Life Sciences 234 (2019) 116751

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Urinary metabolic disturbance in the olfactory bulbectomized rats and the modulatory effects of fluoxetine

Yun-Feng Zhou[a], Li Feng[c], Xin-Min Liu[a,b], Xue Tao[a], Li-Sha Wang[a], Meng-Di Zhang[a], Zhi Wang[a], Shan-Guang Chen[b], Qi Chang[a,*]

[a] Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100193, China
[b] The State Key Laboratory of Space Medicine Fundamentals and Application, China Astronaut Research and Training Center, Beijing 100094, China
[c] The Open University of China, Beijing 100039, China

ARTICLE INFO

Keywords:
Depression
Olfactory bulbecomy
Fluoxetine
Urinary metabonomics

ABSTRACT

Aims: The present study aims to investigate the impacts of olfactory bulbectomy (OBX) on urinary metabolic profile and tryptophan metabolites in prefrontal cortex (PFC) of rats, and to explore the regulation effects of fluoxetine.

Main methods: OBX model was developed by aspiration of olfactory bulbs. After fluoxetine treatment (10 mg/kg) for 14 days, urine samples were collected and behavior tests were applied. Tryptophan (TRP) metabolites and neurotransmitters in PFC were determined by prominence ultrafast liquid chromatography-QTRAP-mass spectrometry, and tryptophan hydroxylase 2 (TPH2) and indoleamine-2,3-dioxygenase 1 (IDO1) were evaluated by western blot. Urinary metabolites were analyzed by ultra-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry-based metabonomics strategy.

Key finding: OBX rats showed hyperlocomotion in open field, hyperactivity in open arm and despair status, and fluoxetine reserved these behavioral abnormalities. The levels of TRP, 5-HIAA, 5-HIAA/5-HT ratio and DA increased, while kynurenine and 5-HT decreased in PFC of OBX rats. The activities of TPH2 and IDO1were inhibited after OBX. Twenty-six altered metabolites were identified as potential biomarkers in OBX rats involved in tryptophan metabolism, gut microbiota metabolism, energy metabolism, purine metabolism, ascorbate and aldarate metabolism, and tyrosine metabolism. Among them, 15 abnormal metabolites were corrected by fluoxetine to some extent.

Significance: Our results revealed that urinary metabolic profile changed greatly in OBX rats, and identified biomarkers might be helpful for the diagnosis of agitated depression. The regulation effects of fluoxetine on urinary metabolic profile and tryptophan metabolites in PFC might contribute to its antidepressant action in OBX rats.

1. Introduction

Depression is a multicausal and recurrent mental disorder, characterized by anhedonia, hopelessness, feeling of guilt and memory deficit [1], which will be the leading cause of morbidity by 2030 [2,3]. Several hypotheses have been proposed to explain the mechanism of depression, including deficiency of monoamine neurotransmitter [4], hyperfunction of hypothalamus-pituitary-adrenal (HPA) axis [5], and neuroinflammation [6]. Unfortunately, none of them can make clear the pathological processes of depression. Therefore, exploring the pathogenesis is indispensable for the clinical treatment of depression.

Olfactory bulbectomy (OBX) causes a series of behavioral alterations in rats such as sexual behavior reduction, hyperlocomotion, despair status, anhedonia and hyeremotionality [7–9]. These abnormal changes are similar to the major symptoms in depressed patients, especially the patients with agitated depression featured by irritability, restlessness and severe suicidal tendency [3,7,10]. The hyperactivity in OBX model is distinguished from chronic unpredictable mild stress (CUMS)-induced model of depression, in which the decreased locomotion suggests psychomotor retardation [11]. With good face, construct and predictive validities, OBX model is suitable for investigating the molecular mechanisms of depression.

Among many kinds of medicines available for depression treatment, fluoxetine is one of the most widely prescribed selective serotonin

* Corresponding author at: Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College, No. 151, Malianwa North Road, Haidian District, Beijing, 100193, China.
E-mail address: qchang@implad.ac.cn (Q. Chang).

https://doi.org/10.1016/j.lfs.2019.116751
Received 4 June 2019; Received in revised form 2 August 2019; Accepted 10 August 2019
Available online 12 August 2019

reuptake inhibitors (SSRIs). Fluoxetine treatment (3–4 weeks) can increase the 5-HT concentration in synaptic cleft and improve depressive symptoms in patients with safety and tolerability [12]. Furthermore, fluoxetine is usually used as positive control in exploring the antidepressant action of candidate compounds in OBX animal model, and chronic administration of fluoxetine (generally 14 days) can reverse the abnormal behavior changes in OBX rodents [13–16]. The deficiency in monoaminergic neurotransmitter plays a key role in the pathology of depression. It has been proved that OBX induced the downregulation of 5-HT and upregulation of 5-HT turnover [17]. 5-HT synthesis in brain depended on the availability of tryptophan (TRP) which was obtained from peripheral circulation through blood brain barrier (BBB) [18]. The two rate-limiting enzymes, tryptophan hydroxylase 2 (TPH2) and indoleamine-2,3-dioxygenase 1 (IDO1), are essential in the production of 5-HT and kynurenine (KYN) from TRP, respectively. Although the breakdown of TRP metabolism has been reported in patients with depression and stress-induced animal models [19,20], the alterations in 5-HT and kynurenine pathways of TRP metabolism in OBX rats are far more clear.

Metabonomics has become a useful tool in exploring the pathophysiology of various nervous system diseases, including Alzheimer's disease, schizophrenia and depression [21–23]. In present study, metabolomics strategy was firstly employed to assess the metabolite changes in the urine of OBX rats and the modulatory effects of fluoxetine based on ultra-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UPLC-Q-TOF/MS). At the same time, TRP metabolites and neurotransmitters in prefrontal cortex (PFC) were determined.

2. Materials and methods

2.1. Chemicals and reagents

Fluoxetine hydrochloride was purchased from Aladdin Biochemical Technology Co. Ltd. (Shanghai, China). TRP, KYN, kynurenic acid (KYNA), 3-hydroxykynurenine (3－HK), 5-hydroxytryptamine (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), dopamine (DA), norepinephrine (NE) were obtained from Sigma-Aldrich (St. Louis, MO, USA). MS-grade acetonitrile (ACN) and formic acid (FA) were provided by Thermo Fisher Scientific Co. Ltd. (Waltham, MA, USA). Anti-TPH2 and anti-IDO1 primary antibodies were obtained from abcam (Cambridge, UK). ACTB monoclonal antibody and HRP goat anti-rabbit IgG secondary antibody was supplied by ABclonol (Wuhan, China). Other reagents in analytical grade were purchased from Beijing Chemical Works (Beijing, China). Ultrapure water was produced by a Milli-Q Ultrapure Water System (Boston, MA, USA).

2.2. Experimental animals

Twenty-four male Sprague-Dawley rats (200 ± 20 g) were commercially obtained from Beijing Huafukang Biotechnology Co. Ltd. (Beijing, China), and maintained in standard laboratory conditions (temperature of 21–25 °C and relative humidity of 50–60%) with a 12/12 light-dark cycle and free access to filtered water and standard food. The experimental procedures were approved by the Animal Ethics Committee of Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College (Approval No. SLXD-20180912006). All the animals were allowed to adapt to the environment for 7 days prior to any experiments.

2.3. OBX surgery

OBX was performed according to the previously described method with slight modifications [10]. Briefly, rats were anesthetized with 2% pentobarbital sodium (50 mg/kg) through intraperitoneal injection (i.p.) and then fixed in a stereotaxic instrument. The skull was exposed by a 1 cm longitudinal incision and iodine was applied as an antiseptic. Two burr holes were drilled on either side of midline (2 mm in diameter, 8 mm anterior to bregma and 2 mm from the midline). Bilateral olfactory bulbs were removed by suction with a blunt hypodermic needle attached to vacuum pump. The holes were filled with absorbable collagen sponge (Xiang'en, Jiangxi, China) to prevent excessive bleeding. Sham rats underwent the similar procedures, but the olfactory bulbs were left intact. After the scalp was sutured, the animals were placed on the heating pad to maintain body temperature until awake. Penicillin sodium (10,000 units, i.p.) was administered for consecutive 3 days to prevent infection. The rats were given 14 days to recover from surgical procedure. During the rehabilitation period, all the rats were housed separately and handled daily to reduce aggressive behavior. Fig. 1 displayed the brains of rats with intact (A) and missing (B) olfactory bulbs.

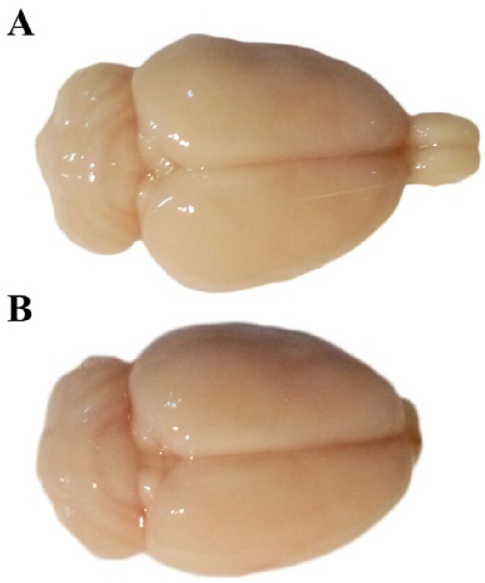

Fig. 1. The brains of rats with intact (A) and missing (B) olfactory bulbs.

2.4. Treatment schedule

On 15th day after surgery, the rats subjected to OBX were randomly divided into OBX group and OBX + Flu group. The rats in both sham and OBX groups were treated with normal saline (NS, p.o.) and the rats in OBX + Flu group were administered with fluoxetine per os (10 mg/kg dissolved in NS, p.o.). The dose and mode of administration of fluoxetine were based on the previous report [24]. The whole experimental procedure including surgery, drug treatment and behavioral tests was exhibited in Fig. 2.

2.5. Urine collection, correction and preparation

The urine collection was performed from the 28th to 30th day post-surgery to avoid the disturbance derived from behavioral tests. All the rats were housed in stainless steel metabolic cages to adapt for 12 h prior to urine collection. Samples of 24-hour urine were collected in clean tubes on ice. One milliliter of 1% sodium azide (w/v) was added to the sampling tube to protect urine from bacterial contamination. After centrifugation (12,000*g* for 15 min, 4 °C), the supernatants were stored at −80 °C until analysis.

