Molecular Neurobiology (2025) 62:4221–4236
https://doi.org/10.1007/s12035-024-04550-5

RESEARCH

Understanding the Antidepressant Mechanisms of Acupuncture: Targeting Hippocampal Neuroinflammation, Oxidative Stress, Neuroplasticity, and Apoptosis in CUMS Rats

Jianguo Li[1,2] · Xinhong Wu[3] · Simin Yan[1] · Junliang Shen[4] · Tao Tong[2] · Muhammad Shahzad Aslam[5] · Jingyu Zeng[1] · Yiping Chen[6] · Wenjie Chen[7] · Meng Li[2] · Zhuoran You[2] · Kaiyue Gong[1] · Jinghao Yang[1] · Maoshu Zhu[3] · Xianjun Meng[1]

Received: 28 December 2023 / Accepted: 11 October 2024 / Published online: 18 October 2024

Abstract

Depression is recognized globally as one of the most intractable diseases, and its complexity and diversity make treatment extremely challenging. Acupuncture has demonstrated beneficial effects in various psychiatric disorders. However, the underlying mechanisms of acupuncture's antidepressant action, particularly in depression, remain elusive. Therefore, this study aimed to investigate the effects of acupuncture on chronic unpredictability stress (CUMS)-induced depressive symptoms in rats and to further elucidate its underlying molecular mechanisms. All rats were exposed to CUMS of two stressors every day for 28 days, except for the control group. One hour before CUMS, rats were given a treatment with acupuncture, electroacupuncture, sham-acupuncture, or fluoxetine (2.1 mg/kg). Behavioral tests and biological detection methods were conducted in sequence to evaluate depression-like phenotype in rats. The findings of this study demonstrate that acupuncture therapy effectively ameliorated depression-like behavior induced by CUMS in rats. Additionally, acupuncture exerted a restorative effect on the alterations induced by CUMS in the levels of malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), interleukin-1β (IL-1β), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), brain-derived neurotrophic factor (BDNF), cyclic AMP response element-binding protein (CREB), postsynaptic density95 (PSD95), gamma-aminobutyric acid (GABA), and acetylcholine (ACh). Additionally, our findings indicate that acupuncture also modulates the ERK and Caspase-3 apoptotic pathways in the hippocampus of CUMS rats. This study suggests that acupuncture may play a potential preventive role by regulating hippocampal neuroinflammatory response, levels of oxidative stress, apoptotic processes, and enhancing synaptic plasticity.

Keywords Depression · Acupuncture · Oxidative Stress · Neuroinflammation · Neuroplasticity · Apoptosis

Jianguo Li and Xinhong Wu have contributed equally to this work and share first authorship.

✉ Maoshu Zhu
1336620278@qq.com

✉ Xianjun Meng
mengxianjun@xmu.edu.cn

[1] Department of Traditional Chinese Medicine, Xiang'an Hospital of Xiamen University, School of Medicine, Xiamen University, Xiamen, China

[2] Second Clinical College, Shanxi University of Traditional Chinese Medicine, Taiyuan, Shanxi, People's Republic of China

[3] The Fifth Hospital of Xiamen, Xiamen, Fujian, People's Republic of China

[4] Longyan Hospital of Traditional Chinese Medicine Affiliated to Xiamen University, Longyan, Fujian, People's Republic of China

[5] School of Traditional Chinese Medicine, Xiamen University Malaysia, Sepang, Malaysia

[6] First Clinical College, Yunnan University of Chinese Medicine, Kunming, Yunnan, People's Republic of China

[7] The First Affiliated Hospital of Guangzhou University of Chinese Medicine, Guangzhou, Guangdong, People's Republic of China

Introduction

Based on statistical data, approximately 4.7% of the global population experiences depression annually [1]. In recent years, depression has attracted much attention as a global public health problem. The disorder is primarily characterized by persistent low mood, slowed thinking, loss of interest, and decreased energy. At the same time, patients with depression may face loss of appetite, sleep disorders, and even thoughts of suicide [2]. It poses a significant threat to individuals' physical and mental well-being, while also imposing a substantial economic burden on society. Despite notable progress in the field of antidepressant drug research and development, several constraints persist. These limitations encompass the delayed onset of therapeutic effects, elevated rates of drug resistance and relapse, as well as the potential for long-term usage to result in gastrointestinal disorders, cardiotoxicity, sexual dysfunction, and other adverse reactions [3, 4]. Consequently, the pursuit of safer and more efficacious therapies for depression, as well as further exploration of the pathogenesis of depression, has become increasingly crucial.

Acupuncture therapy is a traditional Chinese medicine technique with a long history and unique advantages. Stimulating specific points in the human body regulates the internal qi of the body to achieve the purpose of treating diseases. In recent years, escalating public interest in health and advancements in medical technology have propelled acupuncture therapy into the forefront of research focus. Distinguished by its non-invasive attributes, minimal adverse effects, and notable therapeutic efficacy, acupuncture is a viable intervention. Ancient medical literature extensively documents acupuncture's historical utilization in treating mental diseases, adding depth to its contemporary investigation. Numerous studies have confirmed that acupuncture has a wide range of modulating effects on the pathogenesis of depression and exerts antidepressant effects through multiple targets and pathways [5–7]. In this study, our focus centered on the *Shangxing* (GV23) and *Fengfu* (GV16) appoints, derived from the set of thirteen ghost points intricately delineated in the Thousand Gold Formula, a compilation attributed to Sun Simiao. In the theoretical system of traditional Chinese medicine, these two acupoints belong to the governor vessel, which has the effect of regulating the governor and spirit and has a good therapeutic effect for mental diseases such as depression. Given the historical and theoretical significance of GV23 and GV16 in the treatment of mental disorders, our study aims to explore the potential mechanisms by which these two acupoints exert an antidepressive effect.

In recent years, multiple lines of evidence have shown that neuroinflammation and oxidative stress play a crucial role in the pathogenesis of depression [8–10]. Chronic stress, characterized as a prolonged state of psychological or physiological stress, profoundly impacts multiple bodily systems. In the nervous system, chronic stress may lead to neuronal mitochondrial dysfunction. When mitochondrial dysfunction occurs, oxidative stress is activated, leading to the overproduction of reactive oxygen species and the release of cytokines [11]. An overabundance of reactive oxygen species (ROS) has the potential to stimulate the production of pro-inflammatory cytokines, including IL-1β, IL-6, and TNF-α, by activating the nuclear factor κB (NF-κB) signaling pathway situated downstream of the pattern recognition receptor activated by cytokines [12]. Consequently, this process can intensify the inflammatory response, impede neurotransmitter signaling, and disrupt the synthesis, reuptake, and release of neurotransmitters, potentially culminating in the development of depression. Our previous studies have shown that acupuncture can prevent and improve depression-like symptoms induced by CUMS, possibly through the regulation of inflammation mediated by the HMGB1/TLR4 signaling pathway in the brain-spleen axis and the HPA axis [13].

The hippocampus is considered a critical stress response brain region involved in memory, learning, and emotion regulation and plays an essential role in the pathogenesis of depression [14]. Reduced hippocampal formation volume is associated with stress and depression, and hippocampal neurogenesis buffers stress responses and depressive behaviors. Hippocampal neuron cells are considered to be key cells in enhancing neuroprotection, stimulating neuroplasticity, and repairing neuronal loss [15]. Brain-derived neurotrophic factor (BDNF), a plasticity-related protein, is widely distributed in the central nervous system. BDNF plays a crucial role in modulating synaptic plasticity in the brain by activating the transcription factor CREB through its interaction with the receptor TrkB. This activation leads to the regulation of various genes, such as neurotrophic factors, synaptic proteins, and ion channels, ultimately influencing processes like neuronal differentiation, regeneration, and the establishment and maintenance of neuronal synapses. Additionally, BDNF facilitates long-time potentiation (LTP), further contributing to the plasticity of brain neuronal cells [16]. Animal experimental studies have found that rats exposed to CUMS have reduced synaptic plasticity in the hippocampus, as evidenced by reduced levels of BDNF expression and PSD95. This observation implies that enhancing neuroplasticity may serve as a significant approach to attaining antidepressant effects. Moreover, the release of neurotransmitters is closely related to the onset of depression [17]. Under stress, neurons secrete a variety of neurotransmitters, such as 5-hydroxytryptamine (5-HT), gamma-aminobutyric acid (GABA), and acetylcholine (ACh). These transmitters

play an important role in regulating human emotions, cognition, and behavior [4].

ERK belongs to the classic transmission pathway in the MAPK signaling pathway. It is a serine/threonine protein kinase involved in cell growth, development, proliferation, differentiation, apoptosis, and inflammatory response [18]. In recent years, ERK has gradually become a new focus in the field of depression research. In both human subjects and diverse animal models of chronic depression, there is a notable decrease in ERK signaling within the prefrontal cortex and hippocampus, which are two fundamental regions linked to depression [19]. ERK1/2, as the principal member of the ERK family, exhibits two phosphorylation sites, namely, Thr-Glu-Tyr (TEY) and Thr-Asp-Tyr (TDY). The phosphorylation of these sites assumes a pivotal role in the activation of ERK [20]. A recent study pointed out that paeoniflorin can alleviate chronic restraint stress (CRS)-induced depression-like behavior in mice, related to its effect on up-regulating the expression of p-ERK1/2 and BDNF in the hippocampus [21]. In addition, it has been reported that ERK1/2 can activate NF-κB and inhibit the caspase-3 apoptosis pathway, regulating the expression of apoptosis-related proteins Bcl-2 and Bax, thereby improving depressive-like behavior.

However, the specific mechanisms of how acupuncture treats depression through the above possible mechanisms have not been fully explained and elucidated, and thus, further research and exploration are still needed. The present study aimed to investigate the potential mechanisms of acupuncture on the *Shangxing* and *Fengfu* points in CUMS rat model and to provide a scientific basis for their future development and clinical application.

