Behavioural Brain Research xxx (2013) xxx–xxx

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

FGF2 blocks PTSD symptoms via an astrocyte-based mechanism

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HIGHLIGHTS

- Intraperitoneal administration of FGF2 decreased the freezing time and anxiety behavior in rats.
- Single prolonged stress (SPS) induced astrocytic inhibition in the hippocampus.
- Astrocytic inhibition may be reversed by FGF2 application.
- SPS and FGF2 application had no effect on neurons.
- FGF2 blocks the PTSD symptoms via the astrocyte-based mechanism but not neurons.

ARTICLE INFO

Article history:
Received 8 January 2013
Received in revised form 23 August 2013
Accepted 28 August 2013
Available online xxx

Keywords:
PTSD
Astrocyte
FGF2
Fear response
Open field
Elevated plus maze
Double immunofluorescence western blot

ABSTRACT

Although posttraumatic stress disorder (PTSD) is characterized by traumatic memories or experiences and increased arousal, which can be partly alleviated by antidepressants, the underlying cellular mechanisms are not fully understood. As emerging studies have focused on the critical role of astrocytes in pathological mood disorders, we hypothesized that several 'astrocyte-related' mechanisms underlying PTSD exist. In the present study, using the single prolonged stress (SPS) model, we investigated the effects of intraperitoneal FGF2 on SPS-induced PTSD behavior response as well as the astrocytic activation after FGF2 administration in SPS rats. Behavioral data showed that intraperitoneal FGF2 inhibited SPS-induced hyperarousal and anxiety behavior; however, immunohistochemistry showed that SPS-induced astrocytic inhibition was activated by intraperitoneal FGF2. Quantitative western blotting showed that intraperitoneal FGF2 up-regulated glial fibrillary acidic protein (GFAP), but not NeuN, expression in the hippocampus. We suggest that intraperitoneal FGF2 could block the SPS-induced fear response and anxiety behavior in PTSD via astrocyte-based but not neuron-based mechanisms.

1. Introduction

Posttraumatic stress disorder (PTSD) is characterized by traumatic memories or experiences and increased arousal, which can be partly alleviated by antidepressants [1,2]. However, the underlying cellular mechanisms are not fully understood. As emerging studies have focused on the critical role of astrocytes in pathological mood disorders [3,4], we hypothesized the existence of 'astrocyte-related' mechanisms underlying PTSD. Astrocytes are the largest population of cells in the hippocampus, an important structure associated with stress; therefore, we investigated the role of astrocytes in PTSD.

Fibroblast growth factor 2 (FGF2), a mitogen involved in the molecular cascade of memory on extinction and relapse in rats [5–7], is synthesized in astrocytes, modulates the adult rat hippocampal neurogenesis and activates newborn neurons in acute stress [8], but its role in chronic stress such as PTSD remains unknown. In addition to being generally accepted as an angiogenesis factor [9–12], FGF2 has shown antidepressant effects in animal studies [13–16]. Several studies [17–20] demonstrated that FGF2 administration decreased anxiety or depression-like behavior, and the FGF system itself was altered after FGF2 administration. However, Graham and Richardson (2009) reported that acute systemic administration of FGF2 caused enhanced long-term extinction of

Abbreviations: SPS, single prolonged stress; PTSD, posttraumatic stress disorders; EPM, elevated plus maze; OF, open field; GFAP, glial fibrillary acidic protein; FGF2, fibroblast growth factor 2; PBS, phosphate buffered saline.

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0166-4328/$ – see front matter © 2013 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.bbr.2013.08.048

the fear memory and reduces reinstatement [21]; the fear memory is the main factor causing PTSD [22,23]. In addition, during manipulation of FGF2 and FGFR1 in animal models, FGF signaling in the hippocampus affected anxiety behavior and was correlated with the response to antidepressant treatment [24–26]. A previous study demonstrated that antidepressants such as clomipramine could reverse the stress-induced reduction of hippocampal GFAP expression in the chronic unpredictable stress model of depression [27]. These explanations focus on the effects of FGF2 on mood disorders while neglecting the possible contribution of astrocytes. Because the function of glial cells in the central nervous system has been re-evaluated and these cells are now considered to be involved in information processing [28], their role in the mechanism of mood disorders such as PTSD should be examined.

In the present study, we first confirmed the possible anxiolytic effect of FGF2 on the single prolonged stress (SPS) model via the intraperitoneal (IP) route using the fear response and anxiety behavior parameters. Parallel astrocytic activation following FGF2 treatments was evaluated by investigating astrocyte-specific GFAP by semi-quantitative immunofluorescent labeling and quantitative western blotting.

2. Methods

2.1. Animal preparation

Male Sprague-Dawley rats (180–200 g) were housed in plastic cages, and maintained on a 12:12 h light:dark cycle under an ambient temperature of 22–25 °C with food and water available ad libitum. All experimental procedures received prior approval from the Animal Use and Care Committee for Research and Education of the Fourth Military Medical University (Xi'an, China), and the ethical guidelines for experimental stress in conscious animals were followed. All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Drug administration

FGF2 (Fibroblast Growth Factor-2; R&D Systems) was reconstituted at a concentration of 10 mg/mL in phosphate-buffered saline (PBS) containing 0.1% bovine serum albumin (BSA). FGF2 was dissolved and diluted with PBS to 1% (5 g/100 mL) for IP administration (20 mg/kg, approximately 0.5 mL). Normal saline was used as the negative control. The dosages of FGF2 used in the present study were based on previous research [29] and our pilot experiment.

