



## Research report

# A role for the extracellular signal-regulated kinase signal pathway in depressive-like behavior

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## ABSTRACT

Our recent research demonstrates that the extracellular signal-regulated kinase (ERK) signal pathway is impaired in depressed animals, and such disruption is effectively reversed following antidepressant treatment. These results indicate that the ERK pathway may participate in the molecular mechanism of depression. To provide direct evidence for the potential role of the ERK pathway in depression, the present study using a sub-chronic regimen of ERK inhibition investigated the disparate role for the ERK cascade in two specific brain areas, the dorsal hippocampus (dHP) and the medial prefrontal cortex (mPFC), in the pathophysiology of depressive-like behavior. Rats were bilaterally implanted with cannulas in the dHP or mPFC and were microinjected with U0126, a specific inhibitor of ERK upstream activator, or vehicle for 7 consecutive days. The behavioral effects of the ERK pathway inhibition were examined in the open field, elevated plus maze, and saccharin preference test. The results showed that the inhibition of the ERK pathway in dHP resulted in anhedonia and anxiety-like behavior, and the ERK pathway inhibition in the mPFC induced anhedonia and locomotor impairment in rats. The phosphorylation of the cyclic AMP-responsive-element-binding protein (CREB) was decreased following the ERK pathway inhibition either in dHP or mPFC. These findings demonstrate that the ERK pathway in either the dHP or mPFC participates in the pathophysiology of the depressive-like behavior, and may have pivotal role in human depression.

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## 1. Introduction

Our recent studies demonstrate that the extracellular signal-regulated kinase (ERK) signal pathway is disrupted in depressed animals, and such disruption is effectively reversed following systematical administration of antidepressant [39,40]. These results suggest that the ERK signal pathway may be one of the biological underpinnings of depression. To date, however, there is very little direct evidence indicating that the ERK pathway participates in depression, and the results are not consistent. Thus, the present study was to further confirm whether the direct inhibition of the ERK pathway leads to depressive-like behavior and to explore the causal role for the ERK pathway in depressive-like behavior.

ERK belongs to the family of mitogen-activated protein kinases (MAPKs) that integrate signals received by membrane receptors and transfer them to the nucleus [5,37]. The phosphorylation state and activity of ERK isoforms 1 and 2 (ERK1/2) is specifically modified by MAPK/ERK kinases (MEK1/2) [34]. The ERK pathway is activated by neurotrophins and neuroactive chemicals to produce their effects on neuronal differentiation, survival, and structural and functional plasticity [46,48,56]. Recently, the role for the ERK pathway in the

molecular mechanism of depression is increasingly becoming the focus of much research, and a growing body of evidence indicates that the ERK pathway may participate in the neuronal modulation of depression [12,15,18,33,39,40,49]. However, there is very little direct evidence for the role of the ERK pathway in depression, and the results are not consistent because of the differences in drugs, experimental procedures, and treatment regimens used. For example, employing systemic injections of MEK inhibitors, Duman et al. and Einat et al. provide opposite evidence for the role of the ERK pathway in depression [10,13]. Duman et al. demonstrate that the inhibition of the ERK pathway produces depressive-like responses in learned helplessness, forced swim test, and the tail suspension test [10], but Einat et al. observe the antidepressant effects of the ERK inhibition [13]. Moreover, using different regimens with the systemic ERK inhibition, Todorovic et al. and Tronson et al. report the acute antidepressant effects of the local ERK inhibition in the dorsal hippocampus [50,51]. The role for the ERK pathway in depression therefore still awaits further investigations, especially for the role of the ERK pathway in either brain regions involved in depression. Moreover, it is noted that most of the studies performed so far have observed the acute behavioral effects of the ERK inhibition [10,13,50,51]. According to our observation, however, there is a long-lasting decrease in ERK activity in depressed animals exposed to chronic stress, and the depressive-like behavior is introduced not immediately after but generally 7 days later than the decrease

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of the ERK activity [39,40, and our unpublished data]. Thus, it is intriguing to examine the effects of a sub-chronic (7-day) regimen of ERK pathway inhibition, which may have better ecological validity for depression, in some specific brain regions on depressive-like behavior.

Cyclic AMP-responsive-element-binding protein (CREB) is a transcription factor and downstream target of the ERK pathway [29,53]. The phosphorylation state of the CREB is ERK-dependent in the learning, memory, and formation of long-term potentiation (LTP). When the ERK activation is depressed, the CREB phosphorylation is impaired [21,55]. CREB has emerged as a critical effector of the ERK pathway. Based on the proposed interaction of the ERK and CREB, the present study examined the effects of the ERK inhibition on the CREB phosphorylation.

Brain structures of the hippocampus and prefrontal cortex have been implicated in depression. There are morphological and functional alterations in the both brain regions in humans with major depression, such as the reduced volume of the hippocampus [4], and the altered metabolism in the prefrontal cortex [9,41]. Therefore, the disparate role for the ERK pathway in the two specific brain regions in depressive-like behavior deserves further investigation.

Thus, the present study investigated the disparate role for the ERK pathway in two specific brain areas which are especially important in neuronal plasticity [3,44], the dorsal hippocampus (dHP) and the medial prefrontal cortex (mPFC), in the neural mechanism of depressive-like behavior. We used a pharmacologic inhibitor of MEK, U0126, to systematically (7 days) inhibit the ERK activation in the rat dHP and mPFC, respectively, and investigated the effects of the sub-chronic regimen of ERK pathway inhibition on the depressive-like behavior in the open field, elevated plus maze, and saccharin preference test and on the CREB activity.

## 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). For the intra-hippocampus U0126 infusion experiment, rats were divided into HP-U0126 group and HP-vehicle group; for the intra-prefrontal cortex U0126 infusion experiment, rats were divided into PFC-U0126 group and PFC-vehicle group. All rats were housed individually. They were acclimated to daily 3 min of handling for 7 days before being used in experiment. Rats were maintained on a 12-h light/dark cycle (lights on at 08:00 h) with free access to food and water except for the saccharin preference test time period. The experiment procedures were approved by the International Review Board of the Institute of Psychology, Chinese Academy of Science, and were in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals.

