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RESEARCH

Acupuncture as a modulator of microglial polarization and pyroptosis in depression: evidence from a chronic unpredictable mild stress rat model

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Abstract

Background Depression is a common psychiatric disorder, with stress-induced neuroinflammation playing a crucial role in its pathogenesis. Acupuncture has been demonstrated to be both effective and safe in the treatment of depression, but its precise mechanisms remain unclear.

Method This study investigated acupuncture's effects on neuroglia-associated neuroinflammation in chronic unpredictable mild stress (CUMS)-induced depressive rats. Rats underwent CUMS for 28 days, with the acupuncture group receiving treatment at *Shangxing* (GV23) and *Fengfu* (GV16) every other day. The fluoxetine group received daily intragastric fluoxetine. Depression-like behaviors were assessed through body weight measurements and behavioral tests. Hippocampal pathology was examined using hematoxylin and eosin (H&E) staining, and immunofluorescence was used to detect activated microglia in the hippocampus. Western blotting (WB) analyzed key proteins associated with microglial phenotypes, including CD16, inducible nitric oxide synthase (iNOS), CD206, and arginase-1 (Arg-1). Additionally, nuclear factor kappa B (NF-κB), NOD-like receptor protein 3 (NLRP3), apoptosis-associated speck-like protein containing a CARD (ASC), cysteine-aspartic acid protease-1 (caspase-1), and gasdermin D (GSDMD) levels were measured using WB and reverse transcription-polymerase chain reaction (RT-PCR). Cytokines interleukin-1β (IL-1β), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), interleukin-10 (IL-10), and interleukin-4 (IL-4) were quantified via enzyme-linked immunosorbent assay (ELISA).

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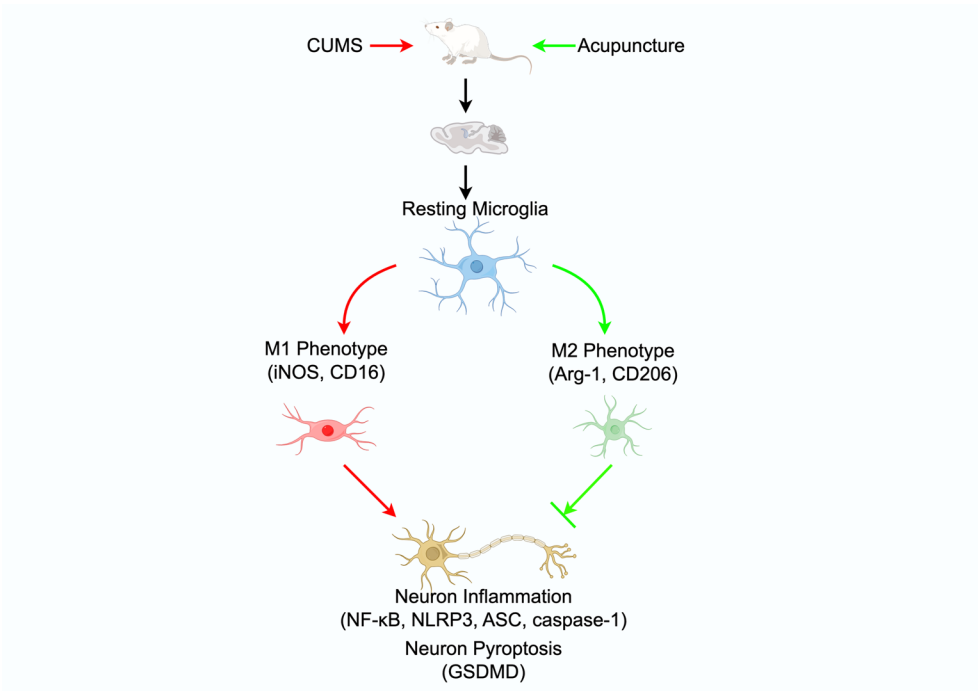
Result Acupuncture significantly alleviated CUMS-induced depression-like behaviors, enhancing sucrose preference and reducing immobility time. It inhibited microglial M1 polarization, downregulated NF-κB and NLRP3 expression, and suppressed pyroptosis-related proteins like caspase-1 and GSDMD.

Conclusion Acupuncture alleviates depression-like behaviors in CUMS-induced rats by modulating microglial polarization and inhibiting neuroinflammation-mediated pyroptosis. Our findings provide further evidence supporting acupuncture as a viable strategy for depression treatment.

Graphical abstract



Keywords Depression · Acupuncture · Chronic unpredictable mild stress · Microglia · Neuroinflammation · Pyroptosis

Introduction

As a globally prevalent mental disorder, depression influences around 350 million people and typically presents with sustained depressive mood, anhedonia, and diminished daily functioning (Martins-Macedo et al. 2023; McCarron et al. 2021). The World Health Organization predicts that by 2030, depression will become the leading global cause of disability, resulting in profound suffering for individuals and their families, as well as imposing a substantial socioeconomic burden (Chisholm et al. 2016). Research has shown that depression is influenced by multiple factors, including genetics, stress, monoamine deficiency, hypothalamic-pituitary-adrenal (HPA) axis dysfunction, immune dysregulation, and neurotransmission impairments (Jesulola et al. 2018). Although selective serotonin reuptake inhibitors (SSRIs), such as fluoxetine, paroxetine, and sertraline, are widely prescribed, their clinical efficacy is limited by several significant drawbacks, including delayed onset, adverse side effects, and high non-responsiveness rates (30–50%) (Cui et al. 2024; Kopschina Feltes et al. 2017; Zhang et al. 2016). These limitations underscore the need for alternative and complementary therapies to address the complex pathophysiology of depression.

As a fundamental component of traditional Chinese medicine with over 3000 years of history, acupuncture has been increasingly recognized as a non-invasive and efficacious intervention for depression (Smith et al. 2018). Given its high efficacy and minimal adverse reactions, it is regarded as a reasonable and effective treatment choice. A growing body of evidence supports the notion that acupuncture regulates the complex mechanisms underlying depression and produces antidepressant effects via various biological targets and pathways. Moreover, acupuncture combined with antidepressant medication has a greater therapeutic efficacy than SSRIs alone (Chan et al. 2015). Despite its growing

popularity as a complementary therapy, the mechanisms through which acupuncture exerts antidepressant effects remain unclear. *Thousand Golden Prescriptions* is an ancient Chinese medical book, a compilation attributed to Sun Simiao, which documents the thirteen ghost acupoints, a group of acupoints traditionally used for treating mental disorders. GV23 and GV16 are two of these acupoints, belonging to the Governor Vessel (GV), which is believed to influence brain function. Modern studies suggest that acupuncture at these sites may modulate neurobiological processes, including reducing neuroinflammation and regulating immune cell activity (Li et al. 2024; Shen et al. 2024). This research aims to explore the antidepressant mechanisms of these acupoints in a CUMS-induced depression model, focusing on their effects on microglial polarization and related inflammatory pathways.

The pathophysiology of depression is complex, with hypotheses encompassing monoamine system injury, HPA axis dysfunction, neuroinflammation, disruptions in neurogenesis, and structural and functional changes in the brain (Peng et al. 2015). Among these, stress-induced neuroinflammation plays a pivotal role in depression pathogenesis (Beurel et al. 2020). Persistent neuroinflammation may contribute to the development of depressive-like behaviors (Yao et al. 2023; Zhang et al. 2022a, b) or accelerate the progression of major depressive disorder (Won et al. 2021). Neuroinflammatory responses are primarily mediated by glial cells, especially microglia, the intrinsic immune cells of the central nervous system (CNS) (M. M. Zhang et al. 2022a, b). Changes in microglia in different brain regions, including the prefrontal cortex, hippocampus, anterior cingulate cortex, and amygdala, are involved in the development of depression. Emerging evidence identifies microglia-mediated neuroinflammation as a hallmark of depression, leading to its recognition as a microglia-associated disorder, or microgliopathy (Troubat et al. 2021; Wu et al. 2022; Xian et al. 2022; Yirmiya et al. 2015). Postmortem studies of individuals with depression have revealed microglial activation and associated neuroinflammation in the brain (Wang et al. 2022). The limbic system, comprising the hippocampus and prefrontal cingulate gyrus, involves memory, learning, and emotion regulation and is severely affected in psychiatric disorders. Studies increasingly show excessive accumulation and activation of microglia in the limbic region of individuals with depression (Gaire et al. 2019; Kaifosh and Losonczy 2016). Minocycline, a semisynthetic tetracycline antibiotic, has been shown to alleviate depressive-like behaviors by suppressing microglial activation in the prefrontal cortex and hippocampus (Pae et al. 2008). During CNS injury caused by infection, traumatic brain injury, or ischemic events, microglia transition from a quiescent state to an amoeba-like activated state. This activation, referred to as microglial polarization, involves both morphological and functional changes. Depending on environmental stimuli, microglia can adopt distinct phenotypes, performing either neurotoxic (pro-inflammatory) or neuroprotective (anti-inflammatory) functions (Yao and Zu 2020). The polarization phenotypes can be divided into the pro-inflammatory M1 phenotype, which causes neuroinflammation, and the anti-inflammatory M2 phenotype, which inhibits inflammatory factors and contributes to neuroprotection (Wang et al. 2022). Evidence has indicated that antidepressant drugs effectively attenuate lipopolysaccharide (LPS)-induced inflammatory responses and inhibit microglial polarization toward the M1 phenotype (Kalkman and Feuerbach 2016). Conversely, the M2 phenotype has been associated with alleviating depressive symptoms (Duan et al. 2020). Therefore, modulating microglia activity and/or regulating the M1/M2 polarization of microglia represents a promising therapeutic strategy for treating depression.

