

# Tetrahydropalmatine protects against methamphetamine-induced spatial learning and memory impairment in mice

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**Abstract: Objective** The purpose of this study was to investigate the effect of methamphetamine (MA) on spatial learning and memory and the role of tetrahydropalmatine (THP) in MA-induced changes in these phenomena in mice. **Methods** Male C57BL/6 mice were randomly divided into eight groups, according to different doses of MA, different doses of THP, treatment with both MA and THP, and saline controls. Spatial learning and memory were assessed using the Morris water maze. Western blot was used to detect the expression of extracellular signal-regulated protein kinase (ERK) in the mouse prefrontal cortex (PFC) and hippocampus. **Results** Repeated MA treatment significantly increased the escape latency in the learning phase and decreased the number of platform site crossings in the memory-test phase. ERK1/2 expression was decreased in the PFC but not in the hippocampus of the MA-treated mice. Repeated THP treatment alone did not affect the escape latency, the number of platform site crossings or the total ERK1/2 expression in the brain. Statistically significantly shorter escape latencies and more platform site crossings occurred in MA+THP-treated mice than in MA-treated mice. **Conclusion** Repeated MA administration impairs spatial learning and memory in mice, and its co-administration with THP prevents this impairment, which is probably attributable to changed ERK1/2 expression in the PFC. This study contributes to uncovering the mechanism underlying MA abuse, and to exploring potential therapies.

**Keywords:** methamphetamine; tetrahydropalmatine; Morris water maze; ERK; prefrontal cortex; hippocampus; drug addiction

## 1 Introduction

The use of methamphetamine (MA) has become a

worldwide problem of substance abuse and dependence and threatens human health and social stability<sup>[1-4]</sup>. Repeated administration of MA is associated with strong addictive effects in humans and animal models<sup>[5]</sup>. Growing evidence indicates that repeated administration of MA may affect the mitogen-activated protein kinase (MAPK)–extracellular signal-regulated protein kinase (ERK) signaling pathway<sup>[6,7]</sup>,

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which is among the important dopamine receptor signaling pathways involved in the regulation of cell proliferation and differentiation, cell transformation, as well as learning and memory performance<sup>[6, 8-10]</sup>.

Tetrahydropalmatine (THP), a major component of *Corydalis*, is a traditional Chinese medicine. Previous studies have shown that THP is a dopamine receptor antagonist and does not have an addictive effect in animal models<sup>[11-14]</sup>. On the contrary, it reduces the excitability and toxicity of amphetamine and inhibits spontaneous activity in animal models<sup>[12-14]</sup>. More recent work shows that THP suppresses the central excitatory effects of amphetamine<sup>[15]</sup>, and plays a protective role against neuronal injury in animal models<sup>[16,17]</sup>.

It is important to investigate whether the administration of MA inhibits ERK expression and function in the hippocampus and prefrontal cortex (PFC), along with the impairment of spatial learning and memory. In this study, we investigated whether THP plays a protective role in preventing this impairment in mice treated with MA. To this end, we used both behavioral and molecular biological techniques to study the effect of MA on spatial learning and memory performance and further explore the molecular mechanisms of MA-dependence.

## 2 Materials and methods

**2.1 Drugs and animals** Methamphetamine hydrochloride (Chinese Pharmaceutical and Biological Products, Lot: 171212-200603, Beijing, China) was dissolved at 2.0 mg/mL in saline for stock solution, and further diluted to 0.5 or 1.0 mg/mL before experiments. THP (purity 98%) was from Kexiang Biological Technology Co., Ltd. (Shanghai, China) and dissolved in diluted H<sub>2</sub>SO<sub>4</sub>. The pH was adjusted to 5.5 using 2 mg/mL NaOH. THP was prepared as 0.5 or 1.0 mg/mL.

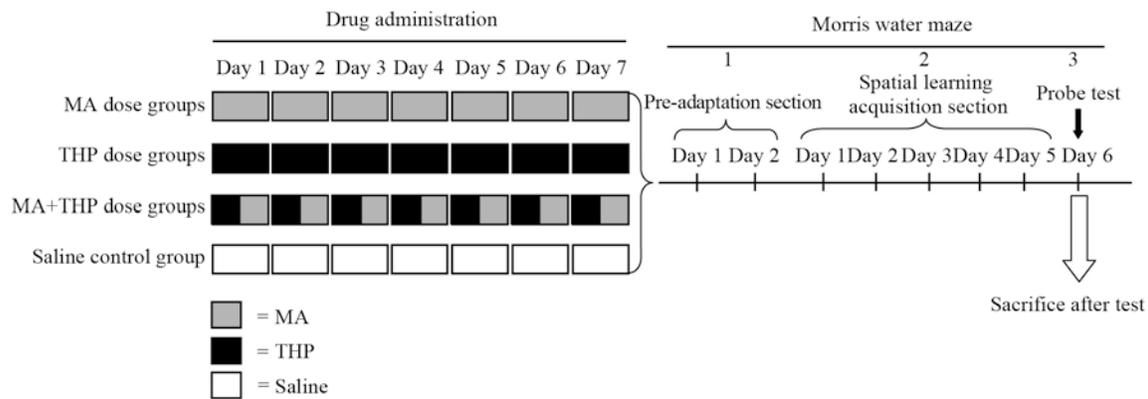
A total of 72 male C57BL/6 mice (8–10 weeks old, weighing 22 ± 0.2 g) were used. Mice were housed at 23 ± 2°C in (50 ± 5)% humidity under a 12:12 h light/dark cycle, and were fed standard chow with water *ad libitum*. They were divided into eight groups ( $n = 8-12$ /group): MA5 (5 mg/kg MA administration), MA10 (10 mg/kg MA), THP5 (5 mg/kg THP), THP10 (10 mg/kg THP), MA5 + THP10

