Neuroinflammation-induced parvalbumin interneuron and oscillation deficits might contribute to neurobehavioral abnormities in a two-hit model of depression

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ABSTRACT

Depression is a common neuropsychiatric disorder that causes profound disability worldwide, yet the underlying mechanism remains unclear. Thus, the present study aimed to evaluate the effects of a two-hit model of depression on glial activation, parvalbumin (PV) interneuron, oscillation activity, and behavior alternations, and whether chronic fluoxetine treatment can reverse these abnormalities. Male mice were submitted to lipopolysaccharide (LPS) injection, followed by a modified chronic unpredictable stress (CUS) protocol. In our study, we showed that mice exposed to LPS and CUS exhibited reduced body weight, anhedonic-like behavior as well as cognitive and anxiety symptoms. These behavioral alternations were related to enhanced neuroinflammation, as reflected by significantly increased IL-1β and IL-6 levels and microglia activation in the pre-frontal cortex (PFC). In addition, mice exposed to LPS and CUS displayed significantly decreased PV expression and disturbance of theta and gamma oscillations in the PFC. However, chronic fluoxetine treatment reversed most of these abnormalities. In conclusion, our study suggests that neuroinflammation-induced PV interneuron and oscillation deficits might contribute to neuro-behavioral abnormalities in a two-hit model of depression.

1. Introduction

Depression is a common neuropsychiatric disorder that causes profound disability worldwide, affecting ~15% of the general population [1]. Symptoms of depression include depressed mood, sleep disturbances, decreased drive, low mood, and anhedonia [2]. Patients with depression have reduced quality of life, impaired social integration, and a high mortality rate, which burdens society significantly [3]. Among specific populations such as survivors from the intensive care unit (ICU), the prevalence of depression is even higher and may be as high as 41% at 3 months following discharge [4]. Critically ill patients in the ICU not only have primary etiologies such as sepsis but also face tremendous physical and psychological stressors [5]. Thus, from a translational perspective, it is reasonable to use the two-hit model combined with immune stimulation and an environmental component to better mimic depression pathophysiology.

Sepsis induced by bacterial infection remains the leading cause of ICU admission [6]. Lipopolysaccharide (LPS), a component of the outer membranes of Gram-negative bacteria, can induce neuroinflammation with commitment neurobehavioral alternations [7,8]. Of

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note, a significant interaction between psychological stressors and immune challenges has been reported previously [9,10]. It has been demonstrated that stress elicited by chronic unpredictable stress (CUS) can exacerbate LPS-induced inflammation in the mouse hippocampus [9]. In addition, the combination of LPS and stress exacerbates depressive behaviors [10]. These results suggested LPS exerts a synergistic effect with CUS to contribute to toxic effects on the brain. However, little information is available on the behavioral, inflammatory, and neurochemical changes in a two-hit animal model of depression.

In the present study, we thus sought to integrate inflammation with a relevant environmental component to mimic clinical depression pathophysiology by using a combination of initial LPS injection and subsequent CUS exposure. We focused our interest on the prefrontal cortex (PFC) because this brain region is significantly affected by stress and plays a key role in various mental disorders including depression [11].

2. Materials and methods

2.1. Animals

Ninety four male C57BL/6 mice, aged 3–4 months, were purchased from the Animal Center of Jinling Hospital, Nanjing University, Nanjing, China. Mice were maintained at a constant room temperature (22–25 °C), with a relative humidity of 30–50%, controlled illumination (normal 12:12 h light/dark cycle), and food and water were available *ad libitum*. All experiments were conducted following international standards on animal welfare and the guidelines of the Institute for Laboratory Animal Research of Nanjing Medical University. In addition, our study has been posted as a preprint on Research Square Platform (doi.org/10.21203/rs.3. rs-373,197/v1).

2.2. Stress protocol

Animals were randomly divided into the following four groups: control group (n = 21), control + fluoxetine group (n = 19), combined stress group (n = 27), or combined stress + fluoxetine group (n = 27). For the combined stress group, the animals were exposed to a single dose of LPS (*E. coli* 0111:B4, Sigma-Aldrich) diluted in sterile saline (0.9%) and injected intraperitoneally (*i.p.*) at 3 mg/kg in a volume of 0.1 ml. Control animals received a single *i. p.* Dose of saline (0.1 ml) to control for injection stress. After then, mice were submitted to a modified CUS protocol. Briefly, the mouse was housed singly and then randomly subjected to four of the eight different stressors: light on overnight (12 h), physical restraint for 6 h, cage tilt 45 °C for 12 h, lights-off for 3 h during the daylight phase, wet bedding overnight, odor overnight, noise in the room for 12 h, or food and water deprivation overnight (Supplementary Table 1). These stressors were administered on randomly selected days over one week and were repeated over 5 weeks of the experiment. In our study, we selected stressors that are frequently observed in the ICU.

To test whether fluoxetine treatment can reverse the behavioral abnormalities in this two-hit animal model of depression, 20 mg/kg fluoxetine (Tocris Bioscience, Bristol, UK) was given in the drinking water 1 week after LPS injection until the end of the behavioral tests in combined stress + fluoxetine group. The solutions were prepared according to the mouse's average weight and daily water consumption to provide an average daily intake of 20 mg/kg.

2.3. Neurobehavioral tests

Behavioral tests were performed as previously described [12]. All apparatus used in tests were purchased from the Shanghai Softmaze Information Technology Co., Ltd., China. The behavior of mice was recorded using a video camera. A well-trained investigator who was blinded to the animal grouping performed the behavioral tests.

2.3.1. Open field test

The open field apparatus is a cube chamber consisting of white non-reflective plastic (40 cm × 40 cm × 50 cm). The floor of the open field was equally divided into 16 squares. Mouse was placed separately in the center of the open field (the center four squares) and allowed to freely explore for 5 min. The behavior of the animals was videotaped, tracked, and analyzed with the behavioral tracking system. The activity was evaluated based on time spent in the center zone and the total distance traveled in the open field arena. At the end of each test, the arena was cleaned with 75% alcohol to avoid the presence of olfactory cues.

