Psychiatry Research 271 (2019) 476–483

Contents lists available at ScienceDirect

Psychiatry Research

journal homepage: www.elsevier.com/locate/psychres

Withdrawal from chronic treatment with methamphetamine induces anxiety and depression-like behavior in mice

Ru Qin[a,*,1], Xiong Qi[a], Zhou Mei[a], Chen Lin[a], Tian Xiang[a], Xiao Huqiao[b], Li Chaoying[a], Li Yi[c,**,1]

[a] Wuhan Institutes of Biomedical Sciences, Jianghan University, Wuhan 430056, China
[b] Wan Ji Psychiatric Hospital, Wuhan 430051, China
[c] Wuhan Mental Health Center, Wuhan 430022, China

ARTICLE INFO

Keywords:
Methamphetamine
Withdrawal
Anxiety
Depression
BDNF pathway

ABSTRACT

Methamphetamine (METH) is an illicit psychostimulant that is widely abused. After producing extreme pleasure, METH abuse leads to negative emotional states during withdrawal in clinical survey. However, the mood behavioral consequences of withdrawal from chronic METH exposure in animal experiments and related mechanisms have not been clarified yet. The aim of this study was to investigate the anxiety and depression-like phenotype in mice induced by withdrawal from chronic METH treatment and the potential molecular mechanism. We found that withdrawal from chronic METH treatment increased the immobility time during the forced swimming test and decreased central activities in open field test, indicating increased anxiety and depression-like behavior. Additional experiments showed that expression of brain-derived neurotrophic factor (BDNF), phosphorylated tropomyosin receptor kinase B (p-TrkB), phosphorylated extracellular signal-related kinase 1/2 (p-ERK1/2) and phosphorylated cAMP-response element binding protein (p-CREB) were decreased in the hippocampus and prefrontal cortex of mice in METH group and the level of mitogen activated protein kinase phosphatase-1 (MKP-1) was increased. Combined, our data show that withdrawal from chronic METH exposure induces anxiety and depression-like behavior associated with aberrant changes of proteins in BDNF-ERK-CREB pathway, providing new evidence for the involvement of BDNF pathway in the negative emotional states induced by withdrawal from METH.

1. Introduction

As a worldwide abused illicit psychostimulant, methamphetamine (METH) has significant toxicity on the central nervous system when taken repeatedly or at higher doses (Wood et al., 2014). Following an initial phase of psychostimulation, withdrawal from METH produces psychiatric symptoms including anhedonia, sleep disturbances and irritability (Marshall and Werb, 2010; McKetin et al., 2016b), which are similar to the symptoms of anxiety and depressive disorder. Clinical investigations showed that METH abusers were at greater risk to experience particular psychiatric symptoms such as anxiety and depression (DiMiceli et al., 2016; Hellem, 2016; Ma et al., 2018; McKetin et al., 2016a). Among 1277 METH users in China, 57.6% participants had psychiatric symptoms including depression and anxiety, and a dose-response relationship was found between duration of METH use and risk of psychiatric symptoms (Ma et al., 2018). 34.3% of METH users had anxiety symptoms during METH withdrawal, including 11.9% with moderate anxiety and 2.4% with severe anxiety (Su et al., 2017). Another American epidemiological survey demonstrated that the incidence of lifetime major depressive disorder was 38.5% among METH adult users compared with 21.5% among non-users (Casaletto et al., 2015). Moreover, depressed METH users had fewer benefits from long-term psychotherapy compared with non-depressed subjects (Glasner-Edwards et al., 2009; Zhang et al., 2014), and failure to manage METH withdrawal symptoms may lead to the high rates of relapse (Brecht et al., 2000). However, the related mechanism has not been clarified yet.

Brain-derived neurotrophic factor (BDNF) contributes to axonal

Abbreviations: METH, methamphetamine; BDNF, brain-derived neurotrophic factor; ERK1/2, extracellular signal-related kinase 1/2; MKP-1, mitogen activated protein kinase phosphatase-1; CREB, cAMP-response element binding protein; TrkB, tropomyosin receptor kinase B; BCA, bicinchoninic acid; SDS, sodium dodecyl sulfate; PVDF, polyvinylidene difluoride; TBS, tris buffered saline; MWM, morris water maze

* Corresponding author at: Jianghan University, Wuhan Economic and Technological Development Zone, Wuhan, Hubei Province 430056, China.
** Corresponding author at: Wuhan Mental Health Center, Wuhan, Hubei Province 430022, China.
E-mail addresses: ruq.whibs@aliyun.com (Q. Ru), psylee@163.com (Y. Li).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.psychres.2018.11.072
Received 20 December 2017; Received in revised form 20 August 2018; Accepted 30 November 2018
Available online 03 December 2018
0165-1781/

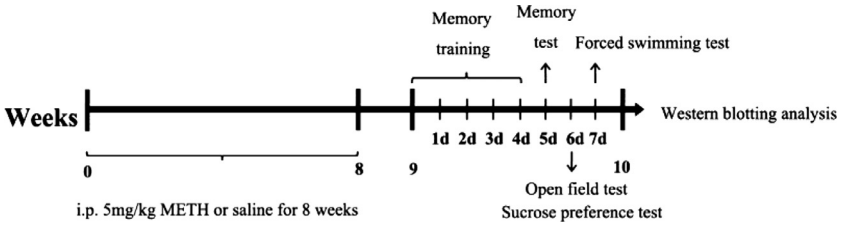

Fig. 1. Experimental procedure.

growth, neuronal survival and synaptic plasticity. Accumulating evidence from animal, postmortem and clinical studies demonstrates that BDNF and its receptor, tropomyosin-related kinase B (TrkB), are involved in the pathophysiology and treatment of anxiety and depression (McGregor et al., 2018; Thompson Ray et al., 2011; Youssef et al., 2018; Zheng et al., 2017). Most, but not all studies in patients indicated that BDNF levels were decreased in depressed patients and long-term antidepressant treatment was able to reverse this deficit (Ghosh et al., 2015; Ren et al., 2017). BDNF conditional knockout animals could display depression-like behaviors (Monteggia et al., 2007), while infusion of BDNF in the brain of rodents and intraperitoneal administration of TrkB agonist could produce antidepressant-like effect, and the effects of BDNF on these behavioral paradigms were lasting much longer than classic antidepressants (Hoshaw et al., 2005; Shirayama et al., 2002). The extracellular signal-regulated protein kinase1/2 (ERK1/2) pathway is a downstream pathway of BDNF that regulates cellular responses, including neuronal survival and plasticity (Callaghan and Kelly, 2012). The ERK1/2 is activated by phosphorylation and selectively inactivated by mitogen activated protein kinase phosphatase (MKP). Evidence implicates changes in ERK1/2 and MKP activities in both drug-induced behavioral changes and aberrant emotional states. For instance, withdrawal from morphine significantly induced depressive-like behavior in mice and correlated with increased MKP-1 and decreased levels of phospho-ERK1/2 (p-ERK1/2, the active form) in the hippocampus, and MKP-1 blockade in the dorsal hippocampus inhibited ERK dephosphorylation and prevented the development of depressive-like symptoms (Jia et al., 2013). Additionally, ERK1/2 can regulate transcription by controlling the phosphorylation of the transcription factor cAMP-response element binding protein (CREB). Chronic antidepressant treatment has also been shown to up-regulate the phosphorylation of CREB, and the increased CREB activation in rodent models of depression resulted in antidepressant-like effects (Li et al., 2015). This evidence suggested that the BDNF-ERK-CREB pathway might be involved in METH induced anxiety and depression-like behavior.