(co-administration of 5 mg/kg MA and 10 mg/kg THP), MA10 + THP5 (10 mg/kg MA and 5 mg/kg THP), MA10 + THP10 (10 mg/kg MA and 10 mg/kg THP), and a control group (saline treatment). All drugs were injected intraperitoneally (i.p.) at 10 mL/kg body weight. Each group was given the fixed dose of MA once a day for 7 days. The drugs were administered at the same time and mice were weighed every day. The experimental procedures are shown in Fig. 1. All procedures were approved by the Laboratory Animal Administration Committee of Xi'an Jiaotong University and performed according to the Guidelines for the Care and Use of Laboratory Animals published by the National Institutes of Health, USA.

**2.2 Morris water maze test** Morris water maze tests were conducted to assess the learning and memory of the animals. This system comprised a circular pool, an automatic camera and an analyzing system. A 120-cm diameter Morris water maze (Shenzhen Rui Wode Life Technology Co., Ltd., Shenzhen, China) was filled with water to a depth of 60 cm. The water temperature was adjusted to 21–23°C. White pigment was dissolved in the pool to make the water opaque. A cylindrical platform, 14 cm in diameter, was placed into the maze, 1–1.5 cm below the water surface. The platform was placed within 35 cm from the pool wall. The doors, lights and cabinets around the pool served as fixed references for mouse positioning. The automatic camera recorded mouse movement in the water, and Smart Version 2.5 software automatically analyzed the relevant parameters.

After 7 days of drug administration, the animals were placed into the system where measurements of spatial learning were evaluated. The training started at 08:30, and mice were placed into the environment 30 min prior to training. At the end of each training day, the pool was cleaned to eliminate olfactory cues. The tests were conducted according to the description by Vorhees *et al.*<sup>[18]</sup>.

The data from the Morris water maze system were obtained from previously-trained mice. In the pre-adaptation section, the platform was submerged 1 cm below the water surface, and both the goal and start positions were changed in each trial (Table 1). This ensured that the mice recog-



**Fig. 1.** Schematic representation of experimental procedures. Drugs were administered at 09:30, and in the co-administration groups the appropriate dose of THP was given 30 min before MA administration.

**Table 1.** Pre-adaptation trial pattern in the Morris water maze test (start position–goal position)

Day	Trial 1	Trial 2	Trial 3	Trial 4
1	North–Southeast	East–Northeast	South–Northwest	West–Southwest
2	North–Southwest	East–Northwest	South–Southeast	West–Northeast

nized the platform to escape but did not use distal cues to locate it. The animals were released into the water from the desired start position facing the tank wall at water-level (not dropped). The process lasted for 2 days, with 4 trials per day.

After pre-adaptation, spatial learning was evaluated (Fig. 1). In this section, the platform was also submerged 1 cm below the water surface but was placed at a fixed location in the northwest quadrant. Each trial had a different start position, the same sequence was used for each mouse on a given day, and the sequence was different for each day (Table 2). When the mouse climbed and remained on the platform for 3 s, it was considered to have found it and the time spent in finding it was recorded as the escape latency. Each mouse was allowed 60 s to locate the platform. If it failed within that time, escape was manually guided, and the escape latency was recorded as 60 s. After a trial ended, the mouse was immediately dried and returned to its homecage for at least 30 min prior to the next trial. Each mouse had 4 trials per day for 5 consecutive days and data were recorded as the arithmetic mean of the 4 trials.

**Table 2.** Spatial start positions in the Morris water maze test

Day	Trial 1	Trial 2	Trial 3	Trial 4
1	East	South	Southwest	Northeast
2	Southwest	East	Northeast	South
3	Northeast	Southwest	South	East
4	South	Northeast	East	Southwest
5	East	Southwest	South	Northeast

Note: The goal (platform) was located in the northwest quadrant during acquisition.

Spatial memory was tested on day 6 with the platform removed and the time limit set at 60 s. Platform site crossings, time in platform site and time in target quadrant were analyzed to assess the effects of MA and THP on spatial memory.

