Various BDNF administrations attenuate SPS-induced anxiety-like behaviors

Jun-Bin Yin a,b,1, Hai-Xia Liu c,1, Wei Shi d,1, Tan Ding b,e, Huai-Qiang Hu a, Hong-Wei Guo a, Shan Jin a, Xiao-Ling Wang a, Ting Zhang b, Ya-Cheng Lu a, Bing-Zhen Cao a,e

a Department of Neurology, The 960th Hospital of Joint Logistics Support, PLA, Jinan 250031, PR China
b Department of Human Anatomy, Histology and Embryology, The Fourth Military Medical University, Xi’an 710032, PR China
c Department of Obstetrics and Gynecology, Shandong Provincial Hospital, Jinan 250021, PR China
d Department of Neurosurgery, The 960th Hospital of Joint Logistics Force, PLA, Jinan 250031, PR China
e Institute of Orthopedics, Xijing Hospital, The Fourth Military Medical University, Xi’an 710032, PR China

A R T I C L E   I N F O
Keywords:
Post-traumatic stress disorder (PTSD)
Brain-derived neurotrophic factor (BDNF)
Single-prolonged stress (SPS)
Tyrosine kinase receptor B (TrkB)
Anxiety-like behaviors

A B S T R A C T
Post-traumatic stress disorder (PTSD) has become epidemic following severely stressful incidents. Previous studies have shown that brain-derived neurotrophic factor (BDNF) has anxiolytic effects on various anxiety or depression disorders including PTSD. However, the detailed mechanisms of BDNF for treating PTSD were rarely investigated. In the current study, single-prolonged stress (SPS) was used as an animal model recapitulating specific aspects for a PTSD-like phenotype. The effects of BDNF on SPS-induced anxiety-like behaviors were investigated. We showed that the levels of BDNF in the cerebro-spinal fluid (CSF) were significantly reduced after the rats experienced SPS. The SPS-induced reductions of percentages of time spent in the central area to total time in the open field test, were dose-dependently mitigated after BDNF intracerebroventricular (i.c.v.) injections. BDNF i.c.v. administration also dose-dependently increased the preference of the light box in the light–dark box test. Both expressions of tyrosine kinase receptor B (TrkB) protein and mRNA in the prefrontal cortex (PFC) and amygdala were significantly increased after SPS challenges. BDNF i.c.v. administration attenuated these compensatory increases of TrkB. At last, the anxiolytic effects of BDNF on SPS model were also observed by using other two injection methods. These results inspired us to study that different administrations of BDNF were used in patients with PTSD in the future, in-depth.

1. Introduction

Post-traumatic stress disorder (PTSD) refers to psychological trauma caused by catastrophic, threatening events, which resulting in delayed or long-lasting mental disorders [11]. PTSD has drawn much attention due to its high incidence and long course of disease. Aberrant susceptibility of emotion- and fear-related brain nuclei, including the amygdala, prefrontal cortex (PFC), and hippocampus may contribute to the development and retention of PTSD symptoms [31], which may be the sensitive regions response to PTSD. The single-prolonged stress (SPS) model generates behavioral and neurobiological alterations that resemble the clinical features of PTSD, and thus it is commonly used to model the disease in rodents [7,31]. The SPS evoked c-fos (one immediate early gene, also one marker for the activation of neuron) expressions in several brain regions of mice that control the stress-anxiety response, including the central and medial amygdala, hypothalamus, thalamic nuclei and several nuclei in midbrain [2].

Brain-derived neurotrophic factor (BDNF) is widely expressed in the central nervous system and plays an important role in synaptic regulation, learning, memory and neuroprotection [14,32]. BDNF and its receptor, tyrosine kinase receptor B (TrkB) play a key role in the pathophysiology of depression and in the therapeutic mechanisms of antidepressants [35]. Electro-acupuncture (The needles were inserted on specific points along the body, which were then attached to a device that generates continuous electric pulses using small clips) obviously ameliorated SPS-induced anxiety-like behaviors, which was accompanied with increased the expressions of BDNF and TrkB in the amygdala and hippocampus [12,13]. The SPS induced decreased expressions of