Materials and Methods

Animals and Grouping

Sixty-six healthy male adult specific pathogen-free (SPF) Sprague–Dawley (SD) rats were purchased from the Beijing Vital River Laboratory, weighing 100-120 g at the beginning of the experiment. The rats were housed in the animal laboratory of Medical College of Xiamen University under 22 ± 2 ℃ room temperature and 55 ± 5% relative humidity with a 12-h light/dark cycle (lights on at 08:00am). All experimental protocols were approved by the Animal Care Ethics Committee of Xiamen University and strictly followed the guidelines of animal ethics (License No. XMULAC202110062). After a 7-day acclimatization period, body weight measurements and sucrose preference tests were performed, and 60 rats with the same baseline were selected for behavioral evaluation. The animals were randomly divided into six groups ($n = 10$/group): control group (CON), chronic unpredictable mild stress group (CUMS), acupuncture group (AP), fluoxetine group (FLX), sham acupuncture group (SAP), and electroacupuncture group (EA). This study was conducted in strict adherence to the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines. The comprehensive checklist provided by ARRIVE (https://arriveguidelines.org/resources/author-checklists) was followed meticulously throughout the design, execution, and reporting phases of this research to ensure transparent and rigorous reporting of in vivo experiments involving animal subjects.

Chronic Unpredictable Mild Stress (CUMS) Model Procedure

The CUMS procedure was performed as previously described with minor modifications [22]. In addition to the control group, the experimental protocol used 4 consecutive weeks of stress stimulation, including (1) water deprivation for 24 h, (2) fasting for 24 h, (3) wet bedding for 24 h, (4) circadian reversal, (5) tail clamping for 3 min, (6) stroboing for 24 h, (7) restraint for 3 h, (8) alcohol odor stimulation, (9) crowding (six or seven per cage), and (10) cages without bedding. Rats were housed in standard plastic cages (three or four per cage). Two stressors were imposed randomly everyday but not repeatedly within 3 continue day for 4 weeks.

Treatment

In this experiment, all intervention methods started 1 h before modeling. Acupuncture needles (0.25 mm × 13 mm, Hanyi, Beijing, China) were administered to rats in the AP group and EA group every other day for a total of 4 weeks (depth 5 mm, angle 15°). The acupuncture points used are GV23 and GV16. The anatomical localization of the selected acupoints, GV23 and GV16, was determined in accordance with the *Experimental Rat Acupuncture and Moxibustion Shu Point Atlas*, coupled with precise guidance from Acupuncture Manipulation. In EA group, an electrical current of 0.5 mA and a frequency of 2 Hz were delivered for 20 min, by an acupoint nerve electrostimulator (SDZ-II, Suzhou Medical Supplies Co, Ltd., Jiangsu, China). Similarly, the sham-AP group uses the same type of needle and acupuncture method, but the acupuncture location is 3 mm to the left of *Shangxing* and *Fengfu*. During the experiment, to ensure that the acupuncture needles remained stable inside the rats and to keep the rats calm, we used specialized experimental rat coat and secured the rats to a restraint board (Fig. 1). All groups, except for the control group, were dressed in the coats during the experiment. Rats in FLX group were intragastrically injected with fluoxetine solution (0.21 mg/ml, 2.1 mg/kg, PHR1394-1G, Sigma-Aldrich, USA).

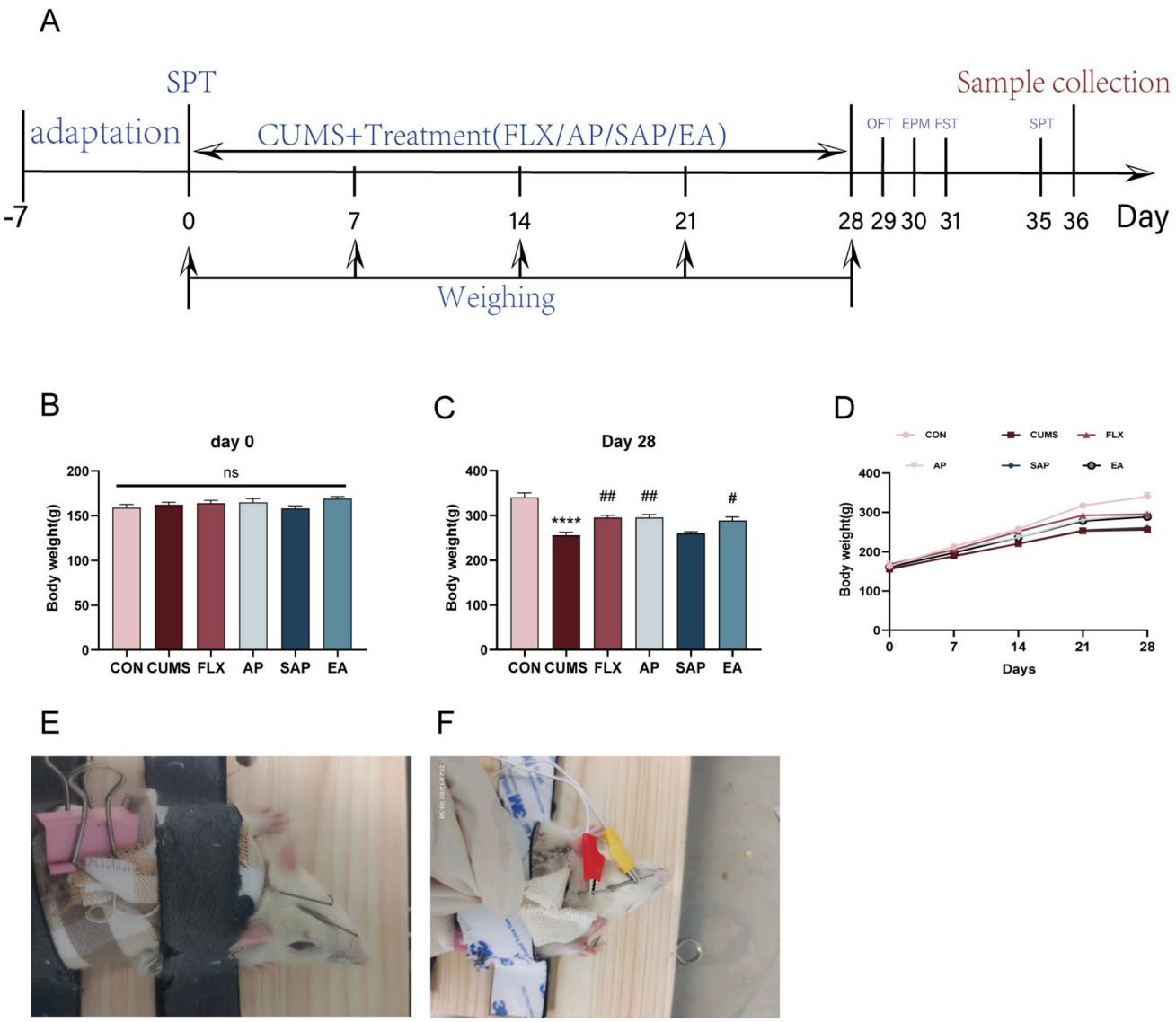

Fig. 1 Schematic representation of experimental protocol and body weight measurement. **A** Flow chart of experiment. **B** The body weight of rats on 0 days. **C** The body weight of rats on 28 days. **D** The body weight changes of rats in 6 groups. **E** Rat restraint device and acupuncture with *Shangxing* (GV23) and *Fengfu* (GV16). **F** Rat restraint device and EA with Shangxing (GV23) and Fengfu (GV16). Results are the mean ± standard error of the mean ($x \pm s$), $n = 9$ per group. $^{*}P < 0.05$, $^{****}P < 0.0001$, compared to the control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, compared to the CUMS group

Behavioral Tests

Body Weight Monitoring (BW)

On days 0, 7, 14, and 28, the body weight of experiment rats was weighed in each group.

Open-Field Test (OFT)

As previously described, the spontaneous locomotor activity in rats was assessed using OFT following the CUMS protocol and after treatment [23]. The Open Field Test (OFT) apparatus consisted of a square box (100 cm long × 100 cm wide × 40 cm high) with black inner walls, with lines dividing the floor into 16 equal squares. Before the experiment, the rats were placed in a dark, quiet test room for 10 min to acclimate to the environment. Rats were placed in the central grid for OFT, and the total movement distance, grid crossing times (all three paws entered a square), and central and peripheral movement time were recorded within 5 min. Data were analyzed using the video analysis software SMART v.3.0 (Harvard Instruments, UK). Before testing the next rat, it is essential to clean the equipment with 75% alcohol to remove any odor the previous rat may have left behind.

Elevated Plus Maze Test (EPM)

As a measure of anxiety-like behavior in rats, we used the elevated plus maze (EPM) test [24]. The elevated cross labyrinth comprised two open arms (50 cm × 10 cm × 40 cm) and two closed arms (50 cm × 10 cm × 40 cm), connected by a central platform (10 cm × 10 cm). The labyrinth was positioned 80 cm above the floor. Rats were individually placed on the central platform of the maze, facing the open arms, and allowed to freely explore the apparatus for 5 min. The open and closed arm entries (all four paws in an arm) and the time spent in the open arms were recorded using Smart v3.0 (Panlab Harvard, MA, USA). The maze was cleaned thoroughly with 75% ethanol before each rat was tested.

Forced Swim Test (FST)

The FST is a classic method for assessing depression-like behavior (behavioral despair) in rats [25]. Rats were placed in a transparent cylindrical tube (diameter 0.2 m, height 0.45 m, water depth 0.3 m, water temperature 22 ± 2 °C) for 6 min, and the immobility time of the last 5 min was recorded. The standard of immobility is that the rat stops struggling in the water and is in a floating state, with only slight limb movements to keep the head above the water.

Sucrose Preference Test (SPT)

The sucrose preference test was performed to assess anhedonia in these rats as described previously [26]. In the first 24 h, all rats were housed in single cages from 9 am, and each rat received 2 bottles of sucrose sugar water (1%). In the second 24 h, each rat was given 2 bottles of liquid: one bottle of sucrose sugar water (1%) and another bottle of pure water. To avoid the formation of positional preferences affecting the experiment results, we changed the position of the two bottles after 12 h. After deprivation of food and water for 24 h, the sucrose preference test was conducted (09:00 am), and each rat was freely provided access to drink pre-weighed sucrose sugar water (1%) and pure water for 12 h. After 12 h (09:00 pm), the bottles containing water and sucrose were weighed at the end of this period. Sucrose preference (%) = sucrose intake (g)/[sucrose intake (g) + pure water intake (g)] × 100%. Immediately after the end of the test, all rats were allowed free access to water and food.

