Inhibition of GALR1 in PFC Alleviates Depressive-Like Behaviors in Postpartum Depression Rat Model by Upregulating CREB-BNDF and 5-HT Levels

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Expression level of GALR1 mRNA was significantly increased in PFC of estrogen withdraw-induced PPD rats. Injecting GALR1-siRNA into PFC alleviated depressive-like behavior and reversed the decrease of 5-HT level and CREB/BDNF signaling in PFC of PPD rats.

HIGHLIGHTS

1 Expression level of GALR1 mRNA was significantly increased in PPF of estrogen withdraw-induced PPD rats.
2 Injecting GALR1-siRNA into PFC alleviated depressive-like behavior and reversed the decrease of 5-HT level and CREB/BDNF signaling in PFC of PPD rats.

Keywords: PPD, PFC, GALR1, CREB, BDNF, 5-HT

INTRODUCTION

Postpartum depression (PPD) is a severe mental disorder that affects both mother and their babies, with an estimated prevalence of 10–15% worldwide (1). The DSM-5 defines postpartum depression as the depressive episode that begins 4 weeks after delivery (2) and may last for 12 months, clinically (3). In some serious scenarios, patients may tend to commit infanticide and are more likely to abuse
their babies (4). The widely accepted hypothesis of PPD is that the withdrawal of estrogen (E2) plays a cortical role in the onset of PPD (5). Clinical studies have shown that women tend to exhibit more symptoms of depression during times of large hormonal changes. Thus, the onset of PPD is thought to arise, at least in part, from the dramatic fluctuations in the levels of the gonadal hormones during the postpartum period, and patients showed strong correlation between lower estradiol level in umbilical cord blood and depressive mood during the postpartum period (4, 6). Moreover, the E2 therapy has a greater improvement in depression scores compared with placebo among patients with severe PPD. However, interfering with breastfeeding, is a major concern for E2 therapy in PPD (6, 7) and searching for a new therapeutic target is emergency for PPD treatment. Galanin (GAL) is an estrogen-inducible neuropeptide, which is widely distributed in central and peripheral nervous system, as well as endocrine system (8). It has been demonstrated that GAL system plays an important role in depression, and drugs that target galanin receptors can modulate stress-related behaviors (9–11). GAL exerts its function via its receptors: galanin receptor 1 (GALR1), galanin receptor 2 (GALR2), and galanin receptor 3 (GALR3). It’s signaling via multiple transduction pathways, including inhibition of cyclic AMP/protein kinase A (GALR1, GALR3) and stimulation of phospholipase C (GALR2) (8). Our recent study revealed that the knockdown of GALR1 in the ventral periaqueductal gray reverses depressive-like behavior of chronic mild stress (CMS) rats (12). Meanwhile, it has been reported that GAL neurons in the medial preoptic area govern the parental behavior in female rats (13). Moreover, the expression of GALR1 mRNA varies across the estrous cycle in the preoptic area and is elevated in females more than males (14). However, it still remains unclear which GAL receptors (GALRs) are involved in PPD pathologic process.

In the present study, utilizing an ovarian-steroid withdrawal-induced PPD rat model, we examined the change of GALRs expression in several brain regions associated with mental disorders, including prefrontal cortex (PFC), central amygdala (CeA), and ventral hippocampus (VH). In addition, we explored whether or not there was a causal link between change of GALR1 expression and depression-like behaviors in PPD model rats and possible signaling mechanisms involved.

METHODS

Animals and Housing
Female Sprague Dawley rats (160–180 g) were used in the present study (Capital Medical University, China). To minimize the stress, the female rats were acclimatized for 1 week before ovariecotmy. The rats were group housed in a room with controlled temperature (22–24°C) and light (12-h light/dark cycle). All rats had free access to food and water. The study was approved by the Animal Care Committee at Capital Medical University. It was the minimal animal number for meeting statistical analysis requirements.