However, the mood behavioral consequences and the underlying molecular mechanism of withdrawal from chronic METH exposure have not been fully clarified yet. In this context, it has been reported that exposure to METH during pre-adolescence resulted in sustained deficits in spatial memory as well as depressive-like behaviors in adult life (Mouton et al., 2016). A single high dose or repeated low dose of METH induced depression in adult rodents (Iijima et al., 2013; Silva et al., 2014). Nevertheless, the administration time in previous literatures was relatively short, mostly single or a few days, and was different from the clinical patterns of METH abuse. Therefore, the aim of this study was to investigate the anxiety and depressive-like phenotype in mice induced by withdrawal from eight weeks chronic METH treatment. Additionally, we assessed the alterations of the BDNF-ERK-CREB pathway in the hippocampus and prefrontal cortex, aiming to explain the underlying mechanism.

2. Materials and methods

2.1. Animals

Male C57BL/6 mice (18–22 g) were provided by Beijing vital river laboratory animal technology co., ltd. The animals were housed five per cage with free access to water and food, a 12 h light/dark cycle, and a temperature-controlled environment. Animals were adapted to these conditions for at least 3 days before experiments. All animal use procedures were carried out in accordance with the Regulations of Experimental Animal Administration issued by the State Committee of Science and Technology of the People's Republic of China, with the approval of the Ethics Committee in Jianghan University.

2.2. Reagents

METH was obtained from the Hubei Public Security Bureau. Protein extraction buffer, protease and phosphatase inhibitors were obtained from Wuhan boster biological technology co., ltd (Wuhan, China). The mouse serum corticosterone detection kit was purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Antibodies against p-ERK1/2 (9101S), ERK1/2 (4695S), p-CREB (9198S), CREB (9197S), GAPDH (5174S) and goat anti-rabbit IgG (7074) were purchased from Cell Signaling Technology (Beverly, MA, USA). Antibodies against mature BDNF (sc-546) and MKP-1 (sc-1199) were purchased from Santa Cruz (California, USA). Antibodies against p-TrkB (abs131087) and TrkB (abs131948) were purchased from Absin Bioscience Co. Ltd (Shanghai, China). All other chemicals were of standard analytical grade.

2.3. Animals treatment

The experimental procedure was detailed in Fig. 1. Mice were randomly divided into two groups: saline group and chronic METH exposure group. In chronic METH exposure group, mice were injected with METH (5 mg/kg, i.p., once a day, 5 days per week) for 8 weeks as previously reports with modification (Andersen et al., 2013; Hong et al., 2015; Wang et al., 2016). The chronic METH exposure paradigm was designed to replicate long-term patterns of METH abuse, including occasional interruptions of drug use after binges. Mice in saline group were injected with equivalent volume of saline. Sucrose preference test and forced swim test (FST) were used to evaluate depression-like behaviors, while open field test was used to evaluate anxiety-like behavior.

2.4. Morris water maze

Mice were trained on the spatial reference memory version of the Morris water maze (MWM) hidden platform task to assess their associative, long term spatial memory as previously report with slight modification (Akiba et al., 2017). Briefly, a circular pool (120 cm in diameter and 50 cm in deep) was filled with 23 ± 2 °C water to a depth of 21 cm. A hidden circular platform (6 cm in diameter) was located in the center of the target quadrant (quadrant II), submerged 1.5 cm beneath the surface of the water. The animal escape latency, swimming speed and the amount of time spent in the target quadrant were measured automatically using a computer-based image analyzer Morris water maze tracking system XR-XM101 (Shanghai Xinruan Information Technology Co. Ltd). For Morris water maze training, mice were subjected to a session of four trials per day with four different starting positions for 4 consecutive days. In each trial, animals were given a maximum of 60 s to find the platform. After mounting the platform, the

mice were allowed to remain there for 30 s. If the mice failed to find the platform in 60 s, it was placed on the platform and allowed to rest for 30 s. The interval of time between the two training session was 15–20 min. On day 5, Morris water maze test probe consisting of a 60 s free swim period without the platform was performed to test spatial memory. After the last trial, the mice were towel dried and placed in a holding cage under a heating lamp before it was returned to the home cage.

2.5. Open field test

Locomotor activity was studied using an open-field test, and the open-field apparatus was similar to those described previously (Jiang et al., 2012; Li et al., 2017). The mice were gently placed in the center of a white plastic chamber (50 cm × 50 cm × 50 cm) for 6 min while exploratory behaviors were automatically recorded by a video tracking system XR-XZ301 (Shanghai Xinruan Information Technology Co. Ltd). The total distance and the time spent in the center of the open field were recorded. At the end of each test, the surface of the arena was cleaned thoroughly with 75% alcohol to avoid the presence of olfactory cues.

2.6. Forced swimming test

The forced swimming test was carried out in mice according to previous reports with slight modification (Jiang et al., 2014). Briefly, mice were individually placed into a glass cylinder (25 cm in height, 10 cm in diameter) filled with 10 cm high water (25 ± 1 °C). The water was exchanged after each trial. All animals were forced to swim for 6 min, and the immobility time during the final 5 min interval of the test was recorded. Immobility time was defined as the time spent by the mouse floating in the water without struggling, and making only those movements necessary to keep its head above the water. Immobility time was measured automatically using a computer-based image analyzer system XR-XQX201 (Shanghai Xinruan Information Technology Co. Ltd).

2.7. Sucrose preference test

The sucrose preference test was conducted over a 48 h period using a two-bottle test, and one with 1% sucrose solution while the other with water (Jiang et al., 2014; Li et al., 2018; Zhang et al., 2016). To prevent potential location preference of drinking, the position of the bottles was changed after 24 h. The mice were deprived of food and water for 24 h prior to the test. On each test, bottles were pre-weighed and the position of the bottles was interchanged, and the preference for the sucrose solution was calculated as the percentage of sucrose solution ingested relative to the total amount of liquid consumed.

2.8. Measurement of serum corticosterone level

The mice were sacrificed by cervical dislocation after anesthesia. The blood samples were collected and kept on ice, then centrifuged at 1000 g at 4 °C for 15 min. The serum was kept at −80 °C until analysis. The corticosterone levels were measured using a commercially available ELISA kit.

2.9. Western blotting analysis

The mice were sacrificed by cervical dislocation after anesthesia. Prefrontal cortex and all hippocampus were rapidly dissected respectively and homogenized in lysis buffer containing protease and phosphatase inhibitors for 30 min. The homogenate was centrifuged at 12,000 g for 15 min (at 4 °C), and the supernatant was collected. Total protein was estimated by BCA Protein Assay Kit. Then, the samples were mixed with SDS sample buffer and boiled for 5 min. Equal amounts of protein samples (30 μg) were separated by gel electrophoresis in a Criterion Cell Electrophoresis System and electroblotted onto polyvinylidene difluoride (PVDF) membrane. After blocking with 5% non-fat dry milk in TBS plus 0.5% Tween for 1 h, blots were incubated with primary antibodies followed by a goat anti-rabbit horseradish peroxidase conjugated secondary antibody. The following antibodies were used: anti-BDNF, anti-MKP-1 (1:500), anti-pERK1/2, anti-ERK1/2, anti-p-CREB, anti-CREB, anti-p-TrkB, anti-TrkB (1:1000), anti-GAPDH (1:10,000). All the primary antibodies were rabbit anti-mouse antibodies. Bands were visualized with enhanced chemiluminescence (Thermo Fisher, USA) by using the imaging analysis system (Gene Company, Hong Kong). The intensity of each band was quantitatively determined by Image J. The density ratio indicated the relative intensity of each band against GAPDH, since GAPDH was used as a loading control in this experiment. Results are shown as normalized of the saline group.

