

Research article

Effects of acupuncture on regulating the hippocampal inflammatory response in rats exposed to post-traumatic stress disorder

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ABSTRACT

Data from clinical and experimental studies have verified the efficacy and safety of acupuncture in the treatment of post-traumatic stress disorder (PTSD). However, the concrete mechanism has not been well elucidated. The stress-induced activation of inflammatory response is involved in the development and pathogenesis of PTSD. Here, we aimed to investigate the effects of acupuncture on regulating the hippocampal inflammatory response in rats exposed to PTSD. Forty male rats were randomly divided into control, model, acupuncture and sertraline group. Within 1 day after adaptive feeding, all rats were exposed to single prolonged stress (SPS), except for the rats in the control group. Rats in acupuncture group were exposed to acupuncture intervention at the acupoints of Baihui (GV20) and Yintang (GV29), 20 min once per day for 15 days. Rats in sertraline group were exposed to a suspension of sertraline and distilled water (0.2 mg/ml), once per day for 15 days continuously. Body weight and elevated plus maze experiment were detected at different time-points to evaluate the behavioral changes of rats. HE staining method was used to observe the basic pathological morphological changes in hippocampus. Immunofluorescence staining method was used to observe the activation of hippocampal microglia. The content of IL-6 and IL-1 β in serum were detected by ELISA method. Compared with the control group, the body weight of rats in model group significantly decreased on 8 days, and the percentage of time in open arms and open arm entries decreased significantly on 15 days after SPS procedures, which indicated that SPS induced PTSD-like behavior in rats. Acupuncture exerted therapeutic effect. Simultaneously, the result of HE staining confirmed that SPS induced hippocampal morphological changes in SPS rats. Notably, acupuncture reversed the reduction and pathological injury to some extent. The results have also shown that acupuncture intervention effectively reversed the activated microglia of the hippocampus in rats. Moreover, the expression of IL-1 β in serum was significantly decreased by acupuncture intervention. In summary, the present study demonstrated that the role of acupuncture in eliminating PTSD-like behavior might be connected with reversing the pathological process of the inflammatory response mediated by the activation of microglia induced by SPS.

Abbreviations: PTSD, post-traumatic stress disorder; SPS, single prolonged stress; IL-6, interleukin-6; IL-1 β , interleukin-1 β ; IBA-1, ionized calcium binding adaptor molecule-1.

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1. Introduction

Post-traumatic stress disorder (PTSD) is considered to be the stress response in individuals who have experienced a traumatic or sudden major disaster such as war, earthquake, or COVID-19 attack [1]. It has been reported that there is inherent heterogeneity in the presentation of PTSD symptoms. The manifestations of PTSD are characterized by hyperarousal, impairment in extinction learning of fear memory, recurring symptoms and intrusive memories, negative cognition and emotions, anxiety, depression, a mental disease with or without cognitive or learning disabilities [1–3]. It has shown that there is obviously heritable characteristic for PTSD approximately from 40 % to 50 % [4]. The prevalence of PTSD in the general population ranges from 6.4 % to 7.8 % [5]. And the lifetime prevalence of PTSD is around 3.9 % all over the world [6,7]. Currently, the treatment for PTSD mainly includes psychotherapy and the selective serotonin reuptake inhibitors (SSRIs) pharmacotherapy, such as paroxetine, sertraline, venlafaxine and nefazodone. However, SSRIs in the treatment of PTSD has been identified with side effects, including anxiety symptoms, restlessness, headache or insomnia [8]. Up to now, there is no universally efficacious therapy for PTSD clinically. And the underlying mechanism is not fully illuminated. Accordingly, there is an indispensable challenge for us to illustrate the pathological mechanism of PTSD and explore continuously more extensive strategies for the prevention and treatment of PTSD.

The primary role of hippocampus is considered to be involved in learning and memory function. The changes of hippocampus and hippocampal volumes have been identified to play a crucial role in PTSD [9]. Simultaneously, the structural and functional impairments in hippocampus are closely related to PTSD [10]. A previous study has shown that PTSD was associated with reduced hippocampal volume [4]. The clinical data and neuroimaging from 794 patients with PTSD were analyzed, suggesting that smaller hippocampal volume in current PTSD were observed when compared with control subjects [11]. Interestingly, study have shown that the hippocampus and other brain regions of patient suffered from PTSD exhibited abnormal resting-state function connectivity, and the function was associated with forecasting the severity of PTSD symptoms [12]. Notably, PTSD patients have been identified with immune activation and increase of inflammatory response clinically [13]. Numerous studies have also shown that stress-induced immune inflammatory activation is involved in the pathological process of PTSD [6,7,14–16]. Microglial activation and blood-brain barrier damage have been linked to cognitive dysfunction associated with PTSD. And minocycline inhibited the activation of microglia and attenuated the poor cognitive performance and blood-brain barrier damage [16]. A systematic review of studies have showed that concentrations of C-reactive, interleukin 6 and tumor necrosis factor- α were significantly increase in patients with PTSD, and the expression of interleukin 1 β levels almost reached the threshold for significance [17]. Hence, inflammatory markers were considered to be the potential therapeutic targets for PTSD. Studies have also verified that the obvious changes of morphology, structure and function in the hippocampus were involved in the pathogenesis of psychological disorders, including PTSD [11,16,18]. Acupuncture has been identified to exert therapeutic effect on patients with PTSD by inducing general relaxation, reducing hyperarousal, inflammation, and sleep deprivation [19]. Clinical studies have preliminarily confirmed the efficacy and safety of acupuncture in the treatment for PTSD, but the specific mechanism is still unclear [8,19].

