

Original Research

GluN2A Mediates PS-Induced Depressive-Like Behavior by Activating CaMKII and Inhibiting Myelinization

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Abstract

Background: Prenatal stress (PS) can induce depression in offspring, but the underlying mechanisms are still unknown. **Objective:** The aim of this work was to investigate the mechanism that underlies PS-induced depressive-like behavior in offspring. **Methods:** A prenatal restraint stress procedure was developed in which pregnant rats at GD14 to GD20 were placed head-first into a well-ventilated bottle three times each day and for 45 min each time. Depressive-like behavior in the male offspring was examined using the sucrose preference test (SPT) and the forced swim test (FST). The level of glutamate and the expression levels of GluN2A, p-CaMKII and myelin basic protein (MBP) in the hippocampus of PS-susceptible (PS-S) offspring were also evaluated. To clarify the mechanism by which PS leads to depression in offspring, the effects of excessive corticosterone were also investigated using an *in vitro* "injured neuronal" model. **Results:** The glutamate level in the hippocampus of PS-S male offspring was significantly elevated compared to controls. The expression levels of GluN2A and p-CaMKII were also altered. In addition, the optical density of MBP staining and the expression levels of MBP mRNA and MBP protein were decreased, demonstrating impaired myelinization in the hippocampus. Treatment of PS-S offspring with the GluN2A receptor antagonist NVP-AAM077 resulted in antidepressant-like effects in the FST, as well as rescue of the MBP and p-CaMKII abnormalities. **Conclusions:** These findings indicate that GluN2A is a promising target in the development of pharmacotherapies for PS-induced depression.

Keywords: depressive-like behavior; prenatal stress; GluN2A; myelinization; MBP

1. Introduction

Depression is one of the most prevalent behavior-debilitating conditions and the most common psychiatric disease amongst patients with mood disorders [1]. The adolescent offspring of women who experience psychosocial stress during pregnancy, referred to as prenatal stress (PS), are at increased risk of depressive symptoms [2,3]. This clinical phenomenon is supported by laboratory data from animal models of depression, in which PS leads to depressive-like behavior in the offspring of rats, mice and primates [4–7]. In a rat model of PS, susceptible offspring show depression-related behavior including anhedonia, despair, and increases in immobility time in swim tests [4,8]. Some of these depression-related behaviors respond to classical antidepressants such as fluoxetine, and to fast-acting agents such as ketamine [8]. Offspring rats with different susceptibility to depression-related behavior following PS have been used to study the biological mechanisms underlying stress and resilience. Such studies may be important for understanding the vulnerability to depression in humans.

Currently, theories regarding the abnormal development of offspring following PS include alternation of hypothalamic-pituitary-adrenal (HPA) axis regulation,

changes to neurotransmitters, changes to inflammatory factors, changes in brain structure and connectivity, alterations in the gut microbiome, and epigenetic alterations [9]. Using high performance liquid chromatography, we previously showed that PS significantly increases the glutamate level in the hippocampus of juvenile offspring [10]. Glutamate that is released from presynaptic neurons interacts with postsynaptic glutamate receptors, such as N-methyl-D-aspartate (NMDA) receptors, kainate, and α -amino-3-hydroxyl-5-methyl-4-isoxazole propionate (AMPA). A large body of literature exists on PS and glutamate, GABA and NMDA in the hippocampus of mice and rats [11–13]. Previous studies have used different stress models, species and biomolecules to investigate the mechanism of stress-related depression. However, the detailed molecular mechanisms that underlie depressive-like behavior in offspring following PS remain to be elucidated. The current study further investigates the roles of GluN2A and the myelin sheath in depressive-like behavior.

Oligodendrocyte lineage cell dysfunctions and changes in myelin have recently been implicated in the etiology and treatment of depression and of various stress-related disorders [14]. Myelin is produced when



plasma membrane extensions from mature oligodendrocytes wrap spirally around discrete axon segments known as internodes during ontogenesis of the central nervous system (CNS) [15]. Myelination is a major contributor of evolutionary success in vertebrates and is essential for CNS development and function [16]. Myelin basic protein (MBP) is the main myelin protein and plays a major role in adhering membranes within the myelin sheath [17,18].

A recent report showed that targeted delivery of glutamate to the white matter of adult spinal cord led to reduced MBP expression and to localized disruption of myelin compaction [19]. MBP expression could also be reduced by blocking the NMDA receptor. GluN2A is the most abundant GluN2 NMDA receptor subunit in the mammalian CNS [20] and has high permeability to Ca^{2+} . Postsynaptic influx of cellular Ca^{2+} binds to calmodulin to form Ca^{2+} /CaM complexes, as well as regulating a variety of protein kinases including CaMKII, ERK1/2, PKA and PKC [21]. In addition, GluN2A can induce changes that affect learning and memory function through various signaling pathways. However, it remains unclear whether GluN2A has a major role in myelination in the PS-induced depressive-like behavior of offspring in rats.