Ovariectomy
All surgeries were performed using an aseptic tip technique. Rats were anesthetized with 6% chloral hydrate (6 ml/kg) administrated i.p., and fixed at the prone position. A 1.5 cm longitudinal dorsal incision was made using an aseptic technique (15). The incision was then pulled laterally to open the muscular layer and peritoneum. The ovaries and fallopian tubes were identified and the ovaries were removed. The skin was sutured and penicillin was administered to prevent infection. The control rats were sham-operated. The rats were housed separately following surgery and allowed 1-week recovery to eliminate estrogen and progesterone.

Procedure

Hormones Administration
The rats were divided into five groups: control group (n = 8), PPD group (n = 8), PPD + siRNA group (n = 8), PPD + scramble group (n = 8), and PPD_E group (n = 8). Rats in PPD group, PPD + siRNA group, PPD + scramble group, and PPD_E group were ovariectomized bilaterally. Rats were administrated vehicle or hormones subcutaneously at 9:00 am for 23 days from 1 week after ovariectomy. The control group was injected with 0.3 ml polyethylene for 23 days. The PPD group, PPD + siRNA group, PPD + scramble group, and PPD_E group were injected with 2.5 µg estradiol dissolved in 0.2 ml polyethylene and 4 mg progesterone (0.1 ml) for the first 16 days and 50 µg estradiol dissolved in 0.3 ml polyethylene from day17 to day23. From day 24 till to the behavioral tests were completed, rats in PPD_E group continued receiving a high dose of estradiol (50 mg), while the PPD group, PPD + siRNA group, and PPD + scramble group were injected with 0.3 ml polyethylene during the same time. The first 23 days were considered the “pregnant” period, after which were considered the “postpartum” period (16) (Figure 1).

siRNA Interference
The green fluorescent protein (GFP) reporting lentivirus encoding the siRNA to Galr1 and scramble (12) were injected into the bilateral PFC of rats in PPD + siRNA group or PPD + scramble group, respectively. The surgery was carried out 4 weeks before the behavioral tests. Rats were anesthetized with 6% chloral hydrate and placed on a stereotactic apparatus (Benchmark). A hole was drilled on the skull based on the coordinate: AP+3.2 mm; ML±/−0.6 mm; DV −4.0 mm (below surface of the skull) from Bregma, according to the atlas of Paxinos and Watson (17). Three micro liters of siRNA or scramble was injected with a 10 µl microsyringe (Hamilton) for 15 min. The needle remained in place for another 15 min. The injection site was verified from 30 µm coronal sections under a fluorescence microscope. Rats were allowed one-week recovery.

Behavior Tests

Forced Swimming Test
Forced swimming test (FST) was conducted 3 days after the termination of estrogen administration in PPD group. Rats in PPD_E group were tested 2 h after the injection. The FST was carried out on rats as described in our earlier study (18), with slight modifications. Briefly, rats were individually placed in a transparent glass cylinder (64 cm height and 22 cm diameter) filled with tap water with depth of 30 cm at 25 ± 2°C. Each test session lasted for 5 min and was recorded by a camera connected to ANY-maze video tracking software (Stoelting Co.,
IL, USA) which automatically calculated the climbing time and immobile time. The water was changed and containers were cleaned thoroughly between rats in order to minimize any effects of other subjects. Rats were dried and returned to their cages after the test.

**Sucrose Preference Test**
The sucrose preference test was carried out on rats as described in our earlier study (12). All rats were trained to consume 1% sucrose solution 1 week before the test to habituate to the new solution. Animals were water-deprived 24 h before the test. The test was conducted at 11:00 a.m. 2 h following the estrogen injection in PPD_E group. Rats were placed in separated cages with no access to food. Two pre-weighed bottles were placed on each cage, one filled with tap water, the other one filled with 1% sucrose solution. The placement of two bottles (left/right) was counterbalanced and interchanged 30 min after the test started. After 1 h, bottles were re-weighed to determine the volume of the sucrose solution consumed and the sucrose preference presented by the percentage of the sucrose solution consumed.

