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Research report

Differential expression of mitogen-activated protein kinase signaling pathway in the hippocampus of rats exposed to chronic unpredictable stress

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ABSTRACT

Much research has indicated that the mitogen-activated protein kinase (MAPK)-cAMP response element-binding protein (CREB) signal transduction pathway is involved in the pathophysiological mechanism of depression. But as to the question of which MAPKs are more relevant to stress effects, there is no definite answer. In the present study, 32 male Sprague–Dawley rats were divided into chronic unpredictable stress (CUS) and control groups, with 16 rats in each group. The CUS rats were exposed to 21-day chronic unpredictable stressors, and the controls were stress-free. After stress, 16 rats (8 in each group) were tested for spatial memory using Morris Water Maze, and 16 rats (8 from each group) were decapitated for detection of the three most extensively studied subgroups of MAPKs, ERK1/2, JNK and P38, and CREB in the hippocampus. The results showed that there was no statistical difference in the body weight between the two groups. The CUS rats showed impaired spatial memory in MWM. Western blot of hippocampus showed that CUS significantly decreased pCREB and pJNK levels, but there was no statistical difference between two groups in CREB, ERK1/2, pERK1/2, P38, pP38 and JNK levels. Immunohistochemistry showed that the reduced pCREB occurred in the dentate gyrus, not in the hippocampus proper. In conclusion, this study highlights that the JNK-CREB pathway, not the P38-CREB or ERK1/2-CREB pathway, in the hippocampus played an important role in the 21-day-CUS, and that the impaired spatial memory acquisition in the CUS rats can be restored to the level comparable to the pre-stressed state.

1. Introduction

Mitogen-activated protein kinase (MAPK) signaling cascades are multifunctional signaling networks that influence cell growth, differentiation, apoptosis, and cellular responses to stress [5,19,28]. There are five distinct groups of MAPK in mammals: extracellular signal-regulated kinases 1 and 2 (ERK1/2), c-Jun amino-terminal kinases/stress-activated protein kinases (JNK/SAPK), P38, ERK3/4 and ERK5 [5,19,28]. The most extensively studied groups of MAPK are the ERK1/2, JNK, and P38 kinases. MAPK can be activated by a wide variety of different stimuli. Although in general, ERK1/2 is preferentially activated by growth factors, JNK and P38 are more responsive to stress stimuli [26,28]. Once activated, MAPK phosphorylates target substrates on serine or threonine residues. One of the substrates of MAPK is cAMP response element-binding protein (CREB).

CREB, a leucine zipper transcription factor, can mediate cellular signal transduction [25]. CREB signaling plays a large regulatory role in the nervous system and is believed to be involved in learning and memory [14,31]. CREB is activated through phosphorylation at Ser133 by various signaling pathways including cAMP-PKA, mitogen-activated protein kinases (MAPKs) and Ca^{2+} [25].

To date, much research has indicated that MAPK signaling transduction pathways contribute to various stress-induced synaptic plasticity and are involved in the pathophysiological mechanism of depression [4,8,26,27], but as to which MAPKs are more relevant to the effects of stress, there is no definite answer. In this study, we have examined the differential expressions of the MAPK signaling pathway in the hippocampus of rats exposed to chronic unpredictable stress and we attempted to provide a new insight into the relationship between hippocampus MAPK signaling and depression (Table 1).

2. Materials and methods

2.1. Chronic unpredictable stress induction procedure

Male Sprague–Dawley rats (150–180 g) from the Animal Center of Shantou University Medical College (Shantou, China) were brought into the laboratory one week before the experiment and housed individually in polycarbonate cages. The room temperature was maintained at 22 ± 1 °C with low humidity and food and water freely available. Thirty-two rats were randomly assigned to two groups: chronic unpredictable stress (CUS) group (n = 16) and the control non-stressed group (n = 16). Rats assigned to the CUS group were subjected to 21-day CUS following a previously reported protocol [18] with some modifications. We added some stressors,

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Table 1
Comparison between the two groups in the probe trial of MWM.

