

Original Article

Changes in neurochemical metabolism measured by magnetic resonance spectroscopy and expression of the ERK signaling pathway in a rat model of depression

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Abstract: Objective: To explore the neurochemical metabolism in the brain of rats with chronic unpredictable mild stress (CUMS) using magnetic resonance spectroscopy (MRS). Methods: All rats were anesthetized and scanned in axial, sagittal, and coronal positions with a 7.0 T MRI. The signals of N-acetylaspartate (NAA), choline-containing compounds (CHO), glutamate (GLU), and myo-inositol (MI) were determined by PRESS sequence acquisition ¹H spectra in the hippocampus and prefrontal cortex. After scanning, the target antigen was detected by immunohistochemistry (IHC). Results: The sucrose consumption in the model group was significantly decreased compared with the control group (P<0.05). The level and vertical scores of the model group in an open field experiment were significantly lower than the control group (P<0.05). The ratios of NAA:Cr in the left and right hippocampus, as well as the left and right prefrontal cortex, were decreased in the model group compared with the control group (P<0.05). The ratios of GLU:Cr and CHO:Cr in the left and right hippocampus and the left prefrontal cortex of the model group were both decreased compared with control group (P<0.05). The expression of brain-derived neurotrophic factor (BDNF) and phosphorylated-ERK (p-ERK) were significantly decreased in the model group (P<0.01). Conclusion: Our results showed that the hippocampus and prefrontal cortex are essential in the pathogenesis of depression and that the ERK signaling pathways are important targets for studying the mechanism of action of antidepressants.

Keywords: Depression, rat, magnetic resonance spectroscopy, BDNF, ERK signaling pathway

Introduction

Depression is considered to be the most common mental disorder and comprises a group of mood disorders, which occurs due to various causes, with depressive mood as the main symptom. The pathogenesis underlying depression is complex and involves neural networks and multi-system dysfunction. Although a great deal of research has led to progress, the specific pathogenesis of depression is still not clarified. Therefore, more research and discussion regarding depression are valuable.

Magnetic resonance spectroscopy (MRS) is a method of determining molecular composition and spatial configuration by using the chemical shift phenomenon in magnetic resonance, and

MRS is the only non-invasive technique available for determining the chemical composition of a specific tissue in a living body. MRS plays a critical role in pathophysiological changes, early diagnosis, prognosis, and determination of the curative effect of depression. N-acetylaspartate (NAA), glutamate (Glu), choline-containing compounds (Cho), and myo-inositol compounds (MI) have been studied in the brains of depressed rats by MRS to better understand the intracerebral number and function of neurons, choline metabolism, and cell membrane regeneration, among other things. In recent years, many researchers have successfully used MRS to study depression [1, 2]. Within the complex neural networks, there are many signaling pathways associated with depression. In addition, the sig-

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naling pathways generally correlate with each other. An increasing number of studies have focused on the changes in signal transduction pathways [3].

This study was carried out using the accepted chronic unpredictable mild stress (CUMS) rat model with a focus on the hippocampus and the prefrontal lobe as the encephalic regions for study. Changes in intracerebral substance metabolism of depressed rats were observed by MRS. In addition, the pathogenesis of depression was analyzed and discussed from the perspective of ERK signaling pathway combined with immunohistochemistry (IHC).

Material and methods

Animals

Male Sprague Dawley (SD) rats weighing 220-250 g were purchased from the Beijing Belife Bio-Medical Technology (Beijing, China) and provided by the Experimental Animal Center of the Academy of Military Medical Sciences (certificate number: SCXK [Army] 2012-0004). Animals were housed in a lighted facility at $18^{\circ}\text{C} \pm 4^{\circ}\text{C}$ with water available *ad libitum* for 3 days. Twenty-six rats were randomly divided into the following 2 groups: 16 rats in the CUMS model group; and 10 in the control (CON) group. Rats in the CON group were given ordinary daily care in a safe and calm environment. This study was approved by the Animal Ethics Committee of our institute.

CUMS procedure

The CUMS model was carried out according to the method of Willner et al. [4]. Rats were exposed to different stressors for 6 weeks, including isolated housing, cold water swimming for 5 min at 10°C , overnight illumination, rotation on a shaker for 15 min, tilted cage with 45°C for 24 h, fasting for 24 h, water deprivation for 24 h, tail clipping for 1 min at a 1-cm distance from the tail root, and white noise for 24 h. Each stress stimulus was irregular and discontinuous. After 6 weeks, the sucrose preference and open field tests were performed.

