Neuroinflammation-Induced Parvalbumin Interneuron and Oscillation Deficits Might Contribute to Neurobehavioral Abnormities in a Two-Hit Model of Depression

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Research

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Abstract

Background: 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.

Methods: Male mice were submitted to lipopolysaccharide (LPS) injection, followed by a modified chronic unpredictable stress (CUS) protocol.

Results: 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 prefrontal 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.

Conclusion: Our study of this two-hit model of depression is clinically relevant and suggests the combination of different etiological and pathophysiological components of depression may provide with a more translational value.

1. Introduction

Depression is a common neuropsychiatric disorder that causes profound disability worldwide, affecting ~15% in the general population [1]. Symptoms of depression include depressed mood, sleep disturbances, decreased drive, and low mood and anhedonia [2]. Patients with depression have reduced quality of life, impaired social integration, and a high mortality rate, which imposes a significant burden on society [3]. Among specific populations such as survivors from 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 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, is able to induce neuroinflammation with commitment neurobehavioral alternations [7, 8]. Of note, a significant interaction between the psychological stressors and immune challenge has been reported in previous studies [9, 10]. It has been demonstrated that stress elicited by 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 has a synergistic effect with CUS in exerting 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 [11].

2. Materials And Methods

2.1. Animals. Seventy-five 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 ℃), 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 in accordance with international standards on animal welfare and the guidelines of the institute for Laboratory Animal Research of Nanjing Medical University.

2.2. Stress Protocol. Animals were randomly divided into the following three groups: control group (n = 21), combined stress group (n = 27), or combined stress + fluoxetine group (n = 27). For 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 that, mice were submitted to a modified CUS protocol. Briefly, 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 Digital Content). These stressors were administered in randomly selected days over one week and were repeated over 5 weeks of experiment. In our study, we selected stressors that are frequently observed in the ICU.

To test whether fluoxetine treatment can reverse the behavioral abnormities in this 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 average weight and daily water consumption in order 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 consisted 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 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, 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 were explored two identical objects at different corners of the arena for 10 min. On the fourth day, one of familiar objects was replaced by a novel object. The time that mice spent on 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 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 cage with two bottles, one filled with water and the other one with 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 the 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 Tests. 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 seconds 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 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 Swimming Test. Forced swimming test was conducted to evaluate depressive-like behavior. 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 ℃. All

samples were assayed via a multiplex biomarker assay platform using ECL on the SECTOR Imager 2400 A from MSD. Plates were washed and blocked as per manufacturer instructions. Samples were diluted and applied to the plate, along with standards. Plates were then sealed and incubated at room temperature for 2 h followed by three washes with phosphate buffered saline Tween 20 (PBST). Plates were sealed and incubated for a further hour following adding the secondary detection antibodies. 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 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, proteintech, 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, cover slipped 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. The intensities were calculated by Image J software (National Institutes of Health, Bethesda, MD, USA).

2.6. Local Field Potential (LFP) Recording. LFP recording was performed as previously described [13]. 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 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.). Multiple comparisons were analyzed by one-way ANOVA followed by Tukey's test if the data were normally distributed or Kruskal-Wallis test followed by Dunn's test if the data were not normally distributed. 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 survival rate for 30 days after LPS injection (Fig 1A). As shown in Fig 1B, no animals died in the control group. The survival rate was 81.818% in combined stress group and 86.364% in combined stress + fluoxetine group, respectively. No difference was detected among each group. With regard to weight change, combined stress induced significantly decreased weight compared with control group, which was prevented by chronic fluoxetine treatment ($F_{(2, 33)} = 5.778$, $P = 0.0071$, Fig 1C).

