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Electroacupuncture improves the learning and memory abilities of rats with PSCI by attenuating the TLR4/NF-κB/NLRP3 signaling pathway on the hippocampal microglia

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This study aims to investigate how electroacupuncture regulates the learning and memory abilities of poststroke cognitive impairment (PSCI) rats through the TLR4/NF-κB/NLRP3 signaling pathway on the hippocampal microglia. Thirty male rats were randomly divided into three groups: sham surgery group, PSCI model group, and electroacupuncture group, with 10 rats in each group. Middle cerebral artery occlusion was used to establish the PSCI model. The Zea Longa method was used to score the rats' neurological function. Electroacupuncture was utilized for 21 days to improve PSCI. The learning and memory abilities of rats were tested using the Morris water maze. Hematoxylin–eosin staining and immunofluorescence were used to find the hippocampus' pathological changes. The concentration of interleukin-1β, interleukin-6, tumor necrosis factor-α, and interleukin-18 were detected by ELISA. The mRNA expression levels of associated inflammatory corpuscles were measured by quantitative real-time PCR. The protein expression levels of TLR4, MyD88, NF-κB, and NLRP3 were measured using western blotting. Electroacupuncture improved not only the learning and memory abilities of PSCI rats but also hippocampal morphology. Electroacupuncture inhibited the activation of microglia and the TLR4/NF-κB/NLRP3 signaling pathway. Electroacupuncture also reduced proinflammatory factors and restrained the mRNA levels of NLRP3-associated inflammatory cytokines. Its mechanism was related to inhibiting the expression of the TLR4/NF-κB/NLRP3 signaling pathway, attenuating the release of inflammatory factors, and regulating the activation of hippocampal microglia in the brain. *NeuroReport* **35: 780–789 Copyright © 2024 The Author(s). Published by Wolters Kluwer Health, Inc.**

NeuroReport 2024, **35**:780–789

Keywords: electroacupuncture, hippocampal microglia, neuroinflammatory, post-stroke cognitive impairment, TLR4NF-κB/NLRP3 signaling pathway

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Received 17 April 2024 **Accepted** 3 June 2024.

Introduction

Post-stroke cognitive impairment (PSCI) is a series of syndromes characterized by cognitive impairment that occurs 3–6 months after stroke onset. It involves a range of deficits in cognitive domains, such as cognitive thinking, memory, language, and attention, and can develop into dementia [1]. It is a subtype of vascular dementia and a common complication of stroke [2]. The prevalence of PSCI is estimated to range between 20 and 80% [3]. The occurrence of PSCI not only greatly affects the daily lives of patients but also imposes a heavy economic burden on their families and society [4]. Currently, there is a lack of specific and effective methods and widely accepted interventions for the treatment of PSCI. Some drugs targeting neurodegenerative diseases, including cholinesterase inhibitors, memantine, and other drugs that are commonly used in clinical practice, cannot make a significant effect on the treatment of PSCI [5].

Acupuncture, as a traditional Chinese medicine therapy, has been proven to be safe and effective in the treatment of PSCI and can be considered a viable alternative therapy [6]. Electroacupuncture is a modern modification of acupuncture techniques that adds electrical stimulation to original acupuncture. While enhancing the therapeutic effect of acupuncture, it retains the advantages of acupuncture, such as safety, environmental friendliness, ease of operation, and minimal side effects [7]. Studies have shown that electroacupuncture has significant therapeutic effects on a variety of neurodegenerative diseases (including ischemic stroke, mood disorders, Alzheimer's disease, multiple sclerosis, Parkinson's disease, etc.); however, the potential mechanism by which electroacupuncture improves PSCI remains unclear [8].

The hippocampus within the brain is responsible for learning, memory, and other cognitive abilities, and changes in the hippocampus can have an impact on cognitive function [9]. Research has indicated that the activation and differentiation of microglia in the hippocampus are closely related to the occurrence and recovery of

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PSCI [10]. Microglia are a population of brain-resident macrophage-like cells that participate in the first-line innate immune response of the central nervous system [11]. Toll-like receptors (TLRs) are a group of pattern recognition receptors, among which TLR4 plays a role in regulating the immune system during central nervous system injury. It triggers the activation of microglia and promotes the expression of inflammatory factors [12]. Upon activation of TLR4, the MyD88-dependent pathway triggers a series of signaling cascades. This leads to the phosphorylation and translocation of nuclear factor-kappaB (NF-κB) into the nucleus [13], resulting in the production of numerous proinflammatory cytokines, including tumor necrosis factor-α (TNF-α), IL-6, and IL-1β [14].

NLRP3 (NOD-like receptor pyrin domain 3) is a multiprotein signaling transduction complex that is considered to be one of the main inflammasomes and is involved in various immune and inflammatory-related diseases of the central nervous system. It plays a crucial role in recognizing cellular damage and regulating the inflammatory response in stroke [15]. The TLR4/NF-κB/NLRP3 signaling pathway can further activate the transcription and expression of NLRP3 after participating in the inflammatory response [16]. After the inflammasome NLRP3 is activated, oligomerization occurs to recruit the adaptor protein ASC, which interacts the CARD domain with the PYD domain to further activate caspase-1 and then process pro-IL-1β and pro-IL-18, thereby releasing IL-1β and IL-18 outside the cell and taking effect, expanding the inflammatory range and aggravating neuronal damage in the brain [17].

In recent years, the neuroinflammatory has been confirmed to play an important role in improving PSCI and degenerative brain diseases. There is, however, little research on the TLR4/NF-κB/NLRP3 signaling pathways as a key link in mediating neuroinflammation [18]. Revealing the molecular mechanisms of electroacupuncture in improving PSCI and reducing neuroinflammation by inhibiting the TLR4/NF-κB/NLRP3 signaling pathway transmission is significant. The purpose of this study was to investigate the effect of electroacupuncture on the neurological functions of rats with PSCI based on the TLR4/NF-κB/NLRP3 signaling pathway and the activation of hippocampal microglia in the brain.