### 2.2. Cannulation and injections

After the handling, the rats were anesthetized with 400 mg/kg chloral hydrate and were implanted with bilateral guide cannulas aimed at the dHP (3.4 mm posterior to the bregma, 2.5 mm lateral to the sagittal suture and 3.5 mm in depth relative to the skull) or mPFC (3.6 mm anterior to the bregma, 1.5 mm lateral to the sagittal suture and 3.0 mm in depth relative to the skull). The rats were allowed to recover from surgery in their home cages for 10–12 days. U0126 (Promega, Madison, WI), a specific inhibitor of MEK, was dissolved in 100% DMSO to a stock concentration of 4 mg/ml. The effects of U0126 have been shown to be specific to ERK/MAPK and to have no effect at a range of concentrations on other kinases, such as c-Jun N-terminal kinase (JNK), protein kinase A (PKA), calcium-calmodulin kinase IV, or protein kinase C (PKC) [14,43]. Before infusion, the drug was diluted 1:1 in artificial CSF (aCSF) containing (mM): 130 NaCl, 3 KCl, 1.25 NaH<sub>2</sub>PO<sub>4</sub>, 26 NaHCO<sub>3</sub>, 1 MgCl<sub>2</sub>, 10 glucose, and 2 CaCl<sub>2</sub>. The vehicle was 50% DMSO in aCSF. Rats were given once daily bilateral infusions of 1.0 µg/0.5 µl/side of either drug (HP-U0126 group and PFC-U0126 group) or vehicle (HP-vehicle group and PFC-vehicle group) for seven consecutive days. The dose and timing of the antagonist were selected on basis of pilot experiments and the observations in our lab [23,28,30,39,40,46, and our unpublished data]. During infusion, the injectors extended 1.5 mm beyond the tips of the guides, and the injectors remained in the cannulas for 1 min after drug infusion to allow diffusion of the drug from the tips. All injections were performed in freely moving animals at a rate of 0.25 µl/min using a dual syringe infusion pump (Stoelting). Injections of methy-

lene blue were performed at the end of the experiments for each rat to verify the cannula position. Only rats with correctly inserted cannulas were used for statistical analysis.

### 2.3. Behavioral tests

#### 2.3.1. Open field test

24 h after the last injection, the test was performed between 08:00 and 12:00 h. The procedures were run as previously described [39,40,45]. Briefly, the apparatus was a circular arena, 180 cm in diameter with 50 cm wall, put in a room with a dim illumination (40 W). One rat was placed in the center of the field, and the distance traveled and the number of rearing during 5 min of exploration were recorded and analyzed by a computer-based system (EthoVision, Noldus Information Technology b.v., Netherlands). After each trial, the apparatus was cleaned with 30% ethanol solution.

#### 2.3.2. Elevated plus maze test

The test was performed between 08:10 and 12:10 h immediately after the open field test. When the open field test was finished, the same rat was immediately placed into the elevated plus maze. The maze procedures were run as previously described [39,40,45]. Briefly, the apparatus consisted of two opposite open arms (50.8 cm × 10.2 cm × 1.3 cm) and two opposite closed arms (50.8 cm × 10.2 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 floor and exposed to dim illumination. A rat was placed in the center of the maze facing a closed arm. The number of entries into the open arms and the closed arms, and the time spent in the open arms, closed arms, and the central area within 5 min were assessed and analyzed by a computer-based system (MED-VPM-RS, Med Associate Inc., USA). An individual entry was recorded when the whole body of an animal entered the arm. The shorter the time spent in the open arms, the higher is the anxiety and vice versa [22,32]. The apparatus was cleaned each time after testing a rat.

#### 2.3.3. Saccharin preference test

On the last-injection day, rats were deprived of water (from 20:00 h) for 20 h. Then on the next day, rats were given a 1-h window saccharin preference test (16:00–17:00 h). The rats were given two bottles one containing water and the other containing 0.5% sodium saccharin solution. The amount of each liquid intake was determined by weighing the bottles before and after the 1-h window. The saccharin solution intake, the water intake, and the total liquid intake were computed. Reduced consumption of sweet solutions (sucrose, saccharin) is a measure of anhedonia [6]. The saccharin preference was calculated as the ratio of the saccharin solution intake to total liquid intake. The positions of the bottles on the cages were changed every half-an-hour. At the end of the preference test, rats were given free access to water.

### 2.4. Preparation of brain sections

Immediately after the completion of all the behavioral tests, all rats were decapitated, and brains were rapidly removed on ice. The brains were placed into liquid nitrogen to be frozen for 10 s and then were stored at –80 °C for use. The brains were sectioned on a cryostat into 200-µm-thick sections. Every sixth-section was mounted on gelatin-subbed slides and the location of the infusion site was evaluated and recorded for each animal. According to the atlas of Paxinos and Watson [36], the bilateral dHP were micropunched with a 1 mm punch tool (Fine Science Tools, Foster City, CA) from sections between 7 and 9 mm posterior to the front pole of the cortex. The punches included the CA1 and 2 subregions, and possibly portions of the DG subregion. The bilateral mPFC were micropunched from sections between 0 and 2 mm posterior to the front pole of the cortex. The punches included the PL and IL. The punches of the dHP and mPFC were prepared for protein determination using Western blot.

### 2.5. Western blot

The antibodies of ERK1/2 primary antibody (rabbit monoclonal, 1:2000), P-ERK1/2 primary antibody (Thr202/Tyr204, mouse monoclonal, 1:1000), CREB primary antibody (rabbit monoclonal, 1:1000), P-CREB primary antibody (Ser133, mouse monoclonal, 1:500), β-actin primary antibody (mouse monoclonal, 1:1000), horseradish-peroxidase-labeled goat antirabbit secondary antibody IgG and horseradish-peroxidase-labeled goat antimouse secondary antibody IgG were purchased from Sigma–Aldrich, St. Louis, MO, USA. Secondary antibodies were always used at the dilution of 1:1000. 0.2 µm nitrocellulose filter (NC), polyacrylamide and buffer were also from Sigma–Aldrich, St. Louis, MO, USA. Bicinchoninic acid assay kit (BCA) and enhanced chemiluminescence (ECL) reagent were obtained from Pierce Biotechnology, Rockford, IL, USA. Gel Doc™ XR System and Quantity One® 1-D analysis software were purchased from Bio-Rad, Hercules, CA, USA.

The punches of the dHP and mPFC obtained in the preparation of brain sections 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, 1 µM sodium vanadate, 5 µg/ml pepstatin A 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  $-80^{\circ}\text{C}$  for use.