* Corresponding author at: Department of Neurology, The 960th Hospital of Joint Logistics Force, PLA, NO. 25 Shifan Road, Jinan 250031, PR China.
E-mail address: czxjia2011@163.com (B.-Z. Cao).
1 These authors contributed equally to this work.

https://doi.org/10.1016/j.neulet.2022.136851
Received 3 April 2022; Received in revised form 2 August 2022; Accepted 18 August 2022
Available online 22 August 2022
0304-3940/© 2022 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).
BDNF in the PFC, with severe anxiety-like behaviors on rats [9]. Bilateral injection of BDNF into the PFC induced an antidepressant effect, which was blocked by pretreatment with TrkB inhibitor, K252a [15].

In our study, the effects of injecting BDNF into the lateral ventricle of rats on SPS-induced anxiety-like behaviors in the open field and light–dark box tests were investigated. After the rats experienced SPS, the expressions of TrkB mRNA and protein in the PFC and amygdala were also checked by molecular biological approaches. At last, we tested the effects of other ways for delivering BDNF on SPS-induced anxiety-like behaviors.

2. Materials and methods

2.1. Animals and drugs

All male Sprague-Dawley rats (8–12 weeks, about 200–220 g) were housed five per cage in a temperature-controlled environment on a 12-h light/dark cycle (6:00 ~ 18:00) with access to food and water *ad libitum*. All experimental protocols were in accordance with the ethical guidelines and received prior approval from the Animal Use and Care Committee for Research and Education of the Fourth Military Medical University.

2.2. Experimental design

Forty-five rats were randomly divided into five groups in the first experiment (Fig. 1), which was used to evaluate the effects of BDNF i.c.v. injection on SPS-induced anxiety-like behaviors. SPS procedures were performed 1 day before saline or BDNF administration. Saline (10 μl) or different BDNF dose (1 mmol, 10 mmol, and 30 mmol/10 μl) was administered once per day and behavioral tests were performed on SPS day 7 [23,5]. The other 54 rats were randomly divided into six groups in the second experiment (Fig. 1), which was used to evaluate the effects of different routes of BDNF administration on SPS-induced anxiety-like behaviors. The rats were acclimated to the injection environment for 1 h and handled by technician before every injection. Rats in both experiments were sacrificed on SPS day 7 for molecular test.

2.3. SPS test

The SPS model 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]. 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, for seven days. The rats only received injections during these 7 days after SPS. The food and water were plentiful. For the control procedure, rats remained in their home cages without food and water for the duration of SPS.

2.4. Open field test

The testing room remained quiet and dark with indirect lighting during the experiment. Rats were gently placed at the center of the testing chamber [100 cm (W) × 100 cm (H) × 50 cm (D)] after 1 h acclimation to the testing room. The experimenter left the room after the rats were placed into the center of the testing chamber. Then the experimenter monitored the activity of rats by the automated analyzing system with camera (Shanghai Mobile datum Information Technology Co., Ltd) and recorded the track of rats for 15 min. The open field was cleaned between rats by removing the faeces and then cleaning the chamber with ethanol solutions. After drying, the next rat was placed into the center of the testing chamber. The total distance and time percentage in the central area were served as measures for locomotion and anxiety/depression levels of rats [26,34].

2.5. Light–dark box test

The rats underwent light–dark box testing based on previously described procedures [3,21]. Light-dark box apparatus consisted of a plexiglass box with two separate compartments of equal dimensions (40 cm × 44 cm × 37 cm); a light compartment (light box) with translucent walls illuminated at 300 lx, and a dark compartment (dark box) with blackened opaque walls. An opening in the dividing wall allowed free movement of rats between the compartments. Following pretreatment, each animal was individually placed in the center of the light box and allowed to roam freely for 10 min. Sessions were filmed, and the behaviors were hand scored by an experimenter blind to the conditions.

2.6. Cannula implantation

For microinjection of human recombinant BDNF (78133, STEM CELL Technologies, Vancouver BC, Canada) or saline into the lateral ventricle, a 5.0 mm length guide cannula (6202, OD 0.56 × ID 0.38 mm, RWD, Shenzhen, China) was stereotaxically implanted, fixed to the skull. A dummy cannula was inserted into the guide cannula. After guide cannula implantation and one week recovery, rats were applied to test for SPS model. BDNF or saline was injected into the lateral ventricle by using micro-syringe with connector and micro pump. During the injection period, the rats were free moving. The injection site was confirmed...
after all the behavior tests were finished.

