Propofol alleviates PTSD-like behavioral deficits by downregulating hippocampal Arc associated with GABAergic activation in basolateral amygdala in rats

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Abstract

Propofol (2,6-diisopropylphenol) is one of the most commonly used anesthetics in clinical surgery. However, its role and relevant mechanisms in post-traumatic stress disorder (PTSD)-like behavioral deficits remain largely unknown. In this study, the PTSD-like phenotype was constructed in rats using the modified single prolonged stress (MSPS) procedure. Propofol and GABAergic system antagonist bicuculline alone or combined administration were performed in rats after MSPS. SH-SY5Y cells were treated with different dosages of BDNF (1, 2, 5, 7 and 10 ng/ml), followed by treated with 25 μmol propofol. We first observed that propofol inhibited the protein level of activity regulated cytoskeleton protein (Arc) in the hippocampus of rats exposed to the MSPS procedure and BDNF-induced Arc upregulation in SH-SY5Y cells. Further analysis showed that administration of propofol alleviated fear memory formation in rats exposed to the MSPS procedure using open field, light dark box and contextual fear conditioning tests, accompanied with increased neurons cells in the hippocampal CA3 region by Nissl staining. Finally, administration of bicuculline to the basolateral amygdala (BLA) can significantly reverse the effect of propofol on the Arc expression and behavioral improvement of the modeling rats. In conclusions, our data suggested that propofol could alleviates fear memory formation in rats underwent MSPS by inhibiting Arc expression in the hippocampus in association with GABAergic activation in the basolateral amygdala GABAergic system.

Introduction

Post-traumatic stress disorder (PTSD) refers to a type of psychiatric disorders mainly characterized by avoidance, re-experience, hyperarousal and negative cognitions (Parsons and Ressler 2013, Yang, Gu et al. 2018). Currently, symptoms-relieving antidepressant, antianxiety and anticonvulsant drugs could effectively relieve these symptoms, but these based on cognitive behavioral therapy usually accompany with severe adverse effects (Yang, Gu et al. 2018). It is well-known that the main obstacle for curing PTSD is the abnormally strengthened conditioned fear memory (Paredes Molina, Berry et al. 2018, Alexander, Nalloor et al. 2019). Thus, anesthetic sedatives that generally act through the central nervous system have been inevitably applied to alleviate these symptoms in patients suffering PTSD, of which propofol (2,6-diisopropylphenol) is the most commonly used agent because of its rapid onset, short-acting and minimal side effects (McKeage and Perry 2003). However, the role and relevant mechanisms of propofol in PTSD-like behavioral deficits remain largely unknown.

It has been reported that the amnestic and anesthetic mechanism of propofol is associated with the central mechanism of PTSD formation in the central area and effective targets. For example, propofol can activate GABA receptors (Shin, Germann et al. 2018), whose anesthesia mechanism may via increasing chloride ion conduction, desensitizing GABA receptors, thereby inhibiting the central nervous system. Vallejo et al. (Ana Galarza Vallejo 2019) showed that propofol can interfere the re-consolidation process of negative memories after reactivation, causing amnesia effect on 50 participants. In addition, propofol has amnesia effect, which is manifested by decrease the time for rats re-enter to the dark box with sufficient electric shock (Michael T. Alkire 2001, Yu Ren, Fu-Jun Zhang, Qing-Sheng 2008;). The

GABAergic system in basolateral amygdala (BLA) is also identified as major neurotransmitter system that regulates memory function and its intervention can affect the formation and development of PTSD (Ren, Zhang et al. 2008). Interference with the GABAergic system can reverse the amnestic effect of propofol (Luo, Min et al. 2011). Activity regulated cytoskeleton protein (Arc) is considered to be a marker protein of learning and memory (Messaoudi, Kanhema et al. 2007, Ploski, Pierre et al. 2008, Okuno, Akashi et al. 2012, Gao, Castro-Gomez et al. 2018) and there is certain connection between Arc and memory consolidation (Ploski, Pierre et al. 2008). Interestingly, Arc interacts with the GABA system in BLA, and a previous study indicated that activated GABA receptor could decrease Arc protein expression (Terunuma, Revilla-Sanchez et al. 2014). Therefore, we speculate that propofol may play an amnesic role by activating GABAergic system in BLA and this role may be realized by influencing the expression of Arc protein.

In the present work, a modified single prolonged stress (MSPS) rat model was constructed and used to evaluate the protection of propofol against PTSD-like behavioral deficits and to assess the possible mechanism. Our findings may provide a potential new therapeutic target for the treatment of PTSD.