**Radioimmunoassay for Estradiol**
Before the surgery and sacrifice, the rats were anesthetized with 6% chloral hydrate (6 ml/kg) administrated i.p., (at 9:00–10:00 a.m.). Blood samples were collected from the retroorbital sinus using a heparan capillary tube then put into the anticoagulation tube. 20 min later, the blood was collected in Eppendorf tubes. The samples were centrifuged at 3,000 rpm (rounds per min) for 10 min. Plasma concentration of estradiol was determined by radioimmunoassay kit (Laerwen Co., LTD., Shenzhen, China) following the standard procedures.

**Microdissection of PFC, CeA, and VH Sample Preparation**
At the end of the behavioral tests, animals were sacrificed under anesthesia. Brains were rapidly separated from the skull and sliced using a 1-mm brain matrix. PFC, CeA, and VH were dissected on ice using stereotaxic coordinates. Each brain tissue was put into an Eppendorf tube and then rapidly shock-frozen on dry ice and stored at −80°C for later RNA extraction. The positions of the cannula were verified by crystal violet staining and only the rats with an exact localization were included in the statistical analysis.

**Quantitative Real-Time PCR (Q-PCR)**
Total RNA of brain tissues or PFC neurons was extracted using RNeasy Lipid Tissue Mini Kit (Qiagen, Germany) or RNeasy Micro Kit (Qiagen, Germany) following the manufacturer's instructions, respectively. Total RNA was dissolved in 30 μl RNase-free water provided in the kit. RNA concentrations were assessed using NanoDrop (Thermo Scientific, DE, USA) with 260 /280 nm ratios between 1.9 and 2.1. Transcriptor First Strand cDNA Synthesis Kit (Roche, IN, USA) was used to reverse-transcribe RNA (1 μg) into cDNA according to the manufacturer's instruction.

Quantitative PCR (Q-PCR) was performed following the reverse transcription. The total volume of Q-PCR reactions was 20 μl containing 8 μl distilled water, 10 μl SYBR Green Mix (Applied Biosystems, UK), 1 μl cDNA, and 1 μl primers (forward + reverse). The sequences of the primers used in this study were as followed: GAPDH-specific primers were forward: GACCACCCAGCCAGCAAGG, reverse: TCCCCACGCCCCTCCTGTTG. GALR1-specific primers were forward: TGGGGAGCCAGCAAGCAG, reverse: TGGGATGATTGAGAACCTTGG. GALR2-specific primers were forward: GCCGCCATGGGGCTCATGCT, reverse: GCCGCCCACTGGGCTCATCTG, forward: GCCGCCATGGGGCTCATGCT, reverse: TCCCAGGCCCTCCTGTTG. Amplification reaction protocol included 2 min at 60°C, 10 min at 95°C, followed by 40 cycles reaction as: 15 s of denaturing at 95°C and 1 min of annealing at 60°C. Samples were held at 10°C at the end of each amplification reaction. GAPDH was used as the internal reference for each sample.

**Western Blot**
Total proteins were extracted using Buffer C lysis buffer. The protein concentrations were measured by bicinchoninic acid (BCA) assay. Forty micrograms of proteins were concentrated at 80 mV and separated at 100 mV using 10% sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE). Proteins were then transferred to a polyvinylidene difluoride (PVDF) membrane (Millipore, MA, USA) for 2 h at 300 mA following the electrophoresis. Membranes were blocked with 10% milk at room temperature for 1 h and incubated with the following primary antibodies: c-fos (sc-413, mouse monoclonal, Santa Cruz CA), BDNF (ab226843, rabbit polyclonal antibody; abcome etchnology), CREB and phospho-CREB (sc-377154, sc-81486, mouse monoclonal, Santa Cruz, CA) at 4°C overnight.
Membranes were washed 3 times (10 min × 3) with Tris-buffered saline-Tween (TBST) and incubated with horseradish peroxidase (HRP)-conjugated secondary antibody (1:5,000, Applygen, Beijing, China) for 1 h at room temperature followed by washing 3 times (10 min × 3) with Tris-buffered saline-Tween (TBST). Proteins were developed using an enhanced chemiluminescence (ECL) reagent kit (Applygen, Beijing, China) and radiographic films (Carestream, Xiamen, China). α-tubulin (ABT170, rabbit polyclonal, 1:10,000, Millipore, Temecula, USA) was used as the internal reference.

