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METABOLOMICS IDENTIFIES PERTURBATIONS IN AMINO ACID METABOLISM IN THE PREFRONTAL CORTEX OF THE LEARNED HELPLESSNESS RAT MODEL OF DEPRESSION

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Abstract—Major depressive disorder is a serious psychiatric condition associated with high rates of suicide and is a leading cause of health burden worldwide. However, the underlying molecular mechanisms of major depression are still essentially unclear. In our study, a non-targeted gas chromatography–mass spectrometry-based metabolomics approach was used to investigate metabolic changes in the prefrontal cortex of the learned helplessness (LH) rat model of depression. Body-weight measurements and behavioral tests including the active escape test, sucrose preference test, forced swimming test, elevated plus-maze and open field test were used to assess changes in the behavioral spectrum after inescapable footshock stress. Rats in the stress group exhibited significant learned helpless and depression-like behaviors, while without any significant change in anxiety-like behaviors. Using multivariate and univariate statistical analysis, a total of 18 differential metabolites were identified after the footshock stress protocol. Ingenuity Pathways Analysis and MetaboAnalyst were applied for predicted pathways and biological functions analysis. ''Amino Acid Metabolism, Molecule Transport, Small Molecule Biochemistry" was the most significantly altered network in the LH model. Amino acid metabolism, particularly glutamate metabolism, cysteine and methionine metabolism, arginine and proline metabolism, was significantly perturbed in the prefrontal cortex of LH rats.

Key words: depression, rat, learned helplessness, metabolomics, gas chromatography–mass spectrometry.

INTRODUCTION

Major depressive disorder (MDD), is a serious psychiatric disorder characterized by pervasive and persistent low mood, accompanied by anhedonia, changes in appetite, sleep disturbances, or fatigue, with a lifetime prevalence of up to 16% (Kupfer et al., 2012). MDD is a common and potentially devastating condition, associated with high rates of suicide (Miret et al., 2013), and is a leading cause of health burden worldwide as identified by the Global Burden of Disease (GBD) study in 2010 (Ferrari et al., 2013). However, only individuals who are particularly vulnerable to stressful events develop pathologies and exhibit the signs or symptoms of MDD (McEwen, 2000). Moreover, individual responses to antidepressant drugs are variable and only 60%–70% of patients respond (Warden et al., 2007). The main reasons for this may be that depression is a multifactorial disorder and the underlying molecular mechanisms are still essentially unknown.

Animal models are important tools for investigating the pathogenesis of depression. The learned helplessness (LH) paradigm, in which rats are exposed to uncontrollable and unpredictable electrical footshock stress, is a well-validated model of depression (Ho and Wang, 2010). Animals that develop helplessness in this model show several behavioral changes that have similarities with human depression, including decreased motor activity, altered sleep, and decreased motivation (Pryce et al., 2011). Neurobiochemical changes in the prefrontal cortex (PFC) have also been observed after the induction of LH. Alterations in serotonin transport sites (Wu et al., 1999) and glutamatergic neurotransmitter systems in the PFC (Muneoka et al., 2013), as well as abnormalities in intracellular signaling (Kohen et al., 2003), have been reported. Moreover, proteomic analysis of synaptosomes from the PFC and hippocampus has indicated that energy metabolism and cellular remodeling are dysregulated in learned helpless rats (Mallei et al., 2011). Metabolites give organisms their biochemical characteristics; as most metabolites are generated by enzymatic proteins resulting from gene expression, the metabolome thus links genotype and phenotype (Tachibana, 2014). This makes metabolomics, the youngest field in the ''-omics" family, a more immediate measure of physiopathology than other -omics approaches (e.g., genomics and proteomics).

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Abbreviations: EPM, elevated plus-maze; FST, forced swimming test; GABA, gamma-aminobutyric acid; GC–MS, gas chromatography–mass spectrometry; IPA, Ingenuity Pathway Analysis; LAT, locomotor activity test; LH, learned helplessness; MDD, major depressive disorder; OFT, open field test; PCA, principal component analysis; PFC, prefrontal cortex spectrometry; PLS-DA, partial least-squares discriminant analysis; SPT, sucrose preference test; TCA, tricarboxylic acid cycle.

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Previously, we studied changes in metabolism in the cerebellum (Shao et al., 2013), PFC (Chen et al., 2015; Liu et al., 2016), and peripheral blood mononuclear cells (Li et al., 2014) in the chronic unpredictable mild stress and chronic restraint stress rat models of depression. We have also profiled the serum metabolic phenotypes of naturally-occurring depression in macaques (Xu et al., 2015), and metabolic changes in the urine (Zheng et al., 2013a,b) and plasma (Zheng et al., 2012; Liu et al., 2015) of patients with depression.