2.7. Measurement of BDNF levels

To determine the BDNF levels, the CSF was obtained by the cannula in the lateral ventricle. The CSF in each group was homogenated with 1 ml of perchloric acid containing 0.1% cysteine, then centrifuged at 10,000 × g for 20 min at 4 °C, and the supernatant was collected and stored at −70 °C. The levels of BDNF were measured with a commercially available ELISA according to the manufacturer’s instructions (CSB-E04504r, CUSABIO, Houston TX USA).

2.8. Real-time PCR

RNeasy Plus Mini Kits (74134, Qiagen, Dusseldorf, Germany) were used to extract the total RNA from the PFC and amygdala tissues (approximately 5 mg) [19]. Before those procedure, the rats were sacrificed with deep anesthesia by using pentobarbital (100 mg/kg, i.p.). Then the medial PFC and amygdala were obtained by a hand-done dissection and tissue punch on fresh brains, respectively. The mRNA was then reversely transcribed into first strand cDNA by using an Omniscript RT kit (205111, Qiagen). Finally, we adopted real-time PCR for 40 cycles to detect target gene mRNA levels (CQ596, ComWin Biotech). All procedures were performed in accordance with the corresponding kit instructions. The real-time PCR primers were designed as follows: TrkB, forward primer 5′-GGCCCAAAGATGATGGTTAAG-3′ and reverse primer 5′-TGGACCTGGCTGTTGGTGAT-3′; GAPDH, forward primer 5′-AAGACCCCTTCAATGCAC-3′ and reverse primer 5′-TCCACGACATTACGAC-3′ [22,10]. The TrkB mRNA levels of each well were normalized to GAPDH mRNA levels.

2.9. Western blot

The rats were sacrificed with deep anesthesia by using pentobarbital. The detailed procedures followed protocols in our previous studies [29,33]. Briefly, the medial PFC or amygdala of the rats was rapidly removed and homogenized at 4 °C with a buffer containing 50 mmol/L Tris-HCl, pH 7.4. After centrifugation of the tissue homogenates at 12000 rpm/min, the supernatants were collected. Protein concentrations were quantified by Bicinchoninic acid (BCA) protein kit (Pierce, Rockford, IL, USA), a final concentration of β-mercaptoethanol (BME) and bromophenol blue (3:1) were added. The proteins (50 μg) in the extracts were separated by 10% SDS-PAGE and transferred to nitrocellulose membrane. The membranes were incubated with primary antibodies rabbit polyclonal anti-TrkB (ab18987, Abcam, Cambridge MA USA) or mouse monoclonal anti-β-actin (SAB4200689, Sigma, Poway CA USA) overnight at 4 °C. Then three washes with TBST, the membranes incubated with the secondary antibody (horseradish peroxidase (HRP)-conjugated anti-rabbit or anti-mouse) at room temperature for 1 h. Labworks Software (Ultra-Violet Products, UK) was used to visualized immunoreactive bands. Bands intensity readings were obtained using ImageJ software.

2.10. Intrathecal (i.t.) catheter implantation

I.t. catheter implantation was performed according to our previous study [29]. Briefly, a midline incision (3 cm) was cut on back of the rat at the level of the thoracic vertebrae. A pre-measured PE-10 tube (ID 0.28 mm and OD 0.61 mm) was passed caudally from T8 to L3 level of the spinal cord, fixed at the back of rat’s ears through subcutaneous tunnel, with 2 cm free end exposed in the upper thoracic region. Rats were allowed to recover for 7 days before further use.

2.11. Statistical analysis

The results were expressed as mean value ± standard error of the mean (SEM). For comparing of differences among groups, One-way ANOVA with Dunnett’s post hoc test was performed using GraphPad Prism version 5.01 for Windows (Graph Pad Software, San Diego California USA, www.graphpad.com).