Pyroptosis, a caspase-1-dependent programmed cell death pathway, plays a critical role in the progression of numerous neurodegenerative diseases, such as multiple sclerosis (MS), Parkinson's disease (PD), and Alzheimer's disease (AD) (McKenzie et al. 2018; Wang et al. 2019; Zhao et al. 2019). This process is initiated by the assembly and activation of the NLRP3 inflammasome. NLRP3 activation requires the recruitment of ASC and the cleavage of procaspase-1 into its active form, caspase-1 (Su et al. 2024). Caspase-1 activation subsequently promotes GSDMD maturation, which results in cell rupture and the secretion of pro-inflammatory cytokines. Multiple studies have linked NLRP3-mediated pyroptosis to cognitive impairment associated with neurodegenerative and psychiatric disorders (Fu et al. 2019). Research indicates that stress contributes to the pathology of depression by triggering NLRP3 complex activation, which subsequently induces pyroptosis (Arioz et al. 2019). Pyroptosis is tightly regulated by NF-κB, a pivotal transcription factor that orchestrates immune responses, inflammation, and cell survival. NF-κB activation is a key upstream signal for NLRP3 inflammasome assembly during pyroptosis (Lei et al. 2018). Notably, therapeutic agents such as anfibatide have been demonstrated to mitigate pyroptosis by downregulating the NF-κB/NLRP3 signaling pathway (Li et al. 2022). The CUMS is widely recognized as a reliable animal model for inducing depression-like behaviors. Although acupuncture has been shown to alleviate depressive symptoms, its precise regulatory effects on microglial polarization, particularly its role in pyroptosis, have remained insufficiently explored. To elucidate the antidepressant mechanisms of acupuncture at GV23 and GV16, this study employed a rat model of depression induced by CUMS. Specifically, we aimed to determine whether the

antidepressant effects of acupuncture are associated with the regulation of microglial polarization and the reduction of neuroinflammatory-mediated pyroptosis in the hippocampus subsequent to CUMS. These findings may offer novel experimental insights supporting the development of new therapeutic strategies for depression.

Materials and methods

Animals and groups

Sprague–Dawley male rats, weighing 100–120 g, were sourced from Vital River Laboratory in Guangdong (License: SCXK (YUE) 2022−0063). All experimental procedures were reviewed and approved by the Animal Ethics Committee of Xiamen University, Xiamen, China (Approval No. XMULAC20240339). All rats were kept under standardized environmental conditions (21 ± 2 °C, 55 ± 10% humidity) on a 12-hour light/dark rhythm beginning at 08:00 AM, with food and water available ad libitum. They were housed three per cage for acclimatization over 7 days. Subsequently, 48 rats that met baseline criteria were randomly assigned to one of four groups (n = 12/group): control (CON), chronic unpredictable mild stress (CUMS), acupuncture (AP), and fluoxetine (FLX).

Chronic unpredictable mild stress procedure for the depression model

A modified version of the Willner CUMS protocol was employed to induce depression-like behavior (Willner et al. 1987). Except for the CON group, rats were subjected to ten mild, unpredictable stressors over four weeks (Fig. 1A). There are ten types of stressors (Table 1): crowding (24 h), water deprivation (24 h), wet bedding (24 h), alcohol odor (10 min), restraint stress (6 h), reversed light/dark cycle (24 h), food deprivation (24 h), tail clamping (3 min), strobe stimulation (24 h), and no bedding (24 h). Two stressors were applied daily, avoiding repetition on consecutive days to prevent habituation. Rats in the CON group were not subjected to any stress and had unrestricted access to food and water.

Interventions

The CON group did not receive any treatment and was used to represent the baseline of natural growth and behavior under non-intervention conditions. Interventions were administered 1 h before applying stressors. In the AP group, acupuncture was performed at GV23 (midline of the anterior skull) and GV16 (occipital ridge near the atlas joint) using 0.25 mm × 13 mm stainless steel needles (Beijing Hanyi Medical Instruments Co., LTD), inserted at an oblique angle of 15° to a depth of 5 mm (Chen et al. 2023; Shen et al. 2024). To secure the acupuncture needles for 20 min, the rats were subsequently immobilized on a restraint board using a specially designed jacket (Fig. 1B). Except for the CON group, all animals wore jackets during the experiment to minimize potential interference from this variable. To ensure procedural consistency, all restraint operations were performed by the same experimenter at the same time of day throughout the study. The FLX group, serving as the positive control for antidepressant effects, received fluoxetine solution (0.21 mg/ml, 2.1 mg/kg, PHR1394-1G, Sigma-Aldrich, USA) by gavage once a day for 28 days.

Behavioral tests

To assess depression-like behaviors in rats, we monitored body weight changes and conducted behavioral tests. All tests were performed under relatively quiet and dark conditions.

Body weight

To evaluate alterations in appetite and nutritional condition, body weight measurements were taken on days 0, 7, 14, 21, and 28 for all groups.

Sucrose preference test (SPT)

The sucrose preference test was conducted to assess anhedonia after CUMS, which can reflect whether the rats are depressed (Bekris et al. 2005). The SPT was divided into two stages: the adaptation stage and the testing stage. In the adaptation stage, rats were acclimatized to two bottles of 1% sucrose solution for 24 h. During the following 24 h, each rat received one bottle of water and one bottle of 1% sucrose solution, with the positions of the bottles switched every 12 h. Rats were subsequently subjected to 24 h of food and water deprivation. In the testing stage, rats were housed in individual cages with access to two bottles: one containing water and the other containing 1% sucrose solution for 24 h to measure water and sucrose solution consumption. Finally, the sucrose preference rate of each rat was calculated using the formula: sucrose preference (%) = (sucrose consumption/(sucrose consumption + water consumption) × 100%.

Open field test (OFT)

The open field test was conducted to evaluate anxiety and explore related behaviors (Kraeuter et al. 2019). The