	Control group	CUS group
Number of times of platform crossing	7.0 ± 3.9	1.8 ± 0.5*
Total distance (m)	26.3 ± 3.4	15.4 ± 7.7*
Velocity (cm/s)	21.9 ± 2.8	12.7 ± 6.4*
Time spent in the target quadrant (s)	51.7 ± 5.9	19.0 ± 15.9*

* Compared with the control group, $P < 0.05$.

like reversed light/dark, behavior restraint and ice water swimming to our stress procedure, and omitted such stressors as white noise, stroboscope and group housing, used by Kim et al. [18]. As for the stress intensity, the stressors used in our experiment were compared to the previous study [18]. Our stress procedure was as such: 24 h water deprivation, 24 h food deprivation, 24 h 45° cage tilt, 10 h damp bedding, 24 h reversed light/dark, 2 h behavior restraint, 10 h empty bottle, 5 min ice water swimming, 5 min 45 °C high temperature, 1 min tail clip and 10 min foot shock (10 times/min, 1 s/time). To be completely unpredictable to the rats, the stressors were applied randomly with the last stressor being food and water deprivation. The control group rats were housed in separate individual rooms and had no contact with the CUS group, with food and water freely available other than on day 21 being food and water deprived. The second day after the last stressor, all rats were tested for sucrose consumption. Then 16 rats (8 from each group) were tested for spatial memory using the Morris Water Maze, and thereafter they were deeply anaesthetized and perfused intracardially via the left ventricle with physiological saline followed by 4% ice-cold paraformaldehyde in 0.1 M phosphate buffer solution (PBS; pH 7.4). The brains were removed, post-fixed in 4% paraformaldehyde overnight, and embedded in paraffin. Five-micrometer tissue sections were used for CREB and P-CREB immunohistochemistry. The other 16 rats (8 from each group) were decapitated on ice immediately after the sucrose consumption test to examine the expression of ERK1/2, JNK and P38, and CREB in the hippocampus by Western blot analysis. All the experimental procedures were approved by the Laboratory Animals Care and Use committee of Shantou University Medical College (Shantou, China).

2.2. Body weight measurement

Rats were weighed every day between 8:00 am and 9:00 am.

2.3. Sucrose consumption test

The main concern of CUS model is the induction of anhedonia, which by definition is a loss or reduction in pleasant events. All rats tested for sucrose consumption were individually housed in isolation. The CUS group went through 21 days of CUS with the last stressor being food and water deprivation for 24 h. Rats in the control group were also food and water deprived for 24 h on day 21. Then they were given a bottle of 1% sucrose solution and a bottle of tap water, and allowed to consume the fluids for 3 h. The positions of bottles were counterbalanced across the left or right side of the test cages. Sucrose consumption was monitored by weighing the bottles at the beginning and end of the test. Sucrose consumption percentage was calculated as follows: sucrose consumption percentage = sucrose consumption/(sucrose consumption + water consumption) × 100%.

2.4. Morris Water Maze (MWM) test

The second day after the sucrose consumption test, the spatial memory acquisition or retention of the rats was tested using Morris Water Maze (MWM) according to a previously described method with some modifications [12]. The black water maze (1.8 m in diameter × 0.7 m in height) was filled with tap water and divided into 4 quadrants. In the center of the 2nd quadrant was a removable escape platform (10 cm diameter), which was 1.5 cm below the water level and covered with black plastic to disguise its presence and to create traction for the rat to climb onto the top of the platform.

The navigation training consisted of 8 trials in all. In each trial, each rat was placed in the water facing the perimeter of the pool at a randomly selected quadrant. For each trial, the rat swam in the pool for 120 s until climbing onto the platform. If the rat did not find the platform within 120 s, it was manually guided to the platform. The rat remained on the platform for 15 s in all trials and was then removed from the pool, dried in a clean cage without bedding, and returned to its home cage. The pool was strained of feces and stirred gently between each swim session to disrupt any scent left behind from the rat's path taken to find the platform. On the fifth day of MWM test, there was one probe trial in which the platform was removed from the pool and the animal swam for 120 s.

All swim sessions were videotaped and recorded with DigBehav-Morris Water Maze Video Analysis System (Jiliang Software Technology Co. Ltd., Shanghai, China).