Materials And Methods

Animals

Adult male Sprague-Dawley rats weighting 250-300g were purchased from Jihui Laboratory Animal Feeding Co., LTD (Shanghai, China) and housed in the Laboratory Animals Center of East China Normal University. Animals were maintained in a controlled cages under a 12-hour light/dark cycle with ad libitum access to food and water. All procedures were approved by the East China Normal University Institutional Animal Care and Use Committee (cat. R20200601) and abided by guidelines in the Guide for the Care and Use of Laboratory Animals.

Modified single prolonged stress (MSPS)

The MSPS protocol was performed according to a previous report (Wang, Liu et al. 2008). In brief, the rats were forcibly restrained for 2 h and then immediately underwent a 20-min forced swimming in 22 ± 2℃ water. Following a 15-min recuperation period, the rats were exposed to ether until the loss of consciousness. After recovering for 30 min, rats experienced 4 s of inescapable foot shock at 1mA. Rats were then returned to their cages for 7 days before behavioral testing and tissue collection.

Animal grouping

Rats were randomly divided into control, MSPS, pre-pro, post-pro and pro + Bic groups. In the pre-pro group, 25 mg/kg propofol (Fresenius Kabi Austria GmbH, Austria) was intraperitoneally injected into rats at 5 min before MSPS. Rats in post-pro group received immediate intraperitoneal injection of 25 mg/kg propofol after MSPS. Control and MSPS rats received the same volume of saline vehicle. In the pro + Bic group, MSPS rats immediately received bilateral injection of GABAergic system antagonist bicuculline

(Selleck, S7071) at different dosages of 50, 100 and 150 pmol/0.5 µl as previously described (Ren, Zhang et al. 2008). After 15 min, the rats were intraperitoneally injected with propofol (25 mg/kg). Rats from different groups were then returned to their cages for 7 days before behavioral testing and tissue collection.

Open field test

The chamber was a box (45 × 45 × 45 cm) consists of two infrared sensor coils in the peripheral and bottom zones of polyvinyl chloride box. The rats from different groups were placed in the room for more than 30 minutes to adapt the environment. Then, rats were placed gently in the center of the box. Automated image analysis software (Truscan, Coulbourn, USA) was used for recording total distance and time spent in central areas for 30 min. After each test, 70% ethanol was used to clear the open field.

Light dark box test

Light-dark box apparatus consisted of a light compartment (light box; 45 × 45 × 45 cm) with translucent walls illuminated at 300 lux and a dark compartment (dark box; 45 × 45 × 45 cm) with blackened opaque walls. Each rate was individually placed in the center of the light box and allowed to roam freely for 20 min. Automated image analysis software (Truscan, Coulbourn, USA) was used to analyze the time that rats stayed in the light box and the numbers of entries between compartments. The apparatus was cleaned with 70% ethanol between trials.

Contextual fear conditioning test

Contextual fear conditioning test was conducted as previously described (Lee and Lee 2018). Briefly, all rats were allowed to habituate for 5 min to the conditioning chamber without any stimulation on day 1–3 (habituation stage). One day 4 (acquisition stage), rats were permitted to freely explore for 3 min. After 3 min, they received a total of 3 electric shocks (1.0 mA, 2s duration) at intervals of 60 s through the test chamber floor. Next day (retrieval stage), the rats were remained in the same chamber for 10 min with no shocks delivered. On day 5–9 (extinction stage), rats were placed for 10 min without foot shock in the same chamber to determine the extinction of contextual fear conditioning via calculating the percentage of freezing (dividing the freezing time by the total time).

Nissl's staining

In brief, collected brains were embedded, frozen at -80°C and cut into 10 µm-thick coronal sections. The sections were deparaffinized in 95% ethanol, rinsed in tap water and stained with 0.5% cresyl violet (Sigma-Aldrich, USA) for 3 min. After dehydrated with 95% ethanol for 5 min, 100% ethanol for 10 min and xylene for 10 min, the histopathological changes of the neurons in hippocampal CA3 were visualized with an optical microscope (Olympus, Japan) at 400 × magnification.