Materials and methods

Animals

Thirty healthy adult male Sprague–Dawley rats with specific pathogen-free status (6–8 week old, body weight between 220–280 g) were purchased from Changchun YiSi Experimental Animal Technology Co., Ltd. (ChangChun, China) (Certificate No. 01021681361945000) and raised at the Animal Experimental Center of Changchun University of Traditional Chinese Medicine [SYXK(Ji)2018-0014]. Rats were raised in separate cages and provided with free access to food and water. The temperature in the enclosure is maintained between 20 and 26°C, with a relative humidity of 40 to 70%. The adaptive feeding was performed for 1 week. This experiment had been approved by the Ethics Review Committee for Animal Management and Use.

Animal grouping and processing

Trial grouping

Out of the 30 Sprague–Dawley rats, 10 were randomly selected for the sham surgery group (SHAM), and the remaining 20 rats were subjected to MCAO modeling. After the successful creation of the models, 10 of them were assigned to the PSCI model group (PSCI), while the other 10 were assigned to the electroacupuncture group.

Middle cerebral artery occlusion modeling method

All rats were strictly fasted for 12 h before surgery to prevent postoperative intestinal obstruction. They had access to free drinking water. After weighing, rats were intraperitoneally injected with 10% phenobarbital at 0.3 ml/100 g for surgical anesthesia. Fixing in a supine position, an approximately 2 cm incision was made slightly to the right of the midline of the neck. Vascular forceps were used to bluntly separate the fascia and muscles, fully exposing the right common carotid artery (CCA), external carotid artery (ECA), and internal carotid artery (ICA). Rats in the sham surgery group only separated the CCA, ECA, and ICA; they didn't need to insert the suture bolt. The remaining rats were treated with the improved Longa method. After separating vasculars, the right ECA and the proximal end of the CCA were ligated, and the ICA was clipped with an artery clip. The operator used the ophthalmic scissors to cut a 'V'-shaped small opening at a distance of 5 mm from the intersection of the CCA and inserted the suture bolt (Beijing Xinong, China) into the CCA through this incision. The operator then released the arterial clamp on the ICA and sent the suture bolt into the brain. The process was able to be stopped when the operator encountered noticeable resistance after inserting the suture bolt into the main cerebral artery (which is around 18–20 mm in length). Tightening the live knot, which was reserved at the CCA previously, and ligating the ICA to fix the suture bolt. Finally, the operator treated all rats with routine sutures and used penicillin to prevent infection. Attention should be paid to exposing the remaining tail of the suture plug outside the wound when suturing. Next, time counting. When the ischemic time reached 120 min, a little section of the suture bolt was gently pulled out of the intersection of the main cerebral artery to achieve reperfusion. The rats were placed on a temperature-controlled heating pad to observe their physical condition and wait for their recovery from anesthesia. Rats were single-cage managed, and after wake-up, their neurological function scores were measured according to the method of Zea Longa 5-scores (0: normal neurological function; 1: mild neurological

deficit, left forelimb flexion during tail lifting; 2: moderate neurological deficit, turning to the left side when walking; 3: moderate neurological deficit, tilted to the left; 4: no spontaneous walking, decreased consciousness; 5: ischemia-related death). The scoring system for experimental inclusion ranged from 1 to 3 points, while the rejection score ranged from 0, 4, to 5 points [19]. If there were cases of rats' deaths before the observation time points, such as improper anesthesia before the operation, subarachnoid hemorrhage, lack of nutrition, and so on, it was necessary to supply rats with similar body weights from the same manufacturer to ensure the number of samples kept pace with each group.

Intervention measure

The rats in the electroacupuncture group received their first acupuncture treatment immediately after the successful modeling process. The 'Baihui' (GV20, on the center of the parietal bone) acupoint and bilateral 'Neiguan' (PC6, on the palmar side of the forearm, between the ulnar-radial suture about 3 mm from the wrist joint) acupoints were chosen and acupoints' selection was done by referring to the research of experimental acupuncture [20]. The 'Baihui' acupoint was inserted obliquely, stabbing forward at a 2 mm length. Bilateral 'Neiguan' acupoints were perpendicularly inserted at a 1 mm length. The electroacupuncture instrument (Huatuo, China, SDZ-II) was added to the needle handles. The grade specification selection was the disperse-dense wave with a frequency of 20 Hz, current intensity of 1–2 mA, and a 20-min lasting time. Rats were anesthetized with an animal respiratory anesthesia machine (Matrx, Ohio, USA) and isoflurane/O_2 (3% for induction and 2% for maintenance) so that they were able to keep easy to control during the treatment. The treatment frequency is once in the morning and once in the evening, 6 days a week, continuously for 3 weeks. The PSCI model group and the sham surgery group were given the same time of bundling and mock capture without any other treatment when the electroacupuncture group received electroacupuncture treatment. After the completion of the treatment, all rats were subjected to a 6-day Morris water maze test (Shanghai Xinruan Information Technology, China, XR-Xmaze).

Morris water maze test

Place navigation test: One day before the beginning of the formal test, all rats were permitted to swim freely in the pool without the platform for 2 min to familiarize themselves with the experimental environment. At the beginning of the training, the rats were randomly put into the water, facing toward the wall of the pool, from any of the four quadrants. Observing and recording the time required for the rats to find the platform and climb up (escape latency). Every time the rats were allowed to search for the platform within 120 s. If they successfully found the platform, they would get 15 s of free time. If they didn't find it, they would be guided to the platform and stay for 15 s. The training lasted continuously for 5 days.

Spatial probe test: After the place navigation test, the platform was dismantled on the 6th day. The swimming trajectory of rats within 120 s was recorded and analyzed. The number of times the rats passed through the platform was recorded to detect their spatial memory ability of the rats.

