proportion of iba-1$^+$/iNOS$^+$ cells in the hippocampal region (**Figure 3**).

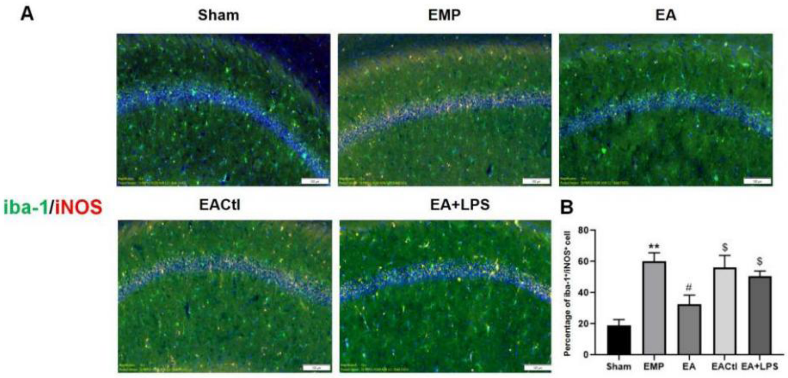

Figure 3. Comparison of activated microglia in the hippocampal region of mice from different groups. (A) Immunofluorescence staining, with red fluorescence labeling iba-1, green fluorescence labeling iNOS, and blue labeling cell nuclei; (B) Percentage of iba-1$^+$/iNOS$^+$ cells in the hippocampal region of mice. ** represents $P < 0.01$ compared to the Sham group; # represents $P < 0.05$ compared to the EMP group; $ represents $P < 0.05$ compared to the EA group.

3.4. Comparison of proinflammatory cytokines IL-1β, IL-6, and TNF-α concentrations in the hippocampal tissue of mice from different groups

Compared to the sham group, the concentrations of proinflammatory cytokines IL-1β, TNF-α, and IL-6 in the hippocampal tissue of mice were increased. Compared to the EMP group, the concentrations of these cytokines were decreased in the EA group, while there was no statistically significant difference between the EACtl and EA+LPS groups. Compared to the EA group, the concentrations of IL-1β, TNF-α, and IL-6 were increased in both the EACtl and EA+LPS groups (**Figure 4**).

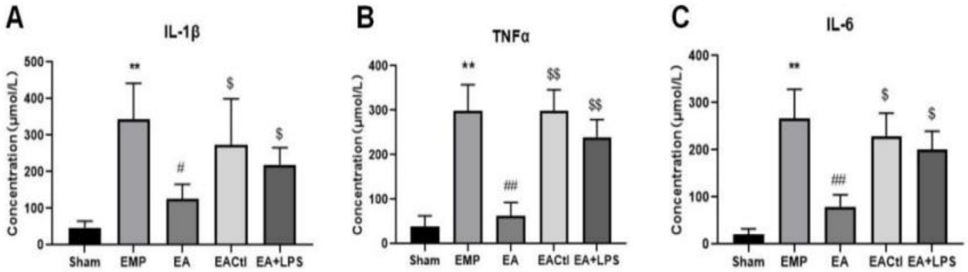

Figure 4. Comparison of proinflammatory cytokines IL-1β, TNF-α, and IL-6 concentrations in the hippocampal tissue of mice from different groups. Detection of (A) IL-1β, (B) TNF-α, and (C) IL-6 concentrations in the hippocampal tissue of mice from each group. ** represents $P < 0.01$ compared to the Sham group; # represents $P < 0.05$ compared to the EMP group; ## represents $P < 0.01$ compared to the EMP group; $ represents $P < 0.05$ compared to the EA group; $$ represents $P < 0.01$ compared to the EA group

4. Discussion

This study aimed to investigate the effects of electroacupuncture stimulation at “Baihui” and “Shenting” acupoints on learning and memory impairment and anxiety-like behaviors induced by EMP in mice, and to further explore its possible mechanisms. The experimental results showed that EMP irradiation could lead to significant learning and memory impairment and anxiety-like behaviors in mice, manifested as shortened stay time in the novel arm of the Y-maze and reduced activity time in the central area of the open field. Simultaneously, microglia in the hippocampal brain region were activated, and the concentrations of proinflammatory factors IL-1β, IL-6, and TNF-α were significantly increased, indicating that EMP-induced brain damage is closely related to microglia activation and inflammatory response. Electroacupuncture stimulation at “Baihui” and “Shenting” acupoints could improve EMP-induced learning and memory decline and anxiety-like behaviors by inhibiting glial cell activation and limiting the inflammatory response.

According to traditional Chinese medicine theory, the Baihui acupoint is the intersection point where the yang qi of the three yang meridians of the hand and foot and the Du meridian intersect. It has the effects of awakening the brain, calming the mind, elevating yang, and stabilizing epilepsy [6]. The Shenting acupoint is the intersection of the Du meridian, the foot Taiyang bladder meridian, and the foot Yangming stomach meridian. It has the effect of awakening the brain and regulating qi and blood in the head [7]. Studies have shown that electroacupuncture stimulation at Baihui and Shenting acupoints can improve learning and memory function in vascular dementia rats and alter synaptic structure in the hippocampal brain region [8]. It has been reported that electroacupuncture stimulation at Baihui and Shenting acupoints has a therapeutic effect on post-stroke mild cognitive impairment. After a certain period of treatment, patients’ memory and attention were significantly improved. Furthermore, it could also alleviate patients’ depression and anxiety, and promote the process of rehabilitation [9]. In this study, “Baihui” and “Shenting” acupoints were selected for electroacupuncture intervention. The results showed that the stay time of mice in the electroacupuncture group in the novel arm of the Y-maze and the central area of the open field was significantly longer than that in the EMP group, indicating that electroacupuncture could effectively improve learning and memory impairment and alleviate anxiety-like behaviors induced by EMP. Simultaneously, the activation of microglia in the hippocampal brain region of mice in the electroacupuncture group was reduced, along with the significantly reduced concentrations of proinflammatory factors IL-1β, IL-6, and TNF-α. These results further confirmed that electroacupuncture may improve EMP-induced brain damage by inhibiting microglia activation and inflammatory response in the hippocampal brain region.

To verify the specific effect of electroacupuncture, this study also set up a non-acupoint group and an electroacupuncture + LPS group as controls. The non-acupoint group received electroacupuncture intervention at 10 mm above the bilateral subcostal iliac crest. The results showed no significant difference in behavioral performance and hippocampal inflammation between this group and the EMP group, indicating that the therapeutic effect of electroacupuncture is acupoint-specific. The electroacupuncture + LPS group received an intraperitoneal injection of the microglia activator LPS before electroacupuncture intervention. The results showed that the improvement in behavioral performance and hippocampal inflammation in this group was significantly lower than that in the electroacupuncture group, indicating that the activation state of microglia is an important factor affecting the efficacy of electroacupuncture.

The results of this study support the potential application value of electroacupuncture in improving EMP-induced brain damage and provide novel insight for the application of acupuncture therapy in electromagnetic radiation-related diseases. However, this study still has some limitations, such as a small number of sample and the optimal time window and acupoint combination for electroacupuncture intervention have not been explored. These issues need to be further investigated in future studies.

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Disclosure statement

The author declares no conflict of interest.

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