Heliyon

Establishment and evaluation of a novel mouse model of peri/postmenopausal depression

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Abstract

Women are believed to be more vulnerable to develop depressive symptoms during the perimenopause compared to postmenopause. The traditional bilateral ovariectomy and chronic mild stress (CMS) stimulation animal model produces a postmenopausal depressive-like state but the transition from perimenopausal period to postmenopausal period was ignored. Thus we establish a novel animal model in which the mice were stimulated by CMS for three months and removed the ovaries by two-step operation, and then evaluate whether this novel model could be much better for preclinical study used as a peri/postmenopause depressive model. The present study systemically evaluated the changes induced by two-step ovariectomy plus CMS in the mice. The depression-like behaviors, the levels of corticosterone, estrogen, pro-inflammatory factors, neurotransmitters, as well as brain-derived neurotrophic factor were determined; the changes of estrogen receptors, serotonin receptors, uterine weight and bone microarchitecture were also observed. The results show that the behaviors and biochemical indexes of mice changed gradually over time. Our study suggests that this two-step ovariectomy operation plus CMS successfully establishes a more reasonable peri/

postmenopausal depression animal model which effectively simulates the clinical symptoms of peri/postmenopausal depressive women.

Keywords: Neuroscience, Endocrinology

1. Introduction

Depression is a chronic, reoccurring neuropsychiatric disease that currently affects 350 million people worldwide and is ranked as the second leading cause of disability worldwide. Epidemiological studies of depression have demonstrated that women are 2 times more likely to experience major depression than men [1], and this risk is particularly higher during reproductive years [2]. In women, the period of hormonal fluctuations or extremely drops in estrogen levels can be classified into three broad stages: premenopause, perimenopause and the postmenopause which follows the final menstrual period [3]. Moreover, several clinical studies have suggested that the risk of symptoms of depression during perimenopause is higher than in the premenopausal stage [4, 5].

Estrogens have neurotrophic and neuroprotective actions on certain brain regions such as hippocampus, striatum and cortex [6]. Estrogen's role in neurotransmitter systems dysfunction was associated with depressive and anxious symptoms during reproductive period [7]. With the numerous data supporting the beneficial impact of estrogens on affective disorders, there is a growing interest in the two different subtypes of estrogen receptors (ERs), ERα and ERβ, have been detected in the hippocampus of many species [8, 9]. The natural hormone 17β-estradiol binds to estrogen receptors, and ovariectomy may influence the ERs expression in brain [10]. Decreased expression of brain-derived neurotrophic factor (BDNF), a mammalian neurotrophin, has been shown to be associated with in ovariectomized animals which was reversed by estradiol treatment [11]. Furthermore, the hormonal status can considerably influence the BDNF signaling on the BDNF-synthesizing neurons in the forebrain, suggesting a relationship between estrogen and BDNF in depressive condition [12]. In addition, the studies have demonstrated that the bone loss at menopause increased along with estrogen deficiency [13, 14].

At present, bilateral removal of the ovaries is commonly used to simulate menopause [15]. However, this animal model results in a drastic decline in hormone levels mimicking human postmenopausal period, without addressing the fluctuations during perimenopausal period condition [16]. To simulating the perimenopausal and postmenopausal period of female, the present study was to establish a new mouse model which removes the ovaries unilaterally two times for two months. We found that this new peri/postmenopausal depression model had better similarities to the endocrinological and pathological changes in the women during the perimenopausal and postmenopausal period.

2. Material and methods

2.1. Animals

Seven to 8-week-old female C57BL/6 mice weighing 20 ± 2 g were obtained from the Animal Resource Center of the Faculty of Medicine of Nanjing Medical University for using in the experiment. Stressed group mice were maintained in small individual cages before and during unpredictable chronic mild stress states in small individual cages ($29\,cm \times 18\,cm \times 16\,cm$), and non-stressed group mice were housed at 4−5 mice per cage. All mice were kept in a standardized environment (temperature $25 \pm 2\,°C$, humidity $55 \pm 10\%$, a reversed 12-h light/dark cycle: lights on from 20:00 to 08:00) and housed in the Animal Resource Centre of the Faculty of Medicine, Nanjing Medical University. All of the experimental mice were allowed to adapt to the new environment for 1 week before the experiment and handled 1−2 min per day before surgeries and behavioral tests. All animal experimentation was approved by the Ethical Committee on Laboratory Animal Welfare of Nanjing Medical University, and conducted in conformity with the rules of the Experimental Animal Application Criteria and Institutional Animal Care and Use Committee (IACUC).

2.2. Experimental design

As described in Fig. 1, after a 7-day adaptation period, Seven to 8-week-old female C57BL/6 mice were randomly divided into: 1) sham group; 2) chronic mild stress (CMS) with sham surgery; 3) bilateral ovariectomy group (CMS + BO) with exposure to chronic mild stress; 4) unilateral ovariectomy group (CMS + UO) with exposure to chronic mild stress; and 5) the ovaries were removed in the first and second month (CMS + UO + UO) with exposure to chronic mild stress. The detailed process was shown in Fig. 1. In the second and third month, the animals from each experimental group were behaviorally assessed. Animals were submitted to the tail suspension test (TST) on the first day and forced swimming test (FST) the next day. After modeling stress, mice were sacrificed and their serum, brain, uterus
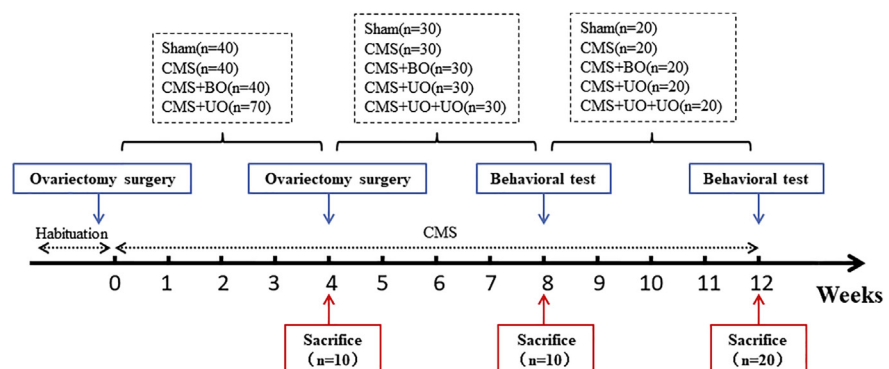

