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✉✉ (Qian Chen)

Renmin Hospital of Wuhan University

Ting Sun

Renmin Hospital of Wuhan University

Jingru Cheng

Renmin Hospital of Wuhan University

Yufeng Mei

Renmin Hospital of Wuhan University

Bohao Liu

Renmin Hospital of Wuhan University

Yan LI (✉ cq15771182933@163.com)

Renmin Hospital of Wuhan University

Research Article

Keywords: Neuroinflammation, Major depressive disorder, gp130, estradiol, estrogen receptor

Posted Date: August 10th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1367437/v2>

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17 β -Estradiol (E2) alleviates depressive-like behaviors by inhibiting gp130/JAK1/STAT3 signaling pathway through estrogen receptor- β

Qian Chen^{1,*}, Ting Sun^{1,*}, Jingru Cheng¹, Yufeng Mei¹, Bohao Liu^{2,#}, Yan Li^{1,#}

¹Department of Clinical Laboratory, Renmin Hospital of Wuhan University, Wuhan 430060, China

²Department of Thoracic Surgery, Renmin Hospital of Wuhan University, Wuhan 430060, China

*These authors contributed equally to these work

#Corresponding Authors at:

Yan Li

Department of Clinical Laboratory , Renmin Hospital of Wuhan University, Wuhan, 430060, China

Telephone number: +8613886079252

E-mail address: yanlitf1120@163.com

Bohao Liu

Department of Thoracic Surgery, Renmin Hospital of Wuhan University, Wuhan 430060, China

Telephone number: +8618743068233

E-mail address: 1325467742@qq.com

Abstract:

Major depressive disorder (MDD) is a worldwide life-threatening disorder with high incidence and high mortality. Besides, the neurotrophic effects of estrogen have been demonstrated in many neurodegenerative disorders including Alzheimer's disease (AD) and Parkinson's disease (PD). However, the mechanistic interplay between depression, neuroinflammation and estrogen remain unclear. Here, this study investigated the effect of E2 on neuroinflammation and depressive-like behaviors induced by ovariectomy (OVX), as well as the underlying mechanism. We found the anti-depressant effect of E2 through behavioral experiments and verified the anti-apoptotic effect of E2 on the depressive hippocampus in mice, and finally we showed that E2 alleviated inflammatory response and reversed depressive-like behaviors by inhibiting gp130/JAK1/STAT3 signaling pathway via combining with estrogen receptor- β (ER- β) in vivo and vitro. These results suggested that E2 possesses potent anti-depressive action, which might be mediated via inhibition of neuroinflammation and apoptosis impairment by promoting the interaction between gp130 and ER- β .

Key words: Neuroinflammation; Major depressive disorder; gp130; estradiol; estrogen receptor

1. Introduction

Major Depressive Disorder (MDD) is a worldwide mental disease characterized by recurrent episodes, high incidence and high mortality(1). There is a gender bias in the prevalence of depression(2). Many clinical studies had indicated that the circulating estrogen levels were associated with anxiety and depression symptom in menopausal women(3, 4). Multiple lines of evidence suggested that hormonal alterations play a crucial role in the pathophysiology of the disease. The risk of depression and the probability of mood abnormalities were elevated in menopausal women or childbirth(5). A cross-sectional clinical study showed that the lower the estradiol level, the higher the Beck Depression Scale score(6). At the molecular level, studies had shown that elevated G protein coupled estrogen receptor (GPER) levels were correlated with depression scale scores in MDD patients compared to healthy control. . In addition, estrogen had shown significant utility in the treatment of patients with perimenopausal depression in improving mood and their cognitive ability and memory(7). The phenomenon was further elucidated in animal studies, female mice showed increased feelings of despair and depressive-like behaviors induced by OVX, and E2 treatment could reverse these behaviors (8).

Some pathophysiological mechanisms of MDD had been elucidated including the disturbance of neurotransmitter system, the disruption of blood-brain barrier and immune imbalance(9). Studies have demonstrated that neuroinflammation plays a crucial role in the etiology and progression of depression(10). Previous study found that serum IL-6 levels are elevated in MDD patients, and the elevated serum IL-1 β and TNF- α were associated with an increased probability of depressive (11). Studies have shown that estradiol can affect the expression of hippocampal inflammatory factors through ER- α /Sirt1/NF- κ B (12). E2 deficiency leads to activation of NLRP3 inflammatory vesicles leading to hippocampal neuroinflammation and depression-like behavior, and this estrogen modulation is ER- β -dependent(13). As the resident immune cells of the brain, microglia play a front-line role in immune defense in the central nervous system(14). Microglia can be activated through TLR-like or Nod-like

receptors and then release immune molecules, including proinflammatory cytokines, chemokines, and reactive oxygen species, leading to the development of depressive symptoms(15).

