A large dose of methamphetamine inhibits drug-evoked synaptic plasticity via ER stress in the hippocampus

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Abstract. Drug addiction is a chronic and recurrent disease associated with learning and memory. Shaped by drug use and cues from the environment, drug memory serves a key role in drug-seeking behaviour. Methamphetamine (MA), a globally abused drug, causes cognitive impairment, and endoplasmic reticulum (ER) stress is one of the mechanisms via which this occurs. In the current study, it was hypothesized that ER stress may serve a role in the disturbance of drug memory. The present study demonstrated that 5 mg/kg MA inhibited conditioned place preference behaviour via ER stress, which caused a disruption in long-term potentiation in the hippocampus. When mice were pre-treated with the ER stress inhibitors 4-phenyl butyric acid or tauroursodeoxycholic acid, drug-evoked synaptic plasticity was induced. Western blotting results indicated that treatment with 5 mg/kg MA enhanced the expression of cyclin-dependent kinase-5 and decreased the expression of Ca^{2+}/calmodulin-dependent protein kinase II α via ER stress. Collectively, the present results suggested that a large dose of MA inhibited drug-evoked synaptic plasticity and disrupted drug memory by inducing ER stress.

Introduction

Drug addiction, which is defined as the chronic and compulsive use of drugs despite adverse consequences (1), is characterized by uncontrolled drug-seeking behaviour (2), accompanied by the functional alteration of specific neural circuits caused by changes in neurotransmitters (3) and synaptic plasticity (4). As drug addiction is a cyclical, chronic and recurrent disease (5), the central problem in the treatment of drug addiction is identifying the underlying mechanisms of relapse. According to previous studies, a special type of memory, known as drug memory (6,7), is formed by the association of drug-induced euphoria with contextual cues, termed pathological learning (8). Drug memory shapes behaviours associated with drug addiction by stimulating the desire or craving for drugs (9,10). Moreover, re-exposure to drug-conditioned stimuli precipitates the recurrence of previously extinguished drug-seeking behaviour (11) by recalling drug memory, while the inhibition of drug memory prevents drug relapse (12). Drug addiction is therefore regarded as a disease of learning and memory (13), and an overlap in the involved neural circuitry and underlying molecular mechanisms between normal memory and drug memory has been demonstrated (14).

A typical example of this overlap is long-term potentiation (LTP) (15), described as a lasting enhancement of synaptic transmission efficiency and intensity induced by a transient high-frequency stimulus (HFS). LTP was first considered to be a laboratory phenomenon; however, it has been suggested that an LTP-like phenomenon is induced during memory formation in an inhibitory avoidance model in rats (16), and LTP maintenance is also involved in spatial information storage (17). Furthermore, drug-evoked plasticity, which is a HFS-independent increase in synaptic strength and connectivity, is observed in addiction (18) and is considered to be associated with addictive behaviour (19). Therefore, it was hypothesized that methods disrupting LTP induction may be applied to inhibit drug memory and drug-seeking behaviour.

Methamphetamine (MA), a globally abused psychostimulant (20), induces memory impairment (21). It has been identified that endoplasmic reticulum (ER) stress, which results from the accumulation of unfolded proteins (22), may be one of the underlying mechanisms for MA-induced memory loss (data not shown). As ER stress serves a role in the inhibition of normal memory, it was hypothesized that ER stress may also serve a role in drug memory inhibition. To validate this hypothesis, the present study first tested whether MA-induced ER stress disrupted the formation of drug memory using the conditioned place preference (CPP) test. Next, the effects of MA-induced ER stress on hippocampal LTP induction were investigated and the expression levels of several molecules underlying both normal and addiction memory formation

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were measured, which were used to elucidate the mechanisms underlying drug memory disruption due to MA-induced ER stress.

Materials and methods

Animals. A total of 166 male C57BL/6 mice (age, 6-8 weeks; weight, 25-30 g) were obtained from SPF (Beijing) Biotechnology Co., Ltd. Animals were housed with free access to water and food in a standard experiment room at 22-24˚C and 50±5% humidity, with a 12-h light/dark cycle. All animal experimental procedures were conducted following the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH) (23), and were approved by the Animal Care and Use Committee of the Beijing Institute of Pharmacology & Toxicology. Different mice were used for each experiment in the present study.

Drugs. MA was obtained from Beijing Institute of Pharmacology & Toxicology. The ER stress inhibitors 4-phenyl butyric acid (PBA) and tauroursodeoxycholic acid (TUDCA) were purchased from Sigma-Aldrich; Merck KGaA (cat. no. P21005) and Shanghai Aladdin Bio-Chem Technology Co., Ltd. (cat. no. S101371), respectively. All drugs were dissolved in saline and prepared at a concentration of 20 mg/ml. The animals were divided into six groups: i) Normal saline (S group; n=31 mice); ii) TUDCA (n=15 mice); iii) PBA (n=15 mice); iv) MA (n=69 mice); v) TUDCA+MA (n=18 mice); and vi) PBA+MA (n=18 mice). In the MA group, mice were administered intraperitoneal (i.p.) injections of 1, 2 or 5 mg/kg MA. In the TUDCA+MA or PBA+MA group, mice received i.p. injections of 200 mg/kg TUDCA or 100 mg/kg PBA 60 min before receiving 5 mg/kg MA injections. In the S, TUDCA and PBA groups, mice were administered i.p. injections of saline, 200 mg/kg TUDCA and 100 mg/kg PBA, respectively.

