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Original Article

Effects of BDNF-ERK-CREB signaling pathways on cognitive function and neural plasticity in a rat model of depression

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Abstract: Objective: The aim of the current study was to investigate the effects of BDNF-ERK-CREB signaling pathways on cognitive function and neural plasticity in a rat model of depression. Methods: A total of 60 clean-grade male Wistar rats were obtained, with 10 randomly selected as the normal group. The remaining rats were used to establish a rat model of depression. They were divided into 4 groups, including the model group (without any treatment), negative control (NC) group (intraperitoneally injected with saline), PMA group (intraperitoneally injected with Phorbol 12-myristate 13-acetate (PMA), which is an activator of BDNF-ERK-CREB signaling pathway), and U0126 group (intraperitoneally injected with U0126, which is an inhibitor of BDNF-ERK-CREB signaling pathway). Behavioral indicators of depression, including body weight changes, sugar water preferences, and immobility times in the tail suspension test were measured. H&E staining was used to detect pathological changes of hippocampal neurons. Moreover, qRT-PCR and Western blotting were performed to detect expression levels of extracellular regulated protein kinases 1/2 (ERK1/2), phosphorylated ERK1/2 (p-ERK1/2), cAMP-response element binding protein (CREB), phosphorylated CREB (p-CREB), brain-derived neurotrophic factor (BDNF), glutamate receptor 1 (GluR1), glial fibrillary acidic protein (GFAP), and soluble protein-100β (S100β). Expression levels of inflammatory factors (5-hydroxytryptamine (5-HT), BDNF, interleukin-6 (IL-6), tumor necrosis factor α (TNF-α), and interferon-γ (IFN-γ)) were measured by ELISA. Results: After 28 days of continuous stimulation, body weights and sugar water preferences of depression model rats decreased more significantly, compared with normal rats. Immobility times in the tail suspension tests increased significantly (all $P<0.05$), indicating that modeling was successful. Compared with the normal group, body weights and sugar water consumption and preferences, as well as mRNA and protein expression levles of ERK1/2, p-ERK1/2, CREB, p-CREB, GluR1, GFAP, 5-HT, and BDNF, were reduced in other groups (all $P<0.05$). However, pure water consumption, immobility times in the tail suspension test, and mRNA and protein expression levels of S100β, IL-6, TNF-α, and IFN-γ were elevated in the other groups (all $P<0.05$). Compared with the model group, body weights, sugar water consumption and preferences in the PMA group, as well as mRNA and protein expression of ERK1/2, p-ERK1/2, CREB, p-CREB, GluR1, GFAP, 5-HT, and BDNF, were significantly increased (all $P<0.05$). In contrast, pure water consumption, immobility times in the tail suspension test, and mRNA and protein expression levels of S100β, IL-6, TNF-α, and IFN-γ were decreased in the PMA group (all $P<0.05$). Compared with the model group, results in the U0126 group were opposite to those in the PMA group (all $P<0.05$). There were no significant differences in indicators between the model group and NC group (all $P>0.05$). Conclusion: Activation of BDNF-ERK-CREB signaling pathways inhibited the release of inflammatory factors in a rat model of depression. Furthermore, it enhanced neural plasticity, improved cognitive function, and promoted recovery from depression. Contrarily, inhibition of BDNF-ERK-CREB signaling pathways aggravated depression in a rat model.

Keywords: BDNF-ERK-CREB signaling pathway, depression rat, cognitive impairment, neural plasticity

Introduction

Depression is a mental disorder, characterized by high prevalence, recurrence rates, disability rates, and mortality rates [1, 2]. Depression not only leads to negative emotions, but also damages normal human physical functions. It may even cause death. Statistics have shown that

nearly 20% of people around the world suffer from depression. By 2030, depression is expected to be the main cause of the worldwide burden of disease [3]. At present, there are many problems with using antidepressant medications, including low efficiency, slow onset of action, and considerable side effects. Therefore, development of new antidepressant treatments is a top priority.

In addition to persistently low moods, cognitive impairment is a common symptom of depression. Patients with cognitive impairment may manifest impaired thinking ability, difficulties in concentration, and memory loss [4]. At present, cognitive impairment has become one of the criteria for diagnosis of depression, according to American Diagnostic and Statistical Manual of Mental Disorders (5th Edition) (DSM-5) and International Classification of Diseases (10th Edition) (ICD-10) [5]. Previous studies have found that causes of cognitive impairment in depression may be related to structural changes in the nervous system and neurotransmitters, as well as other factors [6, 7].