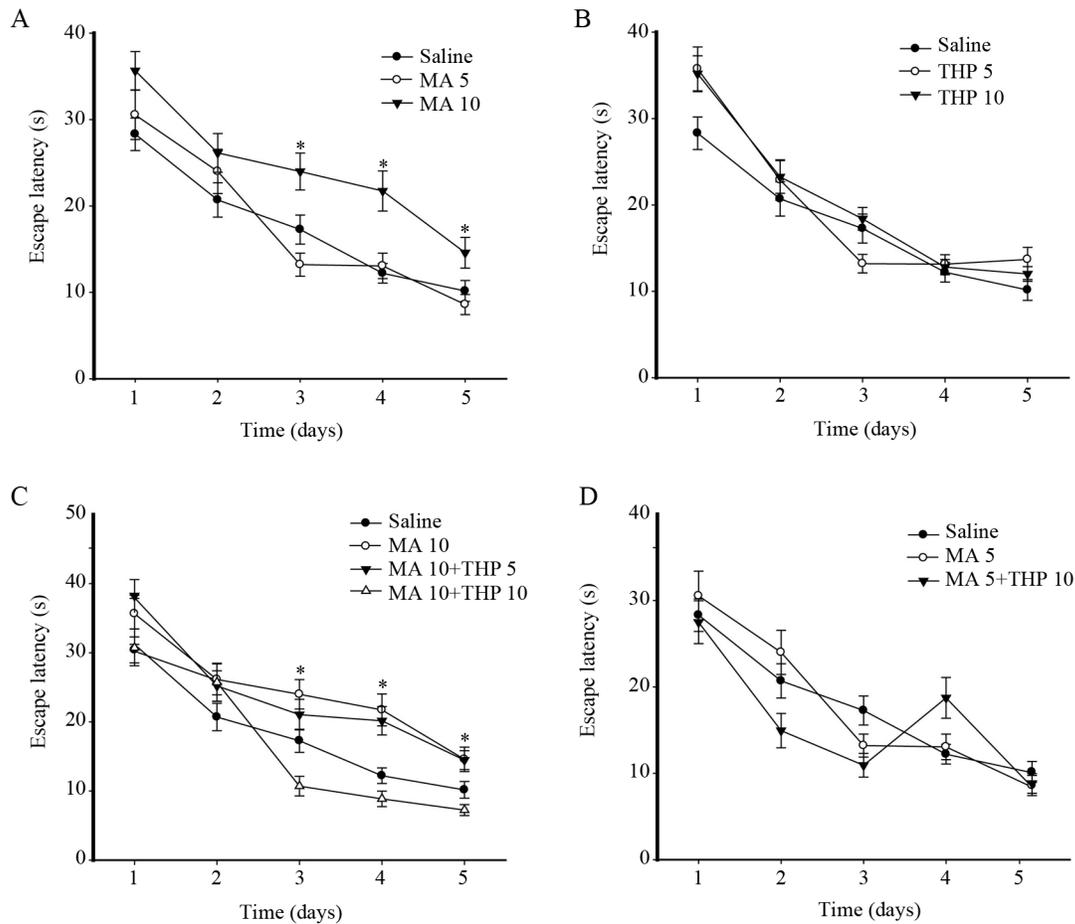
**2.3 Western blotting** Twenty-four mice in the MA10, THP10, MA10 + THP10 and saline groups were randomly chosen for Western blotting (6 mice per group) after the Morris water maze test. The mice were decapitated 20

min after the final behavioral experiment. The bilateral PFC (mainly medial orbital cortex and prelimbic cortex) and hippocampus were dissected out on an ice-cold plate according to stereotaxic coordinates<sup>[19]</sup>. Tissues were collected and kept at  $-80^{\circ}\text{C}$  until use.

Total protein of each dissected tissue was extracted according to the instructions with the kit (Biochain Institute Inc., Newark, CA), and protein concentration was determined with a Coomassie brilliant blue UV spectrophotometer (UV-1601, SHIMADZU, Tokyo, Japan). A sample of 20  $\mu\text{g}$  total protein was size-fractionated by 10% SDS-

polyacrylamide gel electrophoresis and immunoblotted with antibodies against ERK1/2, phospho-ERK1/2 and  $\beta$ -actin (Cell Signalling, Danvers, MA).

**2.4 Data analysis** Data are presented as mean  $\pm$  SEM. All differences in drug effects among groups were tested by two-way repeated measures analysis of variance (ANOVA) with *post hoc* multiple comparisons for analysis of differences in the entire observation time or at each time point among different groups (Fig. 2, Tables S1–S4) or one-way ANOVA (Figs. 3 and 5–7, Table S5) by SPSS 13.0<sup>[8]</sup>. Statistical differences were considered significant at  $P < 0.05$ .



**Fig. 2.** Effects of repeated MA and THP administration on escape latency in the training phase of the Morris water maze test. Male C57BL/6 mice were given MA (A), THP (B), or both MA and THP (C and D), and the escape latency was measured from days 1 to 5. A: The saline and MA10 groups differed on days 3, 4 and 5 ( $P < 0.05$ ), while no significant difference was observed between saline and MA5 groups. B: No significant differences were observed among saline, THP5 and THP10 groups. C: The escape latency differed between the MA10 and MA10 + THP10 groups on days 3, 4 and 5 ( $P < 0.05$ ). There were no significant differences for saline vs MA10 + THP10 and MA10 vs MA10 + THP5. D: No significant differences were observed among saline, MA5 and MA5 + THP10 groups.