Therefore, based on the concept of “treating different diseases with the same treatment” in mental disorders and previous research, the present study established the rat model by single prolonged stress (SPS) to simulate the process of PTSD. We hypothesized that the potential mechanism of acupuncture in the treatment of PTSD might be relied on regulating the inflammatory response mediated by the activation of microglia in the hippocampus induced by SPS. The basic pathological and morphological changes in the hippocampus was observed by HE staining method. Immunofluorescence staining method was used to

observe the activation of hippocampal microglia. And the content of IL-6 and IL-1 β in serum were detected by the enzyme-linked immunosorbent assay (ELISA) method. We aimed to elucidate the effects and potential mechanisms of acupuncture on regulating the hippocampal inflammatory response in rats exposed to PTSD, which might provide new experimental evidence for the treatment of PTSD.

2. Methods

2.1. Animals

Forty Sprague Dawley (SD) rats, seven-week-old, male, were obtained from Weitong Lihua Experimental Animal Center (Beijing, China). All experimental procedures were approved by the Animal Ethics Committee, and were carried out in the Laboratory of Barrier System, Institute of Psychology, Chinese Academy of Sciences (Permission number: SYXK(Beijing)2016–0038). The rats were housed in the comfortable environment (23–26 °C, 50 % $\pm$ 2 % humidity) and ad libitum acquire to water and food, two rats in each cage. Forty rats were randomly divided into control group, model group, acupuncture group and sertraline group (see Fig. 1), with 10 rats in each group.

2.2. Establishment of the animal model of PTSD

Single prolonged stress (SPS) has been proved to be an effective pre-clinical model for simulating PTSD, manifesting behavioral, molecular, and physiological changes that were observed in PTSD [20,21]. Simultaneously, it has shown that SPS-induced apoptosis of the hippocampal neurons was involved in the occurrence of PTSD [22]. In the present study, except for rats in the control group, all rats were exposed to SPS procedures. The rat model was established by the procedures from the previous study within 1 day after adaptive feeding [20]. The concrete SPS procedures were as follows. First, the rats were exposed to restraint stress for 2 h in a self-made cylindrical transparent plastic shell and kept breathing freely. After the restraint stress, rats were exposed to forced swimming immediately for 20 min in a cylindrical transparent tank (high: 1 m, diameter: 20 cm, water depth: 70 cm, 24°C freshwater). The cylindrical transparent tank were covered with the black plastic film surrounding the tank to avoid social interaction. After the rats were removed and recuperated for 5 min, rats were exposed to the ether until unconsciousness. Finally, the awakened rats were put back into their cage. Behavioral assessment was used to evaluate the establishment of the animal model.

2.3. Acupuncture and sertraline intervention

After the SPS procedures, rats in acupuncture group were exposed to acupuncture treatment at the acupoints of Baihui (GV20, located at the bregma, or on the junction of coronal suture and sagittal suture) and Yintang (GV 29, located at the midway between the medial ends of the two eyebrows) at 2:00 PM for 20 min, once per day for 15 days. The acupuncture needles were obtained from Suzhou Medical Instrument Co., Ltd. (0.25 $\times$ 13 mm, No.: 200135). The concrete procedures of acupuncture intervention were as follows. Firstly, the rats were gently immobilized in the palm by the operator's left hand and the acupoint area was quietly kept fully exposed for acupuncture intervention. Following disinfection of the acupoint sites with 75 % alcohol, the acupuncture needles were inserted transversely (keeping the angle between the needle and the skin surface at 15 angle) into Baihui (GV 20) and Yintang (GV 29) to a depth of 5 mm. Then rats were gently placed in the separate room under the condition of free activities for retaining the needles for 20 min. During the therapeutic cycle, rats were exposed to be rotated the needle twice per second for 1 min every 10 min after the rats were gently immobilized in the palm by the operator's left hand. After acupuncture intervention, the needles were slightly removed and rats had access ad libitum to standard rat chow and tap water. When

acupuncture intervention was conducted, additional stress was strictly avoided during each procedure. Due to the operation of being immobilized in the palm by the operator's left hand for 3 times during acupuncture treatment, rats in other group received grasping intervention once per day to ensure the principle of balance among groups.

Sertraline is one of the recommended medications by the Food and Drug Administration (FDA) to be used to treat PTSD clinically [8]. The rats in sertraline group were exposed to sertraline (Pfizer Pharmaceuticals limited, H10980141) pharmacological intervention at 3.00 PM. Sertraline was dissolved in distilled water to prepare 0.2 mg/ml suspension and administered by gavage (10 mg/kg) once a day for 15 days. Additional stress was strictly avoided during the experiment.

2.4. Behavioral assessment

All behavioral assessment were conducted at a dark and sound-isolated environment. The growth and nutrition status of rats in each group were observed by body weight assessment at different time points, and the anxiety state of PTSD rats were evaluated by elevated plus maze test.

2.4.1. Body weight assessment

The differences of body weight in each group were observed at different time-points. The data was collected at 0 day, 8 days and 15 days.