In recent years, studies have found that structural changes and functional impairment of some brain regions of the limbic system have also occurred in animal models of depression. The area closely related to depression is the hippocampus. Astrocytes, fundamental neural cells in the hippocampus, play an important role in hippocampal neuronal regulation, nerve regeneration, and synaptic remodeling. Astrocytes are characterized by cell markers, such as glial fibrillary acidic protein (GFAP) and central nervous-specific protein (S100β). Antidepressants can activate intracellular signaling pathways that promote neural plasticity and reverse pathological changes caused by depression. This suggests that roles of neural plasticity mechanisms in the pathogenesis and treatment of depression. Brain derived neurotrophic factor (BDNF), a member of the neurotrophic factor family, can induce neuronal structural changes by stimulating the hippocampus, amygdala, and prefrontal cortex through related pathways [8, 9]. BDNF-ERK-CREB signaling pathways play an important role in neural differentiation and development of synaptic function, while maintaining normal physiological functioning of central and peripheral nervous systems. It also contributes to learning, memory formation, and maintenance [10, 11]. At the same time, BDNF is involved in the pathogenesis of various mental disorders, such as schizophrenia. However, the roles of BDNF-ERK-CREB signaling pathways in the pathogenesis of depression are not fully understood, requiring further investigation.

The current study explored treatment mechanisms of depression based on cognitive impairment and neural plasticity through BDNF-ERK-CREB signaling pathways, aiming to enrich treatment methods for depression.

Materials and methods

Animal modeling

A total of 60 clean-grade male Wistar rats (Shanghai Slack Laboratory Animals Co., Ltd., China), weighing 205.25±6.27 g at 7-8 weeks old, were obtained. All rats were individually housed in mesh cages at a temperature of 25°C under 12-hour light (06:00-18:00) and 12-hour dark (18:00-06:00) cycles. Ten of them were randomly selected as the normal group. The rest were used for depression modeling. The modeling method was based on the study by Chen [12]. Rats experienced continuous stimulation for 28 days, including food and water deprivation (24 hours), wet bedding (24 hours), cage tilting (45°C, 24 hours), light and dark reversal (24 hours), and heat stress (45°C, 5 minutes), followed by ice water baths (4°C, 5 minutes), tail pinching (1 minute), and horizontally cage shaking (80 times/min, 20 minutes). Only one stimulation method was used each day and the same stimulation method did not appear for 2 consecutive days. To avoid influencing the other animals, the rats were individually brought to the procedure room to receive the stimuli, except for food and water deprivation. All rats were returned to the animal breeding room at the same time upon completion of stimulation. Rats in the normal group were given free access to drinking water and diet without any stimulation. This study was approved by the Laboratory Animal Ethics Committee of School of Pharmacy, Binzhou Medical University, complying with the principles of animal care and use.

Animal grouping

After 28 days of continuous stimulation, body weight changes, sugar water preferences, and

immobility times in the tail suspension test were measured. Compared with normal rats, body weights and the sugar water intake of the model rats decreased significantly. Immobility times in the tail suspension test significantly increased, indicating that modeling was successful. Successfully modeled depression rats were divided into 4 groups, including the model group (without any treatment), NC group (intraperitoneally injected with 10 mg/kg saline (Qingdao Nisuvi Sehuu Biotechnology Co., Ltd., China)), PMA group (Intraperitoneally injected with 10 mg/kg Phorbol 12-myristate 13-acetate (PMA) (Beijing Biolab Technology Co., Ltd., China), which is an activator of BDNF-ERK-CREB signaling pathway), and U0126 group (intraperitoneal injection of 10 mg/kg U0126 (Shanghai GeGene Biotechnology Co., Ltd., China), which is a BDNF-ERK-CREB signaling pathway inhibitor). The next day, after successful modeling, the rats received intraperitoneal injections according to the protocol. They were received once a day for 3 consecutive days. On the 37th day of the experiment, all rats underwent behavioral tests [13]. A total of 5 mL of tail blood was collected before they were euthanized by cervical dislocation under anesthesia. Tissues were harvested for subsequent experiments.

Body weight gain

Body weights of the rats were measured 1 day before the experiment and on the 37th day of the experiment. Weight gain = (body weight at 37th day-body weight at 1 day before the experiment).

Sugar water intake testing

Sugar water intake testing was conducted based on the study by Duan et al. [14]. On the 37th day, the animals were trained to adapt to sugary drinking water by placing 2 bottles of 1% sugar water in each cage. After adaptation, each group of rats was subjected to sugar water intake testing, after food and water deprivation for 24 hours. The rats were given one bottle of 1% sugar water and one bottle of pure water at the same time. After 24 hours, the two bottles were retrieved and weighed. Sugar water consumption, pure water consumption, and sugar water preferences were calculated for each animal. Sugar water preference (%) = sugar water consumption/total liquid consumption * 100%.

Tail suspension testing

On the 40th day after completion of sugar water intake testing, the rats were suspended 35 cm from the ground by taping 1.5 cm-2 cm tail to the suspension rack with their heads pointing downwards. They were left dangling for 6 minutes. Times from the rats ceasing efforts to get to an upright position to the end of the test (immobility times) were measured.

Tissue harvesting

After completion of all behavioral tests, 5 mL of tail vein blood was taken and stored at -80°C. The rats were anesthetized with pentobarbital sodium (Hubei Hongyun Long Biological Technology Co., Ltd., China) and sacrificed by cervical dislocation. The brain was removed. The hippocampus was isolated. One part of the hippocampus was frozen at -80°C. the other part was used for paraffin sections, which were 4 μm thick.