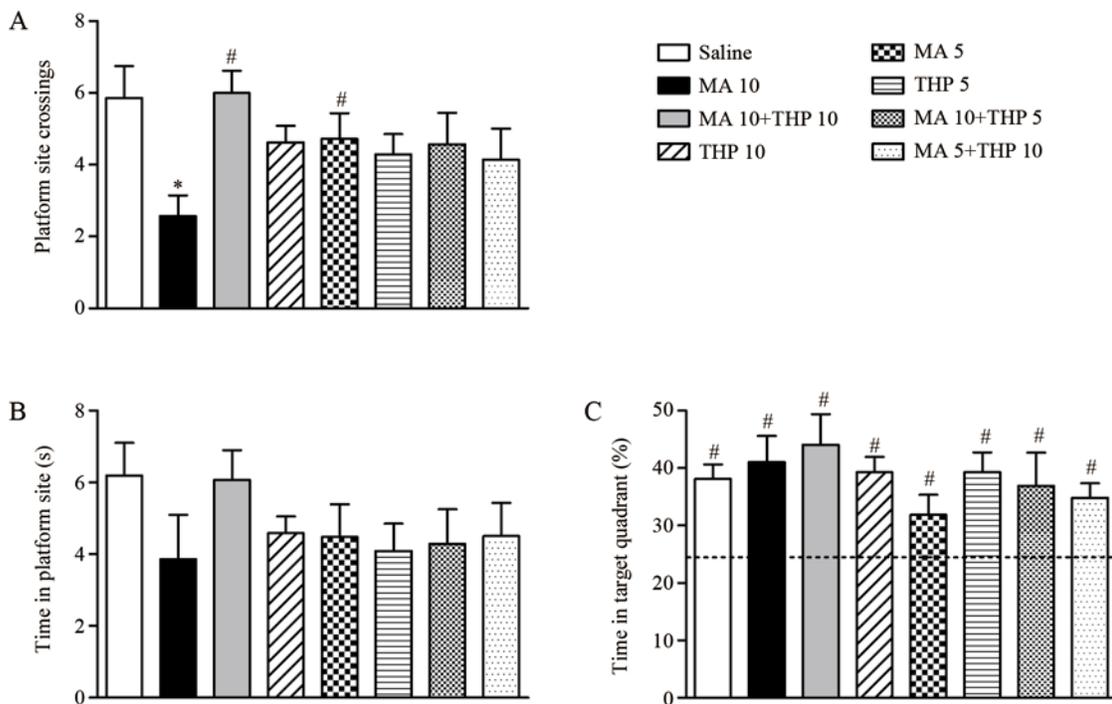
### 3 Results

Repeated ANOVA analysis revealed that the escape latencies were significantly different among the MA5, MA10 and saline groups ( $F_{[2,101]} = 4.821$ ,  $P = 0.01$ ) (Fig. 2A). *Post hoc* multiple comparisons showed that the escape latency of the MA10 group was longer than that of the saline group (Fig. 2A,  $P = 0.006$ ). In addition, there was no difference in escape latency between the MA5 and saline groups (Fig. 2A), suggesting that only the higher dose of MA induced changes in learning and memory performance. The escape latency in the MA10 group was markedly longer than the saline group on days 3, 4 and 5 (Fig. 2A, Table S1,  $P < 0.05$ ).

To investigate the effects of THP on learning and memory performance in MA-treated mice, those that received MA or saline were treated with 5 or 10 mg/kg

THP. We found that repeated treatment with THP alone did not affect the learning performance when compared to the saline-treated mice (Fig. 2B, Table S2). Repeated ANOVA analysis showed that the escape latencies differed among the four groups ( $F_{[3,132]} = 6.084$ ,  $P = 0.001$ ; Fig. 2C, Table S3). *Post hoc* multiple comparisons showed that the escape latencies in the MA10 + THP10 group, but not in the MA10 + THP5 group, were shorter than those in the MA10 group on days 3, 4 and 5 ( $P = 0.002$ ). These results suggest that the lower dose of THP did not prevent the MA-induced impairment of learning and memory. In addition, the escape latency did not differ among the saline, MA5 and MA5 + THP10 groups (Fig. 2D, Table S4), which indicates that the specific effect of MA only started at the higher dose and THP treatment prevented the MA-induced impairment.

Spatial memory was evaluated on day 6 and impair-



**Fig. 3.** Effects of repeated MA and THP administration on mouse performance in the memory phase of the Morris water maze test. The probe test was carried out 24 h after the last training session. **A:** The MA10 group had fewer target crossings than the saline group ( $*P < 0.05$  compared with saline). MA10-induced impaired spatial memory was prevented by 10 mg/kg THP treatment. MA5 mice also had better memory than MA10 mice ( $*P < 0.05$  compared with MA10 group). **B:** The time spent in the platform site showed a similar tendency of change, although no statistical difference was observed. **C:** All mice developed a spatial bias for the target quadrant. Dashed line indicates 25% chance level,  $#P < 0.05$  compared with 25% by chance.