Cell treatment

Human neuroblastoma SH-SY5Y cell line was obtained from American Type Culture Collection (CRL-2266, ATCC) and cultured in DMEM/F-12 medium supplemented with 10% fetal bovine serum (Life Technologies, Darmstadt, Germany), 1% penicillin/streptomycin (Life Technologies, Darmstadt, Germany) under standard conditions (5% CO_2, 37°C). The stock solution of BDNF (100 μg/mL, Sigma Aldrich, B3795) was prepared with double distilled water, which was further diluted into a series of concentrations (1, 2, 5, 7 and 10 ng/ml) with double distilled water. In the *in vitro* assays, SH-SY5Y cells were treated with different dosages of BDNF (1, 2, 5, 7 and 10 ng/ml) for 2 h. After confirmed the optimal concentration, SH-SY5Y cells were further treated with 25 μmol propofol for 2 h.

Cell immunofluorescence

SH-SY5Y cells from different groups were washed in PBS three times and fixed with 4% paraformaldehyde. After washed with PBS, cells were permeabilized with 0.1% Triton X-100 diluted in PBS for 10 min and immersed in blocking buffer (5% BSA in 0.1% Triton X-100) for 30 min. Subsequently, cells were incubated with the primary Arc antibody diluted in PBS overnight at 4°C, followed by incubation with fluorescent secondary antibody conjugated to AlexaFluor 488 for 2 h at room temperature. Stains were washed with PBS three times before nuclear staining with DAPI. Finally, laser confocal microscope was applied for image acquisition and analysis.

Quantitative real time PCR

Total RNA was extracted from tissues from rats of each group and cells with TRIzol reagent (Invitrogen, Carlsbad, USA) and reverse transcribed into cDNA by PrimeScript RT reagent Kit (Takara, Japan) following the manufacturer's guides. Relative mRNA expression levels were performed with SYBR Green detection system (Roche, Germany) with the following primer sequences: Arc forward: 5'-CCCTGCAGCCCAAGTTCAAG-3' and reverse: 5'-GAAGGCTCAGCTGCCTGCTC-3'; GAPDH forward: 5'-GTGGAGTCATACTGGAACATGTAG-3' and reverse: 5'-AATGGTGAAGGTCGGTGTG-3'. All samples were performed in triplicate and normalized with GAPDH level.

Western blot analysis

Total protein from tissues from rats of each group and cells was extracted using ice-cold RIPA lysis buffer containing protease inhibitor, which was quantified with BCA kit according to the product manual. Protein sample (30 μg) was loaded to a 10% SDS-polyacrylamide gel (Beyotime Biotechnology, China) and transferred onto a poly-vinylidene difluoride membrane (Invitrogen) by electroblotting. After blocked with 20% skimmed milk at room temperature for 2 h, the membranes were incubated with primary antibody against Arc (1:100) and GAPDH (1:1000) overnight at 4 ℃, followed by incubation with horseradish peroxidase-labeled secondary antibodies (1:2000, Abcam) for 2 h. Following washed with TBST, the immunoreactive bands were detected using an enhanced chemiluminescence detection kit (Beyotime Biotechnology, China). Meanwhile, the protein bands were semi-quantified by densitometric analysis with Bio Rad.

Statistical analysis

Statistical analysis was performed using Grahpad Prism 8.0. All the results were expressed as mean ± SEM and analyzed by unpaired Student' t-test (two groups) and one-way or two-way ANOVA (more than two groups). Statistical differences were accepted when *p*-values less than 0.05.

Results

Administration of propofol inhibits the protein level of Arc in rats exposed to the MSPS procedure

To investigate whether propofol regulated PTSD-related behaviors was correlated with Arc expression level, rats was used to construct PTSD model via MSPS procedure and administration of propofol was performed before and after modeling. After deeply anesthetized with chloral hydrate, the hippocampus tissues were separated from rats to extract RNA and protein samples. As shown in Fig. 1A, there was no significant difference in Arc mRNA level in all groups. Notably, the results from western blot analysis showed a significant increase in Arc protein in rats exposed to MSPS produce compared with control group, which was notably reversed by administration of propofol after modeling (Fig. 1B). These data indicated that propofol could affect MSPS-induced Arc changes at translational level.

Treatment of propofol downregulates Arc protein level in BDNF-induced SH-SY5Y cells

Subsequently, we utilized an *in vitro* model of BDNF-induced Arc expression in SH-SY5Y cells to reveal whether propofol can also have the suppressive effects on Arc protein expression in cells. The results showed that the expression of Arc was positively correlated with the dosage of BDNF and endogenous BDNF expression was not significantly changed in the SH-SY5Y cells, as determined by western blot analysis (Fig. 2A) and quantitative real time PCR (Fig. 2B-C). Here, the amount of BDNF was finally determined to be 2 ng/ml. Next, cell immunofluorescence experiment showed that 2 ng/ml BDNF could significantly increase the Arc protein level in SH-SY5Y cells, which was obviously reversed after treatment with 25 μmol propofol (Fig. 2D-E).