H&E staining

Paraffin sections were routinely dewaxed and stained with hematoxylin (Beijing Leagene Biotechnology Co., Ltd., China) for 10 minutes at room temperature. Sections were rinsed with tap water for 60 seconds, then dipped in 1% hydrochloric alcohol for 1 minute. Sections were rinsed with tap water again for 1 minute and stained with eosin (Shanghai Bogoo Biotechnology Co., Ltd., China) at room temperature for 5 minutes. Sections were washed with distilled water, then dehydrated using a gradient of ethanol for 1 minute. Sections were deparaffinized in xylene twice, for 1 minute each time, then sealed with Permount. An optical microscope (Boshida Optical Instrument Co., Ltd., China) was used to observe and photograph morphological changes.

Quantitative real time PCR (qRT-PCR)

Total RNA was extracted from the hippocampus of each animal, according to manufacturer instructions of TRIzol Reagent (Invitrogen, USA). Extracellular regulated protein kinase (ERK), cAMP-response element binding protein (CREB), BNDF, glutamate receptor 1 (GluR1), GFAP,

Table 1. qRT-PCR primer sequences

Gene	Primer sequence
ERK1	F: 5'-CTGGCTTTCTGACCGAGTATGTG-3'
	R: 5'-CAATTTAGGTCCTCTTGGGATG-3'
ERK2	F: 5'-GACATGGAGCTGGACGACTTAC-3'
	R: 5'-GGGACACCGACATCTGAACG-3'
CREB	F: 5'-GCAGTGACTGAGGAGCTTGT-3'
	R: 5'-ACTCTGCTGGTTGTCTGCTC-3'
BDNF	F: 5'-GTGACAGTATTAGCGAGTG-3'
	R: 5'-TATCCTTATGAACCGCCAGCC-3'
GluR1	F: 5'-CCATCAATGAAGCCA TACG-3'
	R: 5'-TCTGCTCCAGTTACAATCC-3'
GFAP	F: 5'-CAGAGAACAACCTGGCTGCG-3'
	R: 5'-AGCGTCTGTCGAGTCTGCAA-3'
S100β	F: 5'-TTTTAAGCTTCACCATGTGTGAGCTGGAGAAGGC-3'
	R: 5'-TTTTCTCGAGTTGCATGACCGTCTCTGTTACAG-3'
GAPDH	F: 5'-GGTGTGAACCACGAGAAATATGAC-3'
	R: 5'-TCATGAGCCCTTCCACAATG-3'

Note: ERK1: Extracellular regulated protein kinase 1; ERK2: Extracellular regulated protein kinase 2; CREB: cAMP-response element binding protein; BDNF: Brain-derived neurotrophic factor; GluR1: Glutamate receptor 1; GFAP: Glial fibrillary acidic protein; S100β: Soluble protein-100β; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

S100β, and GAPDH primers were designed and synthesized by Takara Bio USA, Inc. (**Table 1**). RNA was reverse transcribed into cDNA according to manufacturer instructions of the Reverse Transcription Kit (Fermentas, USA). The master mix was subjected to qRT-PCR in accordance with instructions of SYBR® Premix Ex TaqTM II Kit (Xingzhi Biotechnology Co., Ltd., China). The total reaction volume was 50μL: SYBR® Premix Ex TaqTM II (2×) 25 μL, PCR upstream primer 2 μL, PCR downstream primer 2 μL, ROX Reference Dye (50×) 1 μL, DNA template 4 μL, and ddH_2O 16 μL. Moreover, qRT-PCR was performed using the ABI PRISM® 7300 system (Shanghai Kunke Instrument Co., Ltd., China). Reaction conditions were pre-denaturation at 95°C for 10 minutes, denaturation at 95°C for 15 seconds, and annealing at 60°C for 30 seconds. After 32 cycles, extension at 72°C was applied for 1 minute. GAPDH was used as an internal reference. Furthermore, $2^{-\Delta\Delta Ct}$ indicates the ratio of gene expression of each group to the normal group. The formula is as follows: $\Delta\Delta CT = \Delta Ct_{experimental\ group} - \Delta Ct_{control\ group}$.

Western blotting

Hippocampus tissues of each animal were freshly frozen in liquid nitrogen and ground to fine powder. Next, 1 mL of tissue lysis buffer (Beijing Solarbio Technology Co., Ltd., China) was added and the mixture was homogenized on ice. Cell lysis buffer (Beijing Solarbio Technology Co., Ltd., China) was added and the mixture was incubated at 4°C for 30 minutes. Samples were centrifuged for 15 minutes at 4°C and 12,000 r/min. The supernatant was collected to determine protein concentrations, using the BCA Protein Assay Kit (Pierce, USA). Protein concentrations were adjusted to 1 μg/μL and loaded to the sample well with a volume of 20 μg per well. Protein was separated by electrophoresis on a 10% SDS-PAGE gel (Beijing Solarbio Technology Co., Ltd., China). Separated protein was transferred to PVDF membranes (Millipore, United States). Membranes were washed with tris buffered saline tween (TBST) twice for 10 minutes each time. Afterward, 5% skim milk was used to block membranes at room temperature for 2 hours. Membranes were washed with TBST 3 times for 10 minutes each. The following mouse primary antibodies were added, respectively: p-ERK1/2 (1:1,000, Abcam, UK), p-CREB (1:5,000, Abcam, UK), BNDF (1:100, Abcam, UK), GluR1 (1:2,000, Abcam, UK), GFAP (1:10,000, Abcam, UK), S100β (1:1,000, Abcam, UK), and GAPDH (1:1,000, Abcam, UK). Membranes were incubated at 4°C overnight. They were rinsed with TBST 3 times for 10 minutes each. HRP-labeled goat anti-mouse I gG secondary antibody (1:2,000, Abcam, UK) was added and the membranes were incubated for 2 hours at room temperature. They were rinsed with TBST 3 times, for 10 minutes each time, before immersion in the DAB color development solution. A gel imaging system (Bio-Rad, USA) calculated the ratio of the gray value of the protein of interest to the internal reference, calculated as the relative expression level of the protein.