Urine samples were normalized by creatinine calibration, to eliminate the individual difference in the volume of urine among rats [25]. The creatinine level was quantified by high performance liquid chromatograph with UV-detector (Waters Corporation, Milford, MA, USA)

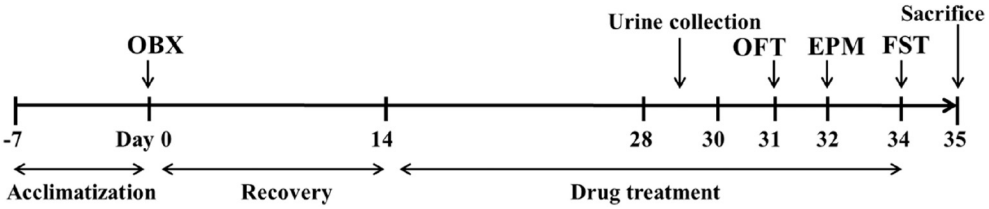

Fig. 2. Schematic representation of the experimental procedure in rats exposed to OBX and fluoxetine treatment. EPM: elevated plus maze test; FST: forced swimming test; OBX: olfactory bulbectomy; OFT: open field test.

at the wavelength of 234 nm. All the urine samples were diluted with ultrapure water to achieve the equal concentration of urine creatinine. Diluted urine samples were centrifuged at 12,000*g* for 15 min at 4 °C, and an aliquot of 5 μL supernatant was injected for UPLC-Q-TOF/MS analysis in both positive and negative modes. A total of 100 μL urine sample from each rat was mixed together to obtain the quality control (QC) sample which was used for method validation.

2.6. Behavioral tests

2.6.1. Open field test (OFT)

All the rats were subjected to OFT to evaluate the locomotor and exploratory activity. The apparatus consisted of a black round area with a diameter of 80 cm, surrounded with 50 cm-high black wall. Each rat was gently put into the center of open field and allowed to explore for 5 min. The total movement distance during the test period was recorded by the video, and the number of fecal pellets was counted. After each test, the apparatus was cleaned with 10% alcohol to eliminate residual odor.

2.6.2. Elevated plus maze (EPM) test

The EPM test was carried out adapted to the previous method [26] with minor modifications. The EPM apparatus comprised of a plus-shaped maze, elevated 60 cm above the floor, with two opposed open arms (43 × 15 cm) and two opposed enclosed arms (43 × 15 × 23 cm). The four arms were joined by a central square platform (15 × 15 cm). Rats were placed into the central platform of the maze facing an open arm at the beginning of each test. During 5-min test period, the number of entries into and time spent on the open and enclosed arms, and movement distance were recorded by video. The 10% alcohol was sprayed to clean the apparatus after each test.

2.6.3. Forced swimming test (FST)

The FST test was conducted in accordance with the method described previously [27]. In brief, on the day before test, the rats were put into a glass cylinder (20 cm in diameter and 50 cm in height), containing 30 cm of clean water (25 ± 1 °C), for 15-min pretest to eliminate the acute stress by water. Twenty-four hours after the pretest, 5-min swimming session was carried out. Total immobility time was recorded by a trained observer who was blind to the experimental conditions. The immobility was defined as the status when the rats only made the subtle necessary movements to keep their heads above the water. After each test, the water was replaced and the rats were carefully dried with clean blanket before returning to home cages.

2.7. Quantitative analysis of TRP metabolites and neurotransmitters in PFC and TRP in serum

On the 35th day after surgery, the rats were anesthetized with 2% pentobarbital sodium (50 mg/kg, i.p.). The blood samples were collected through abdominal aorta and placed at 4 °C for clotting. After centrifugation at 3500 rpm for 15 min at 4 °C, the serum was obtained from the supernatant and stored at −80 °C until analysis. After blood collection, the rats were transcardially perfused with 100 mL NS. The brains of rats were removed on ice, and then PFC was isolated, put into liquid nitrogen immediately, and stored at −80 °C until assay. One side of the PFC was used for LC/MS detection and the other side was applied for western blot analysis.

TRP metabolites and neurotransmitters in PFC including TRP, KYN, KYNA, 3-HK, 5-HT, 5-HIAA, DA and NE, and TRP in serum were analyzed by prominence ultrafast liquid chromatography (UFLC) (Shimadzu, Kyoto, Japan) coupled with a QTRAP 5500 mass spectrometer (AB SCIEX, Framingham, MA, USA). The Restek Ultra Aqueous C18 column (100 mm × 2.1 mm, 3 μm, Bellefonte, PA, USA) was applied in UFLC system for the metabolites separation and 1 μL of prepared sample was injected for the analysis. The preparation procedures of PFC and serum samples, and the detection conditions of chromatographic and mass spectrometry were in according to the previous method reported by our laboratory [28].

2.8. Western blot analysis

The PFC was weighted and homogenized in tissue lysate solution containing 1% protease and phosphatase inhibitor cocktails (CWBIO, Jiangsu, China) with 10 volumes. After centrifugation at 12,000 rpm for 15 min at 4 °C, the supernatant was used for protein quantification by BCA protein assay kit (Solarbio, Beijing, China). A total of 50 μg protein was separated in 10% SDS-PAGE gels and transferred onto nitrocellulose (NC) membrane (Millipore, MA, USA). Subsequently, the NC membrane was blocked with 5% skim milk at room temperature for 1 h, and then incubated with specific primary antibodies overnight at 4 °C. The membrane was washed three times with washing buffer (1 × TBS containing 0.2% Tween-20, TBST) and incubated with HRP-conjugated anti-rabbit IgG secondary antibodies (1:5000) for 2 h at room temperature. Following three washings in TBST, the protein signal was visualized by eECL kit (CWBIO, Jiangsu, China) in accordance with the manufacturer's instructions. The grey intensity values of protein bands were measured by Image J 1.46r software.

2.9. UPLC-Q-TOF/MS analysis of urine samples

The UPLC-Q-TOF/MS analysis was carried out using a Waters Acquity™ UPLC system (Waters Corporation, Milford, MA, USA) coupled with a Q-TOF analyzer in SYNAPT HDMS system (Waters Corporation, Milford, MA, USA). The UPLC system was equipped with a HSS T3 column (2.1 mm × 100 mm, 1.8 μm) with column temperature of 40 °C. The mobile phases were composed of 0.1% FA in water (A) and 0.1% FA in ACN (B). The solvent gradient program was employed as follows: 1% B for 0–1 min, 1%–10% B for 1–7 min, 10%–50% B for 7–15 min, 50%–100% B for 15–16 min, 100% B for 16–17 min, 1% B for 18–20 min. The flow rate was 0.4 mL/min and the samples were kept at 4 °C during the analysis.

The electrospray ionization source was used for MS analysis, and the optimized mass parameters were set as follows: the source temperature was set at 100 °C with a cone gas rate of 50 L/h and a cone voltage of 40 V. Desolvation gas rate was 600 L/h at a temperature of 400 °C. The capillary voltage was set as 3.0 kV for positive ion mode and 2.5 kV for negative ion mode. Centroid data were collected from *m/z* 50 to 1200 and leucine-enkephalin (2 ng/μL) was used as the lock mass (*m/z* 556.2771 and 554.2615 in positive and negative modes, respectively).

2.10. Method validation

QC samples were analyzed to evaluate the method repeatability, instrumental precision and sample stability. The precision of the instrument was measured by 6 replicated injections from the same QC sample, and the method repeatability was assessed using 6 replicates of the QC sample. The sample stability was evaluated by injecting the same QC sample (maintaining in 4 °C) at 0 h, 8 h, 16 h and 24 h after preparation.

2.11. Data preprocessing and multivariate statistical analysis

The raw MS spectra were recorded by MarkerLynx Applications Manager package of MassLynx software version 4.1 (Waters Corporation, Milford, MA, USA). The raw data set was converted to CDF format files by DataBridge software package. Open-source R-language XCMS (version 3.4.1) and CAMERA (version 1.38.0) were used for peak extraction and matching, nonlinear retention time alignment and peak annotation [29]. After optimization, the parameters of XCMS and CAMERA were set as follows: profmethod, binlin; method, centWave; ppm, 10; peakwidth, 1–15 s; noise threshold, 8; mzdiff, 0.025 amu; minfrac, 0.3. Three-dimensional matrix was exported by XCMS into Microsoft Excel, and then the matrix involving sample names (observations), peak index (accurate *m/z* and retention time pairs) and normalized peak intensities was introduced into SIMCA-P 13.0 software (Umetrics, Umea, Sweden). Mean-centering and Pareto-scaling were performed prior to multivariate statistical analysis. Unsupervised principal component analysis (PCA) can display an overview of the general clustering, trends and potential outliers among the observations. Supervised partial least squares discriminant analysis (PLS-DA) was carried out to maximize the separation of sample classes and identify the potential biomarkers. To test the validity of the model, 200-iteration permutation test was performed to estimate the fitting degree of PLS-DA model [30]. The fragment, isotope and adduct ions were excluded according to the annotation information supplied by CAMERA.

2.12. Statistical analysis

The data in this study was expressed mean ± standard error of the mean (SEM). A two-tailed *t*-test was performed to find the discrepant metabolites between groups. One-way analysis of variance (ANOVA) followed by post hoc Tukey's test were used to evaluate the results from behavioral tests and UFLC-MS by SPSS Statistics 18.0 software (SPSS Inc., Chicago, IL, USA). Statistical significance was defined as $p < 0.05$.

3. Results

3.1. Behavioral assessments

In comparison to sham group, the OBX rats showed a characteristic hyperactivity in OFT, displayed by the increased movement distance ($p < 0.01$) (Fig. 3A) and the number of fecal pellets ($p < 0.05$) (Fig. 3B). Compared to OBX group, fluoxetine treatment (10 mg/kg, p.o.) decreased the locomotor activity in open field during 5-min test session ($p < 0.05$), but fluoxetine didn't show obvious effect on the number of fecal pellets counted after OFT. In EPM test, OBX rats exhibited significant increase in the percent of open arm entries ($p < 0.05$) (Fig. 4A) and the time spent in open arm ($p < 0.05$) (Fig. 4B), compared to sham group. The total locomotor activity of OBX rats in EPM had a trend of increase (Fig. 4C), but with no significance. Fluoxetine treatment (10 mg/kg, p.o.) significantly reduced the open arm entry ratio ($p < 0.01$) and the movement distance ($p < 0.05$) in OBX rats. In addition, apparently increased immobility time ($p < 0.01$) in FST was observed in rats after OBX (Fig. 4D), compared to sham group. Fluoxetine treatment (10 mg/kg, p.o.) decreased the immobility time ($p < 0.01$) in rats exposed to OBX.

3.2. The levels of TRP metabolites and neurotransmitters in PFC

The contents of 8 neuroactive substances in the PFC of rats were determined (Fig. 5) in the present study. The levels of TRP, 5-HIAA, 5-HIAA/5-HT ratio and DA in the PFC of OBX rats were markedly increased ($p < 0.05$ or $p < 0.01$), while the levels of KYN and 5-HT in OBX rats were significantly decreased ($p < 0.05$), compared to the sham group. The concentrations of KYNA, 3-HK and NE in PFC were not influenced by the removal of olfactory bulbs. The downregulation of KYN and 5-HT suggested the TRP metabolism was blocked in the PFC of OBX rats. After administration with fluoxetine, the levels of KYN, 5-HT, 5-HIAA and 5-HIAA/5-HT ratio were adjusted to normal level ($p < 0.05$ or $p < 0.01$), while the level of TRP was not affected by fluoxetine. These results indicated that fluoxetine partly regulated the levels of TRP metabolites and neurotransmitters in the PFC of OBX rats.

