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Research report

The depressive-like behaviors are correlated with decreased phosphorylation of mitogen-activated protein kinases in rat brain following chronic forced swim stress

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Abstract

In the present study, 40 Sprague–Dawley rats were divided into forced swim stress group and controls, with 20 rats in each group (10 for behavioral tests, 10 for protein detection). The forced swim stress group received swim stress for 14 consecutive days, and the controls were stress-free. After stress, 20 rats were tested for behavioral observation using body weight gain, open field, elevated plus-maze and saccharine preference test, and 20 rats were decapitated for protein detection. The extracellular signal-regulated kinase (Erk) and phospho-Erk (P-Erk) in the hippocampus and prefrontal cortex were determined using western blot. It was found that the body weight gain of stressed animals during the 7 stressed days and the 14 stressed days was significantly decreased compared to that of controls. Stressed animals spent less time in open arms and longer time in closed arms. The stressed animals demonstrated decreased locomotor activity and increased grooming in open field. The saccharine solution intake and the ratio of saccharine solution intake to total liquid intake were both decreased in the stressed group. Stressed animals showed decreased P-Erk2 and decreased ratio of P-Erk2 to total Erk2 in the hippocampus and prefrontal cortex, but their Erk1/2 was increased in the prefrontal cortex with no change in hippocampus. The saccharine solution intake positively correlated with the P-Erk2 in the hippocampus and negatively correlated with the Erk2 in the prefrontal cortex. In conclusion, chronic forced swim stress was a good animal model of depression, and it induced depressive-like behavior and decreased P-Erk2 in the hippocampus and prefrontal cortex in rats. The depressive-like behaviors were correlated with decreased phosphorylation of Erk, which suggested that the dysfunction of Erk activity might be one of biological mechanisms underlying depression induced by stress.

Keywords: Stress; Open field; Anhedonia; MAPKs; Hippocampus; Prefrontal cortex

1. Introduction

Increasing evidence implicates stress as an important factor in the vulnerability to depression and other behavioral disorders [18,26], however, the neuronal mechanisms that stress signals are transduced into behavioral disorders are far from understanding. Although current clinical therapies for depression usually focus on modulating serotonergic or noradrenergic activity in brain [38], all available antidepressants exert their mood-elevating effects only after several weeks to months of administration [36], which means that enhanced serotonergic or noradrenergic neurotransmission is not directly responsible for the clinical actions of these drugs. Rather, some neuronal synaptic plasticity induced by this enhanced neurotransmission would appear to mediate drug actions. More recent studies have extended neurotransmitters to an examination of the postreceptor intracellular targets that mediate the action of antidepressants, and informed us that signal transduction pathway may be the mechanisms of antidepressant action and the pathophysiology of depression [30,49].

The generic term mitogen-activated protein kinases (MAPKs) is used to denote a family of signal transduction mediators that regulate a diverse of cellular functions via activation of a sequential phosphorylation cascade involving a three-protein cassette [45]. Several MAPKs cascades have been characterized, of which the best-studied and most important MAPKs are the

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extracellular signal-regulated kinases 1 and 2 (Erk1/2). When activated, the phosphorylation state of the Erk1/2, phospho-Erk1/2 (P-Erk1/2) can translocate into nucleus carrying extracellular stimuli primarily regulating the neuronal growth, differentiation and apoptosis [24,51]. The Erk1/2 signal pathway is considered as the major convergence point in all signaling pathways [8], and increasingly becomes a hot topic in many research fields. Erk1/2 is extensively distributed throughout the central nervous system and prominently found in the hippocampus and prefrontal cortex [17,37], which are brain regions most likely to be implicated in response to stress and depression [19,40]. At present, Erk1/2 is being extensively studied in learning and memory. Many results have demonstrated that P-Erk2 has vital role in hippocampus-dependent learning and memory and prefrontal cortex-dependent conditioning. It can facilitate learning [3,47] and memory consolidation [22,46], and regulate neuronal plasticity [14,56]. However, to date, there is little work concerning the role of Erk1/2 in emotional disorders, such as depression, especially in depression induced by stress. One recent research indicates that brain-derived neurotrophic factor (BDNF) can ameliorate depression via activating the Erk MAPKs pathway to increase the signaling [49]. In addition, acute systemic treatment of rats with selective 5-HT1a agonist elicits rapid time- and dose-dependent changes in P-Erk1/2 levels in the brain [7]. Erk1/2 is downstream target of 5-hydroxytraptamine (5-HT) which is deficient in depression, and the downstream signaling pathways of BDNF and 5-HT demonstrate a high degree of overlap [32]. These data suggest that Erk1/2 is involved in the molecular pathophysiology of depression. Moreover, stress studies in experimental animals report that acute swim stress increases Erk1/2 phosphorylatin in the prefrontal cortex [48], and acute restraint stress increases the P-Erk2 level in the hippocampus and prefrontal cortex [35], which indicates stress can exert effect on brain levels of P-Erk1/2 and the Erk1/2 signal pathway participates in the response to stress. According to the above evidence, we could find that Erk1/2 pathway is involved not only in the response to stress but in the molecular pathophysiology of depression, so the Erk1/2 might be a significant target for us to examine the neuronal mechanisms of depression induced by stress.