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 for  $10\text{ min} \times 3$  in TTBS. Blots were incubated with P-ERK1/2 primary antibody overnight at  $4^{\circ}\text{C}$  and then washed for  $10\text{ min} \times 3$  in TTBS. Blots were incubated with secondary antibody IgG for 1 h at RT, washed for  $10\text{ min} \times 3$  in TTBS, treated with ECL reagents and exposed to film. Then blots were stripped of antibodies by incubation for 10 min at  $50^{\circ}\text{C}$  with stripping buffer, re-blocked, washed, incubated for 3 h at RT with ERK1/2 primary antibody which recognizes total antigen protein, incubated with secondary antibody 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  $\beta$ -actin was visualized to film. The P-CREB, CREB and their corresponding  $\beta$ -actin were determined in a different NC using the same procedures.

Immunoblots were analyzed using Quantity One<sup>®</sup> 1-D analysis software. For each blot of P-ERK1/2, ERK1/2, P-CREB and CREB, the relative protein level was calculated from the ratio of the absorbance of P-ERK1/2, ERK1/2, P-CREB and CREB to their corresponding  $\beta$ -actin to correct for small difference in protein loading.

### 2.6. Statistical analysis

The statistical analysis was performed using the "Statistical Package for Social Sciences" (SPSS, Version 11.5). Difference between two groups was compared using Student's *t*-test. The significant level was set at  $P \leq 0.05$ .

## 3. Results

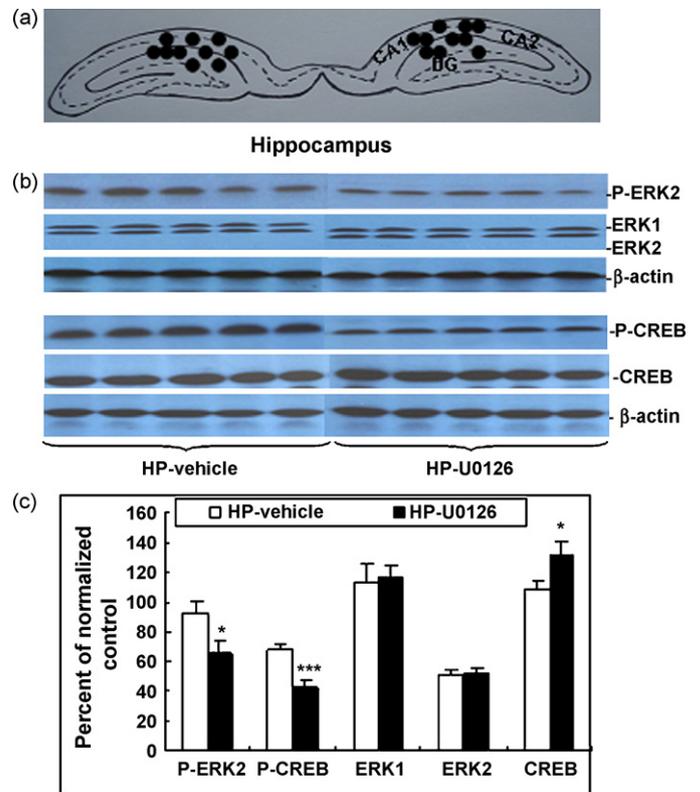
### 3.1. Intra-hippocampus U0126 infusion inhibited ERK activation and decreased CREB phosphorylation

After the completion of all the behavioral tests, animals were decapitated, and the locations of the infusion sites were verified. Only animals with the infusion sites inside the boundaries of the dHP (Fig. 1a) were used. The tissue of the dHP was collected and processed for P-ERK1/2 determination. Consistent with our previous experiments [39,40], the Western blot showed that the signals of the P-ERK1 were too weak to be detected, so only the P-ERK2 signals were quantified in the present study (Fig. 1b). Fig. 1c shows that repeated U0126 injections significantly decreased the basal P-ERK2 level in the dHP ( $t = 2.28$ ,  $P < 0.05$ ), but did not change the total protein level of ERK1/2. Repeated U0126 injections also decreased the CREB phosphorylation in the dHP ( $t = 4.78$ ,  $P < 0.001$ ), and the total protein level of CREB was compensatively increased ( $t = -2.28$ ,  $P < 0.05$ ).

### 3.2. Intra-hippocampus U0126 infusion induced anxiety and anhedonia but no locomotor impairment

The locomotor activity of animals in an open field is thought to reflect general activity [2,38]. Fig. 2 shows that there was no significant difference in the distance traveled and the number of rearing between the HP-U0126 group and HP-vehicle group ( $P > 0.05$ ), suggesting that the blockade of the ERK pathway in dHP exerts no effect on rat locomotor activity.

Based on the demonstrated comorbidity of depression with anxiety [35,57], the present study evaluated the anxiety-like behavior of animals. The elevated plus maze is a commonly used test of anxiety in mice and rats [20,52]. The reduced number of entries into the open arms and the decreased time spent in the open arms are used to assess a state of anxiety [32]. Fig. 3 shows that bilateral hippocampal U0126 infusion decreased the time spent in open arms and increased the time spent in closed arms in rats ( $t = 3.40$ ,  $P < 0.01$  and  $t = -2.95$ ,  $P < 0.01$ , respectively). The U0126-treated animals also exhibited decreased number of entries into the open arms compared to the vehicle-treated animals ( $t = 3.78$ ,  $P < 0.001$ ). There was no difference in the number of entries into closed arms and the time spent in central area between the two groups. These data suggest



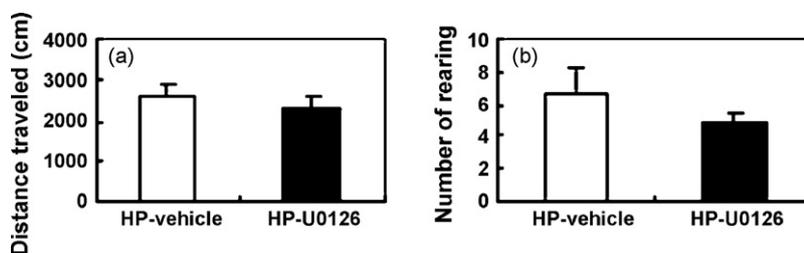
**Fig. 1.** The effect of the inhibition of ERK pathway in dHP on expression of P-ERK2, ERK1/2, P-CREB, and CREB in rat hippocampus. (a) Drawing of a coronal section of the hippocampus indicating infusion sites (black circles) for animals used in analysis. Each circle represents a novel infusion site. (b) Representative Western blots of P-ERK2, ERK1/2, P-CREB, CREB and their corresponding normalized control  $\beta$ -actin in hippocampus. (c) The comparison of P-ERK2, ERK1/2, P-CREB, and CREB in rat hippocampus between the U0126- and vehicle-treated animals. Data expressed as mean  $\pm$  S.E.M.,  $N = 10$ –12 per group. \* $P < 0.05$  and \*\*\* $P < 0.001$ .

that the blockade of the ERK pathway in dHP induces anxiety-like behavior in rats.