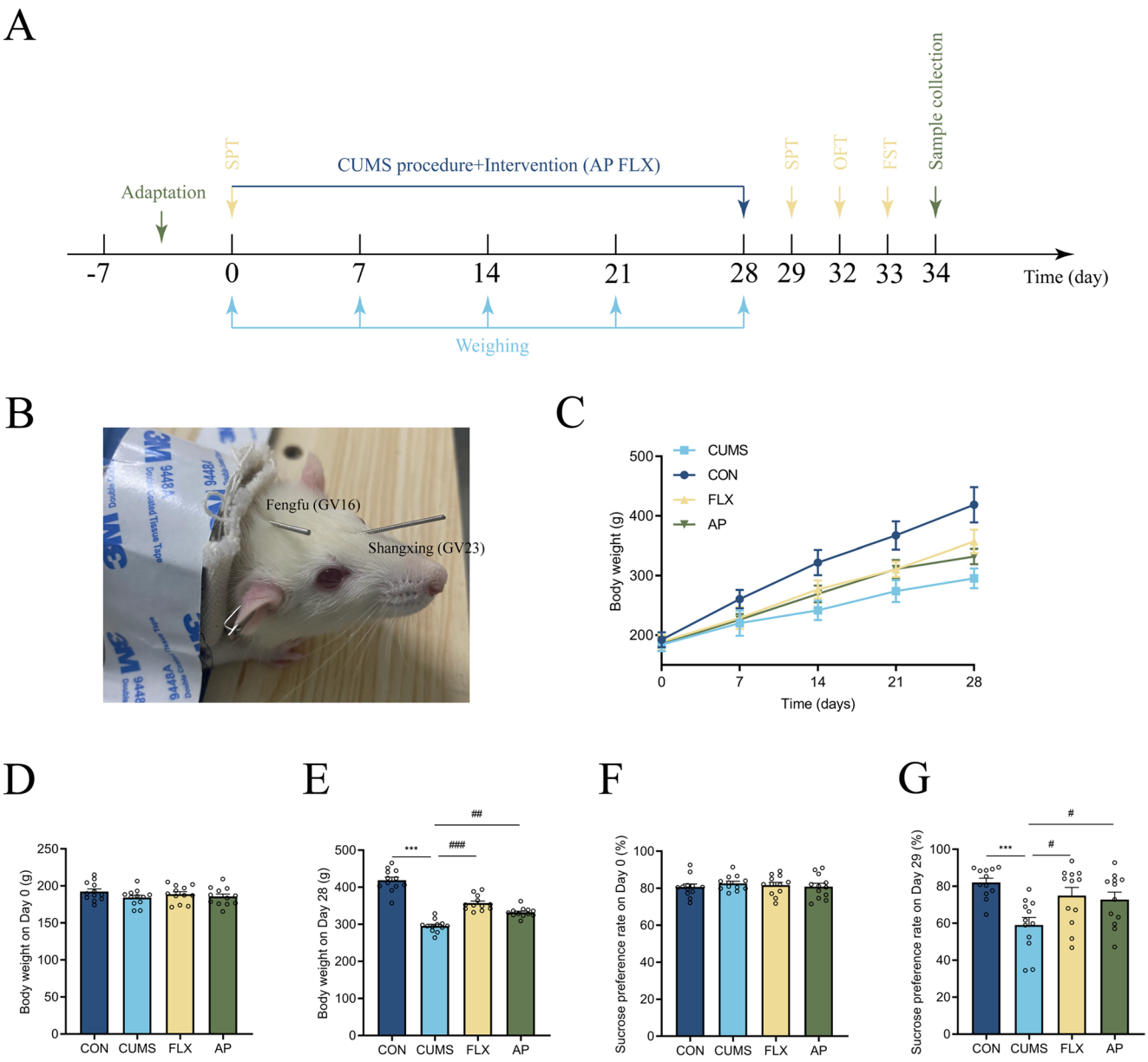


Fig. 1 Schematic flowchart of the experimental protocol, body weight measurement and sucrose preference test. (**A**) Experimental flowchart. (**B**) Schematic diagram of GV23 and GV16. (**C**) The body weight changes of rats. (**D**) The body weight of rats on day 0. (**E**) The body weight of rats after 28 days. (**F**) The sucrose preference rate of SPT on day 0. (**G**) The sucrose preference rate of SPT on day 29. All results are expressed as the mean ± standard error of the mean, with individual data points overlaid ($\bar{x} \pm s$), $N = 12$ per group, $^{***}P < 0.001$, compared to the CON group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$, compared to the CUMS group

open-field box was a square area consisting of a black resin box (100 cm × 100 cm × 40 cm). The floor of the arena was segmented into sixteen squares of equal dimensions, and the central four were defined as the central region. The locomotor activity of each rat was monitored for 5 min after being individually positioned in the center of the open-field arena, using SMART 3.0 software (Panlab, Harvard, MA, USA). The number of grid crossings (defined as the animal moving across the lines between adjacent squares, counted when three claws entered a new grid) and rearings were recorded to evaluate horizontal and vertical locomotor activities, respectively. To minimize odor influence, the open-field apparatus was cleaned with 75% ethanol after each test.

Forced swimming test (FST)

Despair behavior in rodents exposed to stressful environments was evaluated using the forced swimming test (Socała et al. 2017). Each rat was briefly placed in a cylindrical tank (20 cm diameter, 45 cm height) containing fresh water at a

Table 1 Chronic unpredictable mild stress schedule

DAYS	STRESSOR 1	STRESSOR 2
1	Strobe stimulation (24 h)	Alcohol odor (10 min)
2	Water deprivation (24 h)	Wet bedding (24 h)
3	No bedding (24 h)	Tail clamping (3 min)
4	Crowding (24 h)	Reversed light/dark cycle (24 h)
5	Restraint stress (6 h)	Water deprivation (24 h)
6	Food deprivation (24 h)	Tail clamping (3 min)
7	Alcohol odor (10 min)	Strobe stimulation (24 h)
8	Wet bedding (24 h)	Water deprivation (24 h)
9	No bedding (24 h)	Reversed light/dark cycle (24 h)
10	Strobe stimulation (24 h)	Food deprivation (24 h)
11	Crowding (24 h)	Alcohol odor (10 min)
12	Tail clamping (3 min)	Water deprivation (24 h)
13	Food deprivation (24 h)	Reversed light/dark cycle (24 h)
14	Alcohol odor (10 min)	Restraint stress (6 h)
15	Wet bedding (24 h)	Strobe stimulation (24 h)
16	Water deprivation (24 h)	No bedding (24 h)
17	Crowding (24 h)	Alcohol odor (10 min)
18	Food deprivation (24 h)	Restraint stress (6 h)
19	Water deprivation (24 h)	Tail clamping (3 min)
20	Alcohol odor (10 min)	Reversed light/dark cycle (24 h)
21	Strobe stimulation (24 h)	Crowding (24 h)
22	Water deprivation (24 h)	Wet bedding (24 h)
23	No bedding (24 h)	Reversed light/dark cycle (24 h)
24	Alcohol odor (10 min)	Food deprivation (24 h)
25	Strobe stimulation (24 h)	Water deprivation (24 h)
26	Tail clamping (3 min)	Reversed light/dark cycle (24 h)
27	Alcohol odor (10 min)	Food deprivation (24 h)
28	Restraint stress (6 h)	Water deprivation (24 h)

depth of 30 cm and a temperature of 22 ± 2 °C. They were first allowed to adapt for 1 min, and the immobility time was recorded over the subsequent 5 min using a behavioral recorder (Smart 3.0). The immobility time (brief movements to stay afloat) represents the state of behavioral despair in the animal. After each test, rats were quickly wiped dry and gently returned to their respective cages. To eliminate any odor left by the previous rat, the container was cleaned, and the water was changed.

Tissue collection and processing procedures

No rat mortality occurred during the entire experimental period. Following the experiment, rats were anesthetized with an intraperitoneal injection of 1% sodium pentobarbital (50 mg/kg) for tissue collection. Transcardial perfusion with ice-cold saline followed by 4% paraformaldehyde (G1101-15ML, Servicebio) was performed prior to the animals being sacrificed. Blood was collected from the abdominal aorta, and the brain was extracted from each rat. Then, the hippocampus was immediately harvested on ice, cleansed with phosphate-buffered saline (PBS) (G0002, Servicebio), dried, and weighed before being stored at − 80 °C for further examination.

Western blot analysis (WB)

The expression of key proteins related to microglial polarization markers and pyroptosis was detected by Western blot analysis. Hippocampal tissue was collected, and proteins were extracted using RIPA (R0010, Solarbio) lysis buffer. After centrifugation at 12,000 rpm for 20 min, protein levels were quantified using a BCA Protein Assay Kit (PC0020, Solarbio) according to the manufacturer's protocol. A 10% SDS-PAGE gel was used to separate the protein samples, which were then transferred to PVDF membranes (IPVH00010, Merck Millipore) by electrotransfer. After blocking with 5% skimmed milk powder for 1 h at room temperature, the membranes were incubated overnight at 4 °C with the corresponding primary antibodies: anti-NF-κB-p65 (1:5000, 80979-1-RR, RRID: AB_2918923, Proteintech); anti-NLRP3 (1:2000, 68102-1-Ig, RRID: AB_2923634, Proteintech); anti-ASC (1:5000, 67494-1-Ig, RRID:AB_2882718, Proteintech); anti-pro-caspase-1 (1:1000, ab179515, RRID:AB_2884954, Abcam); anti-caspase-1 (1:2000, 22915-1-AP, RRID:AB_2876874, Proteintech); anti-GSDMD(1:2000, 20770-1-AP, RRID:AB_10696319, Proteintech); anti-iNOS (1:2000, 80517-1-RR, RRID:AB_2918898, Proteintech); anti-CD16 (1:500, 16559-1-AP, RRID:AB_2878279, Proteintech);anti- Arg-1 (1:5000, 16001-1-AP, RRID:AB_2289842, Proteintech); and anti-CD206 (1:500, 18704-1- AP, RRID:AB_10 597232, Proteintech).