Gp130 (Glycoprotein 130) is a signaling subunit shared by the IL-6 family of cytokine receptors, which share gp130 for common biological effects while possessing their own specificity. Gp130 itself has no tyrosine kinase activity, but in response to IL-6 family cytokines, gp130 forms homo- or heterodimers, which in turn activating the cytoplasmic tyrosine kinase to which it is bound. In contrast, JAK1 (the Janus kinase 1) is thought to be the main kinase in this family that activates the STAT3 (Signal Transducer and Activator of Transcription) pathway via gp130(16, 17). Mutational analysis showed that JAK1 binds to the proximal membrane region of gp130 and activates it, subsequently causing phosphorylation of tyrosine residues at the distal membrane of gp130, which in turn leads to the recruitment and activation of STAT3, which is essential for growth inhibition and cell differentiation. Studies demonstrated that IL-6 is a stress-induced cytokine that activates the downstream JAK1/STAT3 signaling cascade through gp130 and IL-6R in certain depression subtypes, and studies showed that IL-6 could cause depression behaviors by effecting on the HPA axis (18). IL-6 has been shown to promote gp130 expression in antibody-secreting cells, and E2 could downregulated gp130 levels. Studies showed that E2 inhibited the expression of IL-6 in breast cancer cells and osteoblasts and downregulates gp130, thereby inhibiting downstream inflammatory signaling pathways(19, 20). Furthermore, in Human umbilical vein endothelial cells (HUVECs), E2 reduced gp130 protein expression through co-mediation by ER- α and ER- β (21).

It's the first time to explore the potential mechanisms by which estrogen regulates gp130/JAK1/STAT3 signaling pathway in MDD. We observed the effect of E2 on gp130/JAK1/STAT3 signaling pathway and depressive-like behaviors in vivo and vitro. In addition, we further verified whether the E2 inhibited the activation of gp130/JAK1/STAT3 signaling pathway via the interaction between ER and gp130.

2. Materials and methods

2.1. Animals and modelling

Female C57BL/6 mice (6-8 weeks old, weight: 18~22 g) were purchased from Zhejiang Weitong Lihua Experimental Aluminum Technology Co., Ltd (Zhejiang, China). All animals were raised under following standard conditions: temperature ($22 \pm 1^{\circ}\text{C}$), humidity ($50 \pm 10\%$) and 12 h light-dark cycle. All the experimental procedures received approval from the Animal Care and Use Committee of Renmin Hospital of Wuhan university (certificate no. 20210305).

The procedure used for the bilateral ovariectomy (OVX) under anesthesia with isoflurane (0.2% - 0.5%). The control group was conducted sham surgery. The specific operation process of animals is shown in Figure 1a.

2.2. Drug administration and experimental design

E2 (purity > 98.6%) purchased from Med Chem Express (Monmouth Junction, NJ, U.S.A.) was dissolved in dimethyl sulfoxide (DMSO, sigma, St. Louis, MO) and then diluted in saline to a concentration of 0.2 $\mu\text{g/kg/day}$ (22). All mice were randomly assigned to three groups ($n = 7$ in each group), two of which were conducted the bilateral ovariectomy (OVX) under anesthesia with isoflurane (0.2% - 0.5%) (23). These mice were injected intraperitoneally with E2 in one OVX group. After a month, behavioral experiments were conducted in all group and then the mice were sacrificed to collect blood of eyeballs and the brain hippocampus in animal Center. Mouse body weights were recorded weekly to confirm the mouse modelling.

2.3. Behavioral experiments

Behavioral tests were carried out to assess the depressed-like behaviors of mice, including: tail suspension test (TST), forced swim test (FST), splash test (ST), and novelty suppressed feeding test (NSFT). The observer records all the time. The specific experimental operations will be described below.

2.3.1. tail suspension test (TST)

The test should prepare a timer, a length of 30 cm rope in advance. Each mouse was individually suspended by the rope on the ground, and the 1cm of tail was tied by the rope. Next, the observer recorded the frequency of forearms movements and the latency of time before the first moving within 6 min and calculate the time they remain stationary during the last 4 min.

2.3.2. forced swim test (FST)

The test should prepare a timer, a 40 cm × 25 cm rectangular chamber, and a thermometer in advance. Each mouse was placed in the rectangular chamber filtered with 25°C water. Next, the observer recorded the frequency of forearms movements and the latency of time before the first moving in 6 min and calculate the time they remain stationary during the last 4 min.

2.3.3. splash test (ST)

The test should prepare a timer, a sucrose solution (10%) and an empty cage. the splash test consisting squirting sucrose solution on the dorsal coat of a mouse in its home cage. Because of its viscosity, the sucrose solution dirties the mouse fur and animals initiate grooming behavior. After applying sucrose solution, the observer recorded the time of latency to grooming, frequency, and the total time spent grooming during 6 min. The test was taken as an index of self-care and motivational behavior.

2.3.4. novelty suppressed feeding test (NSFT)

Mice were deprived of food for 24 h before being placed in a novel environment, a white methacrylate box (100 cm ×100 cm ×40 cm). A single food pellet (regular fodder) was placed on a piece of filter paper (10 cm), positioning in the center of the container that was brightly illuminated. The mouse was placed in a corner of the box and the latency to feed was calculated during 10 min. Then these animals were moved into their home cages and the amount of food consumed over the subsequent 5 min period were observed.

2.4. Sacrifice and sample preparation

After completion of behavioral tests, all mice were sacrificed by decapitation. Part of hippocampus were dissected and flash-frozen in liquid nitrogen immediately after decapitation. The remaining were rapidly fixed in 4% paraformaldehyde for one week. Finally, the brains were embedded in paraffin and serial 5 μ m sections were cut in the coronal plane. In addition, the blood was centrifuged at 3500 rpm at 4 °C for 30 min, and was stored in the -80 °C refrigerator for measurement.

