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Research report

The identification of metabolic disturbances in the prefrontal cortex of the chronic restraint stress rat model of depression

Lanxiang Liu[a,b,1], Xinyu Zhou[a,b,1], Yuqing Zhang[a,b,1], Yiyun Liu[a,b,1], Lining Yang[a,b], Juncai Pu[a,b], Dan Zhu[a], Chanjuan Zhou[a,b], Peng Xie[a,b,*]

[a] Department of Neurology and Psychiatry, The First Affiliated Hospital of Chongqing Medical University, Chongqing, China
[b] Institute of Neuroscience and the Collaborative Innovation Center for Brain Science, Chongqing Medical University, Chongqing, China

HIGHLIGHTS

- CRS induces depression-like behaviors and not anxiety-like behaviors.
- A total of 36 differentially expressed metabolites were identified in the PFC after the CRS protocol.
- The pathways commonly perturbed by CRS are mainly involved in amino acid metabolism, energy metabolism and lipid metabolism characterized by disturbed glutamate metabolism, TCA cycle and fatty acid degradation, and neurotransmitter synthesis.

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ABSTRACT

Major depressive disorder, with serious impairment in cognitive and social functioning, is a complex psychiatric disorder characterized by pervasive and persistent low mood and a loss of interest or pleasure. However, the underlying molecular mechanisms of depression remain largely unknown. In this study, we used a non-targeted metabolomics approach based on gas chromatography–mass spectrometry of the prefrontal cortex in chronic restraint stress (CRS)-treated rats. CRS was induced in the stress group by restraining rats in a plastic restrainer for 6 h every day. This stress paradigm continued for 21 days. Body weight measurement and behavior tests were applied, including the sucrose preference test for anhedonia, the forced swimming test for despair-like behavior, and open field test and the elevated plus-maze to test for anxiety-like behaviors in rats after CRS. Differentially expressed metabolites associated with CRS-treated rats were identified by combining multivariate and univariate statistical analysis and corrected for multiple testing using the Benjamini-Hochberg procedure. A heat map of differential metabolites was constructed using Matlab. Ingenuity Pathways Analysis was applied to identify the predicted pathways and biological functions relevant to the bio-molecules of interest. Our findings showed that CRS induces depression-like behaviors and not anxiety-like behaviors. Thirty-six metabolites were identified as potential depression biomarkers involved in amino acid metabolism, energy metabolism and lipid metabolism, as well as a disturbance in neurotransmitters. Consequently, this study provides useful insights into the molecular mechanisms of depression.

1. Introduction

Major depressive disorder, with a lifetime prevalence of up to 16% [1], is a complex psychiatric disorder characterized by pervasive and persistent low mood that is accompanied by a loss of interest or pleasure, feelings of guilt or low self-worth, and disturbed sleep and/or appetite [2]. According to the World Health Organization, depression is predicted to be the leading cause of disease burden by 2030 [3]. Persistent symptoms of depression are associated with impairments in cognitive and social functioning, which has considerable impact on individuals, their partners and

Abbreviations: CRS, chronic restraint stress; CMS, chronic mild stress; PFC, prefrontal cortex; NMR, nuclear magnetic resonance; LC–MS, liquid chromatography–mass spectrometry; GC–MS, gas chromatography–mass spectrometry; SPT, sucrose preference test; BW, body weight; LAT, locomotor activity test; FST, forced swimming test; OFT, open field test; EPM, elevated plus-maze; PCA, principal components analysis; PLS-DA, partial least-squares discriminant analysis; IPA, ingenuity pathway analysis; TIC, total ion current chromatograms; NAA, *N*-acetyl-L-aspartic acid; TCA, tricarboxylic acid cycle; GABA, 4-aminobutyric acid; GSH, glutathione; BCAAs, branched chain amino acids.

* Corresponding author at: Department of Neurology and Psychiatry, The First Affiliated Hospital of Chongqing Medical University, 1 Youyi Road, Yuzhong District, Chongqing 400016, China.
E-mail address: xiepeng973@126.com (P. Xie).
[1] These authors contributed equally to this work.

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families, and wider society. Depression often co-occurs with other psychiatric disorders, such as anxiety [4], attention-deficit hyperactivity disorder [5], substance abuse disorders [6], and other physical and mental health problems. Despite the abundance of published research into this disease, the underlying molecular mechanisms of depression remain largely unknown.

Chronic stress plays an important role in the development of depression. Pre-clinical and clinical studies suggest that repeated stress (e.g. 21 days of chronic restraint stress; CRS) causes functional and structural changes in certain brain regions such as the prefrontal cortex (PFC) [7]. The PFC is associated with emotion regulation, cognition, and learning [8]. There is an abundance of neuroscientific evidence from functional imaging, lesioning, and brain stimulation studies that has implicated the PFC in depression [9]. For example, data from neuroimaging indicated that decreased activity and gray matter volume in the subgenual region of the PFC is an important change in the pathology of depression [10,11]. In particular, dysfunction in the PFC's glutamatergic neurotransmission has been implicated in the pathophysiology of depression [12,13].