In the present study we therefore investigated this issue by we conducting the following experiments. First, a rat model of PS using a restraint procedure was used to simulate stress in the daily life of humans during pregnancy. Second, the level of depressive-like behavior in male offspring was evaluated using the sucrose preference test (SPT) and forced swim test (FST). Third, we quantified the level of glutamate and the expression levels of GluN2A, p-CaMKII and MBP in the hippocampus of PS-susceptible (PS-S) offspring. The lasting impact of PS on HPA axis function may be due to excessive exposure of the fetus to maternal corticosterone, since these abnormalities are prevented by maternal adrenalectomy and restored by the administration of corticosterone [22]. Therefore, to clarify the possible mechanisms of PS leading to depression, we also studied the effects of excessive corticosterone using an *in vitro* “injured neuronal” model.

2. Material and Methods

2.1 Animals and PS Procedure

Rats (Sprague-Dawley) were kept in standard conditions at room temperature ($23 \pm 1^\circ\text{C}$) and a 12:12 h light/dark cycle with light (7:00 AM–7:00 PM). *Ad libitum* access to food and water was provided, except during preparation for the SPT. Ten male rats were mated at a ratio of 1:3 with 30 female rats (230–300 g weight) at 20:00–22:00 PM. At 8:00 AM next morning, female rats underwent vaginal smear testing. Gestational day (GD) zero was designated as the day at which the first positive detection of sperm in the vagina was made. Pregnant female rats were raised separately and were divided randomly into control and experimental groups. Pregnant dams in the experimen-

tal group ($n = 21$) underwent exposure to PS as described previously [23]. Briefly, PS dams were placed head-first into well-ventilated bottles with an adjustable cap. This procedure was carried out three times daily and for 45 min each time between GD14 to GD20 inclusive. The interval between procedures was never <2 h or >4 h. Pregnant dams from the control group ($n = 8$) were placed in identical new cages but without exposure to the PS procedure. Approximately 30 days after birth, male offspring were selected at random for the subsequent experiments. Not more than two male siblings were used in the same experimental group. To avoid “litter effects”, the number of rats selected from each litter was between 6 and 12. The animal experiments received approval from the Animal Ethics Committee of Xi'an Jiaotong University (No. 2020-449) and were performed according to standard guidelines and protocols.

2.2 Drug Preparation and Treatment Schedule

NVP-AAM077 (Cat# 459836-30-7) was purchased from Millipore Sigma (St. Louis, MO, USA) and prepared in a saline solution. PS pregnant dams ($n = 8$) were treated once daily for 8 days (GD7-GD14) with NVP-AAM077 (10 mg/kg, intraperitoneal administration) at 30 min prior to PS exposure and at a volume of 2 mL/kg of body weight [24]. Control animals ($n = 8$) were administered an appropriate vehicle.

2.3 Hippocampal Neuron Culture

Rat hippocampal neurons (Cat# CM-R107, Wuhan, Hubei, China) were purchased from Procell Life Science & Technology and identified by immunofluorescence for β -Tubulin-III. The degree of purity was $>90\%$ and there was no evidence of HIV-1, hepatitis B virus (HBV), hepatitis C virus (HCV), mycoplasma, bacteria, yeast or fungi. Cells for subsequent experiments were grown at 37°C according to instructions from the supplier.

2.4 Model of Corticosterone-Induced Neuronal Injury and the Assessment of Neuronal Cell Viability

The effect of corticosterone on neuronal cell viability was investigated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay as described below. First, rat hippocampal neurons were added to 96-well plates and cultured overnight at 37°C . These were divided into 6 groups and treated with increasing concentrations of corticosterone (0, 0.01, 0.1, 1, 10 and 100 μM) for 24 hours (Table 1). Ten μL of MTT was added and the cells cultured for another 4 h at 37°C . The culture medium was then aspirated, 150 μL of DMSO added, and the cells shaken for 10 min. Measurement of cell viability was performed using the MTT assay (Biofroxx, Cat# 3580MG250, Jiangsu, Nanjing, China) as recommended by the manufacturer. The absorbance value of all wells was measured with a microplate reader (Elx 800, Bio-TEK Instruments, Beijing, China). Data for the experimental

Table 1. Effect of corticosterone on hippocampal neuron cell growth.

Corticosterone concentration (μM)	Cell proliferation (%)
0	100
0.01	106.48
0.1	95.84
1	91.49
10	81.17
100	72.48

groups were expressed as a percentage value relative to the controls. For drug treatment experiments, hippocampal neurons were treated with NVP-AAM077 (5 μM) for 12 h prior to treatment with NMDA. Appropriate vehicle was administered to the controls.