CPP test. The protocol of the CPP test was based on a previous report (24), with some necessary modifications (Fig. 1A). The experiment box, which was manufactured by Anilab Software & Instruments Co., Ltd., consisted of two distinct chambers (17.4x13.5x15 cm^3) separated by a corridor (9.8x13.5x15 cm^3). Different colours (one was white, and the other was black) and different floors (one was made of circular holes, and the other was made of strips) in the two chambers were used so that mice could distinguish one chamber from the other. The chamber in which the mice stayed for a shorter duration in Test 1 was chosen as the drug-paired chamber. The whole experiment included four stages: Habituation, Test 1, Conditioning and Test 2. During the 3-day habituation, mice moved freely in the chambers for 20 min both in the morning and in the afternoon. The 8-day conditioning was conducted with daily injections of drugs and saline, administered alternately in the morning or the afternoon. The mice were placed in the drug-paired chamber immediately following administration of different does of MA (n=10/group) or TUDCA/PBA+5 mg/kg MA (n=10/group) for 30 min. When the mice were administered saline, they were placed in the other chamber. During Test 1 and Test 2, mice were allowed to explore the chambers freely for 15 min.

Electrophysiology. LTP of the perforant path (PP)-dentate gyrus (DG) pathway in the hippocampus was recorded *in vivo* as previously described (25), with some modifications. Mice were first anaesthetized with urethane (1.5 g/kg; i.p.) and then a pair of recording electrodes were implanted into the DG of the left hemisphere at A/P: -2.0 mm, M/L: -1.4 mm, D/V: -1.5 mm (from the dura), while a pair of stimulating electrodes were implanted into the PP of the left hemisphere at A/P: -3.8 mm, M/L: -3.0 mm, D/V: -1.5 mm (from the dura). A population spike (PS) was induced using monopolar pulses (duration, 400 μsec; frequency, 1/30 Hz) using an Isolated Pulse stimulator (A-M SYSTEMS Ltd.) and reported using a Differential AC amplifier (A-M SYSTEMS Ltd.) and Axon Digidata 1550A Data Acquisition system (Molecular Devices LLC). When the stabilized PS lasted for 60 min, the stimulating current was regulated to yield a PS that was 30-50% of the maximum amplitude, and the PS was recorded for 30 min as the baseline. Subsequently, mice were administered with MA (1 or 5 mg/kg; i.p., n=5/group) or MA combined with pre-treatment of 200 mg/kg TUDCA or 100 mg/kg PBA (n=5/group). HFS, consisting of three trains of 10 bursts (duration, 400 μsec; frequency, 300 Hz) with an interval of 10 sec between each train, was given 30 min after drug injection. The PS was recorded for 60 min using formerly single monopolar pulses post-HFS. Data were obtained using pClamp10.0 software (Molecular Devices LLC).

Western blotting. Mice were euthanised using 5% isoflurane with an oxygen flow rate of 1 l/min according to a previous report (26). After the complete cessation of the heartbeat, the hippocampal tissue (n=3/group) was dissected on ice to extract the whole protein fraction. Western blotting was performed as previously reported (27) to examine the expression levels of ER stress markers, including binding immunoglobulin protein (BIP), phosphorylated (p)-eukaryotic translation initiation factor 2α (EIF2α), cyclic AMP-dependent transcription factor-4 (ATF-4), ATF-6 and CHOP, as well as the expression levels of Ca^{2+}/calmodulin-dependent protein kinase II α (CaMKIIα) and cyclin-dependent kinase-5 (Cdk5), which are two proteins associated with the formation of drug-evoked plasticity and drug memory (28,29). The detailed information for antibodies used in western blotting are presented in Table I. Values of these proteins (except for p-EIF2α) were normalized to that of actin. The value of p-EIF2α was normalized to that of total EIF2α. Bands were semi-quantified using ImageJ 1.8.0.112 software (NIH).

Statistical analysis. In each part of the present study, three independent experiments were performed. Data are presented as the mean ± SEM, and statistical analysis was performed using GraphPad Prism 8.0 software (GraphPad Software, Inc.). For the CPP test, a two-way mixed ANOVA followed by Bonferroni's multiple comparison test was used to analyse the difference in the percentage of time spent in the drug-paired chamber between Test 1 and Test 2. For the electrophysiological tests, a paired t-test was used to analyse the difference in PS amplitude between the baseline and post-HFS measurements. For western blotting results, one-way ANOVA followed by Bonferroni's multiple comparison test was used for analysing the difference in protein expression levels between different groups. $P<0.05$ was considered to indicate a statistically significant difference.

Table I. Information of antibodies used for western blotting.

Antibody	Host	Supplier	Working dilution
Primary antibody			
Actin	M	Applygen Technologies, Inc. (cat. no. C1313)	1:5,000
BIP	R	Abcam (cat. no. ab21685)	1:1,000
ATF-4	R	Abclonal Biotech Co., Ltd. (cat. no. A18687)	1:500
ATF-6	R	Abclonal Biotech Co., Ltd. (cat. no. A0202)	1:500
p-EIF2α	R	CST (cat. no. 3398S)	1:1,000
EIF2α	R	CST (cat. no. 5324S)	1:500
CHOP	R	Abclonal Biotech Co., Ltd. (cat. no. A0221)	1:200
Cdk5	M	Santa Cruz Biotechnology, Inc. (cat. no. sc6247)	1:200
CaMKIIα	M	Santa Cruz Biotechnology, Inc. (cat. no. sc13141)	1:500
Secondary antibody			
Anti-mouse HRP	G	Beyotime Institute of Biotechnology (cat. no. A0216)	1:5,000
Anti-rabbit HRP	G	Beyotime Institute of Biotechnology (cat. no. A0208)	1:5,000

M, mouse; R, rabbit; G, goat; CST, Cell Signalling Technology, Inc.; ATF, cyclic AMP-dependent transcription factor; BIP, binding immunoglobulin protein; p-, phosphorylated; EIF2α, eukaryotic translation initiation factor 2α; CaMKIIα, Ca^{2+}/calmodulin-dependent protein kinase II α.