3. Results

3.1. Effects of BDNF i.c.v. injection on the SPS-induced anxiety-like behaviors

To examine the effects of BDNF on the SPS-induced anxiety-like behaviors, rats were subjected to the open field and light–dark box tests. The rats experiencing SPS spent less time in the central area (5.03 ± 1.13 %) and unchanged total distance (8835.89 ± 1727.52 cm) in the open field test. Administration of BDNF into the lateral ventricle did not affect the total distance of rats walked in the open field test (Fig. 2A). i.c. v. injection of BDNF reversed the SPS-induced reduced percentages of time spent in the central area to total time in the open field test, which were dose-dependent (Fig. 2B). The rats experiencing SPS presented obvious preference of the dark box (13.99 ± 4.57 %), rather than the light box. The percentages of time spent in the dark box dose-dependently reduced following BDNF i.c.v. injection (Fig. 2C). The latencies to the light box were also dose-dependently reduced after BDNF i.c.v. injection (Fig. 2D). These results indicate that BDNF i.c.v. injection certainly ameliorated SPS-induced anxiety-like behaviors.

3.2. BDNF levels in the CSF

To better understand the underlying mechanisms for the anxiolytic effects of BDNF on the SPS-induced anxiety-like behaviors, the concentrations of BDNF in the CSF were examined. The BDNF levels in the CSF were calculated by using ELISA method (Fig. 3A). The levels of BDNF in the SPS + saline group were significantly reduced compared with those in control group (Fig. 3B). Whereas i.c.v. injection of different dose of BDNF did dose-dependently increase the levels of BDNF in the CSF, even if SPS models were established. These results indicate that SPS-induced anxiety-like behaviors accompanied with reduced BDNF levels in the CSF. However, the increased BDNF levels ameliorated SPS-induced anxiety-like behaviors.

3.3. The expressions of TrkB in the PFC and amygdala

Then, we checked the expressions of TrkB mRNA and protein in the PFC and amygdala. SPS significantly increased the expressions of TrkB mRNA in both the PFC and amygdala (Fig. 4A, B). BDNF i.c.v. administration attenuated these increases of TrkB mRNA in the two nuclei. The expressions of TrkB protein were increased in both the PFC and amygdala after the rats experienced SPS (Fig. 4C-F). BDNF i.c.v. administration also attenuated these increases of TrkB protein. The above results suggest that the TrkB expressions in the PFC and amygdala were involved into the SPS-induced anxiety-like behaviors.

3.4. Effects of BDNF i.t. or i.p. injection on SPS-induced anxiety-like behaviors

The results that BDNF i.c.v. injection attenuated SPS-induced anxiety-like behaviors, were inspiring. However, few patients accepted the invasive procedures of i.c.v. injection in the clinical application. It was worth looking forward to check some non-invasive BDNF injections on those anxiety-like behaviors. BDNF either i.t. or i.p. injection did not affect the total distance in the open field test (Fig. 5A, E). I.t. or i.p. injection of BDNF mitigated the SPS-induced reducing percentages of time spent in the central area to total time in the open field test (Fig. 5B, F). The percentages of time spent in the dark box certainly reduced following BDNF i.t. or i.p. injection (Fig. 5C, G). The latencies to the light box were also significantly reduced after BDNF i.t. or i.p. injection...
These results indicate that BDNF i.t. or i.p. injection also had the anxiolytic effects for ameliorating SPS-induced anxiety-like behaviors.

4. Discussion

4.1. Effects and mechanisms of BDNF on SPS-induced anxiety-like behaviors

Pharmacotherapy and cognitive behavioral therapy, fail to treat PTSD in a considerable number of populations [8]. Currently, SPS was chosen as an effective animal model for PTSD and used to scan useful
drugs. In our study, BDNF i.c.v. injection certainly ameliorated SPS-induced anxiety-like behaviors. There was few study to investigate the effects of BDNF i.c.v. injection on anxiety-like behaviors. What they did was direct local nucleus BDNF injection [15]. Actually, the anxiolytic effects of BDNF were not specific to the SPS animal model. BDNF has anxiolytic effects on various kinds of stressed animals [24,23]. BDNF also produced anxiolytic effects on chronic unpredictable mild stress (CUMS) animal model [36].

