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Distinct roles of methamphetamine in modulating spatial memory consolidation, retrieval, reconsolidation and the accompanying changes of ERK and CREB activation in hippocampus and prefrontal cortex

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ABSTRACT

Drugs of abuse modulated learning and memory in humans yet the underlying mechanism remained unclear. The extracellular signal-regulated kinase (ERK) and the transcription factor cAMP response element-binding protein (CREB) were involved in neuroplastic changes associated with learning and memory. In the current study, we used a Morris water maze to examine the effect of methamphetamine (METH) on different processes of spatial memory in mice. We then investigated the status of ERK and CREB in the hippocampus and prefrontal cortex (PFC). We found that 1.0 mg/kg dose of METH facilitated spatial memory consolidation when it was injected immediately after the last learning trial. In contrast, the same dose of METH had no effect on spatial memory retrieval when it was injected 30 min before the test. Furthermore, 1.0 mg/kg dose of METH injected immediately after retrieval had no effect on spatial memory reconsolidation. Activation of both ERK and CREB in the hippocampus was found following memory consolidation but not after retrieval or reconsolidation in METH-treated mouse groups. In contrast, activation of both ERK and CREB in the PFC was found following memory retrieval but not other processes in METH-treated mouse groups. These results suggested that METH facilitated spatial memory consolidation but not retrieval or reconsolidation. Moreover, activation of the ERK and CREB signaling pathway in the hippocampus might be involved in METH-induced spatial memory changes.

1. Introduction

Psychostimulants was shown to enhance learning and memory (Carmack et al., 2010; Huang et al., 2009; Iñiguez et al., 2011; Kennedy et al., 2010), yet the underlying mechanism remained unclear. Previous studies focused on effects of amphetamine-type stimulates on different forms of memories (Blaiss and Janak, 2007; Kennedy et al., 2010; McGaugh, 2000; Simon and Setlow, 2006; Wiig et al., 2009), which suggested that amphetamine and methamphetamine (METH) could improve cognitive function in schizophrenia (Barch and Carter, 2005; Sahakian and Morein-Zamir, 2007), addictive (Berke and Hyman, 2000; Mahoney III et al., 2010) and healthy individuals (Barch and Carter, 2005; Greely et al., 2008; Sahakian and Morein-Zamir, 2007; Silber et al., 2006). Amphetamine was therefore illicitly used as cognitive enhancers in both university students (Greely et al., 2008; Sahakian and Morein-Zamir, 2007) and military pilots (Caldwell et al., 2003). Animal studies also found that acute low-dose amphetamine, METH or cocaine could facilitate memory performance (Kennedy et al., 2010; Wood et al., 2007), especially the consolidation of memory (Iñiguez et al., 2011; Wiig et al., 2009). Memory impairment was also detected in both humans and rodents which had been exposed to METH (Belcher et al., 2007; Kalechstein et al., 2003; Nordahl et al., 2003; Simon et al., 2000; Vorhees et al., 2009). Nevertheless, the effects of METH on specific memory processes and the molecular mechanism remained unclear.

Learning and memory was thought to include three processes which were attention/encoding, storage/consolidation

Abbreviations: CREB, cAMP response element-binding protein; ERK, extracellular signal-regulated kinase; METH, methamphetamine; PFC, prefrontal cortex; MWM, Morris water maze.

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and retrieval/recall (Clayton, 2000; Dudai, 2004; Horn, 2004). Briefly, pre-encoded events or information were then stabilized as a memory trace in the brain which was defined as the process of consolidation (Dudai, 2004), and this process was followed by retrieval of memory which referred to the subsequent re-accessing of an encoded and stored memory trace (Muzzio et al., 2009). The retrieval of a memory trace could then induce an additional labile phase named reconsolidation which referred to the stabilization of memory after retrieval (Tronson and Taylor, 2007). Consolidation, retrieval and reconsolidation were necessary and critical processes for memory storage or expression and could be distinguished by different behavioral models. Although previous studies demonstrated that METH could modulate learning and memory, the effects of METH on different memory processes remained unclear. Previous studies found that hippocampus and prefrontal cortex (PFC) were involved in the modulation of different processes of spatial learning and memory (Duva et al., 1997; Maviel et al., 2004; Nadel, 1991) and Morris water maze (MWM) was a commonly used behavioral paradigm for studying the neurobiology mechanism underlying distinct processes of spatial learning and memory (Iñiguez et al., 2011; Vorhees and Williams, 2006; Williams et al., 2003). Drugs were usually treated immediately after training (Dudai, 2004; Iñiguez et al., 2011) or memory test (Miller and Marshall, 2005) to study effects of drugs on memory consolidation and reconsolidation, or pretreated 30 min before memory test (Miller and Marshall, 2005) to study effects of drugs on memory retrieval.

The extracellular signal-regulated kinase (ERK) signal pathway was involved in neuroplasticity (Sweatt, 2001; Tronson and Taylor, 2007; Mazzucchelli and Brambilla, 2000) and spatial learning and memory (Xing et al., 2010). METH induced the phosphorylation of ERK in the striatum (Mizoguchi et al., 2004) and inhibition of ERK kinase MEK blocked the establishment of conditioned place preference induced by METH, cocaine and amphetamine (Gerdjikov et al., 2004; Miller and Marshall, 2005; Mizoguchi et al., 2004; Valjent et al., 2000). Once activated, ERK affected cellular function in multiple ways, including phosphorylation of membrane and cytosolic proteins and transcription and translational controls (Kelleher et al., 2004; Sweatt, 2001). CREB was one of the most prominent transcription factors capable of mediating a variety of neuronal functions (Sakamoto et al., 2011). Pharmacological studies indicated that inhibition of ERK was accompanied by attenuated CREB activation and contextual memory (Fricks-Gleason and Marshall, 2010; Miller and Marshall, 2005; Mizoguchi et al., 2004). Taken together, these results suggested that the ERK and CREB signal pathway might contribute to METH-induced memory alterations.

In the current study, we used the MWM to examine the roles of METH on spatial memory consolidation, retrieval and reconsolidation in mice. We also examined the status of ERK and CREB in the hippocampus and PFC in each behavioral context. Our results suggested that METH had distinct role in spatial memory processes and activation of the ERK and CREB signal pathway in the hippocampus might be involved in METH-induced spatial memory changes.

2. Materials and methods

2.1. Animals

Male C57BL/6 mice were purchased from Beijing Vital River Laboratory Animal Technology Co. Ltd. 7–9 week-old mice (weighing about 20–25 g) were used in our experiment. Four animals per cage were housed in a regulated environment (23 ± 1 °C, 50 ± 5% humidity) with a 12:12 h light/dark cycle (lights on at 07:00) with food and water available ad libitum. Animals were allowed to habituate in the room for one week before experimental manipulations were undertaken. All experimental procedures were approved by the Institutional Animal Care and Use Committee of Xi'an Jiaotong University. All efforts were made to minimize the number of animals used and the distress to the animals.

2.2. Drugs

D-methamphetamine hydrochloride was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, PR China), and was dissolved in 0.9% physiological saline in a concentration of 0.1 mg/ml. The volume of intraperitoneal (i.p.) injection was 10 ml/kg.