2.4.2. Elevated plus maze test

All rats were exposed to elevated plus maze test at 0 day and 15 days. The elevated plus maze (Med associates. Inc., ENV-560) was carried out in a square area (5 cm × 5 cm) with two open arms (30 cm × 5 cm) and two closed arms (30 cm × 5 cm) crossed vertically. And the maze was connected to a computer to record the whole observation. First, rats were placed in the center of the square with their heads facing the open arm. Then, Xeye Aba animal behavior video analysis system was used to directly record the percentage of time and times of rats entering the open arms within 5 min.

2.5. Tissue collection and processing procedures

There were no rats that died throughout the current study. After 15 days of experimental intervention, the brain tissue and blood sample were collected immediately after anesthetization. Five rats in each group were randomly selected to be anesthetized with 3 % sodium pentobarbital in saline (30 mg/kg) through the intraperitoneal injection. Paraformaldehyde solution with 4 % followed by 0.9 % ice saline was perfused through the heart until the liver or other tissue hardened. The brain tissues were immediately and quickly removed on ice, and then,

they were placed in 4 % paraformaldehyde solution for 2 h for the next experimental procedure. The other five rats in each group were anesthetized and the blood samples were collected by the abdominal aorta. Storing the blood samples at room temperature for 2 h. Then, hypothermal centrifugation was performed at 3,000 rpm for 20 min. Finally, the supernatant was obtained. After all tissues and supernatant were collected completely, they were transferred to a -80 refrigerator for storage.

2.6. HE staining

The morphological changes of the hippocampus in each group were observed. Slices of the brain were stained using the hematoxylin and eosin (HE) staining kit (Solarbio, No.G1121, Beijing, China). Before HE staining, the frozen slices, which were cut with 10 μm, were placed in the oven at 42°C for 10 min, and rehydrated for 5 min dip in PBS. The slices were stained with hematoxylin for 3–5 min by counter-staining with eosin for 5 min after alcohol gradient dehydration for 5 min separately. Dehydration-sealed slides. The light microscope (Olympus, Japan) was used to observe the sections, and the nucleus was blue, the cytoplasm red. Thereafter, the image was collected.

2.7. Immunofluorescence staining

The brain tissues stored in the 4 % PFA solution were removed and dehydrated in 30 % sucrose solution at 4 °C overnight. And the tissues were embedded in optimum cutting compound (OCT, Tissue-Tek, No.: 4583). Then, the brain samples were cut into 10-μm-thick sections along the coronal plane. The hippocampus were mounted on glass slides for the next step. After the sections were repaired with EDTA antigen repair buffer, BSA were used to block for 30 min at room temperature. And the hippocampal slices were exposed overnight to the following primary antibody mixtures: IBA-1 Rabbit Polyclonal antibody (1:1000, Abcam, ab178846) in BSA at 4°C overnight. The secondary antibodies (1:2000, Abcam, ab205718) were incubated for 50 min at gloomy environment. After the slides were washed with PBS (PH = 7.4) for 3 times, DAPI staining solution (1:1000, Sigma, D9542) was dropped and incubated at room temperature for 10 min under dark conditions. The addition of anti-fluorescence extractant was the last step in section preparation. The hippocampal images were observed and scanned by inverted fluorescence microscope (Nikon Eclipse Ti-SR, Japan) and immunofluorescence scanning instrument (Pannoramic MIDI, 3D Histech). Hippocampal fluorescent images were captured under a 400x microscope by a Case Viewer soft to observe the expression of IBA-1.

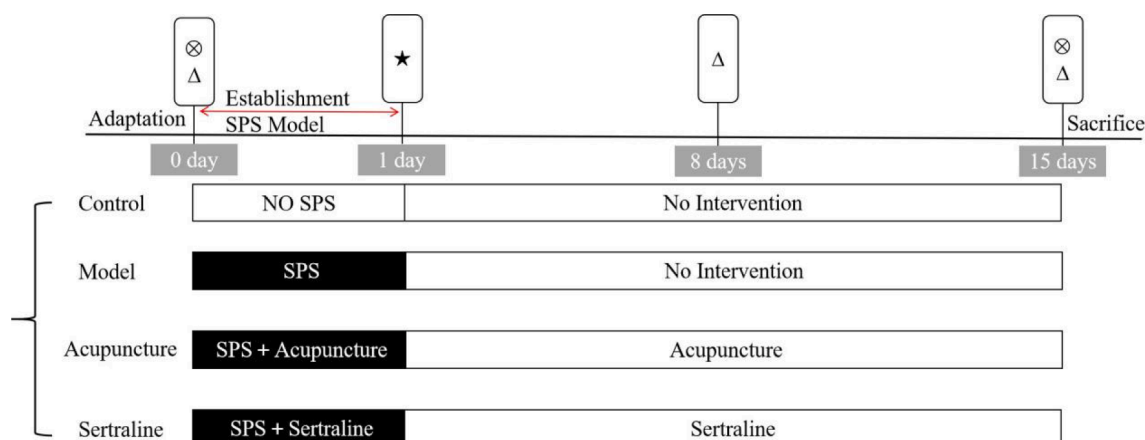


Fig. 1. Experimental procedures. SPS, Single prolonged stress; ★, SPS; Δ, Body weight; ⊗, Elevated plus maze.