Reverse transcription-polymerase chain reaction (RT-PCR)

The mRNA expression of NF-κB-p65, NLRP3, ASC, caspase-1 and GSDMD in the hippocampus was detected by RT-PCR. Following the standard protocol of the RN03 RNA extraction kit (Aidley Biotechnology Co. Ltd, China), total RNA was obtained from the hippocampus. As shown in Table 2, the primer sequences were sourced from the Consensus Coding Sequence (CCDS) dataset available through the National Center for Biotechnology Information (NCBI). Then, 1 μg of RNA from each rat was reverse transcribed to cDNA using a reverse transcription kit (FSQ-101, TOYOBO, Japan). The mRNA expression levels were determined using 1% agarose gel electrophoresis for 10 min.

Enzyme-linked immunosorbent assay (ELISA)

The cytokine contents of IL-6, IL-1β, IL-4, IL-10, and TNF-α in the hippocampus were detected using ELISA kits

Table 2 Primer sequence

Gene	Primer sequence (5′− 3′)	Product length (bp)
Rat-caspase-1-F	CAGGAGGGAATATGTGGG	120
Rat-caspase-1-R	AACCTTGGGCTTGTCTTT	
Rat-GSDMD-F	ATTGGCTCTGAATGGGATA	126
Rat-GSDMD-R	GAGTACGGCAAGCAGACTAA	
Rat-RELA(P65)-F	CCAAAGACCCACCTCACC	236
Rat-RELA(P65)-R	CGCATTCAAGTCATAGTCCC	
Rat-NLRP3-F	CCAGGGCTCTGTTCATTG	287
Rat-NLRP3-R	ACCTTCACGTCTCGGTTC	
Rat-ASC-F	AGGGTCACAGAAGTTGATGG	131
Rat-ASC-R	AGGGTCACAGAAGTTGATGG	
Rat-β-actin-F	CTGGCTCCTAGCACCATGAA	180
Rat-β-actin -R	AAAACGCAGCTCAGTAACAGTC	

in accordance with the provided protocols (Rat IL-6 ELISA Kit, E-EL-R0015; Rat IL-1β ELISA Kit, E-EL-R0012; Rat IL-4 ELISA Kit, E-EL-R0014; Rat IL-10 ELISA Kit, E-EL-R0016; Rat TNF-α ELISA Kit, E-EL-R2856c; Elabscience). Following collection, hippocampal tissues were subjected to centrifugation (12,000 rpm, 10 min, 4 °C), and the resulting supernatant was used for subsequent assays.

Immunofluorescence staining (IF)

The hippocampal tissues were fixed with 4% paraformaldehyde and subsequently cut into 5 μm-thick sections for immunofluorescence staining. The sections were incubated overnight with primary antibodies at 4 °C, including anti-IBA-1 (1:500, GB113502, RRID: AB_3683100, Servicebio). Afterward, PBS (pH 7.4) (G0002, Servicebio) was used to rinse the samples (3 × 5 min), followed by 1-hour incubation at room temperature with a fluorescent-labeled secondary antibody (1:3000, GB21303, Servicebio) in darkness. After adding the DAPI (G1012, Servicebio) staining solution, it was incubated for 10 min under room temperature conditions. The percentage of IBA-1 positive area relative to the total visual field was quantified using ImageJ software (NIH, Bethesda, MD).

Hematoxylin–Eosin (H&E) staining

H&E staining was used to detect the pathological morphology of the hippocampus. The hippocampal tissues of rats in each group were collected, fixed in 4% paraformaldehyde solution for 48 h, dehydrated, and embedded in paraffin. These tissues were sliced into 5-μm thick paraffin sections using a paraffin slicer and then stained with hematoxylin and eosin. The hippocampal pathological analysis was ultimately conducted with the use of a digital slide scanner (3DHISTECH MIDI, Budapest, Hungary).

Statistical analysis

Statistical analyses were performed using GraphPad Prism 9.5 (GraphPad Software Inc., USA). Data are expressed as mean ± standard error of the mean (SEM). Normality and homogeneity of variance were assessed before statistical testing. Data meeting these assumptions were analyzed using one-way ANOVA. When variances were unequal, the Brown-Forsythe or Welch ANOVA was used as appropriate. Statistical significance was defined as a p-value less than 0.05.

Results

Effect of acupuncture on depressive behaviors in CUMS rats

In the present study, in order to evaluate the antidepressant effects of acupuncture, we recorded the body weights of the rats and conducted the SPT, OFT, and FST in each group. As shown in Fig. 1, there were no significant differences in body weight and sucrose consumption among the groups at baseline ($P > 0.05$). After 28 days of intervention, both body weight (FLX: $P < 0.001$; AP: $P < 0.01$) and sucrose consumption (FLX: $P < 0.05$; AP: $P < 0.05$) were significantly higher in the FLX and AP groups compared to the CUMS group. In Fig. 2, compared to the CON group, the number of grid crossings and number of rearings in the OFT were significantly reduced in the CUMS group (all $P < 0.01$). However, after treatment of CUMS rats with FLX or AP, the number of grid crossings (FLX: $P < 0.05$; AP: $P < 0.05$) and number of rearings (FLX: $P > 0.05$; AP: $P < 0.05$) were significantly increased compared to the CUMS group. In the FST, immobility time was significantly increased in CUMS group compared to the CON group ($P < 0.05$). Compared to the CUMS group, immobility time was significantly reduced in the FLX group (FLX: $P < 0.05$). However, there was no statistically significant difference in the AP group, although a trend towards reduction was observed. These findings indicate that acupuncture effectively alleviates CUMS-induced depression-like behaviors.

Effect of acupuncture on CUMS-induced pathological changes in the hippocampus

The hippocampus is a key brain region that plays a crucial role in cognition and emotion regulation. Depression is often associated with pathological damage to the hippocampus. As shown in Fig. 3, histological analysis of the rat hippocampus was performed using H&E staining. The results showed that the neurons in the CON group were