2.10. Statistical analysis

All analyses were performed using SPSS 20.0 software (SPSS Inc., USA) and data were presented as mean ± S.E.M (standard error of the mean). Differences between mean values were evaluated using Independent-Samples t test. Some data about the swimming speed and escape latency during the learning process in Fig. 2 were tested by one-way ANOVA with repeated measures followed by Tukey's HSD post-hoc test. Values of $p < 0.05$ were considered statistically significant.

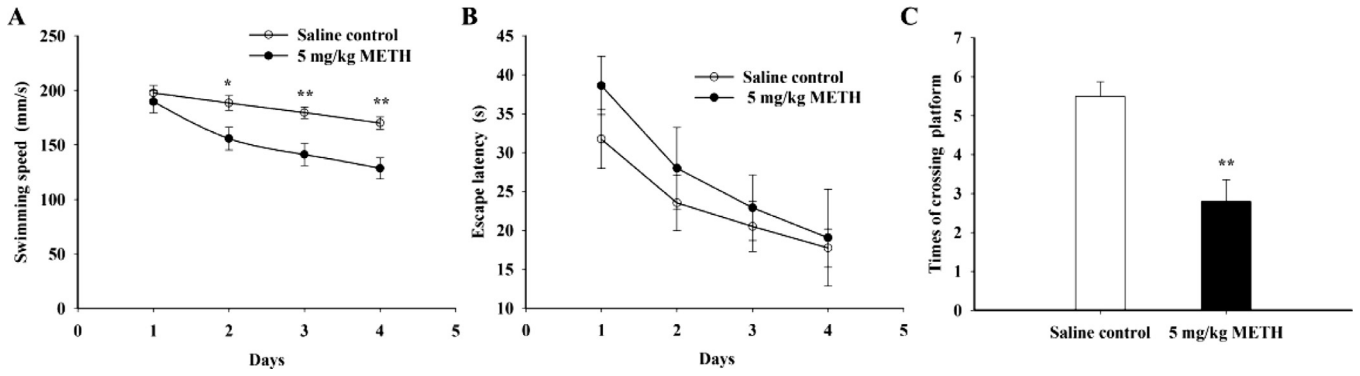

Fig. 2. Effects of withdrawal from chronic METH exposure on spatial reference memory in mice. (A) Swimming speed during training in Morris water maze. (B) The escape latencies during training in Morris water maze. (C) The number of times passing through the platform on the test day. The presented data are mean ± S.E.M. Statistical analysis of swimming speed and escape latencies were carried out by one way ANOVA. Statistical analysis of the times across platform was evaluated using Student's t test. $n = 10$ for per group, $^{*}p < 0.05$, $^{**}p < 0.01$ compared with saline group.

3. Results

3.1. Effect of withdrawal from chronic METH exposure on spatial reference memory

During four training days, the escape latencies and swimming speeds were recorded and analyzed. As shown in Fig. 2, mice in both groups learned this task well and spending less time each day to find the platform. Compared with the saline group, the swimming speeds of chronic METH exposure group decreased significantly after day 1 (Fig. 2A, day 1, $F(1,18) = 0.419$, $p = 0.526$; day 2, $F(1,18) = 6.878$, $p = 0.017$; day 3, $F(1,18) = 11.401$, $p = 0.003$; day 4, $F(1,18) = 13.724$, $p = 0.002$). The latency was increased in chronic METH exposure group, but there was no significant difference compared with the saline group during training days (Fig. 2B, day 1, $F(1,18) = 1.683$, $p = 0.211$; day 2, $F(1,18) = 0.495$, $p = 0.491$; day 3, $F(1,18) = 0.205$, $p = 0.656$; day 4, $F(1,18) = 0.039$, $p = 0.845$), which indicates that chronic METH exposure had no significant effect on spatial learning ability. In spatial reference memory test, the number of times across platform was markedly reduced in chronic METH exposure group than that in the saline group (Fig. 2C, $t(18) = 4.045$, $p = 0.001$). These findings indicate that withdrawal from chronic METH exposure has no significant inhibition of spatial learning ability, but can significantly reduce the reference memory capacity of mice.

3.2. Effect of withdrawal from chronic METH exposure on anxiety and depressive symptoms

The results of open field test showed that there was no difference in total distance between two groups (Fig. 3A, $t(16) = -0.100$, $p = 0.922$), but the time spent in the center square was significantly decreased in chronic METH group than that in saline group (Fig. 3B, $t(16) = 3.335$, $p = 0.004$). After withdrawal from chronic METH exposure, immobility time was noticeably increased in forced swimming test (Fig. 4A, $t(16) = -4.991$, $p < 0.001$). Moreover, the sucrose intakes were also examined as indices of depressive-like behavior. As shown in Fig. 4B, withdrawal from chronic METH exposure induced a significant decline in sucrose consumption ($t(16) = 20.214$, $p < 0.001$). Compared with the saline group, the level of serum corticosterone in chronic METH exposure group was significantly increased (Fig. 4C, $t(10) = -3.328$, $p = 0.008$). All these findings indicate that withdrawal from eight weeks treatment with METH increased anxiety and depressive symptoms.

3.3. Effect of withdrawal from chronic METH exposure on the protein expression in the hippocampus and prefrontal cortex

To investigate the possible mechanism of anxiety and depressive symptoms induced by withdrawal from chronic treatment with METH, several key components of the BDNF pathway in the hippocampus and prefrontal cortex were analyzed by western blotting. As shown in Fig. 5B, the expression of mature BDNF in hippocampus and prefrontal cortex were both decreased in chronic METH exposure group compared with the saline group (hippocampus, $t(10) = 3.982$, $p = 0.003$; prefrontal cortex, $t(10) = 3.647$, $p = 0.004$). Meanwhile, the expression of p-TrkB in hippocampus and prefrontal cortex were both significantly decreased in chronic METH exposure group (Fig. 5C, hippocampus, $t(10) = 10.803$, $p < 0.001$; prefrontal cortex, $t(10) = 9.627$, $p < 0.001$), while the expression of TrkB protein remained unchanged (Fig. 5D, hippocampus, $t(10) = -1.009$, $p = 0.333$; prefrontal cortex, $t(10) = 0.439$, $p = 0.670$). In line with the decreased mature BDNF in the samples, the expression of p-ERK1/2 and p-CREB were also significantly decreased in chronic METH exposure group (Fig. 6B, hippocampus, $t(10) = 6.773$, $p < 0.001$, prefrontal cortex, $t(10) = 5.996$, $p < 0.001$; Fig. 6E, hippocampus, $t(10) = 7.057$, $p < 0.001$, prefrontal cortex, $t(10) = 6.073$, $p < 0.001$), while the expression of ERK1/2 and CREB remained unchanged (Fig. 6C, hippocampus, $t(10) = -0.651$, $p = 0.530$, prefrontal cortex, $t(10) = 1.753$, $p = 0.110$; Fig. 6F, hippocampus, $t(10) = -0.756$, $p = 0.462$, prefrontal cortex, $t(10) = -0.602$, $p = 0.561$). Compared with the saline group, the protein level of MKP-1 (negative regulation factor of ERK signaling pathway) was markedly increased in chronic METH exposure group (Fig. 6D, hippocampus, $t(10) = -3.033$, $p = 0.013$, prefrontal cortex, $t(10) = -4.375$, $p = 0.007$). Together, these results prove that the expression levels of mature BDNF, p-ERK1/2 and p-CREB decreased in the presence of anxiety and depression symptoms, suggesting that BDNF-ERK1/2-CREB pathway in hippocampus and prefrontal cortex may be involved in the behavioral effects induced by withdrawal from chronic METH exposure.