2.8. Enzyme-linked immunosorbent assay

After the blood samples collection, the supernatant was used to detect the content of IL-6 and IL-1 β in serum by enzyme-linked immunosorbent assay (ELISA). The quantifications and procedures of IL-6 and IL-1 β were conducted in accordance with the manufacturer's protocol of IL-6 ELISA kits (Abcam, lot: ab234570) and IL-1 β ELISA kits (Abcam, lot: ab214025). The absorbance was measured at 450 nm for optical density by automatic microplate reader (UV755B, USA).

2.9. Statistical analysis

The statistical analysis was performed by SAS 8.2. The results of measurement data are expressed as mean $\pm$ standard deviation, and One-way ANOVA followed by LSD's post-hoc test. Non-parametric test was used if variance is not uniform or did not conform to normal distribution. A level of $P < 0.05$ was considered to be significant for analysis.

3. Results

3.1. The single prolonged stress induce PTSD-like behaviors in rats

3.1.1. Effects of acupuncture on the changes of body weight of SPS rats

Body weight was observed to assess the physical changes induced by SPS. As shown in Fig. 2A and Table 1, there was no difference in body weight at day 0 among groups. Compared with the control group, body weight of the rats in the model group was significantly reduced at day 8 ($P < 0.01$).

3.1.2. Effects of acupuncture on the percentage changes of time in open arms of SPS rats

As shown in Fig. 2B and Table 2, the data of time in open arms was no significant difference before modeling intervention (0 day). After SPS procedures, the percentage of time in open arms of SPS rats in the model group obviously decreased compared with the control group at day 15 ($P < 0.01$). Compared with the model group, acupuncture intervention showed an obvious upward trend on the percentage of time in open arms of SPS rats ($P < 0.01$).

3.1.3. Effects of acupuncture on the percentage changes of open arm entries of SPS rats

As shown in Fig. 2C and Table 3, there was no significance of open arm entries in each group before the experiment (0 day). After SPS procedures, the percentage changes of open arm entries of SPS rats in the model group decreased significantly at day 15 ($P < 0.01$). Compared with the model group, sertraline intervention reversed the decrease tendency on the percentage of open arm entries of SPS rats ($P < 0.01$).

3.2. Effects of acupuncture on the pathological changes of hippocampal morphology in SPS rats

As shown in Fig. 3, compared with the control group, the results of HE staining indicated that the model group existed the manifestation of disordered cell arrangement, decreased cell number, enlarged cell space, blurred or disappeared cell structure, cell vacuoles, degeneration and necrosis of neuronal cells, and nuclear shrinkage. Compared with the model group, acupuncture intervention reversed the pathological injury to some extent. The cell scatters of the acupuncture group returned to orderly arrangement, and the number of cells increased and the number of cell vacuoles decreased. In the sertraline group, the number of cells increased and the number of necrotic cells decreased as well.

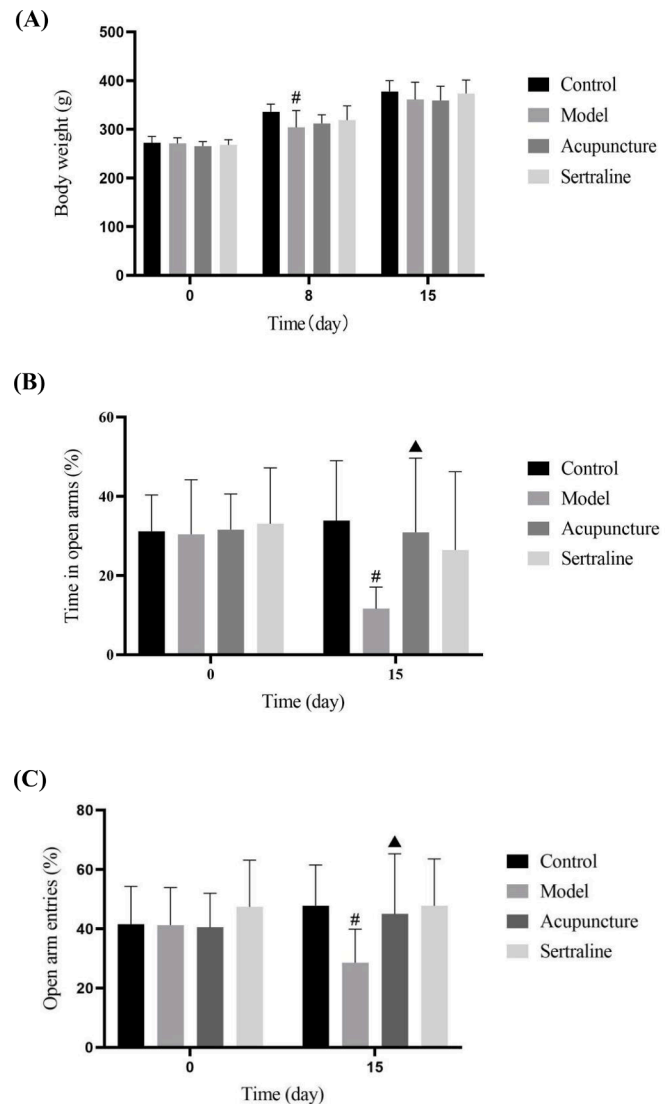


Fig. 2. Effects of acupuncture on the single prolonged stress induces PTSD-like behaviors in rats. (A) Effects of acupuncture on the changes of body weight of SPS rats. (B) Effects of acupuncture on the percentage changes of time in open arms of SPS rats. (C) Effects of acupuncture on the percentage changes of open arm entries of SPS rats. Data were expressed as means $\pm$ s. $^{\#}P < 0.01$, compared with control group; $^{\Delta}P < 0.01$, compared with model group.