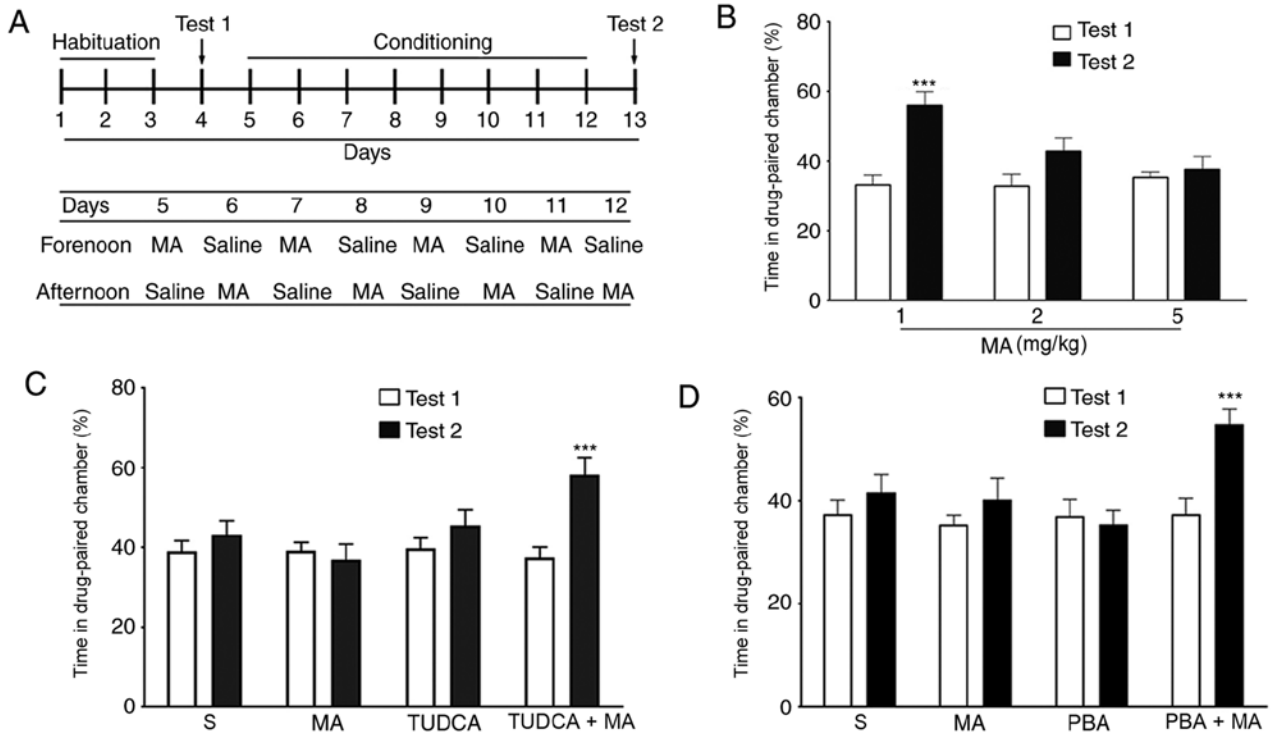

Figure 1. Systemic administration of 5 mg/kg MA induces mouse CPP behaviour following pre-treatment with the ER stress inhibitors TUDCA or PBA. (A) Experimental diagram of the mouse CPP test and the mode of MA or saline administration in the conditioning stage (days 5-12). (B) A dose of 1 mg/kg, but not 2 or 5 mg/kg, MA induced CPP behaviour (n=10/group). A dose of 5 mg/kg MA induced mouse CPP behaviour when animals were pre-treated with (C) 200 mg/kg TUDCA or (D) 100 mg/kg PBA (n=10/group). Data are presented as the mean ± SEM. ***P<0.001 vs. Test 1. PBA, 4-phenyl butyric acid; TUDCA, tauroursodeoxycholic acid; CPP, conditioned place preference; ER, endoplasmic reticulum; MA, methamphetamine; S, normal saline group.

Results

A dose of 5 mg/kg MA inhibits mouse CPP behaviour by inducing ER stress. In the CPP test, 1 mg/kg MA induced a significant increase in the percentage of time spent in the drug-paired chamber (P<0.001), while CPP behaviour was not induced by 2 (P>0.05) or 5 mg/kg MA (P>0.05; Fig. 1B). To examine the role of ER stress in the inhibition of drug memory, 5 mg/kg was selected for further studies. When mice were pre-treated with the ER stress inhibitors TUDCA (200 mg/kg, i.p. Fig. 1C) or PBA (100 mg/kg, i.p; Fig. 1D) 60 min before the injection of 5 mg/kg MA, the percentage of time spent in