Western Blot Analysis (WB)

The hippocampal tissue of rats was collected, and total protein samples were extracted using RIPA lysis buffer. Protein concentration was determined using the BCA protein assay kit (QB214754, Thermo Scientific). Proteins were separated on prepared 10% SDS-PAGE gels and transferred to PVDF membranes (K5NA8023B, Amersham, Sweden) through electrophoresis. Following the transfer, the membrane was blocked with Tris-buffered saline (TBS) containing 0.1% Tween-20 and 5% (w/v) skim milk at room temperature for 1 h. Subsequently, it was incubated with the appropriate primary antibody overnight at 4 ℃ [Antibodies: anti-BDNF (28,205–1-AP, 1:1000, Proteintech), anti-CREB (12,208–1-AP, 1:3000, Proteintech), anti-PSD-95 (20,665–1-AP, 1:3000, Proteintech), anti-ERK1/2 (11,257–1-AP, 1:3000, Proteintech), anti-P-ERK1/2 (28,733–1-AP,1:2000, Proteintech), anti-Caspase-3 (66,470–2-Ig, 1:2000, Proteintech), anti-Bax (60,267–1-Ig, 1:5000, Proteintech), and anti-Bcl-2 (68,103–1-Ig, 1:300, Proteintech)]. After primary antibody incubation, the membrane was rinsed with TBST and subsequently incubated with horseradish peroxidase-conjugated secondary antibody. Samples were washed with TBST three times, and the signals were detected using an enhanced chemiluminescence solution and an Azure Bioimaging system (California, USA). The results were quantified using ImageJ software (U.S. National Institutes of Health, Bethesda, MD, USA).

Enzyme-Linked Immunosorbent Assay (ELISA)

According to the manufacturer's instructions, ELISA kits were utilized to measure the levels of IL-1β, IL-6, and TNF-α in the hippocampus and serum of rats in each group. The optical density was measured at 450 nm using a microplate reader (Thermo Fisher Scientific, Waltham, MA, USA), and the content was calculated based on the standard curve.

Colorimetric Method

As per the provided instructions, the levels of SOD, CAT, GSH-Px, MDA, GABA, and ACh in the hippocampus and serum of rats within each experimental group were assessed using a colorimetric kit. The absorbance of each kit was measured, and the corresponding content was determined by utilizing the standard curve.

Hematoxylin–Eosin (HE) Staining

After decapitating the rats, the entire brain was meticulously extracted and preserved in 4% polyformaldehyde for 24 h. The brains were dehydrated using ethanol, cleared with absolute ethanol and xylene, and then embedded in paraffin. Subsequently, 4-μm sections were obtained from the paraffin-embedded brains using a paraffin microtome (Leica RM2016, Shanghai, China). After drying, the sections were stained with hematoxylin and eosin (HE) using an automated staining machine (Giotto, DIAPATH). Subsequently, a digital slice scanning system (3 DHISTECH MIDI, Budapest, Hungary) was used for pathological analysis of the hippocampus.

Statistical Analysis

All experimental data were analyzed with the GraphPad Prism 10.0.2 (GraphPad Software Inc., USA). After the data were tested for homogeneity with variance, all normally or approximately normally distributed, and meeting the homogeneity of variance were analyzed by one-way ANOVA, and those who did not meet the homogeneity with variance were tested by Brown-Forsythe or Welch ANOVA method. All experimental data were expressed as mean ± standard error (SEM), and $P < 0.05$ was considered statistically significant.

Results

Effect of Acupuncture on CUMS-Induced Depression-Like Behavior in Rats

The body weight of all rats was monitored on days 0, 7, 14, 21, and 28. As shown in Fig. 1, there was no difference in body weight among all groups at day 0. After 28 days of intervention, the weight gain trend of rats in CUMS group was significantly decreased compared with the control group [$F(5, 48) = 18.09$, $P < 0.0001$], while FLX, AP, and EA treatment significantly promoted weight gain compared with CUMS group (FLX: $P < 0.01$; AP: $P < 0.01$; EA: $P < 0.05$). SAP treatment could not significantly reverse the inhibitory effect of CUMS on weight gain. In Fig. 2, compared to the control group, the rearing time, crossing numbers in the OFT [$F(5, 48) = 5.922$, $P < 0.001$; $F(5, 48) = 6.174$, $P < 0.01$], and the open arms time in the EPM test [$F(5, 48) = 3.406$, $P < 0.01$] were significantly reduced in the CUMS group. However, after treatment of CUMS rats with FLX, AP, or EA, the rearing time (FLX: $P < 0.001$; AP: $P < 0.01$; EA: $P < 0.001$) and crossing numbers (FLX: $P < 0.05$; AP: $P < 0.05$; EA: $P < 0.01$) of OFT, as well as the time spent in the open arms in EPM (FLX: $P < 0.05$; AP: $P < 0.05$; EA: $P < 0.05$), were significantly increased compared to the CUMS group. This change did not occur in the SAP group. In FST, immobility time was significantly increased in CUMS group compared with the control group [$F(5, 48) = 5.377$, $P < 0.01$]. However, compared to the CUMS group, immobility time was significantly reduced in the FLX, AP, and EA groups (FLX: $P < 0.05$; AP: $P < 0.05$; EA: $P < 0.05$). There was no significant change in the SAP group compared to the CUMS group. We assessed the degree of anhedonia in rats using SPT and the results showed a significant decrease in sucrose preference in the CUMS group compared to the control group [$F(5, 48) = 7.344$, $P < 0.001$]. Compared with CUMS group, the sucrose preference rate in the FLX group, AP group and EA group was significantly increased (FLX: $P < 0.001$; AP: $P < 0.05$; EA: $P < 0.01$). Compared with CUMS group, SAP group had no significant change.

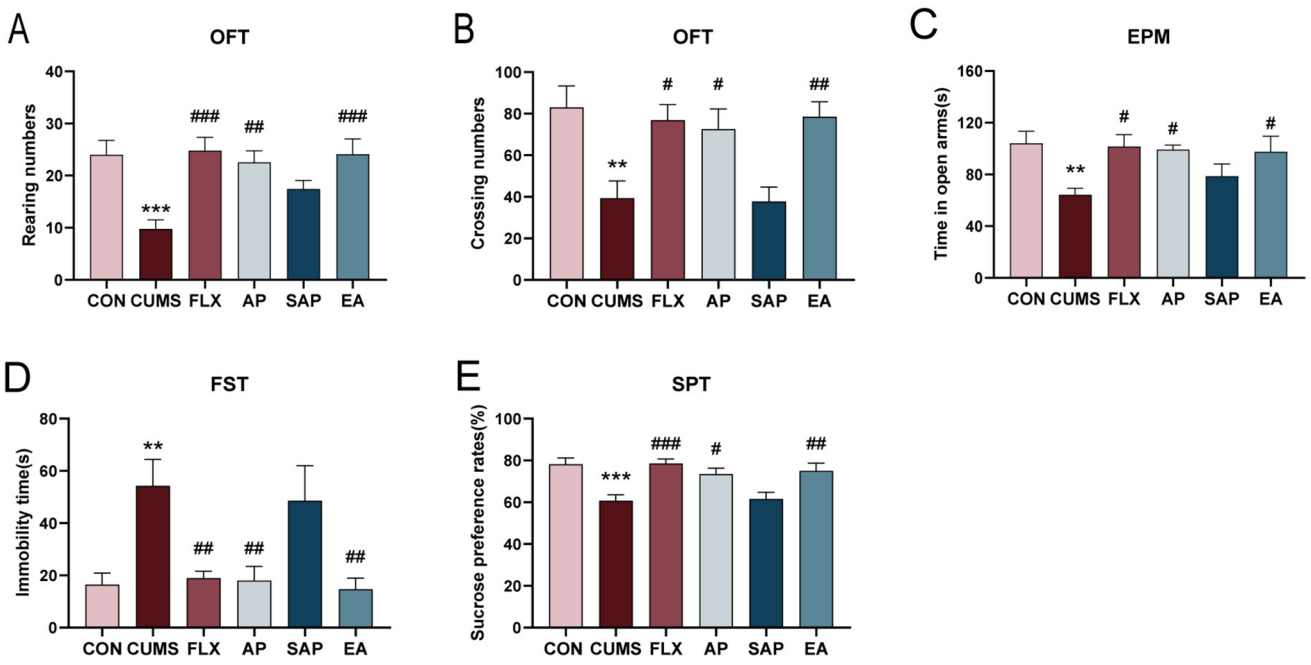

Fig. 2 Acupuncture ameliorates depressive-behavior rats exposed to CUMS. **A**, **B** Rearing numbers and numbers of crossing in the OFT. **C** The time in the open arms of EPM. **D** Immobility time in the FST. **E** The sucrose preference rates in SPT. All the results are expressed as the mean ± standard error of the mean ($x \pm s$), $n = 9$ per group, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, compared to the control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$,compared to the CUMS group

Acupuncture Ameliorates Oxidative Stress in *Hippocampus* and Serum of CUMS Rats

Oxidative stress is a key factor in depression-like behavior. In the present study, we detected the changes of oxidative stress levels in the hippocampus and serum of rats in each group, respectively. In Fig. 3, the results showed that after the CUMS procedure, the levels of SOD, CAT, and GSH-Px ($P < 0.0001$; $P < 0.0001$; $P < 0.001$) were significantly decreased and the level of MDA ($P < 0.001$) was significantly increased in the hippocampus compared with the control group. Interestingly, treatment with FLX (SOD: $P < 0.05$; CAT: $P < 0.0001$; GSH-Px: $P < 0.01$; MDA: $P < 0.001$), AP (SOD: $P < 0.05$; CAT: $P < 0.001$; GSH-Px: $P < 0.01$; MDA: $P < 0.05$), and EA (SOD: $P < 0.05$; CAT: $P < 0.0001$; GSH-Px: $P < 0.01$; MDA: $P < 0.05$) reversed