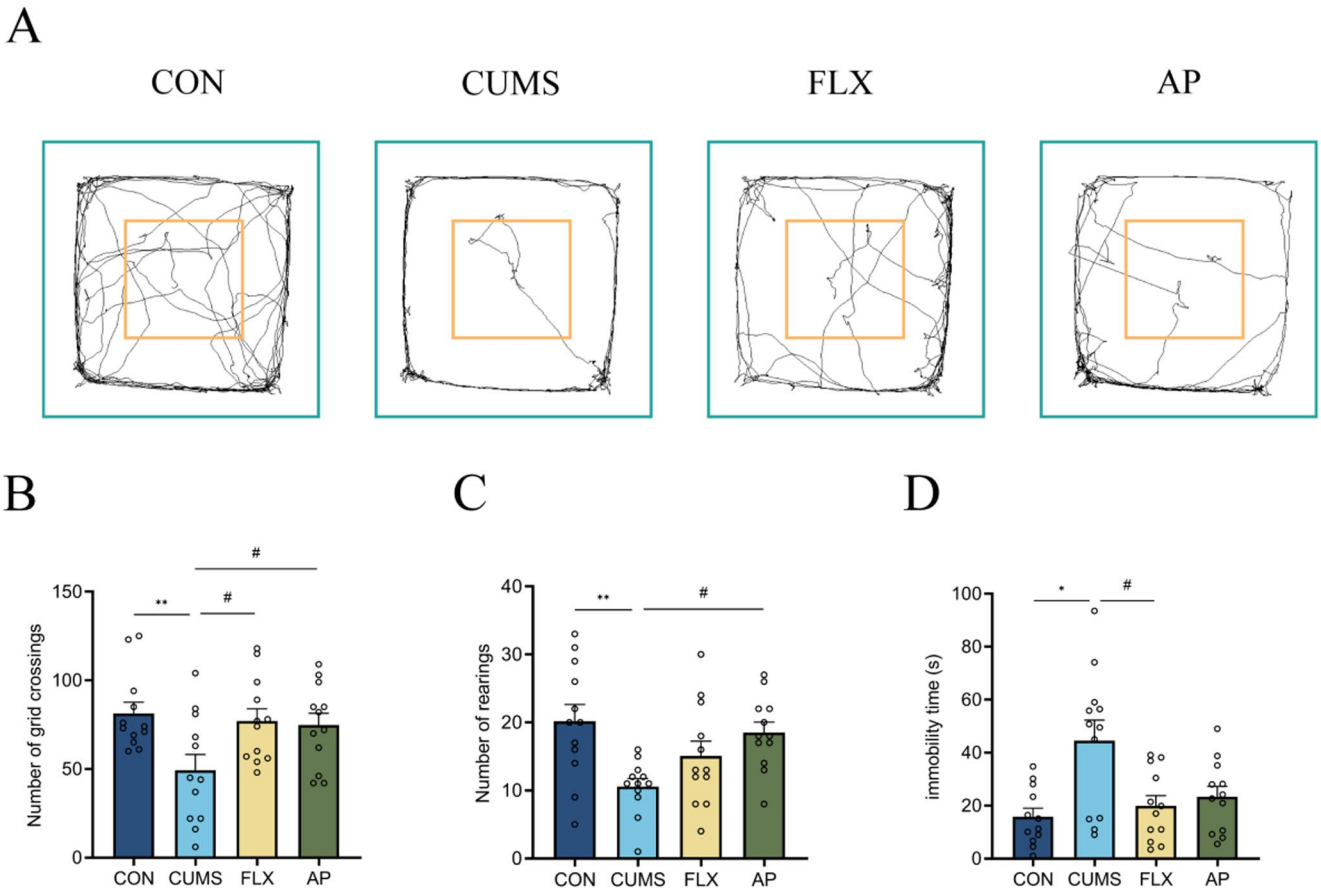


Fig. 2 Effect of acupuncture on depressive behaviors in CUMS rats. (**A**) Representative tracks in OFT. (**B**) The number of grid crossings in OFT. (**C**) The number of rearings in OFT. (**D**) The immobility time in FST. All results are expressed as the mean ± standard error of the mean, with individual data points overlaid ($\bar{x}$ ± s), $N = 12$ per group, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, compared to the CON group; $^{\#}P < 0.05$, compared to the CUMS group

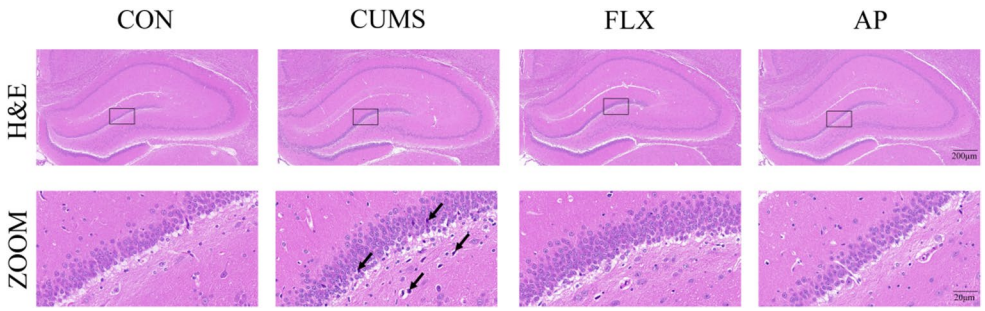


Fig. 3 Effect of acupuncture on the pathological changes in the hippocampus of CUMS rats (bar: 200 μm, 50 μm)

intact, displaying orderly structures and clear nuclei. After the CUMS procedure, rats in the CUMS group exhibited neuronal shrinkage, blurred nucleoli, and an unclear boundary between the nucleus and cytoplasm. Hippocampal neuronal damage was substantially alleviated in the FLX and AP groups compared to the CUMS group. These findings suggest that depression leads to pathological damage in the hippocampal region of rats and that acupuncture reverses this damage and promotes neuronal repair.

Effect of acupuncture on microglial activation and polarization in CUMS rats

To investigate the effect of acupuncture on microglial activation and polarization, we performed both immunofluorescence staining and WB analyses. As shown in Fig. 4, IBA-1 immunofluorescence was used as a microglial marker to assess the overall activation and distribution of microglia in the hippocampus. The levels of IBA-1 positive cells in the

hippocampus of the CUMS group were higher than those in the CON group ($P<0.01$). However, the administration of FLX and AP significantly reversed the increase in IBA-1 positive cells (FLX: $P<0.05$). Given these observations, we further evaluated microglial polarization by WB analyses of specific M1 (iNOS, CD16) and M2 (Arg-1, CD206) markers. The WB results showed that the levels of iNOS and CD16 were significantly increased in the CUMS group compared with the CON group (all $P<0.01$). In contrast, when compared with the CUMS group, FLX and AP significantly reduced the level of iNOS (FLX: $P<0.05$; AP: $P<0.05$) and CD16 (FLX: $P<0.001$; AP: $P<0.05$). In addition, the CUMS group exhibited a significant reduction in Arg-1 and CD206 levels compared to the CON group (all $P<0.01$). The expression of Arg-1 and CD206 after FLX treatment was elevated (Arg-1: $P<0.05$; CD206: $P<0.01$). The AP treatment group increased CD206 ($P<0.05$), but there was no statistical difference in Arg-1 compared to the CUMS group ($P>0.05$). These findings suggest that acupuncture may exert antidepressant effects by modulating microglial activation and promoting polarization toward an anti-inflammatory phenotype.

Effect of acupuncture on inflammatory factors in the hippocampus of CUMS rats

Previous studies have shown that inflammatory factors are closely related to the development of depression. To investigate the impact of acupuncture on inflammatory factors in the hippocampus of rats exposed to CUMS, an ELISA experiment was conducted. As shown in Fig. 5, the levels of IL-1β, IL-6, and TNF-α were significantly elevated in the CUMS group compared to the CON group ($P<0.001$, $P<0.05$, $P<0.01$). Meanwhile, the levels of IL-4 and IL-10 were decreased in the CUMS group ($P<0.01$, $P<0.001$). However, FLX and AP treatment upregulated IL-4, IL-10 (all $P<0.05$) and inhibited IL-1β, IL-6, TNF-α (all $P<0.05$) in the hippocampus. The above findings suggest that acupuncture can inhibit the elevation of CUMS-induced inflammatory factors in the hippocampus.

Effect of acupuncture on neuroinflammatory-mediated pyroptosis in the hippocampus of CUMS rats

To examine the function of acupuncture on neuroinflammatory-mediated pyroptosis, WB and RT-PCR analyses were performed. According to the WB data presented in Fig. 6, levels of NLRP3, ASC, caspase-1, and GSDMD were significantly elevated in the CUMS group compared to the CON group ($P<0.01$, $P<0.001$, $P<0.01$, $P<0.001$). After treatment with FLX and AP, the elevations of NLRP3 (FLX: $P<0.05$; AP: $P<0.05$), ASC (FLX: $P<0.001$; AP: $P<0.01$), caspase-1 (FLX: $P<0.01$; AP: $P<0.01$) and GSDMD (FLX: $P<0.001$; AP: $P<0.01$) were significantly reversed in the CUMS group. RT-PCR analysis indicated that mRNA levels of NLRP3, ASC, caspase-1, and GSDMD were notably increased in the CUMS group compared to the CON group ($P<0.01$, $P<0.001$, $P<0.001$, $P<0.05$). However, treatment with FLX and AP reverted the changes in NLRP3 mRNA (FLX: $P<0.05$; AP: $P<0.05$), ASC mRNA (FLX: $P<0.01$; AP: $P<0.05$), caspase-1 mRNA (FLX: $P<0.01$; AP: $P<0.05$) and GSDMD mRNA (FLX: $P<0.05$) in the CUMS group. Changes in NF-κB, NF-κB mRNA, and procaspase-1 levels showed a trend but were not statistically significant (all $P>0.05$). These results suggest that neuroinflammatory-mediated pyroptosis was significantly alleviated by acupuncture.

Discussion

Acupuncture, a widely accepted traditional Chinese medical therapy, has shown potential antidepressant effects. This study provides novel insights into how acupuncture modulates stress-induced neuroinflammation by regulating microglial polarization in a CUMS rat model. These findings contribute to the growing body of evidence supporting the role of acupuncture as an effective intervention for depression, particularly by targeting neuroinflammatory pathways.