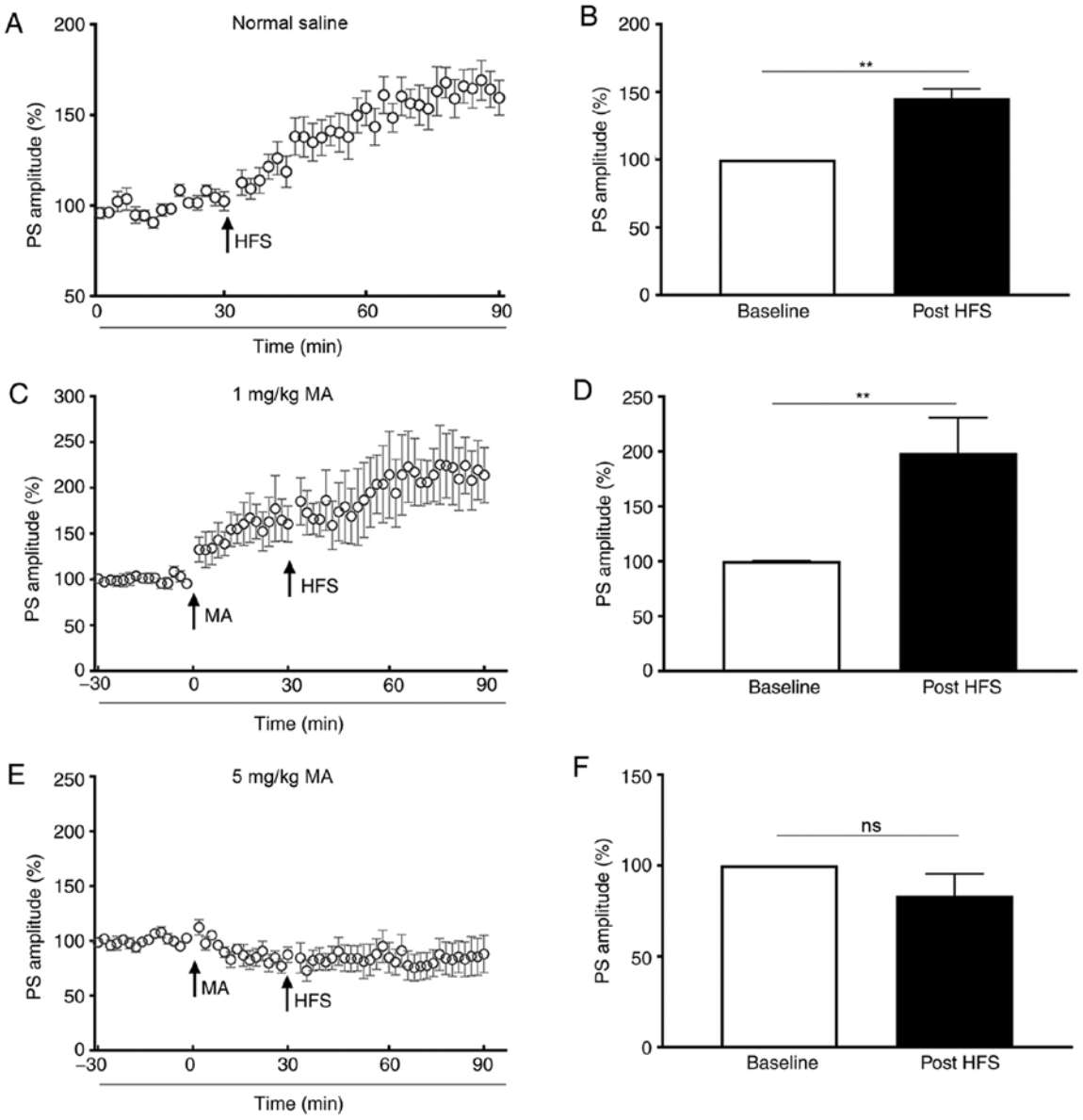

Figure 2. Acute injection of 5 mg/kg MA inhibits hippocampal long-term potentiation in mice *in vivo*. (A) Scatter diagram of the PS amplitude for mice treated with saline (n=5/group). (B) In the S group, the PS amplitude was significantly enhanced post-HFS. (C) Scatter diagram of the PS amplitude for mice treated with 1 mg/kg MA (n=5/group). (D) In the 1 mg/kg MA group, an increase in PS amplitude was observed post-HFS. (E) Scatter diagram of the PS amplitude for mice treated with 5 mg/kg MA (n=5/group). (F) In the 5 mg/kg MA group, the PS amplitude was not enhanced post-HFS. Data are presented as the mean ± SEM. **P<0.01; ns indicates P>0.05. PS, population spike; MA, methamphetamine; HFS, high-frequency stimulus; S, normal saline group.

the drug-paired chamber in Test 2 was significantly increased compared with that in Test 1 (P<0.001). However, when mice were intraperitoneally injected with PBA or TUDCA alone, there was no increase in the percentage of time spent in the drug-paired chamber (P>0.05).

A dose of 5 mg/kg MA disturbs PP-DG LTP in vivo via ER stress. The present study demonstrated that the PS amplitude of saline-treated mice was enhanced to 139.70±6.30% of the baseline post-HFS (P<0.01; Fig. 2A and B). When mice were administered an i.p. injection of 1 mg/kg MA, the PS amplitude increased to 144.20±17.57% of the baseline (P<0.05; bar graph was not shown), and it increased to 206.60±27.53% of the baseline (P<0.01) post-HFS (Fig. 2C and D). However, the PS amplitude of mice treated with 5 mg/kg MA was not increased post-HFS (P>0.05; Fig. 2E and F) in comparison with the baseline.