2.3. SPS

The SPS model created in this study consisted of the application of three stressors (restraint stress, forced swim, and ether exposure) followed by a quiescent period of seven days [30,31]. The rats were restrained for 2 h, followed immediately by 20 min of forced swimming in 20–24 °C water in a plastic tub (24-cm diameter, 50-cm height), filled two-thirds to the top. Following a 15-min recuperation, rats were exposed to ether (using a desiccator) until general anesthesia, defined as loss of toe and tail pinch responses, was induced (<5 min). Immediately after the induction of general anesthesia, rats were removed from the desiccator, placed in their home cages, and left undisturbed for seven days. For the control procedure, rats remained in their home cages without food and water for the duration of SPS.

2.4. Open field (OF) test

Rats were placed at the center of a cubic chamber (100 × 50 × 100 cm). The total distance that the animal traveled in 15 min was measured by an automated analyzing system (Shanghai Mobile Datum Information Technology Co., Ltd). This distance was used as a parameter for the rats' locomotion and the percentage of time spent in the center area (center time percentage) was used to evaluate anxiety levels [32]. All animals were habituated to the testing room for 20 min before the start of the session. The test room was dimly illuminated with indirect white lighting, as rats are nocturnal and their natural exploratory behavior is hindered in well-illuminated conditions.

2.5. Elevated plus maze (EPM) test

Animals were placed in an elevated plus maze (EPM) made of black Plexiglas consisting of two opposite-facing open arms (50 × 10 cm), two opposite-facing closed arms (50 × 10 × 40 cm), and a central area (10 × 10 cm). The walls of the closed arm were of clear Plexiglas. The EPM was mounted on a base and elevated 50 cm above the floor. Rats were placed in the central area facing the open arms. Then, a 5-min test session was started. The numbers of rats entering/climbing onto the open arms and closed arms were recorded by a video recorder, and later scored by two investigators blinded to the experiment. In addition, the amount of time the rats spent on each arm was recorded. An entry was defined as placing four paws onto the open arm [33]. Four measures of behavior in the EPM were scored: (1) time spent on the open arms; (2) time spent on the closed arms; (3) the number of entries onto the open arms; (4) the number of entries onto the closed arms. "OA entries %" = numbers of entries onto the open arms/(numbers of entries onto the open arms + closed arms) and "OA time %" = time spent on the open arms/(time spent on the open arms + closed arms).

2.6. Contextual fear conditioning and extinction

Fear conditioning and extinction as well as retention of the extinction memory were performed according to our previous report [34]. The test chamber consisted of a modified shuttle box (24.2 W × 24.2 D × 30 H cm, Shanghai Mobile Datum Information Technology Co., Ltd, Shanghai, China) constructed of four vertical Plexiglas sides, with a floor consisting of horizontal metal bars (0.5-cm diameter, spaced 1.5 cm apart) connected to an electric shock generator. The test chamber was placed inside a sound-attenuating chamber (Shanghai Mobile Datum Information Technology Co., Ltd.) with a plastic floor. The experiments were performed under dim light (4 lx) at approximately the same time each day.

On day 7, animals were placed in the test chamber and left to explore the environment for 2 min followed by 10 tone-shock paired trainings. The conditioned stimulus (CS) was a tone (amplitude: 80 dB; frequency: 4 kHz, sine wave; duration: 30 s; inter-trial interval (ITI): 1–4 min) and the unconditioned stimulus (US) was an electric shock (0.6 mA, 5 s, co-terminated with tone) delivered through the chamber floor bars.

Extinction trials were administered in box B to animals 24 h after the final conditioning trial. Retention tests were conducted in box B on days 7, 10 and 14. The extinction trials were 30 tones (ITI: 1–2 min; all other parameters were as for fear conditioning) in the absence of electric shock. For the retention test, five tones (ITI: 1–2 min) were administered. The chamber walls, floor, floor bars and tray underneath the floor were cleaned with 70% ethanol between sessions.

Freezing during the presentation of CS alone was defined as complete immobility of the animal in a stereotypical crouching position, except for movements necessary for breathing. The position and shape of rats were dynamically returned based on

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computer-aided contrast-detecting image processing and calculation. Based on the threshold set for immobility, the freezing times were calculated. All image processing and calculations were compiled using the Dr. Rat Rodents' Behavior System (Shanghai Mobile Datum Information Technology Co., Ltd). Freezing behavior during five randomly selected CS was analyzed from a video recorded by a camera positioned above the operant chamber for a 10-min observing period (5–9 CS) after the last CS–US pairing in fear conditioning or during retention tests, by presenting only CS with a random ITI of 1–2 min. After the 10-min video recording, rats were returned to their home cages. Only one rat at a time was present in the experimental room; the other rats remained in their home cages. Each rat was carried to the behavioral room in a new cage that was identical to the home cage. For the contextual conditioning experiments, rats were placed in the conditioning chamber 180 s before the onset of the US (continuous foot shock at 0.8 mA, for 4 s). After the test, rats were placed back in their home cages. Twenty-four hours later, rats were again placed in the same conditioning chamber and contextual freezing was assessed. Conditioning was assessed based on measurements of freezing, defined as the total absence of body and head movement except for that associated with breathing. Freezing behavior of the rat was recorded using a video recorder, and later scored by a well-trained investigator blinded to the experiment. Fear level was quantified as the amount of time (in seconds) spent freezing.

Extinction training was defined as the repetitive exposure to the contextual cue in the absence of foot shock. Twenty-four hours after fear conditioning, rats were placed for 10 min without foot shock in the chamber in which the foot shock was delivered. All image processing and calculations were performed using the Dr. Rat Rodents' Behavior System (Shanghai Mobile Datum Information Technology Co., Ltd).