2.5 Rat Behavioral Studies

Rats were weaned on day 21 after birth. Prepubertal, 30-day-old male offspring were used for the depression-related behavioral studies. Because of the different stress levels caused by the various behavioral tests, the SPT was conducted first, followed by the forced swim test. The two behavioral tests were conducted with a one-day interval between them. As one week was required for the completion of all three behavioral experiments, the rats were almost 40 days old when sacrificed. Experiments were conducted blind to the treatment groups.

2.6 Sucrose Preference Test

For the SPT, rats were selected at random to determine their susceptibility or resistance to PS. They were divided into an experimental group ($n = 121$) and a control group ($n = 16$). The SPT was carried out as described earlier to quantify anhedonia and depression symptoms [25]. Prior to the SPT, rats were habituated for 24 h with 1% sucrose solution and then deprived of food and water for a further 24 h. The SPT was conducted between 8: AM to 9: AM the next day. The animals were concurrently offered normal tap water and a 1% sucrose solution. The drinking bottles were weighed one hour later to measure the consumption of water and sucrose, with the preference for sucrose being calculated as: $\text{sucrose consumption} / (\text{sucrose consumption} + \text{water consumption}) \times 100\%$. The PS-S group was defined as PS offspring that showed a $>30\%$ decrease in sucrose preference relative to controls. The PS resistant (PS-R) group showed a $<10\%$ decrease in sucrose preference, while the PS medium (PS-M) group showed a $<30\%$ but $>10\%$ decrease.

2.7 Forced Swim Test

Following the SPT, 8 rats from each group (PS-S, PS-R, PS-M) were used for the FST. This test was carried out as reported earlier and is used to assess depressive-like behav-

ior [26]. Briefly, rats were put into a circular glass tank (20 cm diameter, 50 cm height) containing 30 cm deep water at 30 °C. The room temperature was 25 °C and fluorescent lighting used throughout. To start the experiment, each rat was put in the tank and allowed to swim for 15 min. It was then taken out, dried, and returned to its cage. After 24 h, the rat was placed in the tank again for 5 min and the immobile time was measured. This was defined as the time spent not struggling, or with minimal movement only to keep its head above water.

2.8 Immunofluorescence

Eight animals from each group were euthanized and underwent cardiac perfusion with ice-cold PBS followed by overnight fixation at 4 °C in paraformaldehyde (4% w/v). A vibratome (VT 1000S, Leica, Shanghai, China) was then used to prepare coronal tissue sections of 4 μm thickness. Antigen retrieval was achieved by incubating for 20 min in a sodium citrate solution (Baxter, Deerfield, IL, C532382) containing Tween (0.05% v/v). The sections were washed thrice with PBS containing Triton X-100 (PBST, 0.1% v/v) for 10 min and then permeabilized at room temperature for 30 min with PBST containing goat serum (10% v/v). The primary antibodies used for immunofluorescence were human monoclonal anti-MBP (1:1000; ab209328, Abcam, Eugene, OR, USA) and mouse monoclonal anti-β-Tubulin-III (1:100; ab78078, Abcam). Tissue sections were incubated overnight at 4 °C with primary antibody, rinsed four times with PBST for 3 min each, and then incubated at 37 °C for 1 h with FITC-conjugated goat anti-mouse immunoglobulin G (IgG) secondary antibody (1:100; BA1101, BOSTER Biological Technology, Pleasanton, CA, USA). After blocking with goat serum, tissue sections were incubated overnight at 4 °C with antibody, washed four times with PBST for 3 min each, and then incubated for 1 h at 37 °C with Cy3-conjugated goat anti-rabbit IgG secondary antibody (1:100; BA1032, BOSTER Biological Technology). The sections were then incubated for 5 min with DNA stain 2-(4-amidinophenyl)-6-indolecarbamide dihydrochloride (DAPI, C1002, Beyotime Biotech, Shanghai, China), washed four times with PBST for 3 min each, and mounted in glycerol (50% v/v). Fluorescence microscopy was used to view slides (BX53, Olympus, Tokyo, Japan) and the fluorescence intensity measured with Image-Pro Plus (Version 6.0, Media Cybernetics, Rockville, MD, USA).

2.9 Quantitative Reverse-Transcription Polymerase Chain Reaction (qRT-PCR)

Following completion of the behavioral experiments, 8 animals from each group were euthanized, the hippocampus sectioned, and the tissue homogenized on ice. TRIzol (Aidlab Biotechnologies, Beijing, China) was used to extract total RNA and the concentration measured at 260 nm with a spectrophotometer (ND-