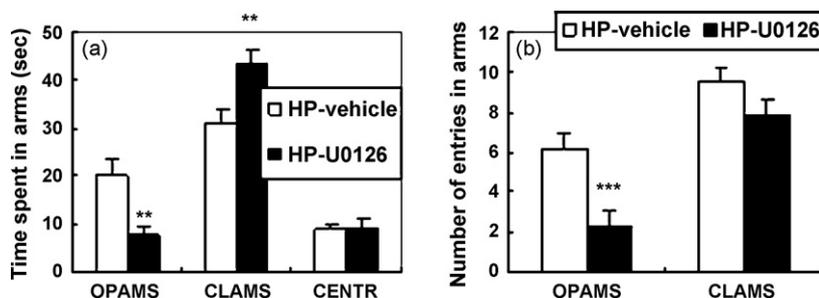
The saccharin preference test is used to measure the hedonic alterations, and the reduced consumption of sweet solutions is an index of anhedonia [6]. Fig. 4 shows that bilateral infusion of U0126 into dHP decreased the saccharin solution intake and the ratio of the saccharin solution intake to total liquid intake ( $t = 2.43$ ,  $P < 0.05$  and  $t = 3.85$ ,  $P < 0.001$ , respectively), but left the total liquid intake unchanged, suggesting that the blockade of the ERK pathway in dHP produces anhedonia.

### 3.3. Intra-prefrontal cortex U0126 infusion inhibited ERK activation and decreased CREB phosphorylation

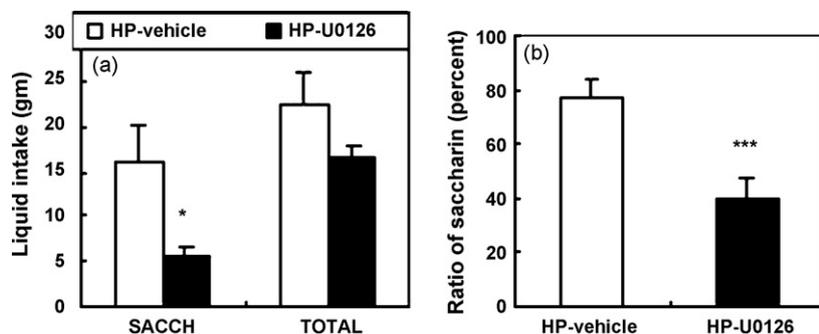
In addition to the hippocampus, the prefrontal cortex is also an important brain structure involved in depression [9,41]. So we investigated here the role of the ERK pathway in the prefrontal cortex in depressive-like behavior. As performed in the intra-hippocampus U0126 infusion experiment, the locations of the infusion sites were verified, and only animals with the infusion sites inside the boundaries of the mPFC (Fig. 5a) were used for analysis. Fig. 5b and c shows that the repeated U0126 infusions inhibited the ERK activation in the mPFC ( $t = 3.49$ ,  $P < 0.01$ ), and produced no effect on the total protein levels of the ERK1 and 2. The repeated U0126 infusions also significantly decreased the P-CREB level in the mPFC ( $t = 2.35$ ,  $P < 0.05$ ), but had no effect on the total protein level of CREB (Fig. 5b and c).



**Fig. 2.** The effect of the inhibition of ERK pathway in dHP on distance traveled (a) and the number of rearing (b) in an open field in rats. Data expressed as mean  $\pm$  S.E.M.,  $N=10-12$  per group.



**Fig. 3.** The effect of the inhibition of ERK pathway in dHP on the time spent in arms (a) and the number of entries into arms (b) in elevated plus maze in rats. OPAMS, open arms; CLAMS, closed arms; CENTR, central area. Data expressed as mean  $\pm$  S.E.M.,  $N=10-12$  per group. \*\* $P<0.01$  and \*\*\* $P<0.001$ .



**Fig. 4.** The effect of the inhibition of ERK pathway in dHP on liquid intake (a) and the ratio of saccharin solution intake to total liquid intake (b) of rats. SACCH, saccharin solution; TOTAL, total liquid. Data expressed as mean  $\pm$  S.E.M.,  $N=10-12$  per group. \* $P<0.05$  and \*\*\* $P<0.001$ .

### 3.4. Intra-prefrontal cortex U0126 infusion induced locomotor impairment and anhedonia but no anxiety

Differing with the effect of the ERK pathway blockade in dHP on locomotor activity, the ERK pathway inhibition in the mPFC decreased the distance traveled ( $t=3.04$ ,  $P<0.01$ ) and reduced the number of rearing ( $t=2.99$ ,  $P<0.01$ ) in an open field in rats, suggesting that the blockade of the ERK pathway in mPFC induces locomotor impairment in rats (Fig. 6).