5-HT, BDNF, IL-6, TNF-α, and IFN-γ expression levels measured by ELISA

Determination of 5-HT and BDNF expression levels: Hippocampal tissues of each animal mixed with 2 mL PBS were homogenized in a tissue homogenizer. The tissue homogenate was centrifuged at 4°C and 3,000 rpm for 20 minutes. The supernatant was transferred into

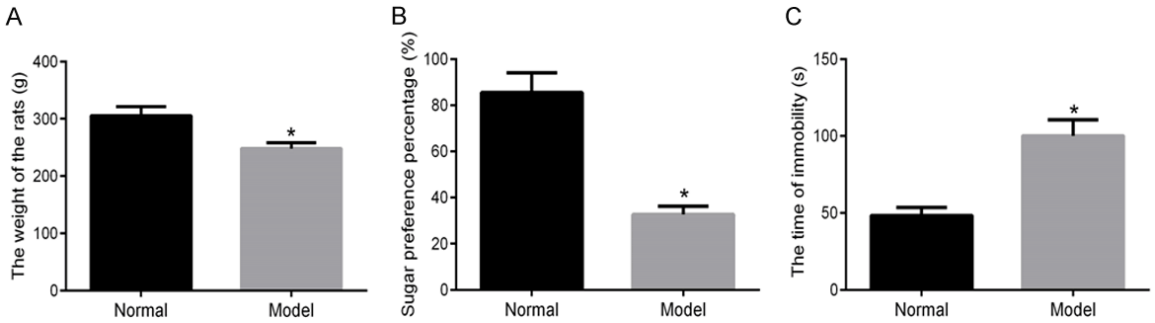

Figure 1. Comparison of body weights, sugar water preferences, and immobility times in the tail suspension test. A: Body weights of normal rats and depression model rats; B: Sugar water preferences of normal rats and depression model rats; C: Immobility times of normal rats and depression model rats in the tail suspension test; *P<0.05 compared with the normal group.

a 1.5 mL centrifuge tube. Expression levels of 5-HT and BDNF in the hippocampus were determined according to manufacturer instructions of the ELISA kit (BD, USA).

Determination of IL-6, TNF-α, and IFN-γ expression levels: Serum of each animal was centrifuged at 3,000 r/min for 30 minutes. The measurement procedure was in accordance with manufacturer instructions of IL-6, TNF-α, and IFN-γ ELISA kits (Shenzhen Jingmei Bioengineering Co., Ltd., China). After measurement, optical density (OD) values of each well, at a wavelength of 450 nm, were read by means of a microplate reader (BioTek Synergy 2, USA). A standard curve was depicted with the concentration of the reference standard as the abscissa and OD values as the ordinate.

Statistical analysis

All data were processed using SPSS 21.0 software. Quantitative data are expressed as mean ± standard deviation ($\bar{x}$ ± sd). Comparisons between multiple groups were analyzed by one-way ANOVA, followed by Bonferroni's post-hoc test. P<0.05 indicates that differences are statistically significant.

Results

Successful modeling

After 28 days of continuous stimulation, compared with the normal group, body weights and sugar water preferences of depression model rats decreased significantly. Immobility times in the tail suspension test increased significantly (all P<0.05), indicating that modeling was successful. See **Figure 1**.

Comparison of body weights

Body weight changes of each group are shown in **Figure 2**. There were no significant differences between groups before the experiment (all P>0.05). Compared with the normal group, body weights and weight gain of the other groups significantly decreased on the 37th day (all P<0.05). Compared with the model group, body weights and weight gain of the PMA group significantly increased on the 37th day (all P<0.05), while body weights and body weight gain of the U0126 group on the 37th day significantly decreased (all P<0.05). There were no differences in body weights and body weight gain between the model group and NC group (all P>0.05). Compared with the PMA group, body weights and weight gain of the U0126 group decreased significantly on the 37th day (all P<0.05).