100, NanoDrop Technologies, Wilmington, DE, USA). Ominiscript® Reverse Transcriptase kit (Vazyme Biotech, Nanjing, China) was used to reverse transcribe RNA into cDNA. qRT-PCR was then performed with ABI 7900HT or QuantStudio 6 System (Applied Biosystems, Grand Island, NY) instruments using the following primer sequences: GluN2A, 5'-ACATTGCAGAAGCTGCCTTT-3' (F), 5'-TTCTGTGACCAGTCCTGCTG-3' (R); MBP, 5'-CACACAGCAGACCCAAAGAA-3' (F), 5'-GTCGCTGTGAGGGTCTCTTC-3' (R); CaMKII, 5'-AACTGGCAGACTTCGGCTTA-3' (F), 5'-ATCCCAGAAGGGTGGGTATC-3' (R); GAPDH, 5'-ACAGCAACAGGGTGGTGGAC-3' (F), 5'-TTTGAGGGTGCAGCGAACTT-3' (R). The $2^{-\Delta\Delta C_t}$ method was used to quantify relative expression, and GAPDH was used as a control to normalize for input. All experiments were carried out in triplicate.

2.10 Western Blotting

Eight rats from each group were euthanized, the hippocampus sectioned, and the tissue homogenized on ice with RIPA lysis buffer containing a cocktail of phosphatase enzyme inhibitors (Beyotime Ins. Biotec, Shanghai, China). Lysates were centrifuged at $12,000 \times g$ for 5 min and the protein concentration in the supernatant quantified using a BCA kit (Beyotime Ins. Biotec). Proteins in the lysates (20 mg) were separated using 5% or 10% SD-SPAGE and then transferred to porous polyvinylidene fluoride (PVDF) membranes (Millipore). Primary antibodies for Western blot were: rabbit monoclonal anti-GluN2A (1:1000; ab124913, Abcam), rabbit monoclonal anti-CaMKII (1:2000; ab52476, Abcam), rabbit monoclonal anti-p-CAMKII (1:1000; I2716, CST Annoron, Beijing, China), and human monoclonal anti-MBP (1:1000; ab209328, Abcam). GAPDH (1:1000; AB-P-R001, Hangzhou Goodhere Biotechnology, Hangzhou, China) was detected as the internal loading control. Detection was achieved using SuperSignal® West Dura Extended Duration Substrate ((Pierce Bio- technology, Rockford, IL, USA) and X-ray Film (Eastman Kodak, Rochester, NY), while signal intensities were measured with BandsScan 5.0 software (Funglyn Biotech, Richmond Hill, Ontario, Canada).

2.11 Measurement of Glutamate Concentration

Six rats from each group were euthanized. The hippocampus tissue was thawed and placed into a glass homogenizer. Frozen formic acid (1 mol/L, 2 mL) was added and the tissue was fully homogenized manually on an ice bath. Homogenates were centrifuged at 4 °C for 30 min and 7000 r/min, and the supernatant stored at -20 °C. Homogenate supernatant (1 mL) was mixed with 4% sodium bicarbonate solution (0.75 mL), centrifuged at 4 °C for 5 min at 3000 r/min and the supernatant collected. This was passed through a 0.45 µm filter membrane (Sigmaaldrich,

St. Louis, MO, USA), and then pack. To the dispensing solution (24 µL) in the sample bottle was added derivative reagent (12 µL) and sodium tetraborate buffer (960 µL, pH 9.18). This was mixed well and stood for 3 min at -20 °C then performed to determine the hippocampus glutamate concentration.

2.12 Statistical Analysis

Results are shown as the mean $\pm$ SD. Kolmogorov-Smirnov test was used to assess whether the data was normally distributed. Student's *t*-test was used to analyze for significant differences in pairwise comparisons. One-way ANOVA was used to compare more than two groups. Bonferroni or Tukey methods were used as appropriate for post-hoc analyses. Statistical significance was assumed at $p < 0.05$. All analyses were conducted using Prism version 5.0 (GraphPad Software Inc., Boston, MA, USA).

3. Results

3.1 Behavioral Studies Show that PS can Induce Depressive-Like Behavior in Offspring

One-way ANOVA found there were significant differences between pre-defined PS groups for behavioral test results (Fig. 1). Offspring from PS-S and PS-M groups (as defined in the Methods and Methods) showed significantly less sucrose preference in the SPT (Fig. 1A). These groups subsequently showed longer immobility time in the FST (Fig. 1B) compared to controls. The PS-R group showed no significant differences compared to controls (Fig. 1A,B).

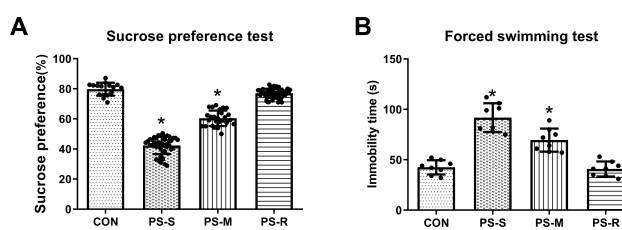


Fig. 1. Results from the behavioral tests. (A) Sucrose preference results for the four groups in the sucrose preference test (SPT). (B) Immobility time results for the four groups in the forced swim test (FST). All results shown are mean $\pm$ standard deviation (SD) ($n = 8$ to $n = 51$ for the different groups). * $p < 0.05$ vs. control. CON, control group; PS-S, PS susceptible group; PS-M, PS medium group; PS-R, PS resistant group.