2.3.2. Novel object recognition test

The novel object recognition test was performed in an open field arena (40 cm long × 60 cm wide × 50 cm tall) with three objects, two of which were almost the same, and the other was different. Animals were habituated in the NOR environment for 5 min without the testing objects for two consecutive days. On the third day, the mice explored two identical objects at different corners of the arena for 10 min. On the fourth day, one of the familiar objects was replaced by a novel object. The time that mice spent exploring familiar and novel objects was recorded. Exploration of an object was defined as the animal's nose being in the zone at a distance of ≤ 2 cm. The discrimination score for the novel object exploration ratio was calculated with the following formula: time exploring novel object/ (time exploring novel object + time exploring familiar object) × 100%. Equipment and apparatus were cleaned using 70% ethanol between trials.

2.3.3. Sucrose preference test

We used the sucrose preference test to measure anhedonia, which is a main symptom of depression. All mice were separately placed in a cage with two bottles, one filled with water and the other one with a 3% sucrose solution. The bottles were weighed before it was placed on the lid of each mouse's cage and reweighed after 24 h of consumption. The positions of bottles were placed in a symmetrical position and changed every 12 h to avoid spatial cues. Sucrose preference was calculated as sucrose consumption/(sucrose consumption + water consumption) × 100%).

2.3.4. Fear conditioning test

Mice were individually placed in the conditioning chamber and left to freely explore for 240 s, without acoustic stimulus. After adaptation for 180 s, mice were subjected to two fear conditioning pairings of 30 s conditioned stimulus (tone at 75 dB, 3000 Hz) and foot shock (0.75 mA) in the last 2 s of the conditioned stimulus. Mice were left in the chambers for an additional 30 s before being returned to the home cage. After 24 h, mice were put back into the original chamber for a contextual conditioning test and allowed to explore freely for 5 min. The auditory-cued fear test was performed 2 h later. Following a 180 s acclimation in the conditioning chamber, mice received a tone stimulus (70 dB, 3000 Hz) lasting 180 s. The rigidity response of mice was recorded.

2.3.5. Forced swim test (FST)

Although one recent study argues that FST actually measures coping strategies to an acute inescapable stress [13], it was often conducted to evaluate depressive-like behavior [14,15]. Mice were placed individually in one transparent cylinder (30 cm height, 15 cm diameter) containing water (22–25 °C) at a depth of 15 cm for 6 min. The immobility duration was recorded by a camera in the final 5 min and was counted by an investigator who was blinded to the animal treatment. The immobility time was defined as the absence of movement except leg kicks to stay afloat.

2.4. Meso scale discovery (MSD)

Animals were sacrificed under general anesthesia with 2% sodium pentobarbital in saline (Sigma Chemical Co., St. Louis, MO, USA, 60 mg/kg, *i. p.*). Then, the PFC was dissected immediately after decapitation, flash-frozen in liquid nitrogen, and stored at −80 °C. All samples were assayed via a multiplex biomarker assay platform using ECL on the SECTOR Imager 2400 A from MSD. The following primary antibodies were added to the plate: anti–IL–1β antibody, anti–IL–2 antibody, anti–IL–4 antibody, anti–IL–5 antibody, anti––IL–6 antibody, anti–IL–10 antibody, anti–12p70 antibody, anti–INF–γ antibody, anti–KC/GRO antibody, and anti–TNF–α antibody (Meso Scale Discovery, USA). Plates were then sealed and incubated at room temperature for 2 h followed by three washes with phosphate buffered saline Tween 20 (PBST). After three washes, read solution was added, and plates were immediately measured using an MSD plate reader. The final protein biomarker concentration was reported in pg/ml.

2.5. Immunofluorescence

Animals were sacrificed under general anesthesia with 2% sodium pentobarbital in saline (60 mg/kg, *i. p.*) and transcardially perfused with phosphate-buffered saline (PBS; pH = 7.4), followed by 4% paraformaldehyde in PBS. The brain was immediately removed, postfixed in the same 4% paraformaldehyde for 2 h, dehydrated in 30% sucrose at 4 °C overnight, embedded in OCT, and stored at −80 °C for further use. Brains were sectioned coronally (30 μm thick) using a cryostat and stored at −20 °C in a cryoprotectant solution. Slices were initially blocked with 1–2% bovine serum albumin and 0.03% Triton X-100 for 2 h at room temperature and then incubated with the primary antibodies: rabbit anti-ionized calcium-binding adapter molecule 1 (IBA1, 1:1000; WAKO, 019–19741), rabbit anti-glial fibrillary acidic protein (GFAP, 1:200, protein tech, 16825-1-AP), rabbit *anti*-PV (1:500; Abcam, ab11427) overnight at 4 °C. Then sections were washed in PBS and incubated with a fluorochrome-conjugated secondary antibody (Alexa Fluor 488). Sections were mounted on slides, coverslipped with Fluoromount G (Beckman Coulter), and stored at −20 °C. Immunofluorescent sections were imaged using confocal laser scanning microscopy (Olympus Fluoview 1000 confocal microscope; Olympus UPlanSapo objective). Samples from each group were processed in parallel to avoid any nonspecific effect of the staining procedure. Detailed images were taken on an Olympus FV1000 confocal microscope at 20 magnifications. For the fluorescence intensity quantification, four sections of the PFC randomly picked from per mouse were analyzed by Image J software (National Institutes of Health, Bethesda, MD, USA) for immunofluorescence analysis. The number of pixels per image with an intensity above a predetermined threshold level was quantified by measurement of the immunoreactive areas for IBA1, GFAP, and PV.