Administration of propofol alleviates depression-like behavior in rats exposed to the MSPS procedure

Next, we determined whether propofol prevented depression-like behaviors in MSPS rats. In the open field test, although there was no significant difference in the total distance of rats, the MSPS-induced reductions of time spent in the central area was remarkedly increased after administration of propofol (Fig. 3A). The results from contextual fear conditioning test (Fig. 3B) revealed that there was no significant difference in the percentage of freezing among different groups, suggesting that propofol did not influence the acquisition of fear memory. With the increasing time, apparently elevated freezing percentage was observed in MSPS rats. By contrast, administration of propofol could significantly prevent the impairment of fear extinction in rats with MSPS. Moreover, we assessed the role of propofol in hippocampus neurons survival by Nissl staining. As shown in Fig. 3C, we observed loosely arranged and shrunken nissomes, as well as decreased neurons in the CA3 region hippocampus after rats

experienced MSPS. After administration of propofol, the number of positive neurons cells in CA3 region of hippocampus was obviously increased compared to the model group. These data suggested that impaired hippocampus neurons were closely related with PTSD-like behavioral deficits.

Bicuculline reverses the effects of propofol on Arc protein expression and PTSD-like behavioral deficits

To further explore whether propofol exerted suppressive effects on PTSD-like behavioral deficits were mediated by activated GABAergic system, we selected a GABAergic system inhibitor bicuculline to inject into BLA of rats, followed by intraperitoneally injected with propofol (25 mg/kg). Western blot analysis first demonstrated that injection of bicuculline to BLA obviously reversed the suppressive effect of propofol on Arc protein expression and 100 pmol/0.5μl concentration obtained maximum effects that was selected for subsequent experiments (Fig. 4A). A series of behavioral tests showed that bicucullinea alleviated the regulatory effects of propofol on the center time (Fig. 4B), the time in light box (Fig. 4C) and the percentage of freezing (Fig. 4D), as determined by open filed, light dark box and contextual fear conditioning test, respectively.

Discussion

It is speculated that excessive consolidation of and failure to extinguish fear memory has been the core mechanism behind the development of PTSD. Consequently, modulating consolidation of fear memory as part of early intervention after a traumatic experience would be a potential strategy to prevent PTSD symptoms from developing later (Matsuoka 2011). Propofol, an agent for the induction and maintenance of anaesthesia, has been widely used for its benefits, including rapid onset, short-acting and minimal side effects. In this study, we found that propofol can inhibit the expression of Arc in the hippocampus, as well as improve the anxiety and fear memory extinction disorder of the rats after MSPS. Similarly, a recent study by Niu et al. (Niu, Duan et al. 2022) showed that propofol treatment can rescue the impaired hippocampus synaptic plasticity after PTSD. In addition, we induced the expression of Arc with BDNF by constructing SH-SY5Y cell model and found that propofol could also inhibit the expression of Arc in cells. However, no one has studied its specific cellular pathway in detail in the current research, which is worthy of further research.

Previous studies have suggested that the hippocampus plays a critical role in contextual fear memory (Maren and Holt 2000). Through Nissl's staining, we found that administration of propofol obviously increased the number of positive neurons cells in CA3 region of hippocampus after rats experienced MSPS. The alteration in structure and functions usually occurred in the hippocampus of PTSD rats (Bonne, Vythilingam et al. 2008, Shucard, Cox et al. 2012). The contextual information encoded in the hippocampus is crucial for extinction learning and the retrieval of fear memory in the fear learning process (Bissiere, Zelikowsky et al. 2011). Consistent with the decreased Arc induced by propofol, Ren et al. (Ren, Zhang et al. 2008) demonstrated that the amnesic effect of propofol seems to involve the modulation of Arc protein expression in the hippocampus. In fact, Arc is implicated as a master regulator of long-term synaptic plasticity and memory formation in mammalian brain (Nikolaienko, Eriksen et al.

2017). Arc/Arg3.1 implicated in the consolidation of memories and its knockout show memory retention deficits (Tzingounis and Nicoll 2006). Furthermore, Gao et al. (Gao, Castro-Gomez et al. 2018) demonstrated that Arc/Arg3.1 mediates a critical period for spatial learning, during which Arc/Arg3.1 fosters maturation of hippocampal network activity necessary for future learning and memory storage. Based on these evidences, it is not difficulty to understand that propofol alleviates PTSD-like behavioral deficits by downregulating Arc protein.

