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Research report

2D-gel based proteomics unravels neurogenesis and energetic metabolism dysfunction of the olfactory bulb in CUMS rat model

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HIGHLIGHTS

- Using 2-D gel electrophoresis coupled with mass spectrometry to characterize the Olfactory Bulb under CUMS treatment.
- 29 out of 47 differential proteins were identified.
- 7 differential proteins were ruled out to validate guided by KEGG and IPA biological analysis.
- Global proteomics Characterization of OB may unravel the dysfunction under CUMS treatment.

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ABSTRACT

Major depression is a devastating psychiatric disease worldwide currently. A reduced olfactory sensitivity in MDD patients was well evidenced. We previously interrogated the mechanism of decreasing hippocampus neurogenesis in CUMS rat model of depression. The Olfactory Bulb (OB) is crucial part of the olfactory system which functions in post-developmental neurogenesis. However, the mechanism of the dysfunction of OB induced by CUMS is still largely unknown. Herein, by using the chronic unpredictable mild stress (CUMS) rat model of depression, differential protein expression between the OB proteomes of CUMS and control group was interrogated through two-dimensional electrophoresis coupling with matrix-assisted laser desorption ionization-time of flight tandem mass spectrometry. Twenty nine differential protein expression was analyzed by Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway over-representation and Ingenuity pathways analysis (IPA). Seven identified differential proteins were selected for Western blotting validation. This study provides insight that neurogenesis and Energy metabolism disorder is involved in OB dysfunction induced by CUMS.

1. Introduction

Major depression is prevailing and devastating psychiatric disorders lately. About 16% of the population is estimated to be suffering major depression (MDD) [1]. Considering the heterogeneity of MDD, multiple clinical manifestations were shown among MDD patients [2]. In particular, a reduced olfactory sensitivity in MDD patients was mentioned in a couple of studies [3]. As a possible reason, we focused on the dysfunction of hippocampus neurogenesis induced by CUMS stress previously [4,5]. The olfactory system is known as a crucial post-developmental neurogenesis brain region,

Abbreviations: OB, olfactory bulb; CUMS, chronic unpredictable mild stress; MDD, major depressive disorder; 2-DE, two dimensional electrophoresis.

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which functions through life time in the adult mammalian brain [6]. In our lab, we studied on the multiple sample levels(in rodent animal and MDD patients), the changes on the olfactory pathway was characterized, the evidences (olfactory epithelium (OE), olfactory bulb (OB), or hub olfactory regions) of rat model of depression may contribute to the olfactory dysfunction (olfactory sensitivity and volume of OB and OE) [3,4,7], however, the dysfunction of OB induced by CUMS is still largely unknown. Recently, a study on neurogenesis using OMICs strategy enables us to employ the OMICs to get a better understanding of OB dysfunction [8].

Affected adult hippocampal neurogenesis may be a candidate mechanism for the etiology of depression [9], besides, subventricular zone (SVZ) participated in the process of neurogenesis [10], given neuronal precursors stem from the SVZ and finally migrate and gather into the OB, the changes of OB are more or less correlated with neurogenesis, newly findings in our group, we revealed the intimate brain region (OE) was affected by CUMS, which gives rise to the survival of olfactory receptor neurons (ORNs) in OE to develop the olfactory dysfunction [11,12]. Together, OB dysfunction induced by CUMS is well evidenced and calls for the further insights to the molecular characterizations.

Proteomics is regarded as a mature and unbiased quantitative analysis of protein expression [13]. Proteomics strategy has been used as a powerful tool to characterize the MDD related biomarkers in patient as well as furthered the mechanisms in rodent model of depression [14,15]. In addition, our group has established solid proteomics based platform [16], which enables us to characterize protein expression of OB in a preclinical rat model of depression.

With this on mind, the chronic unpredictable mild stress (CUMS) rat model of depression has been employed hereby to investigate the differential protein expression in the rat OB. The OB proteomes from two groups (CUMS vs CON) were analyzed by two dimensional electrophoresis (2-DE). Forty Seven differential proteins were identified by matrix-assisted laser desorption ionization-time of flight-tandem mass spectrometry (MALDI-TOF-MS/MS) and analyzed for pathway over-representation via the Kyoto Encyclopedia of Genes and Genomes (KEGG) database and Ingenuity pathway analysis (IPA). Seven differential proteins were finally ruled out for western blotting validation. The findings could aid in further investigation to the pathophysiology underlying OB dysfunction induced by CUMS (Table 1).