2.5. Enzyme-linked immunosorbent assays (ELISA)

Serum gp130, IL-6, TNF- α and E2 levels were measured by ELISA kit (CUSABIO, Wuhan, China) respectively. All procedures were performed according to the manufacturer's instructions.

2.6. Real time polymerase chain reaction (RT-PCR)

Total RNA was completely isolated using Trizol Reagent (Invitrogen Life Technologies) and other necessary reagent in BV2 cells and hippocampus tissue, and the quantified 2 μ g RNA was reverse-transcribed to cDNA by using a Reversion Aid First Strand cDNA Synthesis kit (k1622, Thermo scientific, USA). Next, quantification real time polymerase chain reaction (qRT-PCR, ABI VII7 system, USA) was performed with TB GreenTM Premix Ex TaqTM II (Takara, Tokyo, Japan) and designed primers (Sangon, Shanghai, China) with the optimized condition. All samples were measured for three times. Each sample amplified GAPDH as a PCR reference gene for internal comparisons. Subsequently, mRNA relative expression was analyzed using the $2^{-\Delta\Delta C_t}$ method. In addition, primer sequenced were listed in supplement table 1.

2.7. Western blot analysis

The hippocampus tissues were lysed in Radioimmune-Precipitation Assay (RIPA, Beyotime Biotechnology, China) buffer mixed with PMSF (G2008-1ML, Servicebio, China) and phosphatase inhibitor cocktail (G2007-1ML, Servicebio, China).

Subsequently, protein concentrations were estimated using the bicinchoninic acid (BCA) method (P0010s, Beyotime Biotechnology, China) to ensure the neat internal reference. The Protein extracts were boiled for 10 min with 5 × loading buffer at 4:1 volume ratio. Equal amounts of protein samples were separated on 10 % or 12 % SDS-PAGE gels (Shanghai Epizyme Biomedical Technology Co., Ltd) and transferred to the nitrocellulose membranes (0.22 μm; NC, Millipore, USA) by electroblotting. Then the blots were blocked in 5 % nonfat milk with 0.1% Tween-20 solution (TBS-T) and incubated with primary antibodies: Anti-gp130 (21175-1-AP, Proteintech), anti-IL-6R (66855-1-Ig, Proteintech group, China), anti-JAK1 (CST#3344, Cell Signaling), anti-p-JAK1 (CST#74129, Cell Signaling), anti-STAT3 (10253-2-AP, Proteintech group, China), anti-p-STAT3 (CST#9145, Cell Signaling), anti-ER-β (A2546, abclonal), anti-Bax (CST#5023, cell signaling), anti-Bcl-2 (CST#3498, Cell signaling), anti-Caspase-3 (CST#9662, Cell signaling), anti-β-actin (GB11001, Servicebio) and GAPDH antibody (GB11002, Servicebio, China). The blots were then incubated with the secondary antibody (dilution 1:10000, Servicebio) for 2 h at RT. Immunoreactive proteins bands were visualized using an ECL reagent (P0018S, Beyotime, China) in a chemiluminescence device (Thermo Scientific, USA). For analysis, the levels of target proteins were normalized against those of β-actin or GAPDH.

2.8. Immunoprecipitation

For detecting the interaction of gp130 and estrogen receptor by co-immunoprecipitation (Co-IP) (24). The samples were collected and lysed in lysis buffer for 30 min and centrifuged for 10 min at 4 °C, then the supernatants were incubation with anti-gp130 and anti-ER-β for 12 h at 4°C. Next, 20~40 μL protein A+G Agarose were added into supernatants for 12 h at 4°C with rotors. The immune complexes were boiled in the sample buffer after washing with PBS five times. Later on, the sample were then immunoblotted with anti-gp130 or anti-estrogen receptor, respectively.

2.9. Immunostaining and morphometric analysis

The paraffin-embedded brain sections were washed in PBS (0.01 M, PH 7.3) 3 times (10 mins for each) and blocked in PBS containing 0.02% Triton and 10% normal goat serum for 45 min and overnight at 4°C with primary antibodies: goat anti-Iba-1 antibody (1:200; Abcam, USA), goat anti-ki67 antibody (1:200; Abcam, USA), mouse anti-gp130 (1:400; BIOSS, China), rabbit anti-ER- β (1:500, Abclonal, China) in PBS containing 0.02% Triton-X-100, 10% goat serum. All sections were washed three times in 0.01 M PBS (10 min each) and were incubated for 1.5 h at RT with goat anti- mouse IgG/FITC antibody (1:1000; BIOSS, China), goat anti-rabbit IgG/Cy3 antibody (1:1000; Abclonal, China). Finally, all sections were dried and cover slipped with a mixture of 0.01 M PBS. The confocal images were obtained, and digital images were captured using a fluorescent mounting medium. The immunofluorescence reactivity of the total area assessed relative to a sub-threshold background was measured with Image J (1.4.3.67).

2.10. cell culture and treatment

Mouse microglial (BV2 cells) were provided by the China Cell Line Bank (Beijing, China). The BV2 cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco), supplemented with 10% fetal bovine serum (FBS, Gibco), 1% penicillin (100 U/mL) and streptomycin (100 μ g/mL), and incubated in a humidified 37°C incubator containing 95% air and 5% CO₂. The cells were pretreated with E2 (respectively, 10⁻⁵, 10⁻⁶, 10⁻⁷, 10⁻⁸ mol/L) for 12 h followed by incubation with LPS (1 μ g/ mL) for 0 h, 1 h, 2 h and 4 h. Two estrogen receptor (ER)-selective antagonists and ER- β agonist (ER- α antagonist: MPP; ER- β antagonist: PHTPP; ER- β agonist: DPN; respectively, 10 and 10² pM) were treated with BV2 cells 1 h before E2 treatment respectively.