4. Discussion

Epidemiological survey showed that anxiety and depression often co-occurs with abstinent from amphetamine type stimulant use (DiMiceli et al., 2016; Panenka et al., 2013). Although some papers have showed that depressive and psychotic symptoms could resolve within a few week of abstinence (Zorick et al., 2010), more studies, in contrast, have proved that depression persisting in some METH users

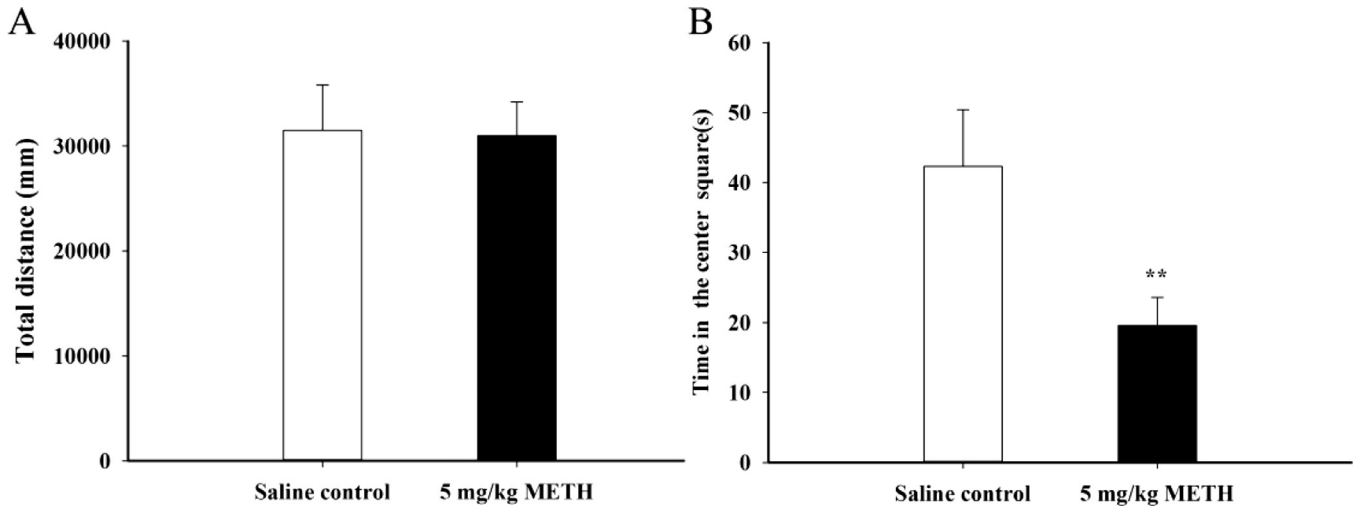

Fig. 3. Effect of withdrawal from chronic METH exposure in open field test. (A) Total distance of mice in open field test. (B) The time in the center square in open field test. The presented data are mean ± S.E.M. Statistical analysis were carried out by Student's t test. $n = 9$ for per group, $^{**}p < 0.01$ compared with saline group.

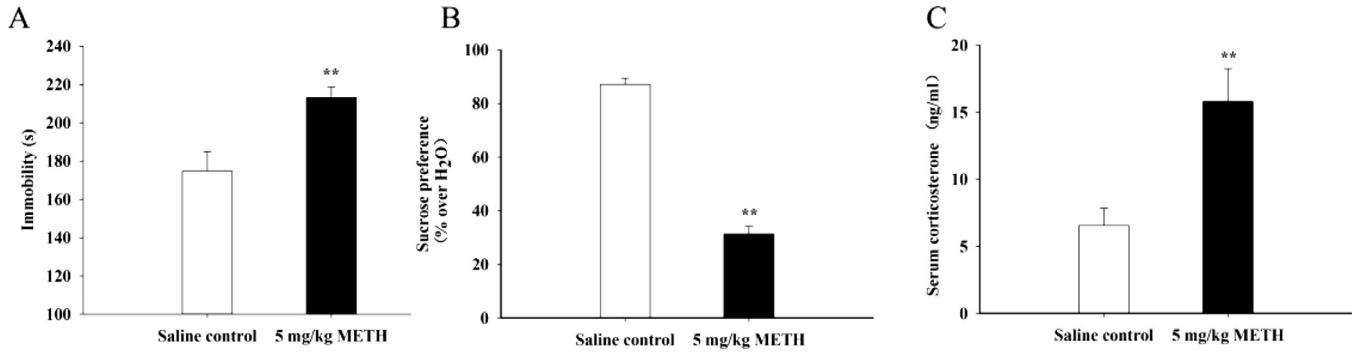

Fig. 4. Effect of withdrawal from chronic METH exposure on depressive symptoms. (A) Immobility time in forced swimming test. (B) Sucrose preference index in sucrose preference test. (C) The level of serum corticosterone in mice. The presented data are mean ± S.E.M. Statistical analysis were carried out by Student's t test. $n = 9$ for per group, $^{**}p < 0.01$ compared with saline group.

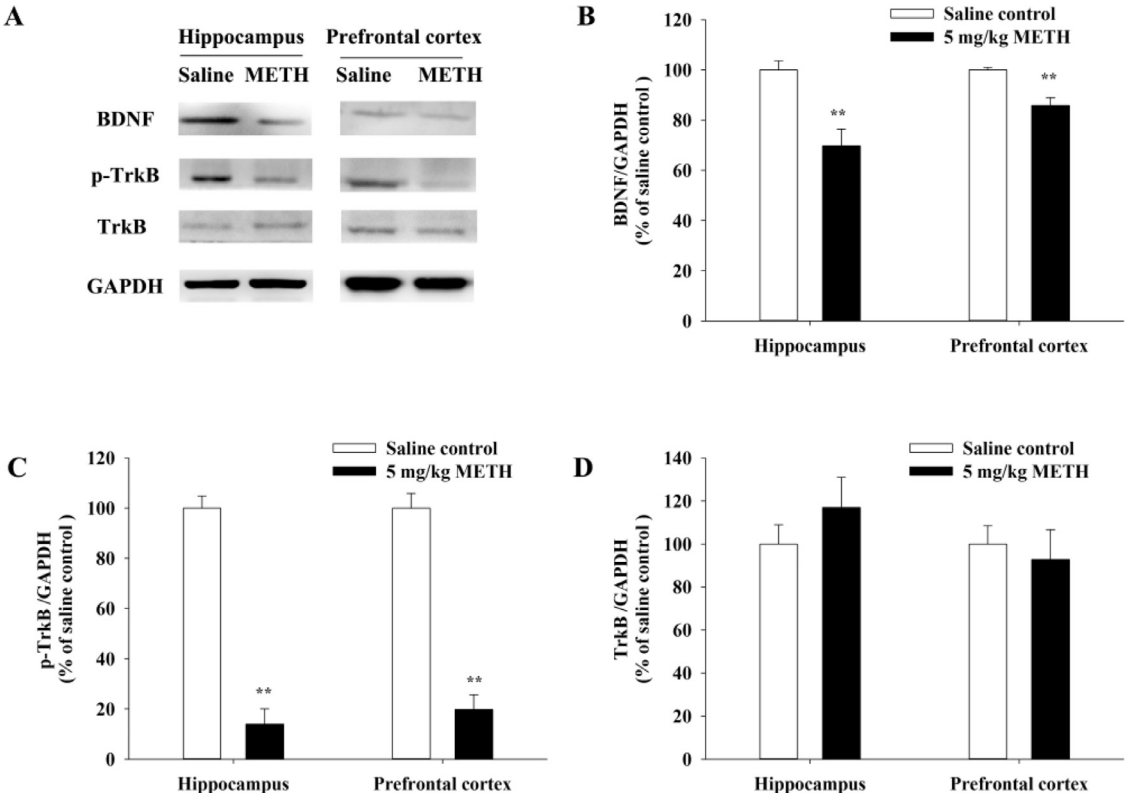

Fig. 5. Effects of withdrawal from chronic METH exposure on the protein expression of BDNF and TrkB. (A) Representative band intensities of the western blotting. The levels of mature BDNF (B), p-TrkB (C) and TrkB (D) in hippocampus and prefrontal cortex were detected using western blotting. The presented data are mean ± S.E.M. Statistical analysis were carried out by independent sample t test. $n = 6$ for per group, $^{**}p < 0.01$ compared with saline group.