2.6. Local field potential (LFP) recording

LFP recording was performed as previously described [16]. Briefly, mice were anesthetized by pentobarbital sodium (40 mg/kg, *i. p.*) and fixed in a stereotaxic apparatus with left and right ear rods. After craniotomy and removal of the dura, an 8-channel microwire electrode array was targeted to the PFC, including prelimbic cortex (PrL), at 3.0 mm rostral to bregma and 0.4 mm lateral to bregma, and vertically lowered to a depth of 3.5 mm from the brain surface. The signals were filtered with a pass-band of 0.3–300 Hz and were further amplified and digitized at 2 kHz. The recorded LFPs were filtered by a 50 Hz notching filter to remove the powerline artifact. For LFP analysis, the wideband recordings were down-sampled at 1000 Hz. All data analyses were performed by Neuroexplorer (Plexon Inc., Dallas, TX) software.

2.7. Data analysis

Data were analyzed and plotted by GraphPad Prism 7.0 (GraphPad Software, La Jolla, CA, USA). Data are presented as mean ± standard error of the mean (S.E.M.). Differences among multiple groups were assessed by one-way or two-way analysis of variance (ANOVA) with Tukey's multiple comparisons. The survival rate was estimated by Kaplan–Meier method and compared by the log-rank test. A $P < 0.05$ was considered statistically significant.

3. Results

3.1. Survival rate and weight changes

To observe the effects of combined stress on mortality, we recorded the survival rate for 30 days after LPS injection (Fig. 1A). As shown in Fig. 1B, no animals died in the control group and control + fluoxetine group. The survival rate was 77.778% in the combined stress group and 81.250% in the combined stress + fluoxetine group, respectively. However, no differences were detected in the control group or control + fluoxetine group as compared with the combined stress group and the combined stress + fluoxetine group ($P = 0.0860$, $P = 0.1202$, respectively). In addition, there was no significant difference between the combined stress group and the combined stress + fluoxetine group ($P = 0.7998$). Concerning weight change, analysis by two-way ANOVA found an interaction effect between groups, but no effect of combined stress challenge or fluoxetine treatment was observed (Interaction: $F_{(1, 47)} = 7.621$, $P = 0.0082$, control/combined stress challenge: $F_{(1, 47)} = 2.824$, $P = 0.0995$, saline/fluoxetine treatment: $F_{(1, 47)} = 1.764$, $P = 0.1905$). Tukey's multiple comparisons showed that combined stress induced significantly decreased weight compared with a control group and control + fluoxetine group ($P = 0.0139$, $P = 0.0228$, respectively, Fig. 1C).

3.2. Combined stress-induced behavioral abnormalities were partially reversed by fluoxetine treatment

In the open field test, the interaction effect between groups and effect of combined stress challenge were observed for total distance, but no effect of fluoxetine treatment was noted (Interaction: $F_{(1, 47)} = 6.628$, $P = 0.0132$, control/combined stress challenge: $F_{(1, 47)} = 7.354$, $P = 0.0093$, saline/fluoxetine treatment: $F_{(1, 47)} = 2.734$, $P = 0.1049$). Combined stress induced significantly increased total distance traveled as compared with the control group and control + fluoxetine group ($P = 0.0027$, $P = 0.0187$, respectively), suggesting combined stress induced the anxiety behavior. This increased total distance traveled was prevented by fluoxetine treatment ($P = 0.0205$, Fig. 2B). However, there was no difference in time spent in the center of the open arena among groups (Interaction: $F_{(1, 47)} = 2.614$, $P = 0.1126$, control/combined stress challenge: $F_{(1, 47)} = 1.353$, $P = 0.2506$, saline/fluoxetine treatment: $F_{(1, 47)} = 0.02076$, $P = $
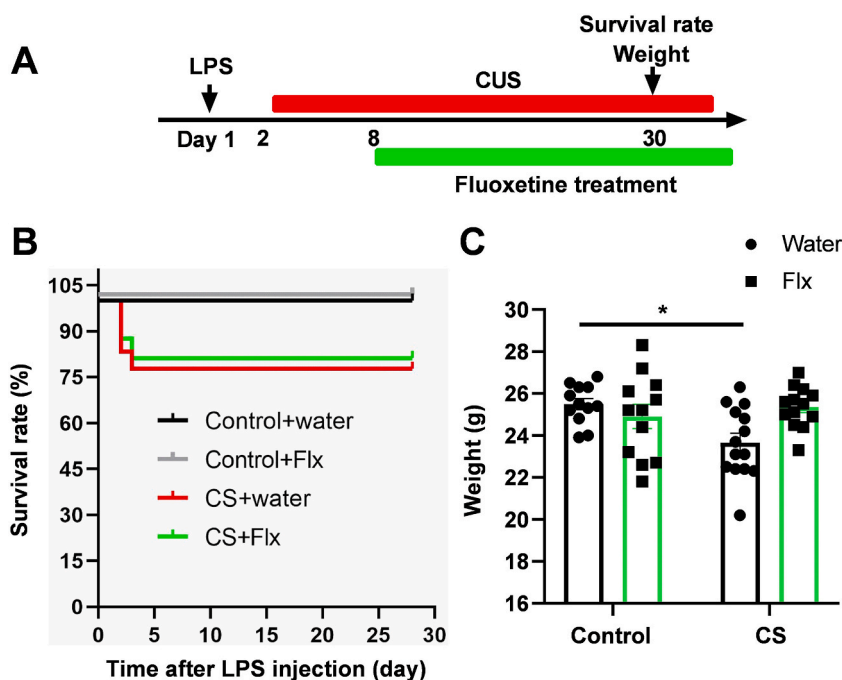