**Determination of 5-HT and 5-HIAA Levels**

The levels of 5-HT and its metabolite 5-HIAA in PFC and VH was measured using high-performance liquid chromatography with an electrochemical detector (HPLC-ECD). Model 5600A CoulArray Detector System and CoulArray for Wingdows® 32 application software (ESA, USA) was used for detection and data analysis. PFC and VH tissues were dissected and stored at −80°C. Pre-weighted tissues were placed into Eppendorf tubes and homogenized in 200 µl of fluid A (0.4 M perchloric acid) for 10 s on ice. Samples were centrifuged at 12,000 rpm for 20 min at 4°C after placing on ice for 1 h, away from light. 80 µl of fluid B (mobile phase) was added into 160 µl of supernatant and vortex mixed. Samples were centrifuged at 12,000 rpm for 20 min at 4°C after placing on ice for 1 h, 200 µl of supernatant was extracted and kept at −80°C away from light. 200 µl of each sample was used for analysis. The flow rate was 1.0ml/min. The voltages of the four CoulArray channels were −150, 100, 220, and 400 mV, respectively.

**Data Analyses**

Data were presented as mean ± SEM (standard error of measurements). Statistical analysis was carried out using SPSS16.0. Animals with incorrect injection positions were excluded from statistical analysis. Data were analyzed with one-way ANOVA and the follow-up Tukey HSD multiple comparison tests were selected to compare mean values in each group. P < 0.05 was considered to be statistically significant.

**RESULTS**

**E₂ Withdrawal-Induced Depressive-Like Behaviors in PPD Rats**

E₂ levels were assessed after the 16-day 2.5 µg E₂+4 mg progesterone treatment and after the 7-day 50 µg E₂ treatment, respectively (Figure 1). There was no significant difference in plasma E₂ level among three groups on the 16th day (p > 0.05) (Figure 2A), while the E₂ level was significantly elevated in PPD_E group after 23-day injection (p < 0.05) (Figure 2B).

Behavioral tests were conducted 1 week after the termination of estrogen administration in PPD group, while rats in PPD_E group kept receiving a high dose of estrogen. In forced swimming test, the climbing time was decreased in PPD group compared with the other two groups (p < 0.01, p < 0.05) (Figure 2C). However, rats in PPD group showed significantly increased immobility time (p < 0.05, p < 0.01). Sucrose consumption test showed that the PPD rats consumed less sucrose than the Ctrl rats, indicating that PPD rats performed anhedonia symptom. Meanwhile, the estrogen treatment reversed sucrose consumption to normal level in PPD_E group (p < 0.01, p < 0.05) (Figure 2E). There was no significant difference between Ctrl group and PPD_E group in forced swimming test and sucrose consumption test (Figure 2D), which is consistent with previous reports (16, 19). Maybe the dose used is not sufficient enough to influence the behavior results, though it is already higher than Ctrl.

**E₂ Withdrawal Elevated GALR1 Expression in PFC in PPD Group**

The protein level of c-fos in PFC was significantly increased in PPD rats compared with Ctrl and PPD_E group (p < 0.05) (Figure 3A), but not in the VH and CeA (data not shown). The expression of GALR1 and GALR2 was analyzed in PFC, CeA, and VH brain regions. The mRNA level of GALR1 in PFC was significantly increased in PPD group compared with Ctrl and PPD_E group (p < 0.05) (Figure 2B), while in VH or CeA, the difference among the groups was not significant (Figures 3C, D) (p > 0.05). There was no difference in the expression of GALR2 in any brain regions tested (p > 0.05) (Figures 3B–D). It needs to be mentioned that the expression of GALR3 in rat brain is low abundance except hypothalamus and pituitary (20), and our data show the CT value of GALR3 in the PFC is near 37 (data not shown). Therefore, GALR3 was not analysis here.