3.2. Combined stress-induced Behavioral Abnormities Were Partially Reversed by Fluoxetine Treatment. In the open field test, combined stress induced significantly increased total distance travelled as compared with the control group, suggesting combined stress induced the anxiety behavior. This increased total distance travelled was prevented by fluoxetine treatment ($F_{(2,33)} = 6.21$, $P = 0.0051$, Fig 2B). However, there was no difference in time spent in the center of the open arena among groups (Kruskal-Wallis statistic $_{(2,33)} = 2.601$, $P = 0.2724$, Fig 2C). In the novel object recognition test, combined stress significantly decreased their exploration time with novel object ($F_{(2,33)} = 5.088$, $P = 0.0119$, Fig 2D) compared with control group, which was prevented by fluoxetine treatment. However, there was no difference in recognition ratio among groups ($F_{(2,33)} = 0.874$, $P = 0.4267$, Fig 2E). In the sucrose preference test, mice displayed significantly decreased preference for sucrose than control animals, which was prevented by fluoxetine treatment ($F_{(2,33)} = 5.377$, $P = 0.0095$, Fig 2F). In the forced swimming test, mice exposed to combined stress had significantly increased immobility time, and this effect was reversed by fluoxetine treatment (Kruskal-Wallis statistic$_{(2,33)} = 14.02$, $P = 0.0009$, Fig 2G), suggesting combined stress induced depression-like behavior. In the fear conditioning tests, there was a significantly decreased freezing time to context in combined stress group compared with control group (Kruskal-Wallis statistic$_{(2,33)} = 7.934$, $P = 0.0189$, Fig 2H), which was not prevented by fluoxetine treatment. There was no difference in freezing time to cue among these groups (Kruskal-Wallis statistic$_{(2,33)} = 4.686$, $P = 0.096$, 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 control group, the intensity of IBA1 positive -cells in the PFC increased significantly in combined stress group, which was reversed by fluoxetine treatment ($F_{(2,9)} = 9.89$, $P = 0.0053$, Fig 3A-B). However, there was no difference in the intensity of GFAP among groups ($F_{(2,9)} = 0.1645$, $P = 0.8508$, Fig 3C-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 control group, combined stress induced significantly increased IL-1β ($F_{(2,15)}$ = 7.793, *P* = 0.0048, Fig 4A) and IL-6 ($F_{(2,15)}$ = 4.26, *P* = 0.034, 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 control group, which was not reversed by fluoxetine treatment ($F_{(2,15)}$ = 4.512, *P* = 0.0292, Fig 4F). These results suggested that combined stress induced a dysrugulated 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$_{(2,33)}$ = 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* = 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 alternation in the PFC after combined stress, we performed immunostaining by antibody raised against PV. As shown in Fig 5, the intensity of PV was significantly decreased in the PFC in combined stress group compared with control group, which were reversed by fluoxetine treatment ($F_{(2, 9)}$ = 5.855, *P* = 0.0235).

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, Fig 6A-B). When the animals explored the novel object, mice in combined stress group displayed significantly decreased theta ($F_{(2,12)}$ = 6.331, *P* = 0.0133, Fig 6C-D) and gamma power ($F_{(2,15)}$ = 5.08, *P* = 0.0252, Fig 6C-D) when compared with control group. However, fluoxetine treatment reversed only theta but not gamma deficit. There was no difference in alpha ($F_{(2,12)}$ = 1.001, *P* = 0.3962, Fig 6C-D) and beta oscillation ($F_{(2,12)}$ = 1.623, *P* = 0.2378, Fig 6C-D) power among groups.

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 inflammation and stress 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 neuroinflammation, 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 of 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 disease such as infection-induced sepsis as well as psychosocial or environmental stress [12]. One earlier study has shown that prior stressor exposure sensitizes LPS-induced cytokine production [14]. In addition, LPS administration to mimic inflammation significantly attenuated the antidepressant action of fluoxetine [15]. However, another study even demonstrated a prior chronic mild stress can protect the brain against the systemic acute and severe stress elicited by sepsis [16]. 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 [17]. LPS is used to induce acute inflammation and depression-like behavior in animal models [18-20]. Our data support the view of depression as an inflammatory disorder [3], as evidenced by significantly increased proinflamamtory mediator 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 [21]. In human study, patients with major depression show increased markers of peripheral inflammation, including IL-6 [22]. Notably, chronic fluoxetine treatment downregulated 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 [23]. Since either LPS or CUS can induce acute and chronic immune activation, thus future study should be performed to investigate whether combined LPS and CUS may contribute to further increase in the inflammatory response. Overall, our study along with previous findings suggests 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 [24]. 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 [25]. In particular, PV interneurons, a subpopulation of GABAergic interneurons has been shown in the regulation of behavioral performance [26]. PV interneurons are highly susceptible to redox dysregulation and implicated in a variety of psychiatric diseases [27]. Reduced PV expression has been observed in the PFC in postmortem studies of human patients diagnosed with schizophrenia, depression, and bipolar disorder [28-31]. Of note, PV interneurons are critically for synchronous activity of principal cells during normal brain function and production of different oscillation activities [25]. Network oscillations cover a wide range of frequencies (~0.05 to 600 Hz), and they play a crucial role in different cognitive and

behavioral states [31]. By contrast, PV interneuron disturbances are consistent with lower gamma oscillation power, these deficits underlie the brain's failure to integrate information and consequent the manifestations of many symptoms of schizophrenia [31]. 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 [32]. 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 [33]. There is accumulating evidence suggesting chronic fluoxetine treatment can increase GABAergic tone in the brain [34, 35]. 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.