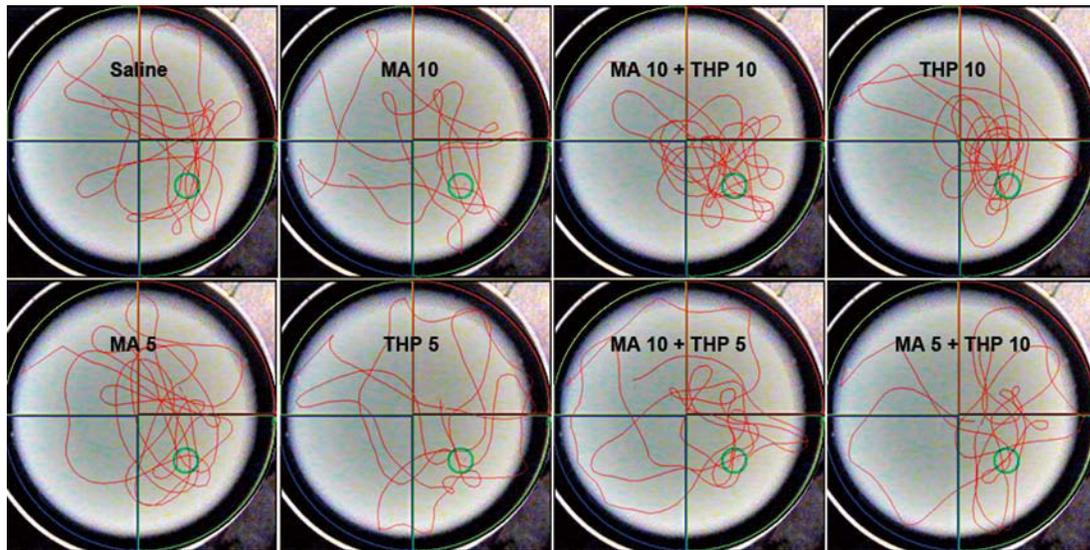


Fig. 4. Representative swim paths of mice in the water maze test. C57BL/6 mice given saline, MA, THP, or both MA and THP were trained to find a hidden platform (green circle). The traces showed paths taken by a representative mouse when the platform had been removed. Mice with poor learning and memory retention spent less time in the quadrant where they had previously learned to find the platform.

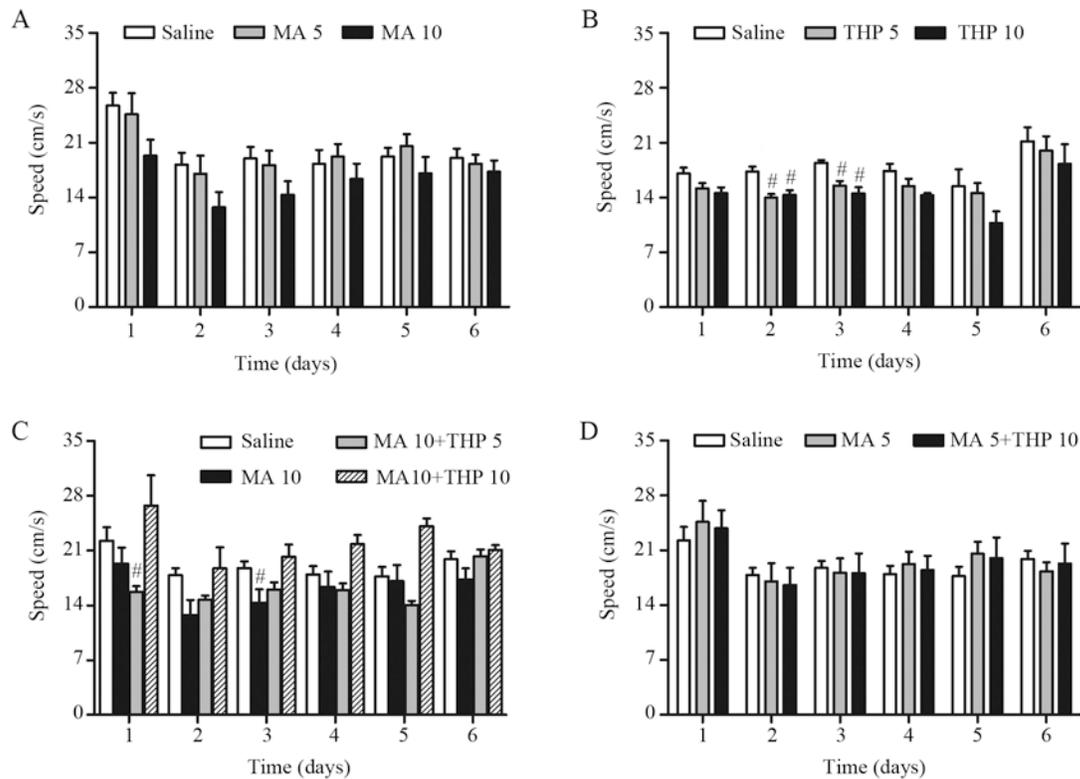
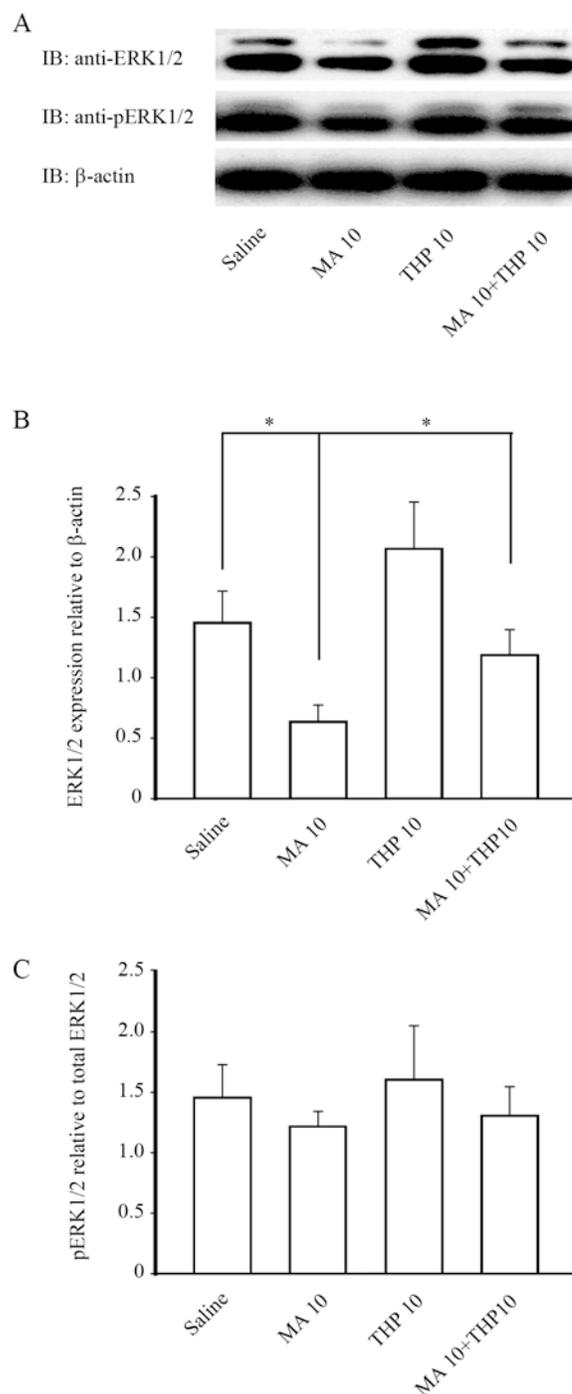


