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Applying ketamine to alleviate the PTSD-like effects by regulating the HCN1-related BDNF

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ABSTRACT

Background: Post-traumatic stress disorder (PTSD) is commonly associated with concurrent anxiety and depression symptoms, and reduce the expression of the Brain-Derived Neurotrophic Factor (BDNF) which promotes the proliferation and survival of neurons. The hyperpolarization-activated cyclic nucleotide-gated channel 1(HCN1) could be inhibited by the ketamine, a drug to alleviate depression and anxiety, and regulated the BDNF expression, however, the effects of ketamine in alleviating PTSD symptoms by regulating the HCN1-related BDNF have been poorly perceived.

Methods: In the present study, the effects of ketamine were examined on the PTSD-like effects in a rat model of PTSD induced by SPS&S procedure. After the SPS&S procedure and model testing, PTSD rats were subjected to behavioral testing and biochemical assessments, followed by single treatment with certain doses of ketamine (5, 10, 15 and 20 mg/kg IP).

Results: The results showed that the SPS&S procedure induced severe PTSD-like behaviors, with lower levels of BDNF protein levels and higher level of the HCN1 protein in the prefrontal cortex (PFC). These were reversed by a single administration of ketamine. The ketamine with dose of 15 mg/kg significantly increased locomotor behavior in the open field test, aggrandized exploratory behavior in the elevated plus maze test, and decreased immobility time spent in the forced swim test. Meanwhile, ketamine with dose of 15 mg/kg could increase the BDNF protein level, while down-regulate the expression of the HCN1. Eventually, there was a negative correlation between the level of BDNF and HCN1 in the PFC.

Conclusion: Ketamine affects the HCN1-related BDNF signaling pathways to alleviate PTSD-like effects in rat.

1. Introduction

Post-traumatic stress disorder (PTSD) is defined as a complex syndrome that is characterized by intrusive re-experiences of traumatic events, preventing situations and stimuli that could serve as reminders as well as feeling jumpy or easily startled. PTSD results from exposure to life-threatening events such as war, childhood adversity, seismic events, and other natural disasters (Turina et al., 1986; Zhang et al., 2015). Selective serotonin reuptake inhibitors (SSRIs) are common drugs used to treat acute and chronic PTSD. However, rate of treatment response scarcely exceeds 60% that is approximately 20–30% of SSRI-treated PTSD patients achieve full remission, indicating a major unmet medical need to find more effective treatment for PTSD (Xu et al., 2011).

Ketamine, an *N*-methyl-D-aspartate (NMDA) receptor antagonist, could induce anxiolytic- and antidepressant-like effects in rodents that are subjected to animal models of anxiety and depression (Ardalan et al., 2017; Kos et al., 2006). A study reported that patients who received perioperative ketamine had significantly lower rates of PTSD than those patients who did not receive ketamine (McGhee et al., 2008). Notably, recent clinical studies demonstrated that ketamine infusions could significantly reduce PTSD symptoms in patients (Feder et al., 2014). Both preclinical and clinical findings reflected that ketamine had potential for treating PTSD symptoms. However, studies that

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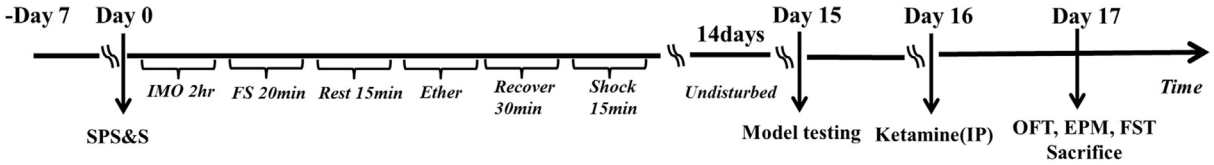

Fig. 1. Experimental schedule for developing PTSD model induced by SPS&S and ketamine treatment. On day 0, rats were exposed to single prolonged stress and electric foot shock (SPS&S), including immobilized for 2 h (IMO 2 h), forced swim for 20 min (FS 20 min), rest period for 15 min (Rest 15 min), exposure to ether until loss of consciousness (Ether), followed by a 30 min recovery. The rats were shocked after 15 min (Shock 15 min). After the SPS&S procedure, rats were left undisturbed in their home cages for 14 days. The PTSD model stability was tested on days 15. After the ketamine treatment, behavioral assessments, including the Open Field Test (OFT), the Forced Swim Test (FST) and the Elevated Plus Maze Test (EPMT) were performed on day 17.

address the anti-PTSD-like effects of ketamine and the mechanism about alleviating the PTSD-like effects of ketamine have still remained unknown.

Brain-derived neurotrophic factor (BDNF) is involved in brain development, plasticity, and maintenance of neurons in adult life (Binder and Scharfman, 2004; Lewin and Barde, 1996). An earlier study found that hippocampal BDNF levels were lower in a rat model of PTSD when compared with controls (Shafia et al., 2017). There was an evidence that the function and production of BDNF were impaired in PTSD patients, where it was revealed that BDNF could mediate the pathophysiology of mood disorders, including PTSD (Andero and Ressler, 2012). Sub-chronic administration of ketamine has been shown to alter the expression of messenger RNA (mRNAs) for BDNF in the prefrontal cortex (PFC) (Broekman et al., 2007). In addition, it has been found that BDNF could be up-regulated by lentivirus-based silencing RNA system (shRNA)-HCN1, which reduced the expression of hyperpolarization activated cyclic nucleotide gated potassium channel 1 (HCN1) protein, increased the neuronal excitability, and produced the anxiolytic-and antidepressant-like behaviors (Kim et al., 2012).