2.11. cell viability analysis

The microglial Cells, were plated in 96-well plates at a density of 1.5×10^3 cells/well and cultured overnight, which was measured by cell counting kit-8 assay (CCK-8, Beyotime, China). Various concentrations of E2 (respectively, 10^{-5} , 10^{-6} , 10^{-7} , 10^{-8} mol/L) were added to each well and incubated for 12 h. according to the manufactures' instructions, the CCK8 solution (5mg/mL) was added to each well and incubated for 0 h, 1 h, 2 h and 4 h in a humidified atmosphere of 5% CO₂ and 95% air at 37°C. Afterward, the cell viability was measured within 0.5 h, 1 h, 2 h, 3 h and 4 h, respectively, therefore, a time-viability curve (t-v curve) will be drawn.

2.12. Statistical analysis

Values were given as Mean $\pm$ SD. The normality of the data was tested using the D'Agostino and Pearson omnibus normality test. *P* values were calculated with one-way ANOVA analysis followed by the Tukey's post hoc Tests and Bonferroni test in GraphPad Prism (8.0.2). *P* $\leq$.05 represented as significant difference.

Results

3.1. E2 alleviated depression-like behaviors in OVX female mice

Wide-type mice were ovariectomized and treated with E2 according to the above-mentioned modelling. A series of behavioral experiments were conducted to verify whether OVX caused depressive-like behaviors and whether E2 alleviated depressive-like behaviors in OVX mice. The results showed E2 markedly reduced immobility time and increased active time in OVX + E2 group compared with OVX group for TST and FST (Fig. 1b-1c); E2 elevated the frequency of grooming in OVX+E2 group compared with OVX group for ST (Fig. 1d); Moreover, E2 shortened the feed latency time in OVX + E2 group compared with OVX group for NSFT (Fig. 1e). By the way, we continuously recorded the body weight of the mice over several weeks and found that the OVX group was slower compared with WT group (supplement Fig 2). Totally, these results suggested that E2 could ameliorate

depression-like behaviors in OVX mice.

3.2. E2 treatment reduced inflammation levels in vivo

We examined the serum levels of inflammatory factors and the mRNA expression of inflammatory factors in the hippocampus of the three groups of mice respectively. The results showed that serum IL-6 and TNF- α levels were significantly elevated in OVX group and decreased in OVX + E2 group (Fig. 2a-2b). Moreover, we assayed the level of serum E2 to verify the success of the pre-experimental OVX procedure in mice (Fig. 2c). We further observed that the mRNA levels of IL-6 and TNF- α in the hippocampus were synchronized with serum inflammation levels, with increased expression of IL-6 and TNF- α in the OVX group and decreased in OVX + E2 group (Fig. 2d-2e).

3.3. E2 suppressed OVX-induced apoptosis in hippocampus

To explore the mechanism that E2 inhibits apoptosis, we next investigated gp130/JAK1/STAT3 signaling pathway proteins and apoptosis-related proteins including Bcl-2, Bax and caspase-3. As shown in figure 3, OVX increased significantly gp130, p-JAK1 and p-STAT3 expression levels compared with sham group, while E2 treatment reversed these changes, gp130, p-JAK1, and p-STAT3 were significantly reduced in OVX+E2 group (Fig. 3a-d). Subsequently, our results demonstrated that E2 suppressed OVX-induced apoptosis in hippocampus, the anti-apoptotic protein Bcl-2 levels increased, while the pro-apoptotic protein Bax and caspase-3 levels decreased in OVX + E2 group (Fig. 3e-h).

3.4. E2 impeded microglial activation in the hippocampus

Microglial activation in the hippocampus is associated with the development of depression. Therefore, we investigated the change of microglia induced by OVX and examined the effects of E2 treatment on the hippocampus. According to immunostaining and morphometric analysis, OVX exhibited significantly increased

expression levels of Iba-1 (a marker of microglia) compared with sham group ($p < .001$), while E2 (0.2 $\mu\text{g}/\text{mL}$) treatment remarkably inhibited the increase of Iba-1 compared with OVX group ($p < .01$) (Fig 4a, 4b). Similarly, OVX inhibited significantly expression levels of ki67 (a marker of cell proliferation) compared with sham group ($p < .05$), while E2 (0.2 $\mu\text{g}/\text{mL}$) treatment increased the expression levels of ki67 compared with OVX group ($p < .01$) (Fig 4c, 4d). The above results show that E2 can impede microglial activation and stimulate cell proliferation in the hippocampus.

3.5. E2 inhibited inflammatory responses in vitro

Before investigating the effects of E2 on inflammatory response of BV2 cells induced by LPS, we first assayed LPS activity by treating BV2 cells with E2 at various concentrations (10^{-5} , 10^{-6} , 10^{-7} , 10^{-8} (mol/L)) at 0 h, 1 h, 2 h and 4 h, and then explored the optimal time and concentration of the intervention (Fig 5a, 5b). As shown in Fig. 5c, we found that E2 of 10^{-6} concentration had the most significant inhibition of gp130 protein expression.