for several years after treatment (Glasner-Edwards et al., 2009; Rawson et al., 2002). The reasons for these discrepancies may be related to the duration of METH use, the severity of depression, the pre-treatment, numbers of relapse after drug abstinence, or gender. Animal studies also back the emergence of negative emotional states upon METH consumption. For instance, depressive behaviors were both observed during withdrawal periods following a single dose (30 mg/kg) or subchronic (5 mg/kg/day × 5 days) of METH administration (Iijima et al., 2013; Silva et al., 2014). Surprisingly, the behavioral profile of laboratory rodents after long-term METH exposure has not been documented to date.

Herein, in the present study, we found that withdrawal from 8 weeks treatment with METH increased the immobility time in the forced swimming test and decreased sucrose consumption, which is consistent with depressive symptoms, such as despair and anhedonia, induced by withdrawal from long term drugs of abuse in clinical survey (Hellem, 2016). A decrease in the time spent in the central square of open-field test, without a change in the total locomotion, is interpreted as an anxiety-like effect (Seibenhener and Wooten, 2015). In our study, METH-treated mice exhibited increased anxiety-like behavior after withdrawal. Our results confirmed previous clinical observations indicating that a majority of patients with major depression also have comorbid anxiety (Hartel-Petri et al., 2017). Importantly, the lack of alteration in total distance in open field test indicates that withdrawal from METH induced anxiety and depressive symptoms without affecting the locomotor activity. In agreement with our finding, previous study also reported that either recent or long-term abstinent METH abusers did not display major motor deficits (Johanson et al., 2006). One major neuroendocrine response to anxiety and depression is the secretion of corticosterone (Jiang et al., 2012; Zhang et al., 2017), and many addicts have elevated cortisol levels in the presence of psychiatric symptoms such as anxiety and depression in the early stage of

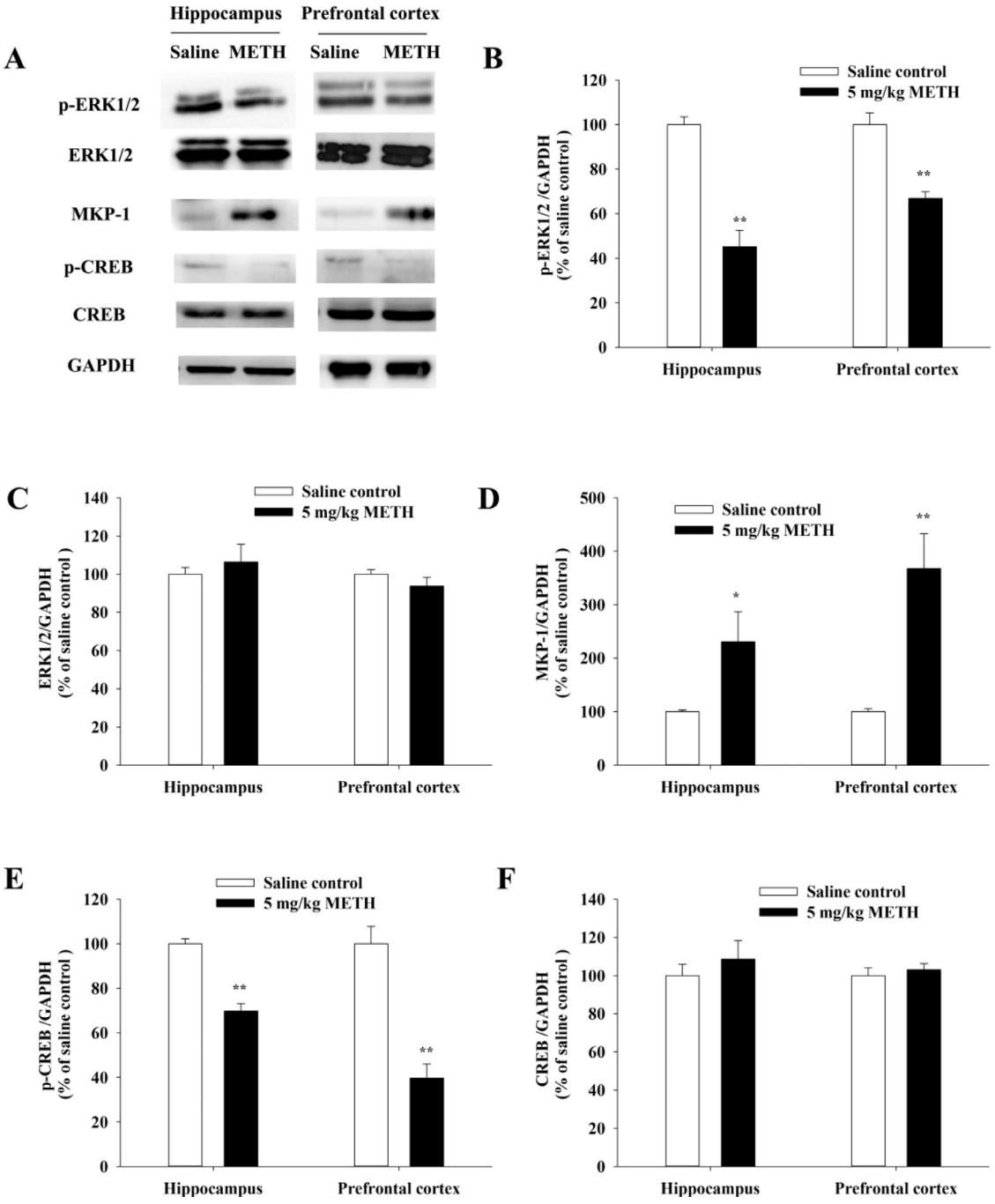

Fig. 6. Effects of withdrawal from chronic METH exposure on the protein expression of ERK1/2, CREB and MKP-1. (A) Representative band intensities of the western blotting. The levels of mature p-ERK1/2 (B), ERK1/2(C), MKP-1 (D), p-CREB (E) and CREB (F) in hippocampus and prefrontal cortex were detected using western blotting. The presented data are mean ± S.E.M. Statistical analysis were carried out by independent sample t test. $n = 6$ for per group, $^{*}p < 0.05$, $^{**}p < 0.01$ compared with saline group.

withdrawal (Motaghinejad et al., 2015; Wichniak et al., 2004). For instance, cortisol levels were increased in early alcohol withdrawal (first week) and normalized during the fourth week (Esel et al., 2001). Consistent with literatures, the level of serum corticosterone in METH-treated mice significantly increased at one week of withdrawal. These findings provide demonstration of anxiety and depressive-like phenotype following a chronic METH exposure paradigm, which is consistent with the high prevalence of psychiatric symptoms in chronic METH users (Ma et al., 2018). However, a minor pitfall of this study is that it used a constant dose of METH. This exposure paradigm may have difference with clinical patterns of METH abuse, which usually includes gradual increases of drug intake. It will be important to consolidate these findings using paradigm with gradually increased dose of METH and other behavioural tests as well as different endpoints to explore psychiatric symptoms in animal models.