3.3. The levels of TRP in serum and the expression of TPH2 and IDO1 in PFC of rats

Serum TRP level and the enzymes in PFC related to TRP metabolism were analyzed to verify the changes in TRP metabolites in PFC. As shown in Fig. 6A, OBX induced significant elevation in serum TRP concentration ($p < 0.05$), compared to sham group. The expressions of IDO1 and TPH2 were remarkable decreased in the PFC of OBX rats ($p < 0.01$). Fluoxetine treatment increased the level of IDO1 ($p < 0.05$), but failed to reverse the abnormalities in serum TRP level and TPH2 expression in PFC.

3.4. UPLC-Q-TOF/MS method validation

The representative based peak intensity chromatograms of QC samples in positive and negative modes were illustrated in Fig. S1. To evaluate the method repeatability, instrumental precision and sample stability, 12 ion chromatographic peaks in the QC samples were extracted. The information of these ions (retention time_*m/z*) was 0.74_114.0658, 0.92_212.1031, 2.87_136.0392, 7.72_105.0332, 8.84_91.0542 and 10.28_473.2285 in positive mode and 1.26_111.0097, 7.74_178.0485, 8.85_74.0257, 9.56_283.0787, 11.06_201.0234 and 11.97_240.9756 in negative mode. The relative standard deviations (RSD, %) of retention times and peak areas were 0.00–0.36% and 1.54–8.71% for method repeatability, 0.02–0.36% and 2.28–9.61% for instrumental precision, and 0.00–0.62% and 2.38–10.66% for sample stability at 4 °C, respectively. The detailed RSD values of selected ions were shown in Table S1. These results suggested that the analytical method was satisfactory for chromatographic separation and MS detection of urine samples.

3.5. Metabolic profiles analysis

Both unsupervised (PCA) and supervised (PLS-DA) multivariate data analysis procedures were carried out to found the potential biomarkers. The score plots of PCA in positive and negative modes were shown in Fig. 7. Although there was some overlap, the separation trend among the three groups was obvious. The separation of sham group from OBX group suggested that the perturbation in urinary metabolites occurred in OBX rats. And OBX + Flu group was separated from OBX group, indicating the antidepressant effect of fluoxetine.

PLS-DA can distinguish the difference among the groups and confirm the important variables. As shown in Fig. 8, sham group, OBX group and OBX + Flu group were separated clearly in PLS-DA score plots in both positive (Fig. 8A) and negative (Fig. 8C) modes, indicating that urinary metabolic profile has been altered notably after OBX and fluoxetine treatment. R2Y and Q2 represented the cumulative

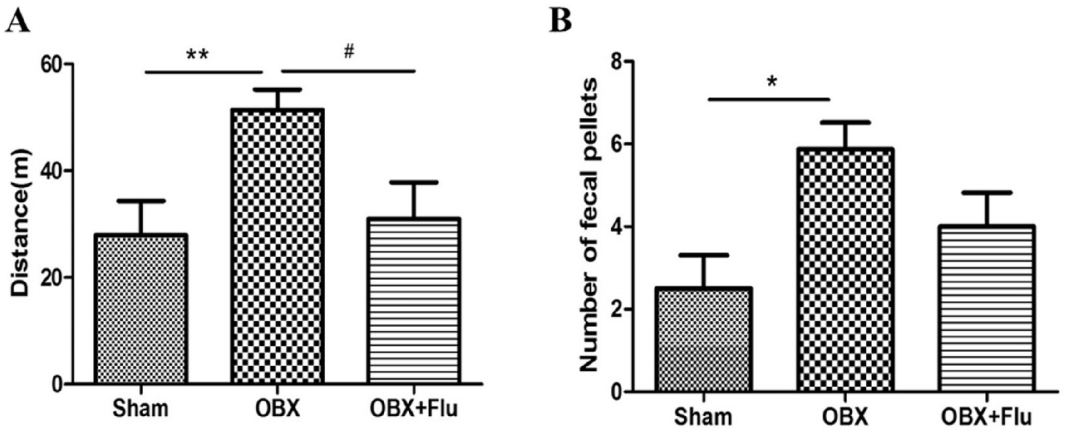

Fig. 3. The effects of OBX and fluoxetine treatment (10 mg/kg, p.o.) on the locomotor activity of rats in open field test. (A) Movement distance in 5 min. (B) The number of fecal pellets. $^{*}p < 0.05$, $^{**}p < 0.01$ compared to sham group. $^{\#}p < 0.05$ compared to OBX group. n = 8 in each group. Data are mean ± SEM. OBX: olfactory bulbectomy; Flu: fluoxetine.

explanatory and predictive power of the current model, respectively, and were calculated in PLS-DA analysis. The parameters were R2Y = 0.845, Q2 = 0.638 in positive mode and R2Y = 0.818, Q2 = 0.61 in negative mode, which implied that the model had high fitness ability and predictability. The intercepts of R2 and Q2 of 200 permutations were 0.502 and −0.273 in positive mode (Fig. 8B), and 0.569 and −0.271 in negative mode (Fig. 8D), suggesting that present models were reliable. The variable importance in the projection (VIP) value reflects the contribution of the variables to PLS-DA model. Only the metabolites with VIP > 1 in PLS-DA and $p < 0.05$ in *t*-test were screened out as potential biomarkers for further identification.

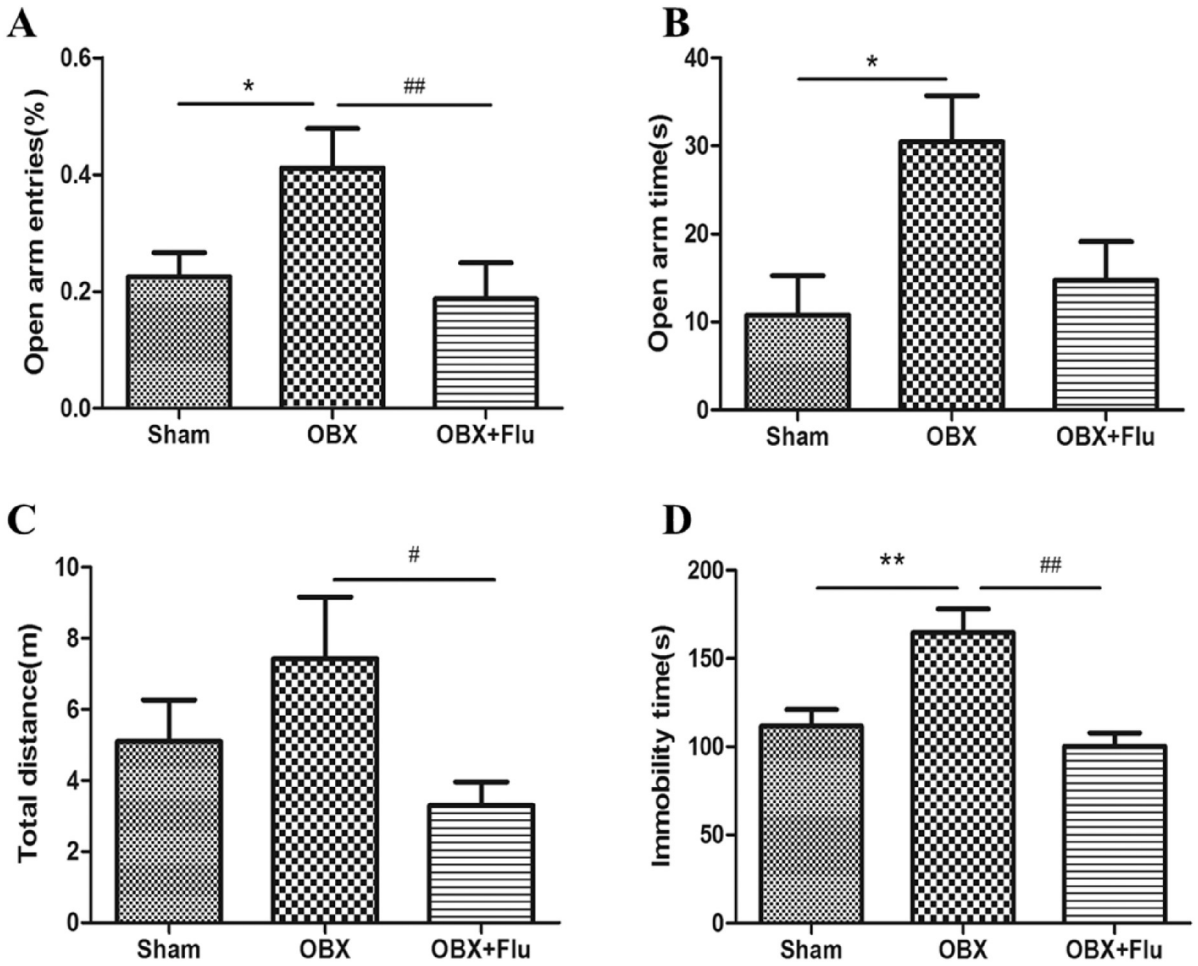

Fig. 4. The effects of OBX and fluoxetine treatment (10 mg/kg, p.o.) on the performance of rats in EPM test and FST. (A) The percent of open arm entries. (B) The time spent in open arm. (C) Movement distance in EPM. (D) The immobility time in FST. $^{*}p < 0.05$, $^{**}p < 0.01$ compared to sham group. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared to OBX group. n = 8 in each group. Data are mean ± SEM. OBX: olfactory bulbectomy; Flu: fluoxetine.