In conclusion, our study showed that this two-hit model is clinically relevant. Thus, the combination of different pathophysiological components of depression may provide with 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.

Declarations

Ethical Approval and Consent to participate

All experiments were conducted in accordance with international standards on animal welfare and the guidelines of the institute for Laboratory Animal Research of Nanjing Medical University.

Consent for publication

Not applicable.

Availability of data and materials

The data used to support the findings of this study are available from the corresponding author upon request.

Competing interests

The authors declare that they have no conflicts of interest.

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Authors' contributions

F.W., J.T., and M.J. designed all of the experiments; J.T. and F.W.performed all behavior tests, imaging, and most of the analysis; F.W. and Y.G.performed Immunofluorescence ,Local Field Potential (LFP) Recording and some imaging; F.W. and S.W. performed some image analysis; Y.G. and S.W. helped in feeding some animals; F.W., M.J., J.T., and Y.G. discussed the results and wrote the manuscript; and all authors discussed the manuscript.

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Table

Table 1 Chronic stress protocol

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Week 1	A, C, F, G	C, D, G, H	B, D, F, H	A, B, C, G	B, D, E, F	B, C, F, G	A, D, F, H
Week 2	A, B, E, H	B, C, D, G	A, C, E, G	A, E, G, H	C, E, F, G	A, B, D, H	A, E, F, G
Week 3	A, D, F, G	B, E, G, H	B, C, F, G	C, D, F, H	A, B, D, E	B,C, D, H	A, C, G, H
Week 4	C, E, F, G	A, B, D, H	A, E, F, G	A, B, E, H	B, C, D, G	A, C, E, G	A, E, G, H
Week 5	A, B, D, E	B,C, D, H	A, C, G, H	A, D, F, G	B, E, G, H	B, C, F, G	C, D, F, H

A, light on overnight (12 h); B, physical restraint for 6 h; C, cage tilt 45 °C for 12 h; D, lights-off for 3 h during the daylight phase; E, wet bedding overnight; F, odor overnight; G, noise in the room for 12 h; H, food and water deprivation overnight.