Fig. 1. Schematic representation of the experimental design. CMS: chronic mild Stress; BO: bilateral ovariectomy; UO: unilateral ovariectomy.

and left tibiae were collected for test. Some mice were euthanized with chloral hydrate (400 mg/kg) and blood was collected from the orbitalsinus. Brains, uterus and left tibiae were rapidly harvested from the decapitated mice. The other mice were transcardially perfused with saline and 4% paraformaldehyde before brains were harvested for immunofluorescence.

2.3. Surgery

Animals were ovariectomized under intraperitoneal chloral hydrate (400 mg/kg) anaesthesia. The abdominal region between the midline at the pelvic level was shaved and cleaned with povidone-iodine. A small median abdominal incision through the skin, connective tissue and the muscle layer was made. The ovaries were exteriorized with the associated fat pad and fallopian tube. After that, the ovaries were cut away and discarded. The muscle and skin layer were sutured and the wound was treated with antibiotic spray. Sham operation consisted of skin and muscle layer incision and suture only. After surgery, mice were allowed to recover for 7 days before undergoing the stress [17].

2.4. Unpredictable chronic mild stress

Unpredictable chronic mild stress is wildly used to induce depression-like behavior. To create a CMS model, mice were subjected to a series of procedures such as pairing (placed two unfamiliar mice in a cage for 2 h), 45 sloped cage (6 h), food and water deprivation (24 h), restrained motion (shut mice in a brown glass bottle with 3 cm radius and 15 cm height for 2 h), wet cage (200 ml sterile water added to the cage; 6 h), switched day/night lights, and tail clamping (20 min), etc. The mice received two or three stressors daily, and there was no repetition of daily stressor combination within a week. At the same time, the weekly stressor plan was also different during a month, and we have also always changed the time and intensity of the stressor, in order to ensure unpredictability.

2.5. Sucrose preference test (SPT)

Anhedonia or loss of interest in activities can be measured by reduction of preference for sucrose in SPT. Initially, mice were habituated for 1 week to a two-bottle choice condition. Sucrose testing was performed once a week throughout the entire experiment. The animals were deprived of water for 12 h before the tests. Then, two drinking bottles containing 1% sucrose water or sterile water were placed in each cage. The sites of the drinking bottles were switched 3 h later to preclude the influence of drinking site preference. After another 3 h, the bottles were weighed. The change in weight of each bottle equaled the sucrose water and sterile water intake. The SPT value was then calculated using the following formula: SPT (%) = sucrose water intake/(sucrose water intake + sterile water intake) × 100%.

2.6. Forced swimming test (FST)

The forced swimming test is a common method for evaluating depression-like behavior [18]. Mice were placed individually into a glass cylinder (19 cm height $\times$ 21 cm diameter) filled to a depth of 13 cm water maintain at 25 ± 2 °C, and the test remained 6 min. The 6-min test was videotaped and total immobility time was recorded during that period with a stopwatch. Immobility time, defined as the time during which the animal ceases to move or making only those movements which were necessary to keep its head above the water. The duration of immobility behavior was recorded during the full testing period by Forced Swim Scan (Clever Sys Inc., USA).

2.7. Tail suspension test (TST)

Mice tails were fixed on the top of the box, and the immobility time of each mouse was recorded over a 6-minute period. Immobility behavior, defined as the mice hung passively and completely motionless [19]. The time of immobility of the tail suspended mice during the last 4 minutes was measured with Tail Suspension Scan (Clever Sys Inc., USA).

2.8. Blood for enzyme-linked immunosorbent assay (ELISA)

Blood samples standed for 30 minutes at 37 °C, centrifugation (3000 rpm for 20 min), collecting the serum, and measuring E2、CORT and BDNF concentration using an enzyme-linked immunosorbent assay (ELISA) kit (Cloud Clone Corp, USA) followed the data sheets.

2.9. Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

Total RNA was extracted by using a TRIzol reagent (Karroten) and prepared for quantitative reverse transcriptase PCR by using MasterMix (TaKaRa, Japan). Primer sequences were designed by Primer Premier 6 as Table 1. Real-time PCR was carried out using the SYBR Green mixture (TaKaRa, Japan) in a QuantStudio 5 system (Thermo Fisher Scientific, United States). The cycling conditions were as follows: denaturation at 95 °C for 30 s, followed by 40 cycles of DNA synthesis at 95 °C for 5 s and 60 °C for 34 s. GAPDH was used as an endogenous control, and the relative expression of target genes were determined using the $2^{(-\Delta\Delta ct)}$ method.

2.10. Western blotting assay

Some Hippocampus tissues were disrupted in a lysis buffer consisting of 1% phenylmethanesulfonyl fluoride (PMSF) and protein concentration was quantified by BCA assay kit (Beyotime Biotech Inc.). The protein (20 μg) was separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS−PAGE) and transferred to

Table 1. Primer sequences of target using qPCR.