We speculated that the BDNF-TrkB signaling in the PFC and amygdala participated in the SPS-induced anxiety-like behaviors. BDNF levels were significantly decreased in amygdala of SPS rats, and reversing these decreases of BDNF ameliorated SPS-induced anxiety- and depression-like behaviors [25]. The expressions of BDNF in other brain nuclei might also be response to the SPS-induced anxiety-like behaviors. One previous study showed that both BDNF mRNA and protein levels in the hippocampus of SPS rats were significantly higher than those in sham rats, accompanied by increased TrkB expressions in the hippocampus [27]. They thought that the increased BDNF and TrkB levels in the hippocampus played a role in the consolidation of fear memory of SPS rats. Danggui Buxue Decoction (a Traditional Chinese Medicine formula composed of Angelica and Astragalus) effectively relieved depression-like behaviors, which also enhanced the protein and mRNA levels of BDNF/TrkB in rat hippocampal cells [28]. We also need to check the expressions of TrkB in the hippocampus of SPS rats in the future.

4.2. The relations between BDNF and PTSD

Based on the anxiolytic effects of BDNF on SPS animal model, it was promising that BDNF would be useful for treating PTSD patients. Serum levels of BDNF and proBDNF in female patients diagnosed with PTSD were significantly lower than those in healthy subjects, which had a role in the etiopathogenesis of PTSD [1]. The single nucleotide polymorphisms C270T of BDNF showed significant differences between PTSD group and control group [4]. Individuals carrying the polymorphic T allele of C270T may be more likely to suffer from PTSD. However, one review analyzed four trial studies and reported that there was no significant changes in BDNF levels in cerebrospinal fluid in patients with PTSD [18]. While they thought these negative results were due to the heterogeneity of PTSD and more high quality randomized controlled trials were needed.

4.3. The effects of peripheral BDNF on anxiety-like behaviors

BDNF i.t. or i.p. injection also had the anxiolytic effects on SPS animal model, which was not investigated in previous studies. In separate work, peripheral, subcutaneous injections of BDNF were reported to produce antidepressant and anxiolytic effects in rodents [20]. Individuals suffering from depression had lower serum BDNF and plasma BDNF (pBDNF) concentrations than those of healthy subjects [19]. For instance, one study reported that pBDNF concentrations were related with the severity of depression [6]. One clinical study demonstrated that six ketamine infusions increased pBDNF concentrations accompanied with the antidepressant effects [37].

BDNF crossed the blood–brain barrier, and pBDNF concentrations were closely correlated with cortical BDNF concentrations, and likely reflect brain BDNF concentrations [17,16]. However, other researchers thought that BDNF was poorly transported across the blood–brain barrier.
barrier [20]. The exact mechanism of anxiolytic effects of BDNF peripherally administered remains to be determined. It was unclear whether the potential effects were due to consequences on brain activity or through BDNF effects on peripheral targets (vascular and cardiac?).

In conclusion, the anxiety-like behaviors accompanied with reduced BDNF levels in the CSF and increased mRNA and protein expressions of TrkB in both the PFC and amygdala, were observed after the rats experienced SPS. BDNF i.c.v., i.t., or i.p. injection all produced anxiolytic effects. The administration of BDNF might be a novel therapy approach for treating PTSD through brain mechanisms. More attention needs to be conducted on other nucleus target in the brain, as well as the molecular mechanisms mediated these anxiolytic effects.

Author contribution

Study concept and design: B-Z Cao and J-B Yin.
Acquisition of data: W Shi, H-X Liu, T Zhang, Y-C Lu, and H-Q Hu.
Analysis and interpretation of data: T Ding, H-W Guo, S Jin, and X-L Wang.

Draft the manuscript: J-B Yin, H-X Liu, and W Shi.
Critical revision of the manuscript for important intellectual content: T Ding, B-Z Cao, W Shi, and H-X Liu.
Study supervision: B-Z Cao.

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.

Data availability

Data will be made available on request.

Acknowledgments

This work was supported by National Natural Science Foundation of China (No.81801099), China Postdoctoral Science Foundation Special
References

[1] S. Aksu, G. Unlu, A.C. Kardesler, B. Cakaloz, H. Aybek, Altered levels of brain-derived neurotrophic factor, proBDNF and tissue plasminogen activator in children with posttraumatic stress disorder, Psychiatry Res. 268 (2018) 478–483.