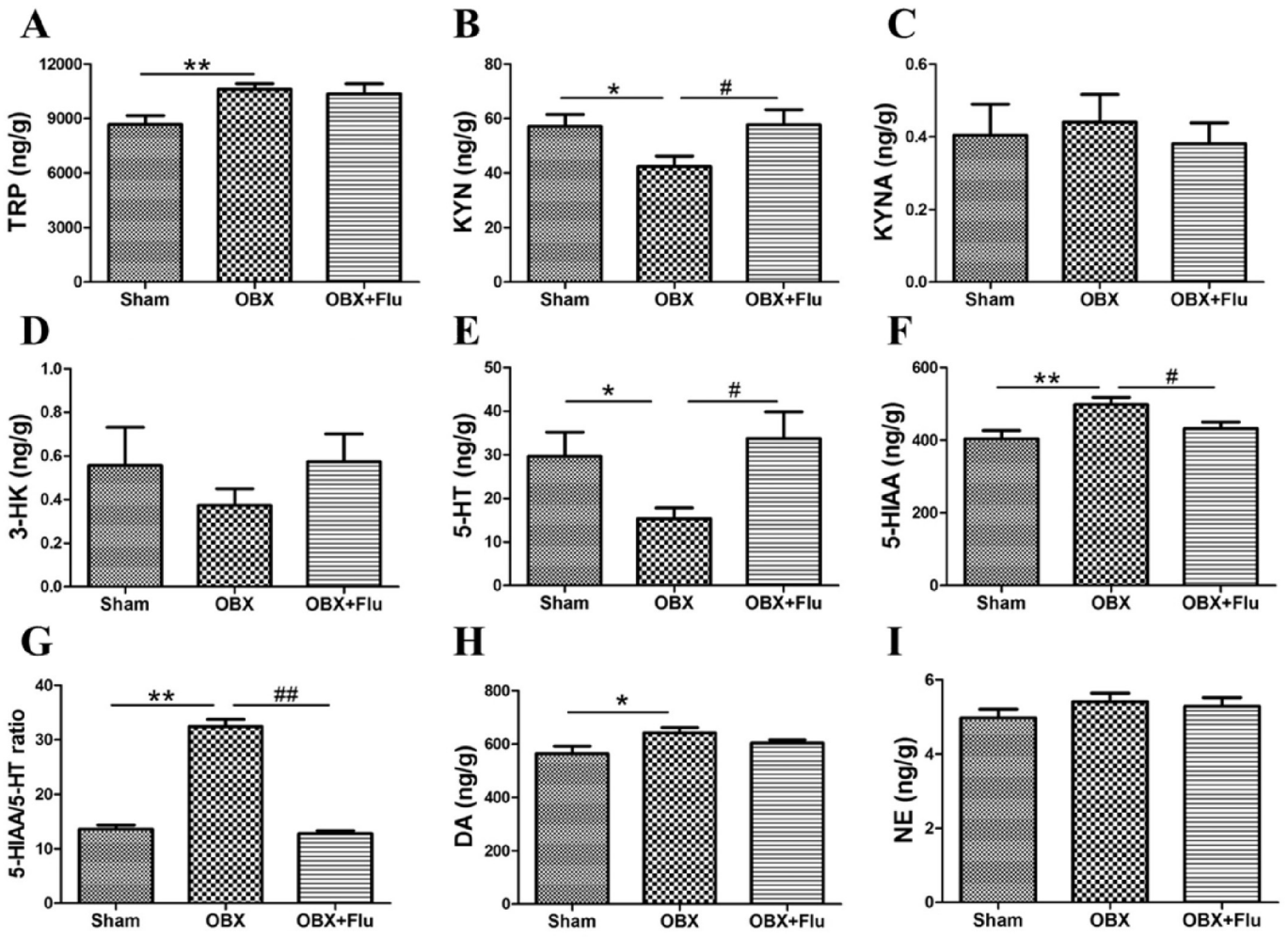

Fig. 5. The effects of OBX and fluoxetine treatment (10 mg/kg, p.o.) on levels of TRP metabolites and neurotransmitters in PFC of rats. (A) TRP levels. (B) KYN levels. (C) KYNA levels. (D) 3-HK levels. (E) 5-HT levels. (F) 5-HIAA levels. (G) 5-HIAA/5-HT ratio. (H) DA levels. (I) NE levels. $^{*}p < 0.05$, $^{**}p < 0.01$ compared to sham group. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared to OBX group. n = 8 in each group. Data are mean ± SEM. OBX: olfactory bulbectomy; Flu: fluoxetine.

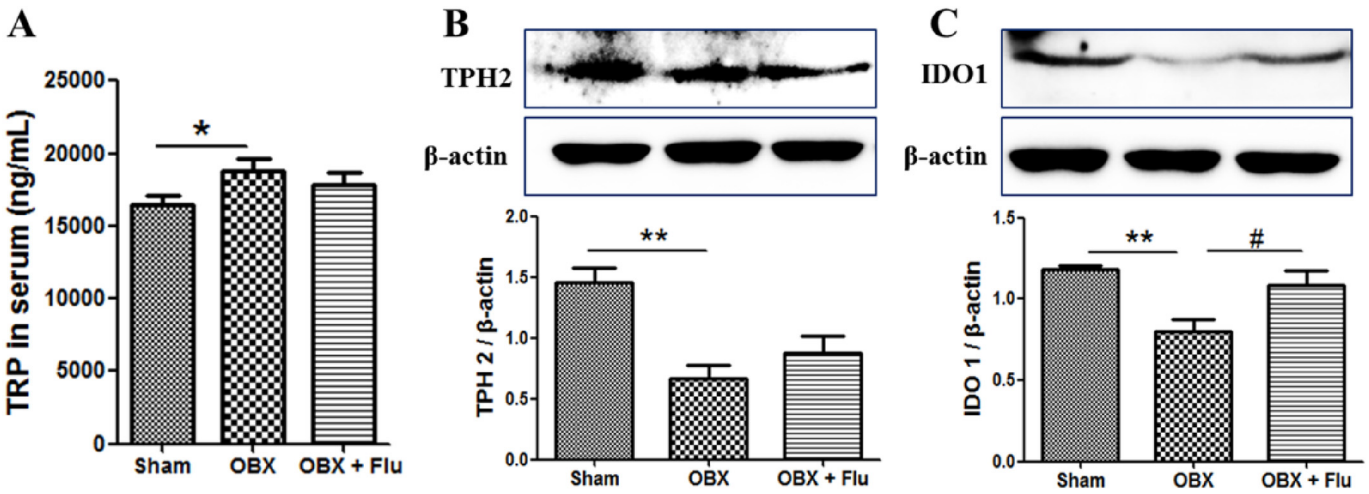

Fig. 6. The effects of OBX and fluoxetine treatment (10 mg/kg, p.o.) on the level of TRP in serum and enzymes related to TRP metabolism in PFC of rats. (A) TRP levels in serum. (B) The expression of TPH2 in PFC. (C) The expression of IDO1 in PFC. $^{*}p < 0.05$, $^{**}p < 0.01$ compared to sham group. $^{\#}p < 0.05$ compared to OBX group. n = 8 for serum TRP measurement, and n = 5 for western blot analysis. Data are mean ± SEM. OBX: olfactory bulbectomy; Flu: fluoxetine.

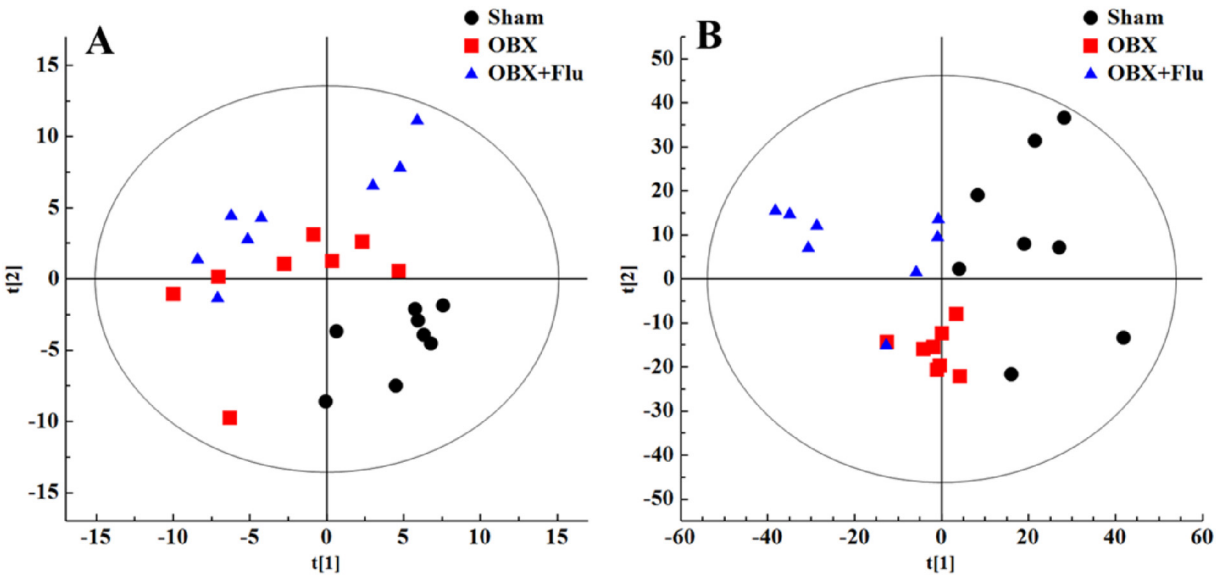

Fig. 7. PCA score plots of urine samples from sham (black circle), OBX (red box) and OBX + Flu groups (blue triangle) assayed by UPLC-Q-TOF/MS in positive (A) and negative (B) modes, respectively. n = 8 in each group. OBX: olfactory bulbectomy; Flu: fluoxetine. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.6. Identification of potential biomarkers

Identification of metabolites was achieved through a mass-based search. Firstly, the accurate mass value of a metabolite was submitted to the online databases including Human Metabolome Database (HMDB, http://www.hmdb.ca/) and MassBank database (http://www.massbank.jp/). Then supposed metabolites acquired by database were verified by comparing the MS/MS fragment ions with the raw data. Here, a potential biomarker with *m/z* 220.1186 at 4.87 min in positive mode was taken as an example to elucidate the identification process. Possible candidate metabolites were acquired by searching the precise *m/z* 220.1186 (positive mode, molecular weight (MW) tolerance: ± 10 ppm, adduct type: [M + H]) in HMDB. Two candidates were obtained with molecular formula of $C_9H_{17}NO_5$ and $C_{10}H_{13}N_5O$ for pantothenic acid and cis-zeatin, separately, and their MW tolerances were both 3 ppm. The fragment ions of the two compounds supplied by HMDB were matched with these obtained by extracting the ion *m/z* 220.1186 at 4.87 min in raw data. There were perfect matching between the metabolite (retention time_*m/z*, 4.87_220.1186) and pantothenic acid, but not cis-zeatin, in the ion fragment at *m/z* 202.1094, 184.0990, 124.0733, 116.0353 and 90.0563. Therefore, the metabolite was speculated as pantothenic acid, whose MS/MS spectrums in HMDB and raw data of urine sample were shown in Fig. S2. With the similar procedures, other biomarkers were identified.

Based on the criterions of VIP > 1 and $p < 0.05$, a total of 26 metabolites were identified as urinary biomarkers for OBX-induced depression in both positive and negative modes (Table 1). It is worth mentioning that xanthurenic acid and uric acid were detected simultaneously in the two modes. Most of the verified biomarkers were downregulated in the urine of OBX rats, expect for 4,6-dihydroxyquinoline and leucine. Fluoxetine treatment could correct 15 abnormal metabolites in the urine of OBX rats to some extent, but 8 metabolites were even worsened by fluoxetine treatment. HemI software (version 1.0.3.3) [31] was used to transform the levels of urine biomarkers into visualized heat map (Fig. 9). The perturbed metabolic pathways associated to candidate biomarkers were recognized by searching KEGG Pathway Database (https://www.kegg.jp/kegg/pathway.html), containing TRP metabolism, phenylalanine metabolism, tyrosine metabolism, purine metabolism, tricarboxylic acid cycle (TCA cycle), ascorbate and aldarate metabolism, and so on (listed in Table 1). The correlation network of altered urinary metabolites in OBX-induced rat model of depression was exhibited in Fig. 10.