The hippocampus and prefrontal cortex are much vulnerable to stress and harmful stimuli [19,40]. There are morphological and functional alterations in the two brain regions in stressed animals and depressed humans. Preclinical studies have demonstrated that stress can cause atrophy or loss of CA3 pyramidal neurons and decrease the neurogenesis of dentate gyrus granule cells in the hippocampus [12,33]. Moreover, clinical brain imaging studies demonstrate that the volume of the hippocampus is reduced in patients with depression [4]. Alterations in blood flow, metabolism, and volume of prefrontal cortex also have been reported in depressed patients [11]. In addition, histopathological evidence demonstrates that depressed subjects have decreases in cortical thickness, neuronal sizes, and neuronal and glial densities in the prefrontal cortex [42]. So the two brain regions deserve further investigation in discovering the neuronal mechanisms of depression.

The forced swim stress is a putative animal model of depression which emulates the behavioral despair paradigm of depression [41]. Currently it is one of the most frequently used behavioral tests for investigating antidepressant potential, and provides the highest degree of pharmacological predictive validity [29]. Because most human depression disorders are induced by chronic stress, but not acute stress, we administrated chronic forced swim stress to establish animal model of depression in our research which can well mimic the development of human depression.

Thus, the purpose of the present study was to investigate the effect of stress on brain levels of Erk1/2 and P-Erk1/2, the effect of stress on behavioral responses of experimental animals, and the relationship between the Erk1/2, P-Erk1/2 and behavior. The levels of Erk1/2 and P-Erk1/2 in the hippocampus and prefrontal cortex as well as the depressive-like behavior of the animals were investigated. Examining both the Erk1/2 activity alterations in the brain and the behavioral changes induced by stress would provide new information for understanding of the neuronal mechanisms of depression induced by stress, and it was the first step for us to further investigate the role of the Erk1/2 in depression.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley (250–275 g) rats were obtained from Wei Tong Li Hua Lab Animal Center (Beijing, China). All rats were housed individually. They were acclimated to 3 min of handling once a day for 7 consecutive days before being used in experiment. Rats were maintained on a 12 h light/dark cycle (with light on, 08:00–20:00 h) with food and water ad libitum except for saccharine preference test time. Forty rats were randomly divided into the swim stress group and the controls with 20 rats in each group. To avoid the possible effect of behavioral tests on the protein level in brain, in each group, 10 rats were tested for behavioral responses, and 10 rats were used for protein detection. The 20 rats in the swim stress group received 14 days of swim stress, and the 20 control rats were free of stress. After stress, 10 stressed rats and 10 control rats were observed using behavioral tests of open field, elevated plus-maze and saccharine preference test. The experimental procedures were approved by the International Review Board of the Institute of Psychology, Chinese Academy of Sciences.

2.2. Stress procedure

Twenty rats were subjected to forced swim stress. The swim stress was performed between 08:00 h and 10:00 h in a stainless steel tank (2.0 mL × 1.0 mW × 1.5 mH) filled with 30 cm depth of water (10 ± 0.5 °C). Each rat was forced to swim individually for 5 min each day for 14 consecutive days. The control rats were given 5 min of handling each day for 14 consecutive days.

2.3. Behavioral tests

2.3.1. Body weight gain

Rats were weighed on the 1st and 7th day of handling, and on the 1st, 7th, and 14th day of experiment. Body weight gain from the 1st to 7th day of handling was marked as CWT, from the 1st to 7th day of experiment was marked as SWT7, and from the 1st to 14th day of experiment was marked as SWT14.

2.3.2. Open field

Twenty-four hours later the last stress, 20 rats were behaviorally tested using open field. The test was performed between 08:00 and 11:00 h. The apparatus

was a circular arena, 180 cm in diameter with 50 cm wall. The test room had a dim illumination (40 W) in order to decrease the averseness of the test [44]. An animal was placed in the center of the field and the following variables were recorded for 5 min and analyzed by a computer-based system (EthoVision, Noldus Information Technology b.v., Netherlands): number of rearing, number of grooming, horizontal activity (distance traveled), and time spent freezing. The fecal boli defecated during open field exploration were counted [21]. The open field was cleaned each time after testing a rat.