To further explore whether E2 has any inhibitory effects on inflammatory response in BV2 cells induced by LPS, we measured the expression levels of IL-6 and TNF- α using ELISA. Our results showed the expression levels of IL-6 and TNF- α were increased significantly following treatment with LPS compared with control (respectively, $p < .05$, $p < .05$). In addition, the expression levels of IL-6 and TNF- α were induced significantly in LPS+E2 group (respectively, $p < .01$, $p < .05$) (Fig. 5d-5e). Similarly, we observed that the mRNA levels of IL-6, TNF- α decreased significantly in LPS+ E2 group ($p < .001$) (Fig. 5f-5g). These results suggested that E2 treatment inhibited inflammatory cytokines in BV2 cells induced by LPS.

3.6. E2 suppressed LPS-induced apoptosis in LPS-activated BV2 microglia

Meanwhile, we investigated gp130/JAK1/STAT3 signaling pathway proteins and apoptosis-related proteins including Bcl-2, Bax and caspase-3. As shown in figure 6,

LPS increased significantly gp130, p-JAK1 and p-STAT3 expression levels compared with sham group ($p < .001$, $p < .01$, $p < .01$), while E2 treatment reversed these changes, gp130, p-JAK1, and p-STAT3 were significantly reduced in LPS+E2 group ($p < .001$, $p < .01$, $p < .05$) (Fig. 6a-6d). Subsequently, our results demonstrated that E2 suppressed LPS-induced apoptosis in BV2 cells, the anti-apoptotic protein Bcl-2 levels increased ($p < .001$, $p < .01$, $p < .001$), while the pro-apoptotic protein Bax and caspase-3 levels decreased in LPS+E2 group ($p < .001$, $p < .01$, $p < .001$) (Fig. 6e-6h).

3.7. E2 inhibited gp130/JAK1/STAT3 signaling pathway mainly depended on ER- β but not ER- α

To further verify whether E2 regulates gp130/JAK1/STAT3 signaling pathway via ER- α /ER- β . We set up ER antagonist groups and ER agonist (ER- α antagonist: MPP; ER- β antagonist: PHTPP; ER- β agonist: DPN). Different ER antagonist concentrations were set (1, 2 pM, respectively). Based on the previous experimental results, we found that the expression of gp130, JAK1 and STAT3 was reduced significantly in LPS+E2+PHTPP group, while the expression of gp130, JAK1 and STAT3 had no change in LPS+E2+MPP group. We further confirmed this result with DPN. The result indicated that E2 inhibited gp130/JAK1/STAT3 signaling pathway via ER- β (Fig. 7a-7l).

3.8. E2 inhibited gp130/JAK1/STAT3 signaling pathway by promoting the interaction between ER- β and gp130

To explore the molecular mechanisms underlying the effect of E2, the mode of binding of gp130 and ER was predicted by an online predictive protein binding website (ZDOCK SERVER) (Fig. 8a). And then double immunofluorescent staining showed that the co-localization of gp130 and ER- β existed in LPS-induced BV2 cells. According to immunoprecipitation, E2 treatment promoted the interaction between gp130 and ER- β (Fig 8b-8c).

These findings supported that E2 may inhibited the activation of the gp130/JAK1/STAT3 signaling pathway through the interaction between ER- β and gp130.

4. Discussion

In the last two decades, number of studies reported the anti-inflammatory effects of estrogen in MDD, but few studies have explored the specific molecular mechanisms by which estrogen exerts its anti-inflammatory effects. This is the first study to investigate the antidepressant effects of estrogen via inhibiting gp130 signaling pathway in MDD.

MDD is one of the major mental illnesses of concern worldwide due to its high morbidity and mortality rates(25). Despite the emergence of a growing body of research, the exact underlying mechanisms of MDD remain unknown. Unlike other disease samples, where there are many uncontrollable factors such as age and drug history, animal models provide a key way to examine neural circuits and molecular and cellular pathways in a controlled environment. Common animal models include learned helplessness, unpredictable chronic mild stress, ovariectomy, etc.(26). Many animal experiments have demonstrated that ovariectomy causes neuroinflammation as well as the emergence of depressive behaviors(27). In this study, the ovariectomy model was applied to verified the antidepressant of estrogen on mice and the anti-inflammatory effect of estrogen on hippocampal neuroinflammation, and LPS-induced model were used to establish a cellular model of neuroinflammation in order to further explore the specific molecular mechanisms.

Estrogen is a steroid hormone that includes estrone (E1), estradiol (E2) and estriol (E3), with E2 playing the primary role in the body and therefore being more commonly studied. Estrogen plays an important role not only in sexual maturation and reproduction, but also in the maintenance and remodeling of brain functions such as cognition and memory. As research into the mechanisms underlying the development of psychiatric disorders continues, studies targeting estrogen and the

estrogen receptors have emerged, demonstrating that estrogen regulates neurotransmitter systems in the brain, such as dopaminergic, 5-hydroxytryptaminergic and glutamatergic, through the estrogen receptor signaling pathway, thereby influencing cognitive, emotional and behavioral responses. Estrogen has also been found to act as an atypical antipsychotic similar to that of neuropsychiatric disorders. Therefore, it is important to study the molecular mechanisms of action between estrogen and the nervous system in order to exploit the therapeutic potential of estrogen. It was reported that MDD patients have higher depression scale scores with lower circulating estrogen in the body. Early clinical trials confirmed that depressive symptoms were relieved following transdermal estrogen treatment in MDD patients. In addition, animal experiments have shown that OVX directly causes depression-like behaviors in female mice. Similarly, we have shown that oestrogen can alleviate depressive-like behaviors in OVX mice. It has also been shown that in depressed mouse brains, the production of depression-like behaviors is accompanied by increased expression of pro-apoptotic factors (e.g. Bax and caspase-3) and reduced expression of the anti-apoptotic factor bcl-2 (28). Nevertheless, the protective effect of E2 treatment on OVX-induced MDD and its associated mechanisms have been explored and validated. The results suggest that E2 replacement can alleviate depressive symptoms in MDD.