Table 1

Effects of acupuncture on the changes of body weight of SPS rats. (n = 10; g).

Groups	0 day	8 days	15 days
Control	272.297 $\pm$ 12.918	336.008 $\pm$ 15.469	377.878 $\pm$ 22.211
Model	270.810 $\pm$ 11.525	303.836 $\pm$ 34.859 [#]	361.106 $\pm$ 35.532
Acupuncture	265.632 $\pm$ 8.939	312.055 $\pm$ 17.593	359.504 $\pm$ 28.984
Sertraline	268.073 $\pm$ 10.631	318.840 $\pm$ 29.092	373.637 $\pm$ 27.718

Notes: Data were expressed as mean $\pm$ s. $^{\#}P < 0.01$, compared with control group.

3.3. Effects of acupuncture on the changes of IBA-1 in the hippocampus of SPS rats

As shown in Fig. 4, compared with the control group, the results of the immunofluorescence staining showed that the morphology of hippocampal microglia cells in the model group was enlarged, the processes were thickened, and the activated microglia cells significantly increased. Compared with the model group, the activated microglia of

Table 2

Effects of acupuncture on the percentage changes of time in open arms of SPS rats. (n = 10; %).

Groups	0 day	15 days
Control	31.183 ± 9.129	33.873 ± 15.133
Model	30.421 ± 13.771	11.611 ± 5.476 [#]
Acupuncture	31.62 ± 8.948	30.942 ± 18.720 [▲]
Sertraline	33.097 ± 14.090	26.438 ± 19.796

Notes: Data were expressed as mean ± s. [#]*P* < 0.01, compared with control group; [▲]*P* < 0.01, compared with model group.

Table 3

Effects of acupuncture on the percentage changes of open arm entries of SPS rats. (n = 10; %).

Groups	0 day	15 days
Control	41.578 ± 12.700	47.817 ± 13.688
Model	41.236 ± 12.658	28.529 ± 11.370 [#]
Acupuncture	40.504 ± 11.450	45.017 ± 20.255
Sertraline	47.376 ± 15.791	47.728 ± 15.810 [▲]

Notes: Data were expressed as means ± s. [#]*P* < 0.01, compared with control group; [▲]*P* < 0.01, compared with model group.

acupuncture group and sertraline group were significantly reduced, which suggesting that both acupuncture and sertraline intervention effectively reversed the activated microglia of the hippocampus of the SPS rats.

3.4. Effects of acupuncture on the content of IL-1 β and IL-6 in serum of SPS rats

As shown in Fig. 5A and B and Table 4, compared with control group, the content of IL-1 β in serum increased significantly in the model group (*P* < 0.05). Compared with the model group, both acupuncture and sertraline intervention reversed the high expression of IL-1 β in serum induced by SPS procedures (*P* < 0.05). However, there was no statistical difference in the content of IL-6 in serum before and after intervention in the current experiment (*P* > 0.05).

4. Discussion

Generally, fear and anxiety are the main clinically psychological sufferings of patients with PTSD [23]. A large number of studies have shown that SPS procedures can simulate the pathological process and occurrence of patients suffered from PTSD. And SPS is an effective preclinical model for modeling PTSD, simulating behavioral, molecular, and some physiological changes [20–22]. Therefore, the present study used the SPS procedure to establish the animal model of PTSD to illustrate the potential mechanism of acupuncture in regulating the hippocampal inflammatory response. In the present study, the changes of behavioral assessment have indicated that SPS could induce PTSD-like behaviors and trigger the neuroinflammation of the hippocampus, which was involved in the pathogenesis of PTSD. Notably, acupuncture exerted therapeutic effect, alleviated the SPS-induced PTSD-like behaviors, ameliorated the pathological damage of hippocampus, reversed the activation of microglia in the hippocampus, and decreased the expression of IL-1 β in serum. The present study has confirmed that the role of acupuncture in eliminating PTSD-like behaviors might be