2.3. Apparatus

The apparatus consisted of a stainless-steel circular tank 124 cm in diameter and 62.5 cm in height (Xing et al., 2010). Water was filled to a depth of 37.5 cm with the temperate at 21 ± 1 °C. Non-toxic, white tempera paint was employed to make the water opaque. A Plexiglas platform 10 cm in diameter was submerged 1–1.5 cm below the water surface and was located in the middle of the second quadrant during the training session. Besides the tank, a camera above the tank and a computer were used to track each mouse's performance automatically by a video-computerized tracking system (SMART, Panlab SL, Barcelona, Spain). Distal cues were placed within the behavioral room.

2.4. Procedure

The MWM protocol and treatment regimens were based on previous studies (Vorhees and Williams, 2006; Xing et al., 2010) with some modifications. Briefly, mice experienced a learning trails phase with the platform hidden in the second quadrant and a probe test phase without the platform. At the beginning of each trial, mice were held facing the tank wall and placed into the water from one of the four fixed entry points randomly. A trial ended when the mouse climbed onto the platform and remained on it for 10 s or when the 60 s time limit had lapsed. Otherwise mice were manually placed on the platform for 15 s if the mouse failed to find the platform within 60 s. After a trial ended, mice were immediately dried and kept in their home cage for at least 10–15 min until the next trial. All mice experienced four trails per day for four or five consecutive days. The probe test was carried out 24 h after the last learning trail for 60 s. Performance of each mouse was tracked and analyzed by a video-computerized tracking system (SMART, Panlab SL, Barcelona, Spain). Latency to the platform site, platform site crossings, time in target quadrant, and time in platform site were recorded to evaluate the animal's learning and reference memory. Data were expressed as the average of four trails on the training days. Different mice were used in each of the following experiments.

2.4.1. Memory consolidation

To assess effects of 1.0 mg/kg dose of METH on spatial memory consolidation, mice experienced four trials per day without any treatment for four consecutive days (day 1–day 4) with the platform hidden in the second quadrant. Immediately after the last trail on day 4, mice were randomly divided into two groups ($n = 7$ per group) and were injected with saline (group 1) or 1.0 mg/kg dose of METH (group 2) respectively. A 60 s probe test was carried out on day 5 with the platform removed.

2.4.2. Memory retrieval

To assess effects of METH on spatial memory retrieval, all mice received four trials per day for four consecutive days (day 1–day 4) without any treatment and were randomly divided into two groups ($n = 7$ per group). The mice were injected with saline (group 1) or 1.0 mg/kg dose of METH (group 2) on day 5 and their memories were tested 30 min later.

Another two groups of mice ($n = 7$ or 8 per group) experienced four trials per day for five consecutive days (day 1–day 5) without any treatment and were then injected with saline (group 1) or 1.0 mg/kg dose of METH (group 2) 30 min before their memories were tested on day 6.

2.4.3. Memory reconsolidation

Another MWM protocol was used to investigate the effects of METH on spatial memory reconsolidation with four groups of mice ($n = 7$–9). All mice were trained for 5 consecutive days (day 1–day 5) without any treatment. Two groups of mice experienced the probe test (test 1) on day 6 after which saline (group 1) or 1.0 mg/kg dose of METH (group 2) was injected immediately and their memories were tested 24 h (test 2) and 48 h (test 3) later (day 7 and day 8). All of the three tests were carried out without the platform. As a control experiment, another two groups were just injected with saline (group 1) or 1.0 mg/kg dose of METH (group 2) on day 6 without reactivation of memory (test 1) which was followed by the two probe tests on day 7 (test 2) and day 8 (test 3) respectively to exclude the memory reactivated effect.

2.5. Tissue preparation

Mice were killed by cervical dislocation immediately after the probe test or reconsolidation test. Their hippocampi and PFC were dissected bilaterally on dry ice. The samples were homogenized in ice-cold RIPA buffer [1 × phosphate-buffered saline (PBS), 1% Nonidet P-40, 0.5% sodium deoxycholate, 1% sodium dodecyl sulfate (SDS)] with protease inhibitors. Homogenates were incubated on ice for 20 min and then centrifuged at 12,000 × g for 20 min at 4 °C (Zhang et al., 2004). Supernatants were collected and protein concentrations were determined by Bradford BCA protein assay and stored at −80 °C until further use.

2.6. Western blotting

Samples treated with β-mercaptoethanol were denatured at 95 °C for 5 min, then 6 μg of extracts for ERK and 25 μg extracts for CREB were electrophoresed on precast 4–12% SDS-PAGE and transferred onto a nitrocellulose membrane (Millipore, Bedford, MA, USA). After blocking with 5% non-fat milk in 1 × Tris-buffered saline with 0.1% Tween-20 (TBST), blots were probed with primary antibodies followed by appropriate secondary antibodies. Signals were detected by enhanced chemiluminescence and quantified by a Quantity One program (Bio-rad, Hercules, CA, USA). We used primary antibodies against phosphor-ERK, ERK, phosphor-CREB, CREB (Cell Signaling, USA) at 1:1000 dilutions, and goat anti-rabbit lgG horseradish peroxidase (HRP)-conjugated secondary antibody (Bioworld, USA) at 1:10,000 dilution. The results for western blot were analyzed using densitometry. Ratios of phospho- to total ERK1, ERK2, ERK1/2 and phospho- to total CREB were calculated respectively for each sample. Saline was set at 1. All western blot analyses were performed at least three times to assure the consistency of the results.

2.7. Data analysis

All data were presented as mean ± SEM. Two way repeated measure ANOVA was used to analyze the effects of time on learning performance and a post-hoc multiple comparison (Bonferroni test) was used to analyze the differences among different days. Other data including probe test, speed and western blot were analyzed by *t*-test. *P*-values < 0.05 was considered to be statistically significant.

3. Results

3.1. Memory consolidation

In order to investigate effects of 1.0 mg/kg dose of METH on spatial memory consolidation, two groups of mice were injected with 1.0 mg/kg METH or saline immediately after four days of training and their memories were tested 24 h later. Two way repeated measures ANOVA of escape latencies revealed significant effects of time [$F(3,36) = 25.347$, $p < 0.001$], but not group [$F(1,12) = 0.078$, $p > 0.05$] and their interaction [$F(3,36) = 0.714$, $p > 0.05$] (Fig. 1A). All mice gradually learned to locate the platform as time went on (Fig. 1A, $+p < 0.05$ compared with day 1) and no difference was found between the two groups because no treatment was given in learning phase. Group 1 represented saline-