Figures

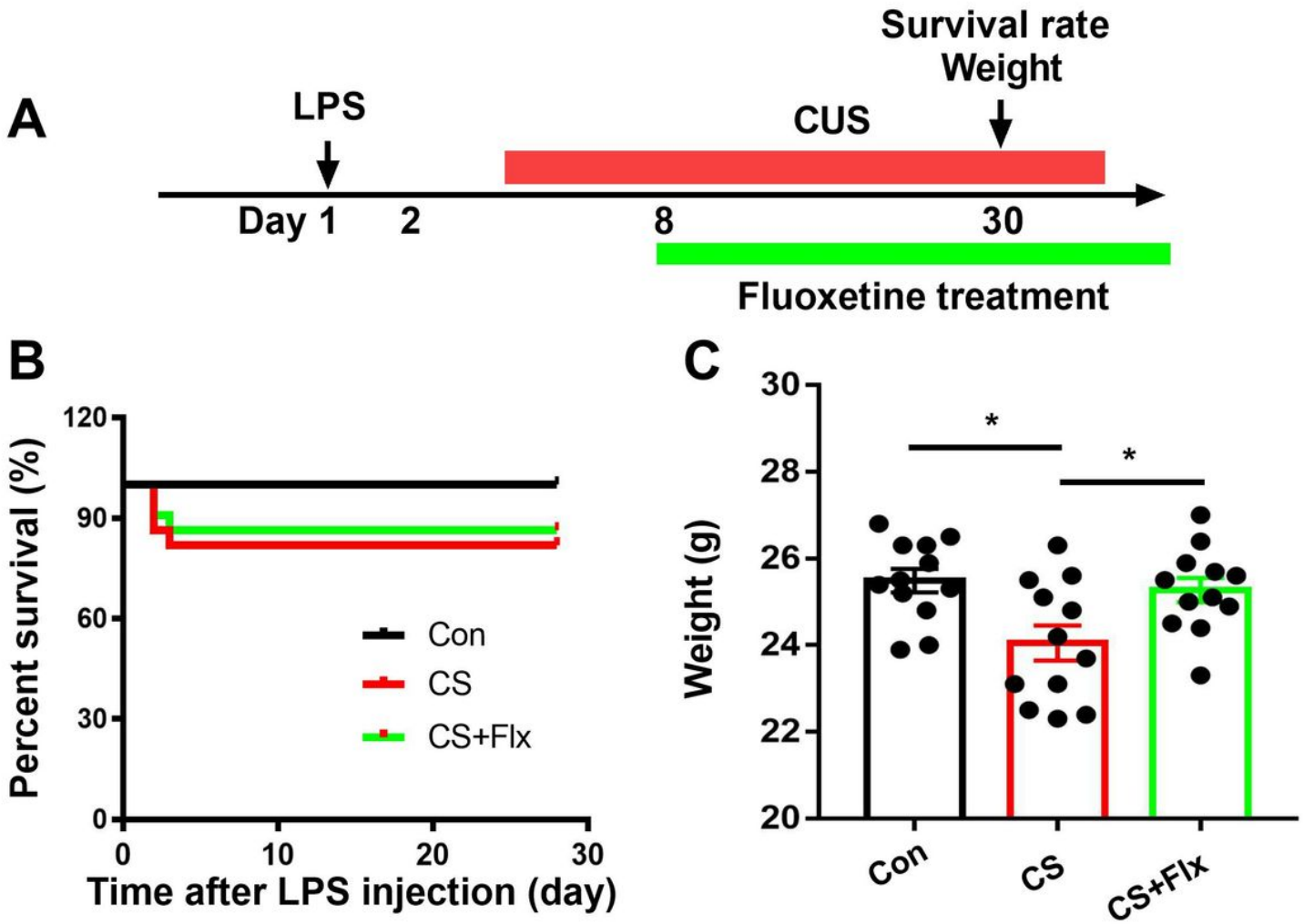

Figure 1

Survival rate and weight changes. (A) Schematic timeline of the experimental procedure. (B) No animals died in the control group, The survival rate was 81.818% in combined stress (CS) group and 86.364% in CS + fluoxetine group, respectively (n = 16-22). (C) CS induced significantly decreased weight compared with control group, which was prevented by chronic fluoxetine treatment. *P < 0.05. Con, control; LPS, lipopolysaccharide; Flx, fluoxetine; CS, combined stress.