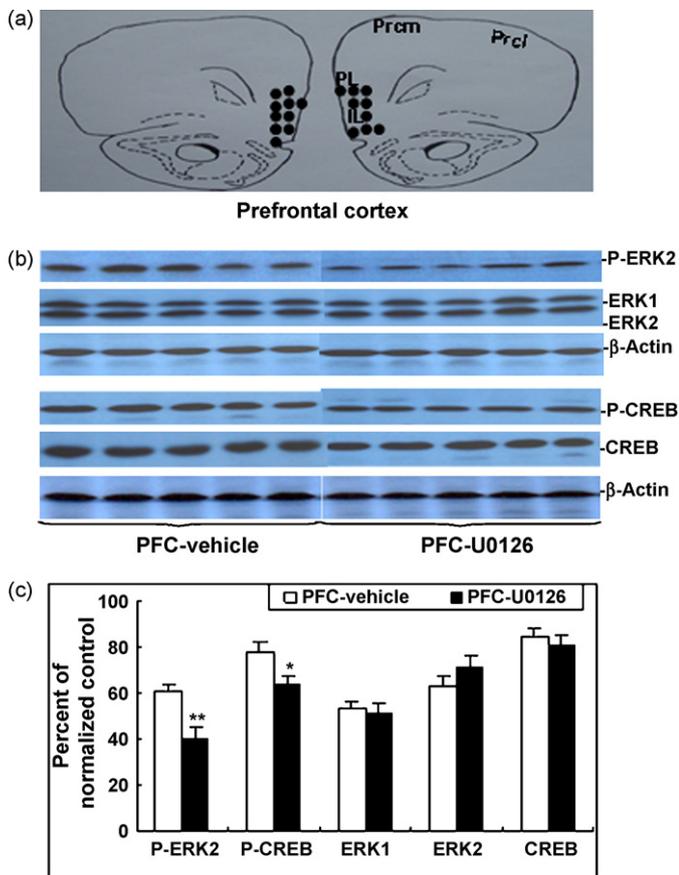
In the elevated plus maze, as shown in Fig. 7, bilateral U0126 infusion into the mPFC produced no difference in the time spent in open arms, closed arms, and the central area between the U0126-treated and vehicle-treated rats. The number of entries into the open arms and the closed arms also did not differ between the two groups. These data suggest that the blockade of the ERK pathway in mPFC induces no anxiety-like behavior in rats.

Fig. 8 shows that the rats bilaterally infused with U0126 in mPFC exhibited the decreased saccharin solution intake and the decreased ratio of saccharin solution intake to total liquid intake ( $t=3.57$ ,  $P<0.01$  and  $t=2.49$ ,  $P<0.05$ , respectively) compared to the vehicle-infused controls. There was no difference in the total liquid intake between the two groups. These data suggest that the blockade of the ERK pathway in mPFC induces anhedonia.

## 4. Discussion

The results presented in this study revealed three novel findings relevant to depression. First, the direct inhibition of the ERK pathway in dHP caused depressive-like behavior. The rats given bilateral U0126 infusion into dHP exhibited the core symptom of depression, anhedonia, and anxiety. Second, the direct inhibition of the ERK pathway in mPFC also induced depressive-like behavior. The rats given bilateral U0126 infusion into mPFC exhibited anhedonia and the decreased locomotor activity. Finally, the phosphorylation of CREB was decreased following the systematic U0126 infusions in either the dHP or mPFC. Taken together, these findings demonstrate a critical role for the ERK pathway in either the dHP or mPFC in the molecular mechanism of depressive-like behavior.

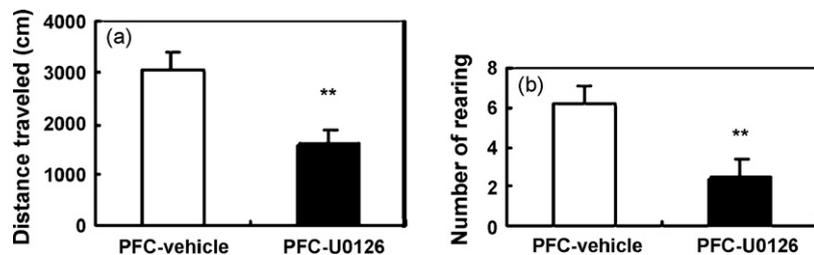
MEK is an upstream activator of the ERK, and the pharmacologic inhibitors of MEK have been useful in identifying roles for ERK signaling in synaptic plasticity, learning, and memory in a variety of systems [17,25,42,47]. In the present study, we systematically infused a MEK inhibitor, U0126, into the dHP and mPFC to observe their effects on depressive-like behavior. Differing with many previous studies investigating the acute effects of ERK inhibition on behavior [28,30,46], the present study employed a sub-chronic regimen of MEK inhibitor injections. Huang and Lin report that



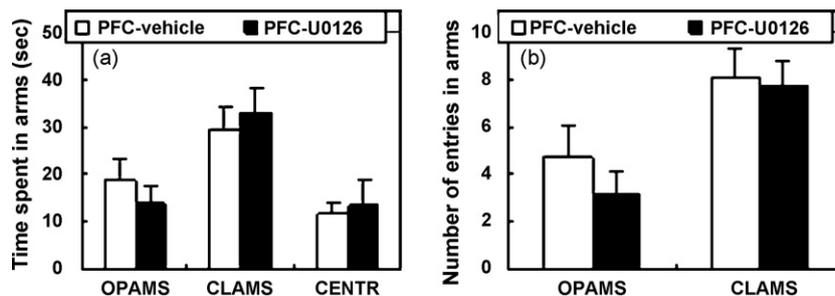
**Fig. 5.** The effect of the inhibition of ERK pathway in mPFC on expression of P-ERK2, ERK1/2, P-CREB, and CREB in rat prefrontal cortex. (a) Drawing of a coronal section of the prefrontal cortex indicating infusion sites (black circles) for animals used in analysis. Each circle represents a novel infusion site. (b) Representative Western blots of P-ERK2, ERK1/2, P-CREB, CREB and their corresponding normalized control  $\beta$ -actin in the prefrontal cortex. (c) The comparison of P-ERK2, ERK1/2, P-CREB, and CREB in rat prefrontal cortex between the U0126- and vehicle-treated animals. Data expressed as mean  $\pm$  S.E.M.,  $N = 10$ – $12$  per group. \* $P < 0.05$  and \*\* $P < 0.01$ .

the MAPK phosphorylation level returns to the original high level within 48 h [23]. We did time course study of the drug's effect on ERK activation, and observed that the P-ERK2 level in the hippocampus obtained 24 h after injection remained at low level (data not presented). Thus, in the present study, the once daily injection of U0126 for 7 days could guarantee that the ERK activity consistently remained at low level before the behavioral tests, and such regimen of ERK inhibition well mimicked the development of long-lasting depressive-like behavior in animals [39,40, and our unpublished data]. The results demonstrated that the repeated U0126 administration decreased the ERK activation and induced depressive-like behavior.