Based on the clinical anesthesia mechanism of propofol, we used GABAergic system antagonist bicuculline and found that the antagonist could reverse the effect of propofol on Arc protein expression and PTSD-like behavioral deficits. In the hippocampus, neuronal excitability is constrained by multiple GABAergic interneurons with highly specialized functions and an extensive repertoire of co-released neuromodulators (Comeras, Hormer et al. 2021). In line with our data, it was found that 100 mg/kg propofol could systematically improve learning and memory disorders in rats through GABA (Luo, Min et al. 2011). In human studies, propofol general anesthesia may cause transient pharmacological "damage" by supporting the neural matrix of early memory consolidation, making the patients forget what they learn before surgery (Moon, Esfahani-Bayerl et al. 2020). Considering the fact that activated GABA receptor could decrease Arc protein expression (Terunuma, Revilla-Sanchez et al. 2014), we thus believed that the inhibitory effect of propofol on memory may be attributed to its role in downregulating Arc protein via activating the GABAergic system.

In summary, we found that administration of propofol suppressed memory consolidation in rats after MSPS. One of the underlying mechanisms of propofol’s neuroprotective effect is its suppressive effect on Arc protein expression via activating GABAergic signaling within the BLA in rats. Our study would provide experimental evidence for the selection of propofol in the treatment of PTSD-like behavioral deficits.

Declarations

Ethics approval

All procedures were approved by the East China Normal University Institutional Animal Care and Use Committee (cat. R20200601) and abided by guidelines in the Guide for the Care and Use of Laboratory Animals.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Authors’ contributions

All authors contributed to the study's conception. ZHY, YH, LLG, LW and MLM contributed to the design of study. ZHY, YH and LLG performed experiments. Material preparation and data analysis were performed by ZHY, YH, LLG, CZ and YH. The preparation, editing and review of the manuscript were performed by ZHY, LW and MLM. All authors read and approved the final version of the manuscript.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Figures

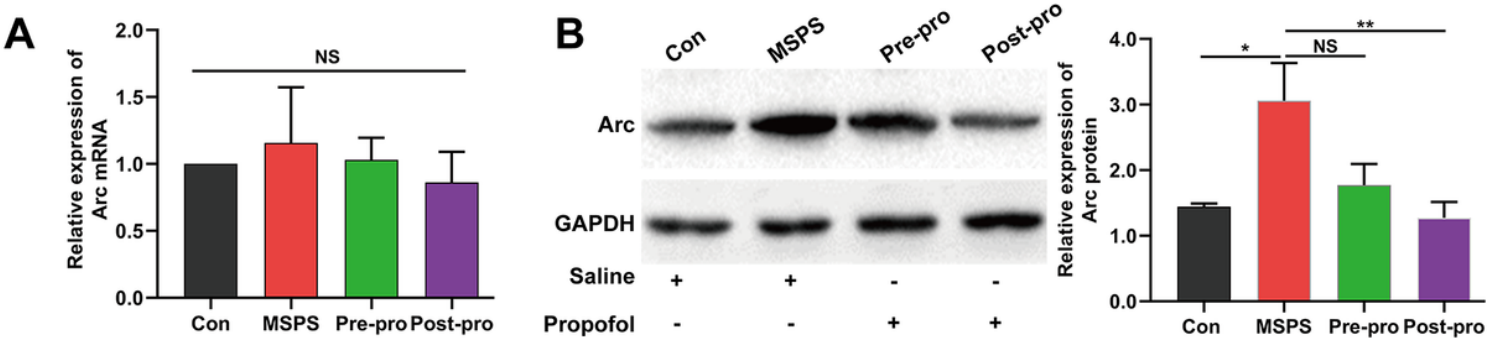

Figure 1

Administration of propofol inhibited the protein level of Arc in rats exposed to the MSPS procedure. (A) Quantitative real time PCR analysis of Arc mRNA levels in the hippocampus of MSPS-induced rats before

and after propofol treatment. Data are means ± SEM (n = 3). NS showed no significant difference, unpaired Student' s t test. (B) Western blot analysis of Arc protein levels in the hippocampus of MSPS-induced rats before and after propofol treatment. Data are means ± SEM (n = 3). *p< 0.05, **p < 0.01 showed significant difference, unpaired Student' s t-test.