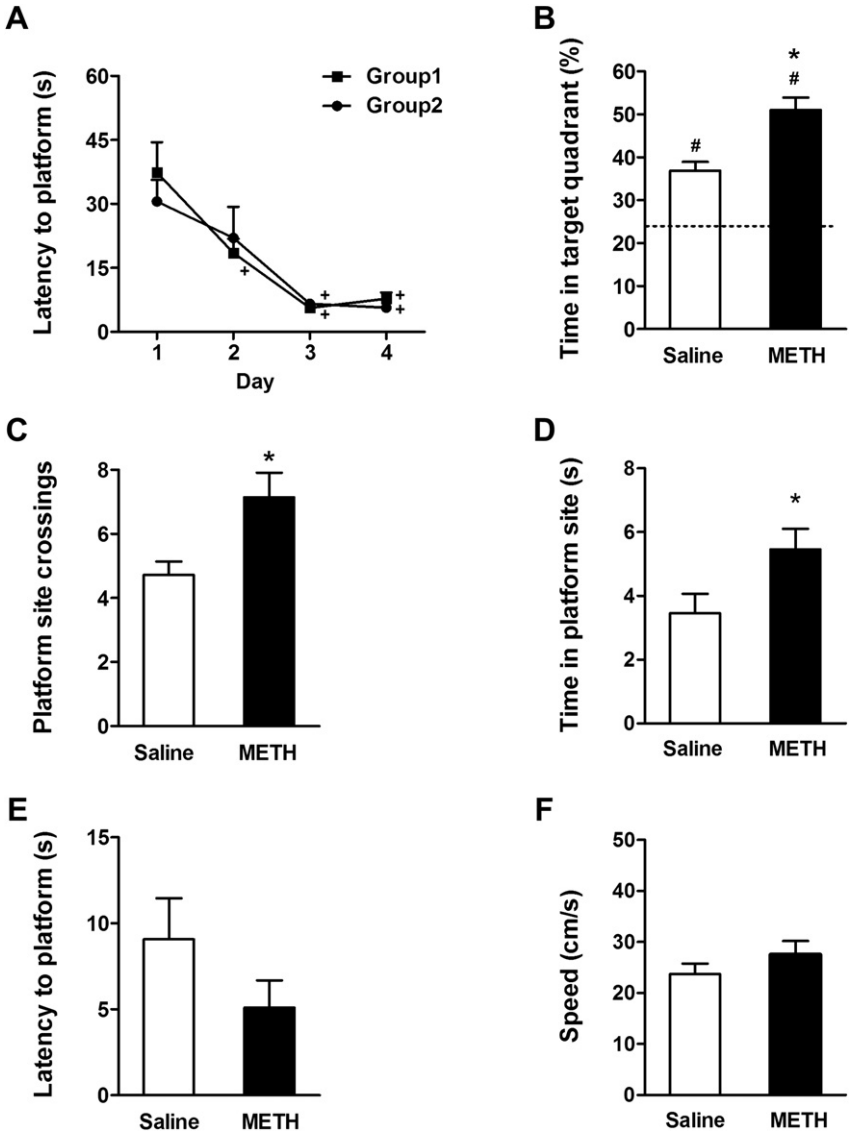

Fig. 1. Effects of methamphetamine (METH) on memory consolidation in mice when it was injected immediately after the last learning trial. Data were presented as mean ± SEM. (A) Latency to platform in mice ($n = 7$ per group). Group 1 represented saline-treated mice and group 2 represented METH-treated mice, respectively. (B–E) 1.0 mg/kg METH facilitated spatial memory consolidation in mice. $^*p < 0.05$ compared with saline group, independent sample *t*-test. $\#p < 0.05$ compared with chance level (25%), one sample *t*-test. (F) Swim speed of mice on day 5 following either saline or METH injections. No difference was found between two groups.

treated mice and group 2 represented METH-treated mice, respectively. In the probe test on day 5, both the METH-treated and saline-treated mouse groups developed significant spatial bias for the target quadrant compared with chance performance (Fig. 1B, $t = 5.811$, df = 6, $p < 0.01$ for saline group and $t = 9.006$, df = 6, $p < 0.001$ for METH group, respectively). However, mice treated with 1.0 mg/kg dose of METH performed significantly better than that of saline-treated mice in probe test [Fig. 1B, $F(1,12) = 15.993$, $^{*}p < 0.01$ for time in target quadrant; Fig. 1C, $F(1,12) = 7.673$, $^{*}p < 0.05$ for platform site crossing; Fig. 1D, $F(1,12) = 5.092$, $^{*}p < 0.05$ for time in platform site, respectively]. Similar tendency was found in measure of latency to platform although the difference failed to reach statistical significance [Fig. 1E, 9.07 ± 2.38 compared with 5.10 ± 1.59, $F(1,12) = 1.925$, $p = 0.191$]. There was no significant difference in the swim speed of mice between the two groups (Fig. 1F). Taken together, these results suggested that exposure to 1.0 mg/kg dose of METH facilitated spatial memory consolidation when it was injected immediately after the last training trial.

3.2. Memory retrieval

We then investigated effects of 1.0 mg/kg dose of METH on spatial memory retrieval using the protocol mentioned above. Two way repeated measures ANOVA of escape latencies revealed significant effects of time [$F(3,36) = 22.351$, $p < 0.001$] and their interaction [$F(3,36) = 4.241$, $p < 0.05$], but not group [$F(1,12) = 3.242$, $p > 0.05$] (Fig. 2A). All mice located the platform faster after training (Fig. 2A, $+p < 0.05$ compared with day 1) and developed spatial bias for the target quadrant compared with 25% in the probe test (Fig. 2B, $t = 7.940$, df = 6, $p < 0.001$ for saline group and $t = 5.647$, df = 6, $p < 0.001$ for METH group, respectively). However, there were no detectable differences between two groups of mice in the learning period and in the memory test after four days of training (Fig. 2). We then conducted a five-day training protocol with another two groups of mice to investigate whether this equal performance was due to an insufficient learning period. Two way repeated measures ANOVA of escape latencies revealed significant effects of time [$F(4,52) = 32.979$, $p < 0.001$], but not