The HCN channel family comprises four subtypes (HCN1-4), with the PFC primarily expressed as HCN1 (Day et al., 2005). HCN1 is known to carry the hyperpolarization-activated current (Ih), playing a fundamental role in controlling several important cellular functions (i.e., cellular excitability, dendritic integration, synaptic transmission, plasticity phenomena, and rhythmic activity in central nervous system (Biel et al., 2009; Nolan et al., 2004). A previous study reported that rats were treated with lentiviral shRNA-HCN1, had a silencing effect on HCN1 and the mRNA displayed antidepressant- and anxiolytic-like behaviors (Kim et al., 2012). Ketamine is a potent inhibitor of HCN1, and it is possible that inhibition of HCN1 could contribute to or be responsible for the antidepressant- and anxiolytic-like behaviors effects (Chen et al., 2009; Kim et al., 2012). It should be noted that there is a lack of research within utilization of ketamine in PTSD by regulating the HCN1-related BDNF signaling pathways in the PFC.

The present study hypothesized that the BDNF levels were decreased due to the increase of HCN1 expression in the PFC, which may associate with improvement of PTSD symptoms. Ketamine could alleviate the PTSD-like symptoms by regulating the HCN1-related BDNF signaling pathways in the PFC. An attempt was carried out to further understand the efficacy of ketamine on PTSD-like symptoms; this study aims to investigate the effects of ketamine on PTSD-like symptoms in the single prolonged stress & electric foot shock (SPS&S) model induced by an inescapable foot electric shock in addition to a classic SPS model (Wang et al., 2008). This included PTSD-like behavior using behavioral tests, as well as variations in expressions of BDNF and HCN1 proteins in the PFC by biochemical tests.

2. Materials and methods

2.1. Animals

This study has utilized male Sprague-Dawley (SD) rats weighing 220–250 g (6 weeks-old, obtained from the animal center at Weifang Medical University). A total of 56 rats were housed in a limited access rodent facility with 8 rats per polycarbonate cage in a room with a 12-h light, 12-h dark cycle (lights on from 7:00 a.m. to 7:00 p.m.), with a relative humidity maintained at 55 ± 15%, and a constant temperature (24 ± 2 °C). Sterilized drinking water and a standard food were available ad libitum. Before conducting the experiments, the rats were habituated to these conditions for seven days. All experiments were performed between 9:00 a.m. and 14:00 p.m. during the light cycle. The experiments were undertaken according to the National Institutes of Health Guidelines (Use of Laboratory Animals) and were approved by the Animal Care and Use Committee of Weifang Medical University.

2.2. SPS&S procedure

The SPS&S procedures were similar to previous literature (Wang et al., 2008). The rats in the experimental group (n = 48) were immobilized for 2 h on restraint tubes where the size was adjusted according to the size of the rat so that they were completely immobilized. After immobilization, they were immediately subjected to the forced swim for 20 min in a glass barrel (46 cm × 20 cm) filled two-thirds full with water at temperature of 24–25 °C. After passing a recovery period of 15 min, the rats were exposed to ether until loss of consciousness (defined as loss of extremity-pinch responses, 2–3 min). The animals were then kept in shock cages for a recovery period of 30 min, and 1 mA current electric shock was applied to the feet via the metal grid for 4 s. The stressed rats remained in the shock cages for the next 60 s. After doing the SPS&S stressors, the rates were left undisturbed in their home cages for 14 days. As a control, the control group (n = 8) did not undergo any of the processing (See Fig. 1).

2.3. Testing the rat model of PTSD

After establishing the rat model of PTSD, 8 rats were randomly selected from 48 stressed rats to form SPS&S group according to random number table. And SPS&S group was tested for the PTSD-like symptoms as well as the control group. PTSD-like symptoms were measured by behavioral tests and the variations in expressions of BDNF and HCN1 proteins were assessed in the PFC by biochemical tests.

2.4. Experimental design and pharmacological treatment

Ketamine hydrochloride was obtained from Jiangsu Hengrui Medicine Co., Ltd. (Lianyungang, Jiangsu, China). After testing the rat of SPS&S model, the other stressed rats were randomly assigned into one of the five groups: SPS&S + vehicle group (saline IP, n = 8); SPS&S + ketamine-5 group (ketamine, 5 mg/kg, IP, n = 8); SPS&S + ketamine-10 group (ketamine, 10 mg/kg, IP, n = 8); SPS&S + ketamine-15 group (ketamine, 15 mg/kg, IP, n = 8); and SPS&S + ketamine-20 group (ketamine, 20 mg/kg, IP, n = 8). Behavioral tests were evaluated 24 h after a single injection of ketamine or saline.

2.5. Behavioral tests

2.5.1. Open field test

The open field test (OFT) is typically performed in order to measure PTSD-like behavior dependent on locomotor activity in rodents (Qiu et al., 2017). The open field area consisted of an enclosed square arena made of dark opaque Plexiglas (100 cm × 100 cm × 50 cm). The field was divided into 25 squares with computer virtual grid lines for analysis using Smart-3.0 software. Rats were individually and gently placed in the open field area in the same corner of the arena, facing the same direction, and were allowed to freely explore the arena for 5 min. The rats' behaviors, including the number of crossings (with all four paws placed into a new square) and up-right postures (with both front paws raised from the floor) were recorded via a digital camera.

2.5.2. Elevated plus maze test

The elevated plus maze (EPM) test is widely utilized to evaluate the PTSD-like anxiogenic behavior in rodents (Wang et al., 2009). The EPM consisted of dark gray plastic and positioned at 100 cm above the floor. The apparatus consisted of four arms (50 cm × 10 cm), arranged in the shape of a cross; two arms had surrounding walls (40 cm, closed arms), the other two opposing arms had no walls (open arms), along with one center platform (10 cm × 10 cm, center area). The individual rats were gently placed in the central platform, facing the closed arms and allowed to freely explore the arena for 5 min. Their behaviors, including the number of open arm entries as well as the time spent in the open arms were recorded via a digital camera as well.