Neural networks, including amygdala, hippocampus, prefrontal cortex, and other regions, participate in the pathophysiology of mood disorder. In this study, we focused on hippocampus and prefrontal cortex (Malykhin and Coupland, 2015). The role of BDNF and its downstream targets ERK and CREB, a pathway mainly regulating protein synthesis and synaptic plasticity were investigated, trying to explain the underlying molecular mechanism of METH withdrawal-

induced mood disorder. As a neurotrophic peptide, BDNF is critical for axonal growth, neuronal survival and synaptic plasticity. Many studies focus on the role of BDNF in neurocircuitry related with anxiety and depression. Previous data showed that BDNF was decreased in serum or the hippocampus of patients or animal models and treatment normalized the expression (Autry and Monteggia, 2012; Chiou and Huang, 2016; Ren et al., 2017; Yi et al., 2014). In agreement with previous studies, we found that withdrawal from eight weeks treatment of METH could induce down-regulation of mature BDNF and p-TrkB in hippocampus and prefrontal cortex. The ERK1/2 is regulated by Ras-Raf cascade in response to BDNF activation. Our results showed that withdrawal from chronic METH exposure reduced the p-ERK1/2 levels in hippocampus and prefrontal cortex. This is in accordance with previous studies which also found a correlation between decreased ERK phosphorylation and depressive-like behaviors in mice (Gourley et al., 2008; Jia et al., 2013). Significantly decreased ERK1/2 activity was also found in the hippocampus of depressed suicide subjects (Dwivedi et al., 2009). Additionally, our results verified the previous findings that depression accompanied by anxiety could decrease the phosphorylation of CREB in the hippocampus and prefrontal cortex (Wang et al., 2007), and they were also consistent with a previous report that 1 week or 6 weeks withdrawal after 6-month chronic alcohol consumption could reduce the level of p-CREB in the prefrontal cortex (Dominguez et al., 2016).

MKP-1 provides a negative feedback mechanism for regulating ERK1/2 activity (Collins et al., 2015). Sustained induction of MKP1 would lead to inhibition of ERK signaling (e.g., decreased levels of phosphoERK), as MKP-1 was significantly upregulated in postmortem hippocampal samples from patients with major depressive disorder (Duric et al., 2010). Our result showed that compared with control group, withdrawal from chronic METH treatment could reduce the p-ERK1/2 levels and increase the MKP-1 levels in hippocampus and prefrontal cortex. This was consistent with a previous study that chronic psychosocial stress induced depression-like behavior, down-regulated the ERK cascade and increased MKP-1 expression in the hippocampus of these rats (Iio et al., 2011). An intraventrolateral orbital cortex infusion of sanguinarine (a selective MKP-1 inhibitor) to rats significantly reduced immobility time in the FST in a dose-dependent manner compared with vehicle-treated controls (Chen et al., 2012). These reports in combination with our experimental results indicate that induction of hippocampal MKP1 may be not only a direct consequence of METH withdrawal-induced depressive-like behaviors but also a key negative regulator of ERK phosphorylation, contributing to the expression of depressive-like symptoms in mice. Taken together, these molecular findings suggested that the BDNF-ERK-CREB signaling in hippocampus and prefrontal cortex is, at least in part, the mechanism underlying the anxiety and depressive behavior induced by withdrawal from chronic treatment with METH. It will be essential to design additional studies to consolidate a possible causal relation between BDNF-ERK-CREB pathway and the emergence of anxiety and depressant-like phenotype induced by withdrawal from METH.

Interestingly, no significant differences were observed between METH and saline groups in the escape latency during training days, indicating that one week withdrawal from chronic METH exposure has no obvious effect on spatial learning. In line with our study, it was shown that a single high METH dose (30 mg/kg) did not alter the procedural memory of mice as assessed by escape latency to find the platform (Silva et al., 2014). Nevertheless, the number of times passing through the platform was markedly less in mice with chronic METH exposure in spatial reference memory test. This was consistent with another study that METH has no effect on the spatial learning process 120 min after injection, but rats exhibited a deficit in spatial reference memory (Bigdeli et al., 2015). The mechanism underlying the spatial learning and memory deficits following METH is unknown. Although it seems, stress-related pathways, oxidative stress and monoamine levels in brain may be involved (Herring et al., 2010; McDonnell-Dowling and Kelly, 2017).

In summary, our data showed that withdrawal from eight weeks METH treatment induced anxiety and depression-like behavior without clear impairment on motor function. We also found that the BDNF-ERK-CREB pathway might account for changed behavior induced by withdrawal from chronic METH exposure. The current findings highlight BDNF pathway as a responsive element in the neurobiological consequences that occur during early time periods of METH withdrawal. Further studies are needed to investigate the mood behavioral and neurochemical consequences following more protracted periods of withdrawal from METH.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgments

This work was supported by Hubei Provincial Department of Education guidance project (B2017273).

References

Akiba, C., Nakajima, M., Miyajima, M., Ogino, I., Miura, M., Inoue, R., Nakamura, E., Kanai, F., Tada, N., Kunichika, M., Yoshida, M., Nishimura, K., Kondo, A., Sugano, H., Arai, H., 2017. Leucine-rich alpha2-glycoprotein overexpression in the brain contributes to memory impairment. Neurobiol. Aging 60, 11–19.

Andersen, M.L., Diaz, M.P., Murnane, K.S., Howell, L.L., 2013. Effects of methamphetamine self-administration on actigraphy-based sleep parameters in rhesus monkeys. Psychopharmacology (Berl) 227, 101–107.

Autry, A.E., Monteggia, L.M., 2012. Brain-derived neurotrophic factor and neuropsychiatric disorders. Pharmacol. Rev. 64, 238–258.

Bigdeli, I., Asia, M.N., Miladi-Gorji, H., Fadaei, A., 2015. The spatial learning and memory performance in methamphetamine-sensitized and withdrawn rats. Iran. J. Basic Med. Sci. 18, 234–239.

Brecht, M.L., von Mayrhauser, C., Anglin, M.D., 2000. Predictors of relapse after treatment for methamphetamine use. J. Psychoact. Drugs 32, 211–220.

Callaghan, C.K., Kelly, A.M., 2012. Differential BDNF signaling in dentate gyrus and perirhinal cortex during consolidation of recognition memory in the rat. Hippocampus 22, 2127–2135.

Casaletto, K.B., Obermeit, L., Morgan, E.E., Weber, E., Franklin, D.R., Grant, I., Woods, S.P., Translational Methamphetamine, A.R.C.G., 2015. Depression and executive dysfunction contribute to a metamemory deficit among individuals with methamphetamine use disorders. Addict. Behav. 40, 45–50.

Chen, Y., Wang, H., Zhang, R., Wang, H., Peng, Z., Sun, R., Tan, Q., 2012. Microinjection of sanguinarine into the ventrolateral orbital cortex inhibits Mkp-1 and exerts an antidepressant-like effect in rats. Neurosci. Lett. 506, 327–331.

Chiou, Y.J., Huang, T.L., 2016. Serum brain-derived neurotrophic factors in Taiwanese patients with drug-naive first-episode major depressive disorder: effects of antidepressants. Intern. J. Neuropsychopharmacol.