Comparison of sugar water intake

Sugar water intake levels of each group are shown in **Figure 3**. Compared with the normal group, sugar water intake and preferences were significantly lower in the other groups. Pure water consumption was significantly increased (all P<0.05). Compared with the model group, sugar water intake and preferences of the PMA group increased significantly. Consumption of pure water decreased significantly (all P<0.05). Contrarily, sugar water intake and preferences of the U0126 group decreased, while pure water consumption increased significantly (all P<0.05). There were no significant differences between the model group and NC group regarding those parameters (all P>0.05). Compared with the PMA group, sugar water intake and preferences in

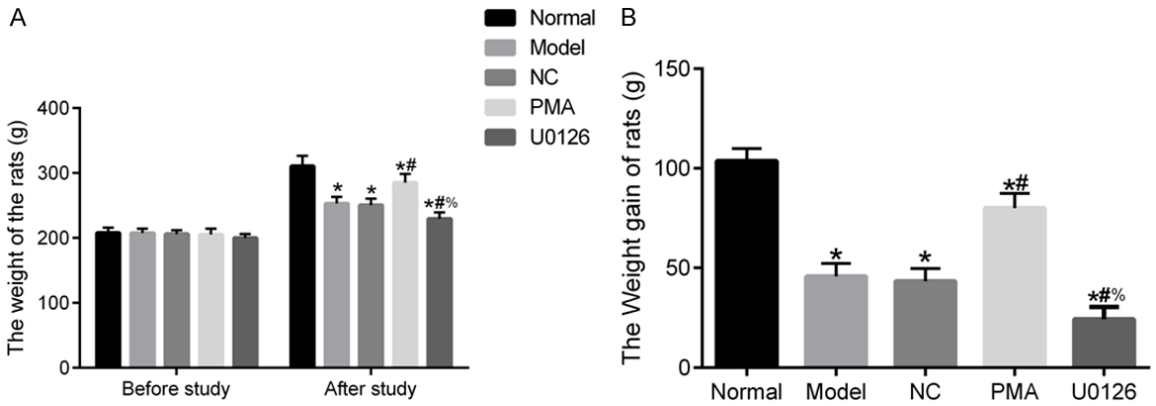

Figure 2. Body weight changes of each group. A: Body weights of each group at 1 day before the experiment and on the 37th day of the experiment; B: Body weight gain of each group; *P<0.05 compared with the normal group; #P<0.05 compared with the model group; %P<0.05 compared with the PMA group.

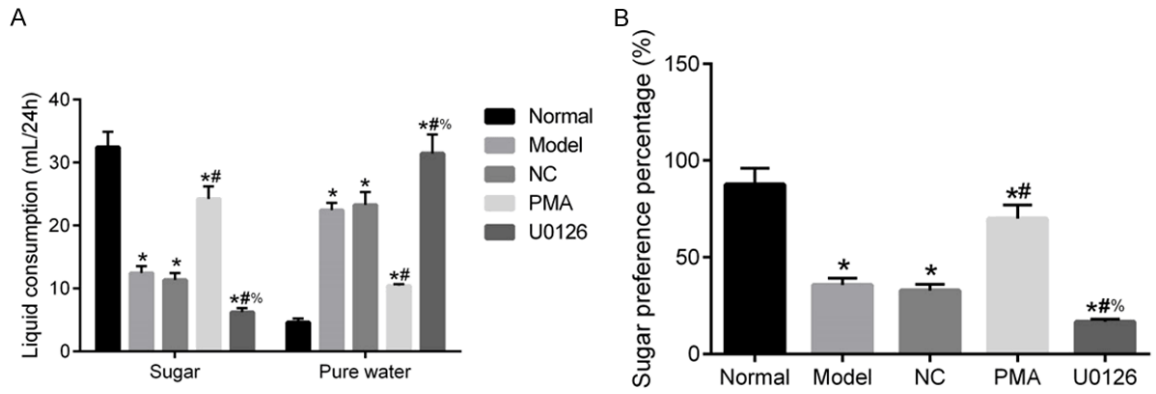

Figure 3. Comparison of liquid consumption and sugar water preferences. A: Liquid consumption of each group; B: Sugar water preferences in each group; *P<0.05 compared with the normal group; #P<0.05 compared with the model group; %P<0.05 compared with the PMA group.

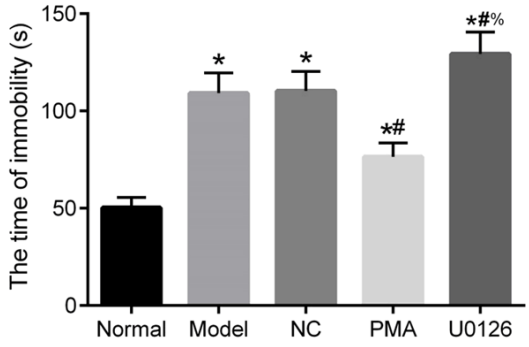

Figure 4. Comparison of immobility times in the tail suspension test. *P<0.05 compared with the normal group; #P<0.05 compared with the model group; %P<0.05 compared with the PMA group.

the U0126 group decreased, while pure water consumption significantly increased (all P< 0.05).

Comparison of immobility times in the tail suspension test

Immobility times in the tail suspension tests of each group are shown in **Figure 4**. Compared with the normal group, immobility times of the other groups significantly increased (all P< 0.05). Compared with the model group, immobility times of the PMA group decreased (P<0.05), while immobility times of the U0126 group significantly increased (P<0.05). There were no significant difference between the model group and NC group (P>0.05). Compared with the PMA group, immobility times of the U0126 group significantly increased (P<0.05).