2.7. Immunohistochemistry

After deep anesthesia induced with urethane (2 g/kg, ip), rats were perfused through the ascending aorta with 100 mL of 0.9% saline followed by 500 mL of 0.1 M phosphate buffer (pH 7.3) containing 4% paraformaldehyde and 2% picric acid. After perfusion, the brain was removed and post-fixed in the same fixative for 2 h and then cryoprotected for 24 h at 4 °C in 0.1 M phosphate buffer containing 30% sucrose. Transverse frozen hippocampus sections (30 μm thick) were cut using a cryostat (Leica CM1800, Heidelberg, Germany) and collected serially in three dishes. Each dish contained a complete set of serial sections that were processed for immunofluorescence staining with one dish selected at random. The sections in the dish were rinsed in 0.01 M phosphate-buffered saline (PBS, pH 7.3) three times (10 min each), blocked with 2% goat serum in 0.01 M PBS that contained 0.3% Triton X-100 for 1 h at 22–25 °C, and then used for immunofluorescence staining. The sections were incubated overnight at 4 °C with the primary antibody, mouse anti-GFAP (1:5000; Chemicon, Temecula, CA, USA). The sections were washed three times in 0.01 M PBS (10 min each) and then incubated for 4 h at 22–25 °C with the secondary antibody, Alexa Fluor 488-conjugated donkey anti-mouse IgG (1:500; Vector, Burlingame, CA, USA). The specificity of the staining was tested on sections in another dish by omission of the primary specific antibodies. No immunoreactive products were found on the sections (data not shown). Confocal images were obtained using a confocal laser microscope (FV1000; Olympus, Tokyo, Japan) and digital images were captured using the Fluoview 1000 software (Olympus, Tokyo, Japan). Consecutive stacks of images (z-step 0.5 μm) were acquired at high magnification (×120) to capture images of the entire astrocyte. The three-dimensional GFAP graphs were then analyzed using Imaris 6.5.0 (Bitplane AG, Zurich, Switzerland).

2.8. Double immunofluorescence western blotting

All animals were sacrificed and the hippocampi rapidly removed and frozen on dry ice. The hippocampus was dissected and the selected region was homogenized with a hand-held pestle in sodium dodecyl sulfate sample buffer (10 mL/mg tissue), which contained a cocktail of proteinase and phosphatase inhibitors. The electrophoresis samples were heated at 100 °C for 5 min and loaded onto 10% sodium dodecyl sulfate–polyacrylamide gels with standard Laemmli solutions (Bio-Rad Laboratories, Hercules, CA, USA). The proteins were electroblotted onto polyvinylidene difluoride membranes (Immobilon-P; Millipore, Billerica, MA, USA). The membranes were placed in a blocking solution, which contained Tris-buffered saline with 0.02% Tween and 5% non-fat dry milk, for 1 h, and incubated overnight under gentle agitation with the primary antibodies, mouse anti-GFAP (1:5000; Chemicon), rabbit anti-NeuN (1:5000; Chemicon) and mouse anti-β-actin (1:5000; Sigma). Bound primary antibodies were detected with the anti-mouse Alexa 488-conjugated secondary antibody and anti-rabbit Alexa 594-conjugated secondary antibody (1:500; AmerControl Pharmacia Biotech Inc., Piscataway, NJ, USA). After 2 h, the bands were detected using the Bio-Rad system. Between each step, the immunoblots were rinsed with Tris-buffered saline containing 0.02% Tween.

2.9. Experimental procedures

All rats were divided into two groups (Fig. 1), each of which was used in a separate experiment. Experiment a (Fig. 1a) evaluated the effects of ip FGF2 application on conditioned fear response and GFAP expression. SPS procedures were performed 2 h before behavioral tests on SPS days 7 and 14. FGF2 was injected from SPS 8 to 10 once per day 30 min before the conditioned fear response test. Rats in experiment b (Fig. 1b) were sacrificed on SPS day 14 for immunohistochemistry or western blotting. Experiment b was designed to assess ip FGF2 application (0.5%, 20 mg/kg) on anxiety behavior and astrocytic activation in the SPS animal model. The open field (OF) and EPM times were recorded seven days after SPS procedures. FGF2 was administered 30 min before the behavioral test on SPS day 7 from 8 to 9 am. Then, from SPS day 7 to 10, drugs were administered once per day and behavioral tests were performed on SPS day 14. Rats in both experiments were sacrificed on SPS day 14 for immunohistochemistry or western blotting.

2.10. Quantification and statistical analysis

The densities of protein blots were analyzed using the Labworks software (Ultra-Violet Products, Cambridge, UK). The densities of GFAP and β-actin immunoreactive bands were quantified with background subtraction. A square of identical size was drawn around each band to measure the density and the background intensity near the band was subtracted. As β-actin levels did not change significantly after inflammation and SPS [35], we used β-actin levels as a loading control, and GFAP levels were normalized against β-actin levels and expressed as a fold change compared to the control.

All data are presented as means ± standard error of mean (SEM) and collected by researchers blinded to the surgery and reagents used. ANOVA followed by the least-significant-difference test was used to evaluate western blot results. Repeated measures ANOVA (with Bonferroni confidence interval adjustment) was conducted for behavioral data, which were confirmed to be normally distributed by one-sample Kolmogorov–Smirnov test (data not shown); $p < 0.05$ was considered to indicate statistical significance.