Draw materials

After the Morris water maze test, all rats were anesthetized by intraperitoneal injection of 10% phenobarbital. After all of the rats were sacrificed by cervical dislocation, the serum of the abdominal aorta was taken for enzyme-linked immunosorbent assay (ELISA) detection, and quickly taking fresh brain tissues off the ice. Some of the brain tissues in each group were frozen in liquid nitrogen and then stored in a refrigerator at –80°C for western blotting and real-time PCR detection. The rest of the brain tissues in each group were fixed in the 4% paraformaldehyde solution (0.0035 ml/g, Biosharp, BeiJing, China, 23067175) overnight for Hematoxylin–eosin and immunofluorescence staining detection.

Hematoxylin–eosin staining

Since fixing fresh brain tissues in 4% paraformaldehyde, the samples were embedded in paraffin, cut into slices with a thickness of 4 μ, dewaxed with xylene and gradient ethanol, and then stained with hematoxylin and eosin (Changchun Semerit Technology Co., Ltd., ChangChun, China Nos. 20170006 and 20170007). After mounting the slices, the pathological changes in hippocampal tissue were observed under a light microscope.

Immunofluorescence staining

After conventional dewaxing of brain tissue slices, they were washed with PBS (Biosharp, BL601A) for 5 min ×3 times and fixed with 4% paraformaldehyde for an hour. Next, a penetration of 0.3% TritonX-100 for 30 min was processed. Endogenous peroxidase was blocked by incubation with the hydrogen peroxide of 3% for 5 min at room temperature. The first primary antibody TLR4 (1 : 500; Cell Signaling Technology, Massachusetts, USA, #13674S), NLRP3 (1 : 500; Abcam, London, UK, ab270449), was added and incubated in a wet box at 4°C overnight, then washed away with PBS 5 min ×3 times. The corresponding second antibody was dropped in a wet box and incubated for an hour in the dark, then washed away with PBS 5 min ×3 times. After completion of all, another primary antibody Iba-1 (1 : 500; Cell Signaling Technology, #17198S) was added, followed by the above steps again. Finally, the nuclei were incubated with 4',6-diamidino-2-phenylindole (DAPI; Boster, BeiJing, China, AR1176) for 15 min in the dark. The expression of fluorescence intensity in the hippocampus

of each group was observed under a fluorescence microscope (Olympus, Tokyo, Japan, CKX53). The ImageJ software was used to evaluate relative images.

ELISA

The collected rat serum was centrifuged, and the supernatant was taken. These samples were analyzed according to the instructions of the ELISA kit (Abcam) to detect the levels of TNF-α (Abcam, ab181421), IL-1β (Abcam, ab242234), IL-6 (Abcam, ab242739), and IL-18 (Abcam, ab282456) in the rats' serum. The standard curve was drawn according to the optical density value of the gradient concentration standard, and the regression equation was obtained to calculate the concentration of TNF-α, IL-1β, IL-6, and IL-18.

Quantitative real-time PCR

The rats' hippocampal tissues were isolated on ice, ground into homogenate, and centrifuged. The collected precipitate was reserved, washed with ethanol, and fully dissolved in order to extract rat hippocampal RNA. Reverse transcription was performed on the isolated RNA to create cDNA according to the reverse transcription kit (SYBR Green quantitative PCR Mix Kit; Mona, BeiJing, China) instructions, and the relative expression levels of NLRP3, ASC, and caspase-1 were detected by real-time quantitative PCR analysis using the above cDNA templates. The target gene was normalized with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as an internal reference. The $2^{-\Delta\Delta Ct}$ method was used to calculate the relative expression levels of mRNA in each group.

And the primers were used: NLRP3, 5'-GATAGGTTTGCTGGGATA-3' and 5'-GGTGTAGCGTCTGTTGAG-3'; ASC, 5'-CAGCAGCGTCTCCGACTTCTTC-3' and 5'-CCGTTCATCTCGTAGCCATTGAGG-3'; aspase-1, 5'-AGGAGGGAATATGTGGG-3' and 5'-AACCTTGGGCTTGTCTT-3'; GAPDH, 5'-CTGGAGAAACCTGCCAAGTATG-3' and 5'-GGTGGAAGAATGGGAGTTGCT-3'.

Western blotting

The hippocampal tissues of rats in each group were ground on ice and homogenized in RIPA lysate buffer (Aladdin, BeiJing, China, R301899) containing protease inhibitors. The homogenate after grinding was centrifuged at 12 000 r/min for 20 min at 4°C, and the supernatant was collected. Protein concentration was determined by the BCA (Thermo Fisher Science, Massachusetts, USA) method. The sample buffer was added to and incubated at a boiling temperature of 95°C for 10 min. Applying 40 ug of protein to the upper sample and operating by SDS-PAGE gel electrophoresis (Bio-Rad, California, USA, DDY-10). Next, the protein strip was charged to the PVDF membrane (Bio-Rad, DDY-7B III), blocked with 5% skimmed milk powder at room temperature for 1.5 h, and incubated with primary antibodies TLR4 (1 : 1000; Cell Signaling Technology, #13674S), MyD88 (1 : 1000; Cell Signaling Technology, #3699S), NF-κB (1 : 1000; Cell Signaling Technology, #8242S), NLRP3 (1 : 1000; Abcam, ab270449), GAPDH (1 : 1000; Cell Signaling Technology, #2118S) at 4°C overnight. Then, tris-buffered saline and tween 20 (Applygen, ShangHai, China, B1009) buffer washed the membrane three times for 10 min every time. The HRP-labeled secondary antibody (1 : 3000; Qiyue Biology, BeiJing, China, 9003990) was added later. Chemiluminescence imaging of protein was detected in the enhanced chemiluminescence darkroom. The protein was analyzed by a gel imaging analysis system (Bio-red, California, USA). The expression of the protein was calculated by Image J software (NIH, Maryland, USA).