Fig. 1. Survival rate and weight changes. (A) Schematic timeline of the experimental procedure. (B) No animals died in the control group, The survival rate was 77.778% in the combined stress (CS) group and 81.250% in CS + fluoxetine group, respectively (n = 16–22). (C) CS-induced significantly decreased weight compared with the control group, which was prevented by chronic fluoxetine treatment. *$P < 0.05$. Con, control; LPS, lipopolysaccharide; Flx, fluoxetine; CS, combined stress.
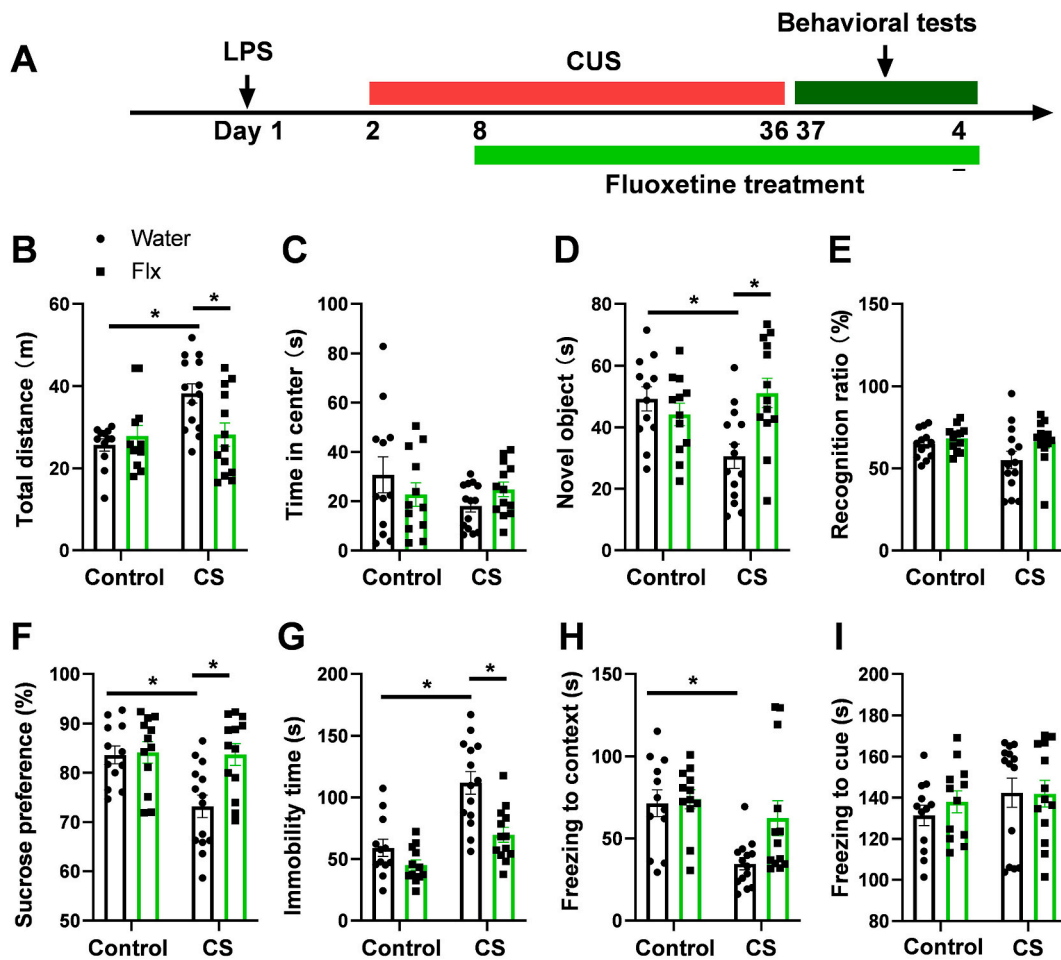
Fig. 2. *CS*-induced behavioral abnormalities were partially reversed by fluoxetine treatment. (A) Schematic timeline of the experimental procedure. (B) *CS*-induced significantly decreased distance traveled in the open field as compared with the control group, which was prevented by fluoxetine treatment. (C) There was no difference in time spent in the center of the open arena among groups. (D) CS significantly decreased their exploration time with a novel object, which was prevented by fluoxetine treatment. (E) There was no difference in the recognition ratio among groups. (F) *CS*-induced significantly decreased preference for sucrose, which was prevented by fluoxetine treatment. (G) Fluoxetine treatment reversed the increased immobility time induced by CS. (H) *CS*-induced decreased freezing time to context was not prevented by fluoxetine treatment. (I) No difference was observed in freezing time to cue among groups. Data are shown as mean ± SEM (n = 12), *$P < 0.05$. Con, control; Flx, fluoxetine; CS, combined stress.

0.8861, Fig. 2C). In the novel object recognition test, an interaction effect for exploration time was noted, but no effect of combined stress challenge or effect of fluoxetine treatment was observed (Interaction: $F_{(1,47)} = 9.597$, $P = 0.0033$, control/combined stress challenge: $F_{(1, 47)} = 2.000$, $P = 0.1638$, saline/fluoxetine treatment: $F_{(1, 47)} = 3.493$, $P = 0.0679$). Combined stress significantly decreased their exploration time with the novel object ($P = 0.0121$, Fig. 2D) compared with the control group, which was prevented by fluoxetine treatment ($P = 0.0039$). However, there was no difference in recognition ratio among groups (Interaction: $F_{(1, 47)} = 1.021$, $P = 0.3175$, control/combined stress challenge: $F_{(1, 47)} = 2.388$, $P = 0.1290$, saline/fluoxetine treatment $F_{(1, 47)} = 3.655$, $P = 0.0620$, Fig. 2E). In the sucrose preference test, analysis by two-way ANOVA found the interaction effect, the effect of combined stress challenge, and the effect of fluoxetine treatment (Interaction: $F_{(1, 47)} = 5.443$, $P = 0.0240$, control/combined stress challenge: $F_{(1, 47)} = 6.453$, $P = 0.0144$, saline/fluoxetine treatment: $F_{(1, 47)} = 6.675$, $P = 0.0129$). Tukey's multiple comparisons showed that mice displayed a significantly decreased preference for sucrose than control and control + fluoxetine animals ($P = 0.0059$, $P = 0.0035$, respectively), which was prevented by fluoxetine treatment ($P = 0.0043$, Fig. 2F). In the forced swimming test, there was no interaction effect between groups, but significant effects of combined stress challenge and the effect of fluoxetine treatment were observed (Interaction: $F_{(1, 47)} = 4.009$, $P = 0.0511$, control/combined stress challenge: $F_{(1, 47)} = 29.77$, $P < 0.0001$, saline/fluoxetine treatment: $F_{(1, 47)} = 15.58$, $P = 0.0003$). Mice exposed to combined stress had significantly increased immobility time ($P < 0.0001$), and this effect was reversed by fluoxetine treatment ($P = 0.0004$, Fig. 2G), suggesting combined stress-induced depression-like behavior. In the fear conditioning tests, there was no interaction effect between groups, but significant effects of combined stress challenge and fluoxetine treatment were observed (Interaction: $F_{(1, 47)} = 2.870$, $P = 0.0969$, control/combined stress challenge: $F_{(1, 47)} = 10.640$, $P = 0.0021$,