H&E staining of hippocampal neurons

H&E staining of hippocampal neurons is shown in **Figure 5**. The number and morphology of

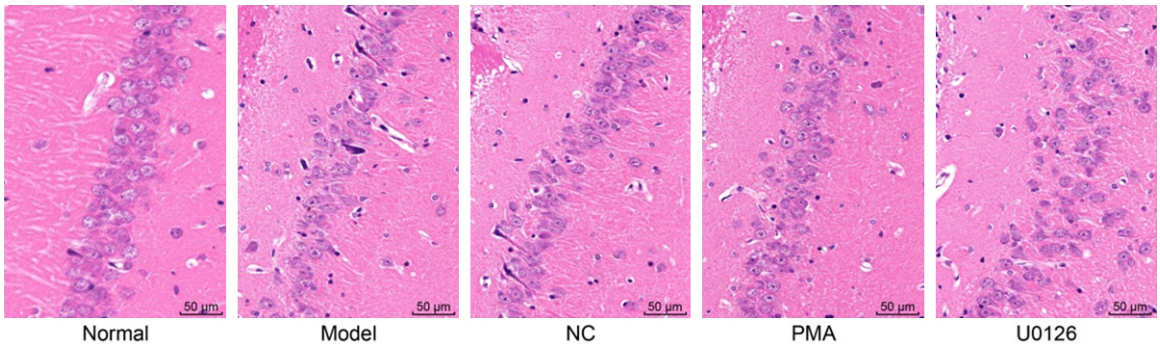

Figure 5. H&E staining of hippocampal neurons.

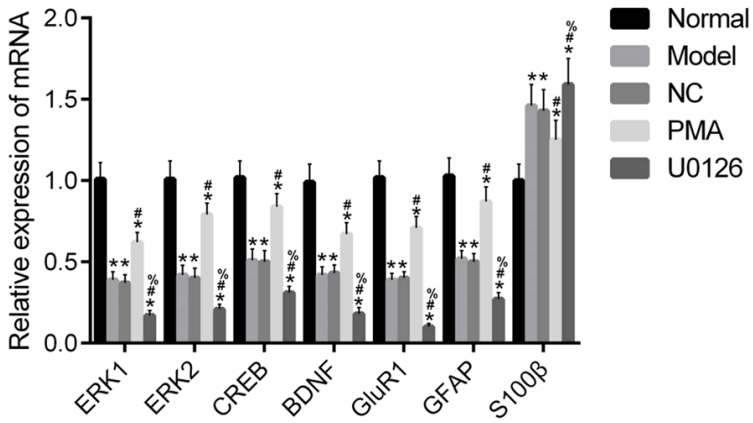

Figure 6. mRNA expression of hippocampus-related genes. *P<0.05 compared with the normal group; #P<0.05 compared with the model group; %P<0.05 compared with the PMA group.

hippocampal neurons in the normal group appeared normal. In the model group and NC group, the number of hippocampal neurons decreased and intercellular space enlarged. The morphology appeared abnormal and there were apparent deformed neurons. The U0126 group showed more obvious morphological changes than the model group. However, the PMA group showed mild neuronal morphological changes and number reduction, compared with the model group.

Related gene mRNA expression measured by qRT-PCR

Results of qRT-PCR are shown in **Figure 6**. Compared with the normal group, mRNA expression of ERK1/2, CREB, BDNF, GluR1, and GFAP in the other groups significantly decreased, while mRNA expression of S100β significantly increased (all P<0.05). Compared with the model group, mRNA expression of ERK1/2, CREB, BDNF, GluR1, and GFAP of the PMA group increased, while mRNA expression of S100β significantly decreased (all P<0.05). Compared with the model group, mRNA expression of ERK1/2, CREB, BDNF, GluR1, and GFAP of the U0126 group decreased, while mRNA expression of S100β increased (all P<0.05). There were no significant differences between the model group and NC group (all P>0.05). Compared with the PMA group, mRNA expression of ERK1/2, CREB, BDNF, GluR1, and GFAP of the U0126 group significantly decreased, while mRNA expression of S100β significantly decreased (all P<0.05).

Related protein expression measured by Western blotting

Western blotting results are shown in **Figure 7**. Compared with the normal group, protein expression of p-ERK1/2, p-CREB, BDNF, GluR1, and GFAP decreased in the other groups, while expression of S100β increased (all P<0.05). The PMA group showed more expression of p-ERK1/2, p-CREB, BDNF, GluR1, and GFAP, but less expression of S100β, compared to the model group (all P<0.05). Contrarily, the U0126 group had less expression of p-ERK1/2, p-CREB, BDNF, GluR1, and GFAP but more expression of S100β, compared with the model group (all P<0.05). There were no differences in expression levels of each protein between the