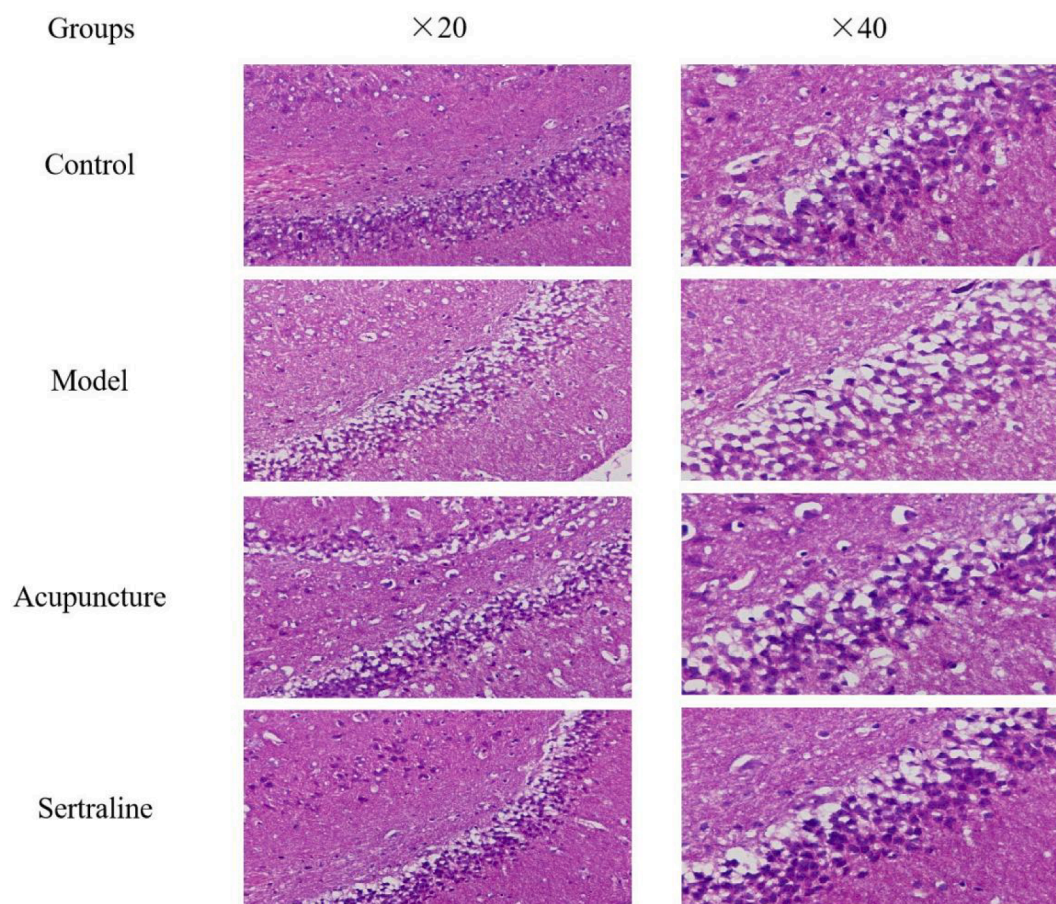


Fig. 3. Effects of acupuncture on the pathological changes of hippocampal morphology in SPS rats.

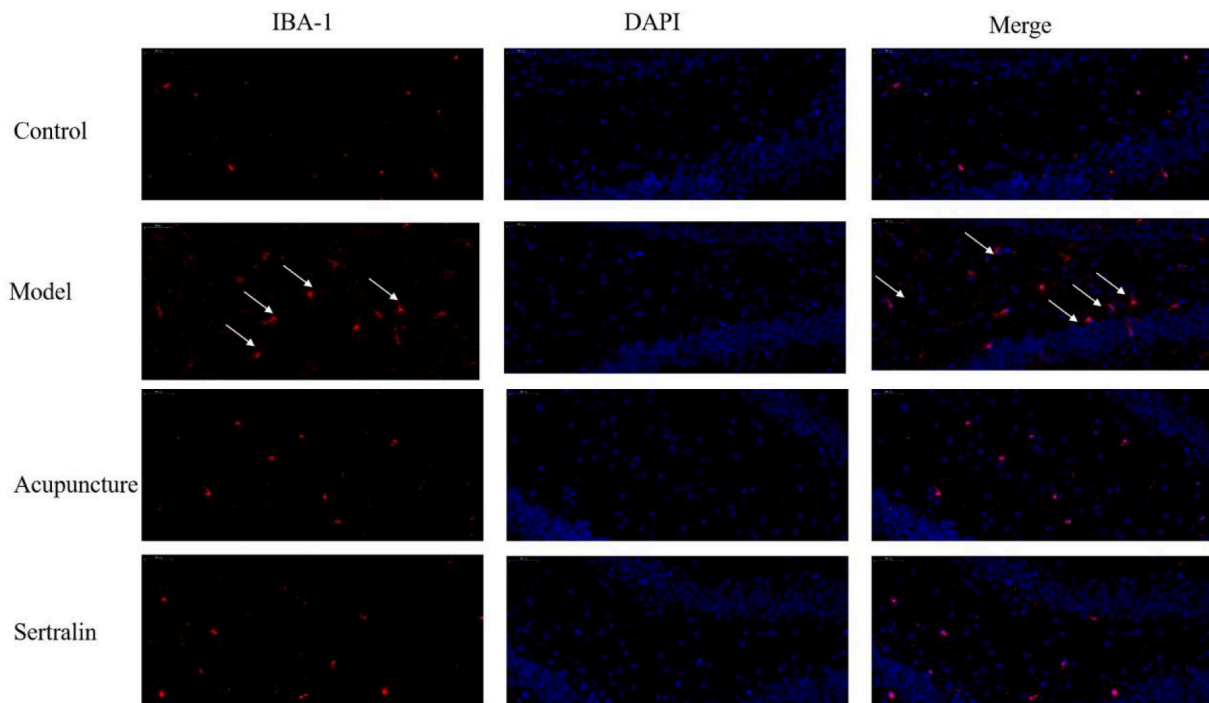


Fig. 4. Effects of acupuncture on the changes of IBA-1 in the hippocampus of SPS rats. Immunofluorescence staining of hippocampal region in different groups. IBA-1, red; DAPI, blue; scale bar, 50 μ m.

connected with reversing the pathological process of the inflammatory response mediated by the activation of microglia, which might provide experimental evidence for the prevention and treatment of PTSD.