[2] H. Azevedo, M. Ferreira, A. Mascarello, P. Osten, C.R.W. Guimarães, Brain-wide mapping of c-fos expression in the single prolonged stress model and the effects of pretreatment with ACH-000029 or prazosin, Neurobiol. Stress 13 (2020) 100226.

[3] J.N. Crawley, Neuropharmacologic specificity of a simple animal model for the behavioral actions of benzodiazepines. Pharmacol. Biochem. Behav. 15 (5) (1981) 695–699.

[4] J.-C. Guo, Y.-J. Yang, M. Guo, X.-D. Wang, Y. Juan, Y.-S. Gao, L.-Q. Fu, X.-L. Jiang, L.-M. Fu, T. Huang, Correlations of Four Genetic Single Nucleotide Polymorphisms in Brain-Derived Neurotrophic Factor with Posttraumatic Stress Disorder, Psychiatry Investig 15 (4) (2018) 407–412.

[5] W. Guo, M.T. Robbins, F. Wei, S. Zou, R. Dubner, K. Ren, Supraspinral brain-derived neurotrophic factor signaling: a novel mechanism for descending pain facilitation, J. Neurosci. 26 (2006) 126–137.

[6] C.N. Halle, J.W. Murrough, D.Y. Josifescu, L.C. Chang, R.K. Al Jurdi, A. Fouleks, S. Iqbal, J.J. Mahoney, R. De La Garza, D.S. Charney, T.F. Newton, S.J. Mathew, Brain-derived neurotrophic factor (BDNF) and response to ketamine in treatment-resistant depression, Int. J. Neuropsychopharmacology 17 (02) (2014) 331–336.

[7] F. Han, B. Xiao, L. Wen, Y. Shi, Effects of fluoxetine on the amygdala and the hippocampus after administration of a single prolonged stress to male Wistar rats: Plasma brain derived neurotrophic factor (BDNF) and response to ketamine in treatment-resistant depression, Int. J. Neuropsychopharmacology 17 (02) (2014) 331–336.

[8] J. Shallcross, P. Hamor, A.R. Bechard, M. Romano, L. Nakosteda, M. Schwendt, The Divergent Effects of CBPDB and Cannabidiol on Fear Extinction and Anxiety in a Predator Scent Stress Model of PTSD in Rats, Front. Behav. Neurosci. 13 (2019) 91.

[9] S.S. Shi, S.H. Shao, B.P. Yuan, F. Pan, Z.L. Li, Acute stress and chronic stress change brain-derived neurotrophic factor (BDNF) and tyrosine kinase-coupled receptor (TrkB) expression in both young and aged rat hippocampus, Yomei Med. J. 51 (2010) 661–671.

[10] Y. Shirayama, A.-H. Chen, S. Nakagawa, D.S. Russell, R.S. Duman, Brain-derived neurotrophic factor produces antidepressant-like effects in behavioral models of depression, J. Neurosci. 22 (8) (2002) 3251–3261.

[11] J.A. Sicilia, S.J. Lewis, S.J. Wiegand, R.M. Lindsay, Antidepressant-like effect of brain-derived neurotrophic factor (BDNF), Pharmacol. Biochem. Behav. 56 (1) (1997) 131–137.

[12] N. Solanki, I. Alkadhi, F. Arrozo, G. Patki, S. Salim, Grape powder prevents cognitive, behavioral, and biochemical impairments in a rat model of posttraumatic stress disorder, Nutr. Res. 35 (1) (2015) 65–75.

[13] Y.-H. Sun, Y.-L. Dong, Y.-T. Wang, G.L. Zhao, G.J. Lu, J. Yan, S.-X. Wu, Z.-X. Gu, W. Wang, C. Sommer, Synergistic analgesia of diltiazem and celecoxib in the mouse formalin test: a combination analysis, PLoS ONE 8 (10) (2013) e76603.