Collins, L.M., Downer, E.J., Toulouse, A., Nolan, Y.M., 2015. Mitogen-activated protein kinase phosphatase (MKP)-1 in nervous system development and disease. Mol. Neurobiol. 51, 1158–1167.

DiMiceli, L.E., Sherman, S.G., Aramrattana, A., Sirirojn, B., Celentano, D.D., 2016. Methamphetamine use is associated with high levels of depressive symptoms in adolescents and young adults in Rural Chiang Mai Province, Thailand. BMC Public Health 16, 168.

Dominguez, G., Dagnas, M., Decorte, L., Vandesquille, M., Belzung, C., Beracochea, D., Mons, N., 2016. Rescuing prefrontal cAMP-CREB pathway reverses working memory deficits during withdrawal from prolonged alcohol exposure. Brain Struct. Funct 221, 865–877.

Duric, V., Banasr, M., Licznerski, P., Schmidt, H.D., Stockmeier, C.A., Simen, A.A., Newton, S.S., Duman, R.S., 2010. A negative regulator of MAP kinase causes depressive behavior. Nat. Med 16, 1328–1332.

Dwivedi, Y., Rizavi, H.S., Zhang, H., Roberts, R.C., Conley, R.R., Pandey, G.N., 2009. Aberrant extracellular signal-regulated kinase (ERK)1/2 signalling in suicide brain: role of ERK kinase 1 (MEK1). Int. J. Neuropsychopharmacol. 12, 1337–1354.

Esel, E., Sofuoglu, S., Aslan, S.S., Kula, M., Yabanoglu, I., Turan, M.T., 2001. Plasma levels of beta-endorphin, adrenocorticotropic hormone and cortisol during early and late alcohol withdrawal. Alcohol Alcohol 36, 572–576.

Ghosh, R., Gupta, R., Bhatia, M.S., Tripathi, A.K., Gupta, L.K., 2015. Comparison of efficacy, safety and brain derived neurotrophic factor (BDNF) levels in patients of major depressive disorder, treated with fluoxetine and desvenlafaxine. Asia J. Psychiat. 18, 37–41.

Glasner-Edwards, S., Marinelli-Casey, P., Hillhouse, M., Ang, A., Mooney, L.J., Rawson, R., Methamphetamine Treatment Project Corporate, A., 2009. Depression among methamphetamine users: association with outcomes from the Methamphetamine Treatment Project at 3-year follow-up. J. Nerv. Ment. Dis. 197, 225–231.

Gourley, S.L., Wu, F.J., Kiraly, D.D., Ploski, J.E., Kedves, A.T., Duman, R.S., Taylor, J.R.,