4.1. SPS induced PTSD-like behaviors and acupuncture exerted therapeutic effect

In general, body weight is an important indicator of physiological health, which is used to assess the state when the animals are exposed to sufficient traumatic stress. Accordingly, the growth and nutritional status of rats in each group were evaluated by body weight in the current study. Meanwhile, the PTSD-like behaviors of rats were evaluated by elevated plus maze method [14,23]. The findings in the present study indicated that the baseline data of behavioral assessment was consistent with each group before intervention. After the SPS procedures, the behavior differences among groups were statistically significant. Compared with the control group, the body weight of rats in the model group decreased at 8 days, and the percentage of time in open arms and open arm entries was significantly decreased in the model group after 15 days. Compared with the model group, acupuncture showed an obvious upward trend on the percentage of time in open arms, and sertraline also reversed the decrease in the percentage of open arm entries of PTSD rats. The results from the behavioral assessment suggested that acupuncture ameliorated SPS-induced PTSD-like behaviors to some extent.

The efficacy and safety of acupuncture in the treatment of PTSD have been continuously verified from various studies [8,19]. And acupuncture can alleviate the symptom of patients suffered from PTSD, such as hyperarousal emotion, inflammation, or sleep deprivation [19]. Studies have shown that acupuncture at Baihui, Neiguan, Shenmen, and Taichong can effectively relieve anxiety-like behavior and alleviate the learning-memory ability in PTSD rats [24]. Meanwhile, there was another study also indicating that acupuncture alleviated the SPS-induced abnormal behaviors in rats and exerted therapeutic effect on PTSD-related symptoms. Of note, the effect of acupuncture and fluoxetine is comparable [25]. Acupuncture can produce neuroprotective, anti-inflammatory, anti-apoptotic and immunomodulatory effects by

regulating brain areas related to emotional and cognitive networks [26,27]. Studies have identified that Baihui (GV20), Dazhui (GV14), Zusanli (ST36), Juliao (ST3) and Hegu (LI4) are the most commonly used acupoints for the nervous system disorders in the clinic [28]. In the present study, the acupoints of Baihui (GV 20) and Yintang (GV 29) were selected to be the acupuncture modulation based on the concept of “treating different diseases with the same treatment” in mental disorders. Our previous studies have also shown that acupuncture at Baihui (GV 20) and Yintang (GV 29) can regulate the hippocampal injury and inflammation response induced by stress [29].

4.2. Acupuncture reversed the SPS-induced neuroinflammation in hippocampus and serum

A large number of studies have confirmed that stress-induced activation of microglia in hippocampus plays an important role in the pathological process of PTSD [14,16,30]. When inflammation, infection, trauma or other neurological diseases occur in the brain, microglia will be activated and initiate phagocytosis, initiating the formation of multiprotein complexes in inflammatory cells, which will further promote the cleavage of the precursor form of IL-1 β into its active form [14,31]. Studies have also shown that the total number of microglia, the density and proportion of activated microglia in the hippocampal CA1, CA2/3 and DG areas were significantly up-regulated after stress procedures. And the mRNA expression of inflammatory factors of IL-1 β and iNOS in the hippocampus was also significantly increased [32]. Studies have indicated that the expression of proinflammatory factors and neurotoxic substances were significantly increased after the microglia were over-activated, followed by less neuroprotective properties and increased release of a large number of immune inflammatory factors, which was considered to be involved in the pathological process of PTSD [33,34]. Other studies have also identified that inflammatory cytokines, such as IL-1 β , IL-6, CRP, and IFN- γ , were involved in the pathogenesis of PTSD [7,17,35]. A previous study has shown that SPS induced the increase of pro-inflammatory cytokines and the decrease of anti-inflammatory cytokines in the hippocampus, which played an important role in the biological process of PTSD [31,36]. However, the other study has