saline/fluoxetine treatment: $F_{(1, 47)} = 4.150$, $P = 0.0473$). There was a significantly decreased freezing time to the context in the combined stress group compared with the control group and the control + fluoxetine group ($P = 0.0050$, $P = 0.0024$, respectively), which was not prevented by fluoxetine treatment ($P = 0.0438$, Fig. 2H). There was no difference in freezing time to cue among these groups (Interaction: $F_{(1, 47)} = 0.3288$, $P = 0.5691$, control/combined stress challenge: $F_{(1, 47)} = 1.490$, $P = 0.2284$, saline/fluoxetine treatment: $F_{(1, 47)} = 0.2522$, $P = 0.6179$, Fig. 2I).

3.3. Combined stress-induced microglia activation but not astrocyte in the PFC was attenuated by fluoxetine treatment

To determine whether combined stress-induced changes in immune response in the PFC, we performed immunostaining by using antibodies of IBA1 or GFAP. Compared with the control group, the intensity of IBA1 positive -cells in the PFC increased significantly in the combined stress group, which was reversed by fluoxetine treatment ($F_{(2, 9)} = 9.89$, $P = 0.0053$, analyzed by Tukey's multiple comparisons, Fig. 3A and B). However, there was no difference in the intensity of GFAP among groups ($F_{(2, 9)} = 0.1645$, $P = 0.8508$, Fig. 3C and D).

3.4. Combined stress-induced abnormal inflammatory mediators in the PFC were partially reversed by fluoxetine treatment

To confirm the inflammatory response after combined stress, we also measured inflammatory mediators in the PFC by MSD. When compared with the control group, combined stress induced significantly increased IL-1β ($F_{(2, 15)} = 7.793$, $P = 0.0048$, analyzed by Tukey's multiple comparisons, Fig. 4A) and IL-6 ($F_{(2, 15)} = 4.26$, $P = 0.034$, analyzed by Tukey's multiple comparisons, Fig. 4E) expressions in the PFC, whereas fluoxetine treatment reversed IL-1β but not IL-6 level. In addition, combined stress induced significantly decreased IL-10 expression in the PFC as compared with the control group, which was not reversed by fluoxetine treatment ($F_{(2, 15)} = 4.512$, $P = 0.0292$, analyzed by Tukey's multiple comparisons, Fig. 4F). These results suggested that combined stress induced a
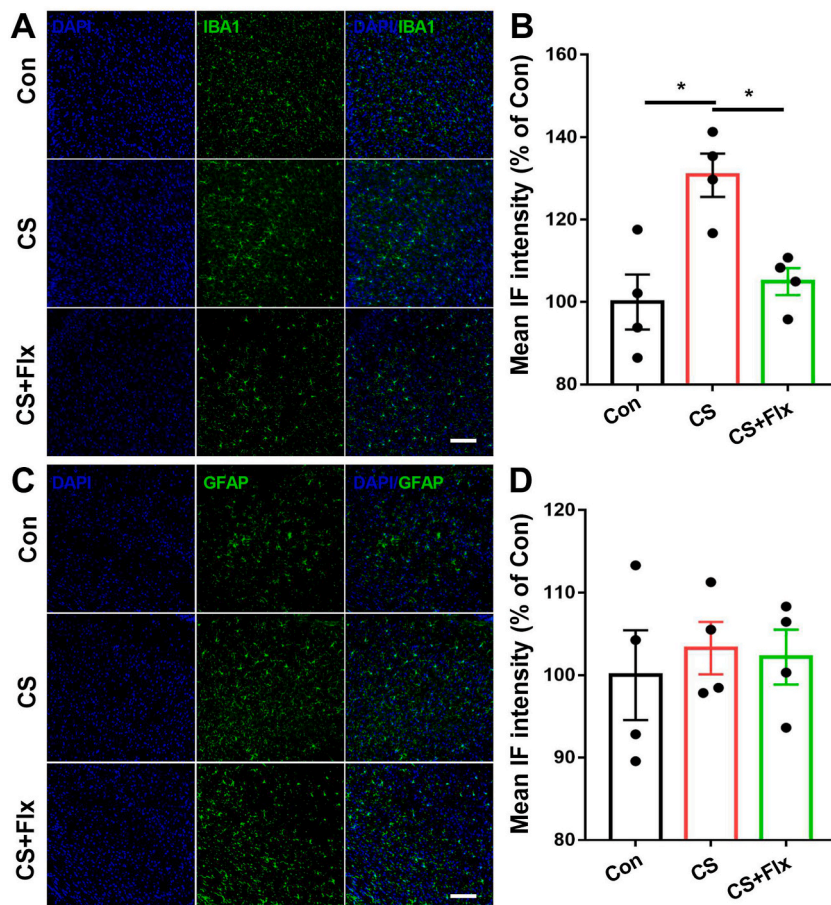