Gene	Sense	Antisense
5-HT$_{1A}$R	GGATGTTTTCCTGTCCTGGT	CACAAGGCCTTTCCAGAACT
5-HT$_{1B}$R	TCACATGGCCATTTTTGACT	CAGTTTGTGGAACGCTTGTT
5-HT$_{2A}$R	AGAACCCCATTCACCATAGC	ATCCTGTAGCCCGAAGACTG
5-HT$_{2C}$R	GAATAATACTACCTGCGTGCTC	AGCGTTCTCTTCCTCATCAC
SERT	TGCCTTTTATATCGCCTCCTAC	CAGTTGCCAGTGTTCCAAGA
IL-1β	TGTCTTGGCCGAGGACTAAGG	TGGGCTGGACTGTTTCTAATGC
IL-6	CTGCAAGAGACTTCCATCCAG	AGTGGTATAGACAGGTCTGTTGG
TNF-α	ACGGCATGGATCTCAAAGAC	AGATAGCAAATCGGCTGACG
GAPDH	TGAGTATGTCGTGGAGTCTAC	TGGACTGTGGTCATGAGCC

polyvinylidenefluoride (PVDF) membranes (Millipore). 5% Skimmilk in TBST was used to block the membranes for 1h at room temperature. Following, the membranes were incubated with the following primary antibodies at 4 °C overnight: anti-ERβ (Proteintech, Chicago, USA 1:3000), and anti-GAPDH (Proteintech, Chicago, USA 1:5000) antibodies. After a washing in TBST, the membranes were incubated with horseradish peroxidase-conjugated goat anti-rabbit secondary antibodies (Proteintech, Chicago, USA 1:5000) for 1 h at room temperature. After washing in TBST for 4 times, protein bands were detected by enhanced chemiluminescence and Image J software.

2.11. High performance liquid chromatography analysis

The monoamine and amino acids neurotransmitter in the hippocampus were measured by high-performance liquid chromatography with electrochemical detector (Ultimate 3000 Autosampler). Hippocampus tissues were homogenized in extract solution, which consisted of 0.1 M HClO$_4$ and 0.1 mM EDTA buffer, and the mixtures were centrifuged at a speed of 20000 rpm for 30 min at 4 °C. Then, 50 μL of the resultant supernatant was injected into the liquid chromatography system equipped with a reversed phase C 18 column (2.2 μm, 120 Å, 2.1 × 100 mm, DIONEX) and was detected by ESA Coulochem III Electrochemical Detector. The detector was set at 350 mV. The mobile phase consisted of 90 mM NaH$_2$PO$_4$, 1.7 mM 1-octanesulfonic acid, 50 mM citrate, 50 uM EDTA-2Na, and acetonitrile (0.05 w/v) (pH 2.6). The identification and purity was evaluated by the chromatographic peaks as well as their quantitative evaluation by comparing their retention times and peak areas with those of standard solutions.

2.12. Micro computed tomography (microCT)

The right tibias of all groups were scanned using Micro-computed tomography (CT) (SkyScan 1176, Bruker-microCT, Kontich, Belgium). The collected right proximal

tibias (cleaned of adherent soft tissues) were placed on the scanning bed. The bone micro architectural properties of the proximal tibias were determined with Micro-computed tomography (SkyScan 1176, Bruker, Germany), using an isotropic voxel size of 9 μm, and a set of scanning parameters consisted of 55 kV, 400 μA, 200 ms exposure time.

2.13. Statistical analysis

The data obtained were presented as the mean ± SEM. Multiple comparisons were conducted using one-way ANOVA with post hoc Tukey's multiple comparisons test. All data were analyzed with GraphPad Prism 6.0 software. A value of $P < 0.05$ indicated that the difference was statistically significant.

3. Results

3.1. The depressive behavioral assessment of mice

Compared with that in the non-stress mice, the percent of sucrose preference of mice significantly decreased after three months of modeling, indicating the absence of delight hedonia (Fig. 2A) ($F_{4,71} = 92.14$, $p < 0.0001$). The weight gain of mice was also decreased in the all model mice (Fig. 2B) ($F_{4,79} = 4.729$, $p = 0.0018$). The TST and FST are extensively used for the evaluation of mouse behavioral
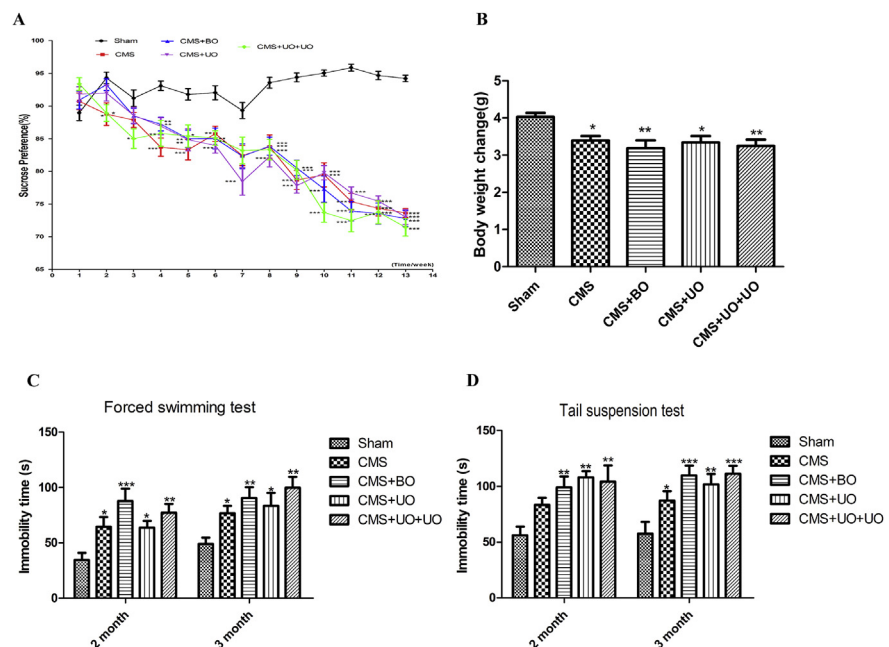