3.2 PS Inhibits Myelination by Increasing Glutamate Levels and Activating GluN2A Receptors

Glutamate levels in the hippocampus of PS-S rats were significantly higher ($p < 0.05$) than in controls (Fig. 2A). Immunostaining showed the optical density of MBP staining in the PS-S group was lower than in controls (Fig. 2B). Moreover, expression levels for GluN2A and MBP protein were lower in PS-S offspring than in con-

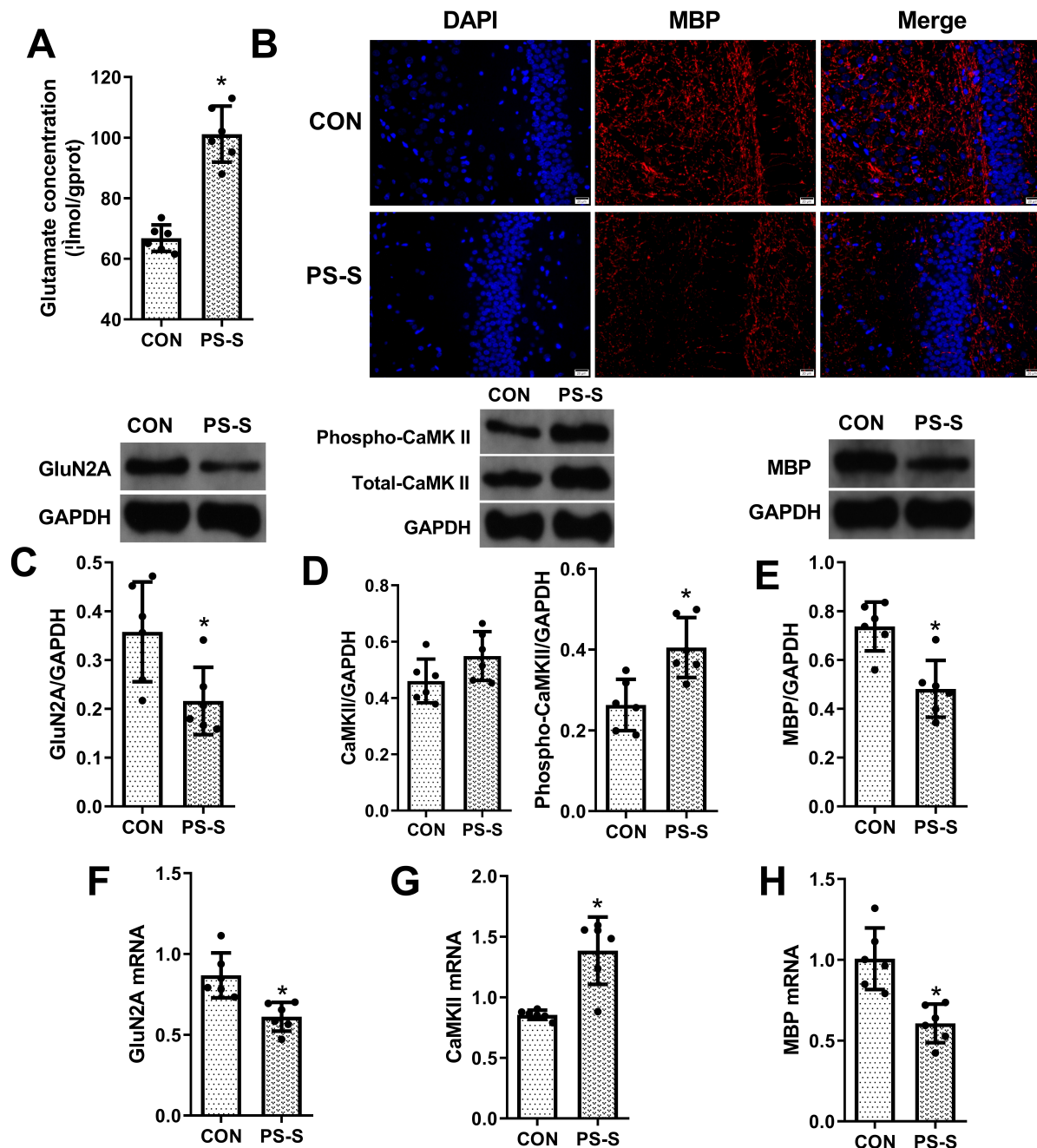


Fig. 2. Effect of PS on the glutamate level and on GluN2A, CaMKII, p-CaMKII and Myelin basic protein (MBP) expression levels in the hippocampus. (A) Glutamate level. (B) MBP staining visualized by immunofluorescence (400×). (C) GluN2A protein expression. (D) CaMKII and p-CaMKII protein expression. (E) MBP protein expression. (F) GluN2A mRNA level. (G) CaMKII mRNA level. (H) MBP mRNA level. Results shown are the mean $\pm$ SD for 6 to 9 rats in each group. * $p < 0.05$ vs. CON. CON, control group; PS-S, PS susceptible group; PS, prenatal stress; MBP, myelin basic protein.