Statistical analysis

GraphPad Prism Version 8.0 (GraphPad software, LLC, USA) was used for the statistical analyses, and the data are expressed as mean values ± SEM. The data from the place navigation test in Morris Water Maze were analyzed by repeated measures analysis of variance; the remaining indicators were analyzed by one-way analysis of variance. The comparison between any two was made by Tukey's test. $P < 0.05$ was considered statistically significant.

Result

Changes in the learning and memory abilities of rats

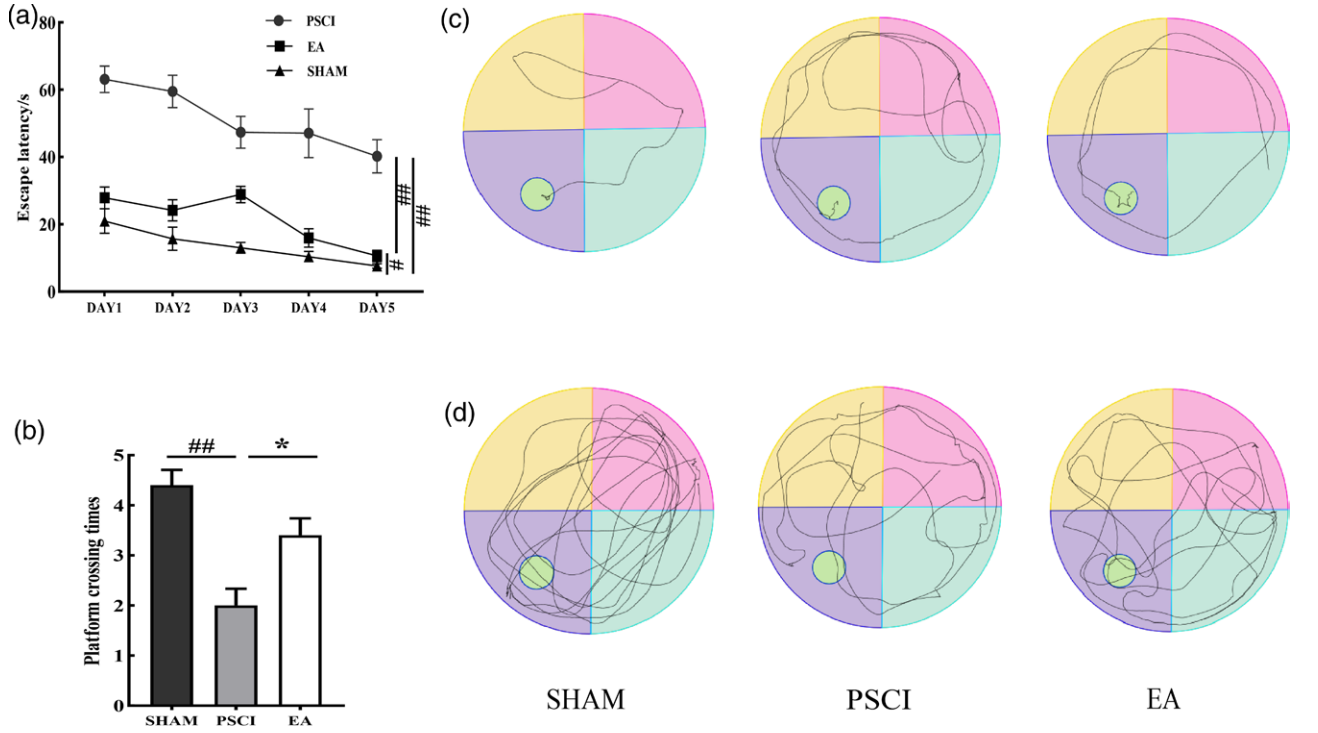
The escape latency data of the three groups of rats in the Morris water maze experiment showed that, compared with the PSCI model group, the escape latency of the sham surgery group and the electroacupuncture group was significantly shortened ($P < 0.0001$); compared with the sham surgery group, the escape latency of the electroacupuncture group was significantly prolonged ($P < 0.01$); compared with the electroacupuncture group, the escape latency of the PSCI model group was significantly prolonged ($P < 0.0001$).

The platform crossing times of the three groups of rats in the Morris water maze experiment showed that, compared with the electroacupuncture group, the platform crossing times in the PSCI model group were significantly reduced ($P < 0.05$); compared with the PSCI model group, the platform crossing times in the sham surgery group were significantly increased ($P < 0.0001$). There was no significant difference in platform crossing times between the electroacupuncture group and the sham surgery group (Fig. 1).

The morphological changes in the hippocampal brain tissue of rats

The hippocampal tissue in rats' brains from the sham surgery group demonstrated clear and intact structural organization with normal morphology. The neuronal cells

Fig. 1



Electroacupuncture improves learning and memory abilities of PCSI rats. (a) The comparison of the escape latency in each group of rats. (b) The comparison of the platform crossing times in each group of rats. (c) Typical trajectory diagrams of the place navigation test in each group of rats. (d) Typical trajectory diagrams of spatial probe test in each group of rats. Data from (a) and (b) are represented as mean ± SEM; ($n = 10$); *$P < 0.05$; #$P < 0.01$; ##$P < 0.0001$.

exhibited faint staining, with centrally located nuclei and clear nucleoli. The hippocampal tissue in rats' brains from the PSCI model group was edema with loosened structure, a decrease in neuronal damage, and shrinking or disintegrated cell nuclei, presenting a foamy appearance. The hippocampal tissue in rats' brains from the electroacupuncture group was clearer than that of the PSCI model group, the neuronal damage was reduced, and they were more similar to the sham surgery group (Fig. 2).