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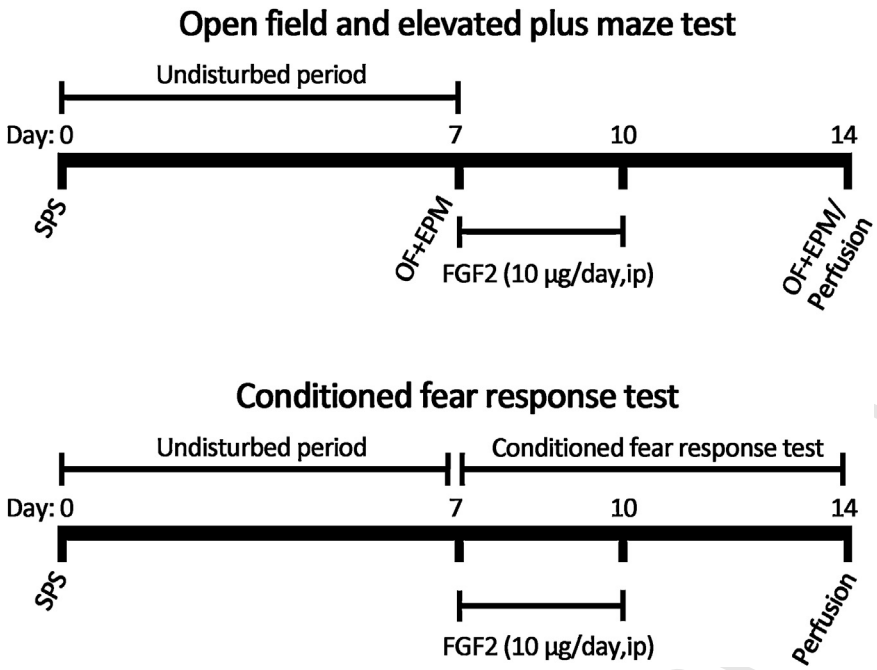

Fig. 1. Experimental procedures: time window displaying the days after single prolonged stress (SPS) or drug application.

All statistical analyses were performed using the SPSS software, version 16.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. IP FGF2 alleviated the conditioned fear response

SPS rats exhibit enhanced freezing in response to contextual fear conditioning, and impaired extinction of fear memory [36,37]. To assess the effect of ip FGF2 on the maintenance of SPS-induced hyperarousal behavior, we applied FGF2 from SPS day 7 to 10, when the hyperarousal was stable according to our previous study [38], and we observed the effect of SPS and SPS + FGF2 at days 7, 10 and 14 in the subject groups (one group for all time points). Compared to the SPS groups, FGF2 administration decreased the freezing time in the SPS + FGF2 group on SPS day 10 in the CS + CS in a neutral context (CSn) test ($p < 0.01$). This decreasing effect in the SPS + FGF2 group was maintained until SPS day 14 (Fig. 2A and B). These results suggested that IP FGF2 alleviated the conditioned fear response in the SPS rats.

On SPS day 7 of experiment a, the freezing time of the SPS group was 63.2 ± 4.8 s, which was significantly higher than the control group (15.0 ± 2.3 s) ($p < 0.001$). On SPS days 10 and 14, the freezing times of the SPS group were 81.8 ± 6.9 s (SPS day 7) and 72.1 ± 4.7 s (SPS day 14) (Fig. 2A). Therefore, SPS increased the freezing time from SPS day 7 to 14, which is consistent with previous reports [38,39].

3.2. Effects of systemic administration of FGF2 on anxiety behavior

To examine the effects of FGF2 administration on anxiety behavior in the SPS model, animals were subjected to the OF and EPM tests. Records of the distance in the central area and the time spent in the central area within 15 min in the OF showed that SPS rats display abnormal hyperactivity, compared with control rats. This is consistent with a previous report that SPS rats spent less time in the central area and covered less distance in the central area in the OF test [40]. Thus, as expected, the rats in the SPS + FGF2 group spent more time in the central area than the SPS day 14 group

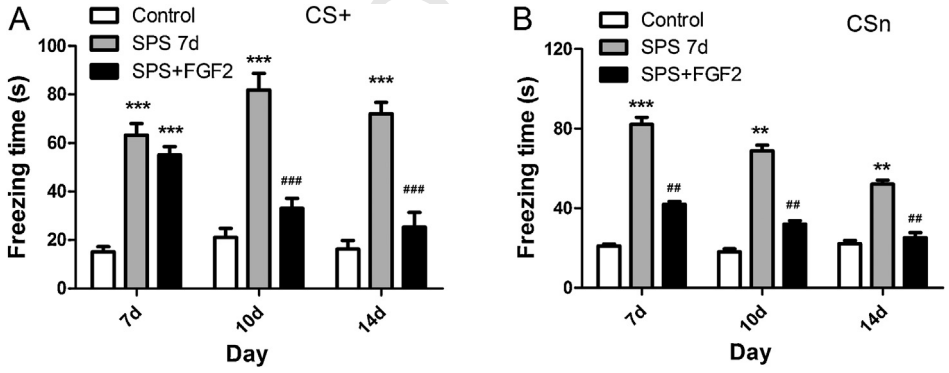

Fig. 2. Systemic administration of FGF2 ameliorated the enhanced fear response in the conditioned fear extinction and sensitized fear tests. CS+: test for conditioned fear response to shock chamber; CSn: test for sensitized fear response to neutral tone in a neutral context. On days 10 and 14, there were significant differences in the freezing levels between the SPS and control, and SPS + FGF2 groups. Data are presented as means ± SEM. $^{***}p < 0.001$ compared to the control group. $^{###}p < 0.001$ compared to the SPS group. Each group consisted of six animals.