trols (Fig. 2C,E), whereas p-CaMKII protein expression was higher (Fig. 2D). Similarly, GluN2A and MBP mRNA levels in PS-S offspring were also lower than in controls (Fig. 2F,H), whereas CaMKII mRNA levels were higher (Fig. 2G).

3.3 GluN2A Mediates PS-Induced Depressive-Like Behavior through CaMKII-Inhibited Myelinization

Compared to the controls, treatment of PS-S rats with the GluN2A receptor antagonist NVP-AAM077 increased immobility time in the FST (Fig. 3A). The optical density for MBP staining in the NVP group was also increased compared to the controls (Fig. 3B). The PS-S group

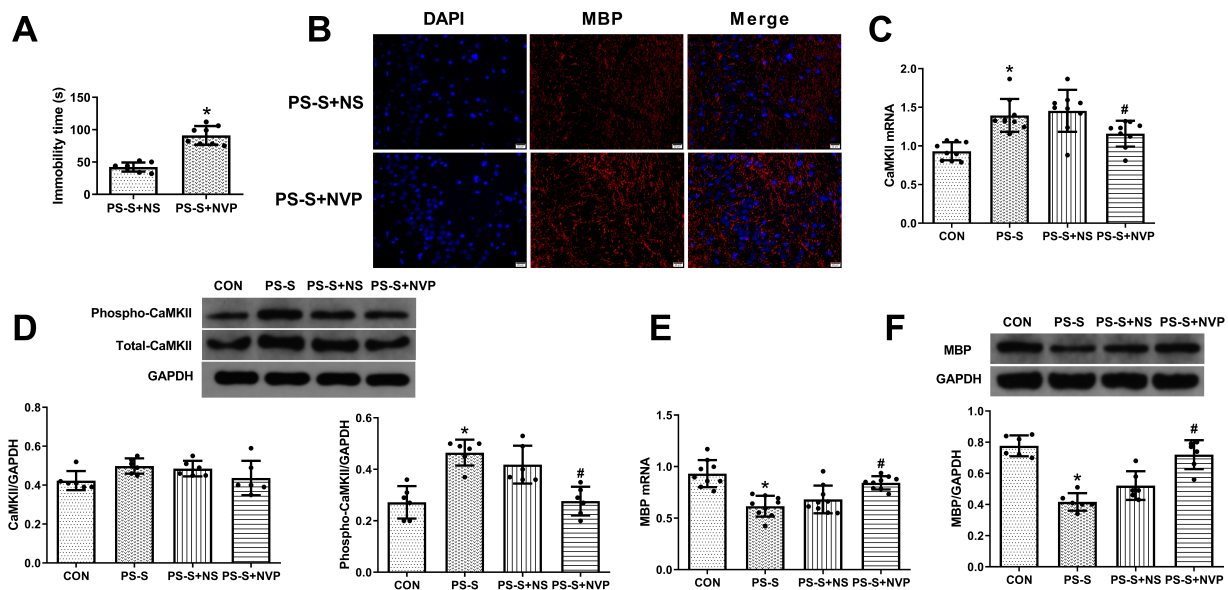


Fig. 3. The effect of the GluN2A receptor antagonist NVP-AAM077 on depressive-like behavior and on mRNA and protein levels for CaMKII and MBP in the hippocampus. (A) Immobility time in the FST. (B) MBP staining visualized by immunofluorescence (400 $\times$). (C) CaMKII mRNA levels. (D) CaMKII and p-CaMKII protein expression levels. (E) MBP mRNA levels. (F) MBP protein expression levels. Results shown are the mean $\pm$ SD for 6 to 9 rats in each group. * $p < 0.05$ vs. CON, # $p < 0.05$ vs. PS+NS. CON, control group; PS-S, PS susceptible group; PS-S+NS, PS susceptible + normal saline group; PS-S+NVP, PS susceptible + NVP-AAM077 group.

showed significantly increased CaMKII mRNA expression compared to controls, but this was attenuated by NVP-AAM077 (Fig. 3C). Similarly, the PS-S group showed significantly elevated CaMKII and p-CaMKII protein expression levels compared to controls that was decreased by NVP-AAM077 (Fig. 3D). The PS-S group showed a significantly lower MBP mRNA level compared to controls, but this was increased by NVP-AAM077 (Fig. 3E). Similarly, the MBP protein expression level was lower in the PS-S group, but this was significantly increased by NVP-AAM077 (Fig. 3F).