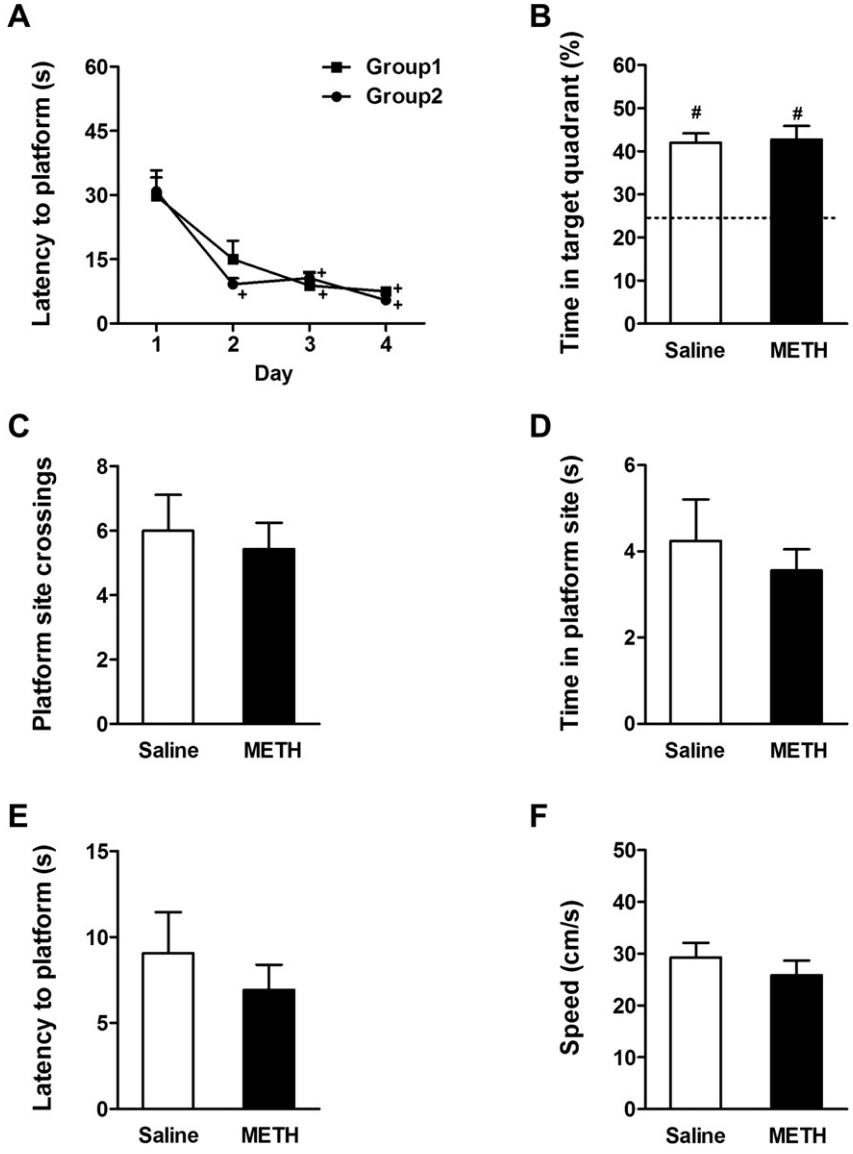

Fig. 2. Effects of methamphetamine (METH) on spatial memory retrieval in mice when it was injected 30 min before the test. Data were presented as mean ± SEM. (A) Latency to platform in mice for 4 consecutive days ($n = 7$ per group). Group 1 represented saline-treated mice and group 2 represented METH-treated mice, respectively. (B–E) 1.0 mg/kg METH did not alter spatial memory retrieval in mice. #$p < 0.05$ compared with chance level (25%), one sample t-test. (F) Swim speed of mice on the test day (day 5, following either saline or METH injections). No difference was found between two differently treated groups.

group [$F(1,13) = 0.014$, $p > 0.05$] and their interaction [$F(4,52) = 2.090$, $p > 0.05$] (Fig. 3A). Similarly, all mice located the platform faster after training (Fig. 3A, $+p < 0.05$ compared with day 1) and developed spatial bias for the target quadrant compared with 25% in the probe test (Fig. 3B, $t = 5.811$, df = 6, $p < 0.001$ for saline group and $t = 6.342$, df = 7, $p < 0.001$ for METH group, respectively), but no significant difference between two groups of mice was found in the learning period and in the memory test after five days of training (Fig. 3), although mice in METH-treated group swam faster than saline-treated group (Fig. 3F). These results indicated that METH at the dose of 1.0 mg/kg was not obviously involved in the modulation of spatial memory retrieval in the paradigms we used.

3.3. Memory reconsolidation

We also investigated effects of 1.0 mg/kg dose of METH on spatial memory reconsolidation using the protocol mentioned above. Two way repeated measures ANOVA of escape latencies revealed significant effects of time [$F(4,56) = 26.670$, $p < 0.001$], but not group [$F(1,14) = 1.290$, $p > 0.05$] and their interaction [$F(4,56) = 0.564$, $p > 0.05$] (Fig. 4A). All mice located the platform faster after training (Fig. 4A, $+p < 0.05$ compared with day 1) and developed spatial bias for the target quadrant compared with 25% even in the probe test 48 h after drug injections (Fig. 4B, $t = 3.175$, df = 6, $p < 0.001$ for saline group and $t = 2.300$, df = 8, $p < 0.05$ for METH group, respectively). No detectable differences were found between the two groups of mice in learning and memory tests (Fig. 4B–E) and swim speed (Fig. 4F). Two-way repeated measures ANOVA was used for the comparison of saline and METH over test 2 and test 3 and no difference was found. These results indicated that METH at the dose of 1.0 mg/kg was not obviously involved in the modulation of spatial memory reconsolidation in the paradigms we used.

As a control experiment, we investigated effects of METH on spatial memory reconsolidation in mice who received no reactivation of memory (test 1) on day 6. Two way repeated measures ANOVA of escape latencies revealed significant effects of time [$F(4,56) = 37.439$, $p < 0.001$], but not group [$F(1,14) = 0.187$,