Sucrose preference test

After the stress stimulation test, rats in the two groups were presented with 1% sucrose water. The amount of sucrose consumption was mea-

sured after 1, 2, and 3 h by weighing the water bottles.

Open field test

All rats were tested in an open field, which consisted of a black square base (80 cm \times 80 cm) and black walls (40 cm each). The base was divided into peripheral and central sectors with an equal area of 25 squares. The score of level and vertical activities was recorded for 3 min.

MRI/MRS acquisition

MRI was conducted on a 7.0 T animal MRI scanner (70/20 PharmaScan, Bruker Biospin GmbH, city, Germany), using a 38-mm birdcage rat brain quadrature resonator for radiofrequency transmission and reception. Rats were anesthetized using isoflurane/O₂ (3% for induction and 1.5%-2.5% for maintenance). During the MRI scan, each rat was in a prostrate position on the bed to minimize head motion, whereas respiration was maintained at a rate of 50-60 breaths/min. Scout T2-weighted imaging (T2 WI) was performed in three planes using a TuberRARE sequence. First, an axial scan of the rat head positioning was acquired using the following parameters: repetition time (TR) = 3500 ms; echo time (TE) = 33 ms; field of view = 32 mm \times 30 mm; matrix size = 256 \times 256; slice thickness = 0.8 mm; slice gap = 0 mm; and acquisition time = 7 min 28 s. Next, coronal and sagittal T2 WI scans were acquired. For single-voxel ¹H MRS of the bilateral hippocampus and prefrontal lobe cortex, a 2 mm \times 2 mm \times 2 mm area was selected. After shimming and water suppression, the PRESS sequence acquisition spectrum was as follows: TR = 2000 ms; TE = 35 ms; sampling points to 1024; and total time = 33 min 28 s. All spectra were initially processed using Topspin 5.0 software provided on the scanner. The data between 0.0 and 4.0 ppm were analyzed in the water-suppressed time domain. Thus, the peaks of the major neurotransmitters were NAA at 2.0 ppm, Cho at 3.22 ppm, Glu at 2.35 ppm, MI at 3.56 ppm, and Cr at 3.0 ppm based on *in vivo* ¹H MRS. The ratios of NAA:Cr, CHO:Cr, GLU:Cr, and MI:Cr were calculated.

IHC procedure

After MR scanning, all of the rats were sacrificed and their brains were removed and fixed

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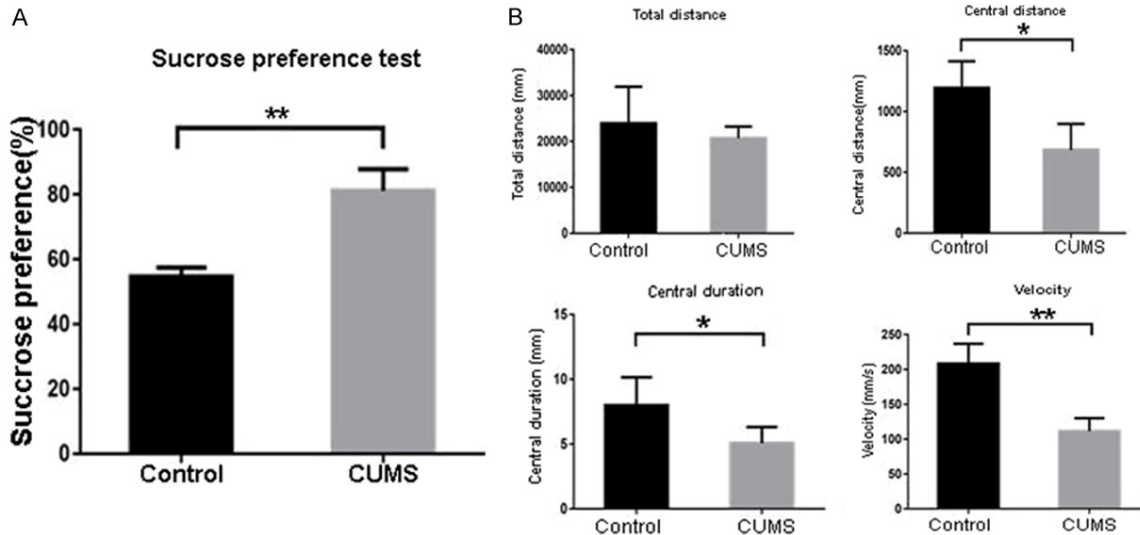