2. Materials and methods

2.1. Animals

Here we chose 18 healthy adult male Sprague-Dawley (SD) rats (weight: 230–280 g; age: 3–4 months), purchased from the animal facility at Chongqing Medical University (China). The rats were housed under standard conditions. After the adaptation to the context for 7 days, the animals were randomly divided into two groups: CUMS group and CON group (n = 9 each group). The CUMS treatment schedule was as previously characterized [16] (Fig. 1). This study was approved by the Ethics Committee of Chongqing Medical University, and all procedures were in accordance with the National Institutes of Health Guidelines for Animal Research (Guide for the Care and Use of Laboratory Animals). Special care was taken to minimize number and suffering of animals.

2.2. Apparatus and procedure

The stress procedure followed our previously described strategy, with minor modifications [4,17]. More details please ref our prior publication or workflow.The content about the mild stressors including cage tilting (24 h), swimming (4 °C cold water/5 min), swimming (45 °C hot water/5 min), fasting (deprivation of food 24 h), water deprivation (24 h), shaking (10 min), nip tail for 1 min, wet bedding (24 h), and inversion of the light/dark cycle. Rats were taken one of these stressors on one day and without repetitive stressors in the 2 consecutive days.

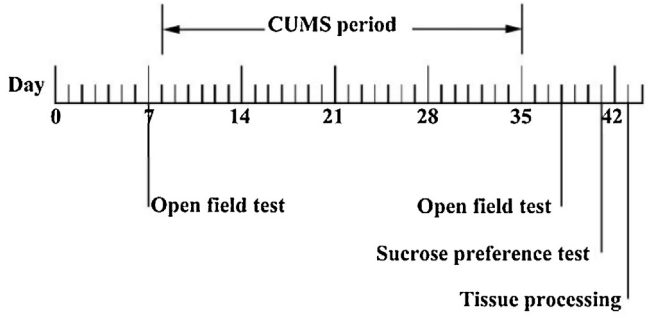

Fig. 1. Time schedule for behavior tests is as followed. Open field tests were conducted on days 7 and 38. Sucrose preference test was completed at day 41.

2.2.1. Behavioral tests

The stress subjects were allowed to habituate for 30 min to the experimental surrounding before we got the CUMS treatment started. The standard working condition is supposed to be in a soundproof room during 8:00 A.M. to 1:00 P.M.

2.2.2. Open field test

The open field test (OFT) was performed to measure spatial exploration behavior. The apparatus consisting of a black square cage (100 cm × 100 cm × 40 cm). A rat was placed in the center of the box. For the first 30 s, it was set to get adaptation. The OFT (the number of locomotion and rearing frequency) were observed for 5 min (Sony DCR-SR45E camera located 190–200 cm above the arena). The box was entirely cleaned by alcohol and dried excluding the interaction of the dejections in the trail. The base was divided into 16 equal squares by a video-computerized tracking system (SMART, Panlab SL, Barcelona, Spain).

2.3. Sucrose preference test and body weight

The Sucrose Preference Test (SPT) was most significant parameter measuring for the anhedonic effects [18], rats were trained to adapting to 1% (w/v) sucrose solution 72 h before testing. In test phase, a two bottle preference test was utilized in the study, rats could get both tap water and a 1% sucrose solution for 24 h. The position of the two bottles (left/right sides of the cages) was changed randomly on purpose of refraining from place preference. SP was calculated as the percentage of sucrose solution ingested relative to the total amount of liquid consumed (SP = sucrose consumption/[sucrose consumption + water consumption]*100%). Following stress exposure, rats showed a SP below 65% were defined as anhedonic

2.4. Protein sample preparation

In accordance with our established proteomics platform [16,19] six rats (n = 3 per group) were decapitated under deep diethylether anesthesia, their OBs were removed as soon as possible, then powderized in liquid nitrogen and suspended in 2 ml of acetone solution containing 0.2% (w/v) DTT and 10% (w/v) TCA. After homogenization, the suspension was left at −20 °C overnight before centrifugation (35,000g for 30 min at 4 °C). The supernatant was decanted, and the pellet was re-suspended in 2 ml of pre-cooled acetone solution containing 0.2% (w/v) DTT, left at −20 °C for 1 h,