Chronic stress, including chronic mild stress (CMS) and CRS, is the most important and widely used animal model of depression [14]. However, the CMS model has been criticized for being difficult to replicate across laboratories [15], and for the high operating costs associated with the long period required for modeling. In contrast, CRS – in which rats are usually placed in a small body-sized device for several hours a day during a long period – has been widely used in stress-induced depressive-like behavior studies, primarily because this stress treatment procedure is readily accessible [16]. Only those animals that are particularly vulnerable – that is, show high sensitivity to chronic stress – develop pathologies [17]. As a result, the subgroup of rats with depressive-like behaviors after CRS can be used for investigating the pathophysiology of depression.

The recently developed "Omics" have been used in the discovery of biomarkers in severe diseases. Following on from genomics [18], transcriptomics [19], and proteomics [20], metabolomics, which can profile the small molecules within biological systems in given biosamples without bias, has become a powerful tool in elucidating the biomarkers and key pathways involved in many diseases [21–23]. Metabolic profiling techniques, such as nuclear magnetic resonance (NMR) [24], liquid chromatography–mass spectrometry (LC–MS) [25], and gas chromatography–mass spectrometry (GC–MS) coupled with multivariate statistical modeling [26] have been used to investigate metabolic changes in depressive disorders. In our laboratory, rat CMS models of depression [27–29] and naturally occurring depression in macaques [30] have been well established for uncovering the molecular mechanisms of depression. We have also utilized biological fluids from depression patients for metabolic analysis [24–26].

In this study, a non-targeted metabolomics approach based on GC–MS technology, with high-throughput, −sensitivity, and −resolution [31], was applied to investigate significant metabolic changes in the PFC of CRS rats. Our primary aim was to identify the CRS-induced metabolic disturbances in information transmission in the PFC, an important brain region in the pathology of depression, in order to better understand the underlying molecular mechanisms of depression.

2. Experimental procedures

2.1. Ethics statement

All procedures in this study were performed in accordance with the guidelines established by the National Institutes of Health for Animal Research [32], and were approved by the Ethics Committee of Chongqing Medical University, Chongqing, China.

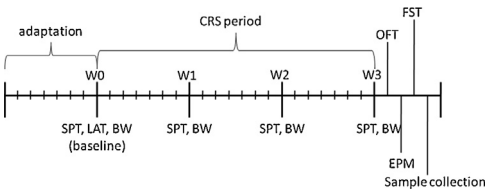

Fig. 1. Time schedule for the CRS protocol.
W0, W1, W2, and W3 represented the baseline, the last day of the first week, the last day of the second week, and the last day of the third week, respectively. LAT, locomotor activity test; SPT, sucrose preference test; BW, body weight; OFT, open field test; EPM, elevated plus-maze; FST, forced swimming test.

2.2. CRS rat model

Thirty-five male Sprague-Dawley rats weighing 200–250 g were purchased from the animal facility at Chongqing Medical University. The rats were single-housed under a reversed 12-h light/12-h dark cycle (lights on at 19:00 h; lights off at 07:00 h) at constant temperature (22 ± 1 °C) and humidity (55 ± 5%). Food and water were provided ad libitum. All rats were allowed to acclimatize to the standard conditions for 7 days before any experimental procedures were initiated. The rats were then weight-matched and randomly assigned to a CRS group or a non-stressed control (CON) group. Rats in the CRS group were repeatedly placed in a plastic restrainer (550 ml cubage water bottle, Nongfu Spring Company Limited, Hangzhou, China) for 6 h (from 09:00 to 15:00) every day. However, rats in the CON group were left undisturbed. During the restraint stress period, rats in both groups were deprived of food and water. This stress paradigm continued for 21 days. The time schedule for the CRS procedure is shown in Fig. 1.

2.3. Behavioral tests

2.3.1. Sucrose preference test (SPT) and body weight (BW)

Anhedonia [33] was assessed via SPT in the dark phase of the light/dark cycle. Briefly, rats were habituated to 1% sucrose solution for 48 h before SPT. On the testing day, rats were water- and food-deprived for 6 h followed by a 1 h test with two pre-weighted bottles filled with either 1% sucrose solution or water [34]. During testing, the side of the bottle used was counterbalanced across animals. Sucrose preference was defined as [sucrose intake/(sucrose intake + water intake)] × 100% [35]. Body weight was measured weekly immediately after the SPT.

2.3.2. Locomotor activity test (LAT), forced swimming test (FST), open field test (OFT), and elevated plus-maze (EPM)

All rats were individually placed in the soundproof experimental room to acclimatize at least 6–7 min prior to LAT, FST, OFT, and EPM testing, alternating between CRS and CON rats. All procedures were conducted between 9:00 and 17:00 h under illumination at ∼2 lx.

The LAT was used to identify the activity ability of the rats before the CRS procedure. Rats were individually placed in four square boxes (44 × 44 × 40 cm) and the total distance (cm) traveled by each rat was recorded for 5 min by a video surveillance system (SMART, Panlab SL, Barcelona, Spain). Learned helplessness was measured by the FST adapted from previous studies [28,36]. The total immobility time in the FST was recorded as an index of depressive-like behavior. Spatial exploration behavior in rodents was tested by the OFT as previously described [27,28], locomotor activity, rearing frequency, and central activity were measured during the 5-min session. The EPM was used to assess the anxiety-like behaviors in rats [37,38]. During the 5-min test period, the total