Fig. 5. Swimming speed of mice in both learning and memory test phases. C57BL/6 mice were given MA (A), THP (B), or both MA and THP (C and D). Swimming speed was measured from days 1 to 6, and one-way ANOVA was used to determine the statistical significance of differences among groups. Swimming speed was not affected by drug treatment although slight differences were found between some groups at a few time points ( $^{\#}P < 0.05$  compared with saline).

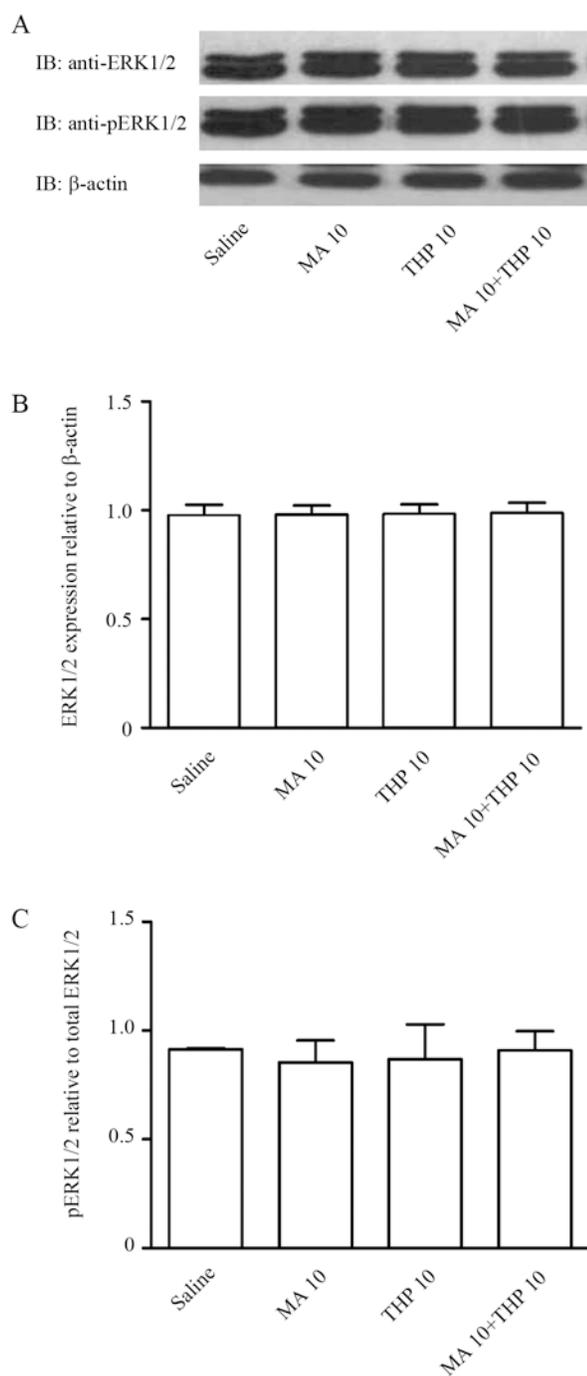
ment was observed in the MA10 group. Repeated ANOVA and *post-hoc* comparisons showed that mice in the MA10 group had fewer platform site crossings than the saline group (Fig. 3A). Consistent with the learning section, a significant difference was found between the MA10 + THP10 group and the MA10 group, indicating that 10 mg/kg THP prevented the MA10-induced spatial memory impairment (Fig. 3A). Representative swim paths in the water maze test are shown in Fig. 4. MA5-treated mice did not have impaired spatial memory compared to the MA10 group (Fig. 3A). Similar results were obtained in the measurement of time in the platform site (Fig. 3B). In addition, all mice developed a spatial bias for the target quadrant (Fig. 3C, #*P* < 0.05 compared with 25% by chance), and no significant difference was observed among these groups on the test day. Raw data in the memory test phase are shown in Table S5.