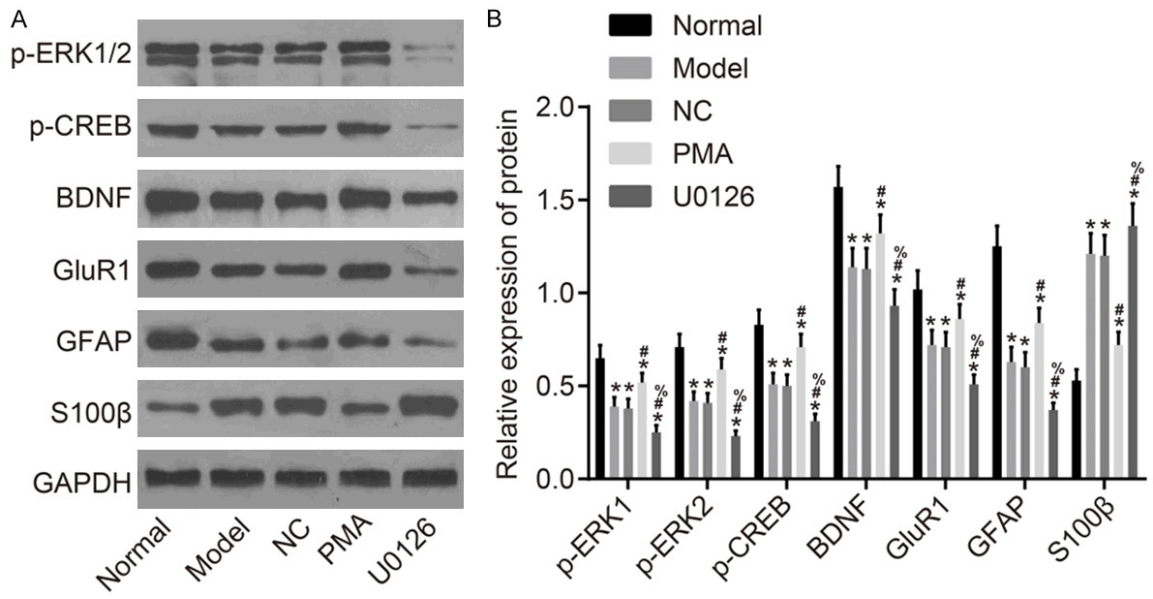

Figure 7. Protein expression of hippocampus-related genes. A: Western blot results of each protein; B: Relative expression of each protein; *P<0.05 compared with the normal group; #P<0.05 compared with the model group; %P<0.05 compared with the PMA group.

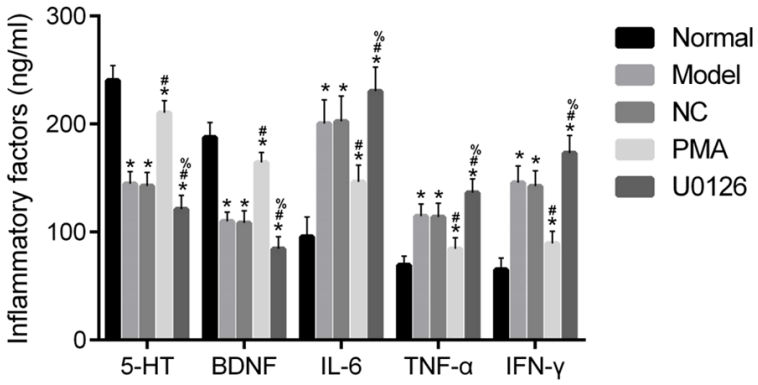

Figure 8. Comparison of inflammatory factors. *P<0.05 compared with the normal group; #P<0.05 compared with the model group; %P<0.05 compared with the PMA group.

model group and NC group (all P>0.05). Compared with the PMA group, expression of p-ERK1/2, p-CREB, BDNF, GluR1, and GFAP in the U0126 group decreased, but expression of S100β significantly increased (all P<0.05).

Serum levels of inflammatory indicators measured by ELISA

Results of ELISA are shown in **Figure 8**. Compared with the normal group, expression of 5-HT and BDNF significantly decreased in the other groups, while expression levels of IL-6, TNF-α, and IFN-γ significantly increased (all P<0.05). The PMA group showed more expression of 5-HT and BDNF but less expression of IL-6, TNF-α, and IFN-γ, compared to the model group (all P<0.05). Contrarily, the U0126 group showed less expression of 5-HT and BDNF but more expression of IL-6, TNF-α, and IFN-γ, compared with the model group (all P<0.05). There were no significant differences in inflammatory factor levels between the Model group and NC group (all P>0.05). Compared with the PMA group, expression of 5-HT and BDNF decreased in the U0126 group, but IL-6, TNF-α, and IFN levels significantly increased (all P<0.05).

Discussion

According to the World Health Organization (WHO), depression is the most common disease, worldwide, and the leading cause of disabilities. About 350 million people, worldwide, suffer from depression [15]. At present, clinical treatment methods available for depression

are relatively scarce. Various viewpoints and hypotheses on the pathogenesis of depression exist, including genetic predisposition, the cytokine theory, the serotonin (5-HT) theory, and psychosocial factors [16, 17]. Researchers have proposed corresponding treatment methods from these aspects. The current study explored mechanisms of BDNF-ERK-CREB signaling pathways on cognitive impairment and neural plasticity in depression model rats, aiming to find alternative treatment methods for depression.