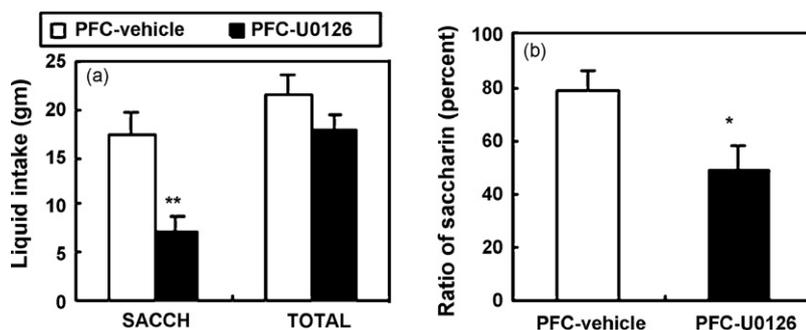
Recently, several studies investigate the direct role for the ERK pathway in the pathophysiology of depressive-like behavior. Einat et al. report that a global ERK inhibition by MEK inhibitor SL327 increases the distance traveled in an open field and decreases the immobility time in forced swim test [13]. In direct contrast to such results, Duman et al. demonstrate that the global inhibition of the ERK pathway by PD184161 produces depressive-like responses in learned helplessness, forced swim test, and the tail suspension test [10]. The reason for the different behavioral effects after ERK inhibition is unclear. Differing with the two studies which make global inhibition on ERK activation using SL327 or PD184161, two structural analogues of U0126 and blood–brain barrier-penetrating manipulations to specific brain regions, and evaluated the role for the ERK pathway in depressive-like behavior specifically in the dHP and mPFC. The results demonstrated that the ERK pathway inhibition in either the dHP or mPFC induced depressive-like behavior in rats. The U0126-treated rats exhibited the core symptom of depression – anhedonia, and the anxiety or locomotor impairment, suggesting that the disruption of the ERK pathway in the dHP and mPFC participates in the molecular mechanism of depressive-like behavior. Opposite to the findings of the present study, Todorovic et al. and Tronson et al. both demonstrate that the acute MEK inhibition in dorsal hippocampus induces antidepressant effect in the forced swim test [50,51]. The discrepancy between our results and theirs may be due to two factors. The first is the treatment regimen. Differing with the multiple injections treatment employed here, they



**Fig. 6.** The effect of the inhibition of ERK pathway in mPFC on distance traveled (a) and the number of rearing (b) in an open field in rats. Data expressed as mean  $\pm$  S.E.M.,  $N = 10$ – $12$  per group. \*\* $P < 0.01$ .



**Fig. 7.** The effect of the inhibition of ERK pathway in mPFC on the time spent in arms (a) and the number of entries into arms (b) in elevated plus maze in rats. OPAMS, open arms; CLAMS, closed arms; CENTR, central area. Data expressed as mean  $\pm$  S.E.M.,  $N = 10$ – $12$  per group.



**Fig. 8.** The effect of the inhibition of ERK pathway in mPFC on liquid intake (a) and the ratio of saccharin solution intake to total liquid intake (b) of rats. SACCH, saccharin solution; TOTAL, total liquid. Data expressed as mean  $\pm$  S.E.M.,  $N=10-12$  per group. \* $P<0.05$  and \*\* $P<0.01$ .

use acute ERK inhibition, and the sub-chronic and acute MEK inhibition may produce opposite effect on depressive-like behavior. The second is the behavioral measures used, such as saccharin preference test and forced swim test. Given the saccharin preference test assesses the anhedonic symptoms of depression while the forced swim test likely evaluates the helplessness behavior, there may be different effects for ERK inhibition on the different components of the depressive-like behavior.

It is noted that the ERK pathway inhibition in the dHP and mPFC both induced anhedonia, however, they showed different effects on anxiety and locomotor activity. The inhibition of the ERK pathway in the dHP induced anxiety-like behavior but not locomotor impairment, but the ERK inhibition in the mPFC induced locomotor impairment but not anxiety-like behavior. This functional segregation of the ERK pathway in the dHP and mPFC may be due to the functional anatomy of the brain structure. Previous lesion studies indicate that some brain region may play an especially important role in some specific behavior, and disruption of the different brain areas may produce different behavioral disorders [8,19,27]. For example, the hippocampus is proposed to be the most important neural substrate underlying anxiety [1,19,26], while the prefrontal cortex is preferably implicated in the modulation of motor activity [16,27]. Then, ERK pathway, as a potential molecular mechanism underlying functional regulations, its disruption in the hippocampus and prefrontal cortex may produce anxiety-like behavior and locomotor impairment, respectively. Thus, it is reasonable to hypothesize that the ERK pathway in the dHP and mPFC may exert differential control over very specific depression reactions, and the control of depression might be executed, in part, by distinct, parallel processing units within the corticohippocampal system, each specialized for a specific phenotype.

A growing body of data demonstrate that the phosphorylation state of CREB is dependent on ERK pathway in many functional regulations such as learning, memory, and the formation of LTP [7,21,55]. In the present study, we also observed that the ERK inhibition induced decrease of CREB phosphorylation. As an important nuclear target of ERK pathway, CREB is crucial for the ERK-dependent stimulus-transcription coupling [24,43]. Thus, it is likely that the ERK pathway exerts its effects on behavior via CREB activation and the expression of CRE sequence-containing genes. But we could not exclude the possibility that ERK induces expression of downstream genes necessary for mood modulation, independent of CREB by phosphorylating other transcription factors such as Elk-1 [54]. Future experiments will help distinguish between these possibilities.

Previous clinical studies have implicated the hippocampus and prefrontal cortex in the central mechanism of depression [4,9,11,41]. There are structural and functional alterations in the two brain regions of depressed humans [4,41], but the underlying molecular mechanism is not elucidated. The results of the present study demonstrated that the ERK inhibition in either the hippocampus or

prefrontal cortex induced depressive-like behavior. Thus, our findings, combined with the demonstrated role of the ERK pathway in neuronal survival, growth, and differentiation, raise the possible mechanism that the inhibition of the ERK pathway induces dysfunction of the hippocampus and prefrontal cortex and ultimately produces depressive-like behavior. Certainly, in addition to hippocampus and prefrontal cortex, abnormalities are also observed in other brain regions of patients with mood disorders, such as amygdala [31]. The possible effects of the ERK pathway on emotional behavior in other potentially relevant brain areas are yet to be investigated.

In conclusion, the findings of the present study demonstrate clearly that the ERK signal pathway in either the hippocampus or prefrontal cortex participates in the molecular pathophysiology of the depressive-like behavior of the animal model. Except for the common effects on the core symptoms of depression, the ERK pathway in the hippocampus and prefrontal cortex exerts differential controls over very specific depression reactions, with the hippocampal ERK pathway specialized for anxiety-like behavior and the ERK pathway in the prefrontal cortex for locomotor activity.