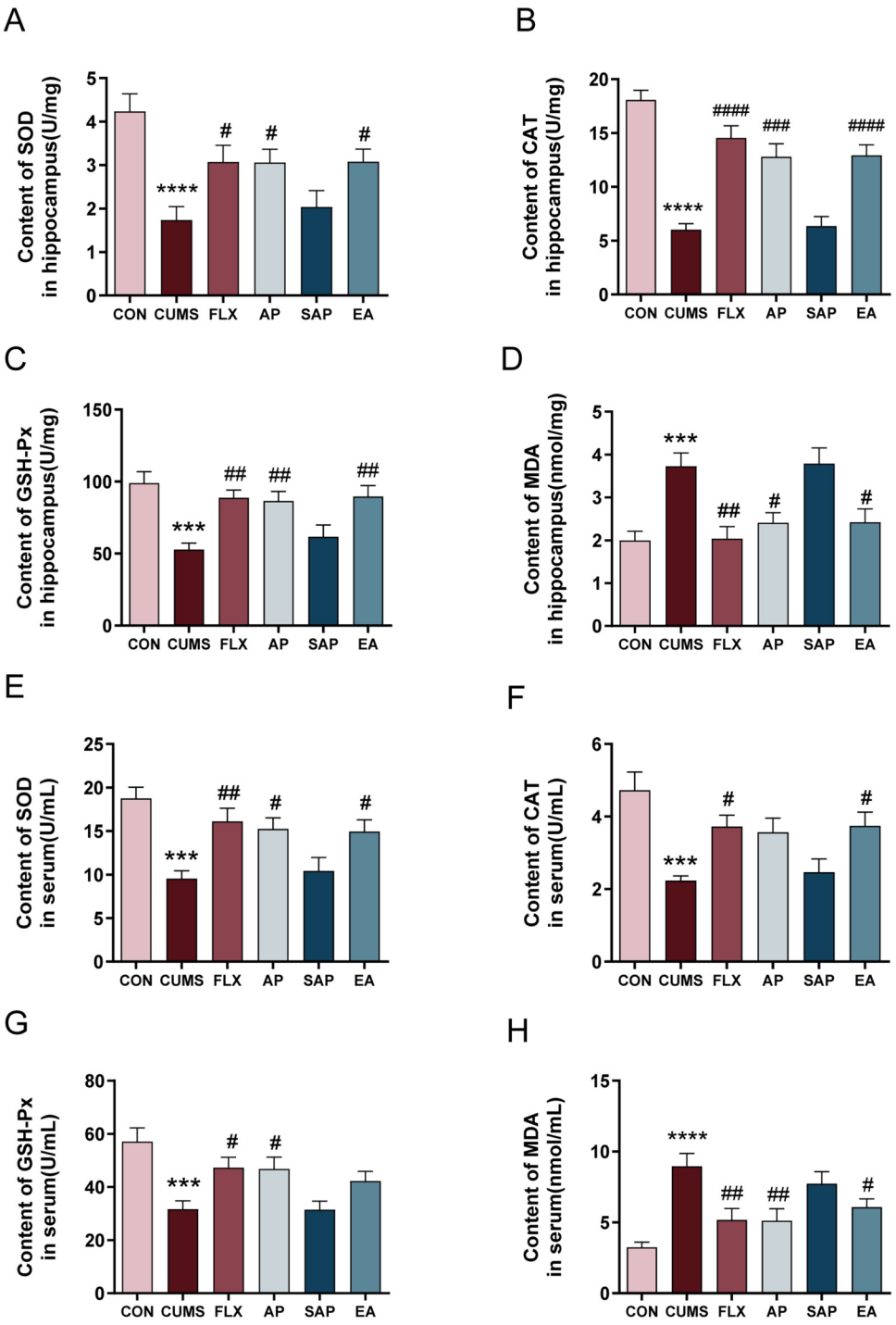

Fig. 3 Acupuncture ameliorates oxidative stress in hippocampus and serum of CUMS rats. **A**, **E** Content of SOD in hippocampus and serum. **B**, **F** Content of CAT in hippocampus and serum. **C**, **G** Content of GSH-Px in hippocampus and serum. **D**, **H** Content of MDA in hippocampus and serum. $n = 6$ per group, $^{***}P < 0.001$, $^{****}P < 0.0001$, compared to the control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, compared to the CUMS group

these changes. However, there were no significant changes in the expression of individual proteins in the SAP group compared to the CUMS group. In addition, we found lower serum levels of SOD, CAT, and GSH-Px (all $P < 0.001$) and higher levels of MDA ($P < 0.0001$) in the CUMS group than in the control group. Compared with the CUMS group, the FLX and AP groups showed significantly higher levels of SOD (FLX: $P < 0.01$; AP: $P < 0.05$), CAT (FLX: $P < 0.05$), and GSH-Px (FLX: $P < 0.05$; AP: $P < 0.05$) and lower levels of MDA (FLX: $P < 0.01$; AP: $P < 0.01$). The expression of SOD and CAT in serum after EA treatment was elevated (SOD: $P < 0.05$; CAT: $P < 0.05$), the expression of MDA ($P < 0.05$) was decreased, and the expression of GSH-Px showed a trend of increase, but the difference was not statistically significant. There was no significant difference in the SAP group compared with the CUMS group. These results suggest that acupuncture attenuated CUMS-induced oxidative stress in the hippocampus and serum.

Acupuncture Inhibits CUMS-Induced Elevation of Inflammatory Cytokines in *Hippocampus* and Serum

Previous studies have shown that inflammatory factors are closely related to the development of depression. In Fig. 4, we found differences in the expression of inflammatory cytokines IL-1β, IL-6, and TNF-α in the hippocampus and serum. The levels of IL-1β, IL-6, and TNF-α were significantly higher in the CUMS group compared to the control group (hippocampus: $P < 0.0001$, $P < 0.0001$, $P < 0.05$; serum: $P < 0.0001$, $P < 0.0001$, $P < 0.001$). After treatment with FLX, AP, and EA, the elevation of IL-1β [hippocampus: (FLX: $P < 0.01$; AP: $P < 0.05$; EA: $P < 0.05$); serum: (FLX: $P < 0.0001$; AP: $P < 0.001$; EA: $P < 0.01$)], IL-6 [hippocampus: (FLX: $P < 0.01$; AP: $P < 0.05$; EA: $P < 0.01$); serum: (FLX: $P < 0.001$; AP: $P < 0.05$; EA: $P < 0.05$)], and TNF-α[hippocampus: (all $P < 0.05$); serum: (FLX: $P < 0.001$; AP: $P < 0.01$; EA: $P < 0.01$)] was significantly reversed in CUMS

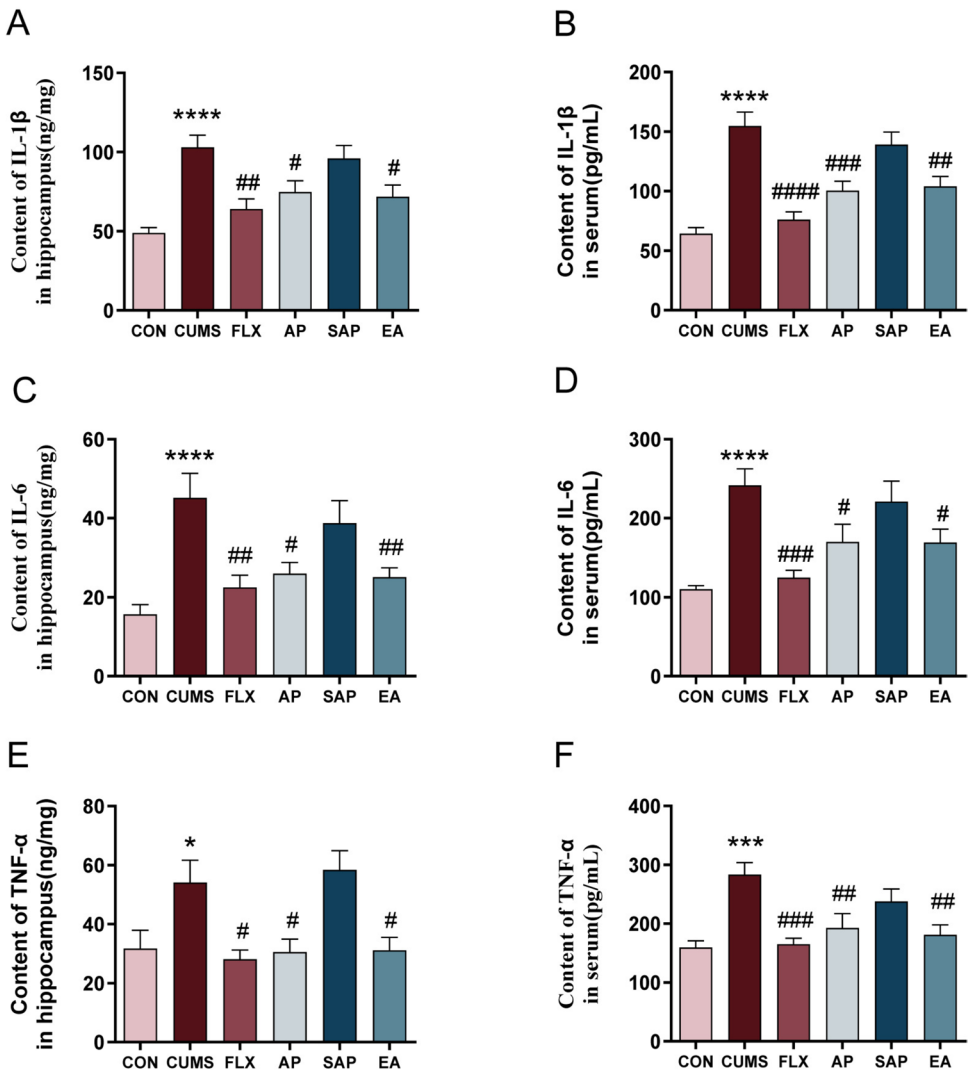

Fig. 4 Acupuncture inhibits CUMS-induced elevation of inflammatory cytokines in hippocampus and serum. **A**, **B** Content of IL-1β in hippocampus and serum. **C**, **D** Content of IL-6 in hippocampus and serum. **E**, **F** Content of TNF-α in hippocampus and serum. $n = 6$ per group, $^{*}P < 0.05$, $^{***}P < 0.001$, $^{****}P < 0.0001$, compared to the control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$, compared to the CUMS group

rats. There was no significant difference in the expression of IL-1β, IL-6, and TNF-α in the SAP group compared to the CUMS group. The above findings suggest that acupuncture can inhibit the elevation of CUMS-induced inflammatory factors in the hippocampus and serum.