Figure 1. A. The CUMS model group showed a significant reduction in sucrose preference. B. There was a significant effect of CUMS in center distance, center duration, and velocity in the open field test.

in 10% formaldehyde. After dehydration and wax embedding, the specimens were cut in the coronal plane (4- μ m sections). The sections were dewaxed and antibodies were added. Finally, all sections were observed under an Olympus microscope (400 \times) after dehydration and mounting.

Statistical analysis

Data are expressed as the mean \pm SEM. Statistical analyses were carried out using SPSS 20.0 software (SPSS, Inc., Chicago, IL, USA). T-tests were used to determine the difference between the model and control groups. The significance level was set at a $P \leq 0.05$.

Results

Sucrose preference test

The CUMS model group had a significant reduction in sucrose preference compared with the CON group (54.64 \pm 2.81 [CON] vs. 81.12 \pm 6.7 [CUMS], $P < 0.05$ [t-test]; **Figure 1A**).

Open field test

The on-center distance in the CUMS group (684.43 \pm 213.4) was significant compared with the CON group (1195.91 \pm 217.96, $P < 0.05$). The center duration in the CUMS group (5.07 \pm 1.25) was significant compared with the CON group (8 \pm 2.16; $P < 0.05$). The velocity in the CUMS

group (208.72 \pm 28.11) was significant compared with the CON group (111.66 \pm 18.48, $P < 0.01$; **Figure 1B**).

Ratios of NAA:Cr, CHO:Cr, GLU:Cr, and MI:Cr in the hippocampus and prefrontal cortex

The ratios of NAA:Cr in the left and right hippocampus, and ratios in the left and right prefrontal cortex were significantly decreased in depressed rats compared with the CON group (1.19 \pm 0.14 [CON] and 1.08 \pm 0.1 [CUMS] in the left hippocampus, $P < 0.05$; 1.14 \pm 0.67 [CON] and 1.08 \pm 0.08 [CUMS] in the right hippocampus, $P < 0.05$; 1.2 \pm 0.23 [CON] and 0.98 \pm 0.19 [CUMS] in the left prefrontal cortex, $P < 0.05$; 1.25 \pm 0.22 [CON] and 1.09 \pm 0.15 [CUMS] in the right prefrontal cortex, $P < 0.05$). The ratios of GLU:Cr in the left and right hippocampus and ratios in the left prefrontal cortex were significantly decreased in depressed rats compared with the CON group (0.41 \pm 0.09 [CON] and 0.28 \pm 0.1 [CUMS] in the left hippocampus, $P < 0.01$; 0.38 \pm 0.13 [CON] and 0.27 \pm 0.05 [CUMS] in the right hippocampus, $P < 0.01$; 0.45 \pm 0.07 [CON] and 0.33 \pm 0.06 [CUMS] in the left prefrontal cortex, $P < 0.01$). The ratios of CHO:Cr in the left and right hippocampus were significantly decreased in the CUMS group compared with the CON group (0.67 \pm 0.12 [CON] and 0.57 \pm 0.11 [CUMS] in the left hippocampus, $P < 0.05$; 0.67 \pm 0.15 [CON] and 0.54 \pm 0.09 [CUMS] in the right hippocampus, $P < 0.05$; 0.64 \pm 0.11 [CON]

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Table 1. Comparison of ¹H-MRS in hippocampus between the CON and CUS

Neurochemical metabolism	L hippocampus		R hippocampus	
	CON	CUS	CON	CUS
NAA:Cr	1.19±0.14	1.08±0.1*	1.14±0.67	1.08±0.08*
Glu:Cr	0.41±0.09	0.28±0.1*	0.38±0.13	0.27±0.05*
Cho:Cr	0.67±0.12	0.57±0.11*	0.67±0.15	0.54±0.09*
MI:Cr	0.24±0.05	0.25±0.05	0.26±0.05	0.25±0.04

*P<0.05.