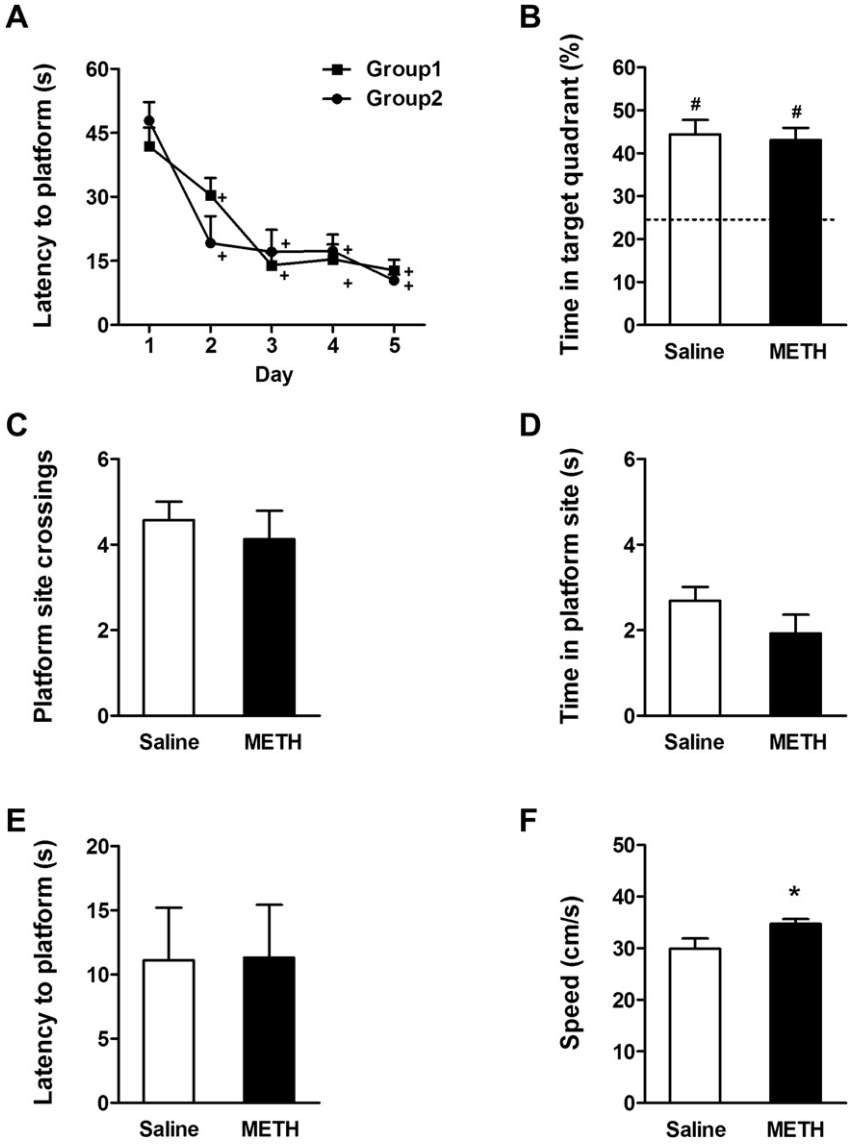

Fig. 3. Effects of methamphetamine (METH) on spatial memory retrieval in mice when it was injected 30 min before the test. Data were presented as mean ± SEM. (A) Latency to platform in mice for 5 consecutive days ($n = 7$–8 per group). Group 1 represented saline-treated mice and group 2 represented METH-treated mice, respectively. (B–E) 1.0 mg/kg METH did not alter spatial memory retrieval in mice. #$p < 0.05$ compared with chance level (25%), one sample t-test. (F) Swim speed of mice on the test day (day 6, following either saline or METH injections, *$p < 0.05$ compared with saline group).

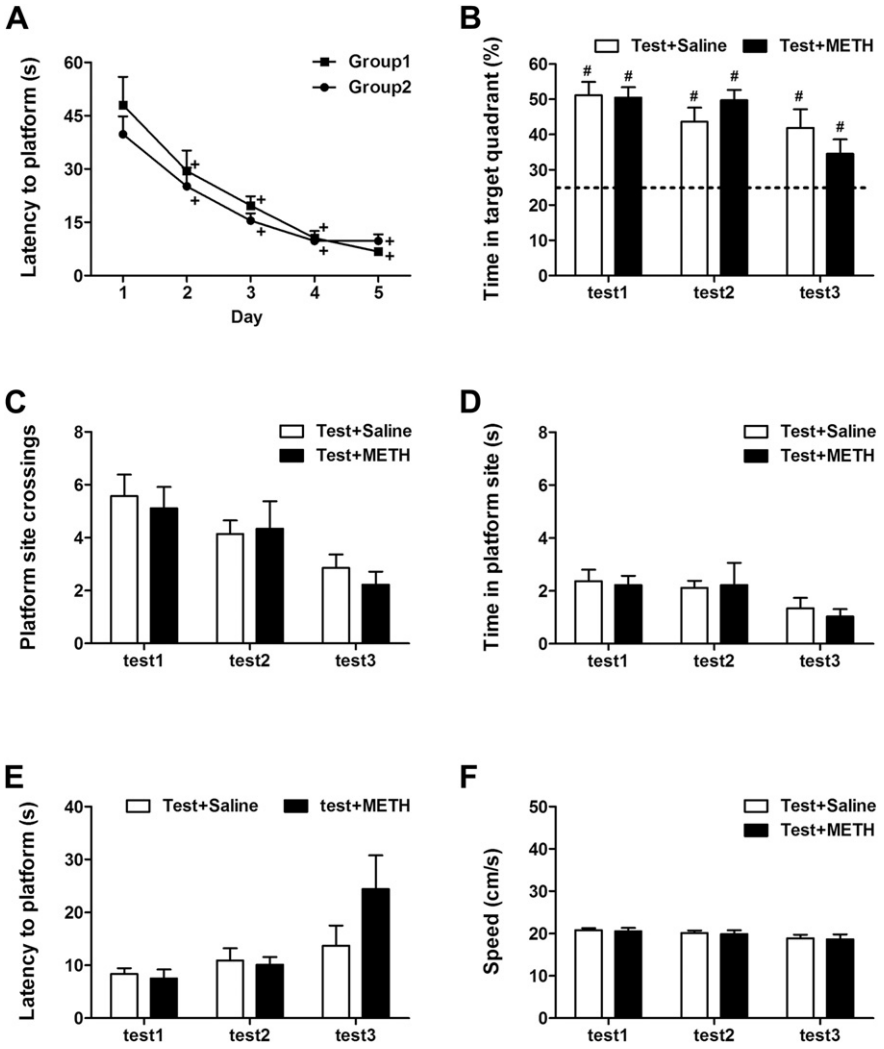

Fig. 4. Effects of methamphetamine (METH) on spatial memory reconsolidation in mice when it was injected immediately after retrieval on day 6. Data were presented as mean ± SEM. (A) Latency to platform in mice without any treatment for 5 consecutive days ($n = 7–9$). Group 1 represented saline-treated mice and group 2 represented METH-treated mice, respectively. (B–E) 1.0 mg/kg METH did not alter spatial memory reconsolidation in mice. #$p < 0.05$ compared with chance level (25%), one sample t-test. (F) Swim speed of mice on probe test days (day 6–8). No difference was found between two treated groups. Test 1, test 2 and test 3 referred to a 60 s period of probe test without the platform on day 6, day 7 and day 8 respectively.

$p > 0.05$] and their interaction [$F(4,56) = 0.205$, $p > 0.05$] (Fig. 5A). All mice located the platform faster after training (Fig. 5A, +$p < 0.05$ compared with day 1) and developed spatial bias for the target quadrant compared with 25% even in the probe test 48 h after drug injections (Fig. 5B, $t = 3.234$, df = 7, $p < 0.05$ for saline group and $t = 3.120$, df = 7, $p < 0.05$ for METH group, respectively). The two groups of mice performed equally in memory test 3 which is 72 h after the last training trial and in swim speed (Fig. 5B–F), although a weak impairment was found in METH-treated mice in test 2 which is 48 h after the trial (Fig. 5B, *$p < 0.05$, compared with saline). Two-way repeated measures ANOVA for the comparison of saline and METH over test 2 and test 3 was used and no difference was found between the two groups.

3.4. METH-induced activation of ERK in hippocampus and PFC

In order to investigate whether hippocampal and PFC ERK (ERK1 and ERK2) activation was involved in METH-induced spatial memory changes, western blotting analysis was used to measure ERK phosphorylation levels after memory tests. Hippocampal ERK was significantly activated in 1.0 mg/kg dose of METH groups of mice who exhibited better spatial memory consolidation than that of in saline-treated groups (Fig. 6A, $p < 0.05$). Mice receiving saline or METH performed similarly in the spatial memory retrieval and reconsolidation tests also showed equal ERK activation (Fig. 6A). Further analysis by separating phosphorylation levels of hippocampal ERK1 and ERK2 found that only ERK2 but not ERK1 was significantly activated in METH-treated mice (Fig. 6A, *$p < 0.05$ compared with saline group) who exhibited better spatial memory consolidation. In contrast, no significant difference was detected in either ERK1 or ERK2 among groups in the retrieval, reconsolidation and reconsolidation-control process (Fig. 6A).