4. Discussion

OBX is regarded as an animal model of agitated depression, and the patients with agitated depression have a high risk of suicide [3]. The hyperlocomotion of OBX rats in open field reflected the psychomotor agitation and was the most prominent characteristic of this model [32]. The locomotor hyperactivity can be interpreted as the lack of integration of sensory inputs (the disinhibition of amygdala) [33] and deficiency in defensive behavior in OBX rats when exposed to unfamiliar environment [34]. Fluoxetine significantly reversed OBX-induced hyperactivity in open field, indicating that fluoxetine could enhance open-field habituation in OBX rats [35]. Rodents prefer to spend less time in open arms than closed arms in EPM test, which can be reversed by anti-anxiety medications [26]. Interestingly, OBX rats exhibited inverse performance displayed by increased open arm entry ratio, open arm time and total distance in EPM test. The increased open arm activity in OBX rats might be attributed to novelty-induced hyperlocomotion, which was consistent with the hyperactivity in open field [36]. In addition, increased immobility time in FST reflects the aggravated despair-like behavior in OBX rats. These behavioral deficits in OBX rats and the reverse effects of fluoxetine indicate that OBX model is established successfully in our study.

An abundance of evidence from neuroanatomy, neuroimaging and brain stimulation demonstrated that PFC was a critical neural substrate in depression [37]. The disorders in TRP metabolism and neurotransmitters have been reported in depressed patients and animals [3,38]. The syntheses of KYN and 5-HT from TRP in brain were mainly depended on the catalysis of IDO1 and THP2, respectively. TRP was transformed from peripheral circulation to brain through BBB [18]. In present study, the levels of KYN and 5-HT were significantly decreased in the PFC of OBX rats, while the TRP levels in both PFC and serum distinctly increased in OBX rats. Consistent with these results, the activities of IDO1 and TPH2 were lower in OBX rats than in sham rats, which implied that the inhibition of IDO1 and TPH2 may lead to the decreased production of KYN and 5-HT from TRP. Increased peripheral TRP and reduced TRP catabolism might contribute to the accumulation of TRP in PFC of OBX rats. Upregulated TRP level was contrary to many

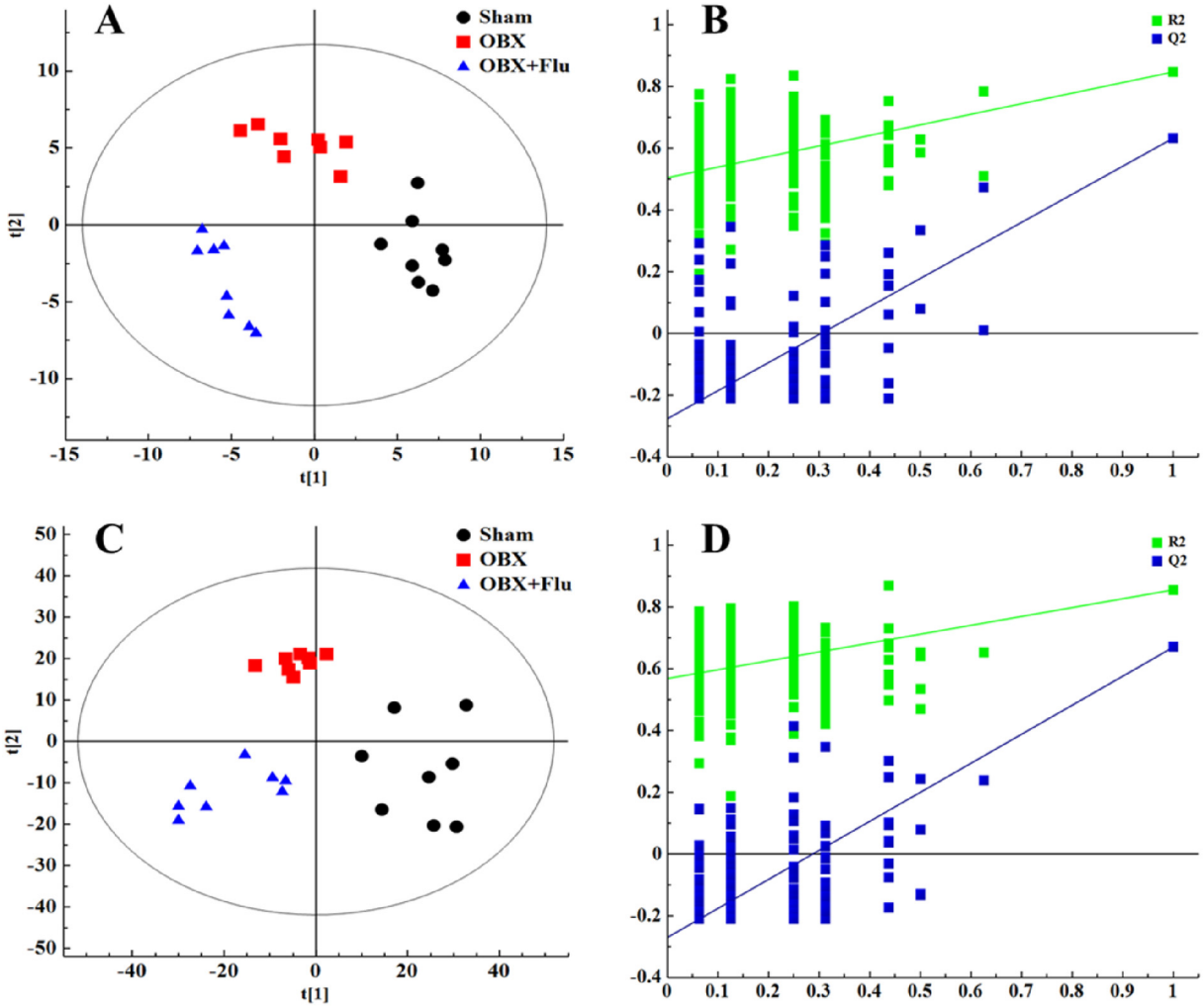

Fig. 8. PLS-DA score plots of urine samples from sham group (black circle), OBX group (red box) and OBX + Flu group (blue triangle) in positive mode (A, R2X = 0.269, R2Y = 0.845, Q2 (cum) = 0.638) and negative mode (C, R2X = 0.261, R2Y = 0.818, Q2 (cum) = 0.61). The results of 200 permutations in PLS-DA models in positive mode (R2 = 0.502, Q2 = −0.273) and negative mode (R2 = 0.569, Q2 = −0.271) were shown in B and D, respectively. n = 8 in each group. OBX: olfactory bulbectomy; Flu: fluoxetine. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

reports which supported the reduced or unchanged TRP in depression [19,39]. However, increased TRP concentration in OBX rats may represent the abnormal TRP metabolism in agitated depression, and the TRP level in this subtype of depression has not been investigated until now. Furthermore, it is still controversial that TRP served as an indicator for depression [40,41]. Therefore, more well-designed studies in patients and animal models are required to assess the relationship between TRP and different subtypes of depression.

5-HT is a key neurotransmitter in mood regulation, the content of which is significantly reduced in the postmortem brain tissue of depressed patients [42]. Decreased 5-HT and increased 5-HIAA were observed in the PFC of OBX rats, and significantly elevated 5-HIAA/5-HT ratio suggests that accelerated metabolism may also cause the decrease of 5-HT level. The increased level of DA in OBX rats was opposite to the previous reports in hippocampus and frontal cortex of OBX rats [27,43]. However, DA overflow increase has been demonstrated in the striatum of OBX rats by microdialysis, which may make an interpretation for the "agitation-like" behavior [44]. Therefore, it is reasonable to speculate that the elevation of DA in PFC may be involved in the hyperactivity in OBX rats. Fluoxetine could normalize the concentrations of 5-HT and 5-HIAA, enhance the activity of IDO1, and elevate the KYN level which indicated the remission of depression [45].

In metabonomics analysis, 26 potential biomarkers identified in present study were not isolated, but interconnected with each other. The abnormal metabolic pathways in OBX rats confirmed by KEGG database were categorized into six main aspects which will be discussed in detail below. Meanwhile, the changing trends of the shared metabolites in urine of OBX-induced (present study) and stress-induced (available literatures) rats were listed in Table S2. Seven of the 12 shared metabolites had the similar variation tendencies, while the 4 shared metabolites (leucine, allantoin, pantothenic acid and indole-3-carboxylic acid) showed inconsistent trends between OBX- and stress-induced rats. Thus, the 4 discrepant biomarkers may distinguish OBX model from stress-induced model of depression, further indicating the different subtypes of depression (psychomotor agitation or retardation).

4.1. TRP metabolism

TRP can be metabolized through three major pathways, namely, KYN pathway, 5-HT pathway and indole pathway. Under the oxidation of tryptophan-2,3-dioxygenase or IDO, TRP is converted to N-formylkynurenine, followed by the formation of KYN [28]. The two branches in KYN pathway result in the syntheses of KYNA and 3-HK, and the latter is further metabolized to 3-hydroxyanthranilic acid and xanthurenic acid [46]. The rate-limiting step in 5-HT synthesis is that TRP is transformed to 5-hydroxy-L-tryptophan (5-HTP) through the

Table 1
The identified biomarkers in the urine of rats exposed to OBX and fluoxetine treatment.

No.	Retention time (min)	VIP value	Measured mass (*m/z*)	Metabolites	Pathway	Trend	
						OBX/Sham	OBX + Flu/OBX
Positive ion mode							
1	5.38	1.03	206.0452	Xanthurenic acid	TRP metabolism	↓**	↓#
2	7.17	1.82	162.0554	4,6-Dihydroxyquinoline	TRP metabolism	↑*	↑#
3	2.86	1.76	154.0506	3-Hydroxyanthranilic acid	TRP metabolism	↓*	↑#
4	2.81	2.78	151.0620	7-Methylhypoxanthine	Purine metabolism	↓***	↓###
5	3.18	1.16	137.0462	Hypoxanthine	Purine metabolism	↓*	↑#
6	1.62	2.76	166.0728	Methylguanine	Purine metabolism	↓***	
7	1.43	4.88	169.0363	Uric acid	Purine metabolism	↓***	↑##
8	2.86	1.52	137.0429	Threonic acid	Ascorbate and aldarate metabolism	↓**	↑
9	4.87	1.03	220.1186	Pantothenic acid	Pantothenate and CoA Biosynthesis	↓*	
10	4.87	1.17	90.0554	Alanine	Alanine, aspartate and glutamate metabolism	↓**	
Negative ion mode							
11	6.38	1.73	204.0322	Xanthurenic acid	TRP metabolism	↓**	↓#
12	6.38	2.48	160.0411	Indole-3-carboxylic acid	TRP metabolism	↓*	↑#
13	6.75	5.78	212.0029	Indoxyl sulfate	TRP metabolism	↓*	↑#
14	9.18	4.85	107.0496	p-Cresol	Phenylalanine metabolism	↓**	↑##
15	9.55	7.28	283.0851	p-Cresol glucuronide	Phenylalanine metabolism	↓**	↑#
16	9.18	8.75	187.0055	p-Cresol sulfate	Phenylalanine metabolism	↓*	↑#
17	8.84	3.14	192.0678	Phenylacetylglycine	Phenylalanine metabolism	↓**	↑##
18	0.74	2.12	157.0391	Allantoin	Purine metabolism	↓**	↓#
19	3.57	1.15	181.0365	Methyluric acid	Purine metabolism	↓*	↑##
20	1.43	4.18	167.0242	Uric acid	Purine metabolism	↓***	↑##
21	9.55	1.19	175.0250	Ascorbic acid	Ascorbate and aldarate metabolism	↓**	↑#
22	4.04	1.97	148.0435	5,6-Dihydroxyindole	Tyrosine metabolism	↓*	↑
23	5.71	2.75	261.0054	Homovanillic acid sulfate	Tyrosine metabolism	↓***	↓###
24	0.88	1.58	117.0211	Succinic acid	TCA cycle	↓**	↓##
25	0.88	1.28	173.0073	Aconitic acid	TCA cycle	↓*	↓
26	11.74	1.02	199.0979	Decenedioic acid	Fatty acid metabolism	↓*	↓
27	9.55	1.51	103.0029	Hydroxypyruvic acid	Glycine, serine and threonine metabolism	↓**	↑##
28	9.37	2.11	130.0873	Leucine	Valine, leucine and isoleucine degradation	↑*	↓#