Activation of TLR4 and NLRP3 in the microglia of hippocampal tissue in each group of rats

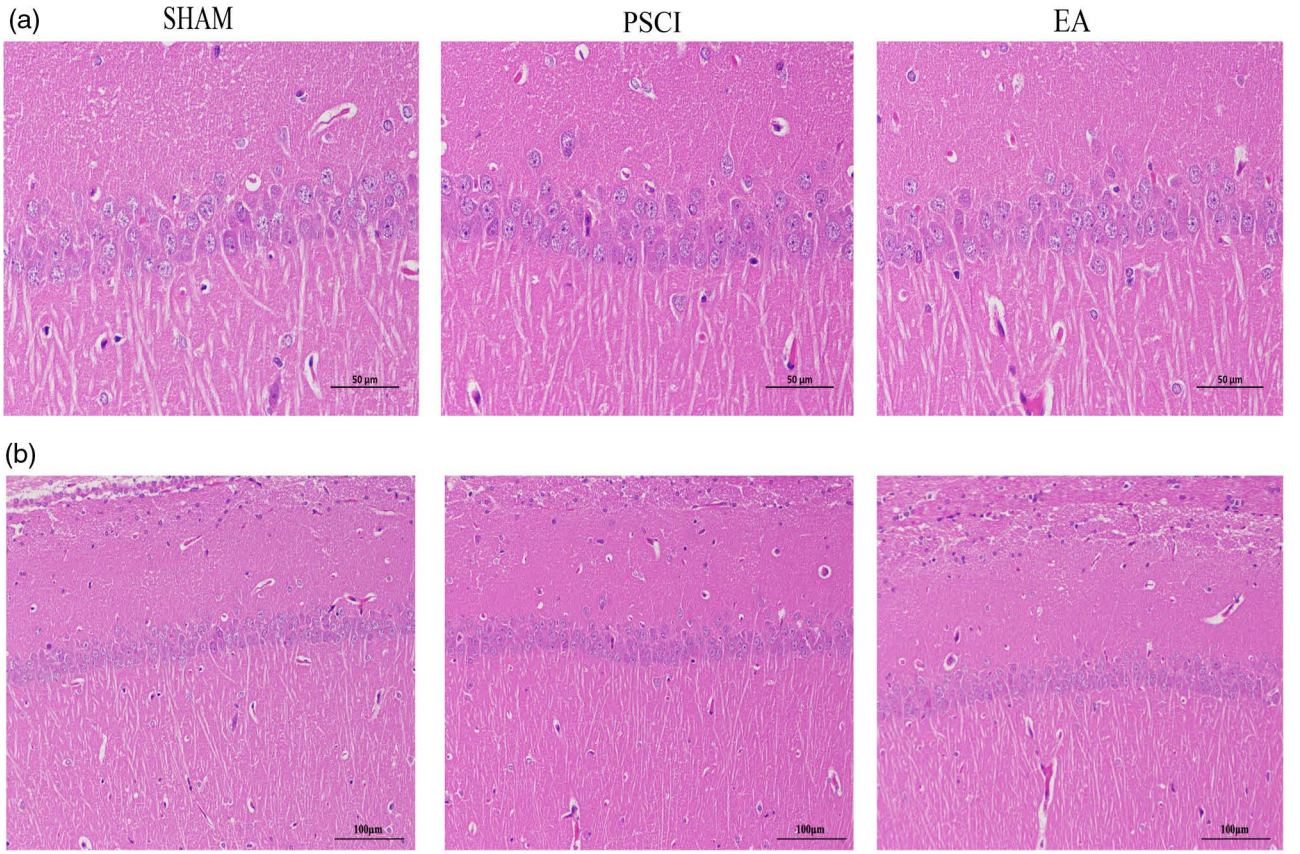
The results of immunofluorescence staining showed that the colocalization of NLRP3$^+$/Iba-1$^+$ positive cells was elevated in the hippocampal microglias of rats in the PSCI model group compared with that in the sham surgery group ($P < 0.001$); compared with the PSCI model group, the colocalization of NLRP3$^+$/Iba-1$^+$ positive cells in hippocampal microglias was decreased in the electroacupuncture group ($P < 0.05$); compared with the electroacupuncture group, the colocalization of NLRP3$^+$/Iba-1$^+$ positive cells in hippocampal microglias was decreased in the sham surgery group ($P < 0.05$).

The colocalization of TLR4$^+$/Iba-1$^+$ positive cells in hippocampal microglias showed a significant difference between the sham surgery group and the PSCI model group ($P < 0.0001$). What's more, the colocalization of TLR4$^+$/Iba-1$^+$ positive cells in hippocampal microglias also showed a significant difference between the PSCI model group and the electroacupuncture group ($P < 0.0001$). Compared with the electroacupuncture group, the colocalization of TLR4$^+$/Iba-1$^+$ positive cells in hippocampal microglias was decreased in the sham surgery group ($P < 0.01$) (Fig. 3).

Changes of proinflammatory cytokines TNF-α, IL-1β, IL-6, and IL-18 in the serum of rats

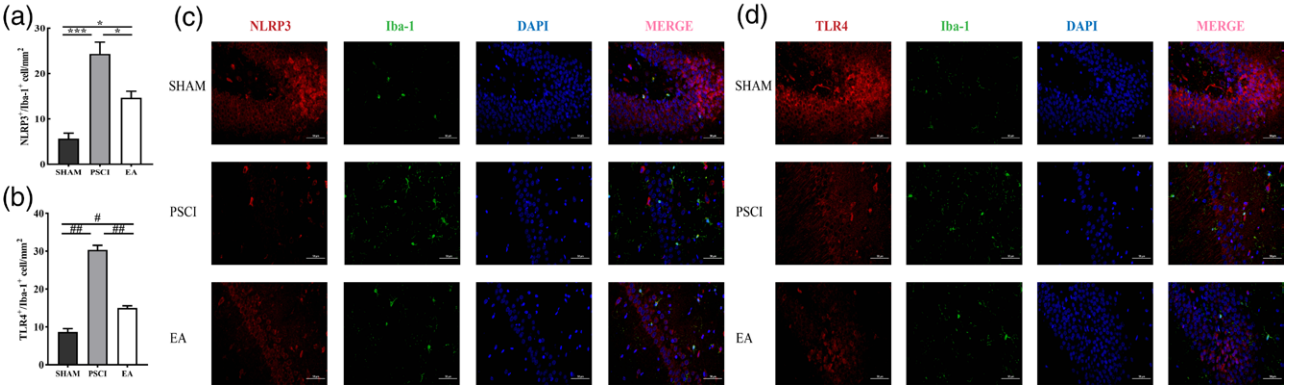
The ELISA results indicated that, compared to the sham surgery group, the rats in the PSCI model group and the electroacupuncture group showed a significant increase in the levels of proinflammatory cytokines TNF-α, IL-1β, IL-6, and IL-18 in their serum ($P < 0.0001$); compared to the PSCI model group, the levels of four proinflammatory cytokines in serum were significantly decreased in the sham surgery group and the electroacupuncture group in rats ($P < 0.0001$); compared with the electroacupuncture group, the levels of four proinflammatory cytokines in the PSCI model group were significantly increased ($P < 0.0001$), and these in the sham surgery group were significantly decreased ($P < 0.0001$) (Fig. 4).