2008. Regionally specific regulation of ERK MAP kinase in a model of antidepressant-sensitive chronic depression. Biol. Psychiatry 63, 353–359.
Hartel-Petri, R., Krampe-Scheidler, A., Braunwarth, W.D., Havemann-Reinecke, U., Jeschke, P., Looser, W., Muhlig, S., Schafer, I., Scherbaum, N., Bothe, L., Schaefer, C., Hamdorf, W., 2017. Evidence-based guidelines for the pharmacologic management of methamphetamine dependence, relapse prevention, chronic methamphetamine-related, and comorbid psychiatric disorders in post-acute settings. Pharmacopsychiatry 50, 96–104.
Hellem, T.L., 2016. A review of methamphetamine dependence and withdrawal treatment: a focus on anxiety outcomes. J. Subst. Abuse Treat. 71, 16–22.
Herring, N.R., Gudelsky, G.A., Vorhees, C.V., Williams, M.T., 2010. (+)-Methamphetamine-induced monoamine reductions and impaired egocentric learning in adrenalectomized rats is independent of hyperthermia. Synapse 64, 773–785.
Hong, S.J., Zhang, D., Zhang, L.H., Yang, P., Wan, J., Yu, Y., Wang, T.H., Feng, Z.T., Li, L.H., Yew, D.T., 2015. Expression of dopamine transporter in the different cerebral regions of methamphetamine-dependent rats. Hum. Exp. Toxicol. 34, 707–717.
Hoshaw, B.A., Malberg, J.E., Lucki, I., 2005. Central administration of IGF-I and BDNF leads to long-lasting antidepressant-like effects. Brain Res 1037, 204–208.
Iijima, M., Koike, H., Chaki, S., 2013. Effect of an mGlu2/3 receptor antagonist on depressive behavior induced by withdrawal from chronic treatment with methamphetamine. Behav. Brain Res. 246, 24–28.
Iio, W., Matsukawa, N., Tsukahara, T., Kohari, D., Toyoda, A., 2011. Effects of chronic social defeat stress on MAP kinase cascade. Neurosci. Lett. 504, 281–284.
Jia, W., Liu, R., Shi, J., Wu, B., Dang, W., Du, Y., Zhou, Q., Wang, J., Zhang, R., 2013. Differential regulation of MAPK phosphorylation in the dorsal hippocampus in response to prolonged morphine withdrawal-induced depressive-like symptoms in mice. PLoS One 8, e66111.
Jiang, B., Wang, F., Yang, S., Fang, P., Deng, Z.F., Xiao, J.L., Hu, Z.L., Chen, J.G., 2014. SKF83959 produces antidepressant effects in a chronic social defeat stress model of depression through BDNF-TrkB pathway. Int. J. Neuropsychopharmacol. 18.
Jiang, B., Xiong, Z., Yang, J., Wang, W., Wang, Y., Hu, Z.L., Wang, F., Chen, J.G., 2012. Antidepressant-like effects of ginsenoside Rg1 are due to activation of the BDNF signalling pathway and neurogenesis in the hippocampus. Br. J. Pharmacol. 166, 1872–1887.
Johanson, C.E., Frey, K.A., Lundahl, L.H., Keenan, P., Lockhart, N., Roll, J., Galloway, G.P., Koeppe, R.A., Kilbourn, M.R., Robbins, T., Schuster, C.R., 2006. Cognitive function and nigrostriatal markers in abstinent methamphetamine abusers. Psychopharmacology (Berl) 185, 327–338.
Li, J., Luo, Y., Zhang, R., Shi, H., Zhu, W., Shi, J., 2015. Neuropeptide trefoil factor 3 reverses depressive-like behaviors by activation of BDNF-ERK-CREB signaling in olfactory bulbectomized rats. Int. J. Mol. Sci. 16, 28386–28400.
Li, M.X., Zheng, H.L., Luo, Y., He, J.G., Wang, W., Han, J., Zhang, L., Wang, X., Ni, L., Zhou, H.Y., Hu, Z.L., Wu, P.F., Jin, Y., Long, L.H., Zhang, H., Hu, G., Chen, J.G., Wang, F., 2017. Gene deficiency and pharmacological inhibition of caspase-1 confers resilience to chronic social defeat stress via regulating the stability of surface AMPARs. Mol. Psychiatry.
Li, M.X., Zheng, H.L., Luo, Y., He, J.G., Wang, W., Han, J., Zhang, L., Wang, X., Ni, L., Zhou, H.Y., Hu, Z.L., Wu, P.F., Jin, Y., Long, L.H., Zhang, H., Hu, G., Chen, J.G., Wang, F., 2018. Gene deficiency and pharmacological inhibition of caspase-1 confers resilience to chronic social defeat stress via regulating the stability of surface AMPARs. Mol. Psychiatry 23, 556–568.
Ma, J., Sun, X.J., Wang, R.J., Wang, T.Y., Su, M.F., Liu, M.X., Li, S.X., Han, Y., Meng, S.Q., Wu, P., Shi, J., Bao, Y.P., Lu, L., 2018. Profile of psychiatric symptoms in methamphetamine users in China: greater risk of psychiatric symptoms with a longer duration of use. Psychiatry Res 262, 184–192.
Malykhin, N.V., Coupland, N.J., 2015. Hippocampal neuroplasticity in major depressive disorder. Neuroscience 309, 200–213.
Marshall, B.D., Werb, D., 2010. Health outcomes associated with methamphetamine use among young people: a systematic review. Addiction 105, 991–1002.
McDonnell-Dowling, K., Kelly, J.P., 2017. The role of oxidative stress in methamphetamine-induced toxicity and sources of variation in the design of animal studies. Curr. Neuropharmacol. 15, 300–314.
McGregor, N.W., Dimatelis, J.J., Van Zyl, P.J., Hemmings, S.M.J., Kinnear, C., Russell, V.A., Stein, D.J., Lochner, C., 2018. A translational approach to the genetics of anxiety disorders. Behav. Brain Res. 341, 91–97.
McKetin, R., Dawe, S., Burns, R.A., Hides, L., Kavanagh, D.J., Teesson, M., Mc, D.Y.R., Voce, A., Saunders, J.B., 2016a. The profile of psychiatric symptoms exacerbated by methamphetamine use. Drug Alcohol Depend 161, 104–109.
McKetin, R., Gardner, J., Baker, A.L., Dawe, S., Ali, R., Voce, A., Leach, L.S., Lubman, D.I., 2016b. Correlates of transient versus persistent psychotic symptoms among dependent methamphetamine users. Psychiatry Res 238, 166–171.
Monteggia, L.M., Luikart, B., Barrot, M., Theobold, D., Malkovska, I., Nef, S., Parada, L.F., Nestler, E.J., 2007. Brain-derived neurotrophic factor conditional knockouts show gender differences in depression-related behaviors. Biol. Psychiatry 61, 187–197.
Motaghinejad, M., Bangash, M.Y., Motaghinejad, O., 2015. Attenuation of alcohol withdrawal syndrome and blood cortisol level with forced exercise in comparison with diazepam. Acta Med. Iran. 53, 311–316.
Mouton, M., Harvey, B.H., Cockeran, M., Brink, C.B., 2016. The long-term effects of methamphetamine exposure during pre-adolescence on depressive-like behaviour in a genetic animal model of depression. Metab. Brain Dis. 31, 63–74.
Rawson, R.A., Huber, A., Brethen, P., Obert, J., Gulati, V., Shoptaw, S., Ling, W., 2002. Status of methamphetamine users 2–5 years after outpatient treatment. J. Addict. Dis 21, 107–119.
Ren, W., Luan, X., Zhang, J., Gutteea, P., Cai, Y., Zhao, J., Gu, Y., Wu, C., Su, H., Tao, J., Xie, Y., Lv, D., Feng, L., He, J., 2017. Brain-derived neurotrophic factor levels and depression during methamphetamine withdrawal. J. Affect. Disord. 221, 165–171.
Seibenhener, M.L., Wooten, M.C., 2015. Use of the Open Field Maze to measure locomotor and anxiety-like behavior in mice. J. Visual. Exp. e52434.
Shirayama, Y., Chen, A.C., Nakagawa, S., Russell, D.S., Duman, R.S., 2002. Brain-derived neurotrophic factor produces antidepressant effects in behavioral models of depression. J. Neurosci. 22, 3251–3261.
Silva, C.D., Neves, A.F., Dias, A.I., Freitas, H.J., Mendes, S.M., Pita, I., Viana, S.D., de Oliveira, P.A., Cunha, R.A., Fontes Ribeiro, C.A., Prediger, R.D., Pereira, F.C., 2014. A single neurotoxic dose of methamphetamine induces a long-lasting depressive-like behaviour in mice. Neurotox. Res. 25, 295–304.
Su, H., Zhang, J., Ren, W., Xie, Y., Tao, J., Zhang, X., He, J., 2017. Anxiety level and correlates in methamphetamine-dependent patients during acute withdrawal. Medicine (Baltimore) 96, e6434.
Thompson Ray, M., Weickert, C.S., Wyatt, E., Webster, M.J., 2011. Decreased BDNF, trkB-TK+ and GAD67 mRNA expression in the hippocampus of individuals with schizophrenia and mood disorders. J. Psychiatry Neurosci. 36, 195–203.
Wang, D., Noda, Y., Tsunekawa, H., Zhou, Y., Miyazaki, M., Senzaki, K., Nabeshima, T., 2007. Behavioural and neurochemical features of olfactory bulbectomized rats resembling depression with comorbid anxiety. Behav. Brain Res. 178, 262–273.
Wang, L., Qu, G., Dong, X., Huang, K., Kumar, M., Ji, L., Wang, Y., Yao, J., Yang, S., Wu, R., Zhang, H., 2016. Long-term effects of methamphetamine exposure in adolescent mice on the future ovarian reserve in adulthood. Toxicol. Lett. 242, 1–8.
Wichniak, A., Brunner, H., Ising, M., Pedrosa Gil, F., Holsboer, F., Friess, E., 2004. Impaired hypothalamic-pituitary-adrenocortical (HPA) system is related to severity of benzodiazepine withdrawal in patients with depression. Psychoneuroendocrinology 29, 1101–1108.
Wood, S., Sage, J.R., Shuman, T., Anagnostaras, S.G., 2014. Psychostimulants and cognition: a continuum of behavioral and cognitive activation. Pharmacol. Rev. 66, 193–221.
Yi, L.T., Li, J., Liu, B.B., Luo, L., Liu, Q., Geng, D., 2014. BDNF-ERK-CREB signalling mediates the role of miR-132 in the regulation of the effects of oleanolic acid in male mice. J. Psychiatry Neurosci. 39, 348–359.
Youssef, M.M., Underwood, M.D., Huang, Y.Y., Hsiung, S.C., Liu, Y., Simpson, N.R., Bakalian, M.J., Rosoklija, G.B., Dwork, A.J., Arango, V., Mann, J.J., 2018. Association of BDNF Val66Met polymorphism and brain BDNF levels with major depression and suicide. Intern. J. Neuropsychopharmacol.
Zhang, J., Xie, Y., Su, H., Tao, J., Sun, Y., Li, L., Liang, H., He, R., Han, B., Lu, Y., Sun, H., Wei, Y., Guo, J., Zhang, X.Y., He, J., 2014. Prevalence and correlates of depressive symptoms during early methamphetamine withdrawal in Han Chinese population. Drug Alcohol Depend 142, 191–196.
Zhang, R., Asai, M., Mahoney, C.E., Joachim, M., Shen, Y., Gunner, G., Majzoub, J.A., 2017. Loss of hypothalamic corticotropin-releasing hormone markedly reduces anxiety behaviors in mice. Mol. Psychiatry 22, 733–744.
Zhang, R.X., Han, Y., Chen, C., Xu, L.Z., Li, J.L., Chen, N., Sun, C.Y., Chen, W.H., Zhu, W.L., Shi, J., Lu, L., 2016. EphB2 in the medial prefrontal cortex regulates vulnerability to stress. Neuropsychopharmacology 41, 2541–2556.
Zheng, Q., Xu, M.J., Cheng, J., Chen, J.M., Zheng, L., Li, Z.G., 2017. Serum levels of brain-derived neurotrophic factor are associated with depressive symptoms in patients with systemic lupus erythematosus. Psychoneuroendocrinology 78, 246–252.
Zorick, T., Nestor, L., Miotto, K., Sugar, C., Hellemann, G., Scanlon, G., Rawson, R., London, E.D., 2010. Withdrawal symptoms in abstinent methamphetamine-dependent subjects. Addiction 105, 1809–1818.