To evaluate the role of ER stress in LTP inhibition evoked by 5 mg/kg MA, TUDCA or PBA were injected 60 min before MA administration. In the TUDCA+MA group, the PS amplitude post-HFS was increased to 155.1±17.09% of the baseline (P<0.05; Fig. 3A and B). For mice in the PBA+MA group, the PS amplitude was increased to 131.90±5.05% of the baseline (P<0.01; bar graph was not shown) when MA was injected and it was elevated to 179.85±12.32% of the baseline post-HFS (P<0.01; Fig. 3C and D), which was significantly higher compared with that of the S group (P<0.01; bar graph was not shown).

A dose of 5 mg/kg MA increases the expression levels of ER stress markers in the hippocampus. Mice were treated with different dosages of MA (1 or 5 mg/kg) for 30 min and then whole hippocampal proteins were extracted for

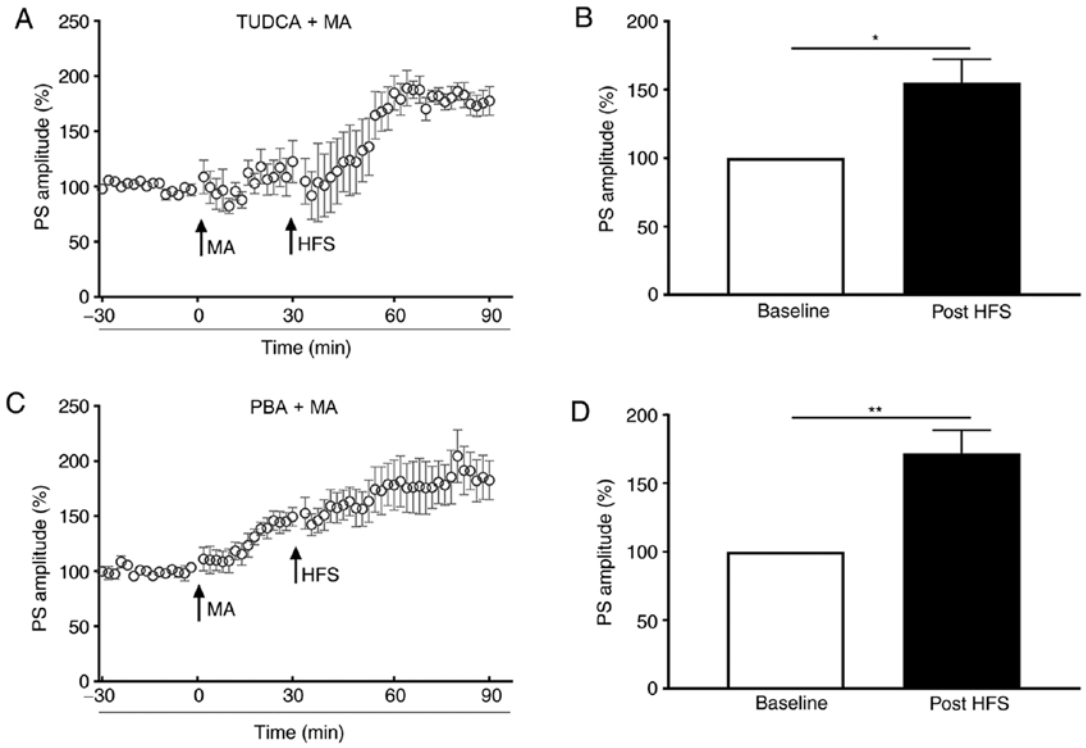

Figure 3. Systemic administration of TUDCA or PBA attenuates the inhibitory effect of 5 mg/kg MA on hippocampal long-term potentiation of mice *in vivo*. (A) Scatter diagram of the PS amplitude for mice in the TUDCA+MA group (n=5/group). (B) When mice were pre-treated with 200 mg/kg TUDCA, 5 mg/kg MA induced a higher PS amplitude post-HFS. (C) Scatter diagram of the PS amplitude for mice in PBA+MA group (n=5/group). (D) When mice were pre-treated with 100 mg/kg PBA, 5 mg/kg MA induced an enhanced PS amplitude post-HFS. Data are presented as the mean ± SEM. *P<0.05, **P<0.01. PS, population spike; MA, methamphetamine; HFS, high-frequency stimulus; PBA, 4-phenyl butyric acid; TUDCA, tauroursodeoxycholic acid.

western blotting. It was found that the expression levels of the ER stress marker proteins BIP (P<0.001), ATF-4 (P<0.01), ATF-6 (P<0.001), p-EIF2α (P<0.05) and CHOP (P<0.05) were significantly increased by 5 mg/kg MA. The ingestion of 1mg/kg had no effect on the expression levels of BIP (P>0.05), ATF-4 (P>0.05), ATF-6 (P>0.05) and CHOP (P>0.05); however, it reduced the expression levels of p-EIF2α (P<0.05; Fig. 4). When mice were pre-treated with TUDCA, the expression levels of BIP (P<0.01), ATF-6 (P<0.01), CHOP (P<0.05) and p-EIF2α (P<0.01) were decreased to normal levels. Similarly, pre-treatment with PBA also decreased the expression levels of BIP (P<0.05), ATF-6 (P<0.001), CHOP (P<0.05) and p-EIF2α (P<0.01), which were increased by 5 mg/kg MA (Fig. 5). Moreover, the expression of Cdk5 (P<0.001) was enhanced, while CaMKIIα expression (P<0.05) was decreased by 5 mg/kg MA administration. In addition, 5 mg/kg MA-induced changes in the expression levels of Cdk5 were reversed by TUDCA (P<0.001) or PBA (P<0.01) pre-treatment. The decreased expression level of CaMKIIα could also be reversed by TUDCA (P<0.01) and PBA (P<0.01) pre-treatment (Fig. 6).