2.5. Immunohistochemistry

Five-micrometer-thick paraffin sections of the right hippocamppus were dewaxed in xylene, rehydrated in graded ethanol, and stained with either pCREB or CREB immunohistochemically as follows: the sections were treated with 3% hydrogen peroxide to block endogenous peroxidase, and antigen retrieval was performed by heating sections to 95 °C in a citric acid buffer (pH 6.0) for 15 min, slowly cooling to room temperature. Subsequently, the sections were incubated with a monoclonal rabbit anti-rat pCREB or CREB antibody (1:50 dilution) (Cell Signaling, USA) overnight at 4 °C. The primary antibody was washed off with PBS-T and the sections were incubated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG (1:50 dilution) (Cell Signaling, USA) for 1 h at room temperature, followed by three changes of PBS-T to remove unreacted secondary antibodies. Finally, the sections were developed in 3, 3′-diaminobenzidine (DAB), counterstained with haematoxylin, and mounted in xylene. The brown-labeled cells (at least 2000 cells per slide) were counted to calculate the labeling index, i.e., the percentage of immunoreactive nuclei in 100 cells.

2.6. Western blotting

Immediately after the sucrose consumption test, 16 rats (8 from each group) were decapitated and the brains were rapidly removed on ice. The whole hippocampus was dissected out from the brain and the right half part was homogenized in cell lysis buffer for Western blotting (Beyotime, Jiangsu, China) supplemented with 1 mM PMSF (Beyotime, Jiangsu, China). Protein content was determined using BCA protein assay kit (Beyotime, Jiangsu, China). Lysates were mixed with 5 × sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) sample loading buffer (Beyotime, Jiangsu, China). Then 50 μg of proteins was separated by SDS-PAGE and was blotted onto nitrocellulose (NC) membranes by electrophoretic transfer. Blots were incubated in blocking buffer (10% non-fat dry milk powder in tris-buffered saline containing 0.5% Tween-20, TBS-T) for 1 h at room temperature and washed 3 times with TBS-T for 10 min each. Blots were then incubated at 4 °C with 1:1000 diluted primary antibodies including mouse anti-β-actin, rabbit anti-ERK1/2, mouse anti-P-ERK1/2, mouse anti-P38, mouse anti-P-P38, rabbit anti- JNK and mouse anti-P-JNK (Beyotime, Jiangsu, China), and rabbit anti-CREB and rabbit anti-P-CREB (cell signaling, USA) and then washed 3 times for 10 min each in TBS-T. Blots were incubated with appropriate HRP-labeled goat anti-mouse or goat anti-rabbit secondary antibody IgG for 2 h at room temperature, washed 3 times for 10 min each in TBS-T, treated with BeyoECL reagents (Beyotime, Jiangsu, China), and exposed to film (Koda, USA). Band intensity was quantified by Bandscan 5.0 analysis software (Glyko Bandscan software). The relative level of each signal protein was calculated as the ratio of total gray of each signal protein/β-actin.

2.7. Statistical analysis

The statistical analysis was performed using the "Statistical Package for Social Sciences" (SPSS, Version 11.5). Group differences for the probe trial of MWM, sucrose intake, sucrose consumption percentage, and immunohistochemical and Western blotting studies were determined using independent sample t tests. Group comparisons in latency to find the hidden platform and swimming velocity during the navigation training of MWM were analyzed with repeated measures analysis of variance (ANOVA) (group × trial) and multivariate ANOVA (group × trial) of the general linear model. Body weight data was also compared with repeated measures ANOVA (group × day). Only $p < 0.05$ was considered statistically significant. All data were reported as means ± SD.

3. Results

3.1. Body weight

Body weight between the groups was analyzed with repeated measures ANOVA. The day before CUS began the weight was 207.8 ± 29.0 g in the CUS group and 201.3 ± 24.1 g in the control group. After 21 days of CUS, body weight was 280.3 ± 33.2 g in the CUS group and 287.3 ± 25.5 g in the control group. No statistical difference in the body weight gain between the two groups was observed ($F(1, 30) = 0.202$, $P > 0.05$) (Fig. 1).

3.2. Sucrose consumption test

The sucrose intake and the sucrose consumption percentage in the CUS group were lower than those in the control group. The difference in the total sucrose consumed between the control and CUS groups was not significant (10.6 ± 5.1 g VS 7.8 ± 3.9 g, $t(30) = 1.744$, $P > 0.05$), whereas the difference in the sucrose consumption percentage was significant (87.1 ± 16.7% VS 65.7 ± 23.3%, $t(30) = 2.986$, $P < 0.05$).