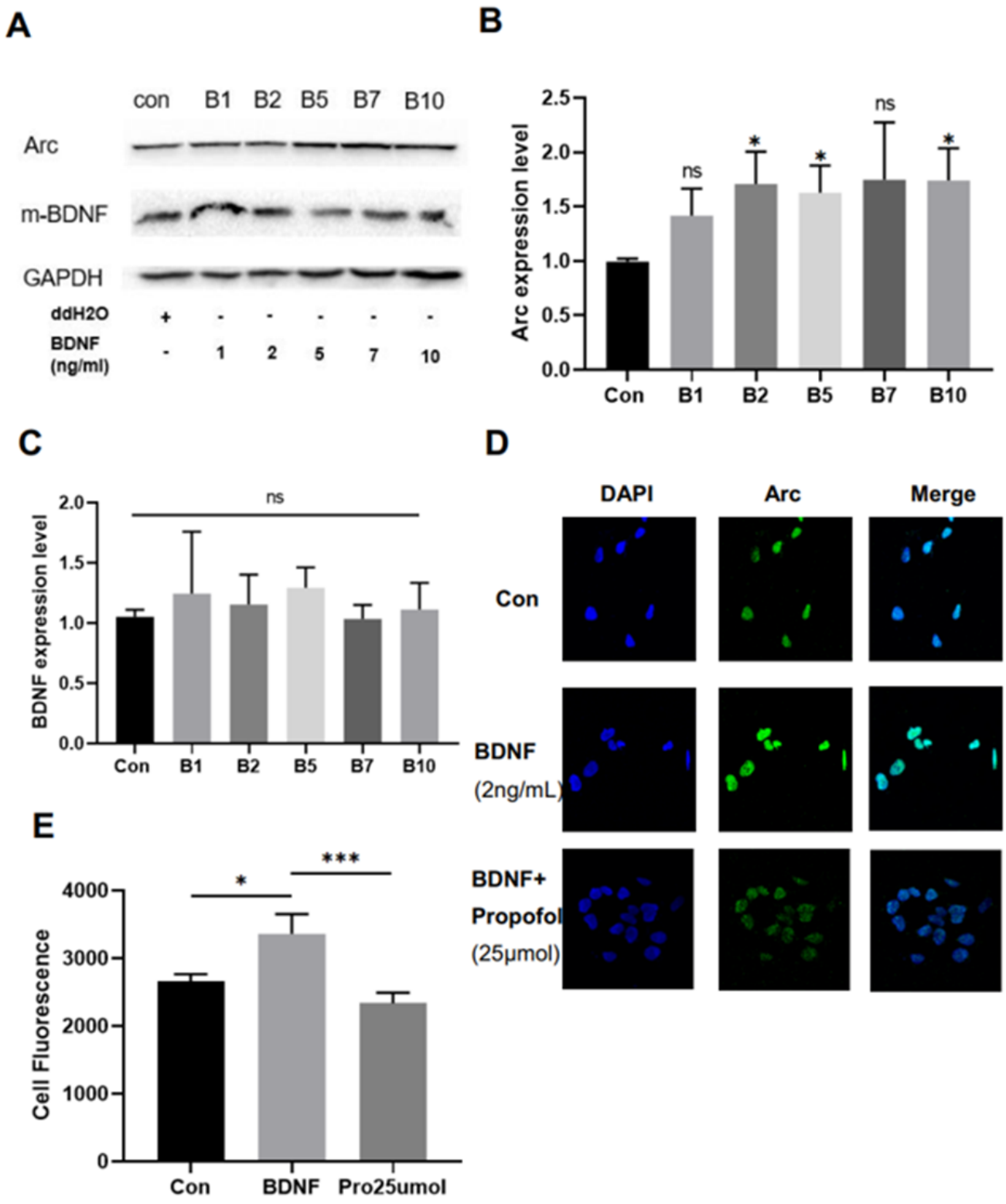

Figure 2

Treatment of propofol downregulated Arc protein level in BDNF-induced SH-SY5Y cells. (A) Western blot analysis and (B-C) quantitative real time PCR were performed to determine the amount of BDNF in SH-SY5Y cells. *$p < 0.05$, compared with control; (D) The representative Arc (green) and DAPI (blue) stained images were captured in the SH-SY5Y cells after treatment with 2 ng/ml BDNF alone or with 25 μmol propofol. (E) Image J was used to analyze the fluorescence intensity of SH-SY5Y cells. Data are means ± SEM (n = 3). *$p < 0.05$, ***$p < 0.001$ showed significant difference, unpaired Student' s t-test.