Acupuncture Enhances Hippocampal Synaptic Plasticity in CUMS Rats

It is well known that the optimization of hippocampal synaptic plasticity is a key prerequisite for the performance of its cognitive functions, whereas the high expression of BDNF, CREB, and PSD95 has a positive effect on promoting synaptic transmission. Recent evidence reveals a strong link between BDNF, CREB, and PSD95 deficiency and the development of depression. Therefore, increasing the expression levels of BDNF, CREB, and PSD95 is a promising strategy for the treatment or prevention of depression. In this experiment, the corresponding values were significantly lower in the CUMS group compared to the expression of BDNF, CREB, and PSD95 in the control group (all $P < 0.001$) (Fig. 5). After fluoxetine and electroacupuncture interventions, the expression of BDNF significantly increased compared to the CUMS group (FLX: $P < 0.01$; EA: $P < 0.05$). However, there was no significant difference between the AP and SAP groups and the CUMS group. Treatment with fluoxetine, acupuncture, and electroacupuncture significantly reversed the decrease in CREB expression caused by CUMS (FLX: $P < 0.01$; AP: $P < 0.05$; EA: $P < 0.05$). The expression of PSD95 was increased in both the FLX and AP groups compared to the CUMS group (FLX: $P < 0.001$; AP: $P < 0.05$), and there was no statistically significant difference in the expression of PSD95 between the EA and CUMS groups.

Acetylcholine (ACh) is a neurotransmitter primarily involved in the regulation of synaptic plasticity. Gamma-aminobutyric acid (GABA) is a compound that plays an important role in the body as an inhibitory neurotransmitter

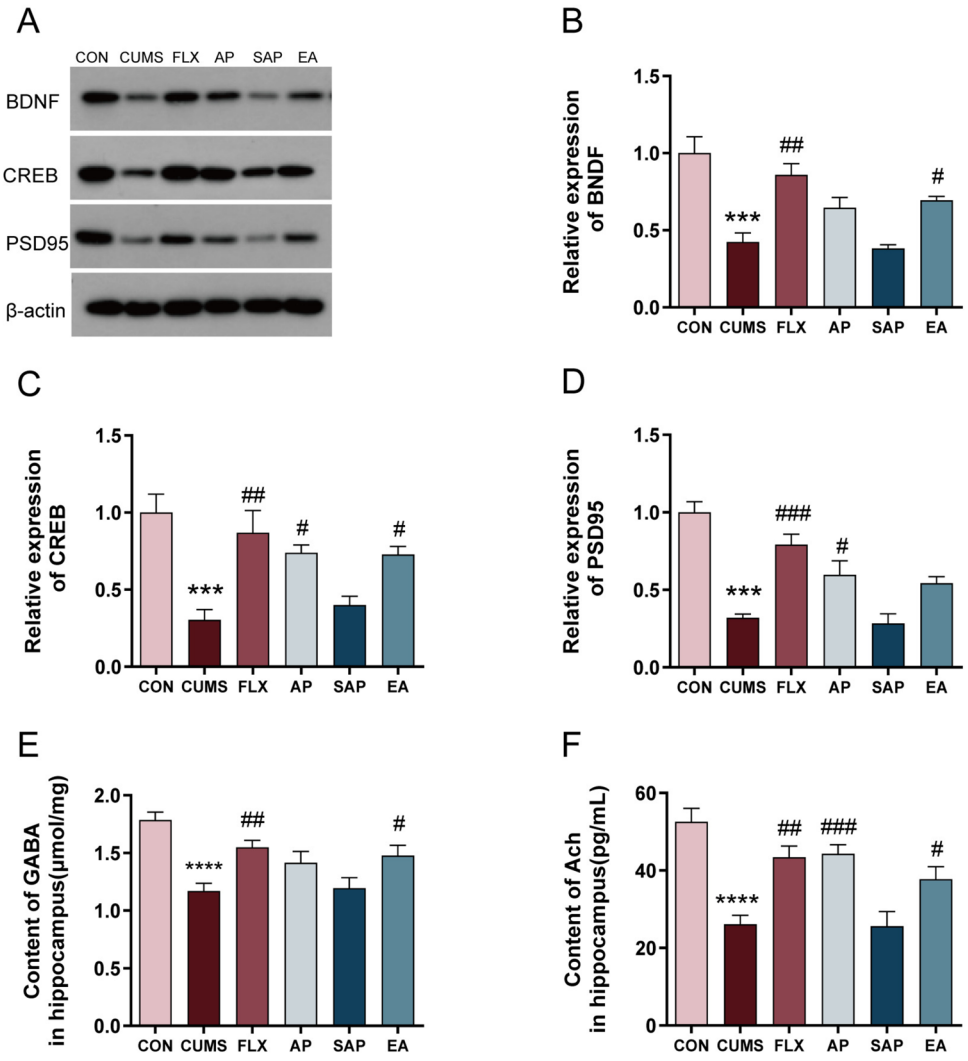

Fig. 5 Acupuncture enhances hippocampal synaptic plasticity in CUMS rats. **A** The expression of BDNF, CREB, and PSD95 protein in each group was detected by Western blot ($n = 3$ per group). **B** Analysis of BDNF proteins. **C** Analysis of CREB proteins. **D** Analysis of PSD95 proteins. **E** Content of GABA in hippocampus was detected by Colorimetry. **F** Content of Ach in hippocampus was detected by Colorimetry ($n = 6$ per group). All values are presented as mean ± standard error of the mean ($x \pm s$), $^{***}P < 0.001$, $^{****}P < 0.0001$, compared to the control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$, compared to the CUMS group

of the central nervous system. They have an important role in regulating CNS function and in ameliorating depression. The results of our study showed that the expression of GABA and ACh was significantly decreased in the CUMS group (all $P < 0.0001$) compared to the control group. After fluoxetine and electroacupuncture treatment, GABA expression increased significantly (FLX: $P < 0.01$; EA: $P < 0.05$). However, there was no significant difference between the AP group and SAP group compared with CUMS group. Interestingly, FLX, AP, and EA interventions all reversed the CUMS-induced decrease in ACh expression(FLX: $P < 0.01$; AP: $P < 0.001$; EA: $P < 0.05$). These data suggest that hippocampal synaptic plasticity is involved in the progression of depression and suggest a potential molecular mechanism by which acupuncture improves depression-like behavior in CUMS rats.

Acupuncture Attenuates Histopathological Damage in the *Hippocampus* of CUMS Rats

The hippocampus is a key region of the brain that plays a crucial role in cognition and emotion regulation, and the development of depression is often accompanied by pathological damage to the hippocampus. The present study consistently demonstrated hippocampal damage after chronic stress (Fig. 6). Overall, in the CUMS group, neurons in the DG area were solidified, with reduced and irregular cell volume, poorly demarcated nuclei and cytoplasm, and more dispersed than in the normal control group. With the treatment of FLX, AP, and EA, the hippocampal pathological damage in CUMS rats was gradually recovered, which was manifested as neurons with regular morphology and neatly arranged and dense.

Acupuncture May Activate Hippocampal ERK and Inhibit Caspase-3 Apoptotic Pathway in CUMS Rats

As shown in Fig. 7, the phosphorylation level of ERK1/2 and the expression level of Bcl-2 in the CUMS group exhibited significant down-regulation (all $P < 0.001$), whereas the expression levels of apoptotic proteins Bax and Caspase-3 showed significant increase (Bax: $P < 0.001$; Caspase-3: $P < 0.01$), in comparison to the corresponding indices in the normal control group. These findings provide robust evidence for the significant inhibition of the ERK signaling pathway activity, alongside the significant enhancement of the apoptotic pathway mediated by Caspase-3. The administration of fluoxetine resulted in a gradual increase in the expression levels of p-ERK1/2 and the anti-apoptotic protein Bcl-2 (p-ERK1/2: $P < 0.05$; Bcl-2: $P < 0.05$). In the AP group, there was an upward trend in the level of p-ERK1/2, and a significant increase in the expression level of Bcl-2 ($P < 0.05$). Furthermore, compared to the CUMS group, the expression level of Caspase-3 was significantly reduced following EA treatment ($P < 0.05$).

Discussion

The current study yielded the following results: (i) Acupuncture treatment in CUMS rats led to an increase in body weight and a decrease in depressive-like behaviors. (ii) The application of acupuncture also led to an up-regulation in the levels of SOD, CAT, and GSH-Px, while simultaneously decreasing the levels of MDA in both the hippocampus and serum of rats subjected to CUMS. Additionally, acupuncture treatment reversed the elevated levels of IL-1β, IL-6, and TNF-α in the hippocampus and serum of CUMS rats. (iii) Acupuncture treatment up-regulated the expression of BDNF, CREB, PSD95, GABA, and ACh in the hippocampus of CUMS rats; (iv) Acupuncture treatment showed an upward trend in the expression level of p-ERK1/2, a significant increase in the expression level of Bcl-2, and a decrease in the expression of Caspase-3 in CUMS rats. From these results, we can conclude that acupuncture administered at the *Shangxing* and *Fengfu* points may ameliorate depression-like behavior induced by CUMS in rats by regulating neuroinflammation, oxidative stress, apoptosis in the hippocampus, and enhancing synaptic plasticity(Fig. 8).