In this study, we have carried out a global metabolomics analysis of the PFC of LH rats. A non-targeted metabolomics approach based on gas chromatography–mass spectrometry (GC–MS) was used to identify differentially expressed metabolites in LH vs. control rats following footshock stress exposure. We aim to identify LH-induced metabolic disturbances in the PFC, a significantly dysfunctional brain region in the pathology of depression, in order to explore the underlying pathogenesis mechanisms of depression.

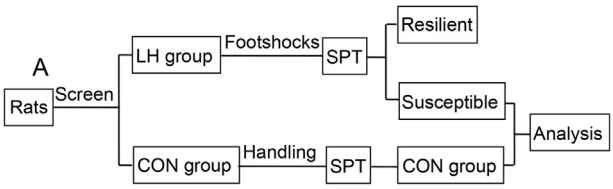
EXPERIMENTAL PROCEDURES

Animals and ethics statement

Thirty-five male Sprague–Dawley rats weighing 200–250 g were purchased from the animal facility at Chongqing Medical University (Chongqing, China). Throughout the experiment, rats were singly housed under standard laboratory conditions (21 ± 1 °C constant temperature, 55 ± 5% relative humidity, and a reversed 12-h light/12-h dark cycle with lights on at 19:00) with free access to food and water. Animal use and procedures were in accordance with the National Institutes of Health guidelines (Clark et al., 1997) and approved by the Ethics Committee of Chongqing Medical University.

Learned helplessness

After acclimating to the standard conditions for 7 days, rats were screened and then randomly assigned to the LH or non-stressed control (CON) group. The experimental design is shown in Fig. 1A and B. Rats in the LH group were individually placed in one side of a shuttle box (Academy of Medical Sciences, Shandong, China) and exposed to 60 inescapable footshocks (0.85 mA intensity, 15 s average duration, 15 s average inter-shock interval). Non-stressed control rats were placed in the chambers for the same time without receiving electric footshocks. On the testing day, each animal was allowed to explore the shuttle box for 5 min. The learned helpless behaviors of latency to escape and escape failures were then evaluated using an active escape test consisting of 30 trials of escapable footshocks (0.8 mA intensity, 10 s maximum duration, 30 s average inter-trial interval). The door separating the two sides of the shuttle box was opened at the onset of the test. After the footshock stress exposure, only the susceptible rats identified as decrease in sucrose preference were used for further analysis.



B

	Day1	Day2	Day3	Day4	Day5	Day6	Day7
Week1	IES	---	---	---	---	---	SPT, BW
Week2	IES	IES	---	---	---	---	SPT, BW
Week3	IES	IES	IES	Test	---	---	SPT, BW
Week4	OFT	EPM	FST	Sacrifice			

Fig. 1. (A) The experimental design and procedure and (B) the time schedule for the learned helplessness protocol. IES, inescapable footshocks; Test, active escape test; SPT, sucrose preference test; BW, body weight; OFT, open field test; EPM, elevated plus-maze; FST, forced swimming test; LH, learned helplessness; CON, non-stress control.

Behavioral tests

Sucrose preference test (SPT) and body weight. The SPT was conducted once a week as previously described (Liu et al., 2016). Briefly, rats were habituated to a 1% sucrose solution for 48 h, followed by 6 h of water and food deprivation and then a 1-h exposure to two pre-weighed bottles filled with either 1% sucrose solution or water. Sucrose preference was calculated as the formula of [sucrose intake/(sucrose intake + water intake)] × 100%. Body weight was measured immediately after the SPT for each week.

Locomotor activity test (LAT), forced swimming test (FST), elevated plus-maze (EPM), and open field test (OFT). The procedures for these behavioral tests were adapted from previous studies (Liu et al., 2016). Briefly, we recorded the total distance (cm) during a predetermined period of 5 min in the LAT for 5 min by a video surveillance system (SMART, Panlab SL, Barcelona, Spain), and used to quantify the rats' activity levels. The total immobility time in the FST, indicative of a depressive-like behavior, was recorded for 5 min (Porsolt et al., 1977). The EPM was adopted to assess the anxiety-related behaviors in rats (Huynh et al., 2011). Two independent observers recorded the total number of open/closed arms entries and time spent in open/closed arms during the 5-min session. The OFT was used to assess the spatial exploration behaviors in rodents. Total distance, central activity and rearing frequency were measured during the 5-min test. Each rat was individually transported into the testing room for acclimation at least 6–7 min prior to FST, OFT, and EPM test, and testing alternated between LH and CON rats. These tests were conducted between 9:00 and 17:00 h with an illumination of ~2 lx.