In addition, swimming speed was measured to evaluate the potential effect of MA and THP on motor activity. Although slight differences were found at a few time points, swimming speed was not significantly affected by MA and THP treatment (Fig. 5). These results indicate that motor activity was not involved in the learning and memory performance changes.

Western blotting was used to assess the expression of proteins that may be involved in the effects of MA and THP in the brain. Our results showed that expression of total ERK1/2 in the PFC did not differ between the THP10 and saline groups (*P* > 0.05). This indicated that repeated treatment with 10 mg/kg THP did not affect the total ERK1/2 expression in the PFC in our model. Repeated treatment with 10 mg/kg MA significantly reduced the total ERK1/2 expression in the PFC, and accompanying treatment with THP10 reversed this reduction (Fig. 6A, B). The ratio of phospho-ERK1/2 to total ERK1/2 remained unchanged in all groups (Fig. 6C). In contrast, no difference was found in either total ERK1/2 or the phospho-ERK1/2-to-total ERK1/2 ratio in the hippocampus (Fig. 7). These results suggest that total ERK1/2 expression in the PFC but not the hippocampus is involved in the changes of learning and memory induced by MA and THP treatment.



**Fig. 6.** Expression of ERK1/2 and phospho-ERK1/2 (pERK1/2) in the prefrontal cortex of MA- and THP-treated mice. The C57BL/6 mice were given MA, THP, or both MA and THP and decapitated 20 min after the last behavioral experiment. **A:** Western blot bands of total ERK1/2, pERK1/2 at Thr202/Tyr204, and  $\beta$ -actin. **B** and **C:** Densitometric analysis of ERK1/2 and pERK1/2 bands in the Western blots (*n* = 6), \**P* < 0.05 compared to the saline group.



**Fig. 7.** Expression of ERK1/2 and phospho-ERK1/2 (pERK1/2) in the hippocampus of MA- and THP-treated mice. C57BL/6 mice were given saline, MA, THP, or both MA and THP. **A:** Representative blots of total ERK1/2, pERK1/2 at Thr202/Tyr204, and  $\beta$ -actin. **B:** Densitometric analysis of ERK1/2 expression relative to  $\beta$ -actin. **C:** Densitometric analysis of p-ERK1/2 expression relative to total ERK1/2.

Together, our data suggest that the mechanisms of the impairment of learning and memory induced by MA and also the protective role of THP in the process might be associated the regulation of total ERK1/2 expression in the PFC.

## 4 Discussion

Drug addiction is a complex process that includes social, psychological, environmental, physiological and biological factors. Previous studies have shown that the mesolimbic dopamine (DA) system plays a positive role in drug-induced reward effects. After repeated administration, MA binds to the dopamine transporter (DAT) on dopaminergic nerve terminals through a pseudo-transmitter role, which promotes DA release but inhibits DA reuptake, thus increasing DA in the synaptic cleft. DA then excites its receptors in specific brain areas, causing complex adaptive changes in the receptors and signal transduction systems in the mesolimbic DA system, resulting in the specific behavioral effects of drug addiction<sup>[20]</sup>. Chronic, long-term or heavy use of MA can damage dopaminergic systems. One example is that decreased DA content leads to neurotoxicity<sup>[6,10,21,22]</sup>. Furthermore, MA strongly affects neuronal excitability, and short-term MA treatment can induce euphoria, increase alertness, cause people to remain in the waking state longer, and enhance attention and motor activity<sup>[23]</sup>. Recent work has shown that the D1 receptor may be specifically involved in the effect of MA on synaptic transmission, thus inducing synaptic plasticity which is associated with adverse cognitive/addictive processes<sup>[24]</sup>.