Note: "↑", upregulation; "↓", downregulation; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared to sham group. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ compared to OBX + Flu group. Flu: fluoxetine, OBX: olfactory bulbectomy, TCA cycle: tricarboxylic acid cycle, TRP: tryptophan.

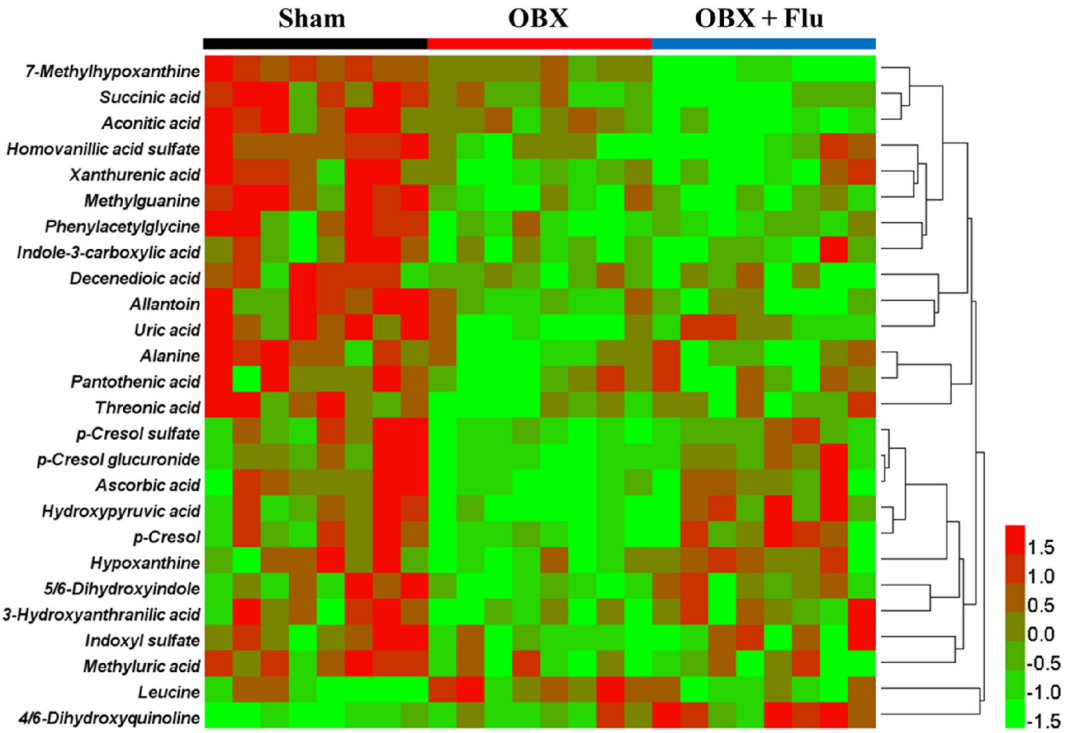

Fig. 9. Heat map of the differential biomarkers in the urine of rats. Increasing values are marked with green to red colors. Rows imply the potential biomarkers and columns represent urine samples of rats. OBX: olfactory bulbectomy; Flu: fluoxetine. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

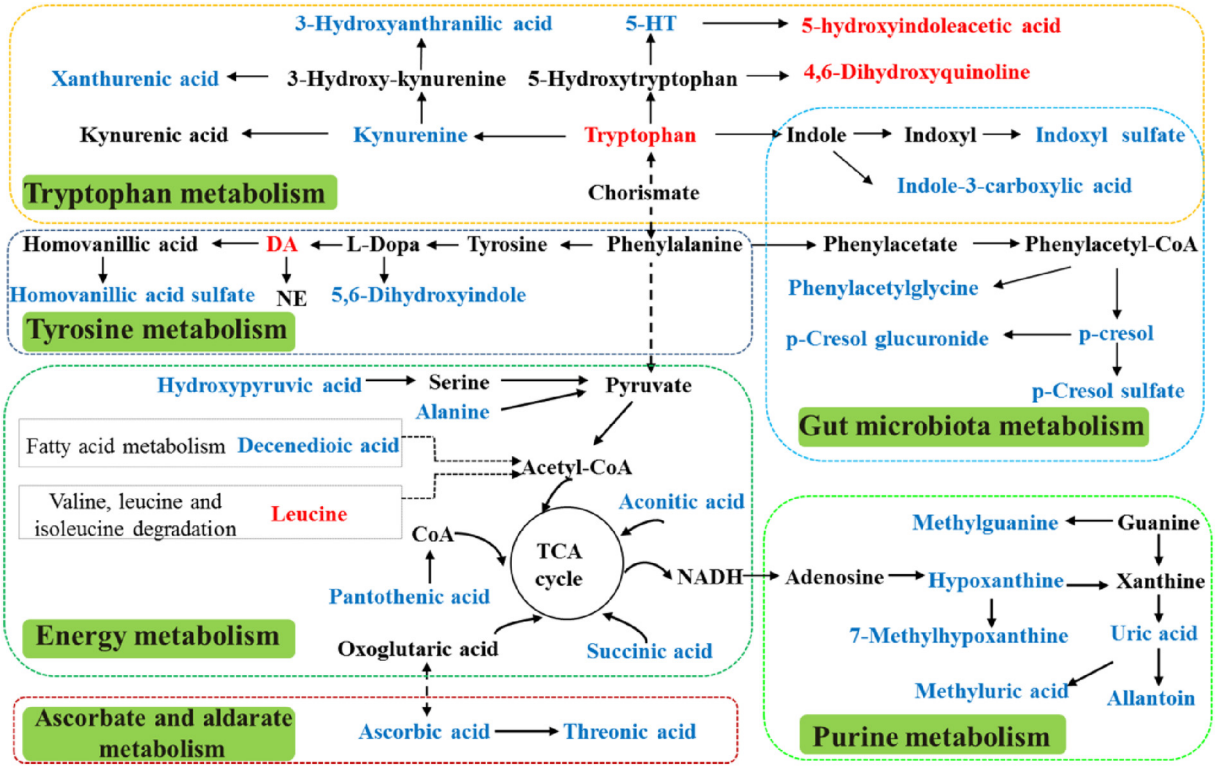

Fig. 10. The correlation network of altered metabolites in OBX-induced rat model of depression. The metabolites marked in blue denote decreased biomarkers and those in red indicate the increased ones. The disturbed pathways are exhibited in green boxes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

hydroxylation. And then 5-HT is metabolized to 5-HIAA. 4,6-Dihydroxyquinoline is generated from 5-HTP by a succession of enzymatic reactions. In the urine of OBX rats, the downregulation of 3-hydroxyanthranilic acid and xanthurenic acid suggests the deficiency of 3-HK, while the upregulation of 4,6-dihydroxyquinoline implies the depletion of 5-HTP, and may reduce the generation of 5-HT. Chronic fluoxetine treatment corrected the level of 3-hydroxyanthranilic acid, but aggravated the levels of xanthurenic acid and 4,6-dihydroxyquinoline. Indole is generated by the hydrolysis of tryptophanase, and indole-containing metabolites (indole-3-carboxylic acid and indoxyl sulfate) closely involving in gut microflora metabolism will be introduced in detail below.

4.2. Gut microbiota metabolism

It has been established that the bidirectional communication between gut microbiota and brain has an important influence on mood and behaviors related to anxiety and depression [47]. The stability and composition of gut microbiota were destroyed in CUMS-induced rats and depressed patients [48,49]. Phenylalanine is the precursor of tyrosine and can be metabolized into phenylacetylglycine and p-cresol by intestinal microorganisms [50]. p-Cresol is absorbed into the body and excreted by kidney in the form of glucuronide and sulfate conjugates (p-cresol glucuronide and p-cresol sulfate). The decreased levels of phenylacetylglycine, p-cresol, p-cresol sulfate and p-cresol glucuronide in the urine of OBX rats suggest that the abnormal phenylalanine metabolism may derive from the dysfunction of gut microflora. Furthermore, indole-containing products as a group of TRP metabolites are generated under the action of intestinal bacteria. These metabolites can promote neuronal function and modify animal behaviors by regulating the voltage-dependent Na^+ channel [51,52]. The significant reduction of indoxyl sulfate and indole-3-carboxylic acid in OBX rats also indicates the imbalance of gut microflora. Consistent with our results, similar variation tendencies have been described in the urine of CUMS-induced rats [53,54], suggesting that both stress- and surgery-induced depression models have paralleled effects on gut microbiota. Fluoxetine treatment elevated the levels of the six metabolites mentioned above. Therefore, the regulatory effect of fluoxetine on intestinal metabolic disorders may contribute to its antidepressant activity. In agreement with this assumption, Macedo et al. considered that the antimicrobial effects might implicate antidepressant effectiveness of antidepressant drugs [55]. Therefore, the strategy to reverse the dysbiosis in gut microbiota may be applied to the clinical therapy of depression.

4.3. Energy metabolism

TCA cycle is the primary approach to generate ATP in body, providing energy for basic vital activity. Pantothenic acid can be used to synthesize coenzyme A (CoA) which is an essential prosthetic group in TCA cycle [56]. Thus pantothenic acid plays an important role in the metabolism and synthesis of carbohydrates, proteins and fats. Aconitic acid as well as succinic acid is the important intermediate in TCA cycle. Moreover, it has been reported that alanine could be converted to pyruvic acid [57] which was further transformed into acetyl-CoA, and thus participated in various reactions in TCA cycle. Decenedioic acid and hydroxypyruvic acid are both closely related to the production of acetyl-CoA through different pathways. In the present research, the levels of pantothenic acid, aconitic acid, succinic acid, alanine, decenedioic acid and hydroxypyruvic acid in the urine of OBX rats were significantly lower than sham group, which implies that OBX induces the energy deficiency in rats.