Fig. 2. The depressive behavioral assessment of mice. (A) SPT and (B) body weight changes of each group of mice. The immobility time of female mice in (C) FST and (D) TST. Data are presented as means ± SEMs, n = 20 for the behavior test. *p < 0.05 vs. Sham, **p < 0.01 vs. Sham, ***p < 0.001 vs. Sham.

despair and fear of new environments. After modeling stress by ovariectomy and CMS for 12 weeks, the immobility time of mice that were exposed to stress significantly increased in FST (Fig. 2C) ($F_{4,77}$ = 5.634, p = 0.0005) and TST (Fig. 2D) ($F_{4,82}$ = 6.252,p = 0.0002) compared with that in the sham group. These behavioral changes reveal that, ovariectomy plus CMS induce a higher degree of anhedonia and desperation in mice. This data show that two-step ovariectomy plus CMS could result in the depression-like behavior in female mice.

In addition, we tested the swimming speed of mice in the Water Morris before stress. There was no difference in the athletic ability of mice among the five groups, indicating that the depression-like behaviors of mice were not influenced by locomotor effect (data not shown).

3.2. The levels of corticosterone (CORT) in the serum and the mRNA expressions, of pro-inflammatory factors in the hypothalamus of mice

Inflammatory processes participate extensively in the etiology of menopausal depression. As shown in Fig. 3A, the levels of CORT ($F_{4, 41}$ = 6.031, p = 0.0007) in serum were gradually elevated during the modeling period. The levels of proinflammatory cytokines including IL-1β ($F_{4,11}$ = 11.68,p = 0.0009), IL-6
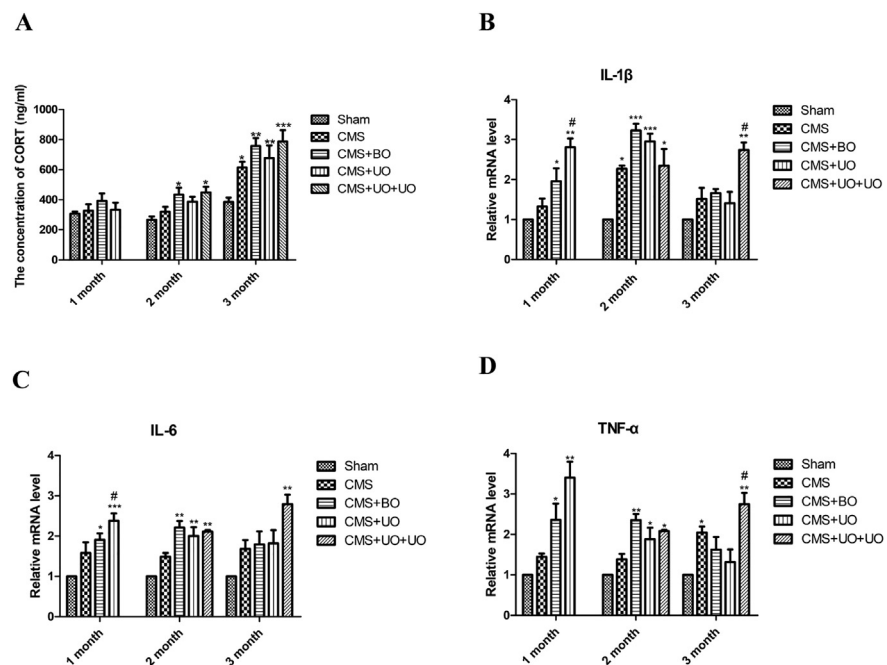
Fig. 3. The levels of corticosterone (CORT) in the serum and the mRNA expressions, of proinflammatory factors in the hypothalamus of mice (A) ELISA analysis of the concentration of CORT. The mRNA level of proinflammatory cytokines IL-1β(B), IL-6(C), and TNF-α(D)were measured by qRT-PCR. Data are presented as means ± SEMs, n = 4–8, *p < 0.05 vs. Sham, #p < 0.05 vs. CMS + BO,**p < 0.01 vs. Sham, ***p < 0.001 vs. Sham.

($F_{4,11} = 7.993$, p $= 0.0028$), and TNF-α ($F_{4,11} = 9.430$, p $= 0.0015$)remarkably increased in the menopausal depression groups compared with the sham group (Fig. 3B−D), and there is difference between BO and UO + UO groups. These results reveal that ovariectomy and CMS treatment has a significant effect in female mice induces increases of CORT and chronic inflammation, and the CMS + UO + UO groups have a stable increases in proinflammatory cytokines during the stress.

3.3. The levels of neurotransmitters and their metabolites, the expressions of 5-HT receptors and serotonin transporters (SERT) in the hippocampus of mice

As shown in Fig. 4A, the 5-HIAA/5-HT ($F_{4, 30} = 12.06$, p < 0.0001), DOPAC/DA ($F_{4, 27} = 12.39$, p < 0.0001) and HVA/NE ($F_{4, 30} = 3.869$, p $= 0.0119$) ratio were decreased in CMS + UO groups and CMS + UO + UO groups compared with sham groups. The similar decreases also existed in the contents of Glu ($F_{4, 30} = 4.487$, p $= 0.0058$), GABA ($F_{4, 31} = 8.146$, p $= 0.0001$), Ser($F_{4, 32} = 16.38$, p < 0.0001) and Asp($F_{4, 32} = 8.007$, p $= 0.0001$) of stressed groups in the two groups (Fig. 4A). However, there were no differences in Tau and Gln among all groups. These results indicate that ovariectomy and CMS cause the neurotransmitter metabolic disorders, especially in CMS + UO groups and CMS + UO + UO groups.

