The distinct roles of various neurotransmitters in modulating methamphetamine-induced conditioned place preference in relevant brain regions in mice

Hongliang Su[a,b,*], Junmei Bai[a,*], Yao Fan[a], Tingting Sun[a], Yan Du[c], Yanhua Li[d], Zhiwen Wei[a,b], Teng Chen[e], Xiangjie Guo[a] and Keming Yun[a,b]

Objectives **Previous studies have shown that methamphetamine (METH) can induce complex adaptive changes in the reward system in the brain, including the changes in the content of neurotransmitters in the signal transduction pathway. However, how the changes of various neurotransmitters in relevant brain reward circuits contribute to METH-induced conditioned place preference (CPP) remains unclear.**

Methods **In this study, first, we designed an animal model of METH-induced CPP. Then we used liquid chromatography-mass spectrometry (LC-MS) to simultaneously determine the contents of various neurotransmitters – dopamine (DA), norepinephrine (NE), 5-hydroxytryptamine (5-HT), 5-hydroxyindole acetic acid (5-HIAA), glutamic acid (Glu) and glutamine (Gln) – in different brain regions of the prefrontal cortex (PFc), nucleus accumbens (NAc), caudate-putamen (CPu) and hippocampus (Hip), which are believed to be relevant to the drug's reward effect.**

Results **The results of the behavioral experiment suggested that 1.0 mg/kg METH could induce obvious CPP in mice. The results about various neurotransmitters showed that: DA significantly increased in NAc in the METH group; Glu increased significantly in the METH group in PFc and NAc and Gln increased significantly in the METH group in PFc.**

Conclusions **These results suggested that the neurotransmitters of DA, Glu and Gln may work together and play important roles in METH-induced CPP in relevant brain reward circuits, especially in PFc and NAc. These findings therefore could help to advance the comprehensive understanding of the neurochemic and psychopharmacologic properties of METH in reward effect, which is important for future improvements in the treatment of drug addiction.** ***NeuroReport*** **33: 101–108**

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[a]Department of School of Forensic Medicine, Shanxi Medical University, Taiyuan, [b]Key Laboratory of Forensic Toxicology, Ministry of Public Security, Beijing [c]Department of Pharmaceutical Science, Shanxi Medical University, [d]Department of Foreign Languages, Taiyuan and [e]Department of Forensic Medicine, Xi'an Jiaotong University Health Science Center, Xi'an, People's Republic of China

Correspondence to Hongliang Su, Department of Forensic Medicine, Shanxi Medical University, Taiyuan 030001, People's Republic of China
Tel: +86 351 3985097; e-mail: hongliangsu@sxmu.edu.cn

*Dr. Hongliang Su and Dr. Junmei Bai contributed equally to the writing of this article.

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Introduction

Drug addiction has been defined as a chronic, relapsing brain disease [1]. Methamphetamine (METH) is a kind of central nervous system stimulant which is highly addictive [2,3]. The World Drug Report 2020 showed that more than a quarter of a billion people worldwide use drugs, and the global METH market remains concentrated in North America as well as East and South-East Asia, though it is expanding worldwide [4]. In China, METH replaced heroin as the 'number one drug' in 2019 and has become the most abused drug. According to China Drug Situation Report 2019 published in 2020, among 2 148 000 existing drug abusers, 1 186 000 were METH abusers, accounting for 55.2% [5]. The potential harm caused by METH is continuously increasing, causing worldwide concern.

Despite the increasing number of studies on METH addiction, the underlying neurobiologic mechanisms remain unclear. Pharmacologic studies showed that METH can change the content of monoamine neurotransmitters, such as dopamine (DA) [2,6]. METH, a pseudo-neurotransmitter, functions as a substrate for DA transporters (DAT) and has high affinity for DAT [7,8]. METH could induce DAT-mediated release of DA via reversal of DAT, eventually leading to the increase of DA content in the synaptic space, through inhibiting its reuptake and promoting its release [8–10]. Increased DA neurotransmitter induces more activation of DA receptors, which plays a key role in the development of addictive