Disturbed energy metabolism has been reported in patients with depression [58], and reduced ATP generation in mitochondria may weaken the neuronal function, leading to the development of depression [58]. Fluoxetine treatment partially redressed the abnormal urine metabolites, such as hydroxypyruvic acid and leucine, which is in

accordance with the findings that antidepressants upregulated mitochondrial energy generation in clinical treatment [59].

4.4. Purine metabolism

Purine takes part in the biosynthesis of macromolecules, oxidative phosphorylation, signal transduction and high energy transfer [21]. Metabonomics-based clinical research revealed that purine-associated metabolites were decreased in the plasma of children and adolescents with depression [60]. In our study, the decreased levels of 7-methylhypoxanthine, hypoxanthine, uric acid, methyluric acid, allantoin and methylguanine suggest that perturbed purine metabolism may play a part in the pathophysiology of depression.

4.5. Ascorbate and aldarate metabolism

Ascorbic acid, as a valuable antioxidant, has a negative correlation with depression [61]. Its antidepressant-like effect has been verified by tail suspension test in mice [62]. In the urine of OBX rats, the levels of ascorbic acid and its metabolite, threonic acid, were significantly downregulated in comparison to sham group, indicating the lowered antioxidant ability in OBX rats. Consistent with this, significantly decreased superoxide dismutase (SOD) and ascorbic acid, and elevated malondialdehyde (MDA) have been confirmed in depressed patients [63] and OBX rats [27]. These studies prove that excessive oxidative stress may promote the development of depression. Fluoxetine treatment reserved the reduction of ascorbic acid, thus the improvement of antioxidant ability may conduce to the therapy of depression.

4.6. Tyrosine metabolism

Tyrosine is the precursor of monoamine neurotransmitter and can be converted to L-dopa, followed by being metabolized to DA and 5,6-dihydroxyindole. Homovanillic acid is a metabolite of DA. In our study, the levels of homovanillic acid sulfate (the derivative of homovanillic acid) and 5,6-dihydroxyindole in the urine of OBX rats were remarkably reduced, suggesting the disruption of tyrosine metabolism. Fluoxetine reversed the level of 5,6-dihydroxyindole, but exacerbated the level of homovanillic acid sulfate.

5. Conclusions

For the first time, metabonomics was used to uncover the urinary metabolic profile in OBX rats which simulated agitated depression. Twenty-six potential biomarkers were identified, and affected pathways were involved in TRP metabolism, gut microbiota metabolism, energy metabolism, purine metabolism, ascorbate and aldarate metabolism, and tyrosine metabolism. The abnormalities in TRP metabolites and related enzymes also were revealed. Fluoxetine corrected some of the abnormal metabolites in urine, and normalized the TRP metabolism in PFC of OBX rats. The OBX model, old but not obsolete, is a valid model in investing the pathological process of depression from the perspective of metabonomics. And potential biomarkers may be helpful for the clinical diagnosis of agitated depression. Further study is still needed to verify these altered metabolites by targeted metabonomics and confirm the biomarkers in depressed patients with psychomotor agitation.

CRediT authorship contribution statement

Qi Chang: Conceptualization, Funding acquisition, Project administration, Supervision, Validation, Writing - review & editing. **Yun-Feng Zhou** Conceptualization, Data curation, Investigatio, Visualization, Writing - original draft. **Li Feng** Formal analysis, Methodology, Software. **Xue Tao**: Formal analysis, Visualization. **Zhi Wang**: Formal analysis, Supervision. **Xin-Min Liu**: Funding acquisition, Methodology, Resources, Validation. **Zhi Wang**: Formal analysis, Supervision. **Li-Sha Wang**: Investigation. **Meng-Di Zhang**: Methodology, Writing - review & editing. **Shan-Guang Chen**: Resources.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

Acknowledgments

This work was supported by the National Science and Technology Major Projects for New Drug Innovation and Development (2017ZX09301029), the Chinese Academy of Medical Sciences Innovation Fund for Medical Sciences (CIFMS, 2016-I2M-1-012) and The National Key Research and Development Program of China (2018YFC1602105).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lfs.2019.116751.

References

[1] B.H. Hidaka, Depression as a disease of modernity: explanations for increasing prevalence [J], J. Affect. Disord. 140 (2012) 205–214, https://doi.org/10.1016/j.jad.2011.12.036.
[2] J.P. Lepine, M. Briley, The increasing burden of depression [J], Neuropsychiatr. Dis. Treat. 7 (2011) 3–7, https://doi.org/10.2147/NDT.S19617.
[3] Y. Bansal, R. Singh, P. Saroj, et al., Naringenin protects against oxido-inflammatory aberrations and altered tryptophan metabolism in olfactory bulbectomized-mice model of depression [J], Toxicol. Appl. Pharmacol. 355 (2018) 257–268, https://doi.org/10.1016/j.taap.2018.07.010.
[4] S.V. Marathe, P.L. D'Almeida, G. Virmani, et al., Effects of monoamines and antidepressants on astrocyte physiology: implications for monoamine hypothesis of depression [J], J. Exp. Neurosci. 12 (2018) 1179069518789149, , https://doi.org/10.1177/1179069518789149.
[5] A. Menke, Is the HPA axis as target for depression outdated, or is there a new hope? [J], Front. Psych. 10 (2019) 101, , https://doi.org/10.3389/fpsyt.2019.00101.
[6] G.E. Hodes, V. Kana, C. Menard, et al., Neuroimmune mechanisms of depression [J], Nat. Neurosci. 18 (2015) 1386–1393, https://doi.org/10.1038/nn.4113.
[7] A.R. Lumia, M.H. Teicher, F. Salchli, et al., Olfactory bulbectomy as a model for agitated hyposerotonergic depression [J], Brain Res. 587 (1992) 181–185.
[8] B. Feng, X.Y. Zhao, Y.Z. Song, et al., Sarsasapogenin reverses depressive-like behaviors and nicotinic acetylcholine receptors induced by olfactory bulbectomy [J], Neurosci. Lett. 639 (2017) 173–178, https://doi.org/10.1016/j.neulet.2016.12.025.
[9] A. Saitoh, K. Yamaguchi, Y. Tatsumi, et al., Effects of milnacipran and fluvoxamine on hyperemotional behaviors and the loss of tryptophan hydroxylase-positive cells in olfactory bulbectomized rats [J], Psychopharmacology 191 (2007) 857–865, https://doi.org/10.1007/s00213-007-0699-x.
[10] J.P. Kelly, A.S. Wrynn, B.E. Leonard, The olfactory bulbectomized rat as a model of depression: an update [J], Pharmacol. Ther. 74 (1997) 299–316.
[11] X.J. Liu, Z.Y. Li, Z.F. Li, et al., Urinary metabonomic study using a CUMS rat model of depression [J], Magn. Reson. Chem. 50 (2012) 187–192, https://doi.org/10.1002/mrc.2865.
[12] S.J. Pan, Y.L. Tan, S.W. Yao, et al., Fluoxetine induces lipid metabolism abnormalities by acting on the liver in patients and mice with depression [J], Acta Pharmacol. Sin. 39 (2018) 1463–1472, https://doi.org/10.1038/aps.2017.207.
[13] A.E. Freitas, D.G. Machado, J. Budni, et al., Fluoxetine modulates hippocampal cell signaling pathways implicated in neuroplasticity in olfactory bulbectomized mice [J], Behav. Brain Res. 237 (2013) 176–184, https://doi.org/10.1016/j.bbr.2012.09.035.
[14] I. Holzmann, L.M. da Silva, J.A. Correa da Silva, et al., Antidepressant-like effect of quercetin in bulbectomized mice and involvement of the antioxidant defenses, and the glutamatergic and oxidonitrergic pathways [J], Pharmacol. Biochem. Behav. 136 (2015) 55–63, https://doi.org/10.1016/j.pbb.2015.07.003.
[15] M. Roche, A. Harkin, J.P. Kelly, Chronic fluoxetine treatment attenuates stressor-induced changes in temperature, heart rate, and neuronal activation in the olfactory bulbectomized rat [J], Neuropsychopharmacology 32 (2007) 1312–1320, https://doi.org/10.1038/sj.npp.1301253.
[16] L. Gotoh, A. Saitoh, M. Yamada, et al., Effects of repeated treatment with a delta opioid receptor agonist KNT-127 on hyperemotionality in olfactory-bulbectomized rats [J], Behav. Brain Res. 323 (2017) 11–14, https://doi.org/10.1016/j.bbr.2016.11.008.
[17] C.B. Filho, C.R. Jesse, F. Donato, et al., Chrysin promotes attenuation of depressive-like behavior and hippocampal dysfunction resulting from olfactory bulbectomy in mice [J], Chem. Biol. Interact. 260 (2016) 154–162, https://doi.org/10.1016/j.cbi.