CUMS is a common and reliable animal model of depression that induces core symptoms of depression, including

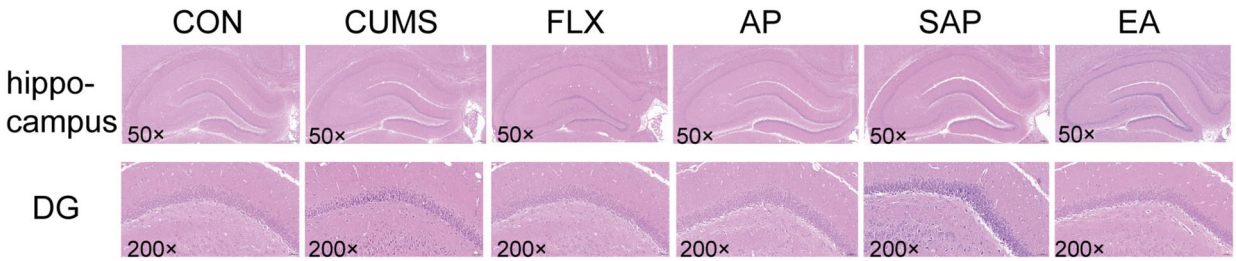

Fig. 6 Acupuncture restored the histopathological injury in hippocampus tissue of CUMS rats. Representative observation of hippocampus sections with H&E staining (×50 and ×200)

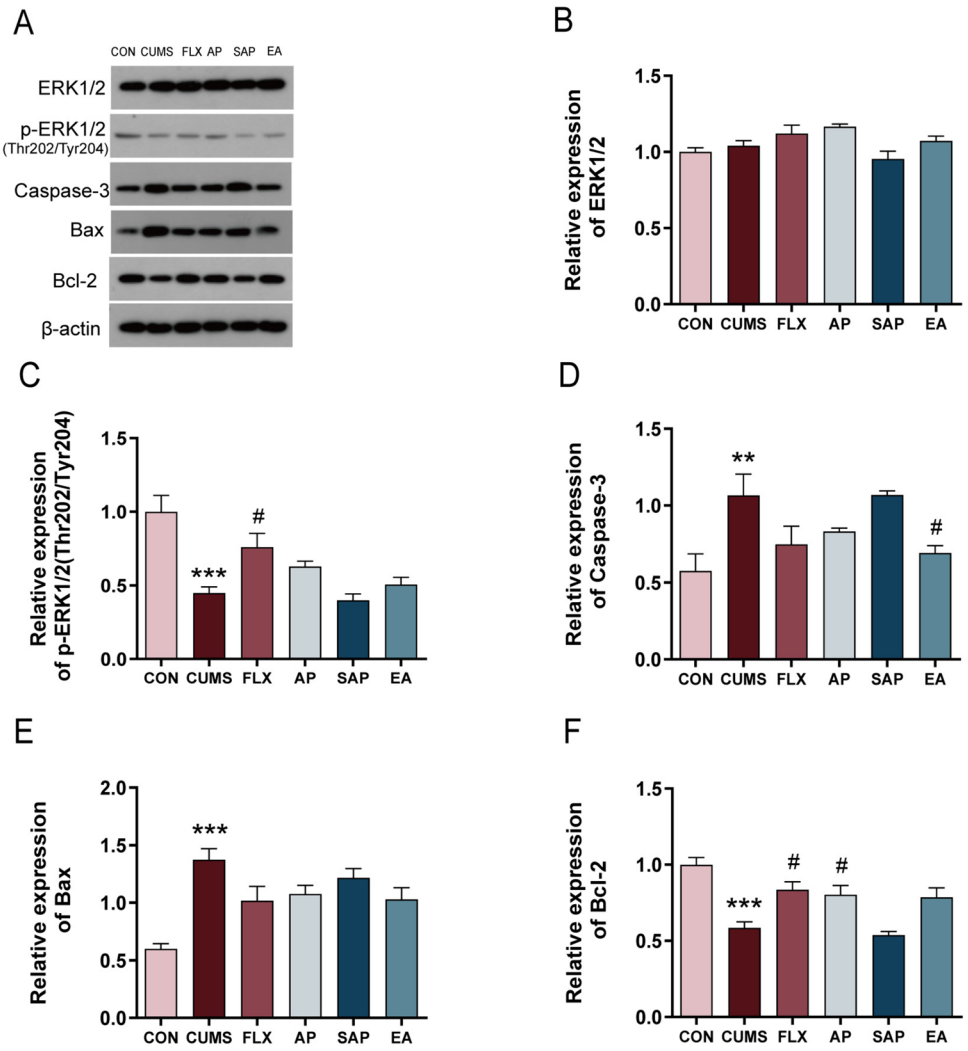

Fig. 7 Effect of acupuncture on ERK and Caspase-3 apoptosis pathways in hippocampus of CUMS rats. **A** The expression of ERK1/2, p-ERK1/2, Caspase-3, Bax, and Bcl-2 protein in each group was detected by Western blot. **B** Analysis of ERK1/2 proteins. **C** Analysis of p-ERK1/2 proteins. **D** Analysis of Caspase-3 proteins. **E** Analysis of Bax proteins. **F** Analysis of Bcl-2 proteins. All values are presented as mean ± standard error of the mean ($x \pm s$), $n = 3$ per group, $^{***}P < 0.001$, compared to the control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, compared to the CUMS group

anhedonia and reduced locomotor activity [27, 28]. The CUMS model has been widely used to study the pathophysiology of depression and its related therapeutic interventions [29]. In the present study, we observed that rats subjected to CUMS for a duration of 28 days exhibited diminished exercise capacity, anxiety-related symptoms, behavioral despair, and anhedonia. However, the application of acupuncture effectively mitigated depressive-like symptoms in rats exposed to CUMS, indicating a beneficial prophylactic impact. These results align with prior research findings [6, 7, 30].

Acupuncture therapy, encompassing traditional needle insertion, electrical acupuncture, moxibustion, and acupoint implantation, is widely recognized as a promising complementary and alternative treatment modality demonstrating positive outcomes in the management of mental health conditions, particularly depression [31]. Both clinical trials and preclinical animal research have demonstrated that acupuncture can improve the quality of life of depressed patients and reduce depressive-like behaviors in animal models of depression [32, 33]. Electroacupuncture, a modern iteration of traditional needle therapy, augments therapeutic outcomes through the application of a mild electrical stimulus to specific acupoints. This technique effectively stimulates the nervous system and modulates neural function, thereby ameliorating symptoms. Prior research has demonstrated that electroacupuncture can mitigate the degeneration of cortical astrocytes in the prefrontal cortex of mice subjected to CUMS and alleviate depressive behaviors [7]. In the present study, both traditional acupuncture and electrical acupuncture were employed as treatment modalities. The findings indicated no statistically significant distinction between the efficacy of the two approaches. Likewise, there was no significant difference compared to the FLX group. Consequently, it is posited that both acupuncture modalities have demonstrated antidepressant effects comparable to those of fluoxetine, consistent with previous reports [34]. Numerous recent studies have demonstrated that the occurrence of neurodegenerative diseases and mental diseases is mainly rooted in the existence of oxidative stress and neuroinflammation [8, 35, 36]. Notably, the onset of depression is often

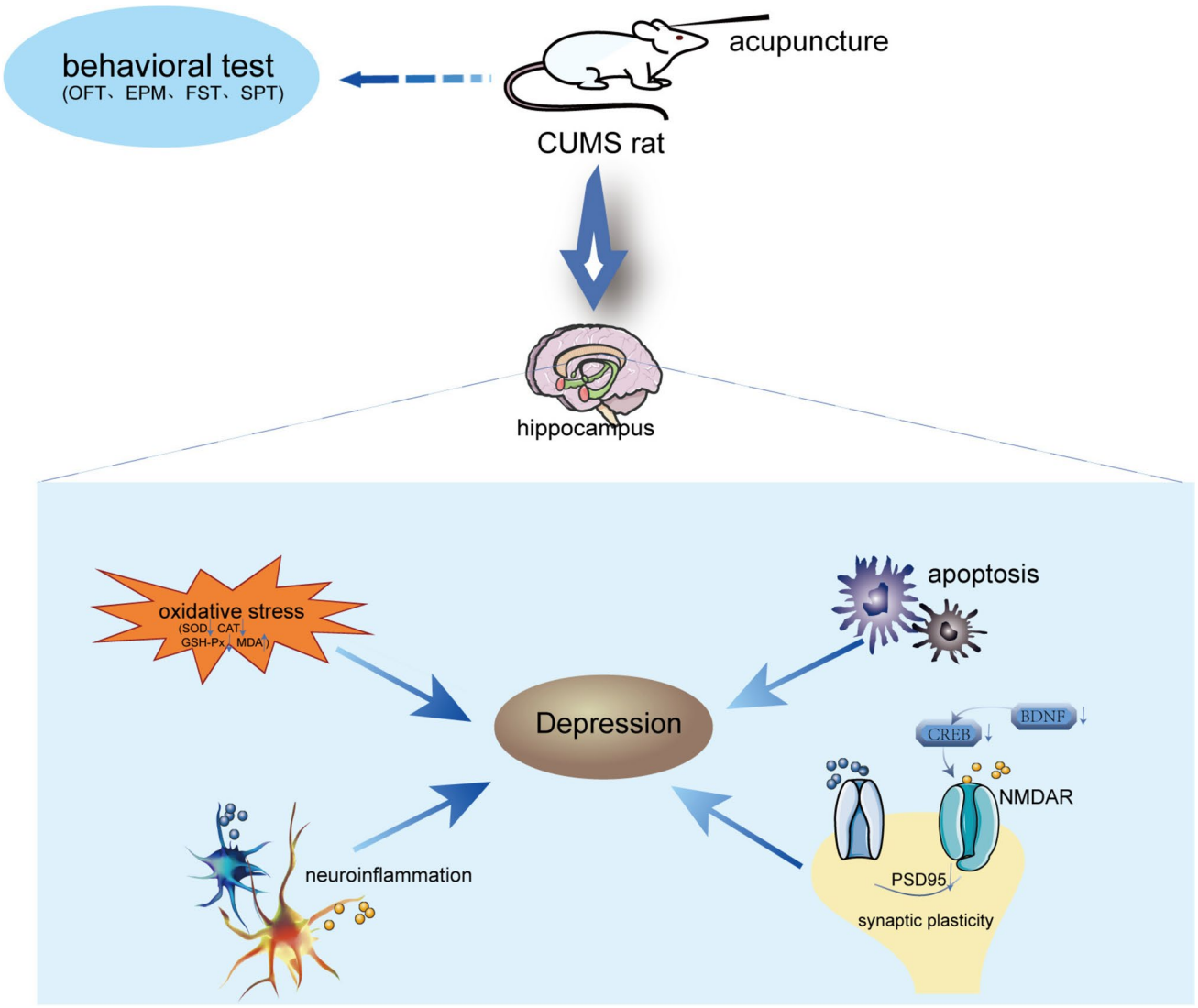

Fig. 8 Schematic diagram of the improvement effect of acupuncture on depression-like behavior in CUMS model

accompanied by increases in biomarkers of oxidative stress and neuroinflammation. Certain investigations posit that the occurrence of lipid peroxidation in the brain constitutes a significant pathological determinant in the progression of depression [37]. Furthermore, the byproducts of lipid peroxidation have the potential to elicit an inflammatory reaction, thereby intensifying the manifestations of depression. Activated microglia have frequently been identified as a prominent indicator of neuroinflammatory activation [38]. Findings from animal studies demonstrate the activation of microglia and the secretion of pro-inflammatory cytokines in models of depressive stress, with NLRP3 and TLR4 potentially serving as pivotal components in this mechanism [33, 39].