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#### References

- [1] Bannerman DM, Deacon RMJ, Offen S, Friswell J, Grubb M, Rawlins JNP. A double dissociation of function within the hippocampus: spatial memory and hypo-neophagia. *Behav Neurosci* 2002;116:884–901.
- [2] Belzung C, Griebel G. Measuring normal and pathological anxiety-like behaviour in mice: a review. *Behav Brain Res* 2001;125:141–9.
- [3] Blum S, Moore AN, Adams F, Dash PK. A mitogen-activated protein kinase cascade in the CA1/CA2 subfield of the dorsal hippocampus is essential for long-term spatial memory. *J Neurosci* 1999;19:3535–44.
- [4] Bremner JD. Structural changes in the brain in depression and relationship to symptom recurrence. *CNS Spectr* 2002;7:135–9.
- [5] Chen Z, Gibson TB, Robinson F, Silvestro L, Pearson G, Xu B, et al. MAP kinases. *Chem Rev* 2001;101:2449–76.
- [6] Cryan JF, Markou A, Lucki I. Assessing antidepressant activity in rodents: recent developments and future needs. *Trends Pharmacol Sci* 2002;23:238–45.
- [7] Davis S, Vanhoutte P, Pages C, Caboche J, Laroche S. The MAPK/ERK cascade targets both Elk-1 and cAMP response element-binding protein to control long-term potentiation-dependent gene expression in the dentate gyms in vivo. *J Neurosci* 2000;20:4563–72.
- [8] Degroot A, Treit D. Anxiety is functionally segregated within the septo-hippocampal system. *Brain Res* 2004;1001:60–71.
- [9] Drevets WC, Price JL, Simpson JR, Todd RD, Reich T, Vannier M, et al. Subgenual prefrontal cortex abnormalities in mood disorders. *Nature* 1997;386:824–7.
- [10] Duman CH, Schlesinger L, Kodama M, Russell DS, Duman RS. A role for MAP kinase signaling in behavioral models of depression and antidepressant treatment. *Biol Psychiatry* 2007;61:661–70.
- [11] Duman RS, Malberg J, Thome J. Neural plasticity to stress and antidepressant treatment. *Biol Psychiatry* 1999;46:1181–91.