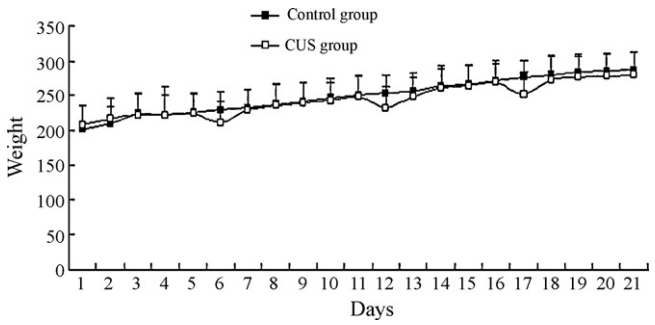

Fig. 1. Body weight comparison between the control and CUS group. Body weight at the baseline was 207.8 ± 29.0 g in the CUS rats and 201.3 ± 24.1 g in the control rats, and after 21-day CUS was 280.3 ± 33.2 g in the CUS rats and 287.3 ± 25.5 g in the control rats. There was no two statistically difference in the body weight at any time point ($P > 0.05$).

3.3. Spatial memory acquisition and retention assessed by Morris Water Maze test

There was a significant effect of group on the latency to find the hidden platform during the navigation training in the MWM ($F(1, 14) = 8.561$, $P < 0.05$). The latency for both the stressed and control rats to find the hidden platform decreased with increasing trials. The rats exposed to CUS had longer latencies to reach the platform than the control rats. When compared through multivariate ANOVA, the latencies of the CUS and control rats differed significantly on trials 1–5 ($F(1, 14) = 5.258$–10.225, $P < 0.05$), but not on trials 6–8 ($F(1, 14) = 4.458$–4.590, $P > 0.05$) (Fig. 2A). There were no significant effects of group ($F(1, 14) = P > 0.05$) on the swimming velocity during navigation training in the MWM, although the swimming velocity of the rats in the CUS group was slower than that in the control group (Fig. 2B).

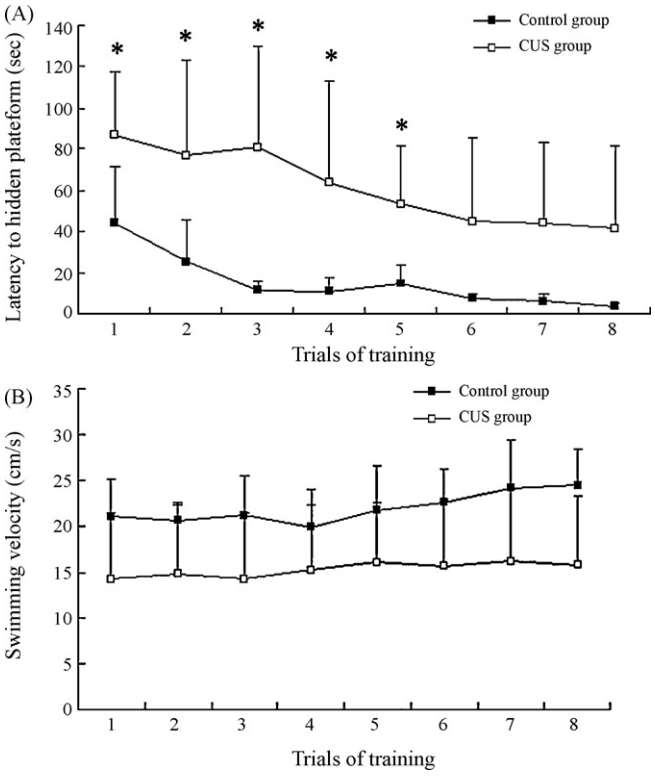

Fig. 2. The latency to locate the hidden platform and the swimming velocity during the navigation training in the MWM. Time to reach the platform was significantly longer in the CUS rats than in the control rats in the first 5 rounds of training, but not in the last 3 rounds (A). There was no significant difference in the swimming velocity during navigation training in the MWM (B).

During the probe trial, the number of times that rats crossed the removed hidden platform in the CUS group was significantly fewer than that in the control group (1.8 ± 0.5 VS 7.0 ± 3.9, $t(14) = 3.741$, $P < 0.05$). Rats exposed to CUS traveled significantly shorter total distance (15.4 ± 7.7 m VS 26.3 ± 3.4 m, $t(14) = 3.663$, $P < 0.05$). The control rats swam at a faster velocity than the CUS rats during the 120 s probe trial (21.9 ± 2.8 cm/s VS 12.7 ± 6.4 cm/s, $t(14) = 3.725$, $P < 0.05$). There was significant difference in the time spent in the target quadrant between groups (51.7 ± 5.9 s in the control group VS 19.0 ± 15.9 s in the CUS group, $t(14) = 5.454$, $P < 0.05$).