Table 2. Comparison of ¹H-MRS in prefrontal cortex between the CON and CUS

Neurochemical metabolism	L prefrontal cortex		R prefrontal cortex	
	CON	CUS	CON	CUS
NAA:Cr	1.2±0.23	0.98±0.19*	1.23±0.24	1.09±0.15*
Glu:Cr	0.45±0.07	0.33±0.06*	0.36±0.11	0.36±0.12
Cho:Cr	0.64±0.11	0.55±0.08*	0.61±0.15	0.52±0.12
MI:Cr	0.28±0.07	0.22±0.09	0.21±0.05	0.22±0.08

*P<0.05.

and 0.55±0.08 [CUMS] in the left prefrontal cortex, P<0.05; **Tables 1** and **2**; **Figure 2A** and **2B**).

Expression of BDNF and p-ERK in the hippocampus and prefrontal cortex

The expression of brain-derived neurotrophic factor (BDNF) in the hippocampus and the prefrontal cortex were significantly decreased in the CUMS group compared with the CON group (0.001518±0.000572 [CON] and 0.000248±0.000278 [CUMS] in the hippocampus, P<0.01; 0.005214±0.001569 [CON] and 0.001734±0.001805 [CUMS] in the prefrontal cortex, P<0.01). The expression of p-ERK in the hippocampus and the prefrontal cortex were significantly decreased in the CUMS group compared with the CON group (0.013495±0.005621 [CON] and 0.000689±0.000564 [CUMS] in the hippocampus, P<0.01; 0.001351±0.001353 [CON] and 0.000106±0.000262 [CUMS] in the prefrontal cortex, P<0.05; **Table 3**; **Figure 3A** and **3B**).

Discussion

There were several major peak signals in ¹H MRS (NAA, Glu, Cho, and MI). Cr is a symbol of energy metabolism *in vivo*, and is commonly used as an internal standard to compare the changes in other metabolites [5]. Cr includes

creatine and creatine phosphate, which are stable in various physiologic and pathologic conditions. NAA has the highest peak within the normal ¹H spectrum, which is primarily located in neurons and synapses and is recognized as a neuronal marker [6]. The NAA level is an indicator of an increase or decrease in the number of neurons. A decrease in the NAA:Cr ratio suggests loss of neurons or a disorder of neurologic function [2]. Various researchers have used ¹H MRS to detect abnormal levels of neurobiochemical metabo-

lites in different regions of the prefrontal lobe and hippocampus in patients with depression [7, 8]. Gonul et al. [9] conducted a study focusing on the medial frontal cortex of patients with depression, indicating that the NAA:Cr ratio in this region was lower than the normal control group. Therefore, the corresponding results suggest that in patients with depression, the level of frontal cortex neuronal activity is decreased, followed by a lower level of neuronal viability or density, eventually resulting in dysfunction in the encephalic region. Xi et al. [10] reported that the NAA:Cr ratio in the hippocampus of rats was significantly lower than the control group using the CUMS model. In this study the NAA:Cr ratio significantly decreased in the left and right hippocampus as well as the left and right prefrontal cortex in depressed rats compared to controls, suggesting neuron damage and dysfunction in the hippocampus and prefrontal cortex of the CUMS model.

CHO reflects the storage of total choline in the brain, which is one of the main components of the cytomembrane phospholipid metabolism. [CHO or choline?] is also the precursor of neurotransmitter acetylcholine and changes reflect renewal of the cell membrane. At present, there is no consensus on the interpretation of the CHO peak, and there are many contradictory studies. An international study [11] document-