- [12] Dwivedi Y, Rizavi HS, Roberts RC, Conley RC, Tamminga CA, Pandey GN. Reduced activation and expression of ERK1/2 MAP kinase in the post-mortem brain of depressed suicide subjects. *J Neurochem* 2001;77:916–28.
- [13] Einat H, Yuan PX, Gould TD, Li JL, Du JH, Zhang L, et al. The role of the extracellular signal-regulated kinase signaling pathway in mood modulation. *J Neurosci* 2003;23:7311–6.
- [14] Favata MF, Horiuchi KY, Manos EJ, Daulerio AJ, Stradley DA, Feeser WS, et al. Identification of a novel inhibitor of mitogen-activated protein kinase. *J Biol Chem* 1998;273:18623–32.
- [15] Feng PF, Guan ZW, Yang XP, Fang JD. Impairments of ERK signal transduction in the brain in a rat model of depression induced by neonatal exposure of clomipramine. *Brain Res* 2003;991:195–205.
- [16] Gentile AM, Green S, Nieburgs A, Schmelzer W, Stein DG. Disruption and recovery of locomotor and manipulatory behavior following cortical lesions in rats. *Behav Biol* 1978;22:417–55.
- [17] Gerdjikov T, Ross GM, Beninger RJ. Place preference induced by nucleus accumbens amphetamine is impaired by antagonists of ERK or p38 MAP kinases in rats. *Behav Neurosci* 2004;118:740–50.
- [18] Gourley SL, Wu FJ, Kiraly DD, Ploski JE, Kedves AT, Duman RS, et al. Regionally specific regulation of ERK MAP kinase in a model of antidepressant-sensitive chronic depression. *Biol Psychiatry* 2008;63:353–9.
- [19] Gray JA, McNaughton N. *The neuropsychology of anxiety*. 2nd edition Oxford: Oxford University Press; 2000.
- [20] Handley SL, Mithani S. Effects of alpha-adrenoceptor agonists and antagonists in a maze-exploration model of 'fear'-motivated behaviour. *Naunyn-Schmiedeberg's Arch Pharmacol* 1984;327:1–5.
- [21] Hardingham GE, Arnold FJ, Bading H. A calcium microdomain near NMDA receptors: on switch for ERK dependent synapse-to-nucleus communication. *Nat Neurosci* 2001;4:565–6.
- [22] Ho YJ, Eichendorff J, Schwarting RK. Individual response profiles of male Wistar rats in animal models for anxiety and depression. *Behav Brain Res* 2002;136:1–12.
- [23] Huang TY, Lin CH. Role of amygdala MAPK activation on immobility behavior of forced swim rats. *Behav Brain Res* 2006;173:104–11.
- [24] Impey S, Obrietan K, Wong S, Poser S, Yano S, Wayman G, et al. Cross talk between ERK and PKA is required for Ca<sup>2+</sup> stimulation of CREB-dependent transcription and ERK nuclear translocation. *Neuron* 1998;21:869–83.
- [25] Kelly A, Laroche S, Davis S. Activation of mitogen-activated protein kinase/extracellular signal-regulated kinase in hippocampal circuitry is required for consolidation and reconsolidation of recognition memory. *J Neurosci* 2003;23:5354–60.
- [26] Kjelstrup KG, Tuvnes FA, Steffenach HA, Murison R, Moser EI, Moser MB. Reduced fear expression after lesions of the ventral hippocampus. *Proc Natl Acad Sci USA* 2002;99:10825–30.
- [27] Kolb B, Whishaw IQ. Dissociation of the contributions of the prefrontal, motor and parietal cortex to the control of movement in the rat. *Can J Psychol* 1983;37:211–32.
- [28] Lewis MC, Gould TJ. Signal transduction mechanisms within the entorhinal cortex that support latent inhibition of cued fear conditioning. *Neurobiol Learn Mem* 2007;88:359–68.
- [29] Lonze BE, Ginty DD. Function and regulation of CREB family transcription factors in the nervous system. *Neuron* 2002;35:605–23.
- [30] Lu L, Dempsey J, Liu SY, Bossert JM, Shaham Y. A single infusion of brain-derived neurotrophic factor into the ventral tegmental area induces long-lasting potentiation of cocaine seeking after withdrawal. *J Neurosci* 2004;24:1604–11.
- [31] Manji HK, Drevets WC, Charney DS. The cellular neurobiology of depression. *Nat Med* 2001;7:541–7.
- [32] Mechan AO, Moran PM, Elliott JM, Young AJ, Joseph MH, Green AR. A comparison between Dark Agouti and Sprague–Dawley rats in their behaviour on the elevated plus-maze, open field apparatus an activity meters, and their response to diazepam. *Psychopharmacology (Berl)* 2002;159:188–95.
- [33] Meller E, Shen C, Nikolao TA, Jensen C, Tsimberg Y, Chen J, et al. Region-specific effects of acute and repeated restraint stress on the phosphorylation of mitogen-activated protein kinases. *Brain Res* 2003;979:57–64.
- [34] Nakiely N, Cohen P, Wu J, Sturgill T. MAP kinase activator from insulin-stimulated skeletal muscle is a protein threonine/tyrosine kinase. *EMBO J* 1992;11:2123–9.
- [35] Nash LT, Hack S. The pharmacological treatment of anxiety disorders in children and adolescents. *Expert Opin Pharmacother* 2002;3:555–71.
- [36] Paxinos G, Watson CR. *The rat brain in stereotaxic coordinates*. 4th edition New York: Academic Press; 1998.
- [37] Pearson S, Robinson F, Gibson TB, Xu BE, Karandikar M, Berman K, et al. Mitogen-activated protein (MAP) kinase pathways: regulation and physiological functions. *Endocr Rev* 2001;22:153–83.
- [38] Prut L, Belzung C. The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. *Eur J Pharmacol* 2003;463:3–33.
- [39] Qi XL, Lin WJ, Li JF, Li HH, Wang WW, Wang DL, et al. Fluoxetine increases the activity of the ERK-CREB signal system and alleviates the depressive-like behavior in rats exposed to chronic forced swim stress. *Neurobiol Dis* 2008;31:278–85.
- [40] Qi XL, Lin WJ, Li JF, Pan YQ, Wang WW. The depressive-like behaviors are correlated with decreased phosphorylation of mitogen-activated protein kinases in rat brain following chronic forced swim stress. *Behav Brain Res* 2006;175:233–40.
- [41] Rajkowska G, Miguel-Hidalgo JJ, Wei J, Dille G, Pittman SD, Meltzer HY, et al. Morphometric evidence for neuronal and glial prefrontal cell pathology in major depression. *Biol Psychiatry* 1999;45:1085–98.
- [42] Ribeiro M, Schofield MG, Kemenes I, O'Shea M, Kemenes G, Benjamin PR. Activation of MAPK is necessary for long-term memory consolidation following food-reward conditioning. *Learn Mem* 2005;12:538–45.
- [43] Roberson ED, English JD, Adams JP, Selcher JC, Kondratieck C, Sweatt JD. The mitogen-activated protein kinase cascade couples PKA and PKC to cAMP response element binding protein phosphorylation in area CA1 of hippocampus. *J Neurosci* 1999;19:4337–48.
- [44] Runyan JD, Moore AN, Dash PK. A role for prefrontal cortex in memory storage for trace fear conditioning. *J Neurosci* 2004;24:1288–95.
- [45] Sarkisova KY, Kulikov MA. Behavioral characteristics of WAG/Rij rats susceptible and non-susceptible to audiogenic seizures. *Behav Brain Res* 2006;166:9–18.
- [46] Schafe GE, Atkins CM, Swank MW, Bauer EP, Sweatt JD, LeDoux JE. Activation of ERK/MAP kinase in the amygdala is required for memory consolidation of Pavlovian fear conditioning. *J Neurosci* 2000;20:8177–87.
- [47] Sharma S, Bagnall MW, Sutton MA, Carew TJ. Inhibition of calcineurin facilitates the induction of memory for sensitization in Aplysia: requirement of mitogen-activated protein kinase. *Proc Natl Acad Sci USA* 2000;100:4861–6.
- [48] Sweatt JD. The neuronal MAP kinase cascade: a biochemical signal integration system subserving synaptic plasticity and memory. *J Neurochem* 2001;76:1–10.
- [49] Tiraboschi E, Tardito D, Kasahara J, Moraschi S, Pruneri P, Gennarelli M, et al. Selective phosphorylation of nuclear CREB by fluoxetine is linked to activation of CaM kinase IV and MAP kinase cascades. *Neuropsychopharmacology* 2004;29:1831–40.
- [50] Todorovic C, Sherrin T, Pitts M, Hippel C, Rayner M, Spiess J. Suppression of the MEK/ERK signaling pathway reverses depression-like behaviors of CRF<sub>2</sub>-deficient mice. *Neuropsychopharmacology* 2008. doi:10.1038/npp.2008.178.
- [51] Tronson NC, Schrick C, Fischer A, Sananbenesi F, Pagès G, Pouyssegur J, et al. Regulatory mechanisms of fear extinction and depression-like behavior. *Neuropsychopharmacology* 2008;33:1570–83.
- [52] Wall PM, Messier C. Methodological and conceptual issues in the use of the elevated plus-maze as a psychological measurement instrument of animal anxiety-like behavior. *Neurosci Biobehav Rev* 2001;25:275–86.
- [53] West AE, Griffith EC, Greenberg ME. Regulation of transcription factors by neuronal activity. *Nat Rev Neurosci* 2002;3:921–31.
- [54] Whitmarsh AJ, Davis RJ. Transcription factor AP-1 regulation by mitogen-activated protein kinase signal transduction pathways. *J Mol Med* 1996;74:589–607.
- [55] Ying SW, Futter M, Rosenblum K, Webber MJ, Hunt SP, Bliss TVP, et al. Brain-derived neurotrophic factor induces long-term potentiation in contact adult hippocampus: requirement for ERK activation coupled to CREB and upregulation of Arc synthesis. *J Neurosci* 2002;22:1532–40.
- [56] Zhu JJ, Qin Y, Zhao M, Van Aelst L, Malinow R. Ras and Rap control AMPA receptor trafficking during synaptic plasticity. *Cell* 2002;110:443–55.
- [57] Zimmerman M, McDermut W, Mattia JL. Frequency of anxiety disorders in psychiatric outpatients with major depressive disorder. *Am J Psychiatry* 2000;157:1337–40.