The use of animal models in depression research facilitates the investigation of its pathogenesis and prevention, serving as a scientific foundation for exploring underlying biological mechanisms. The CUMS paradigm, widely used in depression research, involves exposure to mild, unpredictable stressors that induce depression-like behaviors, including anhedonia, hopelessness, and loss of interest (Ge et al. 2023). An increasing number of studies have shown that exposure to CUMS results in behavioral changes relevant to depressive disorder development (Tong et al. 2023a, 2023b; Zhou et al. 2024). In the present study, we observed that exposure to continuous CUMS procedure significantly reduced sucrose preference, locomotor activity, and exploratory behavior, while increasing immobility time, all of which are indicative of depression-like behaviors. However, acupuncture treatment effectively reversed these changes, indicating a beneficial preventive effect of acupuncture. Additionally, CUMS inhibited weight gain in rats, whereas acupuncture restored normal growth patterns. These results align with findings from previous research (Lin et al. 2023; Ning et al. 2024).

Depression involves complex pathological mechanisms, and neuroinflammation has been widely implicated in its

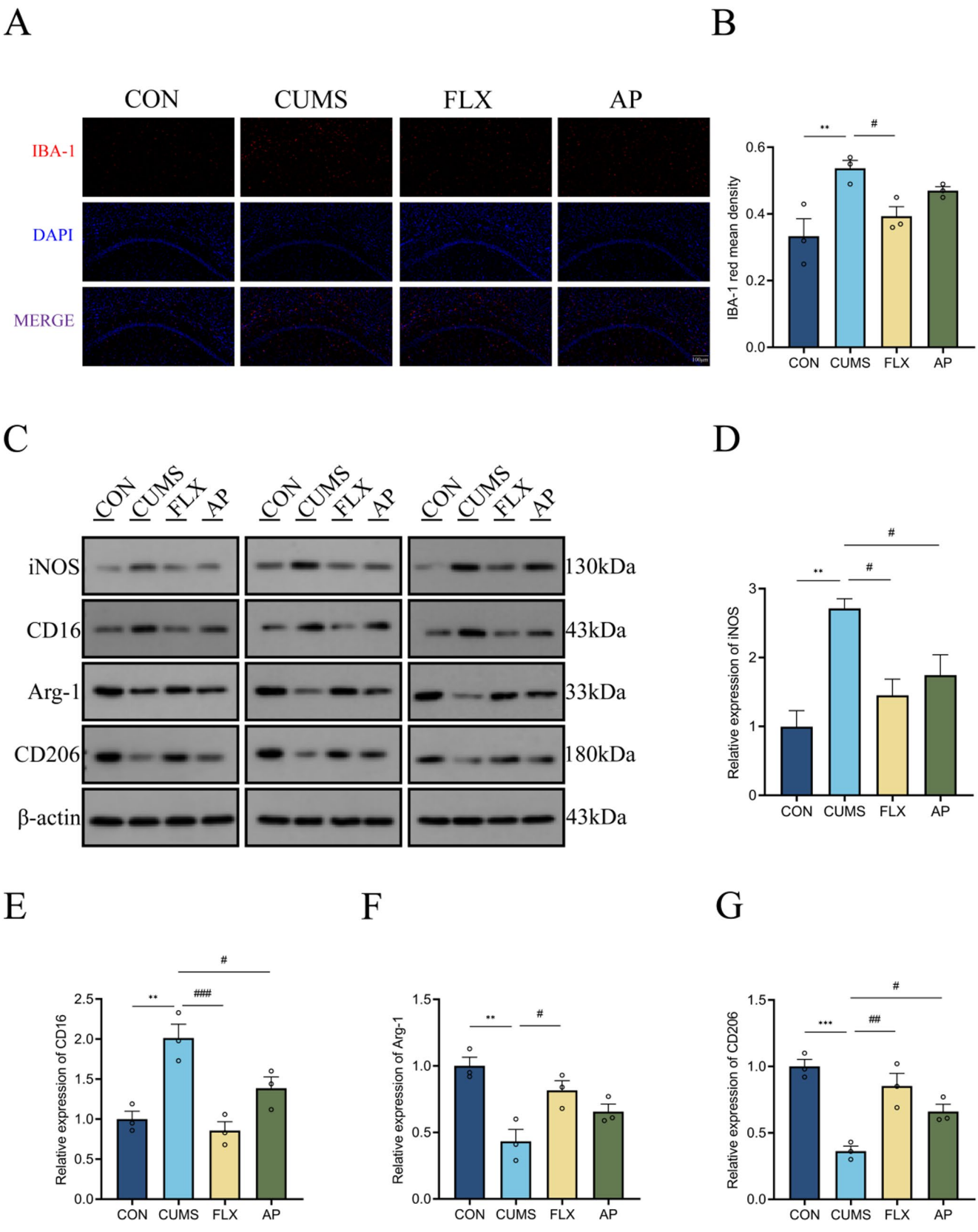

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Fig. 4 Effect of acupuncture on microglial polarization in CUMS rats. (**A**, **B**) Representative images of immunofluorescence staining and quantitative analysis of IBA-1 in the hippocampus (bar: 100 μm). (**C**) The expression levels of microglia polarization markers in each group detected by Western blot. (**D**) Analysis of iNOS proteins. (**E**) Analysis of CD16 proteins. (**F**) Analysis of Arg-1 proteins. (**G**) Analysis of CD206 proteins. All results are expressed as the mean ± standard error of the mean ($\bar{x} \pm s$), $N=3$ per group, $^{**}P<0.01$, $^{***}P<0.001$, compared to the CON group; $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$, compared to the CUMS group

development based on findings from both human and animal studies (Hollis et al. 2022; Miller and Raison 2016). The activation of microglia has been identified as a characteristic indicator of stress-induced neuroinflammation. Microglia are crucial neuroimmune cells in the nervous system and act as resident macrophages within neural tissues. Microglia are vital for sustaining the neural microenvironment by identifying and clearing external pathogens through phagocytosis and promoting tissue repair via the release of cytokines and chemokines that support nervous system development (Weinhard et al. 2018). By regulating immune and inflammatory responses, microglia serve a vital function when triggered by inflammation, infection, or neural injury (Jia et al. 2021). The pathogenesis of depression has been linked to neuroinflammation resulting from microglial activation in the brain (Brites and Fernandes 2015). Microglial activation is commonly assessed using IBA-1, a specific marker. Results demonstrated that the CUMS procedure markedly activated microglia in the hippocampus, accompanied by increased IBA-1 expression. Acupuncture intervention effectively attenuated the accumulation of IBA-1-positive microglia in the hippocampal region of CUMS-induced rats. The findings demonstrated that acupuncture exerts an anti-depressive effect through inhibition of microglial activation. Microglial polarization refers to the distinct functional states that microglia adopt upon activation. Microglia can be categorized into the M1 pro-inflammatory phenotype and the M2 anti-inflammatory phenotype based on their surface markers and intracellular cytokine profiles (Ransohoff 2016). The M1 microglia release pro-inflammatory factors (e.g., IL-1β, IL-6, and TNF-α), participating in the process of CNS injury. The M2 microglia release anti-inflammatory factors (e.g., IL-4 and IL-10), along with neurotrophic factors and nerve growth factors, facilitating nerve repair and regeneration in the brain (Zhou et al. 2019). Increased levels of these pro-inflammatory cytokines are associated with depressive-like behaviors (Batsukh et al. 2022). Elevated serum pro-inflammatory cytokines, activated microglia, and neural dysfunctions in the CNS are commonly observed in depressive patients (Rana et al. 2021). Prior research has shown that CUMS leads to heightened inflammatory responses and promotes microglial polarization toward the pro-inflammatory M1 phenotype, with an increased M1/M2 ratio associated with depressive-like behaviors in rats (Guo et al. 2020). Notably, rosiglitazone has been shown to exert antidepressant effects by shifting microglial polarization toward the M2 phenotype and suppressing the NF-κB inflammatory cascade (Zhao et al. 2016). Therefore, microglial activation and M1/M2 polarization may play a role in the pathogenesis of depression. The CUMS procedure resulted in dysregulated cytokine levels, characterized by suppressed anti-inflammatory and elevated pro-inflammatory mediators, when compared to the CON group. Meanwhile, the expression of CD16 and iNOS increased, while the expression of CD206 and Arg-1 decreased in the CUMS group compared to the CON group. Acupuncture treatment suppressed the elevated levels of IL-1β, IL-6, TNF-α, iNOS, and CD16 induced by CUMS. Acupuncture exerts anti-depressive effects by rebalancing microglial polarization—suppressing the pro-inflammatory M1 phenotype and enhancing the anti-inflammatory M2 phenotype—which leads to reduced neuroinflammation.