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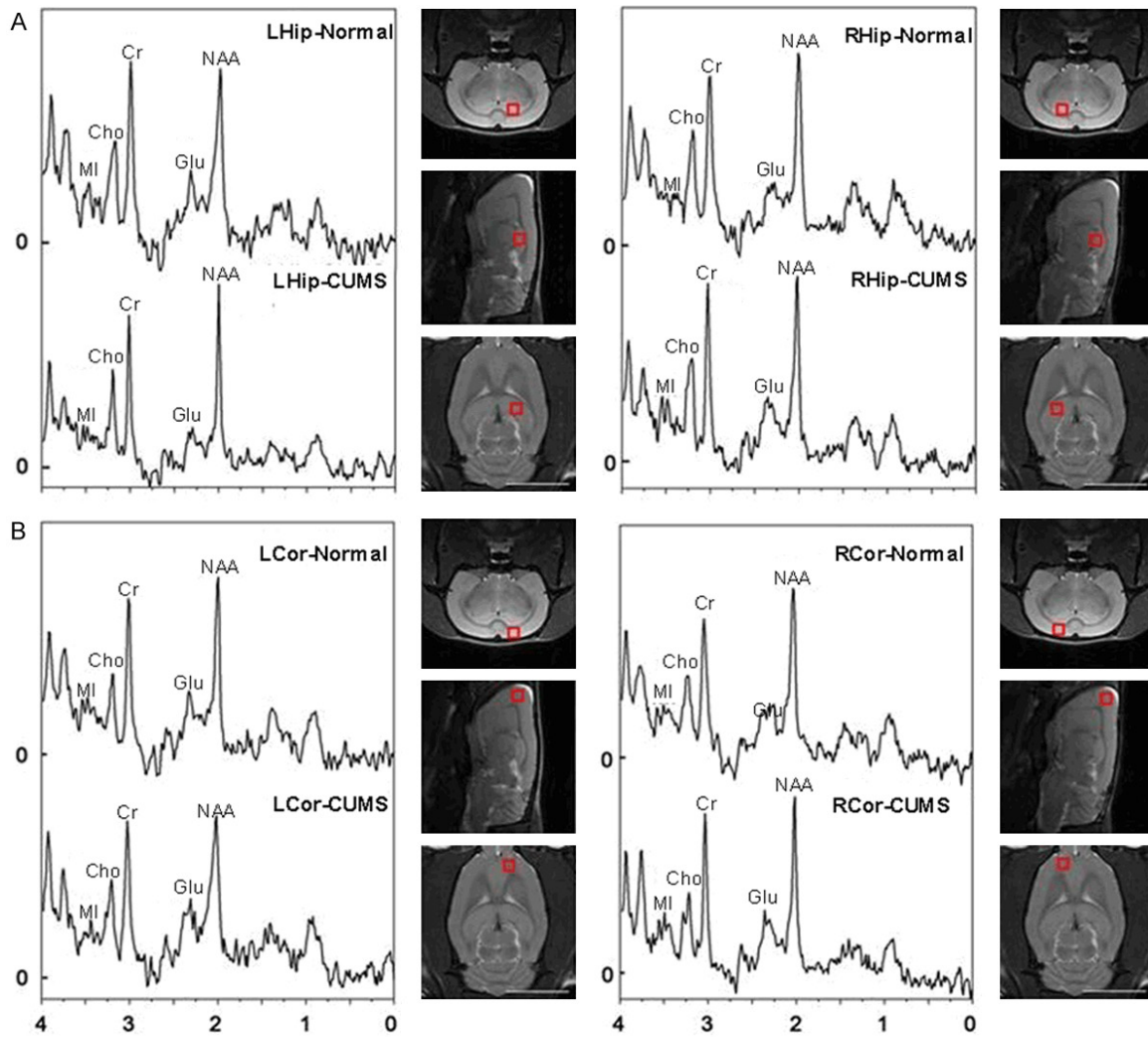


Figure 2. A. Voxel areas for the hippocampus. Typical proton MR spectra in the left and right hippocampus of a rat with depression (lower) and a control rat (upper). B. Voxel areas for the prefrontal cortex. Typical proton MR spectra in the left and right prefrontal cortex of a rat with depression (lower) and a control rat (upper).

Table 3. Comparison of BDNF, ERK1/2, and P-ERK1/2 in the hippocampus and prefrontal cortex between the CON and CUS

	Hippocampus		Prefrontal cortex	
	CON	CUS	CON	CUS
BDNF	0.001518±0.000572	0.000248±0.000278 [#]	0.005214±0.001569	0.001734±0.001805 [#]
ERK1/2	0.004783±0.004454	0.019889±0.018923	0.001648±0.000889	0.001048±0.000817
P-ERK1/2	0.013495±0.005621	0.000689±0.000564 [#]	0.001351±0.001353	0.000106±0.000262 [*]

[#]P<0.01, ^{*}P<0.05.