3.4 Corticosterone Decreases GluN2A and MBP Expression in Hippocampal Neurons

Isolated rat hippocampal neurons were identified by β -Tubulin-III immunofluorescence, with the degree of purity being $>90\%$ (Fig. 4A). Immunostaining revealed the optical density of Ca^{2+} staining was significantly higher in the corticosterone treatment group than the controls, and this elevated level was not reduced by NVP-AAM077 (Fig. 4B). The expression of GluN2A protein in the corticosterone treatment group was significantly less than in controls, but was increased by NVP-AAM077 (Fig. 4C). The levels of CaMKII and p-CaMKII protein expression in the corticosterone treatment group were both higher than in controls, but were attenuated by NVP-AAM077 (Fig. 4D). The GluN2A mRNA level in the corticosterone treatment group was significantly lower than controls, but was increased by NVP-AAM077 (Fig. 4E). The CaMKII mRNA

level was higher in the corticosterone treatment group than controls, but this was decreased by NVP-AAM077 (Fig. 4F).

4. Discussion

The present findings demonstrate using a rat model that PS can induce depressive-like behavior in offspring. The glutamate level was significantly elevated in PS-S offspring rats compared to controls, with these animals also showed altered levels of GluN2A and p-CaMKII expression in the hippocampus. Moreover, the optical density of MBP staining and the expression of MBP mRNA and of MBP protein were lower in PS-S offspring, indicating that myelination in the hippocampus was impaired. Treatment with the GluN2A receptor antagonist NVP-AAM077 caused notable antidepressant-like effects in the FST, as well as rescue of the MBP and p-CaMKII expression abnormalities.

Extensive literature reports have postulated that PS could induce depression or depressive-like behavior in offspring [27–29]. The present results confirmed that PS can lead to depressive-like behavior in the offspring of a rat model, as evaluated by the SPT and FST. However, additional investigation is required to understand the underlying mechanism of PS-induced depressive-like behavior in offspring. We found that PS significantly increased glutamate levels and decreased GluN2A-type NMDA receptor expression in the PS-S offspring. GluN2A may activate CaMKII,