Fig. 3. CS-induced microglia activation in the PFC was attenuated by fluoxetine treatment. (A) Representative images of IBA-1-positive cells in the PFC. (B) Quantification of mean IBA-1 immunofluorescence in the PFC. (C) Representative images of GFAP-positive cells in the PFC. (D) Quantification of mean GFAP immunofluorescence in the PFC. Data are shown as mean ± SEM (n = 4), *$P < 0.05$, scale bar = 100 μm. Con, control; Flx, fluoxetine; IF, immunofluorescence; CS, combined stress.
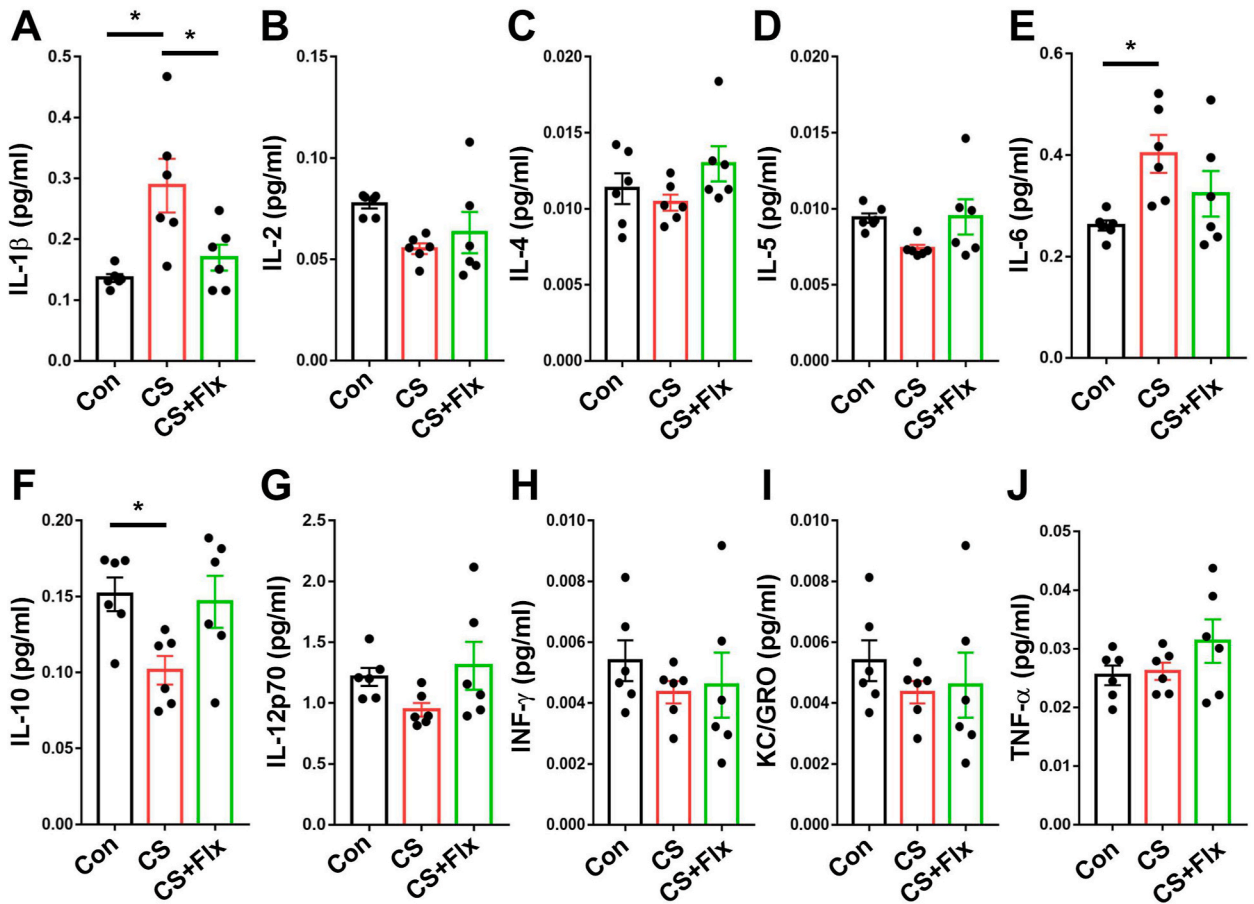
Fig. 4. *CS*-induced abnormal inflammatory mediators in the PFC were partially reversed by fluoxetine treatment. (A–J) Quantification of inflammatory mediators in the PFC. Data are shown as mean ± SEM (n = 6), *$P < 0.05$. Con, control; Flx, fluoxetine; CS, combined stress.

dysregulated inflammatory response. However, there was no difference in IL-2 ($F_{(2, 15)} = 3.229$, $P = 0.0682$, Fig. 4B), IL-4 ($F_{(2, 15)} = 1.896$, $P = 0.1844$, Fig. 4C), IL-5 ($F_{(2, 15)} = 2.769$, $P = 0.0947$, Fig. 4D), IL-12p70 ($F_{(2, 15)} = 2.251$, $P = 0.1396$, Fig. 4G), INF-γ (Kruskal-Wallis statistic$_{(2, 15)} = 1.368$, $P = 0.5264$, Fig. 4H), KC/GRO ($F_{(2, 15)} = 1.367$, $P = 0.2848$, Fig. 4I) or TNF-α ($F_{(2, 15)} = 1.632$, $P =$
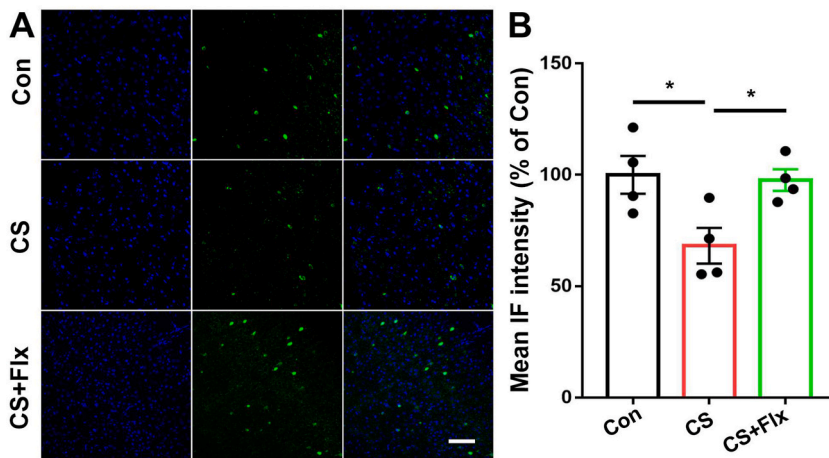

Fig. 5. *CS*-induced PV interneuron deficit in the PFC was attenuated by fluoxetine treatment. (A) Representative images of PV interneurons in the PFC. (B) Quantification of mean PV immunofluorescence in the PFC. Data are shown as mean ± SEM (n = 4), *$P < 0.05$, scale bar = 100 μm. Con, control; Flx, fluoxetine; IF, immunofluorescence; CS, combined stress.