3.4. Expression of CREB and pCREB

CREB antigens were expressed in almost all cells of the hippocampus, including the CA1, CA2, CA3 and CA4 pyramidal cell layers (PCL), and the dentate gyrus (DG) granule cell layer (GCL) (Fig. 3A1–5 and B1–5), whereas pCREB antigens were mainly localized in cells along the innermost region of the GCL (Fig. 3C1–5 and D1–5). There was no significant difference in the labeling index of CREB in the CA1, CA2, CA3, CA4 and DG, and pCREB in the CA1, CA2, CA3 and CA4 subregions of the hippocampus between two groups. However, the labeling index of pCREB in the DG in the control group was significantly higher than that in the CUS group (5.2 ± 1.2 VS 1.9 ± 0.8, $t = 6.472$, $P < 0.05$).

3.5. Western blotting

The signals of β-actin, ERK1/2, pERK1/2, P38, pP38, JNK, pJNK, CREB and pCREB were detected in the hippocampus (Fig. 4). There was no significant difference between two groups in the levels of CREB (48.0 ± 13.0% in the control group VS 44.8 ± 27.4% in the CUS group, $t = 0.298$, $P > 0.05$), ERK1 (126.6 ± 29.1% VS 153.8 ± 45.6%, $t = 1.422$, $P > 0.05$), pERK1 (128.4 ± 75.8% VS 161.4 ± 47.2%, $t = 1.047$, $P > 0.05$), ERK2 (131.2 ± 20.9% VS 153.4 ± 49.7%, $t = 1.165$, $P > 0.05$), pERK2 (136.8 ± 73.1% VS 161.0 ± 41.6%, $t = 0.814$, $P > 0.05$), P38 (77.8 ± 36.5% VS 71.6 ± 38.0%, $t = 0.333$, $P > 0.05$), pP38 (93.2 ± 42.6% VS 139.6 ± 93.9%, $t = 1.273$, $P > 0.05$) and JNK (178.0 ± 21.6% VS 179.8 ± 35.5%, $t = 0.123$, $P > 0.05$) (Fig. 5). However, a significantly decreased level was observed with the CUS group in the pCREB (13.6 ± 8.3% VS 36.0 ± 14.5%, $t = 3.792$, $P < 0.05$) (Fig. 5A) and pJNK (30.8 ± 7.8% VS 113.8 ± 55.3%, $t = 4.204$, $P < 0.05$) (Fig. 5E) in the hippocampus.

The CUS group had a marked decrease in the ratio of pCREB/CREB (27.2 ± 13.4% VS 96.6 ± 38.1%, $t = 4.860$, $P < 0.05$) (Fig. 5A) and pJNK/JNK (17.2 ± 3.1% VS 61.2 ± 24.0%, $t = 5.143$, $P < 0.05$) (Fig. 5E). But there was no significant difference between the two groups in the ratio of pERK1/ERK1 (95.4 ± 39.0% VS 132.1 ± 45.1%, $t = 1.741$, $P > 0.05$) (Fig. 5B), pERK2/ERK2 (101.4 ± 46.6% VS 112.2 ± 39.3%, $t = 0.5.1$, $P > 0.05$) (Fig. 5C), and pP38/P38 (136.4 ± 83.8% VS 215.2 ± 147.5%, $t = 1.314$, $P > 0.05$) (Fig. 5D).

4. Discussion

Cognitive deficit is a core element of depression [7] and depressed People often have some problems in cognitive functioning, such as deficit in remembering and recalling information [6,7]. In this article, we investigate the spatial memory function and the differential expression of MAPK-CREB signaling transduction pathway in the hippocampus in the rat CUS model for depression.

There was no statistical difference in the body weight between the control and CUS group at any time point, which is similar to the results of some studies [8,15,17]. But, in the majority of the published studies, the body weight is significantly lower in the CUS rats than the control rats [12,27]. The inconsistent findings of the