**Injection of GALR1-siRNA Into PFC Reversed Depressive-Like Behaviors in PPD Rats**

GALR1-siRNA was injected into the bilateral PFC of ovariectomized rats, following estradiol withdrawal procedure (Figure 1). The coordinates for the PFC were AP +3.2, ML ±/-0.6, H -4.0 mm from Bregma (17) and the injection sites were confirmed by GFP fluorescence (Figure 4A). The efficiency of GALR1 interference was verified by Q-PCR. The expression of GALR1 was significantly decreased in PPD + siRNA group compared with PPD and PPD + scramble groups in the PFC (p < 0.05), while no significant difference between PPD and PPD + scramble group was observed (p > 0.05) (Figure 4B).

Behavioral tests were carried out 4 weeks after GALR1-siRNA injection. In forced swimming test, PPD and PPD + scramble rats showed less climbing time (p < 0.05) and more immobility time than Ctrl rats (p < 0.01) (Figures 4C, D), while no significant difference was seen between PPD + siRNA and Ctrl rats (p > 0.05) (Figures 4C, D). In sucrose preference test, PPD and PPD + scramble rats consumed less sucrose than Ctrl rats (p < 0.05) while GALR1-siRNA injection reversed sucrose consumption to normal level compared with Ctrl group (p > 0.05) (Figure 4E).

**CREB-BDNF Signaling Involved in the Antidepressant Effect of GALR1-siRNA in the PFC of PPD Rats**

CREB and phospho-CREB protein levels were assessed with WB. The expression of CREB was significantly decreased in PPD and PPD + scramble group, while GALR1-siRNA injection...
FIGURE 2 | Estradiol withdrawal-induced depressive-like behaviors in PPD rats. (A) After the 16-day 2.5 µg E2+4 mg progesterone treatment schedule, all groups had no significant difference in plasma estradiol levels. (B) After the 7-day 50 µg E2 treatment schedule, PPD_E group showed a significant increase of plasma estradiol levels. (C) PPD group showed reduced climbing time than Ctrl and PPD_E group in forced swimming test. (D) PPD group showed increased immobility time than Ctrl and PPD_E group in forced swimming test. (E) PPD group showed a significant decrease of sucrose consumption than Ctrl and PPD_E group in sucrose preference test. Data were represented as mean ± SEM (n = 6–8 in each group)*p < 0.05 compared to PPD_E group (A, B); **p < 0.05, ***p < 0.01 compared to PPD group (C–E). Data were analyzed by one-way ANOVA followed by post-hoc Tukey HSD multiple comparison tests.

FIGURE 3 | The expression of c-fos and GALR1 was significantly increased in the PFC in PPD group. (A) In PFC, c-fos protein level was significantly increased in PPD group compared to Ctrl and PPD_E groups. (B) PPD group showed an increase of GALR1 mRNA level compared to Ctrl and PPD_E group in PFC, while there was no difference in GALR2 expression between groups. (C) There was no difference in GALR1 or GALR2 expression between groups in CeA. (D) There was no difference in GALR1 or GALR2 expression between groups in VH. Data were represented as mean ± SEM (n = 6–8 in each group). *p < 0.05 compared to Ctrl group. Data were analyzed by one-way ANOVA followed by post-hoc Tukey HSD multiple comparison tests.
Injection of GALR1-siRNA into PFC Reversed the Decreased Levels of 5-HT and 5-HIAA in the PFC