In the present investigation, it was observed that CUMS led to a reduction in antioxidant enzymes (e.g., CAT, SOD, and GSH-Px) and an elevation in pro-inflammatory factors (e.g., IL-1β, IL-6, and TNF-α) in the hippocampus and serum of rats. These findings imply that CUMS-induced oxidative stress and neuroinflammation play a role in the development of depression. The outcomes of this study align with previous research findings [40, 41]. In this study, acupuncture demonstrated a significant reduction in oxidative stress and neuroinflammatory activation induced by CUMS when compared to the control group. Both acupuncture and electroacupuncture were found to enhance the expression of antioxidant enzymes in the hippocampus and serum, while simultaneously reducing the expression of inflammatory factors. These results indicate that acupuncture may hold promise as a therapeutic intervention for depression, potentially through its ability to suppress oxidative stress and neuroinflammation.

The hippocampus, a brain region closely associated with memory, learning, and emotion regulation functions,

is considered a crucial component of the stress response. However, stress response has also been confirmed to impair hippocampal plasticity, including negatively affecting its neurogenesis and neuronal apoptosis processes [42]. Dysregulation of hippocampal synaptic plasticity has been considered an important feature in the complex mechanisms of depression [43]. Notably, diminished levels of BDNF, CREB, and PSD95 have been observed in individuals diagnosed with major depression, as well as in animal models of CUMS [44, 45]. Alterations in neurotransmitter release play a crucial role in the development of depression [46]. GABA, as one of the major neurotransmitters, is involved in neuronal migration, differentiation, and synaptic plasticity. Empirical evidence indicates that there are abnormalities in the synthesis and metabolism of GABA in the brains of patients with depression [47]. By modulating the synthesis and metabolism of GABA, it is possible to ameliorate the symptoms experienced by individuals with depression [48]. The aberrant functioning of the cholinergic system exhibits a close association with the pathogenesis of diverse psychiatric and neurological disorders [49]. Acetylcholine (ACh) assumes a pivotal role in the regulation of neuronal activity within both the peripheral and central nervous systems. Findings derived from rodent investigations substantiate that the augmentation of acetylcholine levels through the utilization of physostigmine can elicit anxiety and depression-like behaviors [50]. Based on the research data, it was observed that the levels of BDNF, CREB, PSD95, GABA, and ACh were reduced in rats subjected to CUMS. These findings indicate that CUMS potentially influences neuroplasticity by modulating the expression of these molecules and the release of neurotransmitters, which could be implicated in the development of depression. The results of this study are consistent with reported studies [44, 51, 52]. However, this change caused by CUMS was reversed after acupuncture treatment. Additionally, other studies have demonstrated that EA may ameliorate depression-like behavior in rats subjected to CUMS by upregulating phosphorylation levels of cyclic adenosine monophosphate response element-binding protein and BDNF, facilitating neuronal regeneration [53]. Furthermore, acupuncture has been shown to mitigate the excitatory toxicity of GABAergic interneurons, ultimately ameliorating synaptic dysfunction [54].

Programmed death of nerve cells plays an important role in the development and growth of the nervous system [55, 56]. Nevertheless, the presence of aberrant neuronal apoptosis can contribute to the manifestation of neurological disorders. Recent investigations have revealed that individuals diagnosed with depression exhibit structural impairments in the hippocampus, which are linked to an atypical elevation in hippocampal neuronal apoptosis [57]. The extracellular regulated protein kinase (ERK) is a member of the mitogen-activated protein kinase (MAPK) family, and it plays a crucial role in various cell signal transduction pathways, regulating essential biological processes including cell growth, differentiation, survival, and apoptosis [58]. Research findings indicate a substantial elevation in the phosphorylation level of ERK1/2 within cortical neurons of rats afflicted with depression, accompanied by a notable increase in cellular apoptosis [59]. The inhibition of ERK1/2 activity has been shown to have a potential impact on reducing cell apoptosis, indicating the significant involvement of the ERK1/2 signaling pathway in the apoptosis of cortical neurons in depression. The regulatory molecules Bcl-2 and Bax play pivotal roles in the apoptosis process, with Bcl-2 inhibiting apoptosis and Bax promoting it. Additionally, Caspase-3, as an essential apoptosis execution protein, assumes the final effector role in the apoptosis process [60]. The proteins Bcl-2, Bax, and Caspase-3 collaborate in regulating the apoptotic process. Prior research has demonstrated an imbalance in the expression of Bcl-2 and Bax, as well as an up-regulation of Caspase-3, in animal models of depression [57, 61]. This indicates that hippocampal neuron apoptosis plays a role in the pathogenesis of depression. Our study demonstrates that stress can suppress ERK1/2 phosphorylation, decrease Bcl-2 expression, and enhance the expression of Bax and caspase-3, while acupuncture and electroacupuncture can partially mitigate these alterations. This dual effect of acupuncture exerts an anti-apoptotic influence, thereby alleviating depression-like behavior induced by CUMS in rats. Furthermore, when considering the results obtained from HE staining, it can be inferred that acupuncture plays a partially favorable role in the anti-apoptotic mechanism within the hippocampus of CUMS rats.

Study Limitation

The present study acknowledges certain constraints that warrant consideration. Primarily, the investigation is confined to assessing the impact of acupuncture exclusively on the hippocampus of rats, which may lead to selection bias. Consequently, extending the inquiry to discern the effects on other brain regions associated with depression is imperative for a comprehensive understanding. Secondly, Our study elucidates the underlying mechanisms by which acupuncture alleviates depression, necessitating further investigation. Future investigations employing advanced methodologies such as electrophysiological techniques, functional magnetic resonance imaging (fMRI), diffusion tensor imaging (DTI), magnetoencephalography (MEG), positron emission tomography (PET), and transcranial magnetic stimulation (TMS) are envisaged to delve deeper into the intricate mechanisms. Lastly, this experiment's limited sample size may impact the statistical robustness. Subsequent studies will augment the sample size to enhance the reliability and generalizability of the current findings.

Conclusion

In summary, our study posits that acupuncture may be effective in alleviating depression-like behavior induced by CUMS in rats by modulating hippocampal neuroinflammatory response, oxidative stress levels, apoptotic processes, and enhancing synaptic plasticity. This discovery offers a valuable foundation for future investigations into the underlying mechanisms of acupuncture's anti-depressive effects, as well as valuable insights for the exploration of novel treatment modalities.

Author Contribution Every author participated in the designing, conduction and data collection of the study and contributed to the experimental conduction, and result interpretation. Jianguo Li finished manuscript writing. Xinhong Wu and Simin Yan performed data analysis. Tao Tong, Junliang Shen, Jingyu Zeng, Kaiyue Gong and Jinghao Yang assisted in the experimental conduction. Yiping Chen, Wenjie Chen, Meng Li, and Zhuoran You made revisions to the manuscript. Xianjun Meng, Maoshu Zhu, and Muhammad Shahzad Aslam have edited the manuscript. Xianjun Meng approved the final manuscript.

Funding This study was supported by the Traditional Chinese Medicine Foundation of Xiamen (Nos. XWZY-2023–0604, XWZY-2023–0622), Shenzhen Science and Technology Program (No. JCYJ20230807091359029), the XMU Training Program of Innovation and Entrepreneurship for Undergraduates (No. 2023X654), and Shanxi Traditional Chinese Medicine Administration Traditional Chinese medicine innovation project.

Data Availability No datasets were generated or analyzed during the current study.

Declarations

Ethics Approval All animal experiments and protocols complied with international animal experimental ethics and requirements and were approved by the Animal Ethics Committee of Xiamen University (License No. XMULAC20210062).

Competing Interests The authors declare no competing interests.