behaviors [11]. Additionally, numerous studies revealed that, apart from dopaminergic systems, serotoninergic and glutamatergic systems in the brain could also be altered by drugs of abuse. For example, it was found that METH could increase the levels of DA and 5-hydroxytryptamine (serotonin, 5-HT) significantly and rapidly [12], and repeated exposure to ethanol increases glutamic acid (Glu) level in the nucleus accumbens (NAc) [13]. A recent study about METH-dependent patients reported that METH abusers had lower 5-HT concentrations and higher DA and Glu concentrations in the blood [14]. Previous studies also suggested that METH is an indirect agonist of DA, norepinephrine (NE) and 5-HT receptors [7]. Due to its structural similarity to neurotransmitters, METH substitutes for the DAT, NE transporter (NET), serotonin transporter (SERT) and vesicular monoamine transporter-2 (VMAT-2), and then it reverses their endogenous function, thereby redistributing monoamines from storage vesicles into the cytosol [2,7]. These processes result in the release of DA, NE and 5-HT into the synapse, which then stimulates postsynaptic monoamine receptors. Using PET, researchers observed lower levels of striatum DAT, SERT and VMAT-2 in METH abusers than those in normal people [15].

Throughout the brain, METH has effects on serotonergic and glutamatergic systems as well as dopaminergic systems. It is through these complex neurochemic modulations that addiction reaches its climax, resulting in significant behavioral changes. Most neurotransmitters, such as DA, 5-HT and Glu, are known to be involved in addiction in different ways and they interact with and affect each other instead of acting independently [14]. Although they play important roles in reinforcing the properties of drugs of abuse, how the neurotransmitters contribute to METH addiction in signal transduction remains unclear. Therefore, it is vital to explore the neurochemic effects of METH on biogenic neurotransmitter signaling comprehensively.

Addiction is thought to result from persistent adaptations within brain reward circuits, and the reward system is a collection of brain structures and neural pathways, as well as some relevant projection sites, including ventral tegmental area (VTA), striatum including the ventral striatal nucleus-accumbens (NAc) and dorsal striatal caudate putamen (CPu), prefrontal cortex (PFc) and other structures such as the amygdala and hippocampus (Hip) [16–18]. Despite significant differences in molecular targets and behavioral effects, nearly all rewarding drugs eventually converge their effects onto the mesocorticolimbic circuitry [19]. Among these brain regions, the mesolimbic dopaminergic pathway has received the greatest attention as this circuit mediates the processing of reward-related stimuli via increased DA release: DA is released by the VTA and is directed toward DA receptors established in the NAc via the mesolimbic DA pathway and PFc via the mesocortic pathway [17,18,20]. Other DA pathways such as the mesostriatal pathway (DA cells in the substantia nigra projecting into the dorsal striatum) also contribute to drug reward and addiction [21]. Additionally, VTA is innervated by the glutamatergic projections from the PFc, amygdala and Hip; the striatum receives not only dense DA projections from the VTA and the substantia nigra but also glutamatergic inputs from multiple brain structures (e.g. PFc, amygdala and Hip) [6,19]. Glutamatergic projection provides excitatory control over DA signaling [19,22]. In this cascade, each cerebral structure and neurotransmitter plays a role in inducing addiction. Understanding the complex roles of various regions and neurotransmitters in addiction is critical to improving treatment for drug addiction.

The conditioned place preference (CPP) model is a well-established paradigm that can be used to evaluate the transition from a neutral stimulus to a conditioned stimulus in reward-related behaviors, driving a conditioned response (i.e. behaviors of approaching a drug-paired environmental context) [23,24]. In this model, animals are confined to two distinct environmental contexts, one of which is paired with the rewarding state produced by the drug of abuse. Subsequently, drug reward effects will be verified and measured by comparing the amount of time that an animal chooses to spend in the drug-conditioned context with the time it spends in the unpaired context [25,26].

Based on the above background, this study first used an animal model of METH-induced CPP. Then, liquid chromatography-mass spectrometry (LC-MS) was adopted to detect simultaneously the content of various neurotransmitters – DA, NE, 5-HT, 5-hydroxyindole acetic acid (5-HIAA), Glu and glutamine (Gln) – in the brain regions of PFc, NAc, CPu and Hip. This study aimed to investigate the change patterns of the content of neurotransmitters in the reward-related brain regions and to advance the understanding of the role of biogenic neurotransmitters and relevant reward circuits of the brain in METH addiction, which is important for improving the treatment of drug addiction.