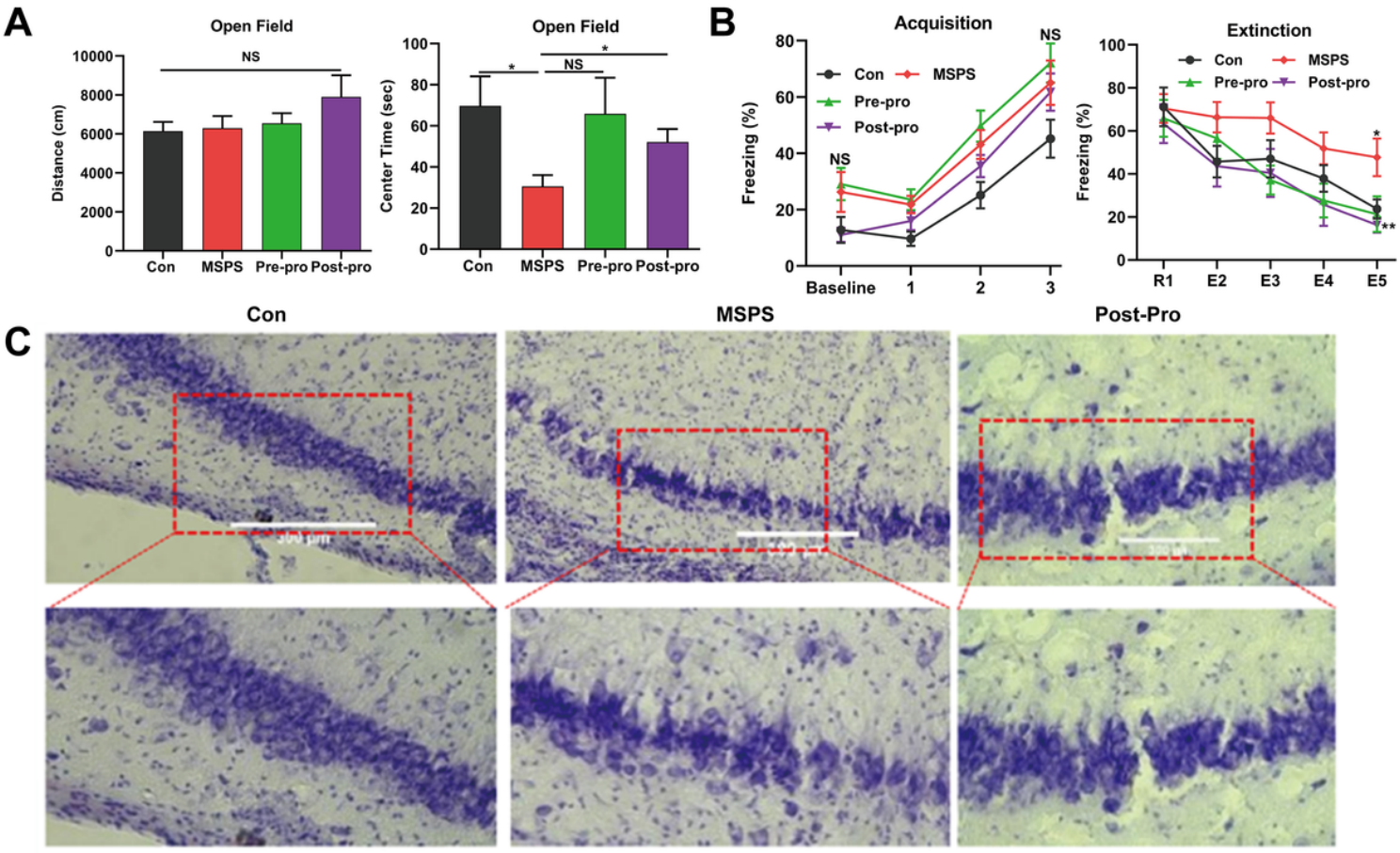

Figure 3

Administration of propofol alleviated depression-like behavior in rats exposed to the MSPS procedure. Rats experienced MSPS procedure, followed by administration of propofol. (A) Open field test was performed to analyze total distance and time spent in central areas for 30 min. (B) Contextual fear conditioning test was conducted to determine the percentage of freezing time in acquisition stage and extinction in rats. (C) Nissl's staining shows the morphology of hippocampus CA3 neurons (scale bar: 300 μm). Data are means ± SEM (n = 6-11 for each). *$p < 0.05$, **$p < 0.01$ showed significant difference; NS showed no significant difference, unpaired Student' s t-test.