2.5.3. Forced swim test

The forced swim test (FST) can reliably predict the alleviation of PTSD-like symptoms after drug treatment (Kochi et al., 2017). The cylindrical tank was made of a transparent Plexiglas cylinder (height: 46 cm, diameter: 20 cm) filled with water (23–24 °C) to a depth of 36 cm. Prior to testing, rats without drug treatment were placed in the water for 15 min (pretest session) to be adapted to the apparatus. Twenty-three and a half hours later, rats were placed in the cylinders for 5 min to perform the FST. The immobility time was recorded via a digital camera. The water in the tank was changed after each trial.

2.6. Biochemical tests

2.6.1. Immunohistochemistry assay

The rats were rapidly dissected for the biochemical tests after the behavioral tests. The Immunohistochemistry is described according to previous reports (Yu et al., 2012). Under 8% chloral hydrate (IP, 400 mg/kg) anesthesia, rats were transcardially perfused with normal saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer. The brains were removed and fixed with 4% paraformaldehyde for 6 h, and then were kept in 30% sucrose. Coronal cryotome sections (20 μm) were cut through the PFC using a cryostat. After quenching endogenous peroxidase by 3% H_2O_2 in methanol for 10 min, the PFC sections were blocked in 5% normal goat serum. The PFC sections were incubated overnight at 4 °C in primary antibody (Anti-HCN1, 1:500, ab84816, Abcam; Anti-BDNF, 1:500, ab108319, Abcam). After repeated washing, the PFC sections were then incubated with the secondary antibody (Peroxidase Affinipure Goat Anti-Mouse IgG (H + L), 1:200 dilutions, ZB-2305, ZSGB-BIO; Peroxidase Affinipure Goat Anti-Rabbit IgG (H + L), 1:200 dilution, ZB-2301, ZSGB-BIO) and treated using Thermo Scientific Pierce DAB Substrate kit (ZLI-0931, ZSGB-BIO) for color reaction. The sections were rinsed, dehydrated, and cleared in xylene prior to being mounted on resin-coated slides. The PFC sections were observed with an Olympus DP80 microscope system (Olympus Corporation, Japan), and photomicrographs of representative PFC areas (400×) were obtained.

2.6.2. Western blot assay

The Western blot assay was conducted in accordance with a previous study (Sun et al., 2011). The PFC was rapidly dissected and the proteins were extracted with the radioimmunoprecipitation assay (RIPA) buffer (P0013B, Beyotime, Shanghai, China) containing the protease inhibitor cocktail (ST506, Beyotime, Shanghai, China) on ice. The protein concentration of the PFC was determined using the bicinchoninic acid (BCA) assay (P0012, Beyotime, Shanghai, China). After heating to 95 °C for 10 min, the protein samples were separated by using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The proteins were then transferred to a polyvinylidene fluoride (PVDF) membrane using Trans-Blot wet transfer system. The samples were blocked with 5% skim milk and incubated overnight at temperature of 4 °C with primary antibodies, including anti-HCN1 (1:1000, ab84816, Abcam) and anti-BDNF (1:1000, ab108319, Abcam). The PVDF membranes were washed and then incubated with the second antibodies, including anti-HCN1 (Peroxidase Affinipure Goat Anti-Mouse IgG (H + L), 1:200 dilutions, ZB-2305, ZSGB-BIO) and anti-BDNF (Peroxidase Affinipure Goat Anti-Rabbit IgG (H + L), 1:200 dilutions, ZB-2301, ZSGB-BIO). They were combined with the Immobilon Western Chemiluminescent HRP Substrate (WBKLS0050, Millipore, Billerica, MA, USA) for color reaction, where the gray value of immune reactivity was quantified with ImageJ software.

2.7. Statistical analysis

An independent investigator blinded to the experimental conditions performed all measurements. Student's *t*-test was used to compare the control and SPS&S groups. Differences within or between normally distributed data were analyzed using an analysis of variance (ANOVA) followed by Dunnett's test using SPSS statistical software (Version 22.0, SPSS, Inc.; Chicago, IL, USA). A correlation between the two variables was assessed by the correlation analysis with the help of GraphPad Prism 6 software (GraphPad Software, Inc., CAPrism6, USA). The level of statistical significance was accepted for all analyses at $P = .05$.

3. Results

3.1. Effect of the SPS&S procedure on the PTSD-like behaviors

The results of the OFT which reflected the effects of SPS&S procedure on the rat's model are illustrated in Fig. 2. As shown in Fig. 2, the number of crossings and up-right postures in SPS&S group remarkably decreased (crossing: $t = 8.340$, $P = .000$**, see Fig. 2A; up-right posture: $t = 4.709$, $P = .000$**, see Fig. 2B). The impact of the SPS&S procedure on different parameters of anxiety-like behaviors are displayed in Fig.2, which revealed that SPS&S animals exhibited significant decrease of the time spent in the open arms ($t = 5.537$, $P = .000$**, see Fig. 2C) and the number of entries in the open arms ($t = 7.591$, $P = .000$**, see Fig. 2D). Data from the pairwise comparison between the control group and SPS&S group demonstrated that exposure to SPS&S procedure considerably increased the immobility time in the FST ($t = 18.935$, $P = .000$**, see Fig. 2E).