Discussion

Drug addiction manifests as compulsive drug-seeking behaviour (30). Addiction is induced by repeated exposure to drugs, and increasing evidence has revealed that drug-seeking is not an uncontrolled behaviour due to a lack of willpower or a character flaw, but drug addiction is rather a chronic disease resulting from complicated neuroadaptations in different encephalic regions (18). Aberrant learning and memory, known as drug memory, are involved during the formation of addiction, and addiction is regarded as a disease of learning and memory (31). Consequently, treatment for addiction by disrupting drug memory has been attempted (32,33).

MA, a man-made psychostimulant that is abused worldwide, is well known for its addictive properties and the damage it causes to multiple organs (20). MA addiction has been a concern for researchers worldwide. Thus, the present study investigated the mechanisms underlying MA addiction by examining it alongside the formation of drug memory. As the ER is an important organelle for protein assembly and folding, and as protein synthesis is of great significance for long-term memory development (34), it was hypothesized that ER stress may participate in the disruption of addiction memory.

In the CPP test, the present study demonstrated that 1 mg/kg, but not 2 or 5 mg/kg MA evoked CPP behaviour, which was consistent with previously published data (35). However, when mice were pre-treated with the ER stress inhibitors TUDCA or PBA, CPP behaviour could be induced by 5 mg/kg MA, indicating that a high dose of MA inhibited drug memory via ER stress.

Next, the mechanisms underlying the disturbance of drug memory formation by MA-induced ER stress were evaluated by studying the effects of MA administration on synaptic plasticity. The hippocampus, a limbic structure, is important for learning to associate specific contexts using reinforcer availability and spatial memory storage (36). Glutamatergic

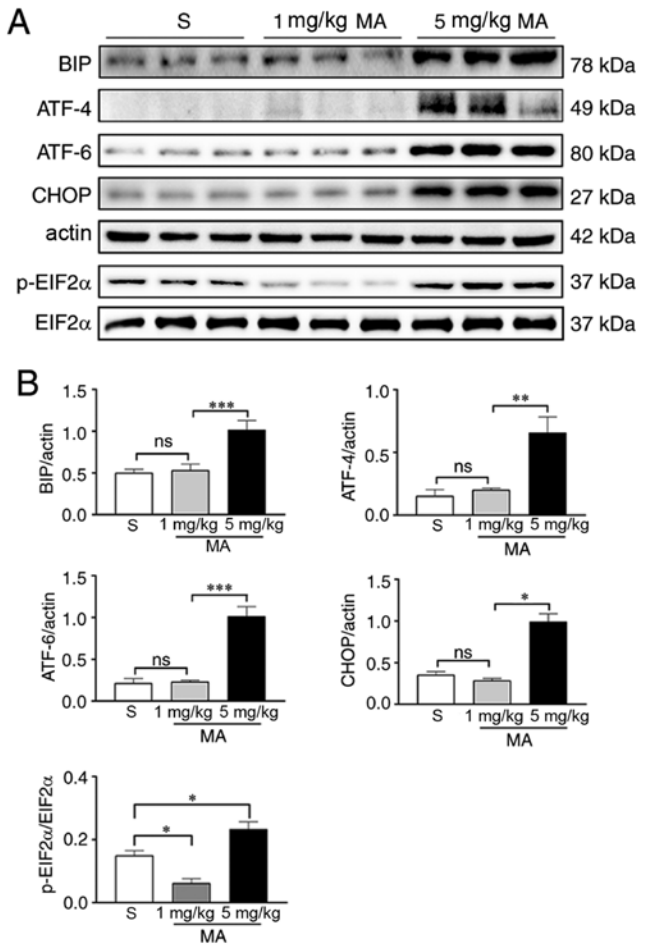

Figure 4. Acute exposure to 5 mg/kg MA induces ER stress in the hippocampus. (A) Western blotting results of the ER stress markers in the hippocampus of mice treated with saline, or 1 or 5 mg/kg MA (n=3/group). (B) Statistical analysis demonstrated that 5 mg/kg MA, but not 1 mg/kg MA, increased the protein expression levels of ER stress markers. Data are presented as the mean ± SEM. *P<0.05, **P<0.01, ***P<0.001; ns indicates P>0.05. MA, methamphetamine; ER, endoplasmic reticulum; p-, phosphorylated; EIF2α, eukaryotic translation initiation factor 2α; ATF, cyclic AMP-dependent transcription factor; BIP, binding immunoglobulin protein; ns, non-significant; S, normal saline group.