As part of the limbic system, the hippocampus—located between the thalamus and medial temporal lobe—is essential for learning, memory, emotion regulation, and susceptibility to chronic stress. Previous research has provided evidence that chronic stress exposure can induce hippocampal NF-κB activation in mice (Wang et al. 2021). NF-κB is an important transcription factor involved in the regulation of inflammation and is closely related to microglial cell activation. Animal studies have consistently demonstrated that chronic stress increases pro-inflammatory factor levels in the hippocampus and activates NLRP3 inflammasomes, which are key components of the innate immune system functioning as pattern recognition receptors (PRR) and recognizing pathogen-associated molecular patterns (PAMP) (Guo et al. 2023). Activation of the NLRP3 inflammasome triggers caspase-1 activation, which subsequently cleaves GSDMD into a GSDMD-N-terminal fragment with pore-forming activity, ultimately resulting in cell rupture and the initiation of pyroptosis. Studies have shown that suppressing NF-κB/NLRP3-mediated neuroinflammation effectively alleviates depressive-like behaviors in rats (Li et al. 2021). A significant increase in pyroptosis was observed in the hippocampus of aged rats with isoflurane-induced postoperative cognitive decline (Wang et al. 2018). Our results indicated that acupuncture significantly inhibited the inflammatory response in the hippocampus of CUMS-induced rats, downregulated NF-κB expression, suppressed the assembly and activation of the NLRP3 inflammasome, and downregulated the expression of NLRP3, ASC, pro-caspase-1, caspase-1, and GSDMD, thereby further inhibiting pyroptosis. These results align with those of our previous research (Chen et al. 2022). Our findings highlight the therapeutic potential of acupuncture in modulating pyroptosis as a pathological

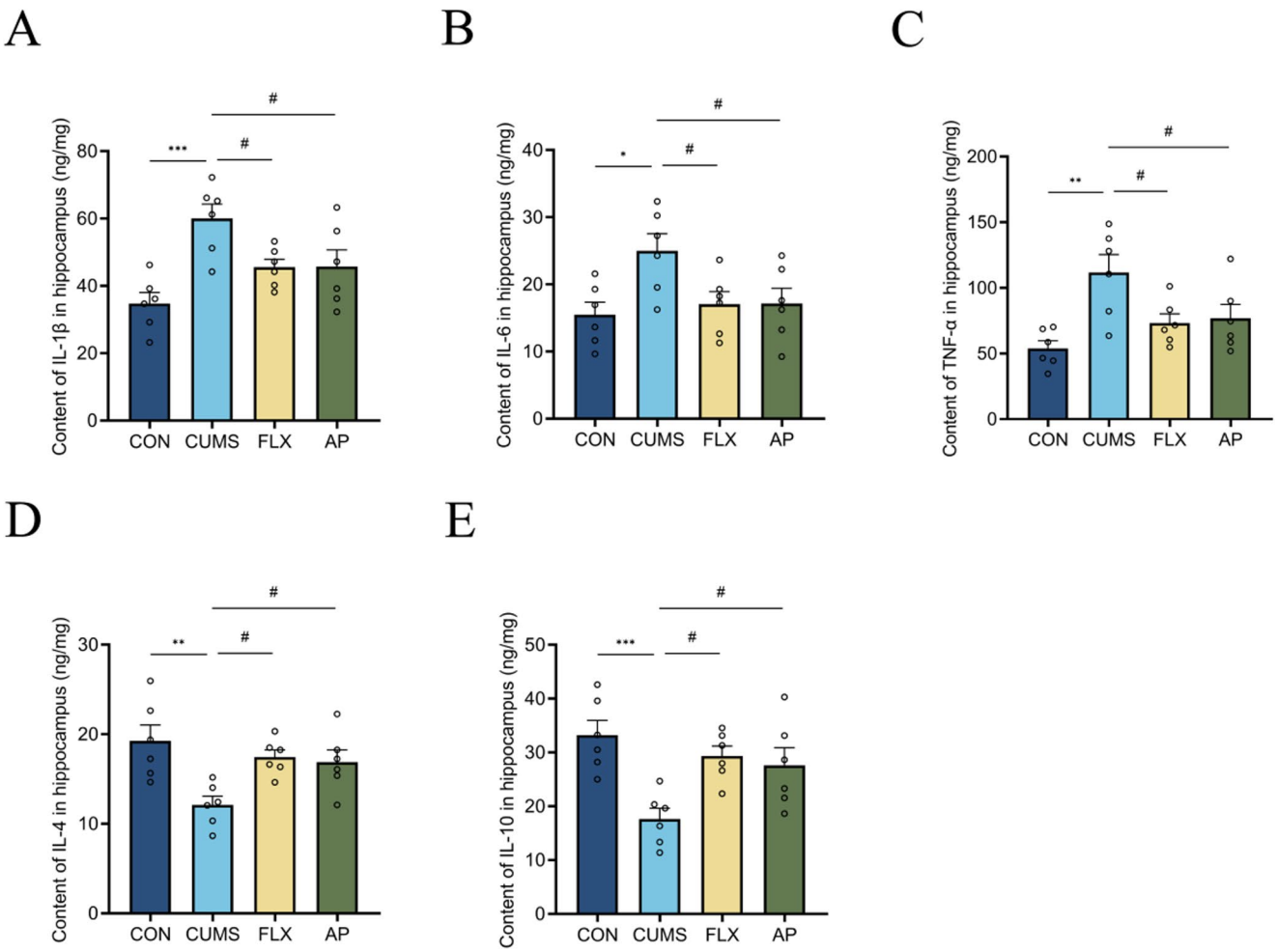
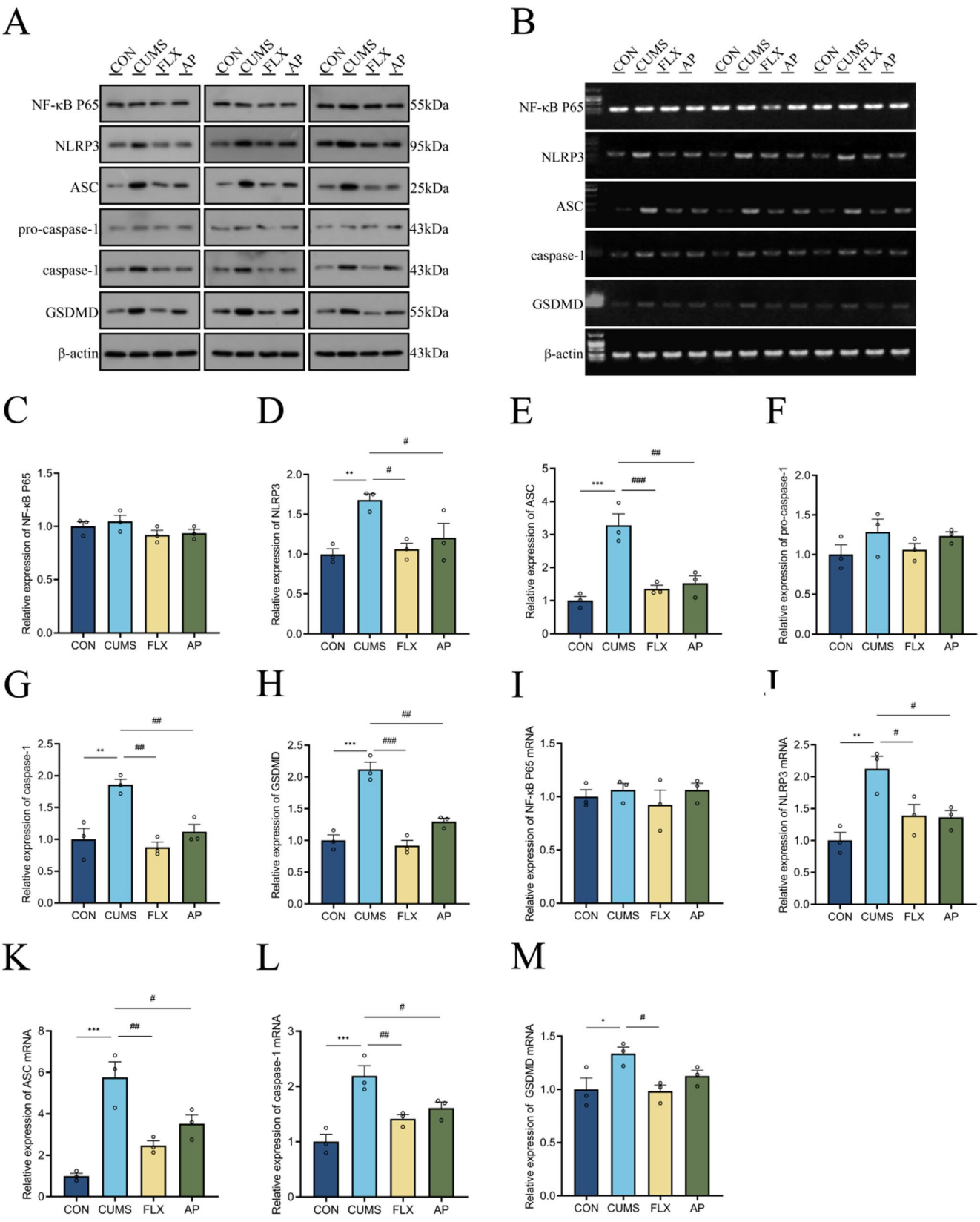