Fig. 2



Electroacupuncture improves hippocampal morphology and reduces neuronal damage in PSCI rats. (a) Representative images of hematoxylin–eosin staining (400×, scale bar = 50 μm); (b) representative images of hematoxylin–eosin staining (200×, scale bar = 100 μm).

Fig. 3

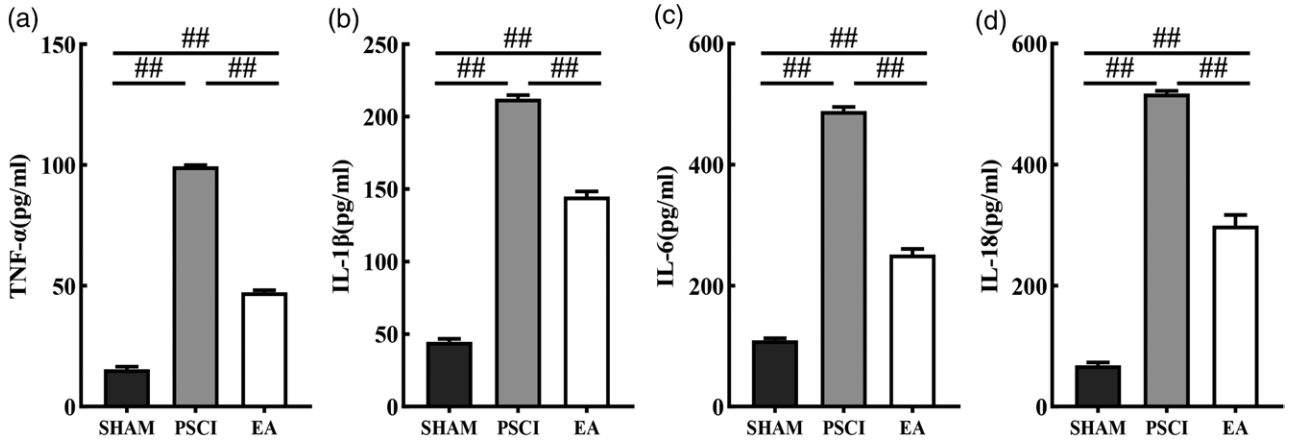


Electroacupuncture alters the activation of TLR4 and NLRP3 in hippocampal microglia of PSCI rats. Quantification of (a) NLRP3+/Iba-1+ and (b) TLR4+/Iba-1+ colabeling cells in different groups. Representative immunofluorescence images showing colocalization of (c) NLRP3 (red) and Iba-1 (green); (d) TLR4 (red) and Iba-1 (green). Scale bar: 50 μm. Data are represented as mean ± SEM. ($n = 5$). *$P < 0.05$; ***$P < 0.001$; #$P < 0.01$; ##$P < 0.0001$.

Changes of the mRNA expression of NLRP3, ASC, and caspase-1 in the hippocampus of rats

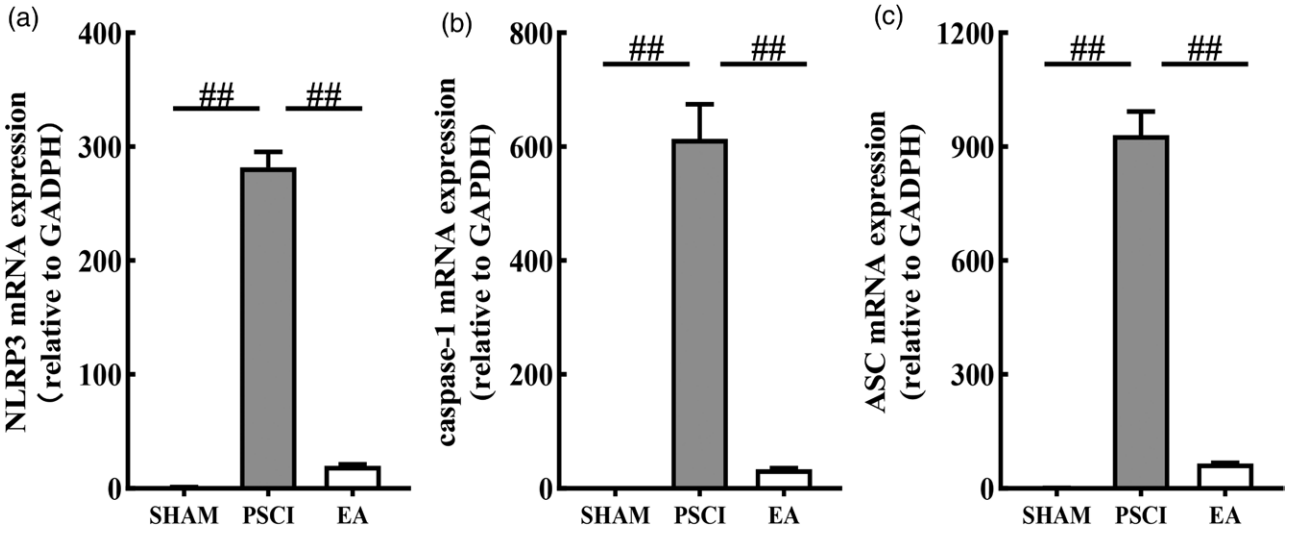
The real-time PCR results demonstrated that significantly increased mRNA expression of NLRP3, ASC, and caspase-1 was observed in the rats in the sham surgery group compared with that in the PSCI model group ($P < 0.0001$). Rats in the electroacupuncture group showed a significant decrease in the mRNA expression