The PFC plays critical roles in short-term/working memory, decision-making, and learning new context-dependent behaviors<sup>[25-27]</sup>. The hippocampus is the key structure of the medial temporal lobe involved in certain types of learning and memory, especially the conversion of short-term to long-term memory, and the formation of long-term memories<sup>[28,29]</sup>. Swant *et al.* have suggested that the administration of MA decreases long-term potentiation (LTP) and induces synaptic maladaptation by affecting excitatory synaptic transmission via activation of DA and serotonin receptor systems in the CA1 sub-field<sup>[24,30]</sup>. It is thought that the pathway from cyclic adenosine monophos-

phate–protein kinase A (cAMP–PKA) to MAPK signaling is closely associated with LTP<sup>[7,31]</sup>. Studies have shown that repeated administration of MA affects the MAPK–ERK signaling pathway and leads to changes in learning and memory<sup>[6]</sup>. During the Morris water maze test, the animal uses information within and outside the maze to learn to find the hidden platform, and through training finally forms a stable reference memory to discriminate its spatial location<sup>[32,33]</sup>. Saab *et al.* have shown that repeated training in the Morris water maze significantly improves short-term memory without interfering with long-term memory in C57BL/6 mice<sup>[33]</sup>.

THP is a new inhibitor and a DA receptor blocker<sup>[11–13]</sup>. Long-term administration of THP has no addictive side-effects<sup>[13]</sup>. Currently, it is widely used in drug treatment research. Swant *et al.* demonstrated that both DAT blockade and a DA agonist increase LTP in CA1 via activation of the D3 subtype<sup>[30]</sup>. Studies show that THP can antagonize the central stimulating and toxic effects of amphetamine and protect neurons from injury<sup>[16]</sup>. However, the target site of THP action remains unclear.

**4.1 Mechanism of MA effects on spatial learning and memory in mice** In order to investigate the effect of repeated MA administration on spatial learning and memory, we recorded the performance of mice in the Morris water maze after administration of MA. The results showed that repeated administration of 10 mg/kg MA prolonged the escape latency in the learning phase, whereas repeated 5 mg/kg administration had no effect, indicating that the high dose of MA impaired spatial learning and memory, while the low dose did not. We speculate that repeated treatment with MA could induce drug-dependence and affect the expression of brain signaling pathways which lead to spatial learning and memory-related changes, or this treatment could be neurotoxic and directly damage neurons and memory function. In addition, Western blotting demonstrated that after 7 days of 10 mg/kg MA administration, mice had lower expression of ERK in the PFC than the saline group. This suggests that the learning and memory changes induced by repeated administration of high-dose MA may be associated with decreased ERK expression in

the PFC. It has been shown that the PFC and hippocampus play different roles in learning and memory: while the PFC is important in short-term memory, the hippocampus is mostly involved in long-term memory<sup>[34]</sup>. Here, we observed a decrease of ERK expression in the PFC but not in the hippocampus, suggesting that short-term memory in the Morris water maze was impaired by repeated MA administration.

Nagai *et al.* reported that the phosphorylation of ERK is abolished in the hippocampus of MA-treated rats<sup>[6]</sup>. In our study, we did not find this change in either the PFC or the hippocampus. However, we found that the protein level of ERK was decreased in the PFC in the MA10 group. A possible reason for this discrepancy is that multiple administration of higher doses of MA is neurotoxic in mice, as reported by several other groups<sup>[35–37]</sup>, while the neurotoxic effect of MA is less in rats<sup>[27]</sup>. Also, since the promoter of the mouse ERK gene contains DNA-binding sites for many transcription factors, such as AP-1, AP-2, p53, Ets-1.32, Myb, Sp1, NF-IL-6, and CTF-NF1<sup>[38,39]</sup>, another possible reason is that repeated MA administration may lead to changes in transcription factors which contribute to the downregulation of ERK expression. Further studies are needed to determine the molecular regulation of ERK expression by MA.

**4.2 Potential mechanism of the effect of THP on impairments induced by MA** To investigate the effect of THP on the impairments induced by MA, we evaluated the performance of mice in the Morris water maze task after THP treatment. The results showed that repeated treatment with THP alone did not affect performance, indicating that it has no effect on spatial learning and memory in mice. These results are consistent with previous reports that THP is not an addictive drug<sup>[12,13]</sup>. Moreover, the escape latency in the MA10 + THP10 group was lower than that in the MA10 group. These results indicate that the THP treatment blocked the effect of MA on learning in mice. Further molecular biological experiments showed that the expression of total ERK1/2 in the PFC of the THP10 group did not differ from that in the saline group. Repeated treatment with MA10 markedly reduced the total

ERK1/2 expression in the PFC, while combined treatment with THP10 reversed this change. These data are consistent with the results of Mizoguchi *et al.*<sup>[40]</sup>. Taken together, our results suggest that total ERK1/2 expression in the PFC, but not the hippocampus, is involved in the learning and memory changes in our experimental model.

In summary, high-dose MA impaired spatial learning and memory in mice, and co-administration with THP reversed these impairments. Changes in learning and memory in MA mice were associated with decreased ERK expression in the PFC, while administration of THP prevented this decrease. THP is thus demonstrated to be a new type of brain DA receptor antagonist that can block the spatial learning and memory impairment effects of MA in the PFC. The protective effect of THP on MA-induced brain impairment may be through its antagonistic effect on these DA receptors. However, further studies are required to clarify the neurobiological mechanisms.

**Supplemental Data:** Supplemental Data include five tables and can be found online at <http://www.neurosci.cn/epData.asp?id=19>.

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