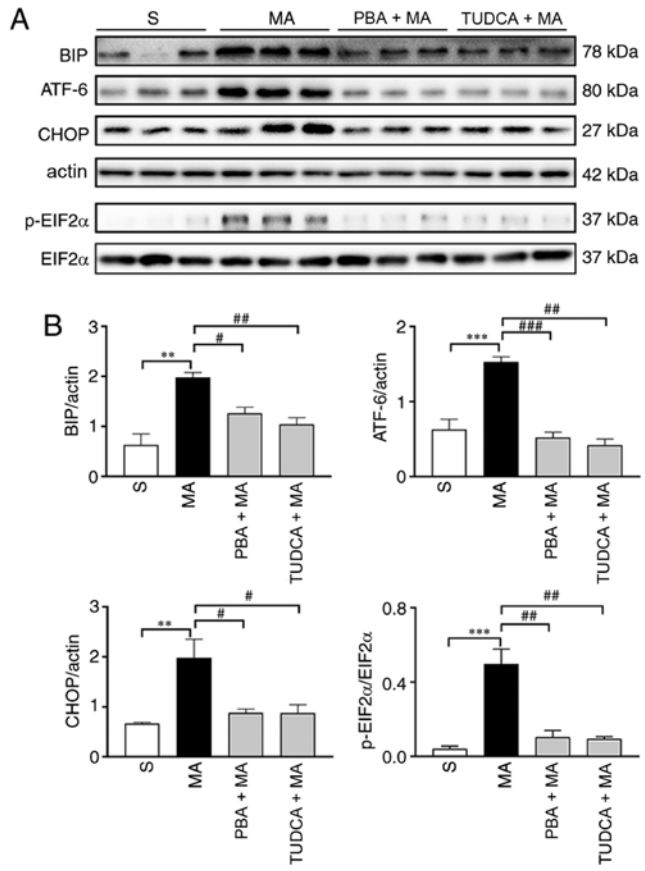

Figure 5. MA-induced ER stress are inhibited by the pre-treatment with PBA or TUDCA. (A) Western blotting results of ER stress markers in the hippocampus of mice treated with saline, 5 mg/kg MA, PBA + 5 mg/kg MA or TUDCA + 5 mg/kg MA, n=3 per group. (B) Statistical results indicated that higher protein expression levels of ER stress markers induced by 5 mg/kg MA were reversed by PBA or TUDCA pre-treatment. Data are presented as the mean ± SEM. **P<0.01, ***P<0.001; $^{\#}$P<0.05, $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001. MA, methamphetamine; ER, endoplasmic reticulum; PBA, 4-phenyl butyric acid; TUDCA, tauroursodeoxycholic acid; p-, phosphorylated; EIF2α, eukaryotic translation initiation factor 2α; ATF, cyclic AMP-dependent transcription factor; BIP, binding immunoglobulin protein; S, normal saline group.

neurotransmission can be projected from the hippocampus to multiple regions within the reward circuitry (37) The hippocampus and the ventral tegmental area (VTA) form a functional loop to control the entry of information into long-term memory (37). This loop is activated when novel information is detected in the hippocampus. Moreover, the hippocampus-VTA loop is involved in MA-mediated place reinforcement learning (38). Consequently, the present study selected the PP-DG pathway in the hippocampus to investigate the influence of MA-evoked ER stress on synaptic plasticity, which is closely associated with the acquisition process of MA addiction (39). As mice underwent a 30-min conditioning period immediately after MA injection in the CPP experiment, the present study examined the acute effect of MA administration on LTP induction and HFS was conducted 30 min post-MA injection. The current results suggested that acute administration of 1 mg/kg MA induced LTP facilitation, while 5 mg/kg MA caused a disturbance in LTP induction. However, this disruption was attenuated when mice were pre-treated with TUDCA or PBA. Additionally, HFS-independent enhancement of the PS amplitude, which was conceptualized as drug-evoked synaptic plasticity (18), was observed when mice were injected with 1 mg/kg MA. Drug-evoked plasticity is also reported to be induced by other addictive drugs, such as ethanol and cocaine (40). It is also considered to be involved in the early stages of the development of drug addiction (41). In the present study, 5 mg/kg MA generated drug-evoked plasticity when mice were pre-treated with PBA, suggesting that ER stress may be involved in the disturbance of drug-evoked synaptic plasticity. However, drug-evoked plasticity was not observed when mice were pre-treated with TUDCA. Nevertheless, the reasons underlying this difference are not obvious based on the present results only.

The present study also evaluated the effects of MA-induced ER stress on proteins involved in memory formation. Western blotting data demonstrated that 5 mg/kg, but not 1 mg/kg, MA induced ER stress in the hippocampus, as indicated by enhanced expression levels of the ER stress marker proteins BIP, p-EIF2α, ATF-4, ATF-6 and CHOP. These increases

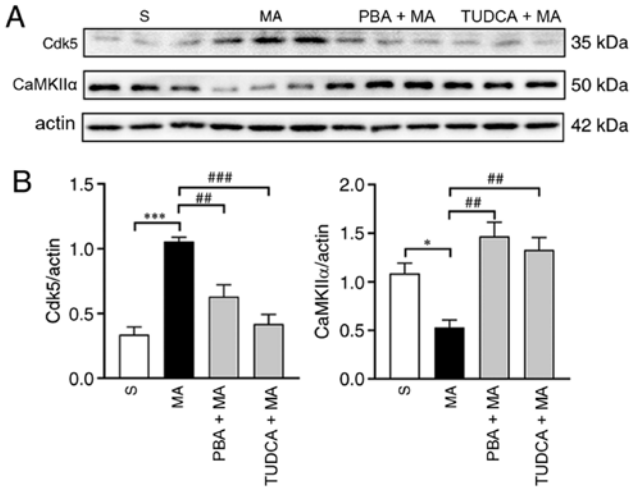

Figure 6. Acute exposure to 5 mg/kg MA increases the protein expression level of Cdk5 and decreases the protein expression level of CaMKIIα, which can be reversed by TUDCA or PBA. (A) Western blotting results of Cdk5 and CaMKIIα in the hippocampus of mice treated with saline, 5 mg/kg MA, PBA + 5 mg/kg MA or TUDCA + 5 mg/kg MA (n=3/group). (B) Statistical analysis demonstrated that 5 mg/kg MA enhanced the protein expression levels of Cdk5 and decreased the protein expression levels of CaMKIIα; these effects were reversed by TUDCA or PBA pre-treatment. Data are presented as the mean ± SEM. *P<0.05, ***P<0.001; $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001. MA, methamphetamine; PBA, 4-phenyl butyric acid; TUDCA, tauroursodeoxycholic acid; Cdk5, cyclin-dependent kinase-5; CaMKIIα, Ca^{2+}/calmodulin-dependent protein kinase II α; S, normal saline group.