2.3.3. Elevated plus-maze

The test was performed immediately after the open field test and between 08:10 and 11:l0 h. When the open field test was finished, the rat was immediately placed into the elevated plus-maze. The maze procedures were run as previously described [44]. Briefly, the apparatus consisted of two opposite open arms (50.8 cm × l0.2 cm × 1.3 cm) and two opposite closed arms (50.8 cm × l0.2 cm × 40.6 cm). The arms were connected by a central square (10.2 cm × 10.2 cm). The apparatus was 72.4 cm above a floor and exposed to dim illumination. An animal was placed in the center of the maze facing a closed arm. The behavior was recorded for 5 min and analyzed by a computer-based system (MED-VPM-RS, Med Associate Inc., USA). Time spent in the open arms, the number of entry into the open and closed arms were assessed. An individual entry was recorded when the animal entered the arm with at least two front paws and half of its body. The shorter is the time spent in open arms, the higher is anxiety and vice versa [23,34]. After each trial the apparatus was cleaned with 30% ethanol solution.

2.3.4. Saccharin preference

On the last-stressed day, rats were deprived of water (from 20:00 h), then from the next day on, rats were given a 3 h window saccharin preference test (14:00–17:00 h) once a day for 4 consecutive days. The rats were given two bottles, one containing water and the other containing 1% sodium saccharin solution. The amount of each solution consumed was determined by weighing the bottles before and after the 3 h window. Total saccharine solution intake, total water intake, average saccharine solution intake and average water intake in 4 days were computed. Reduced consumption of sweet solutions (sucrose, saccharin) by chronic mild stress rats is a measure of anhedonia [53]. The saccharin preference was calculated as total saccharin intake/total (saccharin + water) intake. The positions of the bottles on the cages were changed every day. At the end of the preference test, rats were given free access to water.

2.4. Western blot

2.4.1. Materials

The antibodies of Pan-Erk1/2 primary antibody (rabbit monoclonal), P-Erk1/2 primary antibody (mouse monoclonal), β-actin primary antibody (mouse monoclonal), horseradish-peroxidase-labeled goat antirabbit secondary antibody IgG and horseradish-peroxidase-labeled goat antimouse secondary antibody IgG were obtained from Sigma–Aldrich, St. Louis, MO. A 0.2 μm nitrocellulose filter (NC), polyacrylamide and Buffer were also from Sigma–Aldrich, St. Louis, MO. Bicinchoninic acid assay kit (BCA), enhanced chemiluminescence (ECL) reagent were obtained from Pierce Biotechnology, Rockford, IL. Gel Doc™ XR System and Quantity One® 1D analysis software were purchased from Bio-Rad, Hercules, CA.

2.4.2. Tissue dissection and processing

Twenty rats were decapitated 24 h after last swim stress, and brains were rapidly removed on ice. The brain was placed in a stainless steel brain matrix, and prefrontal cortex was punched according to the atlas of Paxinos and Watson [40]. The whole body of hippocampus was dissected from the brain. All tissues were placed into liquid nitrogen to be frozen. Tissues were homogenized in 20 volumes of Buffer (pH 7.5, containing 50 mM Tris–Cl, 2 mM EDTA, 2 mM EGTA, 0.05 mM okadaic acid, l μM sodium vanidate, 5 μg/ml pepstatinA and 0.5% Nonidet P-40). Protein content of lysates was determined using BCA assay kits. Lysates were mixed with 5× sodium dodecyl sulfate (SDS) to prepare for certain concentration of sample solutions. All the sample solutions were stored at −80 °C for use.

2.4.3. Protein separation and immunoblot

Proteins were separated by SDS-PAGE on 10% polyacrylamide gels. Then proteins were moved to NC by electrophoretic transfer. Blots were incubated in blocking buffer (10% nonfat dry milk powder in Tris–buffered saline containing 0.5% Tween-20, TTBS) for 1 h at room temperature (RT), washed 10 min ×3 in TTBS. Blots were incubated with P-Erk1/2 primary antibody overnight at 4 °C and then washed 10 min ×3 in TTBS. Blots were incubated with horseradish-peroxidase-labeled goat antimouse secondary antibody IgG for 1 h at RT, washed 10 min ×3 in TTBS, treated with ECL reagents and exposed to film. Then blots were stripped of antibodies by incubation for 10 min at 50 °C with stripping buffer, re-blocked, washed, incubated for 3 h at RT with pan-Erk1/2 primary antibody which recognized total antigen protein, incubated with horseradish-peroxidase-labeled goat antirabbit secondary antibody IgG for 1 h at RT, then the total protein of Erk1/2 was visualized by treatment with ECL reagent and exposure to film. Repeat the above procedures, and the antigen of β-actin was visualized to film by binding to the proper primary antibody (β-actin primary antibody) and secondary antibody (horseradish-peroxidase-labeled goat antimouse secondary antibody IgG).

2.4.4. Immunoblots analysis

Immunoblots were analyzed using Quantity One® 1D analysis software. For each blot of P-Erk1/2 and pan-Erk1/2, the relative protein level was calculated from the ratio of absorbance of P-Erk1/2/β-Actin and the ratio of the absorbance of pan-Erk1/2/β-Actin to correct for small difference in protein loading.