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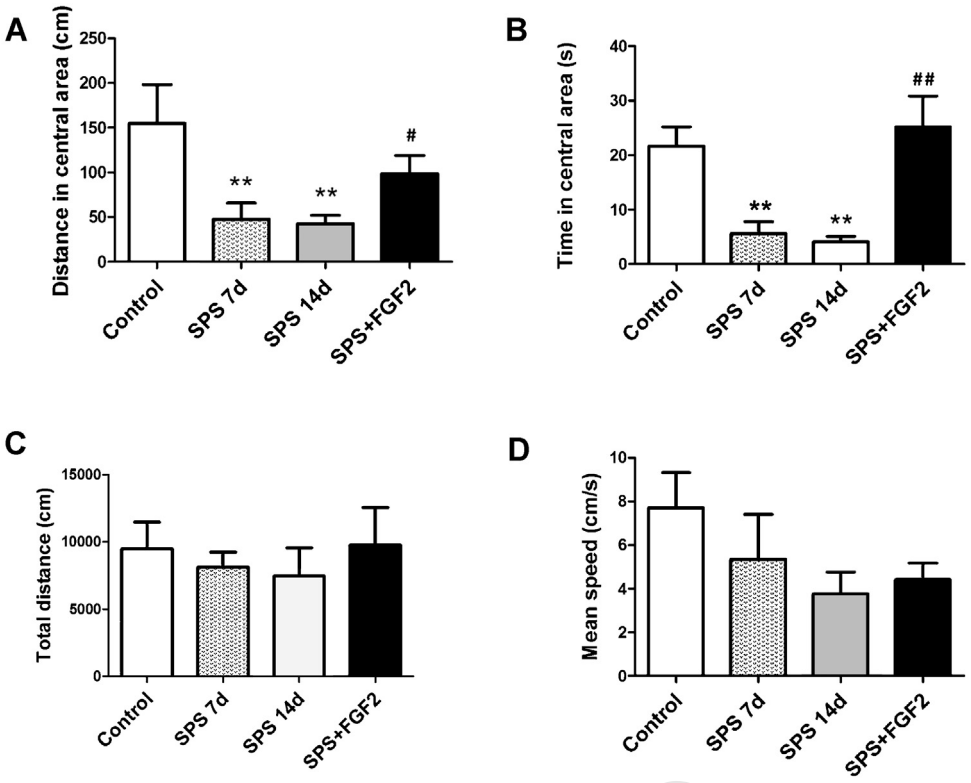

Fig. 3. Systemic administration of FGF2 produced anxiolytic-like behavior in a rodent model of PTSD. Comparison of distance in the central area (A), time in the central area (B), total distance (C) and mean speed (D) in OF among control, SPS day 7, SPS day 14, and SPS + FGF2 rats. Data are plotted as means ± SEM. Quantified performance in the EPM of SPS ($n = 8$), control ($n = 10$) or SPS + FGF2 ($n = 8$) rats. **Indicates statistically significant difference ($p < 0.01$) compared to the control group. #Indicates statistically significant difference ($p < 0.05$) compared to the SPS day 14 group.

($p < 0.01$). Furthermore, the total distance in the central area in the SPS + FGF2 group was significantly greater than that in the SPS day 14 group (Fig. 3A and B, $p < 0.05$), indicating that the FGF2 rats displayed less anxiety behavior than the SPS day 14 rats. The total distance traveled in the OF and mean speed were not significantly different among the four groups. These results further confirmed that SPS can cause a high level of anxiety behavior and that systemic administration of FGF2 can alleviate that symptom.

The time spent on the open arms and the frequency of entry onto the open arms was significantly reduced in the SPS day 7 rats compared to the control group. After FGF2 injection, the time spent on the open arms and the frequency of entry onto the open arms became normal compared to the control group in the SPS day 14 group ($p < 0.01$) (Fig. 4A and B). The time spent on the open arms and the frequency of entry onto the open arms in the SPS + FGF2 group was significantly elevated after FGF2 administration compared to the SPS group, indicating that systemic FGF2 application alleviated the anxiety response. As expected, the percentage of time in the closed arms and the percentage of entries onto the closed arms did not differ among the four groups (Fig. 4C and D).

3.3. SPS-induced significant GFAP inhibition

SPS induced marked GFAP inhibition in the hippocampus and anterior cingulate cortex (ACC), as indicated by GFAP down-regulation in the SPS day 7 group compared with the control group in hippocampus and ACC (Figs. 5 and 6). Immunohistochemistry indicated that inhibited GFAP presented atrophied cell bodies and thinned processes with decreased GFAP immunoreactivity (Figs. 5 and 6). We observed significant GFAP down-regulation on SPS day 1, peaked on SPS day 7, then remained at high levels 14 days after SPS. Using double-immunofluorescence western blotting, we performed quantitative studies to confirm the above results. In the experiment, GFAP expression was 2.6-fold decreased ($p < 0.01$) in the SPS day 7 group and 3.2-fold decreased in the SPS day 14 group compared to the control group (Fig. 7B).

3.4. Effects of ip FGF2 on SPS-induced GFAP inhibition

To assess the effect of systemic administration of FGF2 on the initiation of GFAP activation, we injected FGF2 daily from SPS day 7 to 10 and investigated GFAP expression on SPS day 14 when GFAP activation was assumed to be at a peak level. No significant difference in GFAP immunodensity was observed between the SPS + FGF2 and control groups. In addition, the morphology of GFAP expression in the SPS + FGF2 (FGF2) group differed significantly after systemic FGF2 administration compared to the SPS day 14 group (Figs. 5 and 6). In accordance with the immunohistochemical results, after administration of a high dose of FGF2 on SPS day 7 for three days, the expression of GFAP was reversed at SPS day 14. Western blotting showed that GFAP expression in SPS + FGF2 rats was not different to that in the control group (Fig. 7B), but differed significantly between FGF2 treatment and SPS day 14 rats.