2016.11.005.
[18] M. Franklin, I. Bermudez, H. Murck, et al., Sub-chronic dietary tryptophan depletion—an animal model of depression with improved face and good construct validity [J], J. Psychiatr. Res. 46 (2012) 239–247, https://doi.org/10.1016/j.jpsychires.2011.10.003.
[19] A. Messaoud, R. Mensi, W. Douki, et al., Reduced peripheral availability of tryptophan and increased activation of the kynurenine pathway and cortisol correlate with major depression and suicide [J], World J. Biol. Psychiatry (2018) 1–9, https://doi.org/10.1080/15622975.2018.1468031.
[20] W. Liu, H. Sheng, Y. Xu, et al., Swimming exercise ameliorates depression-like behavior in chronically stressed rats: relevant to proinflammatory cytokines and IDO activation [J], Behav. Brain Res. 242 (2013) 110–116, https://doi.org/10.1016/j.bbr.2012.12.041.
[21] Y. Liu, M. Wei, K. Yue, et al., Study on urine metabolic profile of Abeta25-35-induced Alzheimer's disease using UHPLC-Q-TOF-MS [J], Neuroscience 394 (2018) 30–43, https://doi.org/10.1016/j.neuroscience.2018.10.001.
[22] H.L. Cai, P. Jiang, Q.Y. Tan, et al., Therapeutic efficacy of atypical antipsychotic drugs by targeting multiple stress-related metabolic pathways [J], Transl. Psychiatry 7 (2017) e1130, https://doi.org/10.1038/tp.2017.94.
[23] L. Liu, X. Zhou, Y. Zhang, et al., Hippocampal metabolic differences implicate distinctions between physical and psychological stress in four rat models of depression [J], Transl. Psychiatry 8 (2018) 4, , https://doi.org/10.1038/s41398-017-0018-1.
[24] A. Jindal, R. Mahesh, S. Bhatt, Type 4 phosphodiesterase enzyme inhibitor, rolipram rescues behavioral deficits in olfactory bulbectomy models of depression: involvement of hypothalamic-pituitary-adrenal axis, cAMP signaling aspects and antioxidant defense system [J], Pharmacol. Biochem. Behav. 132 (2015) 20–32, https://doi.org/10.1016/j.pbb.2015.02.017.
[25] T. Xu, C. Lu, L. Feng, et al., Liquid chromatography-mass spectrometry-based urinary metabolomics study on a rat model of simulated microgravity-induced depression [J], J. Pharm. Biomed. Anal. 165 (2019) 31–40, https://doi.org/10.1016/j.jpba.2018.11.058.
[26] S. Pellow, P. Chopin, S.E. File, et al., Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat [J], J. Neurosci. Methods 14 (1985) 149–167.
[27] V.N. Thakare, M.K. Aswar, Y.P. Kulkarni, et al., Silymarin ameliorates experimentally induced depressive like behavior in rats: involvement of hippocampal BDNF signaling, inflammatory cytokines and oxidative stress response [J], Physiol. Behav. 179 (2017) 401–410, https://doi.org/10.1016/j.physbeh.2017.07.010.
[28] L.S. Wang, M.D. Zhang, X. Tao, et al., LC-MS/MS-based quantification of tryptophan metabolites and neurotransmitters in the serum and brain of mice [J], J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 1112 (2019) 24–32, https://doi.org/10.1016/j.jchromb.2019.02.021.
[29] L. Feng, H.W. Wu, G.Q. Song, et al., Chronical sleep interruption-induced cognitive decline assessed by a metabolomics method [J], Behav. Brain Res. 302 (2016) 60–68, https://doi.org/10.1016/j.bbr.2015.12.039.
[30] L. Liu, X. Zhou, Y. Zhang, et al., The identification of metabolic disturbances in the prefrontal cortex of the chronic restraint stress rat model of depression [J], Behav. Brain Res. 305 (2016) 148–156, https://doi.org/10.1016/j.bbr.2016.03.005.
[31] W. Deng, Y. Wang, Z. Liu, et al., HemI: a toolkit for illustrating heatmaps [J], PLoS One 9 (2014) e111988, , https://doi.org/10.1371/journal.pone.0111988.
[32] C. Song, B.E. Leonard, The olfactory bulbectomised rat as a model of depression [J], Neurosci. Biobehav. Rev. 29 (2005) 627–647, https://doi.org/10.1016/j.neubiorev.2005.03.010.
[33] B.E. Leonard, M. Tuite, Anatomical, physiological, and behavioral aspects of olfactory bulbectomy in the rat [J], Int. Rev. Neurobiol. 22 (1981) 251–286.
[34] S.D. Primeaux, P.V. Holmes, Role of aversively motivated behavior in the olfactory bulbectomy syndrome [J], Physiol. Behav. 67 (1999) 41–47.
[35] A. Mar, E. Spreekmeester, J. Rochford, Fluoxetine-induced increases in open-field habituation in the olfactory bulbectomized rat depend on test aversiveness but not on anxiety [J], Pharmacol. Biochem. Behav. 73 (2002) 703–712.
[36] R. Ramamoorthy, M. Radhakrishnan, M. Borah, Antidepressant-like effects of serotonin type-3 antagonist, ondansetron: an investigation in behaviour-based rodent models [J], Behav. Pharmacol. 19 (2008) 29–40, https://doi.org/10.1097/FBP.0b013e3282f3cfd4.
[37] M. Koenigs, J. Grafman, The functional neuroanatomy of depression: distinct roles for ventromedial and dorsolateral prefrontal cortex [J], Behav. Brain Res. 201 (2009) 239–243, https://doi.org/10.1016/j.bbr.2009.03.004.
[38] K. Ogyu, K. Kubo, Y. Noda, et al., Kynurenine pathway in depression: a systematic review and meta-analysis [J], Neurosci. Biobehav. Rev. 90 (2018) 16–25, https://doi.org/10.1016/j.neubiorev.2018.03.023.
[39] A. Watanabe, S. Hasegawa, K. Nishi, et al., Chronic buspirone treatment normalizes regional serotonin synthesis in the olfactory bulbectomized rat brain: an autoradiographic study [J], Brain Res. Bull. 69 (2006) 101–108, https://doi.org/10.1016/j.brainresbull.2005.11.008.
[40] D. Enko, H. Wagner, G. Kriegshauser, et al., Assessment of tryptophan metabolism and signs of depression in individuals with carbohydrate malabsorption [J], Psychiatry Res. 262 (2018) 595–599, https://doi.org/10.1016/j.psychres.2017.09.049.
[41] K. Shaw, J. Turner, C. Del Mar, Are tryptophan and 5-hydroxytryptophan effective treatments for depression? A meta-analysis [J], Aust. N. Z. J. Psychiatry 36 (2002) 488–491, https://doi.org/10.1046/j.1440-1614.2002.01046.x.
[42] M.J. Owens, C.B. Nemeroff, Role of serotonin in the pathophysiology of depression: focus on the serotonin transporter [J], Clin. Chem. 40 (1994) 288–295.
[43] X.R. Chang, L. Wang, J. Li, et al., Analysis of anti-depressant potential of curcumin against depression induced male albino wistar rats [J], Brain Res. 1642 (2016) 219–225, https://doi.org/10.1016/j.brainres.2016.03.010.
[44] C.V. Masini, P.V. Holmes, K.G. Freeman, et al., Dopamine overflow is increased in olfactory bulbectomized rats: an in vivo microdialysis study [J], Physiol. Behav. 81 (2004) 111–119, https://doi.org/10.1016/j.physbeh.2004.01.003.
[45] D. Krause, A.M. Myint, C. Schuett, et al., High kynurenine (a tryptophan metabolite) predicts remission in patients with major depression to add-on treatment with celecoxib [J], Front. Psych. 8 (2017) 16, , https://doi.org/10.3389/fpsyt.2017.00016.
[46] Y. Liu, Z. Liu, M. Wei, et al., Pharmacodynamic and urinary metabolomics studies on the mechanism of Schisandra polysaccharide in the treatment of Alzheimer's disease [J], Food Funct. 10 (2019) 432–447, https://doi.org/10.1039/c8fo02067a.
[47] R.A. Luna, J.A. Foster, Gut brain axis: diet microbiota interactions and implications for modulation of anxiety and depression [J], Curr. Opin. Biotechnol. 32 (2015) 35–41, https://doi.org/10.1016/j.copbio.2014.10.007.
[48] M. Yu, H. Jia, C. Zhou, et al., Variations in gut microbiota and fecal metabolic phenotype associated with depression by 16S rRNA gene sequencing and LC/MS-based metabolomics [J], J. Pharm. Biomed. Anal. 138 (2017) 231–239, https://doi.org/10.1016/j.jpba.2017.02.008.
[49] H. Jiang, Z. Ling, Y. Zhang, et al., Altered fecal microbiota composition in patients with major depressive disorder [J], Brain Behav. Immun. 48 (2015) 186–194, https://doi.org/10.1016/j.bbi.2015.03.016.
[50] Y. Liu, M. Wei, K. Yue, et al., Non-target metabonomic method provided new insights on the therapeutical mechanism of Gancao Fuzi decoction on rheumatoid arthritis rats [J], J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 1105 (2019) 93–103, https://doi.org/10.1016/j.jchromb.2018.11.015.
[51] E.Y. Hsiao, S.W. McBride, S. Hsien, et al., Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders [J], Cell 155 (2013) 1451–1463, https://doi.org/10.1016/j.cell.2013.11.024.
[52] F. Moroni, Tryptophan metabolism and brain function: focus on kynurenine and other indole metabolites [J], Eur. J. Pharmacol. 375 (1999) 87–100.
[53] X.X. Gao, J. Cui, X.Y. Zheng, et al., An investigation of the antidepressant action of xiaoyaosan in rats using ultra performance liquid chromatography-mass spectrometry combined with metabonomics [J], Phytother. Res. 27 (2013) 1074–1085, https://doi.org/10.1002/ptr.4805.
[54] X. Chang, H. Jia, C. Zhou, et al., Role of Bai-Shao towards the antidepressant effect of Chaihu-Shu-Gan-San using metabonomics integrated with chemical fingerprinting [J], J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 1006 (2015) 16–29, https://doi.org/10.1016/j.jchromb.2015.10.007.
[55] D. Macedo, A. Filho, C.N. Soares de Sousa, et al., Antidepressants, antimicrobials or both? Gut microbiota dysbiosis in depression and possible implications of the antimicrobial effects of antidepressant drugs for antidepressant effectiveness [J], J. Affect. Disord. 208 (2017) 22–32, https://doi.org/10.1016/j.jad.2016.09.012.
[56] F. Depeint, W.R. Bruce, N. Shangari, et al., Mitochondrial function and toxicity: role of the B vitamin family on mitochondrial energy metabolism [J], Chem. Biol. Interact. 163 (2006) 94–112, https://doi.org/10.1016/j.cbi.2006.04.014.
[57] L. Li, B. Sun, Q. Zhang, et al., Metabonomic study on the toxicity of Hei-Shun-Pian, the processed lateral root of Aconitum carmichaelii Debx. (Ranunculaceae) [J], J. Ethnopharmacol. 116 (2008) 561–568, https://doi.org/10.1016/j.jep.2008.01.014.
[58] K.G. Kahl, B. Stapel, H. Frieling, Link between depression and cardiovascular diseases due to epigenomics and proteomics: focus on energy metabolism [J], Prog. Neuro-Psychopharmacol. Biol. Psychiatry 89 (2019) 146–157, https://doi.org/10.1016/j.pnpbp.2018.09.004.
[59] G. Scaini, D.D. Maggi, B.T. De-Nes, et al., Activity of mitochondrial respiratory chain is increased by chronic administration of antidepressants [J], Acta Neuropsychiatr. 23 (2011) 112–118, https://doi.org/10.1111/j.1601-5215.2011.00548.x.
[60] X. Zhou, L. Liu, X. Lan, et al., Polyunsaturated fatty acids metabolism, purine metabolism and inosine as potential independent diagnostic biomarkers for major depressive disorder in children and adolescents [J], Mol. Psychiatry (2018), https://doi.org/10.1038/s41380-018-0047-z.
[61] M. Gautam, M. Agrawal, M. Gautam, et al., Role of antioxidants in generalised anxiety disorder and depression [J], Indian J. Psychiatry 54 (2012) 244–247, https://doi.org/10.4103/0019-5545.102424.
[62] M. Moretti, J. Budni, A.E. Freitas, et al., Antidepressant-like effect of ascorbic acid is associated with the modulation of mammalian target of rapamycin pathway [J], J. Psychiatr. Res. 48 (2014) 16–24, https://doi.org/10.1016/j.jpsychires.2013.10.014.
[63] A. Bajpai, A.K. Verma, M. Srivastava, et al., Oxidative stress and major depression [J], J. Clin. Diagn. Res. 8 (2014) CC04–07, https://doi.org/10.7860/JCDR/2014/10258.5292.