2.5. Statistical analysis

The statistical analysis was performed using the "Statistical Package for Social Sciences" (SPSS, Version 11.5). The controls and the swim stress group were compared using Student's *t*-test. Pearson correlation tests (two-tailed) were used for protein-behavioral correlation analysis. $P \leq 0.05$ was the accepted level of significance.

3. Results

3.1. Body weight gain

During 7 handling days, the body weight gain did not differ significantly between controls and stressed animals; after 7 stressed days, stressed animals showed a decreased body weight gain as compared to controls (75.36 ± 6.21 g versus 100.64 ± 9.48 g, respectively; $t = 2.19$, $P < 0.05$), and after 14 stressed days, the body weight gain of stressed group was markedly less than that of controls (70.35 ± 6.49 g versus 114.80 ± 12.49 g, respectively; $t = 3.13$, $P < 0.01$) (Fig. 1).

3.2. Open field

The stressed animals revealed decreased distance traveled and higher number of grooming as compared to controls (distance: 2076.60 ± 393.80 cm versus 2990.00 ± 328.23 cm, respectively; $t = 1.87$, $P < 0.05$; grooming: 1.22 ± 0.22 versus 0.30 ± 0.15, respectively; $t = -3.48$, $P < 0.01$). The number of rearing, the freezing time and the number of fecal boli did not differ significantly between groups (Fig. 2).

3.3. Elevated plus maze

The stressed animals spent shorter time in open arms (90.30 ± 22.71 s versus 127.40 ± 13.97 s, respectively; $t = 2.39$, $P < 0.05$) and longer time in closed arms (209.70 ± 22.71 s

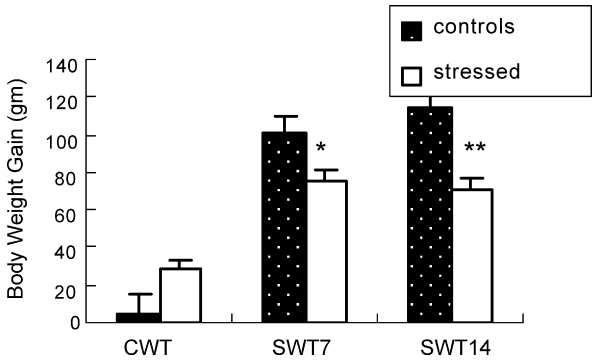

Fig. 1. Body weight gain of controls and stressed group during the period of 7 handling days (CWT), 7 stressed days (SWT7) and 14 stressed days (SWT14). All data were presented as means ± S.E.M. Controls $n = 10$, stressed $n = 10$. $^{*}P < 0.05$; $^{**}P < 0.01$.

versus 172.60 ± 13.97 s, respectively; $t = -2.39$, $P < 0.05$). The open arms entries and the closed arms entries did not differ significantly between the two groups ($t = -0.85$, $P > 0.05$; $t = 0.00$, $P > 0.05$) (Fig. 3).

3.4. Saccharine preference

The stressed animals showed decreased average saccharine solution intake in 4 days compared to controls (10.81 ± 1.65 g versus 14.98 ± 1.22 g, respectively; $t = 2.05$, $P < 0.05$), and the ratio of the total saccharine solution intake to total liquids intake was also significantly decreased (37.30 ± 5.45% versus 46.72 ± 5.22%, respectively; $t = 2.54$, $P < 0.05$); There was no significant difference in average water intake in 4 days between controls and stressed animals (Fig. 4).

3.5. The P-Erk2 and Erk1/2 level in the hippocampus and prefrontal cortex

P-Erk1 and P-Erk2 changed in parallel, but in short exposure, the signals of P-Erk1 were too weak to be detected, so only P-Erk2 signals were quantified. Chronic swim stress significantly decreased P-Erk2 level in the hippocampus and prefrontal cortex (hippocampus: 45.68 ± 3.52% versus 75.51 ± 9.83%, respectively; $t = 3.05$, $P < 0.01$; prefrontal