5-HT induces its wide range of actions through a myriad of receptors, the expression and function of which has also been heavily studied in an attempt to unravel the pathophysiology of menopausal depression. Ovariectomy-induced alterations in hippocampus expressions of 5-HT receptors and SERT were shown in Fig. 4B. 5-HT$_{1A}$R ($F_{4, 14} = 1.338$, p $= 0.3045$) mRNA expressions in hippocampus of model group didn't change among all the groups. The mRNA expressions of 5-HT$_{2A}$R ($F_{4, 14} = 5.911$, p $= 0.0073$) and 5-HT$_{2C}$R ($F_{4, 15} = 4.376$, p $= 0.0153$) in hippocampus of CMS + BO groups and CMS + UO + UO groups were increased remarkably in comparison with those in other groups. However, ovarian resection up-regulated the hippocampal mRNA levels of 5-HT$_{1B}$R ($F_{4, 14} = 1.121$, p $= 0.3860$) and SERT ($F_{4, 14} = 6.041$, p $= 0.0080$) in the CMS + BO groups, but down-regulated 5-HT$_{1B}$R and SERT mRNA in the CMS + UO + UO groups. These data reveal that CMS + UO + UO groups had better similarities to the expressions of 5-HT receptors and SERT in peri/postmenopausal condition.

3.4. The levels of E2 and BDNF in the serum, and the protein expressions of ERβ in the hippocampus of mice

After ovariectomy surgery, the serum levels of E2 ($F_{4, 39} = 7.673$, p $= 0.0001$) in CMS + BO and CMS + UO + UO groups were stable and sustained lower compared to other groups (Fig. 5A), and the low E2 levels remained 3 months after ovariectomy. We also determined the expressions of ERβ, and found that
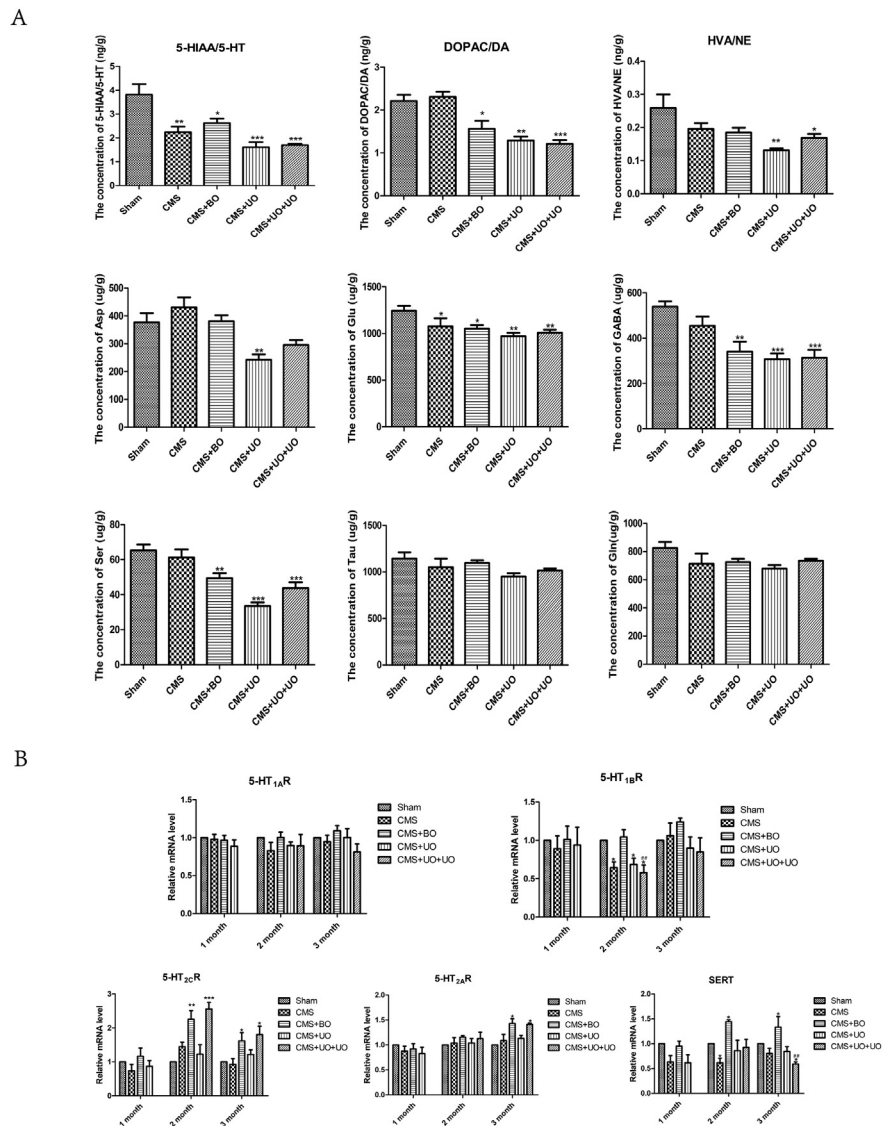
Fig. 4. The levels of neurotransmitters and their metabolites, the expressions of 5-HT receptors and serotonin transporters (SERT) in the hippocampus of mice. The ratio of 5-HIAA/5-HT, DOPAC/DAandHVA/NE, and amino acid neurotransmitter Asp, Glu, GABA, Ser, Tauand Glnin hippocampus were analyzed by high performance liquid chromatography (A). The mRNA expressions of $5\text{-}HT_{1A}R$, $5\text{-}HT_{1B}R$, $5\text{-}HT_{2A}R$, $5\text{-}HT_{2C}R$and SERTwere detected by qRT-PCR(B). 5-HT,5-hydroxytryptamine; 5-HIAA, 5-OH-IH-Indole-3-acetic acid; NE, noradrenaline; DOPAC, 3,4-Dihydroxyphenylacetic; HVA, Homovanillic acid; DA, dopamine; Asp, aspartic acid; Glu, glutamic acid; GABA, gamma-aminobutyric acid; Ser, serine; Tau, tautine; Gln,glutamine. Data are presented as means $\pm$ SEMs, n = 4–8, *p < 0.05 vs. Sham, **p < 0.01 vs. Sham,##p < 0.05 vs. CMS + BO, ***p < 0.001 vs. Sham.