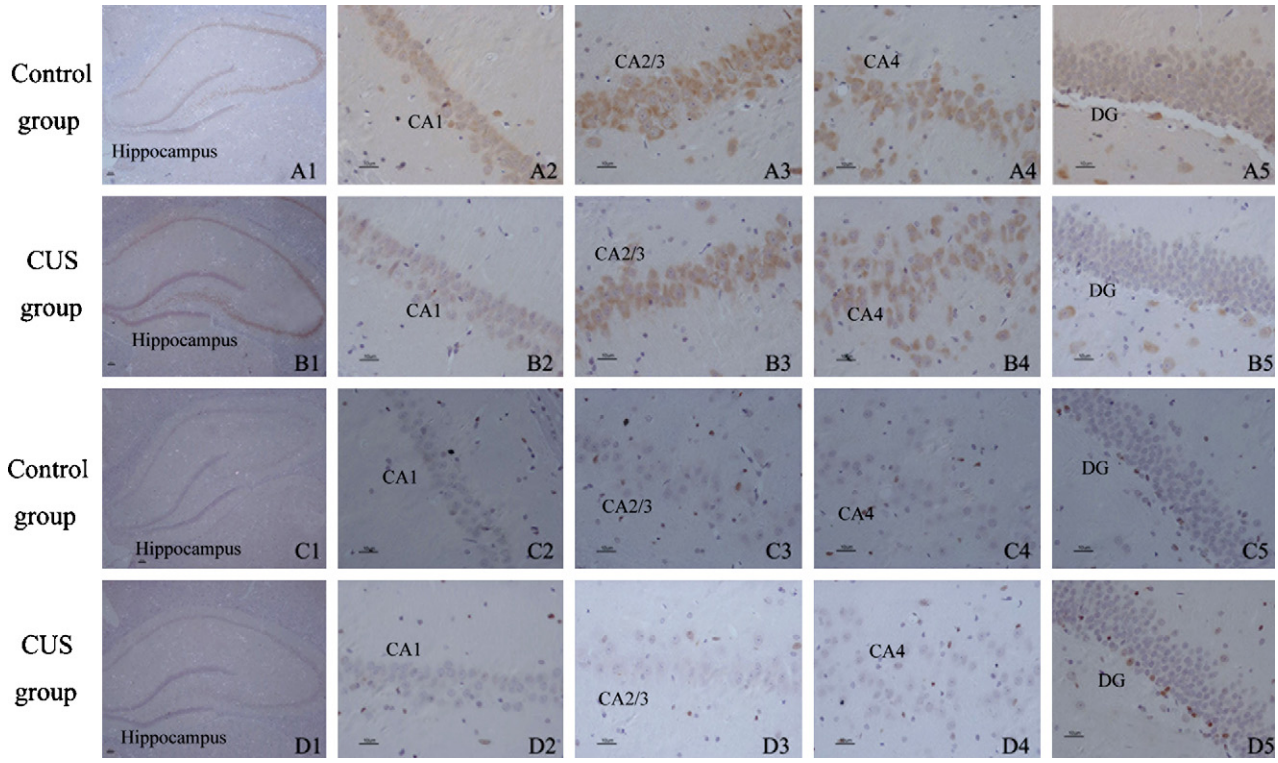

Fig. 3. Immunohistological stainings show distribution of CREB and pCREB in hippocampus. CREB antigen expression was in almost all cells of the hippocampus (A1 and B1) and localized expression of pCREB antigen was in cells along the innermost region of the GCL (C1 and D1). There was no significant difference in the labeling index of CREB in the CA1 (A2 and B2), CA2/3 (A3 and B3), CA4 (A4 and B4), and DG (A5 and B5), and in the labeling index of pCREB in the CA1 (C2 and D2), CA2/3 (C3 and D3), and CA4 (C4 and D4) subregions of the hippocampus between the two groups. However, the labeling index of pCREB in DG (C5, D5) in the control group was significantly higher than that in the CUS group. A1-5 and C1-5 represent the control group; B1-5 and D1-5 represent the CUS group. Left to right represents hippocampus, CA1, CA2/3, CA4 and DG.

body weight between independent laboratories may be due to differences in the CUS protocols, the time points being compared, and the statistic methods.

Although there was no statistical difference in the sucrose intake in our research, the sucrose consumption percentage is significantly different between two groups. The decrease in sucrose intake or in the sucrose consumption percentage represents a loss of preference for the palatable sucrose solution [17] and is an indication of anhedonia [17,27]. The results of sucrose consumption test indicated validate the success of using the CUS model in our study.