ERK in the PFC was significantly activated in 1.0 mg/kg dose of METH groups of mice in memory retrieval (Fig. 6B, *$p < 0.05$ compared with saline group), although they performed equally in behavioral tests. No significant difference was detected between saline and METH injected groups among other behavioral tests (Fig. 6B). Further analysis by separating phosphorylation levels of PFC ERK1 and ERK2 found that both ERK1 and ERK2 were significantly activated in METH-treated mice (Fig. 6B, *$p < 0.05$ compared with saline group) in spatial memory retrieval. In contrast, no significant difference was detected in either ERK1 or ERK2 among

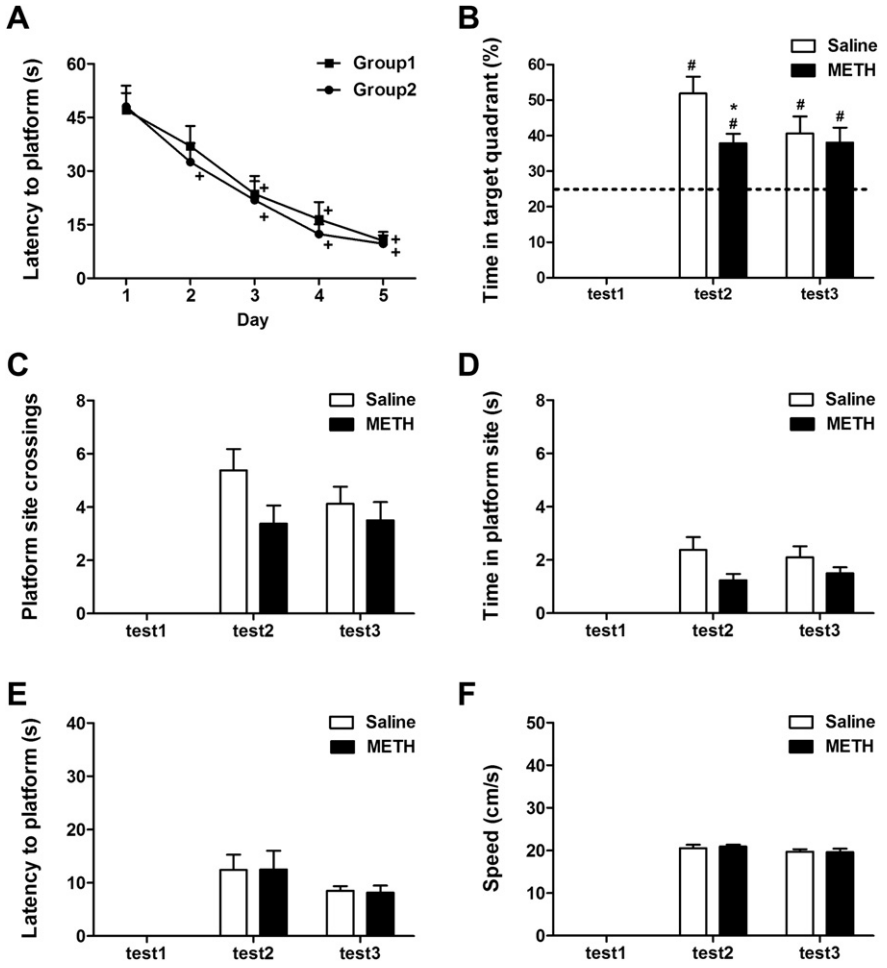

Fig. 5. Effects of methamphetamine (METH) on spatial memory reconsolidation in mice without reactivation when it was injected at a paired time point with the mice experienced memory retrieval on day 6. Data were presented as mean ± SEM. (A) Latency to platform in mice without any treatment for 5 consecutive days ($n = 8$ per group). Group 1 represented saline-treated mice and group 2 represented METH-treated mice, respectively. (B–E) 1.0 mg/kg METH did not alter spatial memory reconsolidation in mice. $^{*}p < 0.05$, compared with saline, independent sample *t*-test. $\#p < 0.05$ compared with chance level (25%), one sample *t*-test. (F) Swim speed of mice on two probe test days (day 7–8). No difference was found between two groups. Test 1, test 2 and test 3 referred to a 60 s period of probe test without the platform on day 6, day 7 and day 8 respectively.

groups in the consolidation, reconsolidation and reconsolidation–control process (Fig. 6B).

3.5. METH-induced activation of CREB in hippocampus and PFC

To further investigate whether CREB activation in the hippocampus and PFC was involved in METH-induced spatial memory changes, western blotting analysis was used to measure ERK and CREB phosphorylation levels after memory tests. CREB activation in mouse hippocampus was similar to ERK, which was accompanied with spatial memory consolidation (Fig. 7A, $^{*}p < 0.05$ compared with saline group) but not retrieval or reconsolidation (Fig. 7A). These results suggested that ERK/CREB signaling pathway in the hippocampus might be involved in METH-induced spatial memory changes. Activation of CREB in the PFC was only found in METH-treated groups in spatial memory retrieval (Fig. 7B, $^{*}p < 0.05$ compared with saline group) while no significant difference was detected in spatial memory consolidation or reconsolidation groups (Fig. 7B).

4. Discussion

As a highly addictive drug (Herrold et al., 2009; Mizoguchi et al., 2004), METH was proved to induce cognitive functional alterations in both humans (Mahoney III et al., 2010) and rodents (Belcher et al., 2007; Kamei et al., 2006; Skelton et al., 2008; Vorhees et al., 2009; Williams et al., 2003). Previous studies mainly focused on the neurotoxic effects of METH, which found that high doses of METH in binge (4–25 mg/kg given 4 times a day) or escalating (10 mg/kg–30 mg/kg) treatment regimens impaired spatial learning ability (Vorhees et al., 2007, 2009), working memory (Simoes et al., 2007) and recognition memory (Belcher et al., 2007). Impaired recognition memory and spatial memory was also detected in animals experienced repeated (once daily for 5–7 days) low dose (1.0 mg/kg) METH treatments (Ito et al., 2007; Kamei et al., 2006; Schutová et al., 2009). On the other hand, previous evidences also suggested that psychostimulants such as amphetamine and cocaine could facilitate memory performance (Wood et al., 2007), especially the consolidation of memory (Iñiguez et al., 2011; Wiig et al., 2009). Besides, low dose METH administration was reported to improve memory in human addicts (Mahoney III et al., 2010) and also in animal models (Kennedy et al., 2010). However, there were few studies about how METH modulates spatial learning and memory when it was given during different phases of memory formation and during reconsolidation. In the current study, we investigated the distinct effects of 1.0 mg/kg dose of METH on spatial memory consolidation, retrieval and reconsolidation in mice.