ovariectomy led to significant changes in ERβ ($F_{4, 11} = 8.303$, p = 0.0032) expression in hippocampus (Fig. 5C). Moreover, the protein expression of ERβ in the CMS + UO + UO groups displayed a more stable downtrend. As shown in Fig. 5B, stressed for three months significantly decreased the levels of BDNF ($F_{4, 34} = 7.249$, p = 0.0002), especially in the CMS + UO + UO groups. The
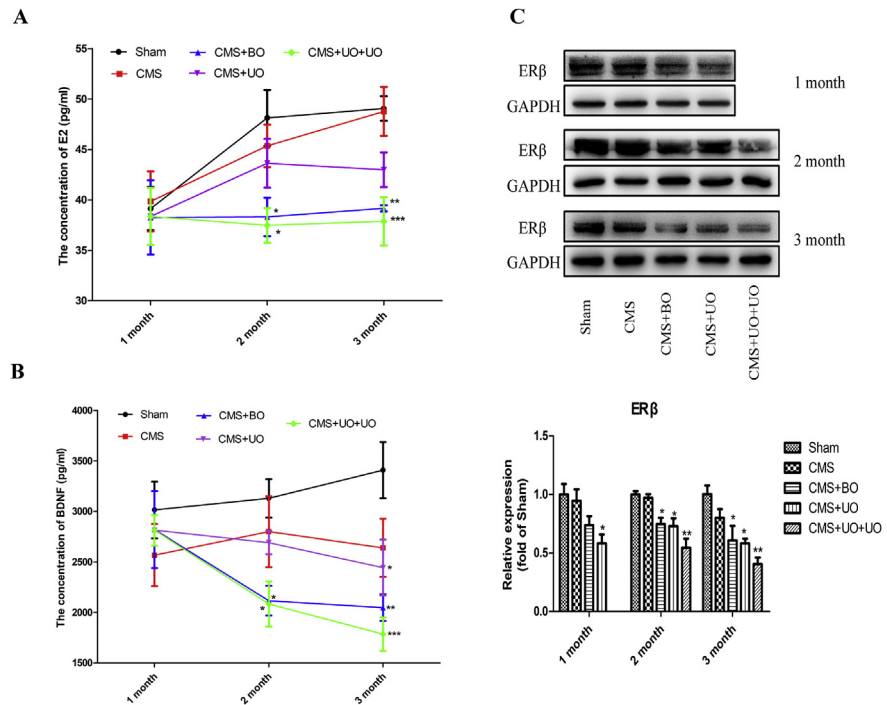
Fig. 5. The levels of E2 and BDNF in the serum, and the protein expressions of ERβ in the hippocampus of mice. The concentration of E2(A) and BDNF(B)in serum was measured by ELISA. The hippocampal ERβ protein expression which were detected by Western blotting using GAPDH as the loading control(C). Data are presented as means ± SEMs, n = 4−8, *p < 0.05 vs. Sham, **p < 0.01 vs. Sham, ***p < 0.001 vs. Sham.

alterations of E2, ERβ and BDNF indicate that CMS + UO + UO group mimics theperi/postmenopausal women's clinical symptoms better than CMS + BO group.