3.2. Effects of the SPS&S procedure on the HCN1 and BDNF level variations in the PFC

As saw in Fig. 2, the expression of the HCN1-positive cells in the PFC were tested (see Fig. 2F), where the expression of the HCN1 notably improved in the SPS&S group. However, that was not enhanced in the control group when compared with the SPS&S group by using the Student's *t*-test ($t = 52.718$, $P = .000$**, see Fig. 2G). The expressions of BDNF-positive cells in the PFC are presented in Fig. 2H. The BDNF-positive cells in the SPS&S group remarkably diminished when compared to the control ($t = 6.014$, $P = .004$*, see Fig. 2I). Additionally, it was found that there was a correlation between expression of BDNF-

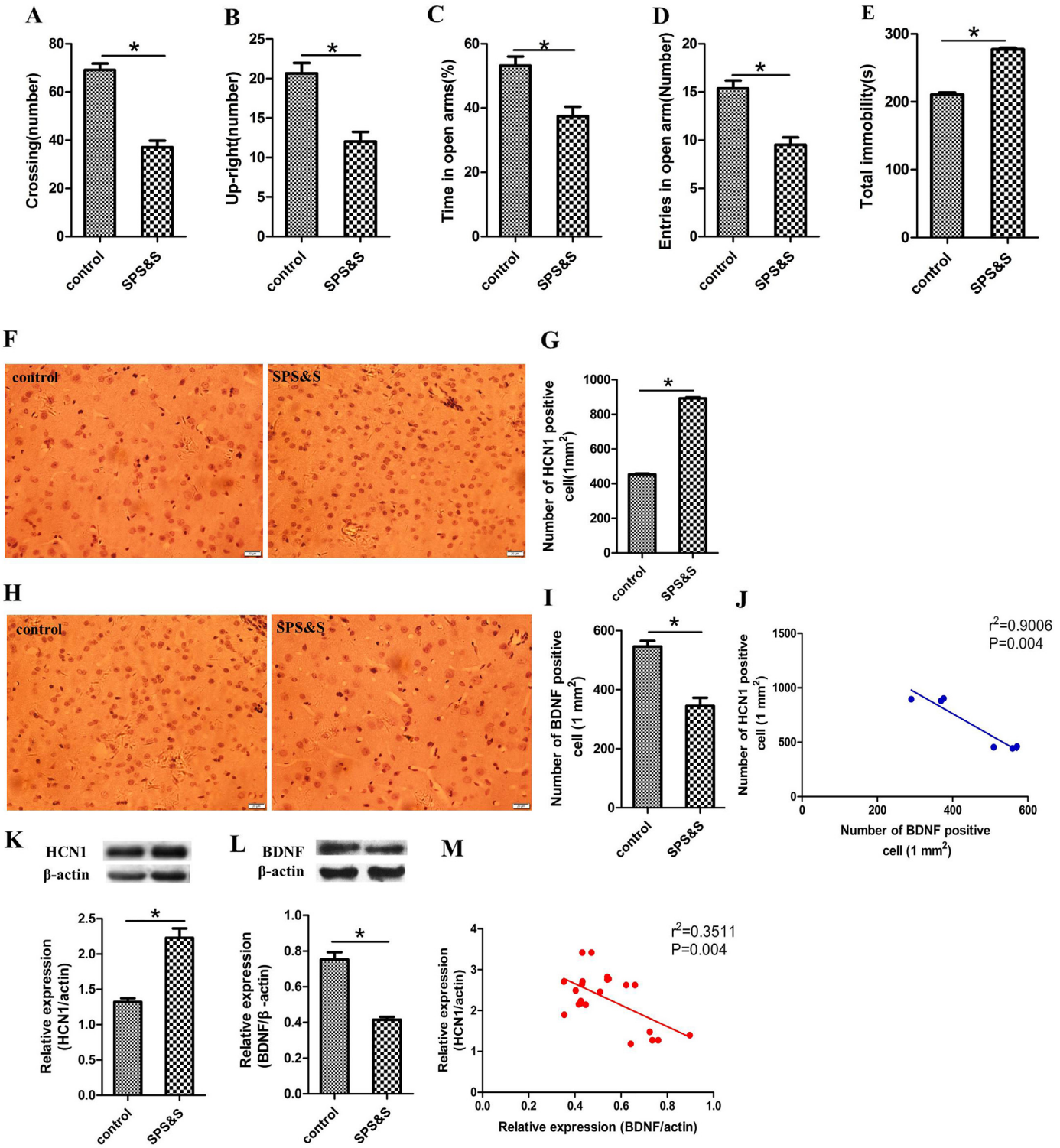

Fig. 2. Effects of the SPS&S procedure. A and B: the number of crossings and up-right in the OFT. C and D: The time spent in the open arms and the number of entries in the open arms in the EPM. E: The duration of immobility in the FST. F: The expression of HCN1-positive cells in the PFC. G and K: The number of HCN1-positive cells and the relative expression of HCN1 protein. J: The correlation between the BDNF- and HCN1- positive cells in the PFC. H: The expression of the BDNF-positive cells in the PFC. I and L: The number of BDNF-positive cells and the relative expression of BDNF protein. M: The correlation between the level of BDNF and HCN1 protein in the PFC. The BDNF and HCN1-positive cells in the PFC are representative by photomicrographs (400 ×). Data are represented as mean ± standard error of mean (SEM). *$P < .05$ was expressed statistically significant.

positive cells and expression of the HCN1-positive cells in the PFC ($r^2 = 0.9006$, $P = .004$*, see Fig. 2J). The results showed that the SPS&S group had more expression of HCN1 compared with the control group ($t = 6.351$, $P = .000$**, see Fig. 2K). The level of the BDNF protein was lower in the SPS&S group than the control group ($t = 7.548$, $P = .000$**, see Fig. 2L). Moreover, there was also a correlation between level of BDNF protein and HCN1 protein in the PFC ($r^2 = 0.6422$, $P = .005$*, see Fig. 2M).