0.2284, Fig. 4J) in the PFC among groups.

3.5. Combined stress-induced PV interneuron deficit in the PFC was attenuated by fluoxetine treatment

To evaluate PV interneuron alteration in the PFC after combined stress, we performed immunostaining by an antibody raised against PV. As shown in Fig. 5, the intensity of PV was significantly decreased in the PFC in the combined stress group compared with the control group, which was reversed by fluoxetine treatment ($F_{(2, 9)} = 5.855$, $P = 0.0235$, analyzed by Tukey's multiple comparisons).

3.6. Combined stress-induced altered neural oscillations in the PFC were partially reversed by fluoxetine treatment

To further assess the possible role of altered oscillatory activities in depression and the possible protective effects of fluoxetine treatment, we recorded LFP when the animals explored the novel object. Although there was no difference in baseline oscillation activities among groups (theta: $F_{(2, 12)} = 0.02858$, $P = 0.9719$; alpha: $F_{(2, 12)} = 0.03326$, $P = 0.9674$; beta: $_{(2, 12)} = 0.002465$, $P = 0.9975$; gamma power: $F_{(2, 12)} = 0.0737$, $P = 0.9294$, analyzed by Tukey's multiple comparisons, Fig. 6A and B). When the animals explored the novel object, mice in the combined stress group displayed significantly decreased theta ($F_{(2, 12)} = 6.331$, $P = 0.0133$, analyzed by Tukey's multiple comparisons, Fig. 6C and D) and gamma power ($F_{(2, 12)} = 5.08$, $P = 0.0252$, analyzed by Tukey's multiple comparisons, Fig. 6C and D) when compared with the control group. However, fluoxetine treatment reversed only theta but not the gamma deficit. There was no difference in alpha ($F_{(2, 12)} = 1.001$, $P = 0.3962$, Fig. 6C and D) and beta oscillation ($F_{(2, 12)} = 1.623$, $P = 0.2378$, Fig. 6C and D) power among groups.
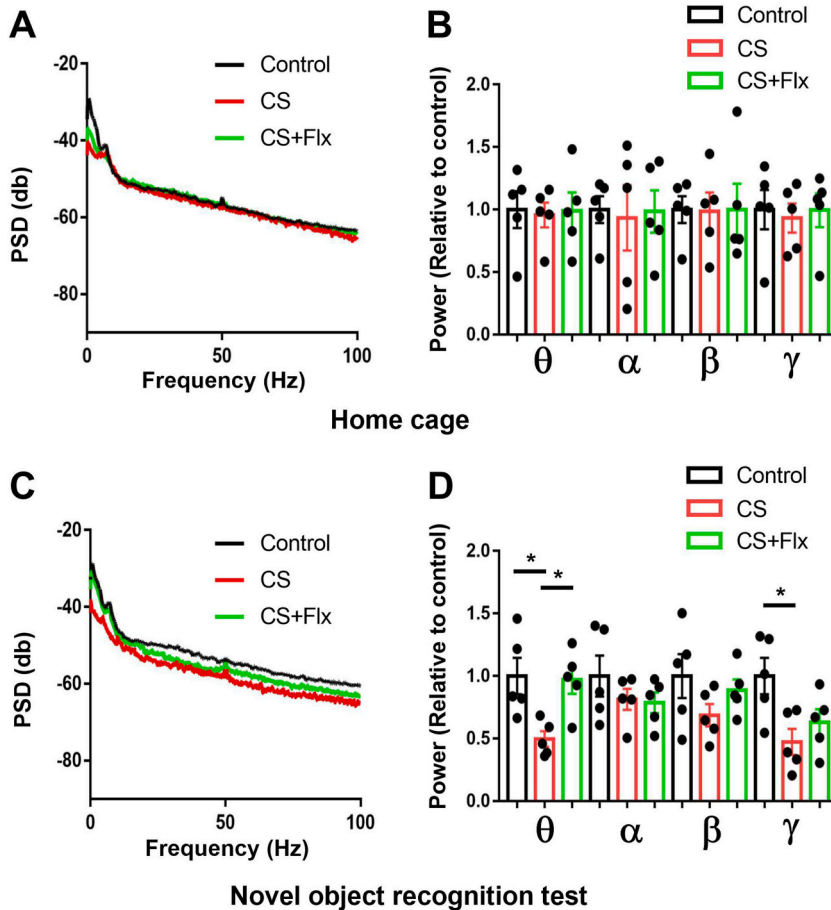

Fig. 6. CS-induced altered neural oscillations in the PFC were partially reversed by fluoxetine treatment. (A) Quantification of local field potential in the PFC at the home cage. (B) Quantification of average theta, alpha, beta, and gamma power in the PFC at home cage. (C) Quantification of local field potential in the PFC when the animal explored the novel object. (D) Quantification of average theta, alpha, beta, and gamma power in the PFC when the animal explored the novel object. Data are shown as mean ± SEM (n = 5), *P < 0.05. Con, control; Flx, fluoxetine; IF, immunofluo-rescence; CS, combined stress.

4. Discussion

In the present study, we applied a new paradigm by exposing mice to LPS and then a CUS. The rationale behind this two-hit model is based on the fact that the combination of the initial LPS-induced inflammation and subsequent stressors would better mimic human depression pathophysiology. By using this paradigm, our study showed that mice exposed to combined stress exhibited anhedonic-like behavior, anxiety symptoms, and cognitive impairment. These behavioral changes were accompanied by enhanced neuro-inflammation, decreased PV expression, and disturbance of theta and gamma oscillation in the PFC. However, fluoxetine, a selective serotonin reuptake inhibitor reversed most of these abnormalities.