3.5. Determination of tibiae osteoporosis and uterine atrophy in mice

Lower levels of estrogen following ovariectomy can seriously impair both bone mass and architecture, and uterine atrophy is another commonly observed phenomenon. As shown in Fig. 6A, morphologic changes of proximal tibiae were determined with micro-CT. BMD ($F_{4, 27}$ = 5.230,p = 0.0030)indicated the bone loss in ovariectomy group, and ovariectomy also altered proximal tibiae architecture which showed that BV/TV($F_{4, 28}$ = 3.377, p = 0.00224) decreased when compared with the Sham. The proximal tibiae of ovariectomy mice presented significantly lower bone mass and fewer trabeculas than that of the sham mice, which indicating that the ovariectomy-induced osteoporosis in female mice. Simultaneously, we observed the gross morphological changes of uterine after stress (Fig. 6B). We noticed that there is adramatic edema and increased weight in the uterus at one-month-stress. However, we found that ovariectomy resulted in the atrophy and decreased weight of uterus ($F_{4, 55}$ = 6.713, p = 0.0002) at 3 month after surgery.
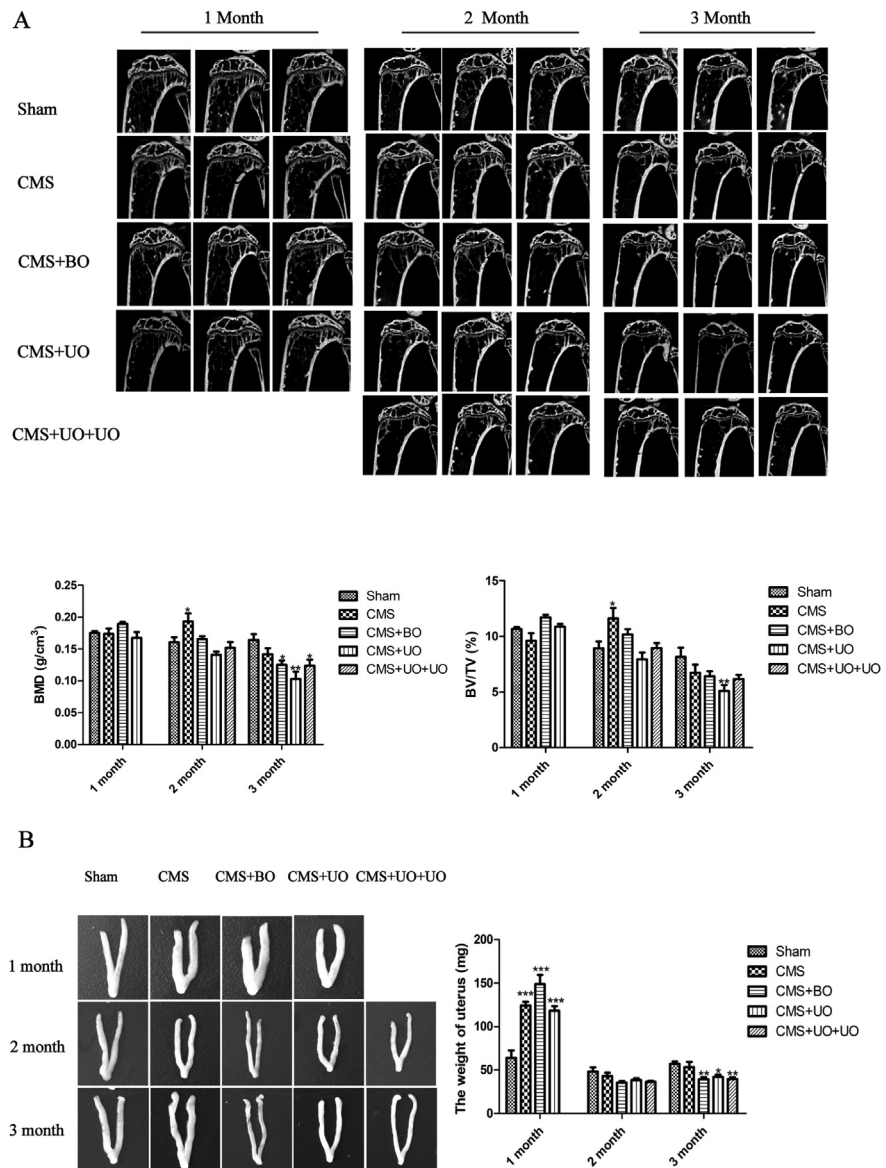
Fig. 6. Determination of tibiae osteoporosis and uterine atrophy in mice. (A) Representative micro-CT images of the tibiae and trabecular morphometric parameters of each group. BMD, bone mineral density; BV/TV, bone volume density. (B) Representative images of uterus of different groups and ovariectomy-induced uterus weight change. Data are presented as means ± SEMs, n = 6−12, *p < 0.05 vs. Sham, **p < 0.01 vs. Sham, ***p < 0.001 vs. Sham.

4. Discussion

The mechanism of menopausal depression is complex and remains incompletely understood. The combined application of CMS and bilateralovariectomy has been demonstrated to cause menopausal depression in female mice and used to study the involved mechanisms. However, this model is limited to mimicking the post-menopausal depression rather than the perimenopausal depression. Therefore, the

present study establish a novel depressive animal model which has more similarities to perimenopausal and postmenopausal depression by two-step ovarietomy plus three-month-CMS.

Several typical tests were always utilized to evaluate depressive behaviors, including FST, TST and SPT. The FST and TST were typical tests utilized to evaluate helplessness or behavioral despair, with longer immobility time indicating greater depressive behaviors [17, 19]. The results of the present study show that ovariectomy mice subjected to CMS exhibited depressive characteristics, including decreases in weight and SPT, and increase in immobility time of FST and TST. Moreover, the immobility time in FST and TST of CMS + UO + UO groups were increased gradually. These results suggested that the two-step ovariectomy plus three-month-CMS could successfully cause the depression-like behaviours in the female mice.

Ovarian failure leads to the dysfunction of hypothalamic−pituitary−adrenocortical (HPA) axis, causes the dysequilibrium of neurotransmitters and as well inflammation in female mice [20]. The abnormal excessive activation of HPA axis by chronic stress results in increased the secretions of CORT, and the production of inflammatory cytokines, such as interleukin (IL-1β, IL-6 and TNF-α) [21]. The levels of CORT in serum and the mRNA expressions of IL-6, IL-1β and TNF-α in CMS + UO + UO groups were continuously elevated, indicating the abnormal excessive activation of HPA axis and the constant inflammatory state in the mouse brain.

The deficiency of 5-HT, NE, and DA in patients with major depressive disorder still is one of the proposed theories regarding the etiology of depression [22]. The levels of monoamine neurotransmitter-metabolites levels have also been altered in the brain by exposure to diverse stressors. The 5-HIAA/5-HT, HVA/NE and DOPAC/DA ratios are reliable index indicating the monoamine neurotransmitter turnover [23]. Ovariectomy plus stress produced an decrease in 5-HIAA/5-HT, HVA/NE and DOPAC/DA ratios especially in CMS + UO and CMS + UO + UO groups, which indicated the imbalance of monoamine neurotransmitters in these groups. In agreement with previous reports, post-partum depression also had the decreased catecholamines turnover in hippocampus [24, 25]. However, there are different changes in the neurotransmitters and their metabolities reported in previous studies [26], which might due to the different protocols used in the stress treatment, including differences in the stressors type, stress duration and the time point of sample collection [23]. We also measured the changes of amino acids neurotransmitters and demonstrated that there was the dysfunction of amino acids in hippocampus. The results showed that Glu, Ser, GABA significantly decreased in hippocampus of menopausal depression mice, compared with that of control group, among which obviously lower levels in the CMS + UO groups and the CMS + UO + UO groups were observed. Gonadal steroids, oestradiol and progesterone have also been shown to have an effect on amino acids, all of which are implicated in the development of

depression [27, 28]. Moreover, 5-HT receptor and SERT can affect the level of GABA in brain [29]. The dysfunction of neurotransmitters indicates the depressive status during the reproductive period.