[14] S. Iqbal, J.J. Mahoney, R. De La Garza, D.S. Charney, T.F. Newton, S.J. Mathew, Neurotrophic factor signaling: a novel mechanism for descending pain facilitation, Acta Neuropsychiatr 31 (03) (2019) 143–170.

[15] V.S. Pereira, A.C.D.R. Suavinha, G. Wegener, S.R.L. Joca, Prelimbic neuronal nitric oxide synthase inhibition exerts antidepressant-like effects independently of BDNF signalling cascades, Acta Neuropsychiatrica 31 (03) (2019) 143–150.

[16] S.-S. Zhang, Y.-H. Tian, S.-J. Jin, W.-C. Wang, J.-X. Zhao, X.-M. Si, L.i. Zhang, M.-Z. Zhai, H.-H. Wu, J.-B. Yin, Y.-Y. Cui, X.-P. Mei, H. Zhang, X. Zhu, X.-F. Shen, Decreased BDNF levels in CSF of drug-naive first-episode psychotic subjects: correlation with plasma BDNF and psychopathology, The International Journal of Neuropsychopharmacology 33 (6 Suppl 5) (2020) 137.

[17] H. Xu, J.-Y. Jin, Isoflurane produces antidepressant effects inducing BDNF-TrkB signaling in response to fear conditioning in an animal model of posttraumatic stress disorder, J. Psychiatr. Res. 45 (4) (2011) 460–466.

[18] W.-K. Yi, Y. Zhou, L.u. Fan, Y. Sun, F. Ge, M. Xue, The antidepressant-like effects of Danggui Buxue Decoction in GR rats by activating CREB/BDNF/TrkB signaling pathway, Phtymocin 89 (2021) 153605.

[19] M. Sagud, M. Nikolac Perkovic, B. Vuksan-Cusa, A. Maravic, D. Svob Strac, Mihaljevic Peles, M. Zivkovic, Z. Kusevic, N. Picav, A prospective, longitudinal study of platelet serotonin and plasma brain-derived neurotrophic factor concentrations in major depression: effects of vortioxetine treatment, Psychopharmacology 233 (17) (2016) 3259–3267.

[20] H.D. Schmidt, R.S. Duman, Peripheral BDNF produces antidepressant-like effects in cellular and behavioral models, Neuropsychopharmacology 35 (12) (2010) 2378–2391.

[21] F. Han, B. Xiao, L. Wen, Y. Shi, Effects of fluoxetine on the amygdala and the hippocampus after administration of a single prolonged stress to male Wistar rats: Plasma brain derived neurotrophic factor (BDNF) and response to ketamine in treatment-resistant depression, Int. J. Neuropsychopharmacology 17 (02) (2014) 331–336.

[22] J. Shallcross, P. Hamor, A.R. Bechard, M. Romano, L. Nakosteda, M. Schwendt, The Divergent Effects of CBPDB and Cannabidiol on Fear Extinction and Anxiety in a Predator Scent Stress Model of PTSD in Rats, Front. Behav. Neurosci. 13 (2019) 91.

[23] Y.-H. Sun, Y.-L. Dong, Y.-T. Wang, G.L. Zhao, G.J. Lu, J. Yan, S.-X. Wu, Z.-X. Gu, W. Wang, C. Sommer, Synergistic analgesia of diltiazem and celecoxib in the mouse formalin test: a combination analysis, PLoS ONE 8 (10) (2013) e76603.

[24] S. Iqbal, J.J. Mahoney, R. De La Garza, D.S. Charney, T.F. Newton, S.J. Mathew, Neurotrophic factor signaling: a novel mechanism for descending pain facilitation, Acta Neuropsychiatr 31 (03) (2019) 143–150.

[25] S.-S. Zhang, Y.-H. Tian, S.-J. Jin, W.-C. Wang, J.-X. Zhao, X.-M. Si, L.i. Zhang, M.-Z. Zhai, H.-H. Wu, J.-B. Yin, Y.-Y. Cui, X.-P. Mei, H. Zhang, X. Zhu, X.-F. Shen, Decreased BDNF levels in CSF of drug-naive first-episode psychotic subjects: correlation with plasma BDNF and psychopathology, The International Journal of Neuropsychopharmacology 33 (6 Suppl 5) (2020) 137.