To explore the mechanisms by which E2 alleviates neuroinflammation and depressive symptoms, we selected another pathway, gp130/JAK1/STAT3, in which gp130 forms a dimer and thus activates intracellular tyrosine kinase in response to IL-6 family cytokines. Subsequently, JAK1 binds to the box1 region at the proximal end of the gp130 cytoplasmic region, further enabling the recruitment and phosphorylation of STAT3, which in turn affects cell growth and differentiation. Encouragingly, our study found that E2 inhibited the production of inflammatory factors through the gp130/JAK1/STAT3 signaling pathway while suppressing apoptosis, and reduced the mRNA levels of gp130.

Further testing of estrogen receptors (ER- α and ER- β) in monocyte-macrophages

confirmed the anti-inflammatory properties of estrogen (29). Mechanistic studies suggest that E2 activates intracellular signaling pathways via estrogen receptors (ER- α , ER- β and GPR30) on microglia, such as intracellular activation of PIK3 to inhibit the transcription factor NF- κ B, which further downregulates nitric oxide synthase (NO) and reactive oxygen species (ROS) (30). Studies have shown that estrogen deficiency leads to activation of NLRP3 inflammatory vesicles, further leading to hippocampal neuroinflammation and depression-like behaviors. Furthermore, it was shown that the effects of estrogen on hippocampal inflammation as well as depressive-like behaviors are ER- β -dependent (13). Previous mechanistic studies have shown that in human umbilical vein endothelial cells (HUVECs), E2 downregulates gp130 mRNA levels and protein expression through a combination of ER- α and ER- β (21). In addition, it was shown that SEMS reduces pancreatic cancer tumor volume with gp130 protein expression through ER- β -mediated effects (31). Encouragingly, in this mechanistic study, we found that E2 downregulated the gp130/JAK1/STAT3 signaling pathway via ER- β , but not ER- α , and alleviated depressive-like behaviors.

In conclusion, our study found that E2 can downregulate apoptosis and neuroinflammation via promoting the interaction between ER- β and gp130 in OVX-induced MDD, thereby downregulating gp130/JAK1/STAT3 signaling pathway and alleviating depression-like behaviors. These provide a new insight and theoretical basis for the clinical application of E2 replacement therapy to alleviate the symptoms of MDD.

Abbreviation

MDD: major depressive disorder

OVX: ovariectomy

E1: estrone

E2: estradiol

E3: estriol

gp130: Glycoprotein 130
JAK1:the Janus kinase 1
STAT3: Signal Transducer and Activator of Transcription
ER- α : estrogen receptor- α
ER- β : estrogen receptor- β
LPS: lipopolysaccharide
MPP:ER- α antagonist, Methyl-piperidino-pyrazole
PHTPP:ER- β antagonist,
DPN: ER- β agonist, Diarylpropionitrile
DAPI: 4',6-diamidino-2-phenylindole
DMSO: dimethyl sulfoxide
TST: tail suspending test
FST: forcing swimming test
ST: splash test
NSFT: novel suppress food test
ELISA: enzyme-linked immunosorbent assay

Data Availability Statement

The data in this study were obtained from experiments, not from the internet, and therefore are not suitable for data sharing.

Funding

The National Natural Science Foundation of China (81772265) have contributed to analysis and interpretation of data.

Competing interests

The authors have declared no conflicts of interest.