Materials and methods

Animals

Male C57BL/6 mice (8 weeks old and weighing 18–25 g) were purchased from the Animal Center of Shanxi Medical University. All mice were housed in cages (four per cage) under a 12-h light/dark cycle (lights on at 7 a.m.) with food and water ad libitum. The room temperature was maintained at 22 ± 2 °C. All experiments were approved by the Institutional Animal Care and Use Committee of Shanxi Medical University and carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978). All efforts were made to minimize the number of animals used and their suffering.

Reagents

Methamphetamine hydrochloride, dopamine hydrochloride, norepinephrine standard, serotonin hydrochloride, 5-hydroxyindoleacetic acid standard, glutamate standard and glutamine standard were purchased from National Institutes for Food and Drug Control (Beijing, People's Republic of China). The volume of intraperitoneal injection was 10.0 ml/kg. The dosage of METH used in this experiment was 1.0 mg/kg, which induced obvious CPP in our previous studies [27,28].

Conditioned place preference apparatus

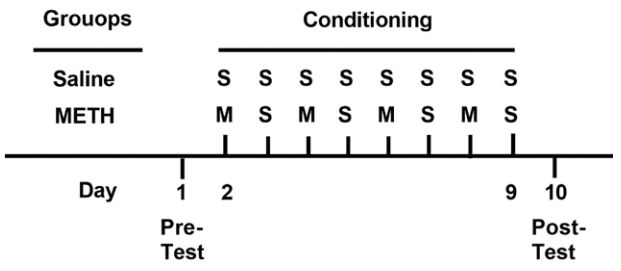
The CPP apparatus (JLBeHv, People's Republic of China) consists of two equal-sized compartments (15 cm × 15 cm × 37 cm) with a sliding door in the center of the base. The two compartments were equipped with different visual and tactile cues: one was black with a metal grid floor, and the other was white with a metal rod floor. The time lengths that each mouse spent in each compartment and the times that each mouse crossed the compartments were recorded by the infrared monitoring system.

Conditioned place preference procedure

The behavioral experiment was divided into three phases as previously described [27,28] (Fig. 1). In the pretest (day 1), no drug was given, and all mice (the Saline group and the METH group) were allowed to explore the two compartments freely for 15 min (900 s) with the door open. The time lengths that each mouse spent in each compartment were recorded and used to determine its initial preference for a particular compartment. To eliminate major individual differences, we excluded those mice that spent over 600 s in the session in either compartment or crossed less than 20 times between the compartments (a total of 36 mice were used in the current study and 4 were excluded, therefore the final $n = 16$ for each group). The following 8 days were the conditioning phase (days 2–9) in which METH was paired with the less preferred side (the white compartment). On day 2, the METH group was confined in the white compartment for 40 min immediately after the mice received intraperitoneal METH injection; meanwhile, the saline group was confined in the white compartment for 40 min immediately after the mice received intraperitoneal saline injection. On day 3, both groups were confined in the black compartment for 40 min immediately after they received an intraperitoneal saline injection. The METH group received METH and saline injections alternatively in two consecutive days; whereas the saline group received saline injections in two consecutive days (from day 2 to day 9). In post-test (day 10), all mice were placed into the CPP apparatus and allowed to explore the two compartments freely for 15 min, and the time lengths that each mouse spent in each compartment were recorded.

Previous studies of amphetamine- and cocaine-induced CPP agreed that the animals more or less showed a more obvious CPP in the inactive (light) phase than in the active (dark) phase [29,30], therefore, we adopted a similar experimental design for the sake of significance level and all our behavioral experiments were carried out in the light phase (8:00 a.m.–6:00 p.m.) of the light/dark cycle, as it has become the routine practice in CPP research.

Fig. 1



The conditioned place preference (CPP) behavioral paradigm. Pretest phase (day 1): all mice were drug free and allowed to explore the two compartments freely for 15 min when the door was open. Conditioning phase (days 2–9): mice received daily intraperitoneal. methamphetamine (METH)/saline or saline injections alternatively and were confined in the white or black compartments respectively for 40 min. Post-test phase (day 10): the operation was the same as pretest phase. M: methamphetamine; S: saline.