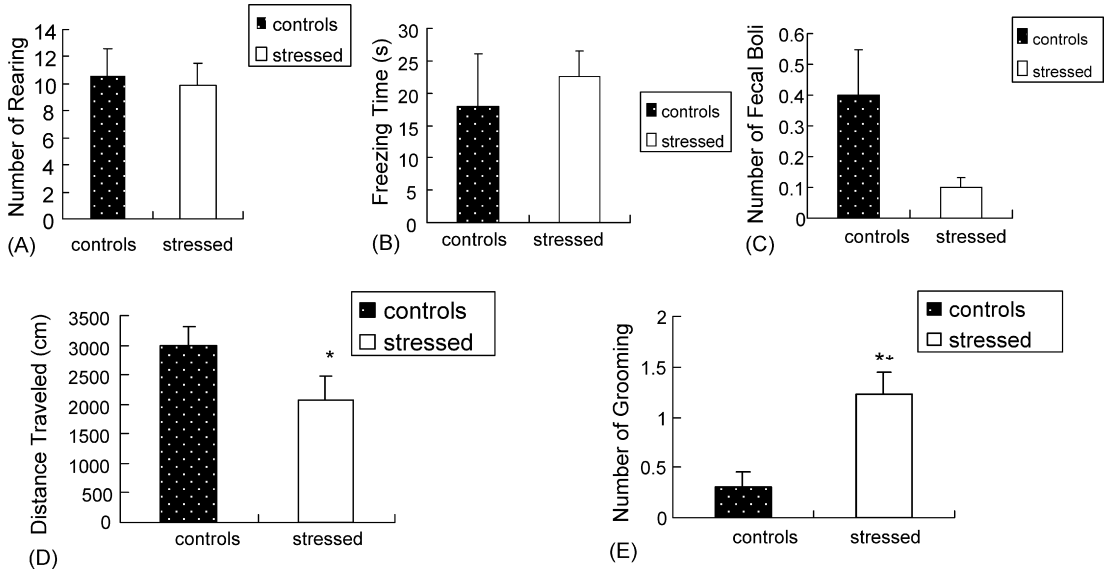

Fig. 2. Behavioral outcome of controls and stressed animals in open field. (A) The number of rearing. (B) Freezing time. (C) The number of fecal boli. (D) The distance traveled. Student's *t*-test analysis for distance traveled revealed the stressed animals showed decreased distance traveled than controls. (E) The number of grooming: the stressed animals had higher number of grooming compared to controls. All data were presented as means ± S.E.M. Controls $n = 10$, stressed $n = 10$. $^{*}P < 0.05$; $^{**}P < 0.01$.

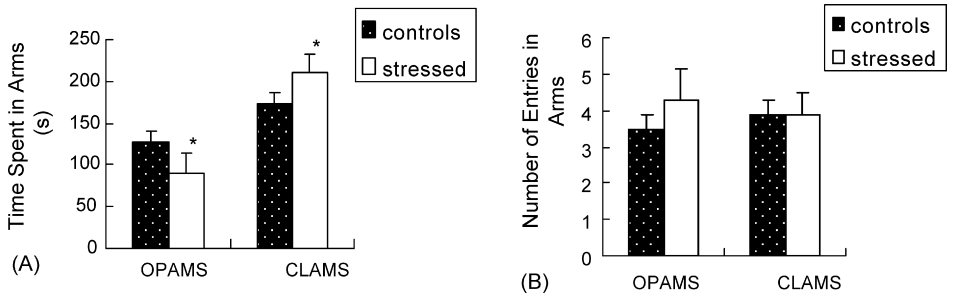

Fig. 3. Behavioral outcome of controls and stressed group in elevated plus-maze. (A) Time spent in open arms and closed arms. (B) The number of open arms entries and closed arms entries. OPAMS, open arms; CLAMS, closed arms. All data were presented as means ± S.E.M. Controls $n = 10$, stressed $n = 10$. $^{*}P < 0.05$.

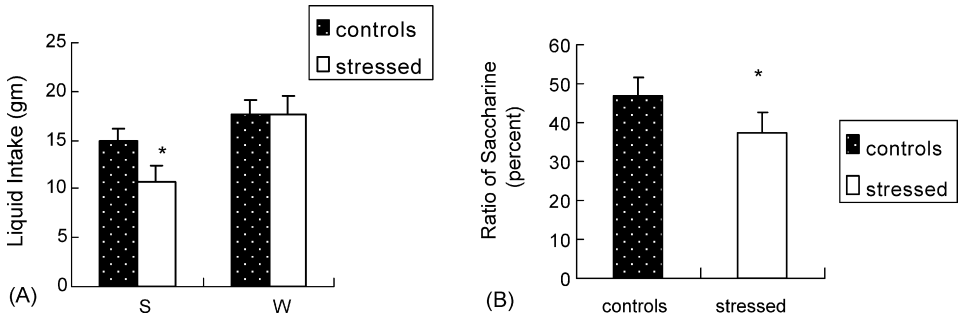

Fig. 4. Saccharine preference of controls and stressed group. (A) The average saccharine solution intake (S) and water intake (W) in 4 days. (B) The total saccharine solution intake/the total (saccharine solution + water) intake in 4 days. All data were presented as means ± S.E.M. Controls $n = 10$, stressed $n = 10$. $^{*}P < 0.05$.