The hippocampus plays an important role in hippocampal-dependent learning and memory and is markedly susceptible to stress [23]. Many studies have demonstrated that chronic stress decreases or inhibits the growth of CA1-PCL, CA3-PCL and DG-GCL, decreases the neurogenesis of DG, causes CA3 dendritic retraction, and reduces the volume of the hippocampus [10,11,16,24,32]. The morphological changes are also associated with hippocampus-dependent spatial memory deficits [24,32]. Therefore, the integrity of structure and function of the hippocampus is important for spatial memory. In the MWM, we observed significant difference between two groups in the latency to the hidden platform in the first five navigation trials, but not in the last three navigation trials. Moreover, there were no significant effects of group and time, as well as their interaction on the swimming velocity during naviga-

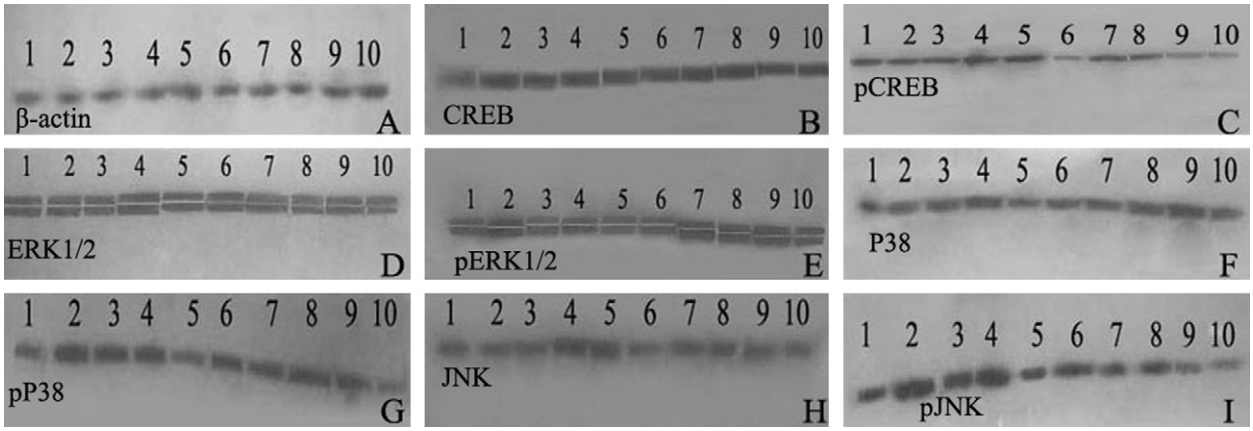

Fig. 4. Western blots showing the expression of β-actin, CREB, pCREB, ERK1/2, pERK1/2, P38, pP38, JNK and pJNK in hippocampus. Protein expression of β-actin (A), CREB (B), pCREB (C), ERK1/2 (D), pERK1/2 (E), P38 (F), pP38 (G), JNK (H) and pJNK (I) in the hippocampus of the CUS (1–5) and control rats (6–10) was detected by Western blot analysis.