Fig. 4



Electroacupuncture inhibites the proinflammatory cytokines in the serum of PSCI rats. (a) TNF-α, (b) IL-1β, (c) IL-6, and (d) IL-18. Data are represented as mean ± SEM. All the experiments were repeated three times ($n = 10$). ##$P < 0.0001$. IL-1β, interleukin-1β, IL-6, interleukin-6, IL-18, interleukin-18; PSCI, post-stroke cognitive impairment; TNF-α, tumor necrosis factor-α.

Fig. 5



Electroacupuncture reduces the mRNA expression of NLRP3-associated inflammatory cytokines in PSCI rats. (a) NLRP3, (b) caspase-1, and (c) ASC. Data are represented as mean ± SEM. All the experiments were repeated three times ($n = 5$). ##$P < 0.0001$.

of NLRP3, ASC, and caspase-1 compared with rats in the PSCI model group ($P < 0.0001$). No difference in the mRNA expression of NLRP3, ASC, and caspase-1, however, was observed between rats in the electroacupuncture group and the sham surgery group (Fig. 5).

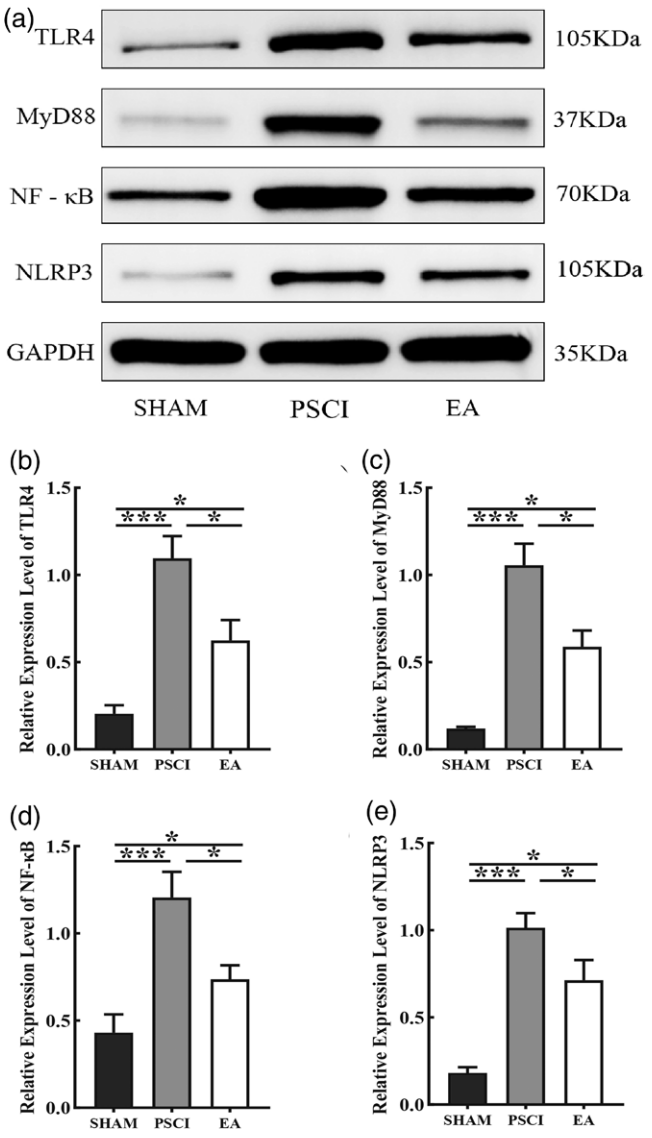
Changes of the protein expression of TLR4, MyD88, NF-κB, and NLRP3 in hippocampus of rats

The western blotting results found that, compared to the sham surgery group, the PSCI model group rats showed a significant increase in protein expression levels of TLR4, MyD88, NF-κB, and NLRP3 ($P < 0.001$), the electroacupuncture group rats also showed an increase in protein expression levels of these four proteins ($P < 0.05$). In comparison to the PSCI model group, the sham surgery group rats exhibited a significant decrease in protein expression levels of the four proteins ($P < 0.001$), while the electroacupuncture group rats also showed a decrease, but to a lesser extent ($P < 0.05$). When comparing the electroacupuncture group to the model group and the sham surgery group, protein expression levels of the four proteins were higher in the PSCI model group ($P < 0.05$) and lower in the sham surgery group ($P < 0.05$) (Fig. 6).

Discussion

With the global population aging, the incidence of stroke is increasing year by year, and the occurrence of PSCI also follows [21]. Currently, there are no more definitive

Fig. 6



Electroacupuncture inhibits the expression levels of TLR4/NF-κB/NLRP3 signaling pathway in hippocampus of PSCI rats. Effects of the relative protein expression levels in hippocampus. (a) Electrophoretogram, (b) TLR4, (c) MyD88, (d) NF-κB, (e) NLRP3. Data are represented as mean ± SEM. All the experiments were repeated three times ($n = 5$). *$P < 0.05$; ***$P < 0.001$.