ed that the CHO signal in the hippocampus region of patients with depression was reduced compared with the normal control group, revealing the occurrence of abnormal membrane phospholipid metabolism. In addition, this abnormality could be improved by electric shock

treatment. Based on research involving adolescents with depression, Ning et al. [7] found that the CHO:Cr ratio in the white matter of the left dorsolateral prefrontal in the depression group was significantly lower than the control group. An experimental study by Hong et al. [12] found

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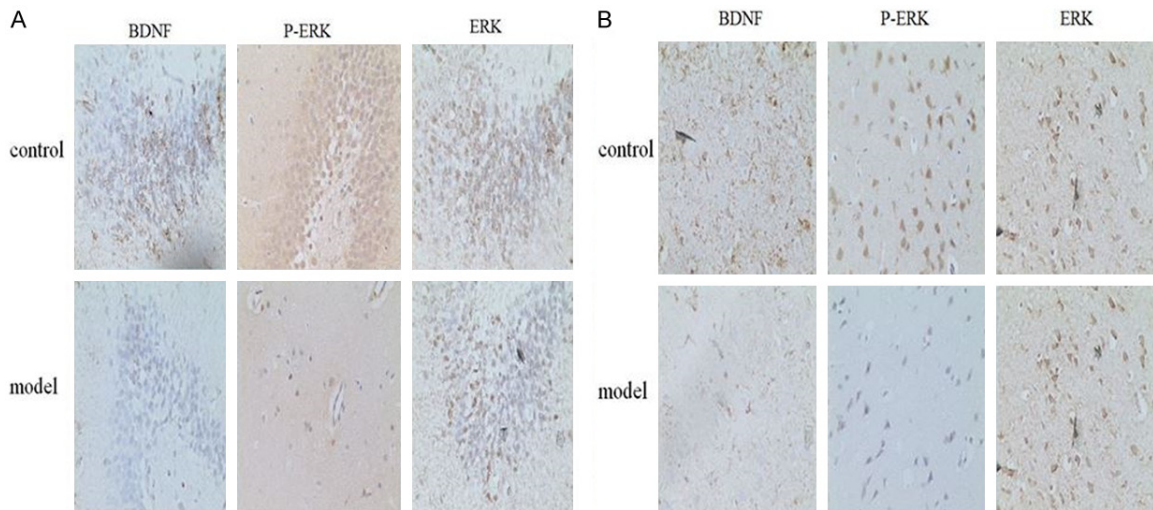


Figure 3. A. Expression of BDNF, P-ERK, and ERK in the hippocampus. Neuron stain is very light; decreased neurons in BDNF and P-ERK. B. The expression of BDNF, P-ERK, and ERK in the prefrontal cortex. Neuron stain is very light; decreased neurons in BDNF and P-ERK.

that the CHO:Cr ratio in the left hippocampus of depressed rats decreased significantly, which was considered to be an essential factor in major pathophysiologic changes of depression. In the current study the CHO:Cr ratios of the left and right hippocampus, and the CHO:Cr ratio of the left prefrontal cortex, were significantly lower than the CON group, suggesting that the regeneration function of the cell membrane was impaired in the hippocampus and the left prefrontal cortex of depressed rats.

GLU is the most important excitatory neurotransmitter in the central nervous system and plays a critical role in the differentiation, migration, growth, and survival of neurons. Glutamic acid can modulate the postsynaptic potentials of some neurons, such as dopaminergic neurons, which affects the physiologic functions of other neurotransmitters. The normal metabolism of glutamic acid depends on the normal function of neurons and neuroglial cells. Therefore, some researchers have suggested that a decrease in glutamic acid levels in the brain of patients with depression coincides with the morphologic changes in the hippocampus and cortex of depressed patients [13], which includes changes in the number of neurons and neuroglial cells, thus indicating dysfunction of the glutamatergic system. Previous results have revealed a significant decrease in glutamate in the hippocampus and prefrontal cortex of CUMS animals [2]. In the current study, the GLU:Cr ratios in the left and right hippocampus,

and GLU:Cr ratio in the left prefrontal cortex were reduced significantly compared with the CON group, indicating that dysfunction of glutamatergic neurons might be the main pathologic change accompanying depression.