Reference

1. Murray CJL, Vos T, Lozano R, Naghavi M, Flaxman AD, Michaud C, et al. Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. *The Lancet*. 2012;380(9859):2197–223. doi: 10.1016/s0140-6736(12)61689-4.
2. Weissman MM, Klerman GL. Sex differences and the epidemiology of depression. *Archives of general psychiatry*. 1977;34(1). PubMed PMID: 319772.
3. Tanabe H, Mutai H, Sasayama D, Sasamoto H, Miyashiro Y, Sugiyama N, et al. Sex differences in serum levels of 5 α -androstane-3 β , 17 β -diol, and androstenediol in the young adults: A liquid chromatography-tandem mass spectrometry study. *PloS one*. 2021;16(12):e0261440. doi: 10.1371/journal.pone.0261440. PubMed PMID: 34910781.
4. Kobayashi M, Sugiyama N, Sasayama D, Sasamoto H, Miyashiro Y, Arima K, et al. Sex differences in the serum level of endogenous ligands for estrogen receptor β in the elderly population. *Sci Rep*. 2016;6:25878. doi: 10.1038/srep25878. PubMed PMID: 27165125.
5. Li F, He F, Sun Q, Li Q, Zhai Y, Wang X, et al. Reproductive history and risk of depressive symptoms in postmenopausal women: A cross-sectional study in eastern China. *Journal of affective disorders*. 2019;246:174–81. doi: 10.1016/j.jad.2018.12.031. PubMed PMID: 30583142.
6. Drozdowicz-Jastrzębska E, Skalski M, Gdańska P, Mach A, Januszko P, Nowak RJ, et al. Insomnia, postpartum depression and estradiol in women after delivery. *Metab Brain Dis*. 2017;32(6):1913–8. doi: 10.1007/s11011-017-0079-0. PubMed PMID: 28791548.
7. Cohen LS, Soares CN, Poitras JR, Prouty J, Alexander AB, Shifren JL. Short-term use of estradiol for depression in perimenopausal and postmenopausal women: a preliminary report. *Am J Psychiatry*. 2003;160(8):1519–22. PubMed PMID: 12900318.
8. Karisetty BC, Joshi PC, Kumar A, Chakravarty S. Sex differences in the effect of chronic mild stress on mouse prefrontal cortical BDNF levels: A role of major ovarian hormones. *Neuroscience*. 2017;356. doi: 10.1016/j.neuroscience.2017.05.020. PubMed PMID: 28527954.
9. Dean J, Keshavan M. The neurobiology of depression: An integrated view. *Asian J Psychiatr*. 2017;27:101–11. doi: 10.1016/j.ajp.2017.01.025. PubMed PMID: 28558878.
10. Mahajan GJ, Vallender EJ, Garrett MR, Challagundla L, Overholser JC, Jurjus G, et al. Altered neuro-inflammatory gene expression in hippocampus in major depressive disorder. *Prog Neuropsychopharmacol Biol Psychiatry*. 2018;82:177–86. doi: 10.1016/j.pnpbp.2017.11.017. PubMed PMID: 29175309.
11. Das R, Emon MPZ, Shahriar M, Nahar Z, Islam SMA, Bhuiyan MA, et al. Higher levels of serum IL-1 β and TNF- α are associated with an increased probability of major depressive disorder. *Psychiatry Research*. 2021;295:113568. doi: 10.1016/j.psychres.2020.113568. PubMed PMID: 33199026.
12. Jiang X, Chen Z, Yu X, Chen J, Sun C, Jing C, et al. Lipopolysaccharide-induced depression is associated with estrogen receptor- α /SIRT1/NF- κ B signaling pathway in old female mice. *Neurochem Int*. 2021;148:105097. doi: 10.1016/j.neuint.2021.105097. PubMed PMID: 34119591.
13. Xu Y, Sheng H, Bao Q, Wang Y, Lu J, Ni X. NLRP3 inflammasome activation mediates estrogen deficiency-induced depression- and anxiety-like behavior and hippocampal inflammation in mice. *Brain, Behavior, and Immunity*. 2016;56:175–86. doi:

- 10.1016/j.bbi.2016.02.022. PubMed PMID: 26928197.
14. Jia X, Gao Z, Hu H. Microglia in depression: current perspectives. *Sci China Life Sci.* 2021;64(6):911-25. doi: 10.1007/s11427-020-1815-6. PubMed PMID: 33068286.
 15. Slowik A, Lammerding L, Hoffmann S, Beyer C. Brain inflammasomes in stroke and depressive disorders: Regulation by oestrogen. *J Neuroendocrinol.* 2018;30(2). doi: 10.1111/jne.12482. PubMed PMID: 28477436.
 16. Ihle JN, Kerr IM. Jaks and Stats in signaling by the cytokine receptor superfamily. *Trends Genet.* 1995;11(2):69-74. PubMed PMID: 7716810.
 17. Murakami M, Hibi M, Nakagawa N, Nakagawa T, Yasukawa K, Yamanishi K, et al. IL-6-induced homodimerization of gp130 and associated activation of a tyrosine kinase. *Science (New York, NY).* 1993;260(5115):1808-10. PubMed PMID: 8511589.
 18. Girotti M, Donegan JJ, Morilak DA. Influence of hypothalamic IL-6/gp130 receptor signaling on the HPA axis response to chronic stress. *Psychoneuroendocrinology.* 2013;38(7):1158-69. Epub 2012/12/12. doi: 10.1016/j.psyneuen.2012.11.004. PubMed PMID: 23218517; PubMed Central PMCID: PMC3609893.
 19. Lin SC, Yamate T, Taguchi Y, Borba VZ, Girasole G, O'Brien CA, et al. Regulation of the gp80 and gp130 subunits of the IL-6 receptor by sex steroids in the murine bone marrow. *J Clin Invest.* 1997;100(8):1980-90. PubMed PMID: 9329961.
 20. Grant SL, Hammacher A, Douglas AM, Goss GA, Mansfield RK, Heath JK, et al. An unexpected biochemical and functional interaction between gp130 and the EGF receptor family in breast cancer cells. *Oncogene.* 2002;21(3):460-74. PubMed PMID: 11821958.
 21. Zhou M, Dai W, Cui Y, Li Y. Estrogen downregulates gp130 expression in HUVECs by regulating ADAM10 and ADAM17 via the estrogen receptor. *Biochem Biophys Res Commun.* 2020;523(3):753-8. Epub 2020/01/19. doi: 10.1016/j.bbrc.2020.01.008. PubMed PMID: 31952790.
 22. Ma Y, Liu M, Yang L, Zhang L, Guo H, Hou W, et al. Loss of Estrogen Efficacy Against Hippocampus Damage in Long-Term OVX Mice Is Related to the Reduction of Hippocampus Local Estrogen Production and Estrogen Receptor Degradation. *Mol Neurobiol.* 2020;57(8):3540-51. doi: 10.1007/s12035-020-01960-z. PubMed PMID: 32542593.
 23. Lasota A, Danowska-Klonowska D. Experimental osteoporosis--different methods of ovariectomy in female white rats. *Rocz Akad Med Bialymst.* 2004;49 Suppl 1:129-31. PubMed PMID: 15638397.
 24. Ponzielli R, Boutros PC, Katz S, Stojanova A, Hanley AP, Khosravi F, et al. Optimization of experimental design parameters for high-throughput chromatin immunoprecipitation studies. *Nucleic Acids Res.* 2008;36(21):e144. doi: 10.1093/nar/gkn735. PubMed PMID: 18940864.
 25. Gu L, Xie J, Long J, Chen Q, Chen Q, Pan R, et al. Epidemiology of major depressive disorder in mainland china: a systematic review. *PLoS One.* 2013;8(6):e65356. Epub 2013/06/21. doi: 10.1371/journal.pone.0065356. PubMed PMID: 23785419; PubMed Central PMCID: PMC3681935.
 26. Wang Q, Timberlake MA, Prall K, Dwivedi Y. The recent progress in animal models of depression. *Prog Neuropsychopharmacol Biol Psychiatry.* 2017;77. doi: 10.1016/j.pnpbp.2017.04.008. PubMed PMID: 28396255.
 27. Kiss A, Delattre AM, Pereira SIR, Carolino RG, Szawka RE, Anselmo-Franci JA, et al. 17 β -estradiol replacement in young, adult and middle-aged female ovariectomized rats promotes improvement of spatial reference memory and an antidepressant effect and alters