HPLC was carried out to test the 5-HT and its metabolite 5-HIAA levels in PFC and VH. The levels of 5-HT and 5-HIAA in PFC were decreased in PPD and PPD + scramble rats compared to Ctrl rats (Figures 6A,B). However, they were significantly increased in PPD+ siRNA rats compared to PPD and PPD + scramble rats (p < 0.05). There is no significant difference between PPD+ siRNA and Ctrl group (p > 0.05) (Figures 6A,B). There was no significant difference of the 5-HT and 5-HIAA levels in the VH between all groups (Figures 6C,D). These results suggested that GALR1 interference may exert anti-depressant effect associated with up-regulation of 5-HT and 5-HIAA levels in the PFC.

**DISCUSSION**

In the present study, E2 withdrawal paradigm induced depressive-like behavior and upregulation of GALR1 and c-fos expression with a region specific pattern in the PFC of PPD rats. Treatment of GALR1-siRNA in PFC reversed depression-like behavior accompanied with the reversion of down-regulated CREB-BDNF and 5-HT levels.

The pathophysiology of PPD is considered to be triggered by the rapid decline in reproductive hormones following pregnancy. Besides post-partum period, lower E2 levels also associated with depression symptoms, it has been shown that CUMS-OVX rats demonstrated longer immobility time in FST test and lower sucrose preference than CUMS-intact female rats (21). However, so far most studies of depression model focus in male rats, the data about estrogen is very limited.

Consistent with other studies (16, 19), our data demonstrated that E2 withdrawal paradigm-induced depression-like behavior including decreased sugar preference, which implied this paradigm was sufficient to produce “anhedonia” in rats, suggesting that the establishment of E2 withdrawal rat model
is an effective approach to investigate the mechanism of PPD. Moreover, the depressed symptoms in rodents is prevented by prolonging exposure to high levels of E2 through the "post-partum period," suggesting that E2 plays antidepressant roles at least partially in this paradigm and further confirmed that the PPD is associated with E2 withdrawal (16). It has been shown...
that E2 complementary treatment plays antidepressant roles in O VX mice. However, it interferes with breastfeeding, which is a major concern in the treatment of PPD (22–24). Therefore, searching for a new therapeutic target is emergency for PPD treatment.

The monoamine-deficiency is one of the hypothesis of major depression (25). Menopausal depressive rats also showed decreased 5-HT levels in cerebral spinal fluid (26). Various studies showed that neuropeptide GAL has direct and indirect inhibitory effect on both NA and 5-HT neurons (27). It has been demonstrated that GAL system is associated with major depression in a postmortem brain study (9). Our recent study has also shown that the increased expression of GALR1 in the ventral periaqueductal gray of chronic mild stress rats is related to depression-like behavior (12). In the present study, we found that in PPD rats, GALR1 was significantly and selectively upregulated in PFC, but not in other brain regions. Meanwhile, c-fos, which is widely used as a marker for the activation of neurons in the brain (28), was also up-regulated only in the PFC in PPD rats. PFC is one of the regions involved in cortico-limbic circuits and is important in the development and treatment of depression (29). A recent proton magnetic resonance spectroscopy study showed glutamatergic dysfunction and neuronal damage occurred in dorsolateral prefrontal cortex in PPD patients (30). It has been shown that GALR1 is high expression in the mPFC (31). Taken together, all those data suggest that upregulation of GALR1 in PFC may be involved in PPD. To further demonstrate the causal link between change of GALR1 expression and depression-like behaviors in the PPD rats model, knocking down GALR1 in the bilateral-PFC with a siRNA technique was carried out and depressive-like behavior was ameliorated in PPD rats after GALR1-siRNA treatment. Moreover, we found that higher expression of GALR1 was accompanied with lower expression of cAMP response element binding protein (CREB) and BDNF in the PFC of PPD rats. GALR1-siRNA injection reversed the decreased levels of p-CREB, CREB, and BDNF. It has been known that activation of GALR1, a Gi protein-coupled receptor, inhibits cyclic adenosine monophosphate synthesis and its downstream molecule CREB (31–33). Meanwhile, chronic administration of antidepressants increases the expression, phosphorylation, and function of CREB, and its downstream target gene BDNF in the limbic brain regions related to depression (29, 34, 35). All those implied that GALR1-siRNA-induced reversion of CREB-BDNF signaling might be involved in the antidepressant role of GALR1-siRNA.