4. Discussion

Clinical studies have shown that GFAP expression is reduced in patients with major depression [41], and emerging studies have demonstrated that astrocytes may be an important underlying antidepressive mechanism [42]. In addition, a reduction in the density of GFAP immunoreactive astrocytes was observed in the amygdala of subjects with major depressive disorders compared to controls [43]. However, to our knowledge, our study is the first to show that the astrocyte-specific biomarker GFAP is reduced in the

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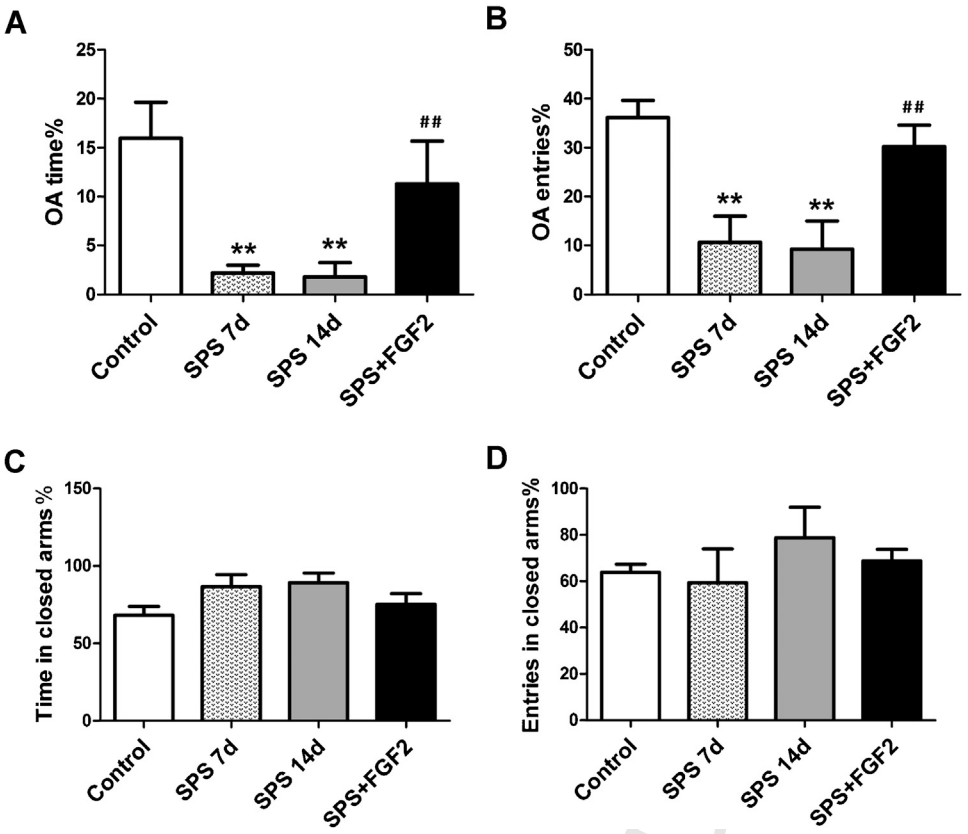

Fig. 4. Effects of systemic administration of FGF2 on SPS rats in the EPM test. Comparison of the percentage of open arm entries (OA entries %) (A), percentage of time spent on the open arms (OA time %) (B), time on closed arms (C) and entries onto closed arms (D) in the EPM test. Data are plotted as means ± S.E.M. Quantified performance in the EPM test of SPS ($n = 8$), control ($n = 10$) or SPS + FGF2 ($n = 8$) rats. **Indicates statistically significant difference ($p < 0.01$) compared to the control group. ##Indicates statistically significant difference ($p < 0.01$) compared to the SPS day 14 group.

SPS, a well-recognized animal model of PTSD [44–46], and systemic FGF2 application up-regulated astrocyte activation and alleviated SPS-induced anxiety behavior in rats, as indicated by the OF/EPM test.

The SPS paradigm is considered a model of PTSD, and the conditioned fear response is a main symptom of PTSD in rats, which is characterized by a high freezing time in the chamber [38,47,48]. Moreover, FGF2 enhanced extinction and reduced renewal of conditioned fear [49], and has been shown to both facilitate long-term extinction of fear and reduce stress-precipitated relapse in rats [2,3]. We found that three days of FGF2 administration decreased the freezing time in the conditioned fear response test in the SPS paradigm, as well as anxiety behaviors in the EPM, consistent with reports of increased anxiety in mice with a FGF2 mutation that decreases secretion. The IP FGF2 administration did not influence the total distance and mean speed in OF tests, and decreased anxiety behavior in the EPM tests, consistent with previous reports of intraventricular FGF2 administration. However, the above-mentioned OF and EPM behavioral test results showed that FGF2 can alleviate the anxiety level. Moreover, there was no effect on activity in locomotor chambers, providing further evidence that FGF2 administration does not influence general activity levels.

Although hippocampal FGF2 is a central integrator of the genetic and environmental factors that modify anxiety [50], how the ip FGF2 injection affects the astrocytes in the hippocampus remains unknown. There are reports that peripheral FGF2 can cross the blood–brain barrier, although this remains controversial [51–55], and additional studies are required to determine if the effects observed in this study are direct or indirect. The findings presented here also raise the possibility that the therapeutic effects of FGF2 could be mediated, in part, by the increased action of astrocytes; FGF2 reportedly can regulate GFAP expression [51]. Moreover, a previous study has demonstrated that animals selectively bred for high anxiety/low novelty seeking, and who express lower FGF2 mRNA levels in the hippocampus, can reverse their highly anxious phenotype by consistent administration of FGF2 [18]. The majority of evidence in animal models suggests that FGF2 plays an important role in mediating anxiety behaviors.

PTSD patients may have atrophy in the hippocampus [56,57]. Because astrocytes are the largest population of cells in the hippocampus, it is plausible that changes in hippocampal astrocytes are reflected in the PTSD animal model. While previous studies suggested the involvement of astrocytes in mood disorders [43,58], emerging studies have suggested a critical role for astrocytes in the efficacy of antidepressant drugs [59,60]. Moreover, astrocytes express receptors for various neurotransmitters, which enable them to respond to neural signals and thus be activated [28,61]. Activated astrocytes produce numerous mediators, such as pro-inflammatory cytokines and growth factors that enhance neuronal activity [62,63]. Reportedly, astrocytes participate not only in the induction but also the maintenance of fear memory [64]. Collectively, research suggests that the action of astrocytes may be an important target in PTSD.