monoamines and BDNF levels in memory- and depression-related brain areas. Behavioural brain research. 2012;227(1):100-8. doi: 10.1016/j.bbr.2011.10.047. PubMed PMID: 22085882.

28. Liu C-H, Tan Y-Z, Li D-D, Tang S-S, Wen X-A, Long Y, et al. Zileuton ameliorates depressive-like behaviors, hippocampal neuroinflammation, apoptosis and synapse dysfunction in mice exposed to chronic mild stress. Int Immunopharmacol. 2020;78:105947. doi: 10.1016/j.intimp.2019.105947. PubMed PMID: 31796384.

29. Crespo-Castrillo A, Arevalo M-A. Microglial and Astrocytic Function in Physiological and Pathological Conditions: Estrogenic Modulation. Int J Mol Sci. 2020;21(9). doi: 10.3390/ijms21093219. PubMed PMID: 32370112.

30. Li P, Gan Y, Xu Y, Wang L, Ouyang B, Zhang C, et al. 17beta-estradiol Attenuates TNF- α -Induced Premature Senescence of Nucleus Pulposus Cells through Regulating the ROS/NF- κ B Pathway. Int J Biol Sci. 2017;13(2):145-56. doi: 10.7150/ijbs.16770. PubMed PMID: 28255267.

31. Wu X, Cao Y, Xiao H, Li C, Lin J. Bazedoxifene as a Novel GP130 Inhibitor for Pancreatic Cancer Therapy. Mol Cancer Ther. 2016;15(11):2609-19. PubMed PMID: 27535971.

Figure legend

Figure 1. E2 alleviated depression-like behaviors induced by OVX and experimental programs. (a) experimental program. (b) Immobility time in the TST. (c) Immobility time in the FST. (d) Grooming time in the ST. (d) The time of latency to feed in the NSFT. The following groups were used: WT, WT+OVX, OVX+E2 (0.2 μ g/ mL). The data (mean $\pm$ SEM) were analyzed by the one-way analysis of variance (ANOVA), * p < .05, ** p < .01, *** p < .001 versus WT group; # p < .05, ## p < .01, ### p < .001 versus OVX group. OVX: Ovariectomy

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Figure 4. The immunostaining and morphometric analysis of Iba-1 and ki67 in the hippocampus. (a) Immunostaining of Iba-1 in the hippocampus. (b) The expression levels of Iba-1 in hippocampus. (c) Immunostaining of ki67 in the hippocampus. (d) The expression levels of ki67 in hippocampus. The data (mean $\pm$ SEM) were analyzed by the one-way analysis of variance (ANOVA), * p < .05, ** p < .01, *** p < .001 versus WT group; # p < .05, ## p < .01, ### p < .001 versus OVX group. OVX: Ovariectomy

Figure 5. Detection of cytokine productions in LPS-activated BV2 cells. (a) The optimal time LPS activity in various E2 concentration. (b) Cell viability in various E2 concentration at 4 h. (c) the expression of gp130 protein for various E2 concentrations (no LPS-activity). (d-e) ELISA analysis of IL-6 (d) and TNF- α (e) expression. (f-g) RT-PCR analysis of IL-6 (d) and TNF- α (e) mRNA levels. The data (mean $\pm$ SEM) were analyzed by the one-way analysis of variance (ANOVA), * p < .05, ** p < .01, *** p < .001 versus control group; # p < .05, ## p < .01, ### p < .001 versus LPS group.

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Figures

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