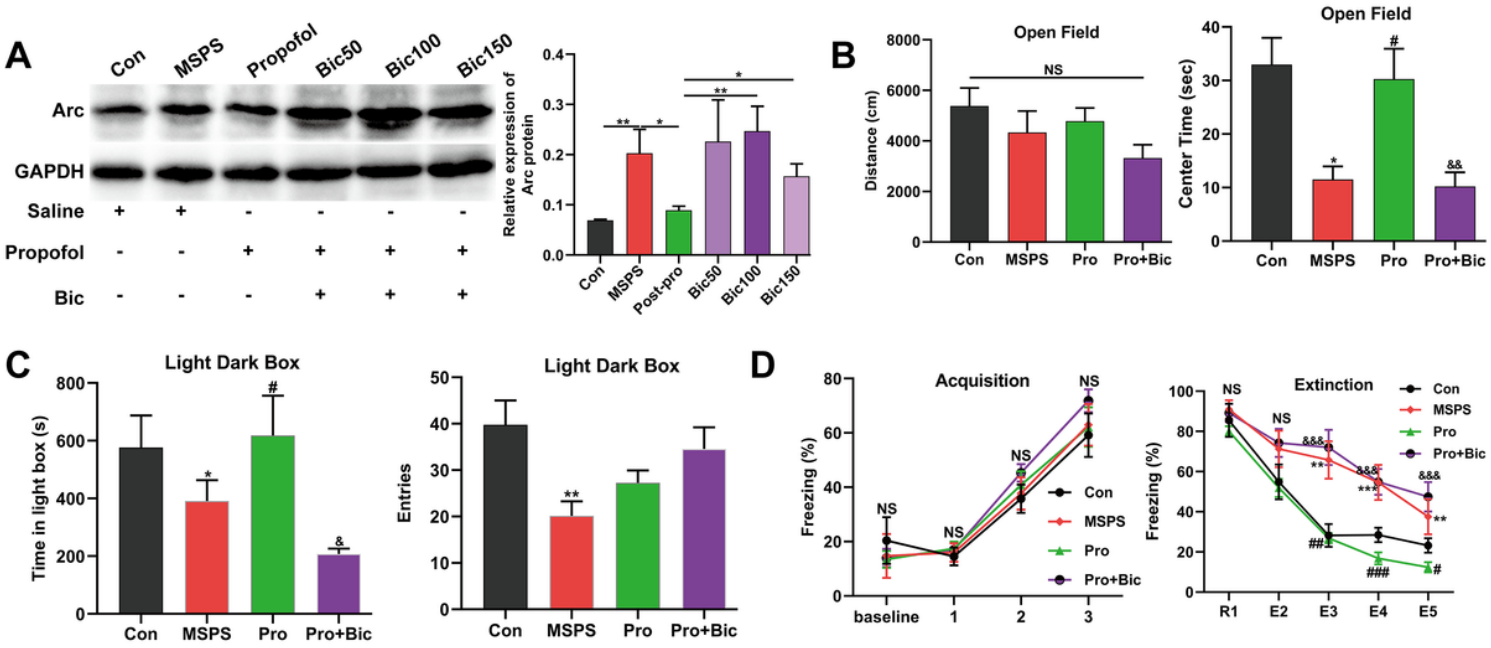

Figure 4

Bicuculline reversed the effects of propofol on Arc protein expression and PTSD-like behavioral deficits. MSPS rats immediately received bilateral injection of GABAergic system antagonist bicuculline at different dosages of 50, 100 and 150 pmol/0.5 μl for15 min, followed by intraperitoneally injected with propofol (25 mg/kg). (A) The protein level of Arc was detected by western blot analysis in rats from different groups. And the bicuculline dose of 100 pmol/0.5μl can restore Arc protein expression level induced by propofol was used in subsequent behavioral test. *$p < 0.05$, **$p < 0.01$ showed significant difference; (B) Open field test was performed to analyze total distance and time spent in central areas for 30 min. (C) Light-dark box test results shows the time rats stayed in light box and light-dark box entries in 20 min. (D) Contextual fear conditioning test results shows fear memory acquisition and extinction of rats of different groups. Data are shown as mean ± SEM (n = 9-12 for each). NS showed no significant difference.; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, compared with Control; #$p < 0.05$, ##$p < 0.01$, ###$p < 0.001$, compared with MSPS; &$p < 0.05$, &&$p < 0.01$, &&&$p < 0.001$, compared with propofol, two-way ANOVA, followed by Tukey's multiple comparisons test.