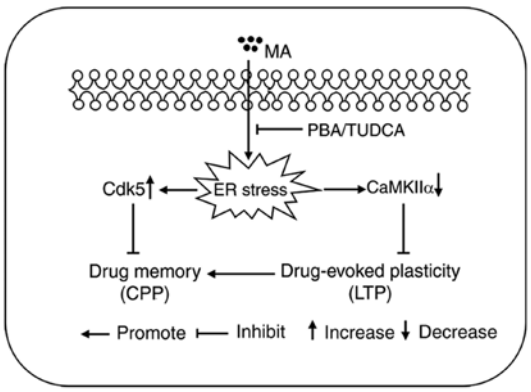

Figure 7. Schematic diagram representing the main findings of the present study. Acute exposure to a large dose of MA induced a higher expression level of Cdk5 protein, and decreased the protein expression level of CaMKIIα, both of which are closely associated with drug-evoked plasticity and drug memory. When mice were pre-treated with the ER stress inhibitors PBA or TUDCA, increased protein expression of Cdk5 and decreased protein expression of CaMKIIα were reversed. When ER stress in the hippocampus was inhibited, 5 mg/kg MA could also induce LTP and CPP behaviour. MA, methamphetamine; Cdk5, cyclin-dependent kinase-5; CaMKIIα, Ca^{2+}/calmodulin-dependent protein kinase II α; ER, endoplasmic reticulum; PBA, 4-phenyl butyric acid; TUDCA, tauroursodeoxycholic acid; LTP, long term potentiation; CPP, conditioned place preference.

could be reversed by TUDCA or PBA. Moreover, 5 mg/kg MA induced an increase in Cdk5 expression, as well as a decrease in CaMKIIα expression. As previously reported, the inhibition of Cdk5 serves a key role in facilitating cocaine-induced CPP behaviour (42). When mice were pre-treated with TUDCA or PBA, 5 mg/kg MA-induced CPP behaviour and Cdk5 expression levels were reversed to normal levels. CaMKIIα, an essential protein in LTP induction, is also involved in the development and maintenance of drug memory (43). Therefore, the decreased expression of CaMKIIα, which was caused by 5 mg/kg MA in the present study, was considered to serve a role in LTP disturbance and the inhibition of drug memory. According to the results of the behavioural, electrophysiological and molecular studies, it was suggested that a large dosage of MA resulted in a disturbance of drug memory formation by inhibiting hippocampal LTP and altering the expression levels of proteins associated with memory formation via ER stress.

To the best of our knowledge, few studies have been reported that directly examine the relationship between addiction and MA-induced ER stress. However, it has been revealed that the expression of p-EIF2α, a marker protein of ER stress, is decreased by multiple addictive drugs, such as cocaine and nicotine (44,45). Moreover, enhanced p-EIF2α-mediated translation control could prevent the maintenance of cocaine-induced LTP in VTA dopamine neurons (46), which underlies compulsive drug-seeking (18). In accordance with this observation, the present study found that p-EIF2α expression was enhanced by treatment with 5 mg/kg MA, which disrupted hippocampal LTP induction and drug-seeking behaviour. It was also observed that CPP and LTP could be induced when mice were pre-treated with the ER stress inhibitors TUDCA or PBA, decreasing the enhanced expression of p-EIF2α, as well as the signalling cascades evoked by this molecule. Furthermore, the present results indicated that the protein expression level of p-EIF2α was declined by acute exposure to 1 mg/kg MA, which could induce both LTP and CPP behaviour, suggesting that inhibiting the expression of p-EIF2α might contribute to the formation of MA-induced drug memory.

Generally, researchers inhibit CPP expression to investigate the role of certain molecules or neural circuits in the addiction process (33,47). In the current study, however, CPP behaviour was first found to be disturbed by 5 mg/kg MA, and it could be induced when mice were pre-treated with ER stress inhibitors, suggesting that MA-evoked ER stress serves a role in drug memory inhibition caused by a high dosage of MA. Therefore, it can be considered that the disruption of drug memory via ER stress in the hippocampus without any adverse effects may serve an active role in blocking the formation of drug memory. However, the detailed mechanisms underlying the inhibition of drug-evoked plasticity and drug-seeking behaviour caused by high-dose MA-induced ER stress are still unclear based on the present findings and require further investigations.

In conclusion, the present study demonstrated that a large dose of MA could disrupt drug memory and hippocampal drug-evoked plasticity by inducing ER stress (Fig. 7).

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

GC conducted all of the experiments with the help of GY and HY. ZY analyzed the results. HW and RS designed the research. ZY and RS confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All animal experimental procedures were conducted following the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH), and were approved by the Animal Care and Use Committee of the Beijing Institute of Pharmacology & Toxicology.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that there are no competing interests.

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