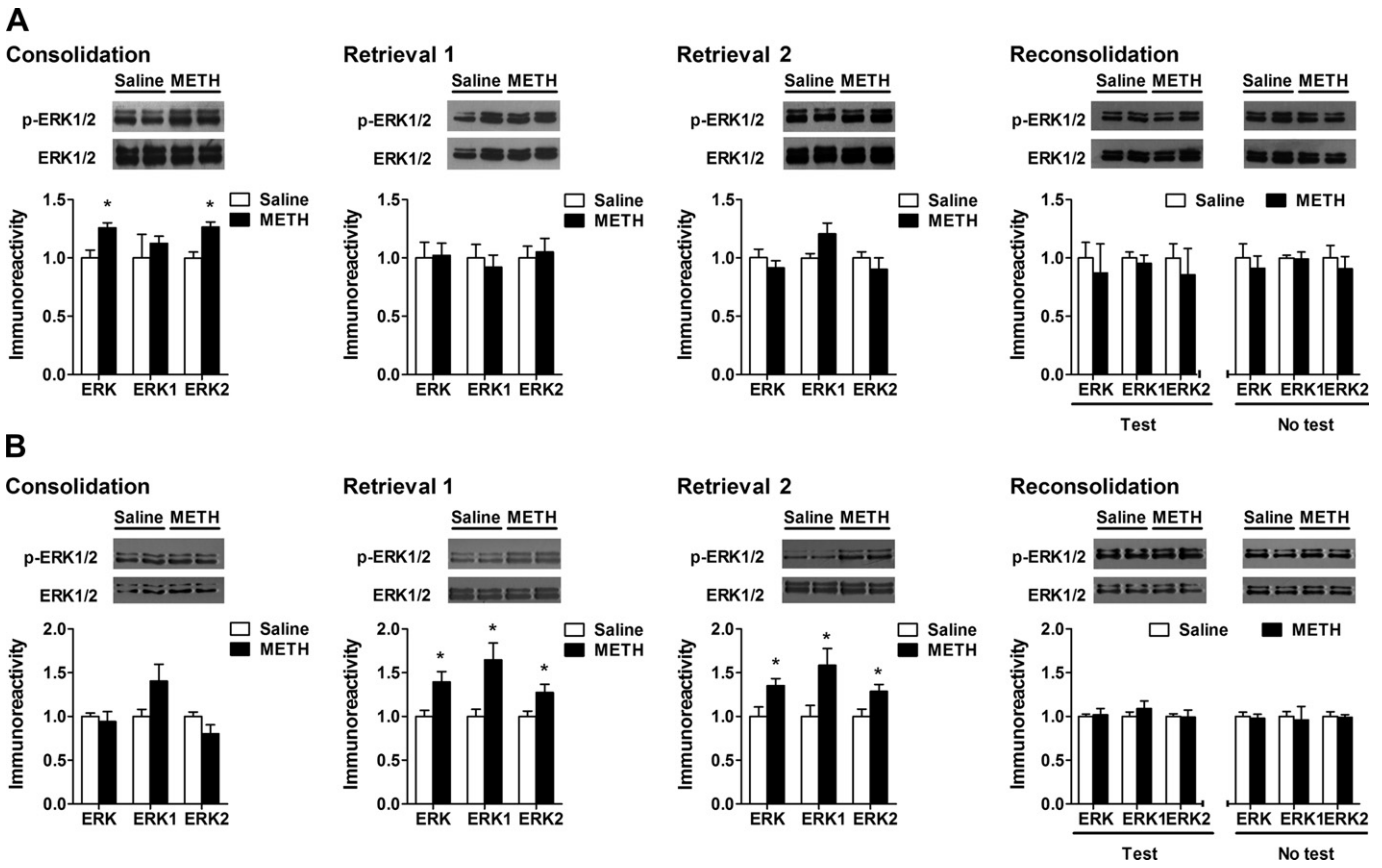

Fig. 6. METH-induced activation of extracellular signal-regulated kinase (ERK) in hippocampus and prefrontal cortex (PFC). Representative blots of phospho-ERK and ERK from hippocampal (A) and PFC (B) tissues of mice and relative levels of phosphorylation of ERK as well as ERK1 and ERK2 accompanied with spatial memory consolidation ($n = 7$ per group), retrieval (retrieval 1 and retrieval 2 represented samples from mice experienced 4 or 5 consecutive days training respectively. $n = 7–8$ per group), and reconsolidation ($n = 7–9$ per group) were shown respectively. Saline group levels were set at 1 for quantifications. Data were expressed as mean ± SEM. Test indicated samples from mice with reactivation, no test represented samples from mice with no reactivation. $^*p < 0.05$, compared with saline, independent sample *t*-test.

To dissect whether METH affected memory consolidation, we gave mice a single METH injection after the last trial on day 4 and then tested the effects of this manipulation on memory consolidation 24 h later (day 5). We found that, in the absence of noticeable differences in swim speed, mice treated with 1.0 mg/kg dose of METH performed significantly better than mice treated with saline in memory tests (Fig. 1). These results were consistent with previous studies which demonstrated that a single low dose cocaine injection after the last trial facilitated spatial memory consolidation in mice (Iñiguez et al., 2011). Immediate post-trial amphetamine injections after daily trials were also reported to improve spatial learning and memory consolidation in rats (Brown et al., 2000). In order to exclude the potential impact of swim speed alterations on MWM performance, we also examined swim speed of mice on the test day and no significant difference was found between the two groups (Fig. 1F). Taken together, the present results suggested that exposure to 1.0 mg/kg dose of METH facilitated spatial memory consolidation when it was injected immediately after the last training trial.

Little is known about the effect of METH on spatial memory retrieval. In the current study, we treated four groups of mice with 1.0 mg/kg dose of METH or saline 30 min before their memory performance were tested on day 5 or day 6 to examine effects of METH on memory retrieval. We found that 1.0 mg/kg dose of METH had no obvious effects on memory retrieval (Figs. 2–3). Whereas additional doses of METH needed to be used in the future, these results suggested that 1.0 mg/kg dose of METH had no effect on spatial memory retrieval. To our knowledge, this was the first description of a distinct role of METH in different memory processes.

We also investigated effects of METH on spatial memory reconsolidation. We found that METH at the dose of 1.0 mg/kg did not obviously alter spatial memory reconsolidation in the paradigm we used (Fig. 4). In the absence of memory reactivation, mice receiving either a METH or a saline injection performed equally in memory test 3 which was 72 h after the last training trial and in swim speed, although a weak impairment was found in METH-treated mice in test 2 which was 48 h after the trial (Fig. 5). We should like to note that this finding is based on the use of METH at a single dose. Whether other doses of METH may affect spatial memory needs to be investigated in the future.