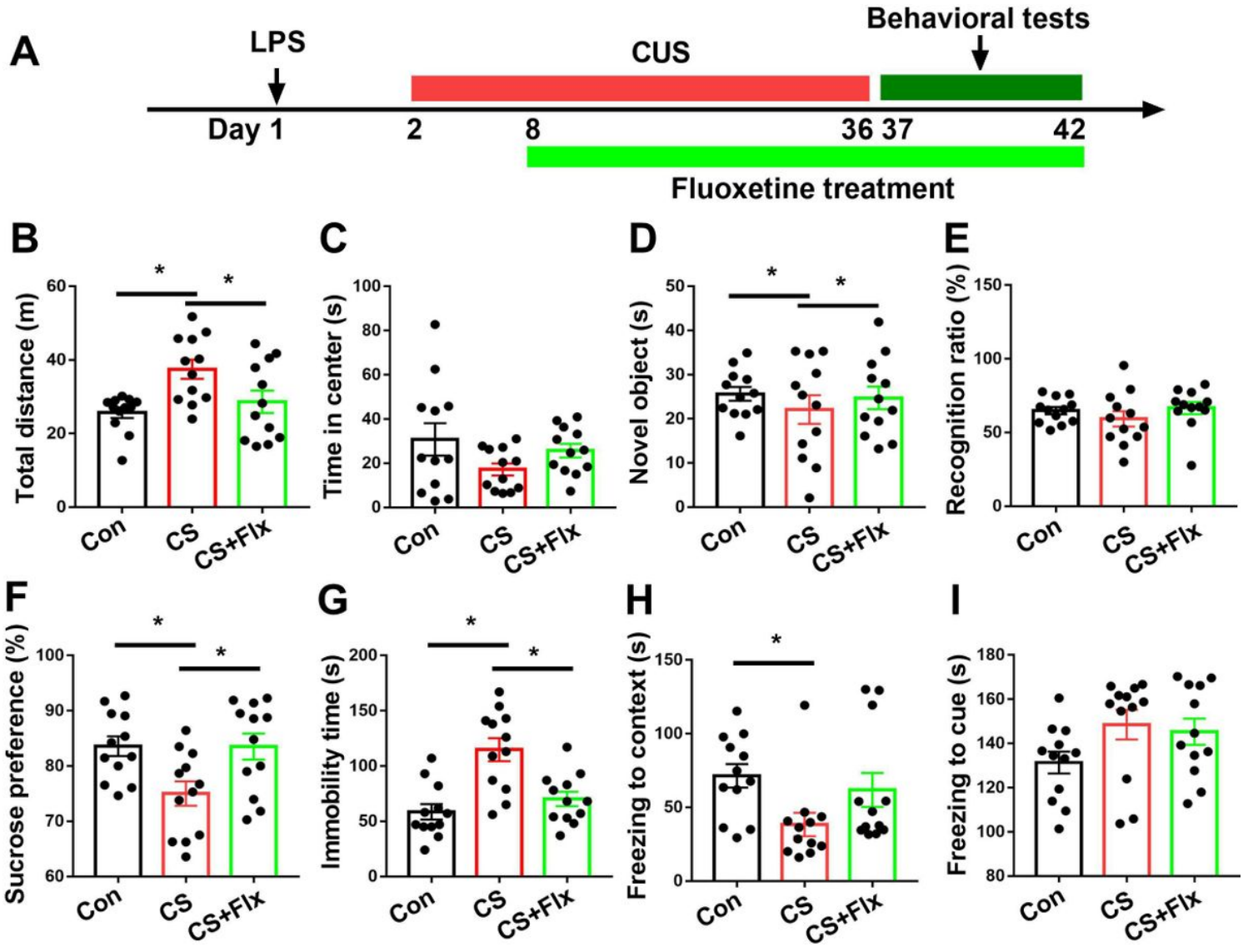

Figure 2

CS-induced behavioral abnormities were partially reversed by fluoxetine treatment. (A) Schematic timeline of the experimental procedure. (B) CS induced significantly decreased distance travelled 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 novel object, which was prevented by fluoxetine treatment. (E) There was no difference in recognition ratio among groups. (F) CS induced significantly decreased preference for sucrose, which was prevented by fluoxetine treatment. (G) Fluoxetine treatment reversed 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.