As a second messenger, MI can balance the functions among various neurotransmitter systems, therefore having a great influence on adjusting nerve signals and downstream cellular and molecular responses. In an analysis conducted by Yildiz et al. [14], the MI:Cr ratio of the left dorsolateral prefrontal cortex was significantly higher than the control group, suggesting that there may be abnormal signal transduction in nerve cells and abnormal phospholipid metabolism in the cell membrane. The results of our study showed that the MI:Cr ratios in the hippocampus and left prefrontal cortex were increased in depressed rats, confirming that an imbalance of the second messenger system has a role in the pathologic mechanism underlying depression.

There is currently an increasing concern about the role of the mitogen activated protein kinase (MAPK)/ERK signaling pathway in the central nervous system. Due to the complex pathogenesis of depression, further study of the ERK signaling pathway may contribute to a deeper understanding, thus providing targets for new diagnostic and therapeutic techniques for the treatment of depression.

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Prior evidence has shown that the level of BDNF in the hippocampus and prefrontal cortex is decreased significantly in patients with depression [15], indicating that BDNF has an antidepressant effect that is achieved via multiple pathways and action sites. ERK is the earliest known kinase in the MAPK family, and is divided into ERK1 and ERK2, which are collectively known as ERK1/2. At the same time, ERK is a key molecule that transmits signals from the surface receptors to the nucleus and requires a cascade reaction of three enzymes in the MAPK signaling pathway to induce extracellular stimulation to cells to stimulate corresponding biological effects [16]. In addition, ERK1/2 activated by phosphorylation is translocated from the cytoplasm to the nucleus, acts on transcription factors, such as Ets-like-protein 1, activating transcription factor, and nuclear transcription factor- κ B, and promotes the transcription and expression of some genes [17, 18]. In addition, Yuan et al. [19] studied the role of the ERK pathway in neuronal plasticity and elasticity in patients with mental disorders. The findings indicated that during autopsy, the protein levels of ERK1/2 in the frontal cortex of patients with bipolar disorders and schizophrenia declined considerably compared with the frontal lobes of healthy subjects without mental illness. In addition, Yu et al. [20] reported that the ERK and phosphatidylinositol 3-kinase (PI3K) signaling pathways were both mediated [modulated?] in the hippocampus, whereas in the prefrontal cortex, ERK signaling pathway was primarily mediated [modulated?] to assume a neurotrophic role in brain neurons. Therefore, activation of the two pathways has an impact on protecting cranial nerves. In contrast, inhibition of the two pathways may produce or aggravate depression. In the current study the results showed that the levels of BDNF, ERK1/2, and p-ERK expression in the hippocampus and prefrontal cortex were decreased significantly compared with the control group, suggesting that a depressed mood is associated with low expression of BDNF, ERK1/2, and p-ERK, and the BDNF-ERK signaling pathway is closely related to depression.

The pathogenesis of depression is complex, involving multiple brain regions in which the hippocampus and frontal lobe can regulate changes in mood and behavior by various mechanisms [21]. Accordingly, the hippocampus

and frontal lobe were the focus of the current study. The hippocampus is a high-level center of mental activities in animals, as well as a susceptible site of stress injury, suggesting that the hippocampus has a strong relationship with the regulation of emotions. Based on autopsies of depressed patients, the hippocampus is atrophied [22]. In addition, Natalia et al. [23] suggested that the nerve regeneration ability of the hippocampus can be restored to normal via short-term stimulation in experimental animals; however, if rats received a long-term unpredictable stimulus, nerve regeneration of the hippocampus is damaged without any possibility of restoration. In addition, important hippocampal proteins are involved in the regulation of emotion through different signaling pathways. The frontal lobe accounts for approximately 40% of the cerebral cortex and is the executive center of brain activity. Structurally, the frontal lobe can be divided into four parts, among which the prefrontal cortex plays an important role as the emotional center.

In the current study MRS and IHC were used to study the bilateral hippocampus and bilateral prefrontal cortex of depressed rats. The changes in spectra were consistent with histologic changes, indicating that both the hippocampus and prefrontal cortex play important roles in the pathogenesis of depression. In addition, the MRS results showed that there was a specific difference in the allogenic metabolites of the bilateral prefrontal cortex, which was consistent with the results of previous studies. Consequently, this study supports the view of an imbalance in hemispheres underlying the pathophysiologic process of depression.

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pared the manuscript. Bin Wang reviewed and revised the manuscript.

Disclosure of conflict of interest

None.

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