In the current study, we examined the activation of ERK and CREB in the mouse hippocampus, which was involved in spatial learning and memory. We found that hippocampal ERK and CREB was significantly activated in 1.0 mg/kg METH groups of mice that exhibited better spatial memory consolidation than saline control groups (Figs. 6–7). Mice performed similarly in the spatial memory retrieval test and reconsolidation test showed equal ERK and CREB activation (Figs. 6–7). METH increased extracellular levels of dopamine (Beauvais et al., 2011; Graham et al., 2012; Mizoguchi et al., 2004; Skelton et al., 2008) that potentiated functions of the dopamine system (Bubenikova-Valesova et al., 2009; Crawford et al., 2003) which played an important role in the modulation of synaptic plasticity and learning. Previous studies demonstrated that activation of dopamine D1 and/or D5 dopamine receptors

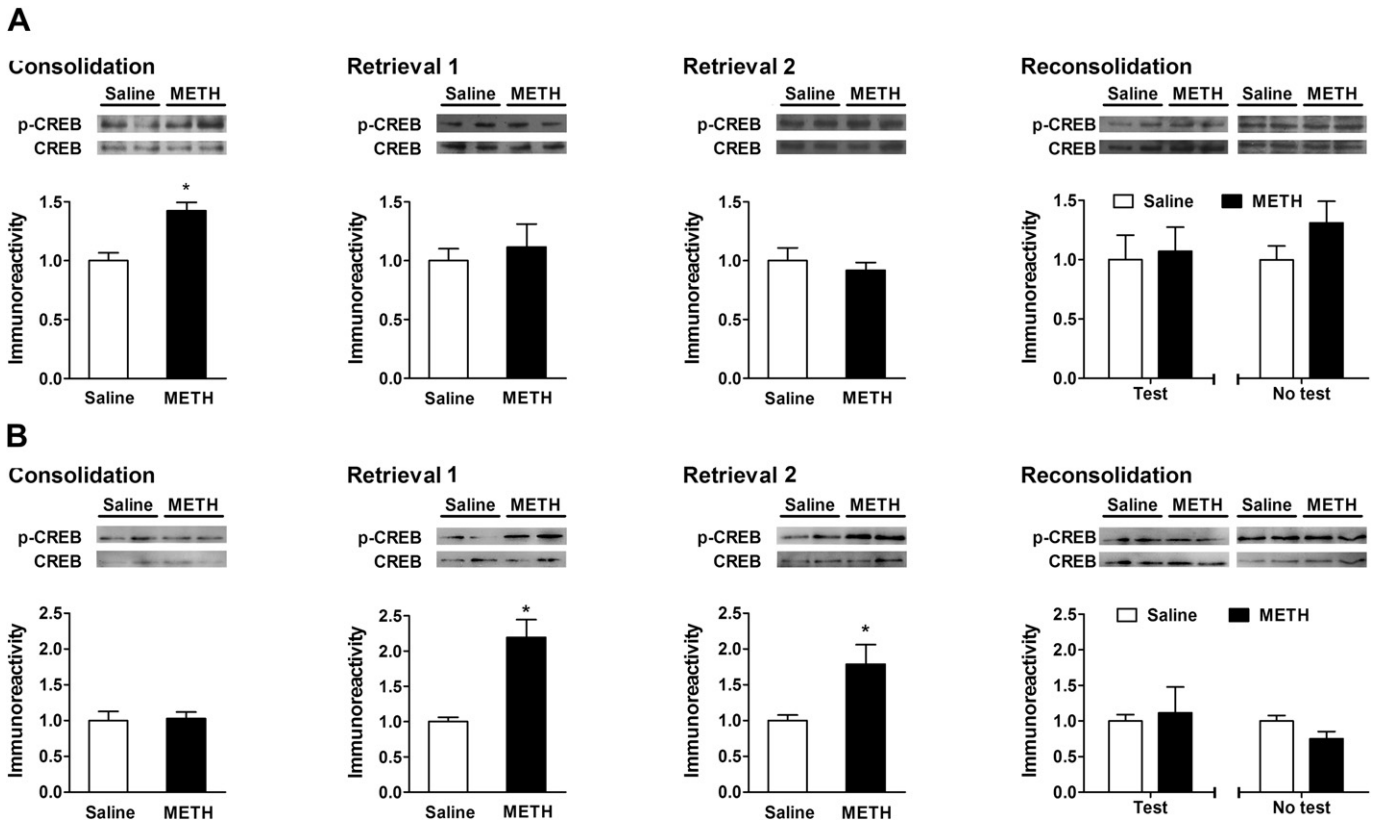

Fig. 7. METH-induced activation of cAMP response element-binding protein (CREB) in hippocampus and prefrontal cortex (PFC). Representative blots of phospho-CREB and CREB from hippocampal (A) and PFC (B) tissues of mice and relative levels of phosphorylation of CREB accompanied with spatial memory consolidation ($n = 7$ per group), retrieval (retrieval 1 and retrieval 2 represented samples from mice experienced 4 or 5 consecutive days training respectively. $n = 7–8$ per group), and reconsolidation ($n = 7–9$ per group) were shown respectively. Saline group levels were set at 1 for quantifications. Data were expressed as mean ± SEM. Test indicated samples from mice with reactivation, no test represented samples from mice with no reactivation. $^*p < 0.05$, compared with saline, independent sample *t*-test.

enhanced spatial memory (Amico et al., 2007; Fricks-Gleason and Marshall, 2010; Jay, 2003), whereas mice lacking D1 receptors had a severe impaired spatial memory (Xing et al., 2010). It was possible that METH enhanced memory consolidation by activating the ERK signaling pathway via dopamine D1 receptors (Ito et al., 2007; Mizoguchi et al., 2004). Activated ERK phosphorylated relative transcriptional regulators such as CREB (Zanassi et al., 2001), which contributed to synaptic plasticity as well as learning and memory (Kelleher et al., 2004; Pittenger et al., 2006; Sweatt, 2001; Xing et al., 2010). Further analysis found that only ERK2 but not ERK1 was significantly activated in METH-treated mice compared with that of in saline injection (Fig. 6). These results were consistent with previous studies which showed that ERK2 but not ERK1 was the predominant isoform in the regulation of synaptic plasticity in hippocampus (English and Sweatt, 1996). Additionally, previous evidence also demonstrated that enhancement of ERK2 activity might be involved in changes of associative learning and memory (Li et al., 2008; Mazzucchelli et al., 2002).

Investigation of the PFC found significant activations of ERK and CREB in 1.0 mg/kg dose of METH groups of mice in memory retrieval (Figs. 6–7), although they performed equally in behavioral tests in the current study. One possible explanation was that PFC was not obviously involved in spatial memory retrieval which was tested 1 day after acquisition of a newly memory. Previous studies demonstrated that both hippocampus and prefrontal cortex engaged in spatial memory changes and they played different role in modulating distinct spatial memory processes (Maviel et al., 2004). For instance, hippocampus engaged in recent spatial memory processing that was tested 1 day after memory acquisition whereas PFC engaged in remote spatial memory processing that was tested 30 days after memory acquisition (Maviel et al., 2004). Alternatively, activations of ERK and CREB in the PFC might be more sensitive to METH than that of behavioral changes. Therefore, although ERK and CREB were significantly activated in the PFC, changes of spatial memory retrieval were not detected in mice in the current study. Future experiments are needed to investigate the unresolved possibilities. Our current results suggested that ERK and CREB signaling pathway in the hippocampus might be involved in METH-induced spatial memory changes.

5. Conclusions

In conclusion, our current results demonstrated that 1.0 mg/kg dose of METH facilitated spatial memory consolidation but not retrieval or reconsolidation, and the ERK and CREB signaling pathway in the hippocampus might be involved in METH-induced spatial memory changes.

Acknowledgments

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