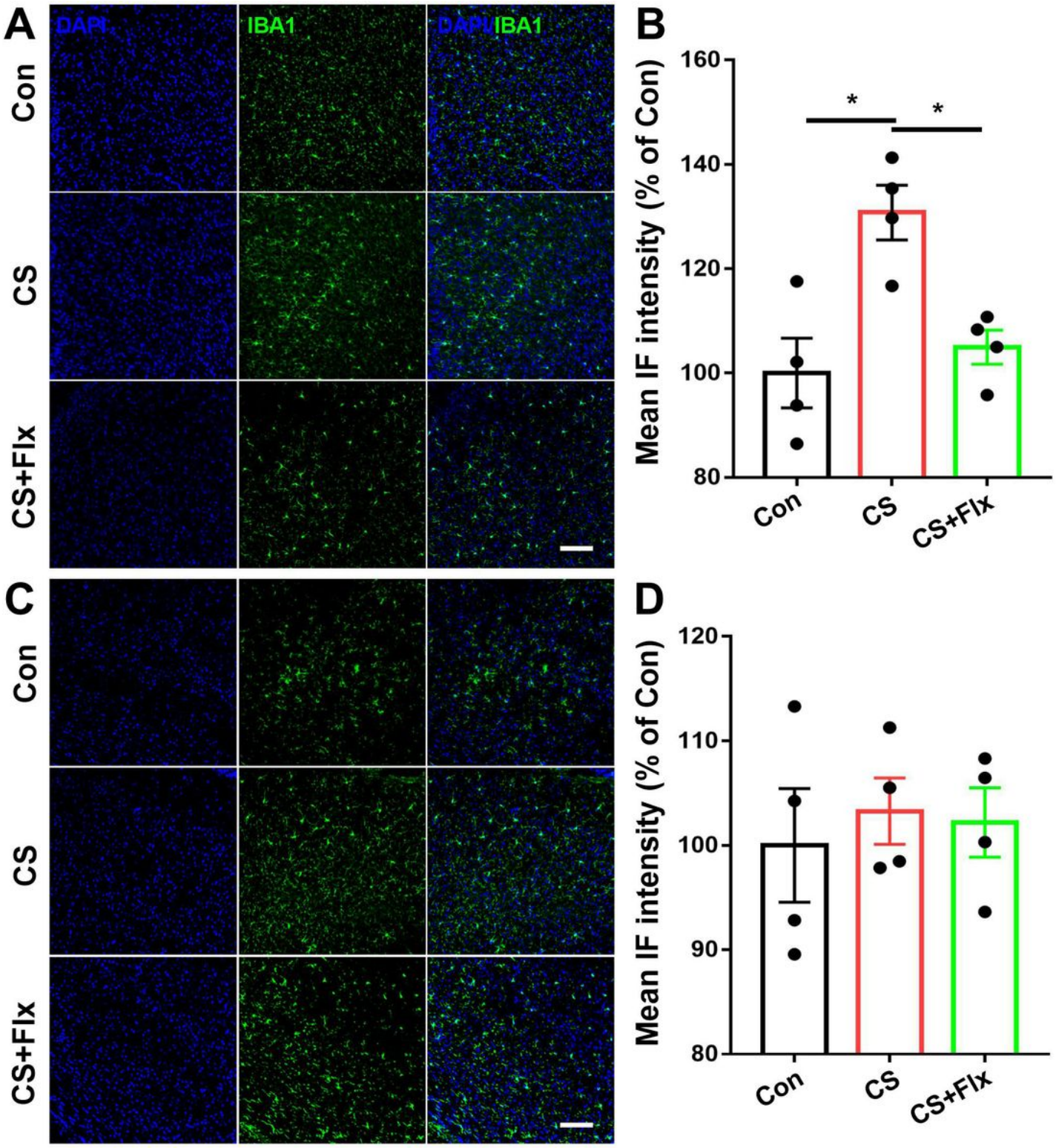

Figure 3

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 immufluorescence in the PFC. (C) Representative images of GFAP-positive cells in the PFC. (D) Quantification of mean GFAP immufluorescence in the PFC. Data are shown as mean ± SEM (n = 4), *P < 0.05, scale bar = 100 μm. Con, control; Flx, fluoxetine; IF, immufluorescence; CS, combined stress.