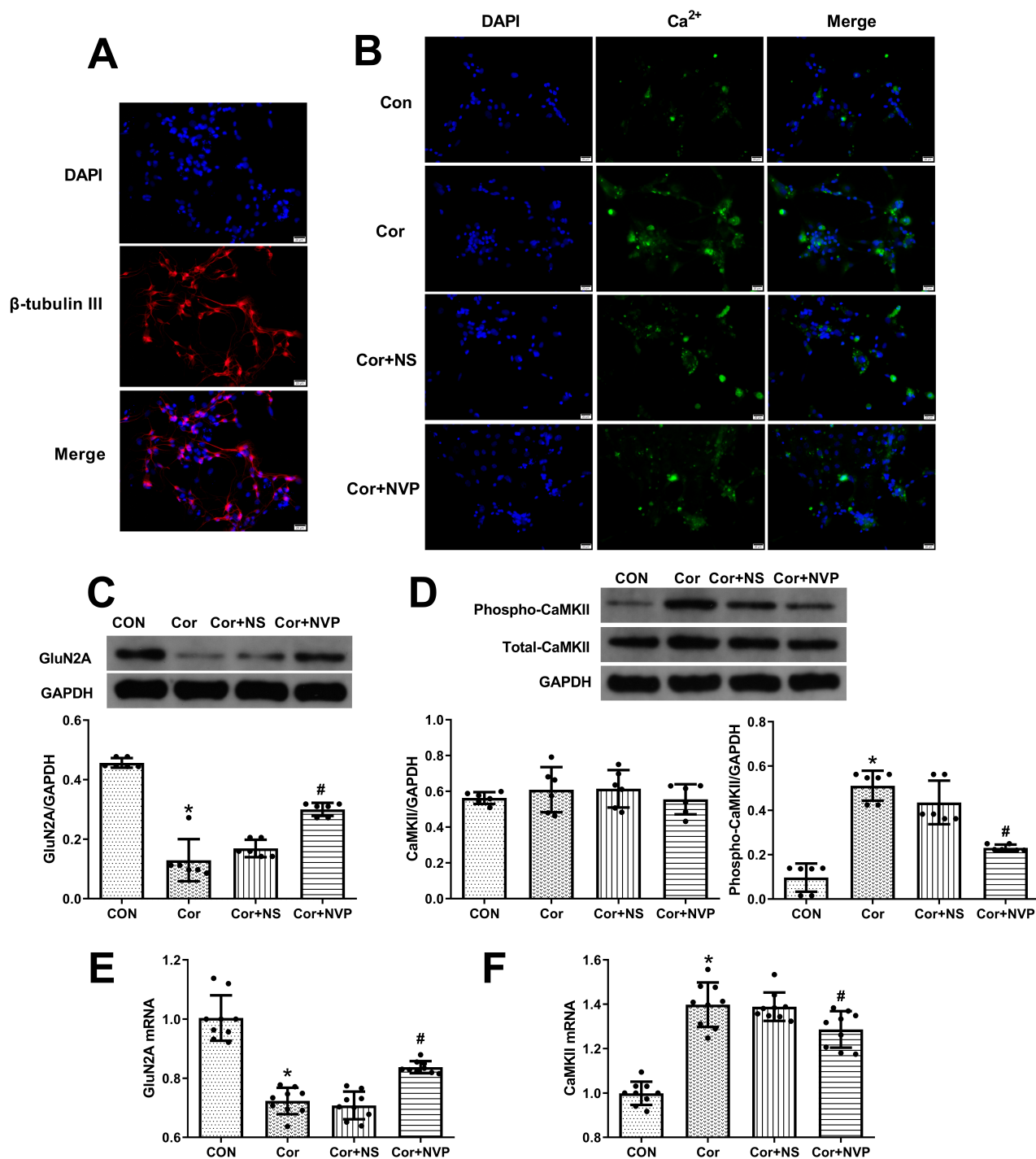


Fig. 4. Effect of the GluN2A receptor antagonist NVP-AAM077 on hippocampal neurons. (A) Identification of hippocampal neurons by immunofluorescence of β -Tubulin-III (400 $\times$). (B) Ca^{2+} staining visualized by immunofluorescence (400 $\times$). (C) GluN2A protein expression. (D) CaMKII and p-CaMKII protein expression. (E) GluN2A mRNA level. (F) CaMKII mRNA level. The results shown are the mean $\pm$ SD for 6 to 9 rats. * $p < 0.05$ vs. CON, # $p < 0.05$ vs. Cor+NS. CON, control group; Cor, corticosterone group; Cor+NS, corticosterone + normal saline group; Cor+NVP, corticosterone + NVP-AAM077 group.

which is then accompanied by increased p-CaMKII protein expression levels. It has been reported that CaMKII is important for normal morphological maturation of differentiating oligodendrocytes, which is mediated mainly through

changes in the cellular cytoskeleton [30]. This supports the hypothesis of the current study that GluN2A mediates PS-induced depressive-like behavior by inducing CaMKII-inhibited myelination.

To evaluate the role of GluN2A, we treated rats with the GluN2A receptor antagonist NVP-AAM077. Our results indicate increase MBP protein and mRNA levels in PS-S offspring. Moreover, the increased expression of p-CaMKII protein in the hippocampus caused by PS also returned to normal following treatment with NVP-AAM077. GluN2A is a primary type of NMDA receptor subunit in the brain and has been implicated in the pathogenesis of several brain diseases including depression, anxiety, cerebral ischemia and seizure disorder [31]. However, causality between GluN2A and these diseases has yet to be established. We have shown for the first time that GluN2A-mediated, PS-induced depressive-like behavior may inhibit myelination through the activation of CaMKII.

To further explore the effect of stress on hippocampal neurons and the role of GluN2A in a model of neuronal injury model, we treated hippocampal neurons with different concentrations of corticosterone. The MTT assay was used to determine the optimal stimulus concentration. The inhibition of hippocampal neuron proliferation gradually increased with increasing corticosterone concentration. The optimal concentration selected was 100 μ M corticosterone, which decreased neuronal cell viability to 72.5%. Our results indicated that corticosterone had a similar effect as PS on GluN2A and CaMKII levels. NVP-AAM077 also rescued GluN2A and p-CaMKII abnormalities in hippocampal neurons. The present results add further support for a pivotal role of GluN2A in depression [31]. Blocking the actions of GluN2A may therefore provide an effective strategy for the treatment of depression.

5. Conclusions

The findings from this research provide evidence that PS can induce depressive-like behavior in offspring from a rat model, as observed by the SPT and FST behavioral tests. Increased glutamate levels activate the GluN2A receptor, which in turn activates CaMKII to inhibit myelination in the hippocampus. The GluN2A receptor antagonist NVP-AAM077 induced marked antidepressant-like effects in the FST, as well as rescue of abnormalities in MBP and p-CaMKII expression, suggesting that GluN2A is an attractive target for the development of pharmacotherapies aimed at PS-induced depressive-like behavior.

Availability of Data and Materials

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author Contributions

HMH and HLJ designed and performed the research study. HLS provided help and advices. HMH and HLS analyzed the data. HMH wrote the manuscript. All authors contributed to editorial changes in the manuscript. All

authors read and approved the final manuscript. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

Ethics Approval and Consent to Participate

The animal study was reviewed and approved by the Experimental Animal Care and Use Committee of Xi'an Jiaotong University (No. 2020-449) and was conducted in accordance with approved guidelines and protocols.

Acknowledgment

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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