Our HPLC results showed that the 5-HT and 5-HTAA levels were decreased in PFC of PPD rats. Moreover, this downregulation of 5-HT and 5-HTAA was ameliorated after GALR1-siRNA injection in the PFC. Decreased 5-HT level in the PFC associated with depression (36, 37). The concentration of 5-HT in PFC is depended on release and reuptake of 5-HT. Though it is well-known that GALR1 mRNA expresses in DR neurons (6) and very likely GALR1 protein also expresses in the terminal of DR neurons. Therefore, GALR1 might be involved in modulating release or/and reuptake of 5-HT. But local injection of GALR1-siRNA in PFC is not able to knockdown GALR1 expressed in the terminal of DR neurons as receptor protein is synthesized in the cell body. On the other hand, PFC neurons project to dorsal raphe and modulate serotonergic neurons activity as well as 5-HT releasing in target regions including PFC (38). GALR1 is highly expressed in PFC, and GALR1 mRNA level was significant increase in PFC of major depression patients (9). Thus, if the PFC-DR neurons expressing GALR1, blocking GALR1 might cause de-inhibition of PFC-DR neurons and in turn, enhance 5-HT release in PFC. However, because of lacking selective antibody against GALR1, it is still unknown whether PFC-DR neurons expressing GALR1. Meanwhile, it has been reported that astrocytes in PFC expresses 5-HT and transporters and might also be involved in modulation of 5-HT concentration in PFC (39). It is also been reported that GALR1 expresses in astrocytes in the brain (40). Thus, local injection of GALR1-siRNA might knockdown astrocytes-expressing GALR1 and enhance release or inhibit reuptake of 5-HT. But the expression of GALR1 on astrocytes is also needed to be determined with selective antibody. In the present study, lower level of 5-HT in PPD model rats and ameliorated depression-like behavior together with reversed 5-HT level after GALR1-siRNA treatment suggesting that antidepressant effect of GALR1-siRNA might be mediated by reversing downregulation of 5-HT in PFC. Thus, GAL regulates 5-HT levels by modulating not only activity of 5-HT neurons (via GALR1/R3) in DR, the main source of 5-HT ascending system (41, 42), but also releasing or/and reuptaking of 5-HT (via GALR1) in PFC, the projection region of 5-HT ascending system.

It has been demonstrated that interaction between BDNF and 5-HT is involved in depression by influencing neuronal plasticity and depression susceptibility (43–45). Our data showed that GALR1-siRNA reversed PPD-induced downregulation of BDNF and 5-HT in PFC, suggesting that the interaction of BDNF and 5-HT might be involved in antidepressant effect of GALR1-siRNA. However, it is needed to be determined in future study.

Meanwhile, the etiology of PPD is a complex interaction of psychological, social and biological factors. It has been reported that, in addition to estrogen system, hypothalamic-pituitary-adrenal (HPA) axis disorder, gestation stress are involved in PPD (18, 46). In future study, we will continue study the effects of GAL signaling in other pathways of PPD.

**CONCLUSION**

The present results, based on the rat PPD depression model, provide evidence for involvement of GALR1 in the PFC in depression-like behavior. Thus, a GALR1 antagonist acting in the PFC may have antidepressant actions in PPD.

**AUTHOR CONTRIBUTIONS**

HL and Z-QX designed experiments. TW and CS carried out behavior and qPCR test. YY carried out WB test. XL carried out HPLC test. YW analyzed experimental results. HL and Z-QX wrote the manuscript.
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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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