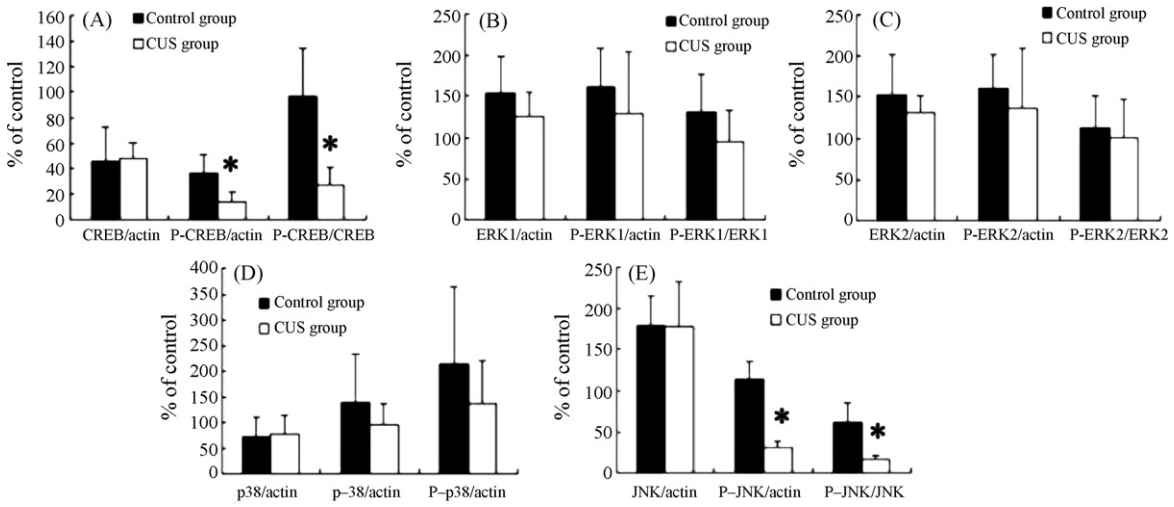

Fig. 5. The level of CREB, pCREB, ERK1, pERK1, ERK2, pERK2, P38, pP38, JNK, and pJNK, and the ratio of pCREB/CREB, pERK1/ERK1, pERK2/ERK2, pP38/ P38 and pJNK/ JNK in hippocampus. No significant difference between two groups in the level of CREB (A), ERK1 (B), pERK1 (B), ERK2 (C), pERK2 (C), P38 (D), pP38 (D), and JNK (E); significantly decreased pCREB level (A) and pJNK level (E) in the hippocampus in the CUS group; marked decrease in the ratio of pCREB/CREB (A) and pJNK/ JNK (E) in the hippocampus of the CUS group; no significant difference between the two groups in the ratio of pERK1/ERK1 (B), pERK2/ERK2 (C), and pP38/P38 (D).

tion training. So we conclude that while the motor ability was not affected by CUS, there was impairment in spatial memory acquisition, which, however, could be restored to the level comparable to the pre-stressed state through training. Besides, the spatial memory retention was impaired in the CUS rats compared to the control rats. Our findings are in accord with some studies [2,16], but not with the study of Gouirand et al., who reported that rats exposed to 10-day-CUS had shorter latencies to reach the hidden platform during training and traveled less overall and less in the outer portion during the probe trial [12]. They explained this behavior as the result of the CUS rats having more efficient search strategies [12].

The neuronal mechanisms that turn stress signals into behavioral disorders are far from understood. Studies thus far, however, have suggested that the abnormal intracellular signal transduction pathways play important roles in the pathophysiology of depression [22,29], and that the MAPK-CREB signal transduction pathway is involved [20,21,30]. By Western blot analysis in this study, no statistical difference between the rats exposed to 21-day-CUS and the control was observed in CREB, ERK1/2, pERK1/2, P38, pP38, and JNK levels in the hippocampus. However, CUS significantly decreased pCREB and pJNK levels in the hippocampus, indicating an attenuated signaling cascade in the hippocampus. The effects of stress on the MAPK pathways have been reported in great variability. The decrease in pERK level in both the rats exposed to chronic forced-swim stress and the post-mortem brains of depressed suicide human subjects has been documented, and the decrease is correlated with depressive-like behavior [9,27]. Different from the effects of chronic stress, it has been reported by Shen et al. that acute swim stress increases ERK level in the neocortex, prefrontal cortex and striatum, and JNK level in the neocortex, prefrontal cortex and striatum, hippocampus and amygdala, whereas p38 levels remained unchanged [30], and by Liu et al. that mitogen-activated kinase 4 (MKK4)-JNK signaling pathway is activated within 5 min following both restraint stress and forced-swim stress in mice [21]; the effects in the former stress model is however smaller than in the latter [21].

Due to the diversity and complexity of the role of CREB in stress, contradictory effects regarding CREB activity have been observed. Böer et al. have found pCREB level is enhanced in many brain regions in mice exposed to chronic psychosocial stress [3] and that chronic rather than acute treatment with the antidepressant imipramine reduces CREB activity [3]. However, Alfonso et al. demonstrated that CREB level was reduced in repeated restraint stressed mice, and tianeptine treatment reversed the stress effects [1]. Similar to our finding, Grønli et al have demonstrated that the reduction of pCREB level of the dentate gyrus, but not the hippocampus proper in rats exposed to be repeated, unpredictable and mild stressor [13].

The discrepancy in the MWM test and in the protein level expression of MAPK-CREB signal pathways in the hippocampus may be due to the type of stress and its duration. These distinct alterations following different durations of stress exposure may be because the beneficial responses to moderate stress exposure are converted into maladaptive responses to prolonged stress exposure.

In conclusion, our study highlights that (i) the JNK-CREB pathway, not the P38-CREB or ERK1/2-CREB pathway played a crucial pathophysiology role in the 21-day-CUS, (ii) the reduced pCREB occured in the dentate gyrus, and (iii) the impaired spatial memory acquisition in the CUS rats could be restored to the level comparable to the pre-stressed state.

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