Serotonin receptors (5-HTR) are part of a complex signaling pathway in the brain and can be divided into 7 different families, each with different subtypes. In the present study, the mRNA expression levels of $5\text{-HT}_{1A}R, 5\text{-HT}_{1B}R$, $5\text{-HT}_{2A}R$, $5\text{-HT}_{2C}R$ in hippocampus were analysed. Several previous studies revealed that ERβ mutant rats demonstrated an significant increase in expression levels of $5\text{-HT}_{2A}R$, but there were no significant changes in the expression of the $5\text{-HT}_{1A}R$ receptor [30]. Our results correspond with previous and clinical findings that have reported an increase in $5\text{-HT}_{2A}R$ expression in the CMS + UO + UO mice [31]. $5\text{-HT}_{2C}R$ is mainly located in choroid plexus, hippocampus and substantia nigra [32]. It is preferentially found on GABAergic interneurons [33]. The expressions of $5\text{-HT}_{2C}R$ were increased in the CMS + BO groups and the CMS + UO + UO groups, which could inhibits serotoninergic neural activity. CMS + BO groups and CMS + UO + UO have opposite trends in SERT and $5\text{-HT}_{1B}R$, and the stability of CMS + BO model has some limitations which contradicts previous reports [34].

Menopausal transition is characterized by irregular cycling coupled with fluctuations in gonadal steroid hormone levels, especially in the levels of E2 [35]. Extensive studies have shown that E2 exerts neuroprotective effects in several neural injury models. It has been demonstrated that E2 is essential for neural development, cognition, synaptogenesis and favors long-term potentiation [36, 37]. Neurotrophic factors including BDNF are important neurotrophins for neurogenesis, neuroprotective and synaptic plasticity [38]. BDNF gene promoter includes an estrogen response element, thus E2 can induces the expression of BDNF in the brain [39]. Moreover, A number of studies have reported decreased BDNF mRNA in ovariectomized animals which was reversed by estradiol treatment administration [11]. In the present study, the E2 levels of the two-step ovariectomized mice were stable and sustained lower which were more similar to that of climacteric women. The fluctuation of E2 level in sham groups might be due to age and physiological cycle factors [10]. Our studies showed that BDNF in serum was significantly decreased in ovariectomized animals, and the CMS + UO + UO groups showed a lower and persistent downward trend compared to that of the CMS + BO groups. Generally, estrogen binds to two primary estrogen receptors, ERα and ERβ, which exerts the majority of its biological effects [40]. ERα and ERβ are differently expressed throughout the body, ERα is mainly expressed in the reproductive system, breast, liver, bone, kidney and liver adipose tissue; ERβ is preferentially expressed in the central nervous system, immune system, urogenital tract, gastrointestinal tract and lungs [41, 42]. There are several studies which indicate that ERβ, but not ERα, plays a major role in mediating the antidepressant effects of estradiol [43, 44]. The present study revealed that the expression of ERβ in hippocampus was significantly

decreased especially in the novel animal model. Our results demonstrate that ERβ plays a key role in menopausal depression and CMS + UO + UO mimicks the feature of depression successfully.

Levels of estrogen are dramatically reduced following ovariectomy which can result in osteoporosis [45]. Bone loss is known to accompany the decrease in estrogen [13, 46]. The results of our study showed that ovariectomy seriously decreased the bone mineral density (BMD), and impaired the bone microarchitecture. The reduction of uterine weight is another commonly phenomenon following ovariectomy operation [47]. Our study also showed that ovariectomy-induced uterine atrophy of mice.

On the whole, the typical animal model results in a drastic decline in hormone levels that only mimicks postmenopausal period, without considering the perimenopausal period condition. Therefore, the results of our study may not exhibit statistically significant differences between CMS + BO groupsand CMS + UO + UO groups. Two-step ovariectomy operation plus chronic mild stress induces a stabilized and gradual transition to peri/postmenopausal depressive status (e.g. FST, IL-6, 5-$HT_{1B}R$, SERT), which has more similarities to clinical menifestation of peri/postmenopausal depression. In addition, the new model may bring more reliability in the study of mechanisms of menopausal depression. Therefore, this novel model could be used as a useful model for simulating peri/postmenopausal status.

5. Conclusions

Taken together, the present study provides a novel animal model for peri/postmenopausal depression. Two-step ovariectomy operation plus CMS induces a stabilized and gradual transition to peri/postmenopausal depressive status, which has more similarities to clinical menifestation of peri/postmenopausal depression. This novel model could be used as a useful model for simulating peri/postmenopausal status and as a new approach for developing new drugs for peri/postmenopausal depression.

Declarations

Author contribution statement

Ling Zhang, Lu-Lu Cao, Dan-dan Yang, Ji-Ye Huang: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Xu-Dong Guo, Teng-Fei Xue, Xiao-Jie Zhao: Performed the experiments.

Jian-Hua Ding: Performed the experiments; Analyzed and interpreted the data.

Xiu-Lan Sun: Conceived and designed the experiments; Wrote the paper.

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The authors declare no conflict of interest.

Additional information

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