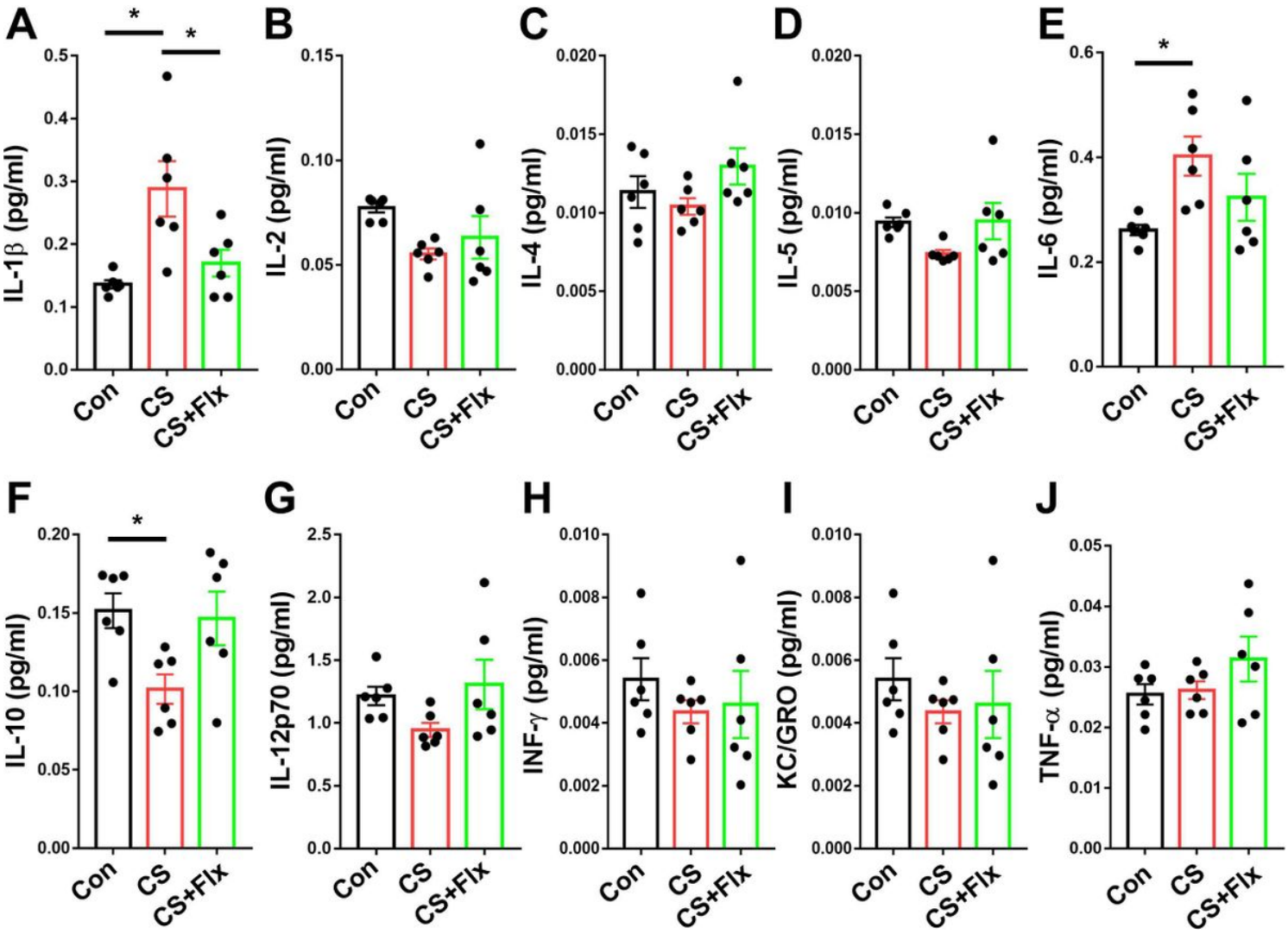

Figure 4

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.

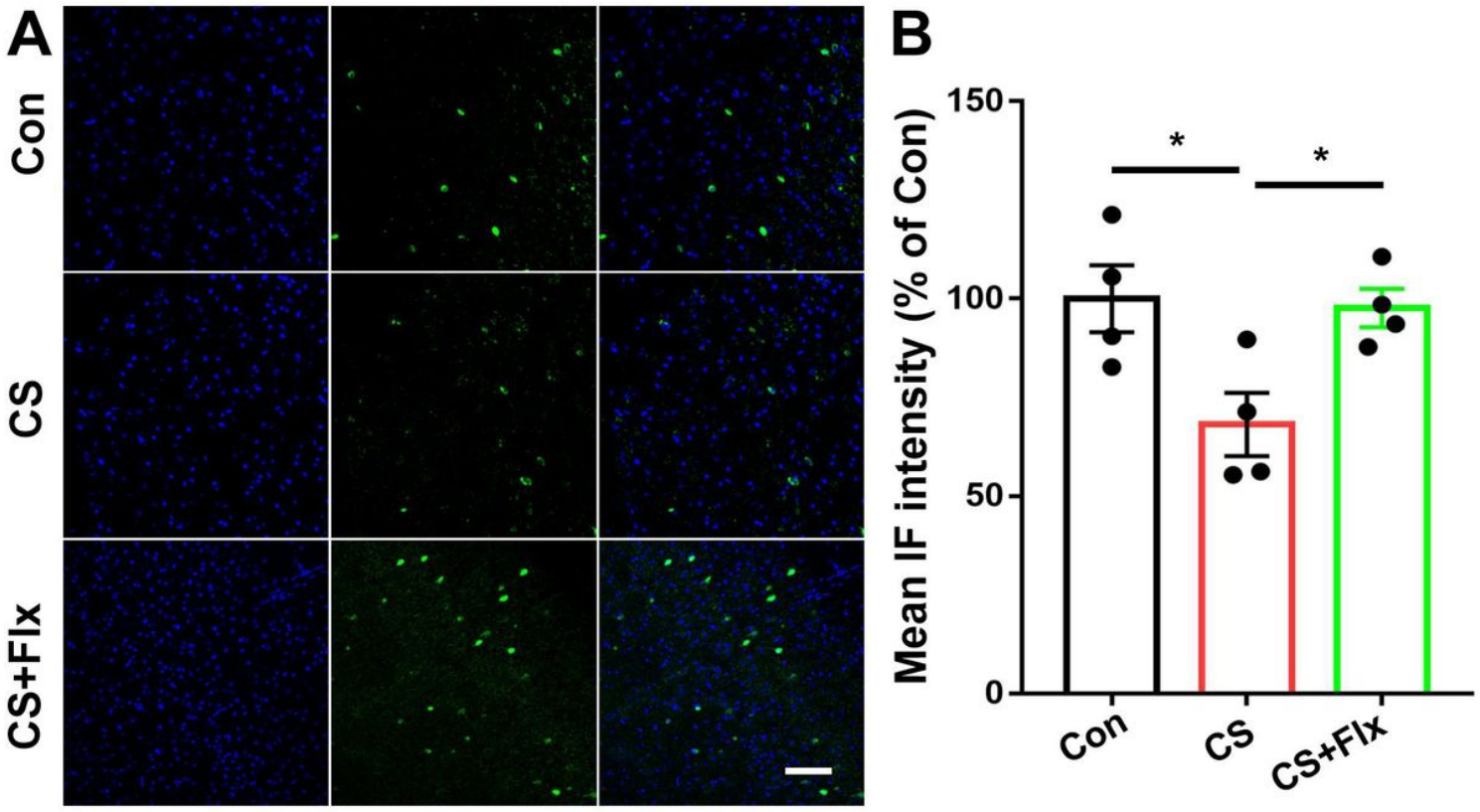

Figure 5

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 immufluorescence in the PFC. Data are shown as mean ± SEM (n = 4), *P < 0.05, scale bar = 100 μm. Con, control; Flx, fluoxetine; IF, immufluorescence; CS, combined stress.