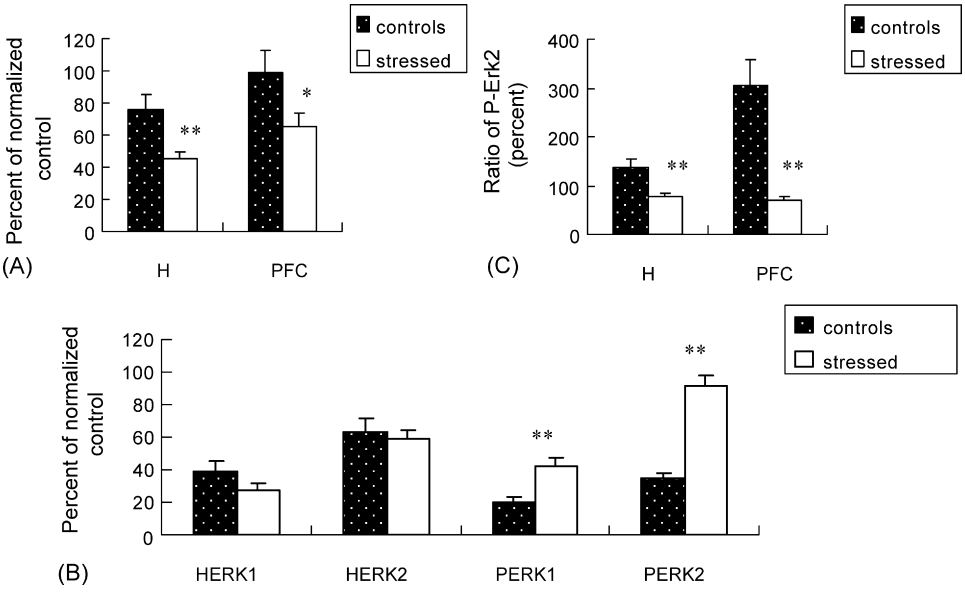

Fig. 5. The level of P-Erk2 (A), Erk1/2 (B) and the ratio of P-Erk2 to total Erk2 (C) in the hippocampus and prefrontal cortex. (A) The level of P-Erk2 in the hippocampus and prefrontal cortex. (B) The total protein of Erk1/2 in the hippocampus and prefrontal cortex. (C) The ratio of P-Erk2 to total Erk2 in the hippocampus and prefrontal cortex. H, hippocampus; PFC, prefrontal cortex; HERK1, Erk1 in the hippocampus; HERK2, Erk2 in the hippocampus; PERK1, Erk1 in the prefrontal cortex; PERK2, Erk2 in the prefrontal cortex. All data were presented as means ± S.E.M. Controls $n = 10$, stressed $n = 10$. $^{*}P < 0.05$; $^{**}P < 0.01$.

cortex: 65.27 ± 8.03% versus 98.61 ± 13.91%, respectively; $t = 2.16$, $P < 0.05$) (Figs. 5A and 6). Erk1 and Erk2 were both significantly increased in the prefrontal cortex in stressed group (Erk1: 42.56 ± 4.73% versus 20.11 ± 3.33%, respectively; $t = -4.12$, $P < 0.01$; Erk2: 91.74 ± 6.57% versus 34.46 ± 3.67%, respectively; $t = -7.93$, $P < 0.01$). There was no change in Erk1/2 in the hippocampus (Figs. 5B and 6). Chronic swim stress markedly decreased the ratio of P-Erk2 to total Erk2 in the hippocampus and prefrontal cortex (hippocampus: 78.20 ± 5.83% versus 135.26 ± 19.43%, respectively; $t = 2.95$, $P < 0.01$; prefrontal cortex: 70.33 ± 5.85% versus 303.56 ± 52.71%, respectively; $t = 4.64$, $P < 0.01$) (Fig. 5C).

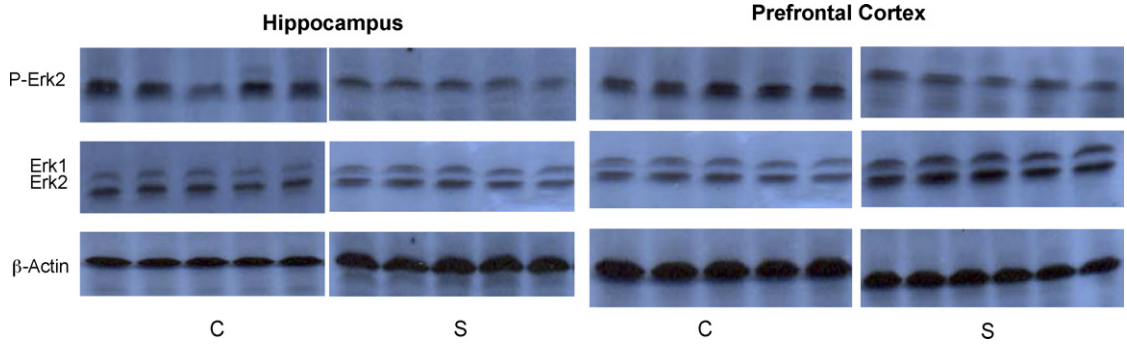

Fig. 6. The P-Erk2, Erk1/2 and β-actin in the hippocampus and prefrontal cortex. C, controls; S, stressed group.