In the current study, compared with the normal group, body weights and sugar water intake and preferences in other depression model groups were reduced. Moreover, mRNA and protein expression levels of ERK1/2 and p-ERK1/2, CREB and p-CREB, BDNF, GluR1, GFAP, and inflammatory factors, such as 5-HT and BDNF, were significantly reduced. This indicates that depression jeopardizes the physiological functions of animals. Other studies have also confirmed that serum BDNF is downregulated in patients with depression and levels of BDNF are correlated with degree of depression. Abnormal expression of BDNF may be involved in the pathophysiological mechanisms of depression [18]. Specifically, under normal circumstances, BDNF is abundant in the brain and higher in serum. When BDNF is reduced, neurotrophic effects are impaired. This may cause depression. Moreover, serum levels of 5-HT in patients with depression are also decreased, correlating with severity of depression.

Furthermore, compared with the model group, mRNA and protein expression levels of ERK1/2, CREB, BDNF, GluR1, and GFAP were significantly increased, while mRNA and protein expression levels of S100β were significantly decreased in the PMA group. Elevated expression of GFAP is a prominent marker of astrocyte activation. Activated astrocytes can synthesize and release a variety of neurotrophic factors, supporting neuron and axon growth and playing an important role in neuron damage repair. S100β can increase the survival rate of neurons, promote the neurite outgrowth of glial cells, and promote the extension of axons. GluR1 reduces damage to synaptic plasticity and memory function induced by inflammatory factors caused by glial cell activation.

Present H&E staining results showed that the number and morphology of hippocampal neurons in the normal group appeared normal. In contrast, the number of hippocampal neurons decreased in the model group and NC group, with enlarged intercellular space, abnormal cellular structures, and apparently deformed cells. These changes reflect the alteration of neural plasticity in the hippocampus of depression model rats. As a part of the limbic system, the hippocampus is currently the most popular brain area in depression research. Its roles in memory, study, and cognitive impairment have been well established. A previous study demonstrated that hippocampal atrophy found in depression patients is associated with cognitive impairment [19].

Many studies have shown that BDNF is a crucial regulator of neuronal plasticity. It has many physiological functions, such as promotion of the development, growth and differentiation of neurons and prevention of neuronal apoptosis [20, 21]. Zhang found that the loss of BDNF and its specific receptor TrkB might be the main pathogenesis of hippocampal neuron atrophy and apoptosis, leading to depression [22]. The role of BDNF is mainly achieved by activating its specific receptors. Receptors for BDNF include TrkB and p75. The former has a high affinity and the latter has a low affinity. When BDNF specifically binds to TrkB receptors, it activates the following two signaling pathways: ERK signaling pathways and PIK3/AKT signaling pathways [23]. ERK includes ERK1 and ERK2. Under normal physiological conditions, both are involved in coordinating the response of neurons to external signals. Activated ERK can phosphorylate kinases, such as RSK and MSK, thereby activating downstream transcription factors, such as CREB, and multiple non-transcription related proteins. Phosphorylation of CREB enhances synaptic plasticity and the formation of new synaptic connections, increases neuronal survival, and alleviates cognitive impairment, thereby achieving antidepressant effects. In the pathogenesis of depression, BDNF may play a role in cell repair and regeneration by inducing expression of CRED and the transcription of induced genes. Increased expression of BDNF and binding to the TrkB receptor activates the downstream protein kinase cascade Raf-MEK-ERK, producoing effects in regulating synaptic remodeling, axonal growth, and neuronal excitation [24].

Clinically, levels of pro-inflammatory cytokines in the serum of patients with depression are often significantly increased, indicating that increased cytokines (including pro-inflammatory cytokines and anti-inflammatory cytokines) can be considered a potential biomarker of depression [25]. Studies have shown that cytokine levels in the peripheral circulation are closely related to degrees of anxiety, depression, and cognitive impairment. Moreover, cytokines, such as IL-1β, IL-6, and TNF-α, can act directly or indirectly on the brain, activating the hypothalamic-pituitary-adrenal (HPA) axis. This affects the release of monoamine neurotransmitters and leads to development of depression [26, 27]. In the current study, expression levels of inflammatory factors IL-6, TNF-α, and IFN-γ were significantly lower in the PMA group, compared with the model group.

In summary, the current study found that antidepressant therapy can increase expression levels of key proteins, such as ERK and CREB, in downstream cascade signaling pathways by increasing expression of BDNF. This inhibits the release of inflammatory cytokines in depression model rats, enhancing neural plasticity and promoting the rehabilitation of depression model rats.

Therefore, medications directly acting on BDNF-ERK-CREB signaling pathways are a new research direction for treatment of depression, with great application prospects. However, results should be further confirmed in clinical practice, examining possible side effects and other problems. However, with the rapid development of medicine, application of BDNF-ERK-CREB signaling pathways fort treatment and diagnosis of depression may lead to new breakthroughs, greatly benefitting those suffering from depression.

Disclosure of conflict of interest

None.

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