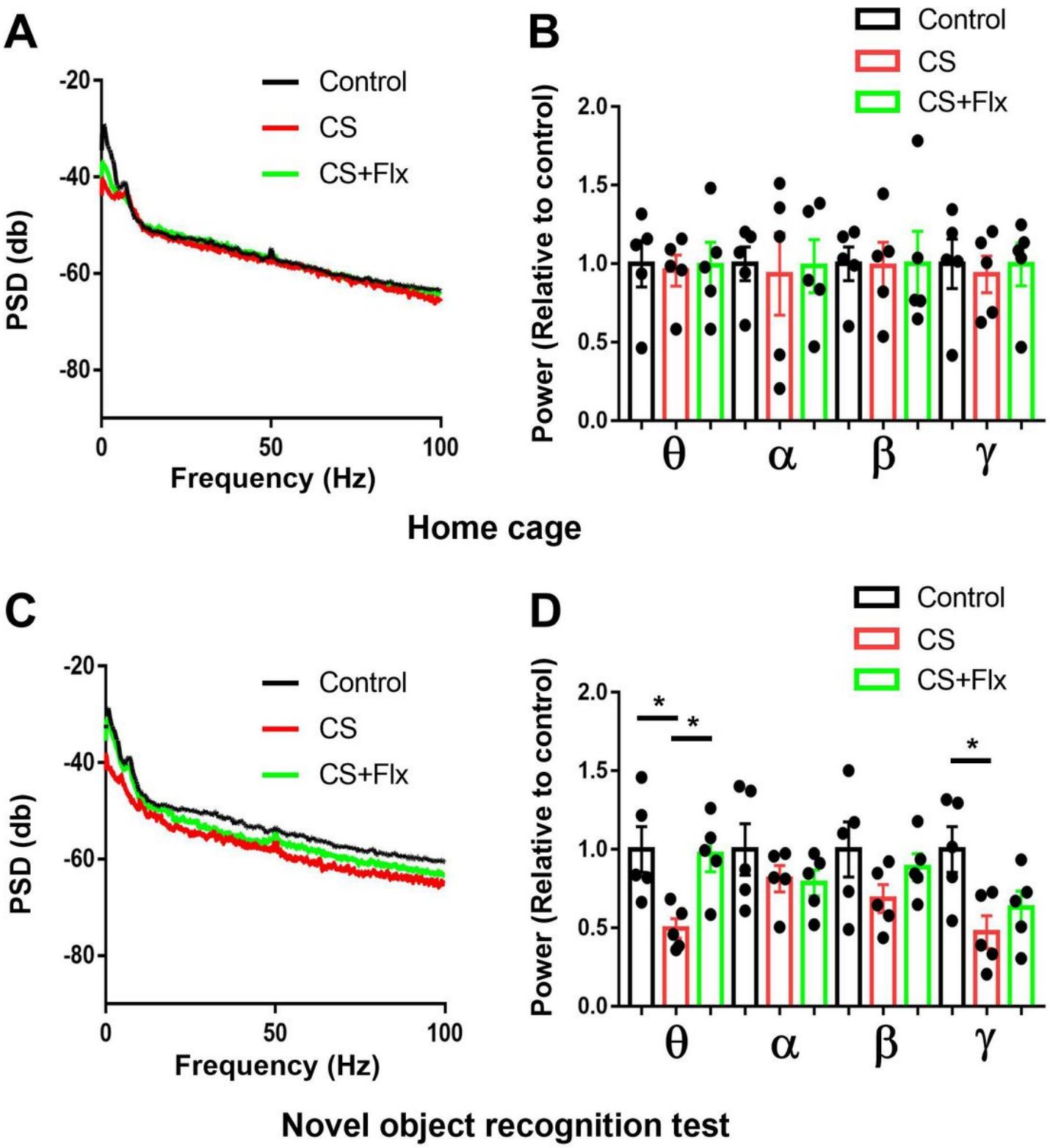

Figure 6

CS-induced altered neural oscillations in the PFC were partially reversed by fluoxetine treatment. (A) Quantification of local field potential in the PFC at 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, immufluorescence; CS, combined stress.