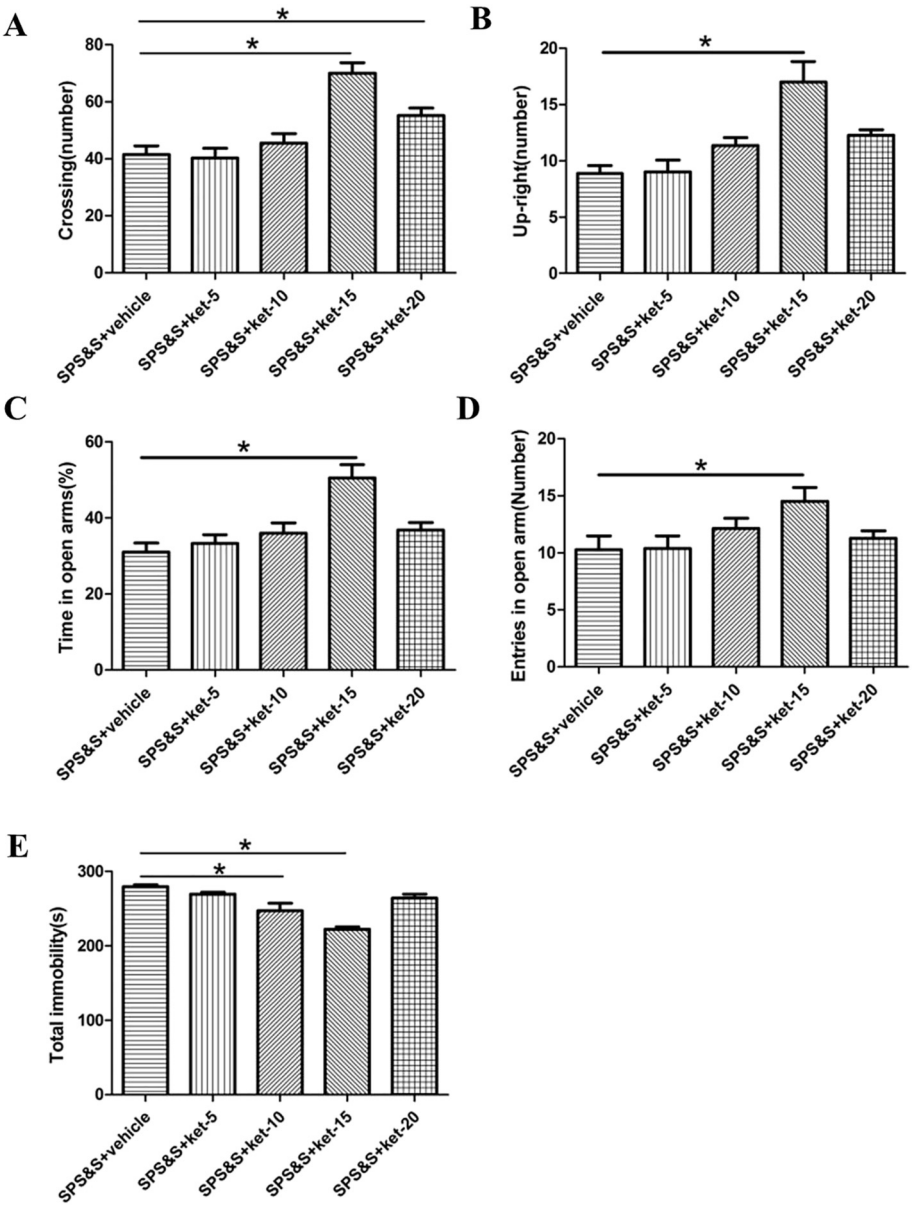

Fig. 3. Effects of single dose of ketamine on PTSD-like behaviors. A and B: the number of crossings and up-right in the OFT. C and D: The time spent in the open arms and the number of entries in the open arms in the EPM. I: The duration of immobility in the FST. Data are represented as mean ± SEM. *$P < .05$ was expressed statistically significant.

3.3. Effects of ketamine on PTSD-like behaviors following SPS&S procedure

The ketamine treatment with the dose of 15 mg/kg resulted in the increase of the number of crossings (ANOVA: $F = 14.253$, $P = .000$**; Post hoc test: $P = .000$**; see Fig. 3A) and up-right (ANOVA: $F = 9.508$, $P = .000$*; Post hoc test: $P = .000$**; see Fig. 3B). Furthermore, the SPS&S + ket-20 group solely enforced the number of the crossings (ANOVA: $F = 14.253$, $P = .000$**; Post hoc test: $P = .020$*, see Fig. 3A). When compared with the SPS&S + vehicle group, the SPS &S + ket-15 group had more time spent in the open arms (ANOVA: $F = 7.158$, $P = .000$**; Post hoc test: $P = .000$**, see Fig. 3C) as well as the number of entries in the open arms (ANOVA: $F = 2.730$, $P = .045$*; Post hoc test: $P = .025$*, see Fig. 3D). A single treatment with ketamine showed a main effect on the PTSD-like behaviors exposed to the SPS&S procedure ($F = 16.411$, $P = .000$**, see Fig. 3I). Based on the performed post hoc analysis, there was an increase of immobility time in rats treated with 10 and 15 mg/kg doses of ketamine (SPS&S + ket-10: $P = .001$*; SPS&S + ket-15: $P = .000$**, see Fig. 3E) compared with the SPS&S + vehicle group.