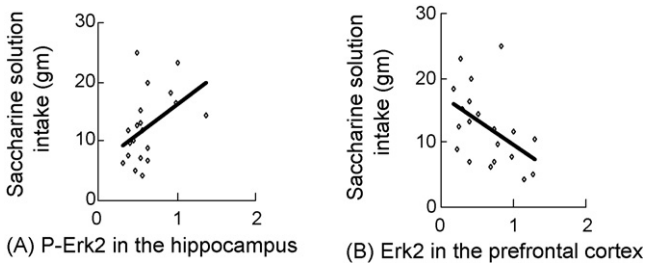

Fig. 7. Correlations between the level of saccharine solution intake and the level of P-Erk2 in the hippocampus (A), or the Erk2 in the prefrontal cortex (B). (A) The P-Erk2 in the hippocampus was positively correlated to the saccharine solution intake. (B) The Erk2 in the prefrontal cortex was negatively correlated to the saccharine solution intake.

3.6. Protein-behavioral correlations

There were significant positive correlations between the P-Erk2 in the hippocampus and body weight gain of 14 stressed days as well as saccharine solution intake ($r = 0.52$, $P < 0.05$; $r = 0.47$, $P < 0.05$). P-Erk2 in the prefrontal cortex was positively correlated to body weight gain of 7 stressed days as well as body weight gain of 14 stressed days ($r = 0.47$, $P < 0.05$; $r = 0.44$, $P = 0.05$). Negative correlations were found between Erk2 in the prefrontal cortex and body weight gain of 7 stressed days ($r = -0.48$, $P < 0.05$), body weight gain of 14 stressed days ($r = -0.57$, $P < 0.05$), saccharine solution intake ($r = -0.45$, $P = 0.05$), the ratio of saccharine solution intake to total liquids intake ($r = -0.44$, $P = 0.05$), and horizontal activity ($r = -0.44$, $P = 0.05$). Erk2 in the prefrontal cortex was positively correlated to the number of grooming ($r = 0.49$, $P < 0.05$) (Fig. 7).

4. Discussion

Forced swim stress is currently one of the most frequently used animal models of depression, which includes the components of anxiety and desperation induced by psychological stress when animals are exposed to novel situations and confronted with life threats. Our study demonstrated that chronic swim stress did significantly induce depressive-like behaviors in animals. The stressed animals exhibited the following behavioral disorders: decreased body weight gain; decreased interest in exploring novel and light places; decreased locomotor activity in open field and decreased saccharine solution intake. Our results are in accord with those from other animal models of depression. For example, animals of maternal deprivation, social deprivation and predator exposure et al. also show decreased time spent in open arms in elevated plus maze and decreased locomotor activity in open field [28,52]. Moreover, the depressive-like behaviors the stressed animals exhibited are analogous to the symptoms presented in human major depression: feelings of hopelessness; weight loss; decreased interest in pleasurable stimuli and low energy (DSM-IV, 2000). Especially, the decreased saccharine solution intake indicated the core symptoms of depression-anhedonia, an inability to experience pleasure from normally pleasurable sources [9]. Thus our results demonstrated, for the first time, that the chronic swim stress had good face validity for animal model of depression. At the same time, the present study indicated that the number of grooming of the stressed animals was increased. Grooming induced by novel environment is a kind of behavior of stress-coping and de-arousal, and the increased grooming means anxiety-like behavior [25,50], so from our results we could found that the anxiety-like behavior was accompanied with the depressive-like behavior. This result is in accord with clinical studies which suggest that anxiety is not only accompanied by symptoms of depression, but may be an expected precursor syndrome in the development of depression [39].

Another important finding of our study is that chronic swim stress impaired the Erk1/2 signal transduction pathway in the hippocampus and prefrontal cortex indicated by (1) the P-Erk2 level was decreased in the hippocampus and prefrontal cortex, and (2) the ratio of the P-Erk2 to total Erk2 was also decreased in the hippocampus and prefrontal cortex. Our findings are partially in accord with observations derived from depressed suicide subjects, in which the levels of P-Erk1/2, the levels of Erk1/2, and the expression of Erk1/2 mRNA are significantly decreased in the hippocampus and frontal cortex [13]. In another related study, researchers find that depressed animals show decreased P-Erk1/2 in the frontal cortex and P-Erk1 in the hippocampus, and a small decrease of Erk2 in the frontal cortex [16]. However, our finding that chronic swim stress increased Erk1/2 in the prefrontal cortex and did not change Erk1/2 in the hippocampus was not consistent with the previous reports. The possible reasons for this discrepancy could be due to the duration of depression-inducing stimuli and the extent of depressive response. Contrast to depression of human subjects and the above animals which is induced by prolonged stress and chronic clomipramine administration respectively, in our experiment, the depressive-like behaviors were induced by 14 days of forced swim stress. With this time profile, animals might experience minor depressive responses and have the capability to increase Erk1/2 to compensate the decrease of the P-Erk2 in the prefrontal cortex. However, there was no change in Erk1/2 in the hippocampus, perhaps because of abundant synaptic connections of hippocampal neurons can compensate for the decrease of the P-Erk2 by other compensatory reactions, such as increase in electrical responsiveness to synaptic activation and possibly by an increase in synaptic efficacy or synaptogenesis [2,31].