References

1. Herrman H et al (2022) Time for united action on depression: a Lancet-World Psychiatric Association Commission. Lancet 399(10328):957–1022
2. Barnett R (2019) Depression. Lancet 393(10186):2113
3. Li K et al (2020) Seahorse treatment improves depression-like behavior in mice exposed to CUMS through reducing inflammation/oxidants and restoring neurotransmitter and neurotrophin function. J Ethnopharmacol 250:112487
4. Li YF (2020) A hypothesis of monoamine (5-HT) - glutamate/GABA long neural circuit: aiming for fast-onset antidepressant discovery. Pharmacol Ther 208:107494
5. Wang J et al (2023) Evaluation of electroacupuncture as a non-pharmacological therapy for astrocytic structural aberrations and behavioral deficits in a post-ischemic depression model in mice. Front Behav Neurosci 17:1239024
6. Ning B et al (2024) The rapid antidepressant effect of acupuncture on two animal models of depression by inhibiting M1-Ach receptors regulates synaptic plasticity in the prefrontal cortex. Brain Res 1822:148609
7. Lin SS et al (2023) Electroacupuncture prevents astrocyte atrophy to alleviate depression. Cell Death Dis 14(5):343
8. Chen B et al (2018) Surface modification of PGP for a neutrophil-nanoparticle co-vehicle to enhance the anti-depressant effect of baicalein. Acta Pharm Sin B 8(1):64–73
9. Wang JY et al (2021) Mechanisms underlying antidepressant effect of transcutaneous auricular vagus nerve stimulation on CUMS model rats based on hippocampal α7nAchR/NF-κB signal pathway. J Neuroinflammation 18(1):291
10 Almulla AF et al (2023) Reverse cholesterol transport and lipid peroxidation biomarkers in major depression and bipolar disorder: a systematic review and meta-analysis. Brain Behav Immun 113:374–388
11. Sahin K et al (2022) Therapeutic effects of a novel form of biotin on propionic acid-induced autistic features in rats. Nutrients 14(6):1280
12. Tian Y et al (2022) Photothermal therapy with regulated Nrf2/NF-κB signaling pathway for treating bacteria-induced periodontitis. Bioact Mater 9:428–445
13. Chen W et al (2023) Acupuncture exerts preventive effects in rats of chronic unpredictable mild stress: the involvement of inflammation in amygdala and brain-spleen axis. Biochem Biophys Res Commun 646:86–95
14 Kaifosh P, Losonczy A (2016) Mnemonic functions for nonlinear dendritic integration in hippocampal pyramidal circuits. Neuron 90(3):622–34
15. Hsu RS et al (2022) Wireless charging-mediated angiogenesis and nerve repair by adaptable microporous hydrogels from conductive building blocks. Nat Commun 13(1):5172
16 Suri D, Vaidya VA (2013) Glucocorticoid regulation of brain-derived neurotrophic factor: relevance to hippocampal structural and functional plasticity. Neuroscience 239:196–213
17 Hansen JY et al (2022) Local molecular and global connectomic contributions to cross-disorder cortical abnormalities. Nat Commun 13(1):4682
18. Kahnamouyi S et al (2018) The role of mitogen-activated protein kinase-extracellular receptor kinase pathway in female fertility outcomes: a focus on pituitary gonadotropins regulation. Ther Adv Endocrinol Metab 9(7):209–215
19. Dwivedi Y, Zhang H (2016) Altered ERK1/2 signaling in the brain of learned helpless rats: relevance in vulnerability to developing stress-induced depression. Neural Plast 2016:7383724

20. Russo R et al (2020) Long-lasting activity of ERK kinase depends on NFATc1 induction and is involved in cell migration-fusion in murine macrophages RAW264.7. Int J Mol Sci 21(23):8965
21. Tang M, Chen M, Li Q (2021) Paeoniflorin ameliorates chronic stress-induced depression-like behavior in mice model by affecting ERK1/2 pathway. Bioengineered 12(2):11329–11341
22. Liang S et al (2020) Iron aggravates the depressive phenotype of stressed mice by compromising the glymphatic system. Neurosci Bull 36(12):1542–1546
23. Dávila-Hernández A et al (2021) Antidepressant-like effects of acupuncture via modulation of corticosterone, sex hormones, and hippocampal BDNF expression in male rats. Brain Res Bull 173:53–65
24. Ogrodnik M et al (2019) Obesity-induced cellular senescence drives anxiety and impairs neurogenesis. Cell Metab 29(5):1233
25. Li Y et al (2020) Ginsenoside-Rg1 rescues stress-induced depression-like behaviors via suppression of oxidative stress and neural inflammation in rats. Oxid Med Cell Longev 2020:2325391
26. Liu MY et al (2018) Sucrose preference test for measurement of stress-induced anhedonia in mice. Nat Protoc 13(7):1686–1698
27. Chen YP, Wang C, Xu JP (2019) Chronic unpredictable mild stress induced depression-like behaviours and glutamate-glutamine cycling dysfunctions in both blood and brain of mice. Pharm Biol 57(1):280–286
28. Antoniuk S et al (2019) Chronic unpredictable mild stress for modeling depression in rodents: meta-analysis of model reliability. Neurosci Biobehav Rev 99:101–116
29. Qiao H et al (2016) Dendritic spines in depression: what we learned from animal models. Neural Plast 2016:8056370
30. Huang W et al (2019) Nitric oxide and cyclic guanosine monophosphate signaling mediates the antidepressant effects of acupuncture in the rat model of chronic unpredictable mild stress. Med Sci Monit 25:9112–9122
31. Hu H et al (2022) The efficacy and safety of acupuncture for depression-related insomnia: protocol for a systematic review and meta-analysis. J Pain Res 15:1939–1947
32. Zhao B et al (2019) Can acupuncture combined with SSRIs improve clinical symptoms and quality of life in patients with depression? Secondary outcomes of a pragmatic randomized controlled trial. Complement Ther Med 45:295–302
33. Chen Y et al (2022) Anti-depressant effects of acupuncture: the insights from NLRP3 mediated pyroptosis and inflammation. Neurosci Lett 785:136787
34. Zhang ZJ et al (2010) The effectiveness and safety of acupuncture therapy in depressive disorders: systematic review and meta-analysis. J Affect Disord 124(1–2):9–21
35. Zhan Q, Wu Y, Liu L (2022) Effects of notoginsenoside R1 on attenuating depressive behavior induced by chronic stress in rats through induction of PI3K/AKT/NF-κB pathway. Drug Dev Res 83(1):97–104
36. Stein MB et al (2019) Risk of posttraumatic stress disorder and major depression in civilian patients after mild traumatic brain injury: a TRACK-TBI study. JAMA Psychiat 76(3):249–258
37. Michel TM, Pülschen D, Thome J (2012) The role of oxidative stress in depressive disorders. Curr Pharm Des 18(36):5890–5899
38. Brites D, Fernandes A (2015) Neuroinflammation and depression: microglia activation, extracellular microvesicles and microRNA dysregulation. Front Cell Neurosci 9:476
39. Jiang H et al (2024) Acupuncture ameliorates depression-like behaviors through modulating the neuroinflammation mediated by TLR4 signaling pathway in rats exposed to chronic restraint stress. Mol Neurobiol 61(5):2606–2619
40. Shen F et al (2021) Polygonatum sibiricum polysaccharide prevents depression-like behaviors by reducing oxidative stress, inflammation, and cellular and synaptic damage. J Ethnopharmacol 275:114164
41. Wang Y et al (2022) Association between increased inflammatory cytokine expression in the lateral habenular nucleus and depressive-like behavior induced by unpredictable chronic stress in rats. Experiment Neurol 349:113964
42. Unno K et al (2020) Theanine, antistress amino acid in tea leaves, causes hippocampal metabolic changes and antidepressant effects in stress-loaded mice. Int J Mol Sci 22(1):193
43. Chen JL et al (2021) Correction to: Normalization of magnesium deficiency attenuated mechanical allodynia, depressive-like behaviors, and memory deficits associated with cyclophosphamide-induced cystitis by inhibiting TNF-α/NF-κB signaling in female rats. J Neuroinflammation 18(1):269
44. Zhao C et al (2023) Obligatory role of microglia-mobilized hippocampal CREB-BDNF signaling in the prophylactic effect of β-glucan on chronic stress-induced depression-like behaviors in mice. Eur J Pharmacol 964:176288
45. Xie XH et al (2023) Altered in vivo early neurogenesis traits in patients with depression: evidence from neuron-derived extracellular vesicles and electroconvulsive therapy. Brain Stimul 17(1):19–28
46. Monday HR, Younts TJ, Castillo PE (2018) Long-term plasticity of neurotransmitter release: emerging mechanisms and contributions to brain function and disease. Annu Rev Neurosci 41:299–322
47. Naaijen J et al (2017) Glutamatergic and GABAergic gene sets in attention-deficit/hyperactivity disorder: association to overlapping traits in ADHD and autism. Transl Psychiatry 7(1):e999
48. Duman RS, Sanacora G, Krystal JH (2019) Altered connectivity in depression: GABA and glutamate neurotransmitter deficits and reversal by novel treatments. Neuron 102(1):75–90
49. Kim SH et al (2017) Beyond symptomatic effects: potential of donepezil as a neuroprotective agent and disease modifier in Alzheimer's disease. Br J Pharmacol 174(23):4224–4232
50. Higley MJ, Picciotto MR (2014) Neuromodulation by acetylcholine: examples from schizophrenia and depression. Curr Opin Neurobiol 29:88–95
51. Wang G et al (2022) Antidepressant-like effect of ginsenoside Rb1 on potentiating synaptic plasticity via the miR-134-mediated BDNF signaling pathway in a mouse model of chronic stress-induced depression. J Ginseng Res 46(3):376–386
52. Shi HJ, et al (2024) Hippocampal excitation-inhibition balance underlies the 5-HT2C receptor in modulating depressive behaviours. Brain https://doi.org/10.1093/brain/awae143
53. Duan DM et al (2016) Antidepressant effect of electroacupuncture regulates signal targeting in the brain and increases brain-derived neurotrophic factor levels. Neural Regen Res 11(10):1595–1602
54. Sun TY et al (2022) Acupuncture improves the structure of spastic muscle and decreases spasticity by enhancing GABA, KCC2, and GABAAgamma2 in the brainstem in rats after ischemic stroke. NeuroReport 33(9):399–407
55. Weinlich R et al (2017) Necroptosis in development, inflammation and disease. Nat Rev Mol Cell Biol 18(2):127–136
56. Nagata S (2018) Apoptosis and clearance of apoptotic cells. Annu Rev Immunol 36:489–517
57. Bai Y et al (2022) Potential anti-depressive effects and mechanisms of Zhi-Zi Hou-Po decoction using behavioral despair tests combined with in vitro approaches. Front Pharmacol 13:918776

58. Zhang L, Mao L, Wang H (2022) The neuroprotection effects of exosome in central nervous system injuries: a new target for therapeutic intervention. Mol Neurobiol 59(12):7152–7169
59. Chen M, He G, Li Q (2018) Maternal deprivation promotes hippocampal neuronal apoptosis via ERK1/2 signaling. Front Biosci (Landmark Ed) 23(10):1923–1932
60. Castro DTH et al (2019) Ethanolic extract of Senna velutina roots: chemical composition, in vitro and in vivo antitumor effects, and B16F10-Nex2 melanoma cell death mechanisms. Oxid Med Cell Longev 2019:5719483
61 Wang X et al (2020) UFL1 alleviates LPS-induced apoptosis by regulating the NF-κB signaling pathway in bovine ovarian granulosa cells. Biomolecules 10(2):260