Our results revealed atrophied cell bodies and thinned processes with decreased GFAP immunoreactivity in the SPS, suggesting that the number of astrocytes may be related to the incidence of PTSD. Antidepressant treatment can stimulate adult neurogenesis in the hippocampal dentate gyrus and that this specific effect may contribute to their therapeutic efficacy [65]. Additionally, FGF2 is widely accepted as an endogenous antidepressant

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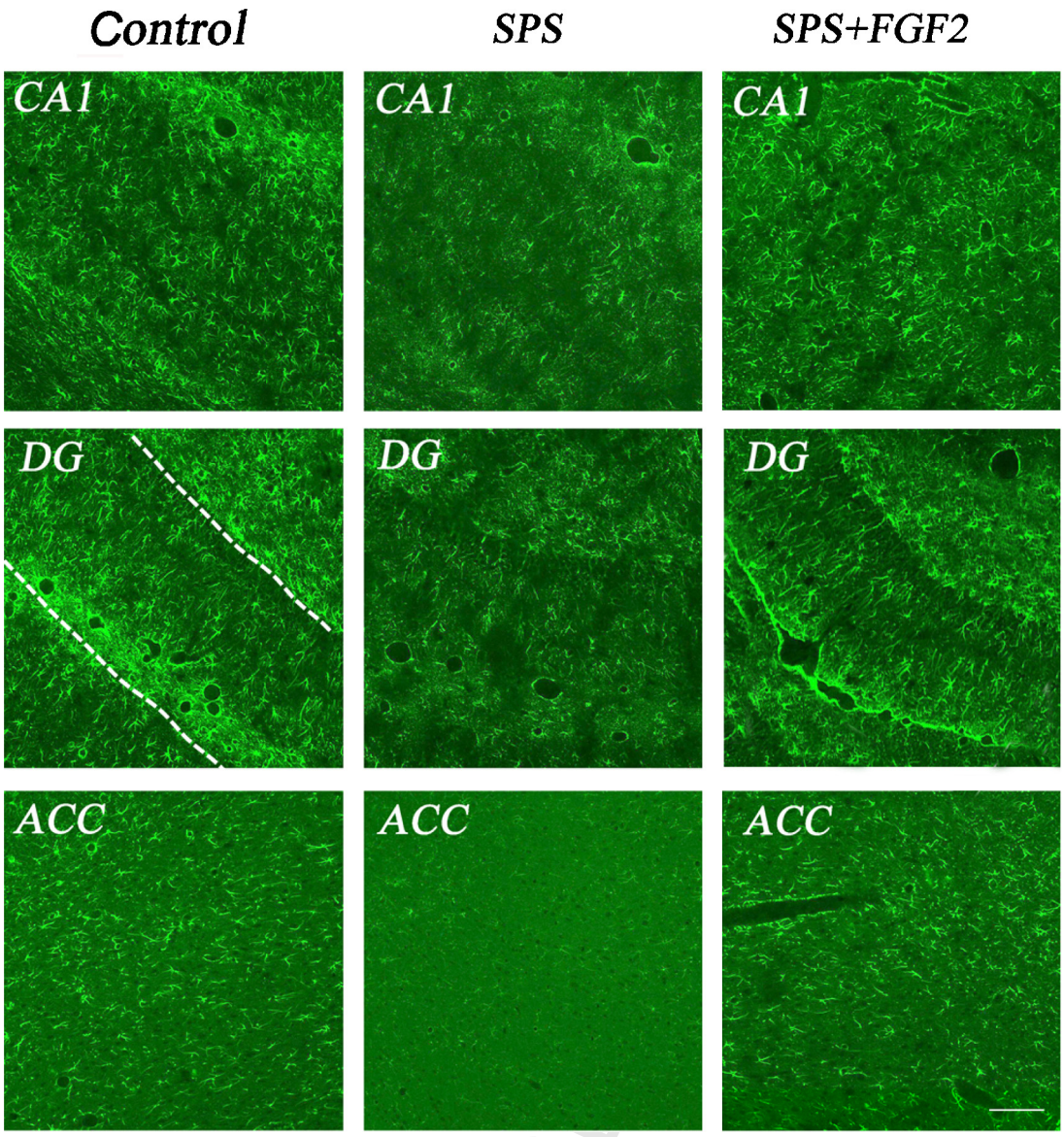

Fig. 5. Effects of SPS and intraperitoneal administration of FGF2 on GFAP expression in the rat hippocampus and ACC. Immunohistochemistry results showed that SPS inhibited the astrocytic action in the CA1, DG and ACC. However, in the FGF2 treatment groups, intraperitoneal FGF2 application from SPS day 7 to 10 activated GFAP immunodensities to the normal level in the hippocampus and ACC. Scale bars = 100 μm.

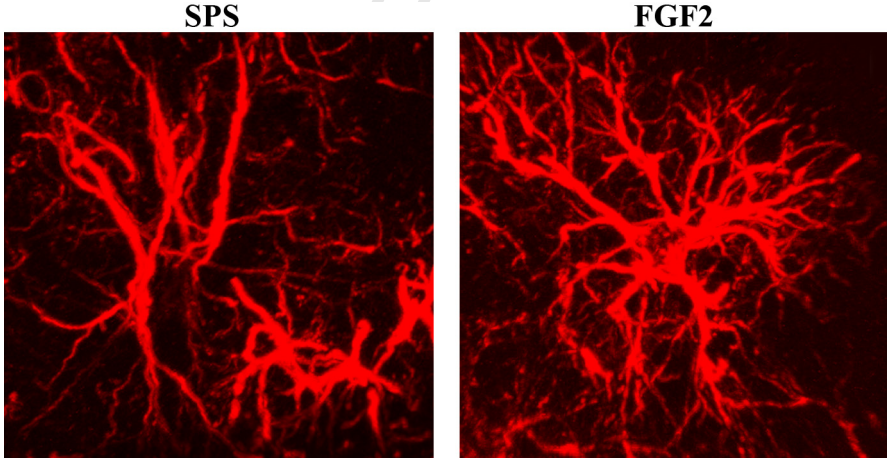

Fig. 6. The effects of FGF2 on GFAP expression in hippocampus induced by SPS. Three-dimensional reconstruction of the complete morphology of GFAP by Imaris 7.5.0 is shown; GFAP is stained red. Scale bars = 10 μm.