It is of interest to note that phosphorylation state of the Erk1/2 is regulated by opposing kinases and various types of Erk-specific protein phosphatases such as serine/threonine protein phosphatase 2A (PP2A) [1,55] and mitogen-activated protein kinase phosphatases (MKP) [27]. In depressed suicide victims, P-Erk1/2 decreases in the hippocampus and frontal cortex are accompanied by increased MKP-2 [13]. But in the depressed rats, the decreased P-Erk1/2 is accompanied by increased PP1, but not MKP-2, in the frontal cortex and hippocampus. So there is difference in the regulation of Erk1/2 dephosphorylation by phosphatases between humans and rats. Further studies are needed to determine which phosphatase is increased in our animal model of depression and whether the increased phosphatase is indeed involved in the reduction of P-Erk1/2 in brain in depressed animals and humans.

The present study demonstrated that the P-Erk2 and Erk1/2 in the hippocampus and prefrontal cortex were closely correlated to depressive-like behaviors of the experimental animals. For example, the P-Erk2 in the hippocampus was positively correlated to saccharine solution intake, the P-Erk2 in the prefrontal cortex was positively correlated to body weight gain of 7 stressed days, and the Erk2 in the prefrontal cortex was negatively correlated to saccharine solution intake and horizontal activity. In the recent studies, researchers have speculated that the Erk1/2 signal transduction pathway may be involved in the neuronal plasticity that is presumed to underlie depression disorders induced by stress, in their studies they find that acute swim stress increases P-Erk2 in the prefrontal cortex [48], acute restraint stress increases P-Erk2 in the hippocampus and prefrontal cortex in rats, and chronic repeated restraint stress decreased the brain level of P-Erk2 [35]. However, there are no behavioral parameters in their studies, so there is no direct evidence concerning whether depressive-like behavior is indeed related to the Erk1/2 pathway in brain. Feng et al. [16] demonstrate, for the first time, that sexual activity is positively correlated with the activation of Erk2 in the frontal cortex but not other brain regions investigated. Our results demonstrated that the depressive-like behaviors were accompanied with the decreased level of P-Erk2 and increased level of Erk2, and indicated, for the first time, that the P-Erk2 and Erk2 in the hippocampus and prefrontal cortex significantly correlated with several behavioral variables such as locomotor activity and interest in exploration. Especially there was a positive correlation between the P-Erk2 in the hippocampus and the saccharine solution intake, which meant the lower was the P-Erk2 level, the less was the saccharine solution intake. Because the decrease of saccharine solution intake indicates the core symptoms of depression-anhedonia [9], we tentatively infer the decrease of P-Erk2 might be at least one of the mechanisms underlying depression induced by stress. It is notable that the Erk2 in the prefrontal cortex was negatively correlated to saccharine solution intake and horizontal activity, but positively correlated with the number of grooming. Because decreased saccharine solution intake and horizontal activity indicates animals are depressed, but increased number of grooming indicates animals are anxious, such results meant the higher was the Erk2 level, the more depressive and anxious were the animals. So we hypothesized that the anxiety and depression might have a common neurobiological substrate.

The Erk1/2 signal pathway has vital role in a wide array of cellular functions. When phosphorylated, Erk1/2 can lead to the activation of CREB, which is crucial for stimulus-transcription coupling: the transmission of events that occur at cell membranes into alterations in gene expression [5,10,46]. It is currently known that activation of the Erk1/2 pathway can lead to activation of c-fos, c-jun, Zif268, Arc gene expression [15,20,43,54]. When Erk1/2 activation is decreased, the genes expression and the corresponding proteins regulated by P-Erk2 will be destroyed, such that necrosis, apoptosis or dysfunction may occur to the neurons which can ultimately affect the function of individual neurons and entire neuronal circuits [6], which may be the mechanism the reduction of phosphorylation of Erk1/2 leads to aberrant brain function and depression.

In summary, chronic swim stress significantly induced depressive-like behavior in animals, decreased the P-Erk2 level and the ratio of the P-Erk2 to total Erk2 in the hippocampus and prefrontal cortex, and increased Erk1/2 in the prefrontal cortex. The P-Erk2 and Erk1/2 in the hippocampus and prefrontal cortex were closely correlated to depressive-like behaviors of the experimental animals. The reduction of Erk1/2 phosphorylation may be the pathophysiology of depression induced by stress.

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