3.4. Effects of ketamine on the variations of HCN1 and BDNF level in the PFC

3.4.1. Immunohistochemistry assay

The SPS&S rats demonstrated the up-regulated expression of BDNF-positive cells with the ketamine treatment ($F = 26.987$, $P = .000$**, see Fig. 4A); it can be observed that these variations were significant in both the SPS&S + ket-15 group ($P = .000$**, see Fig. 4C) and the SPS&S + ket-20 group ($P = .000$**, see Fig. 4C). A one-way ANOVA showed a main change of the response of HCN1-positive cells to the ketamine treatment in the rat model of PTSD ($F = 11.967$, $P = .001$). Further post hoc analysis revealed that ketamine treatment with 15 mg/kg ($P = .002$*, see Fig. 4D) or 20 mg/kg ($P = .001$*, see Fig. 4D) could inhibit the HCN1 level, compared with the SPS&S + vehicle group. Moreover, linear regression analysis from Immunohistochemistry assay results indicated that there was a correlation between expression of

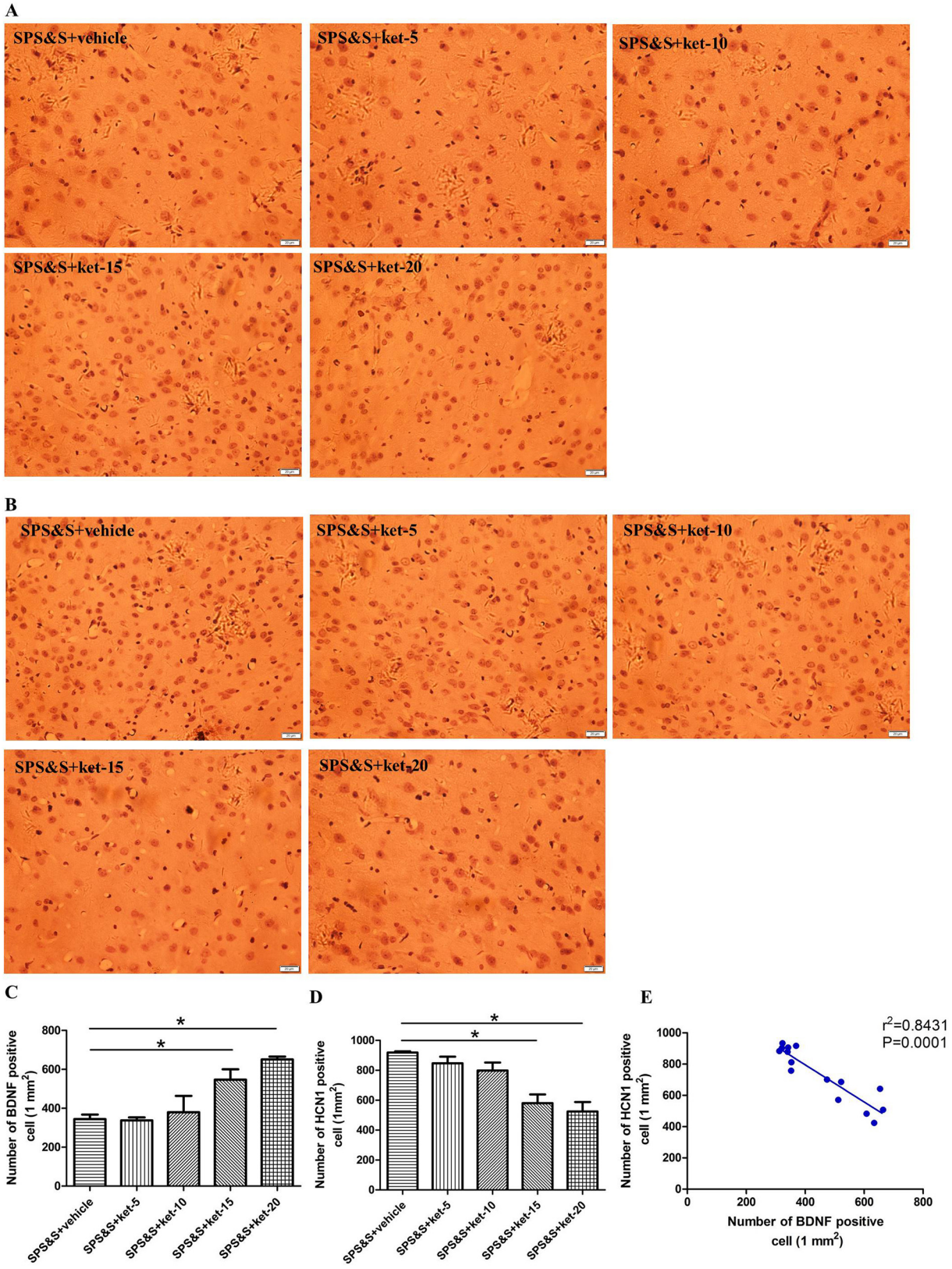

Fig. 4. Effects of single dose of ketamine on the number of BDNF and HCN1-positive cell in the PFC. A: The expression of BDNF positive cells in the PFC. B: The expression of HCN1-positive cells in the PFC. C: The number of BDNF-positive cells. D: The number of HCN1-positive cells. E: The correlation between the BDNF- and HCN1- positive cells in the PFC. The BDNF- and HCN1-positive cells in the PFC are represented by photomicrographs (400×). Data are represented as mean ± SEM. *$P < .05$ was statistically significant.