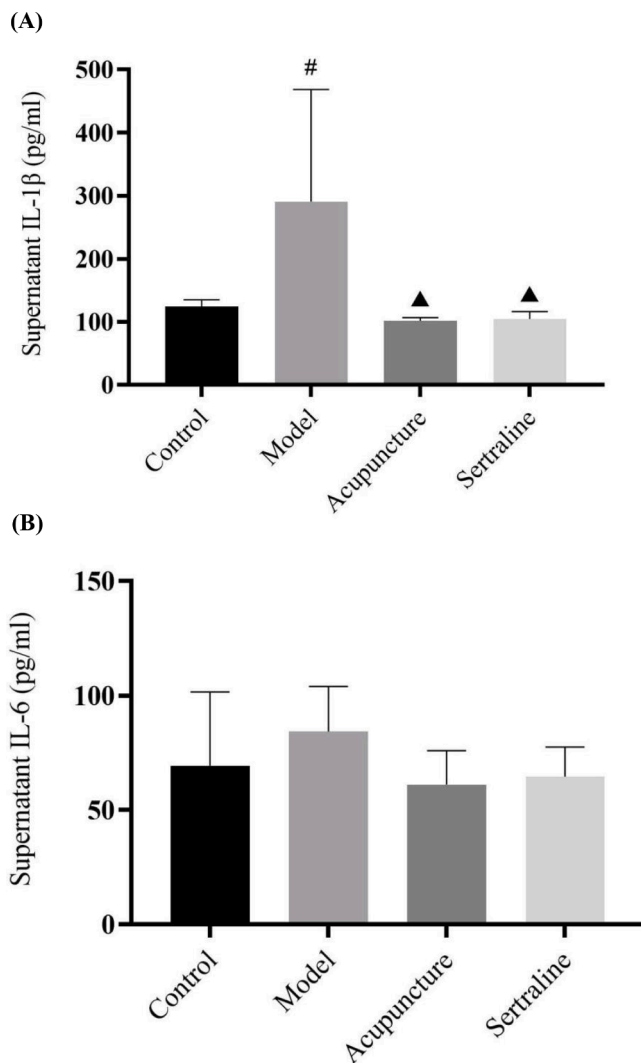


Fig. 5. Effects of acupuncture on the content of IL-1 β and IL-6 in serum of SPS rats. (A) Effects of acupuncture on the content of IL-1 β in serum of SPS rats. Data were expressed as means $\pm$ s. # P < 0.05, compared with control group; ▲ P < 0.05, compared with model group. (B) Effects of acupuncture on the content of IL-6 in serum of SPS rats. Data were expressed as means $\pm$ s.

Table 4

Effects of acupuncture on the content of serum IL-1 β and IL-6 of SPS rats. ($\bar{x} \pm s$).

Groups	n	IL-1 β (pg/ml)	IL-6(pg/ml)
Control	5	124.513 $\pm$ 10.504	69.371 $\pm$ 32.175
Model	5	290.620 $\pm$ 177.783 [#]	84.308 $\pm$ 19.653
Acupuncture	5	101.639 $\pm$ 4.929 [▲]	61.179 $\pm$ 14.814
Sertraline	5	104.705 $\pm$ 11.449 [▲]	64.661 $\pm$ 12.872

Notes: Data were expressed as means $\pm$ s. # P < 0.05, compared with control group; ▲ P < 0.05, compared with model group.

published the different findings. It has reported that the expression of hippocampal IL-6 increased significantly after seven days of SPS procedures, even with no difference in the expression of TNF- α , IL-1 β , and IL-10 in the hippocampus [37]. In addition, the phenomenon of high expression of inflammatory factors has also been demonstrated to exist in PTSD-related symptoms, including depression, anxiety, or sleep disorders [38]. The increase of pro-inflammatory cytokines is considered to be associated with fatigue, negative emotions, anhedonia, cognitive impairment, social withdrawal and other symptoms, which could lead to

the occurrence of PTSD-related symptoms [39]. Notably, the study has found that minocycline reversed the increase of pro-inflammation and nuclear factor kappa-B (NF- κ B), and inhibited the activation of microglia in the prefrontal cortex and hippocampus, which relieved the development of biological process in PTSD [33]. Hence, microglial activation and pro-inflammatory mediators have been considered to be the key factors in the etiological progress of PTSD. Inhibiting the activation of microglia and down-regulating the expression of pro-inflammatory mediators might be the potential therapeutic strategy for the treatment of PTSD [14].

The results from present study have shown that SPS procedures induced the activation of hippocampal microglia and enhanced the degeneration, necrosis, nuclear pyknosis of hippocampal neuronal cells, and even obvious inflammatory cell infiltration and edema cells, which induced obvious PTSD-like behaviors. Meanwhile, SPS-induced pro-inflammatory mediator disorders has also been verified in the present study. After SPS procedures, there was significantly increased expression of IL-1 β in serum. The findings from present study have identified that single prolonged stress played an important role in the pathological process of PTSD by activating hippocampal microglia and inflammatory response. Importantly, acupuncture and sertraline intervention effectively reversed the activated microglia of the hippocampus in rats exposed to SPS. Meanwhile, acupuncture intervention alleviated the damage degree of hippocampal cells, restored the disorder arrangement of cells, increased the number of cells, and reduced vacuoles and the activation degree of microglia. Besides, acupuncture and sertraline intervention reversed the high expression of serum IL-1 β , which indicates that acupuncture could regulate the SPS-induced pro-inflammatory mediator disorders and exhibit the therapeutic effect for PTSD.

4.3. Conclusion

Conclusively, the present study aims to elucidate the potential mechanisms of acupuncture in the treatment of PTSD. All of the findings have indicated that SPS-induced activation of hippocampal microglia and neuroinflammation plays an important role in the pathological process of PTSD. Acupuncture could reverse the pathological process of the inflammatory response mediated by the activation of microglia induced by SPS procedures, which might be the underlying mechanism of acupuncture in the treatment of PTSD and provide experimental evidence for the clinical strategies. However, there are several limitations of the present study. The present study only preliminarily investigated the role of neuroinflammation induced by SPS in the pathogenesis of PTSD, and the underlying mechanism of acupuncture in the treatment of PTSD. More concrete mechanism and pathways that contributes to this process are needed to be fully elucidated. Meanwhile, the efficacy and safety of acupuncture in the treatment of PTSD have been preliminarily demonstrated in both clinical and experimental studies. The present study was the preliminary study on the effects and mechanisms of acupuncture in the PTSD, so shame acupuncture was not set to be the control. In the future, our team will continue to carry out the study of the anti-PTSD effect of acupuncture based on the present findings.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.neulet.2023.137056>.

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