and effective treatments for most neurodegenerative diseases, including PSCI, and there is an urgent need to find new treatment directions [22]. PSCI is classified as 'Dementia' and 'Amnesia' in Traditional Chinese Medicine. It refers to a series of diseases secondary to stroke characterized as 'brain marrow deficiency' and 'mental apraxia', which are mostly caused by impaired Qi and blood, phlegm misting the heart, deficiency of the heart and spleen, as well as weakness of the brain, or deficiency of kidney essence and malnutrition of the brain. It manifests as sluggishness, trance, slow response, loss of memory, and abnormal behavior [23]. Our research team chose the Baihui and Neiguan acupoints based on years of clinical practice combined with the Chinese medicine theory to perform electroacupuncture treatment, which can increase the abundance of the brain marrow and adjust consciousness. In the traditional Chinese medicine theory system, the 'BaiHui' (GV20) acupoint of the Governor Vessel can invigorate vital Qi and warm Yang, fill and nourish the sea of marrow [24]. And the bilateral 'Neiguan' (PC6) acupoints of the Pericardium Meridian of Hand-Jueyiin are capable of 'regulating qi and blood, promoting circulation in the meridians, and nourishing the heart and calming the mind' [25]. Briefly, the aforementioned two acupoints, if matched with electroacupuncture, could potentially alleviate a range of symptoms after stroke including PSCI, while also improving blood circulation and the delivery of essential nutrients to the brain [26]. Modern research has also shown that electroacupuncture is safe and effective in improving cognitive function, and can further enhance efficacy in a short period of time, potentially serving as a complementary treatment for PSCI [27]. In addition, some studies have suggested that electroacupuncture may improve PSCI at multiple levels and targets in various brain regions by improving damage to the central cholinergic system, enhancing synaptic plasticity, promoting the expression of neurotrophic factors, inhibiting inflammatory factors, regulating cellular autophagy, reducing oxidative stress, and establishing collateral circulation [28]. The specific mechanisms of action, however, remain unclear and further investigation is needed to elucidate these effects.

The hippocampus is the brain region that is most closely associated with cognitive function, learning, and memory. It can also be damaged after stroke, resulting in cognitive dysfunction [29]. Research conducted by Yaru Liu and colleagues demonstrated that electroacupuncture may improve cognitive impairment caused by vascular dementia by inhibiting the JNK signaling pathway and reducing apoptosis in the hippocampus [30]. Additionally, through behavioral testing and proteomic analysis, Sa and colleagues found that electroacupuncture can improve the proteome in the hippocampal region of rats with cognitive impairment, which is associated with the upregulation of heat shock proteins in the brain via electroacupuncture treatment [31]. In this study, we also observed an improvement in hippocampal function in the brains of rats with PSCI through behavioral analysis, and found that the performance of rats treated with electroacupuncture was significantly improved compared with that of the untreated rats in the Water Maze test, and the difference between the number of traversing platforms in the late stage of training and that of the sham surgery group of rats was smaller. It suggested that the learning and memory abilities of rats improved and hippocampal function was restored after electroacupuncture treatment. Microscopic observation of the hippocampal

morphology of rats in each group at the end of the experiment also confirmed this viewpoint, and the neuronal cells of rats treated with electroacupuncture tended to be much similar to that in the rats of the sham surgery group, suggesting that electroacupuncture can restore hippocampal damage, repair damaged neuronal cells, and improve learning and memory ability.

Microglia play a crucial role in the neuroinflammatory response following stroke. Once overactivated, microglia promote an inflammatory response that can lead to neurotoxicity and further exacerbate brain damage [32]. The TLR4 is abundantly expressed in microglia and mediates the generation of neuroinflammation in the brain [33]. Relative research had indicated that there was a correlation between the neuroinflammatory response and poststroke cognitive decline, as well as a decrease in learning, memory, and reasoning abilities [34]. In this study, we further investigated the mechanism of action of electroacupuncture in improving PSCI using molecular biological experiments. By detecting the expression of the TLR4/NF-κB/NLRP3 signaling pathway-related proteins and inflammatory factors in the hippocampal tissues of PSCI rats in each group, it was found that proinflammatory cytokines and various types of inflammasomes related to the TLR4/NF-κB/NLRP3 signaling pathway were inhibited, as was the activation of TLR4/NF-κB/NLRP3 signaling pathway proteins on the surface of microglia. Consistent with this study, Zhuang Lihua and other studies had shown that acupuncture could regulate the inflammatory response through the TLR4/NF-κB signaling pathway to treat central nervous system diseases, and its mechanism of action was related to the inhibition of microglia activation and reduction of proinflammatory cytokine release [35]. The research conducted by Liao Dongmei and colleagues has concluded that electroacupuncture could inhibit the expression of the TLR4/NF-κB/NLRP3 signaling pathway in the central nervous system, alleviate neuroinflammatory responses, and improve cognitive and learning abilities in mice with Alzheimer's disease [36]. Li Yunping and other researchers found that the Renshen Shouwu extract could promote the regeneration of neurons and blood vessels in rats after ischemic stroke by inhibiting the TLR4/NF-κB/NLRP3 signaling pathway [37].

This study, however, also has some limitations. As we all know, microglia frequently have dual roles after brain injury. Activated microglia not only have neurotoxicity to expand the inflammatory response but also produce anti-inflammatory factors to repair brain tissue damage and play a neuroprotective role [38]. This research was only focused on the damage that excessive microglia activation causes to the nervous system. Furthermore, the cascade reactions about TLR4/NF-κB/NLRP3 signaling pathwas are complex and diverse [12], and the specific regulatory mechanism of electroacupuncture in improving PSCI still needs further in-depth research.

Conclusion

In summary, this study explained the function, and possible mechanism of electroacupuncture in improving PSCI, which was related to the inhibition of the activation of microglia and the transformation of the TLR4/NF-κB/ NLRP3 signaling pathway. Further confirming the therapeutic effect of electroacupuncture on PSCI, it improved neural function, reduced neuroinflammation, and enhanced the learning and memory abilities.

Acknowledgements

This study was supported by Natural Science Foundation of Jilin Province (20210101214JC) and Jilin Provincial Department of Education (JJKH20241067KJ)..

Conflicts of interest

There are no conflicts of interest.

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