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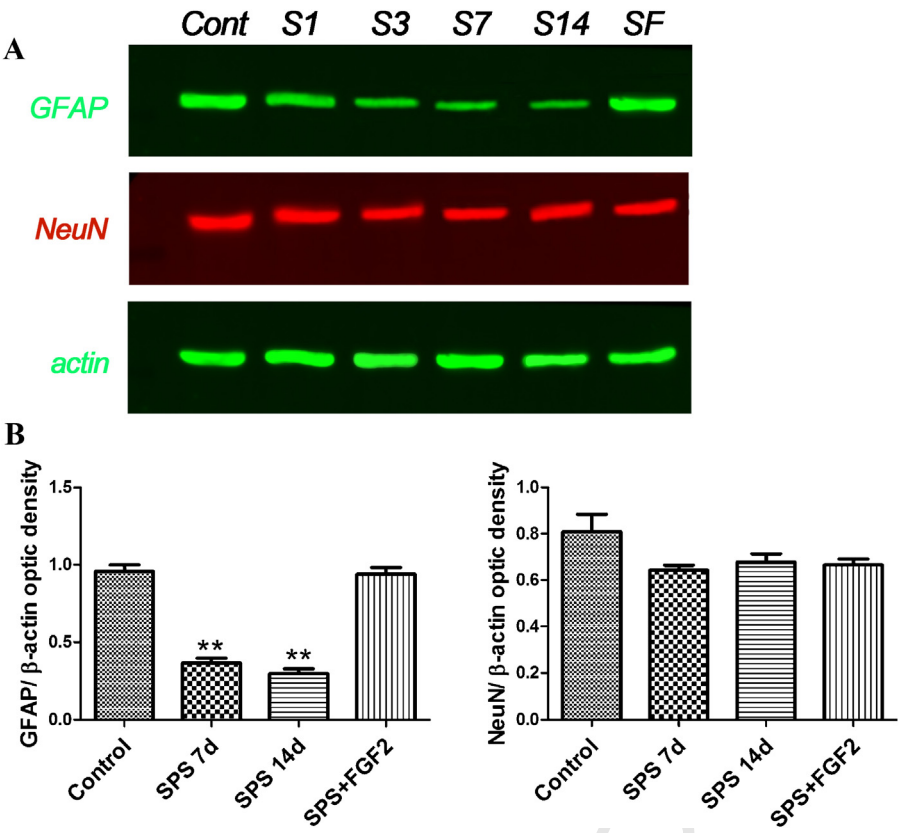

Fig. 7. Effects of intraperitoneal administration of FGF2 on GFAP and NeuN expression in the hippocampus of SPS rats. (A) Schematic overview of the region detected by western blotting. SPS induced a marked astrocytic inhibition indicated by GFAP down-regulation in the hippocampus, but NeuN expression was not altered in any group. (B) SPS-induced astrocytic inhibition was confirmed by the lower expression of GFAP in the SPS groups in each experiment. SF: SPS + FGF2 group; S1: SPS day 1 group; S3: SPS day 3 group; S7: SPS day 7 group; S14: SPS day 14 group. **Indicates statistically significant difference ($p < 0.01$) compared to the control-saline group. ###Indicates statistically significant difference ($p < 0.001$) compared to the SPS day 14 group.

and anxiolytic molecule [66,67]. Chronic antidepressant treatment increased levels of FGF2 and FGF-binding protein in hippocampal neurons [25], but whether FGF2 can be used as an anti-PTSD drug and affect astrocyte action remains unknown. Moreover, many theories of the abnormal functioning of glia cells contributing to the pathophysiology of major psychiatric disorders exist [68,69], and antidepressant therapies regulating the expression of GFAP and other astroglia-specific proteins [27,70]. We showed that treatment with FGF2 increases the activation of astrocytes and alleviates the symptoms of PTSD behavior. However, the molecular signaling pathway underlying the effect of FGF2 on astrocytes needs further study.

Our data indicate that ip administration of FGF2 decreased the freezing time and anxiety behavior, suggesting that FGF2 application may be a novel therapeutic for PTSD, and that SPS-induced astrocytic inhibition may be reversed by FGF2. To our knowledge, this is the first report that astrocytes may be the cellular targets underlying the mechanisms of PTSD. Our study suggests that astrocyte-related mechanisms are important in the incidence and treatment of PTSD.

Role of the funding source

The study was supported by grants from the Natural Science Foundation of China (Nos. 81171052 and 31070976) and intramural funds of the Fourth Military Medical University (China). The sponsors had no role in the design and conduct of the study; in the collection, analysis, and interpretation of the data; or in the preparation, review, or approval of the manuscript.

Contributors

Author WW, ZX designed the study and wrote the protocol. Author XL, Miao DM managed the literature searches and analyses. Authors XL, Wang LY and Zhai MZ undertook the statistical analysis, and author XL wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

Acknowledgement

We thank Dr. Liao Yonghui, who kindly provided the data necessary for our analysis, and Dr. Chen Lei, who assisted with the preparation and proof-reading of the manuscript. The manuscript was strictly checked by the professional company.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbr.2013.08.048.

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