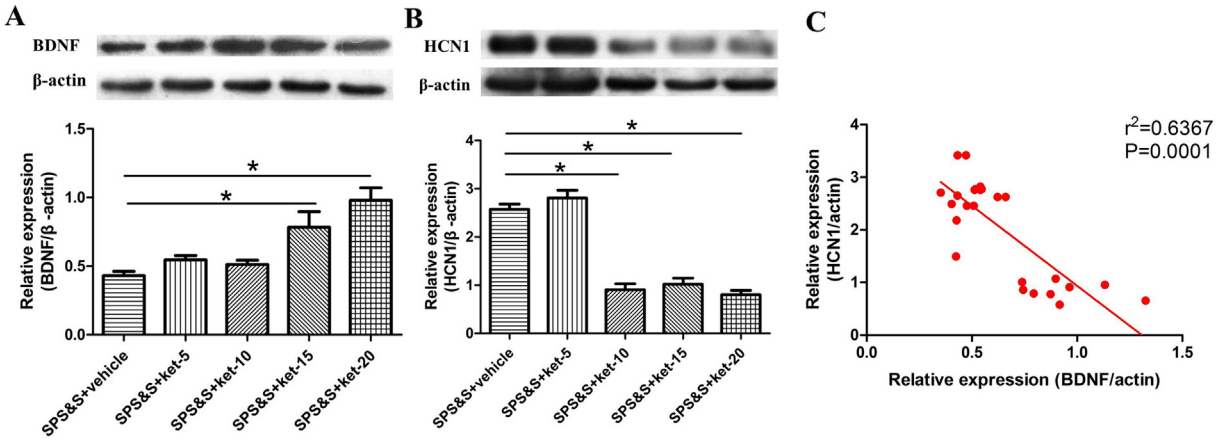

Fig. 5. Effects of single dose of ketamine on the levels of BDNF and HCN1 in the PFC. A: the relative expression of BDNF protein B: the relative expression of HCN1 protein. C: The correlation between the level of BDNF and HCN1 protein in the PFC. Values are shown as mean ± SEM of three rats per each group; the western blot data are expressed as percentage of corresponding optical density (normalized to β-actin) in the vehicle control samples. The data are represented as mean ± SEM. *$P < .05$ was statistically significant.

BDNF-positive cells and HCN1-positive cells in the PFC ($r^2 = 0.8431$, $P = .011$*, see Fig. 4E).

3.4.2. Western blot assay

The main changes regarding the level of the BDNF protein responses in the PTSD rats, after treatment by the ketamine, were analyzed using one-way ANOVA ($F = 10.510$, $P = .000$**, see Fig. 5A). The results of Dunnett's *t*-test demonstrated that the levels of BDNF protein in the SPS &S + ket-15 group ($P = .007$*, see Fig. 5A) and SPS&S + ket-20 group ($P = .000$**, see Fig. 5A) were significantly higher than that in the SPS &S + vehicle group. One-way ANOVA findings showed that the levels of the HCN1 protein were remarkably elevated in the SPS&S + ket-10 group ($P = .000$**, see Fig. 5B), SPS&S + ket-15 group ($P = .000$**, see Fig. 5B), and SPS&S + ket-20 group ($P = .000$**, see Fig. 5B) compared with the SPS&S + vehicle group when analyzed by Dunnett's t-test. In addition, according to the achieved results by correlation analysis, there is a negative correlation between the level of BDNF protein and HCN1 protein in the PFC ($r^2 = 0.6367$, $P = .000$, see Fig. 5C).

4. Discussion

This present study was designed to assess the effectiveness and modulation of ketamine's properties in order to alleviate the PTSD-like symptoms in SPS&S model. Behavioral results implied that SPS&S animals develop PTSD-like behaviors; however, a single treatment with ketamine (15 mg/kg) could lead to significant suppression of these symptoms. Furthermore, ketamine could down-regulate the expression of HCN1 and raise the level of BDNF in the PFC of SPS&S rats. Meanwhile, there was a negative correlation between expression of BDNF and HCN1, which indicated that the behavioral effects might be likely based on the modulation of BDNF expression by regulating HCN1 expression in the PFC of SPS&S animals.

The SPS&S procedure is a reliable-established animal model for PTSD studies, mimicking a portion of the neuroendocrine and variations of behavior associated with PTSD (Liberzon et al., 1999). A recent research showed that the inescapable foot electric shock after a classic SPS model (SPS&S) considerably enhanced the fear responses, and it presented a novel PTSD model (Wang et al., 2008). This modified model had shown significant changes in OFT, EMP, and Morris water maze performance, which reflected the increase of anxiety-like behaviors that are characteristic features of the PTSD (Yamamoto et al., 2009). This study also revealed that the SPS&S model could produce more robust symptoms, including the decrease of the locomotor activity in the OFT, the aggrandized exploratory behavior in the EPM, as well as increasing the immobility time in the FST. These are typical of the characteristics from traumatically cued PTSD-like behaviors. The level of the BDNF was lower in the SPS&S group than the control, and the expression of the HCN1 significantly increased in the SPS&S group compared with the control group. This not only demonstrated that the SPS&S was effective in inducing PTSD-like behaviors, but also showed that the SPS&S model in our study was successfully established.

The decrease of exploratory activity, including the higher number of the crossings and up-right movements in the OFT, the more entries and explore the open arms in the EPM, and the increase of immobility time in the FST can be interpreted as a typical symptom associated with PTSD-like behavior (Murrough et al., 2013; Pynoos et al., 1996; Shang et al., 2015). In this study, ketamine with dose of 15 mg/kg could improve the exploratory activity in the OFT or EPM, and reduce significantly the immobility time in the FST at 24 h after administration which could be interpreted as a reduction in the degree of PTSD-like behavior in these ketamine-treated rats (aan het Rot et al., 2010; Shang et al., 2015). These findings disagreed with previous reports that PTSD associated with multiple foot shocks did not affect exploratory activity due to the fear conditioning caused by the multiple foot shocks rather than the fewer foot shocks (Jin et al., 2016a,b).