Fig. 5 Effect of acupuncture on inflammatory factors in the hippocampus of CUMS rats. (**A**) The levels of IL-1β. (**B**) The levels of IL-6. (**C**) The levels of TNF-α. (**D**) The levels of IL-4. (**E**) The levels of IL-10. All results are expressed as the mean ± standard error of the mean, with individual data points overlaid ($\bar{x} \pm s$), $N=6$ per group, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, compared to the CON group; $^{\#}P<0.05$, compared to the CUMS group

mechanism underlying depression. Interestingly, our study also found that fluoxetine, a classical SSRI, significantly attenuated hippocampal neuroinflammation in CUMS rats. Fluoxetine primarily acts by blocking serotonin reuptake at presynaptic terminals, thereby increasing the concentration of 5-hydroxytryptamine in widespread brain regions (Perez-Caballero et al. 2014). However, accumulating evidence suggests that SSRIs also exert anti-inflammatory and neuroprotective effects beyond their serotonergic mechanisms (Bleibel et al. 2025; Caiaffo et al. 2016). Fluoxetine has been shown to inhibit p38 MAPK and NF-κB signaling, reduce the release of pro-inflammatory cytokines, and modulate microglial activation, thereby influencing neuroinflammatory processes (Liu et al. 2011). Despite their distinct upstream mechanisms, both fluoxetine and acupuncture may share a common therapeutic pathway involving the regulation of microglial activity and inflammatory cytokine production. Together, these findings suggest that attenuation of neuroinflammation represents an essential mechanism

Fig. 6 Effect of acupuncture on neuroinflammatory-mediated pyroptosis in the hippocampus of CUMS rats. (**A**) The expression of NLRP3-mediated pyroptosis proteins in each group detected by WB. (**B**) The expression of NLRP3-mediated pyroptosis mRNA in each group detected by RT-PCR. (**C**) Analysis of NF-κB P65 proteins. (**D**) Analysis of NLRP3 proteins. (**E**) Analysis of ASC proteins. (**F**) Analysis of pro-caspase-1 proteins. (**G**) Analysis of caspase-1 proteins. (**H**) Analysis of GSDMD proteins. (**I**) Analysis of NF-κB P65 mRNA. (**J**) Analysis of NLRP3 mRNA. (**K**) Analysis of ASC mRNA. (**L**) Analysis of caspase-1 mRNA. (**M**) Analysis of GSDMD mRNA. All results are expressed as the mean ± standard error of the mean, with individual data points overlaid ($\bar{x} \pm s$), $N=3$ per group, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, compared to the CON group; $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$, compared to the CUMS group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underlying the antidepressant effects of both pharmacological and non-pharmacological interventions.

Acupuncture acts on specific acupoints, stimulating the meridian system to promote the circulation of qi and blood, thereby restoring the balance of yin and yang. Acupuncture has been identified as an alternative intervention for depression with fewer side effects. Growing evidence has supported the efficacy of acupuncture in relieving depressive symptoms in patients and reducing depression-like behaviors in animal models (Chen et al. 2024; Li et al. 2020). While acupuncture's efficacy in treating depression has been well-documented, limited studies have focused on the specific role of GV23 and GV16 in modulating microglia polarization-mediated neuroinflammation. GV23 and GV16 are two of the thirteen ghost points, and both belong to the GV. Based on the foundational theories of traditional Chinese medicine, these two acupoints are traditionally used for managing neurological and emotional disorders, as they are believed to regulate qi circulation and modulate brain function. Our previous studies have demonstrated that acupuncture at GV23 and GV16 can improve the symptoms in CUMS rats by regulating neuroinflammation and microglial activation (Chen et al. 2023; Shen et al. 2024; Tong et al. 2023a, 2023b, 2024). The present study extends these findings by elucidating the role of these acupoints in regulating microglial polarization and pyroptosis, thereby providing a more comprehensive mechanistic framework for their antidepressant effects. The findings suggest that acupuncture at GV23 and GV16 ameliorated depression-like behaviors in CUMS rats by regulating the M1/M2 polarization of microglial cells and pyroptosis. These findings suggest that acupuncture might offer antidepressant efficacy comparable to fluoxetine, which aligns with previous findings (Zhang et al. 2010). This novel mechanistic insight further supports the clinical relevance of acupuncture and its potential as an alternative treatment for depression.

Limitations

While this study used a well-established rat model (CUMS) to simulate depression, the findings may not fully translate to humans due to biological and behavioral differences. Future clinical trials are needed to confirm the results in human populations. The research specifically examined chronic unpredictable stress-induced depression, which represents only one type of depression. Other forms, such as depression linked to hormonal imbalances or genetic predispositions, were not explored. The study only investigated the effects of acupuncture at two specific points (GV23 and GV16). The potential impact of other acupuncture points or combinations remains unknown. A placebo or sham acupuncture group was not included, meaning we cannot completely rule out placebo effects or confirm that the therapeutic benefits are specific to these acupoints. The study covered a 28-day period, which limits our understanding of the long-term effects and safety of acupuncture for depression. Only male rats were used, leaving unanswered questions about whether female subjects would respond similarly to both stress and treatment. Although fluoxetine was used as a positive control, the study did not evaluate how combining acupuncture with fluoxetine might enhance or alter therapeutic outcomes. Additionally, while we assessed microglial activation and polarization using IBA-1 immunofluorescence and molecular markers, the study did not quantify or analyze detailed morphological changes of microglia, which could provide further insight into microglial functional states. Moreover, specific blockers or agonists targeting microglial polarization and the NLRP3 signaling pathway were not employed, which limits the ability to definitively confirm the causal role of these mechanisms in the antidepressant effects of acupuncture. Future studies incorporating pharmacological or genetic interventions are necessary to further validate these findings.

Conclusions

In summary, this study demonstrates that acupuncture exerts antidepressant effects by modulating neuroinflammation through the regulation of microglial polarization and inhibition of pyroptosis in a CUMS-induced depression model. These findings provide novel insights into the neuroimmune mechanisms underlying acupuncture's therapeutic effects and highlight its potential as a promising intervention for depression. Further research is warranted to validate these findings in clinical settings and explore additional molecular targets involved in acupuncture-mediated neuroprotection.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11011-025-01785-6.

Author contributions Xinhong Wu: Funding acquisition. Zhuoran You: Writing – original draft, Formal analysis, Data curation. Tiansheng Zhang: Methodology, Data curation. Jingyu Zeng: Formal analysis. Meng Li: Data curation. Simin Yan: Formal analysis. Jianguo Li: Data curation. Peng Li: Formal analysis. Junliang Shen: Formal analysis. Siyu Liu: Formal analysis. Muhammad Shahzad Aslam: Visualization. Jingxuan Li: Data curation. Lianlian Ning: Data curation. Hui Fang: Data curation. Yizheng Li: Data curation. Dong Yao: Resources, Data curation. Chongyao Hao: Writing – review & editing, Project administration. Xianjun Meng: Writing – review & editing, Project administration, Funding acquisition.

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Data availability The raw data and materials applied in the study will be available for all qualified researchers without any reservation.

Declarations

Competing interests The authors declare no competing interests.

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