Survivors from ICU are known to be at increased risk of developing long-term psychiatric comorbidity [5]. Among them, depression is a common complication that is challenging to cope with. Although most current animal models of depression use psychosocial or environmental stress as the inducing manipulation [2], it should be noted that other factors should also be considered. In sepsis survivors, patients may experience existing diseases such as infection-induced sepsis as well as psychosocial or environmental stress [12]. One earlier study has shown that prior stress exposure sensitizes LPS-induced cytokine production [17]. In addition, LPS administration to mimic inflammation significantly attenuated the antidepressant action of fluoxetine [18]. However, another study even demonstrated a prior chronic mild stress can protect the brain against the systemic acute and severe stress elicited by sepsis [19]. Despite this discrepancy, our study suggested that LPS may have a synergistic effect with CUS in exerting toxic effects on the brain.

Accumulating evidence has suggested that immune dysregulation, in particular inflammatory processes, has been identified as one of the major pathophysiological mechanisms underlying various neuropsychiatric disorders, including depression [3]. Indeed, the incidence of depression is higher in patients with chronic inflammatory illnesses, including cardiovascular disease, diabetes, and cancer [20]. LPS is used to induce acute inflammation and depression-like behavior in animal models [21–23]. Our data support the view of depression as an inflammatory disorder [3], as evidenced by significantly increased proinflammatory mediators such as IL-1β and IL-6 expressions as well as microglia activation in the PFC, confirming the findings reported previously that microgliosis in the PFC following chronic stress modality [24]. In human studies, patients with major depression show increased markers of peripheral inflammation, including IL-6 [25]. Notably, chronic fluoxetine treatment down-regulated the enhanced inflammatory response, concomitant with improved neurobehavioral outcomes. In support, the anti-inflammatory effects of fluoxetine have been reported in a rat model of depression [26]. Since either LPS or CUS can induce acute and chronic immune activation, thus future studies should be performed to investigate whether combined LPS and CUS may contribute to a further increase in the inflammatory response. Overall, our study along with previous findings suggested that neuroinflammation is a shared mechanism underlying depression. However, the mechanism by which neuroinflammation induces depression remains to be elucidated.

Alterations in the balance between neuronal excitation and inhibition have been implicated in the neural circuit activity–based information processes [27]. GABAergic neurons provide the main inhibitory control of neuronal activity in the brain. Accumulating evidence has demonstrated that alterations in inhibitory interneurons contribute to neurobehavioral associated with various psychiatric and neurological diseases [28]. In particular, PV interneurons, a subpopulation of GABAergic interneurons have been shown in the regulation of behavioral performance [29]. PV interneurons are highly susceptible to redox dysregulation and are implicated in a variety of psychiatric diseases [30]. Reduced PV expression has been observed in the PFC in postmortem studies of human patients diagnosed with schizophrenia, depression, and bipolar disorder [31–34]. Of note, PV interneurons are critical for the synchronous activity of principal cells during normal brain function and the production of different oscillation activities [28]. Network oscillations cover a wide range of frequencies (~0.05–600 Hz), and they play a crucial role in different cognitive and behavioral states [34]. By contrast, PV interneuron disturbances are consistent with lower gamma oscillation power, these deficits underlie the brain's failure to integrate information and consequently the manifestations of many symptoms of schizophrenia [34]. In an animal model of depression, disturbance of low-gamma oscillations caused by local defects of PV interneurons is impaired in the prelimbic cortex and inversely correlated with the extent of behavioral despair [35]. In our study, we showed that mice exposed to LPS and subsequent CUS displayed significantly decreased PV expression with lower theta and gamma oscillations in the PFC, whereas the defects of PV expression and theta oscillation can be reversed by chronic fluoxetine treatment. In support of our data, restoration of gamma activity at the network level is associated with behavioral remission in a mouse model of depression [36]. There is accumulating evidence suggesting chronic fluoxetine treatment can increase GABAergic tone in the brain [37,38]. Surprisingly, our study found that chronic fluoxetine treatment did not reverse gamma activity. These results suggested the antidepressant effects of fluoxetine are not restricted to gamma oscillation. Interestingly, somatostatin interneurons, another type of GABAergic interneurons, have also played a key role in major depressive disorder [39]. In addition, somatostatin interneurons in the medial prefrontal cortex mediate the rapid antidepressant responses to scopolamine [40]. Together, these results suggested that GABAergic interneuron dysfunction is critically involved in the development of depression.

Our study has several limitations. Due to technical reasons, we did not determine the serum concentration of fluoxetine in the treated groups. Thus, further studies are required to observe the effective doses of fluoxetine in this two-hit model of depression. In addition, our model of combined immune stimulation and subsequent stressors can only mimic some characteristics of depression in ICU patients, which do not ideally reflect clinical situations. However, our study provides a better understanding of the interactions between immune stimulation and stressors, as well as their contribution to depression.

In conclusion, our study suggests that neuroinflammation-induced PV interneuron and oscillation deficits might contribute to neurobehavioral abnormalities in this two-hit model of depression. Thus, the combination of different pathophysiological components of depression may provide a more translational value. However, there is much to be learned about the complexities of immune/environment interactions by using more specific approaches in our future studies.

Author contribution statement

Qing-Ren Liu: Conceived and designed the experiments; Performed the experiments; Wrote the paper.
Cui-na Shi: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.
Fei Wang: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.
Jian-hua Tong: Conceived and designed the experiments; Wrote the paper.

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Data availability statement

Data included in article/supplementary material/referenced in the article. The article was previously published as a preprint (https://doi.org/10.21203/rs.3.rs-373197/v1).

Additional information

No additional information is available for this paper.

Declaration of competing interest

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

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e18468.

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