However, it is noteworthy that there is trivial or no significant dose response to ketamine except the dose of 15 mg/kg. In general, subanesthetic dose of ketamine may produce antidepressant, analgesic, and propsychotic effects for animals in a dose-related manner from low-dose to high-dose. For instance, ketamine at the doses of 5, 10, and 15 mg/kg was known to improve depression-like behaviors without psychomotor adverse reactions (Garcia et al., 2008). Moreover, the ketamine with dose of 14 or 16 mg/kg exerted robustly the anti-PTSD-like effects (Pietersen et al., 2006; Qiu et al., 2017). However, ketamine could provide analgesia for evoked or spontaneous pain, even propsychotic effects when the dose is higher than 20 mg/kg (Wang et al., 2011). Therefore, the reason for this lack of dose response and partial inverted U-shaped dose-effect could be attributed to the higher dose of the ketamine that could elicit stereotyped behaviors by causing loss of parvalbumin (PV)-immunoreactivity in the PFC (Zhou et al., 2015).

Furthermore, the achieved results revealed that ketamine could improve the number of the BDNF-positive cells in the PFC, although ketamine with certain doses (i.e., 5, 10, and 20 mg/kg) does not alter BDNF-positive cells. The ketamine with dose of 15 mg/kg could increase the level of the BDNF protein in the PFC. This was consistent with ketamine's antidepressant effects, which may depend on rapid activation of the mammalian target of BDNF protein in the PFC (Autry et al., 2011). This is possibly involved in the pathophysiology of mood

disorders, including depression, anxiety, and PTSD. The correlation between the PTSD-like effects and the decreased concentration of BDNF protein is biologically plausible. Stress is known to impair various endocrine, physiological, and neuronal functions, and it is often associated with higher vulnerability to psychiatric disorders. The evidence represented that BDNF might play a role in stress-related psychiatric disorders.

Some preclinical studies reported the variations of BDNF in different stress conditions, which strongly support the role of the BDNF in stress reactions (Duman, 2002). These preclinical studies disagree the paucity of clinical data on BDNF alterations in patients who had faced with traumatic or stressful experiences. The serum BDNF protein levels are lower in depressed patients with an early life stress than the healthy volunteers (Grassi-Oliveira et al., 2008). The present study draws a conclusion that ketamine could alleviate the PTSD-like effects by increasing the BDNF in the PFC, based on the behaviors test, immunohistochemistry and western blotting results.

There has been a previous research showed the mechanism how a single injection of ketamine increase BDNF protein levels in the rat model of PTSD (Zhang et al., 2015). The antagonist NMDA receptor could up-regulate BDNF protein levels by interacting with voltage sensitive Ca^{2+} channels that facilitate the phosphorylation of cAMP-response element-binding protein in clinical patients (Lonze and Ginty, 2002). While it was generally accepted that the mechanism of ketamine was due to the effects on the NMDA receptors, there was not substantial behavioral evidence to support this hypothesis. Relevant literature presented an interesting theory, demonstrating that HCN1-null mice could encourage antidepressant behavior and ketamine caused subunit-specific inhibition of recombinant HCN1 (Chen et al., 2009; Lewis et al., 2011).

In this study, doses of ketamine at 10, 15, and 20 mg/kg could reduce the number of the HCN1-positive cells as well as the expression level of the HCN1 protein in the PFC. The HCN1 effectively activated the lower expression of the BDNF protein in the PFC. What's more interesting was that the results of the BDNF and HCN1 showed that there was a negative correlation between the level of BDNF and HCN1 in the PFC, which expressed that the ketamine up-regulated the BDNF signaling by reducing the HCN1 protein to alleviate the PTSD-like effects. This was in agreement with regulating the HCN1-related BDNF that produced anxiolytic- and antidepressant-like behaviors in rats (Kim et al., 2012). Mala also reviewed the inhibition of HCN1 channels which might led to antidepressant-like effects and enhanced the BDNF protein levels (Shah, 2012).

The cellular mechanism (or mechanisms) that reduces the HCN1 channel function, leading to an increase of BDNF synthesis is unknown (Shah, 2012). In this study, it was found that ketamine could improve BDNF levels, which alleviated the PTSD-like behavior through inhibiting the expression of HCN1 protein. Meanwhile, it is suggested that targeting the activity of the HCN1 might provide an alternative therapy for treating PTSD-like effects (Zhang et al., 2016). It should be mentioned that few studies have assessed the role of the HCN1 in ketamine treatment for PTSD-like symptoms (Li et al., 2014).

The probability that ketamine could up-regulate the BDNF protein level by binding to the NMDA receptor was not excluded. Meanwhile, whether there was an association between the activity of the HCN1 Channel and expression of the HCN1 protein during the ketamine administration was not clear. Therefore, we intend to test the BDNF protein level after reducing the expression level of HCN1 protein by lentivirus-based silencing RNA system expressing shRNA against HCN1 mRNA. And the activity of the HCN1 Channel and expression of the HCN1 protein were assessed simultaneously in the future.

Besides, despite the significant role of prefrontal cortex in mediating PTSD-like effects, the lesion of amygdala and the hippocampus are also the pathogenic factor in the PTSD-like effects. We did not evaluate BDNF or HCN1 protein levels in these areas. Therefore, we cannot discard the idea that BDNF protein levels and the activity of the HCN1 might also be altered in the amygdala and hippocampus which is also we will explore one of the important point furthermore.

5. Conclusions

Overall, this study is a novel research that has used ketamine with dose of 15 mg/kg in order to up-regulate the BDNF protein levels via inhibiting the expression of HCN1 to alleviate PTSD-like effects in the rat model induced by SPS&S. The achieved results extended earlier reports and suggested that HCN1-related BDNF signaling pathways might be a potential target for treatment of PTSD disorders and ketamine might be a useful alternative medicine for treating PTSD-like effects.

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The authors have no financial interests or conflicts of interest to declare.

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Ethical statement

All analyses were based on previous published studies, thus no ethical approval and patient consent are required. And all the authors declare no conflict of interest.

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