Tissue preparation

To investigate the relationship between reward behavior and neurotransmitter changes in relevant brain regions, we used the METH group as the experimental group and the saline group as the control group because the METH group showed obvious CPP. The mice were euthanized with cervical dislocation immediately after the behavioral test was completed on day 10. By reference to the map of the mouse brain tissue by Paxinos and Franklin [31], the brain tissue was rapidly extracted, and then the PFc, NAc, CPu and Hip were dissected bilaterally on an ice-cold plate and stored at −80 °C immediately. Subsequently, all neurotransmitters and relevant substances in different brain regions were detected with LC-MS. To meet the requirements of sample loading of the instrument, the same brain regions in each group of mice were combined and weighed precisely. In total 1 mL of cold 0.1 mol/L perchloric acid solution was added into brain tissue and the homogenate was prepared with a glass homogenizer. After vortex oscillation for 10 s, the homogenate was centrifuged at 12000 × g for 20 min at 4 °C. The supernatant was filtered with a filter membrane and stored at −20 °C.

Liquid chromatography-mass spectrometry quantification of neurotransmitters

DA, NE, 5-HT, 5-HIAA, Glu and Gln were determined by LC-MS. Chromatographic conditions: ACQUITY UPLC BEH C18 chromatographic column (2.1×100 mm, 1.7 m),

sample injection amount 5 µl, column temperature 40 °C, mobile phase A-10% methanol water (containing 0.1% formic acid), B-50% methanol water (containing 0.1% formic acid). Gradient elution conditions were 0–1 min, 20–100% B; 1–7 min, 100% B; 7–7.5 min, 100–20% B; 7.5–11 min, 20% B. Flow rate: 0.4 ml/min. Mass spectrometry conditions: electrospray ionization source and positive ion ionization mode. Ion source temperature was 500 °C; ion source voltage was 5500 V; collision gas was 6 psi; air curtain gas was 30 psi; atomization gas and auxiliary gas were 50 psi. Multi-reaction monitoring was used for scanning and detection.

LC-MS was carried out for detecting the working standard solutions of DA, NE, 5-HT, 5-HIAA, Glu and Gln, respectively. According to previous studies, Glu and Gln need to be processed with precolumn derivatization [32]. The content of the working standard solution was taken as the abscissa, and the ratio of the peak area to the internal standard was taken as the ordinate. The linear range was investigated and the standard curve was drawn, with a linearity expected to be >0.99. The signal-to-noise ratio (SNR) method was used to determine the quantitative limit. We compared the measured signals from samples with known low concentrations with the measured signal from the blank sample, and the corresponding concentration when the SNR was 10:1 (S/N = 10) was determined as the quantitative limit. The standard solutions of different concentrations were injected into the capillary six times in a row, and the intra-day precisions were calculated. The sample injection was carried out 3 days, and the inter-day precisions and recovery rates were calculated. The supernatants were 100 times diluted and used as testing samples of compounds with a high content of Glu and Gln, and the supernatants were directly used as testing samples of compounds with a low level of DA, NE, 5-HT and 5-HIAA, without being diluted. Each sample was tested three times.

Statistical analysis

The CPP score refers to the difference between the time lengths that each mouse spent in the drug-paired compartment in the pretest phase and post-test phase. All data were represented by mean ± SEM and analyzed with SPSS software (version 25; IBM Corp., Armonk, New York, USA). The paired-samples *t*-tests were conducted to compare CPP scores between the METH group and the saline group. Two-way analysis of variance (ANOVA) was used to analyze the effects of drug treatment (METH vs. Saline) and brain region on the content of various neurotransmitters with a post hoc comparison test least significance difference to determine significant differences between groups. A value of $P < 0.05$ was considered to be statistically significant.

Results

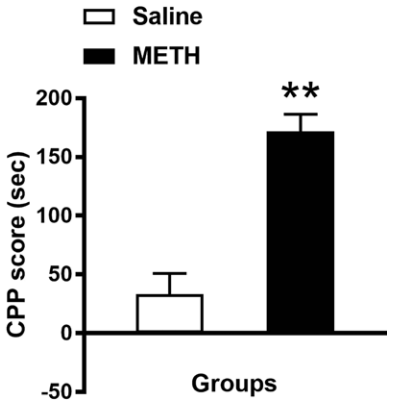
Effects of METH on place conditioning

In the conditioning phase of CPP (Fig. 1, days 2–9), the METH group was injected with 1.0 mg/kg METH. After the conditioning sessions, on day 10, the results of *t*-tests revealed that the CPP scores of the METH group were significantly higher than those of the Saline group [$t(7) = 4.474$; $P = 0.003$] (Fig. 2, $^{**}P < 0.01$), indicating that METH (1.0 mg/kg) can induce obvious CPP in mice, which was consistent with our previous studies [27,28].

Effects of METH on transmitters in relevant brain regions

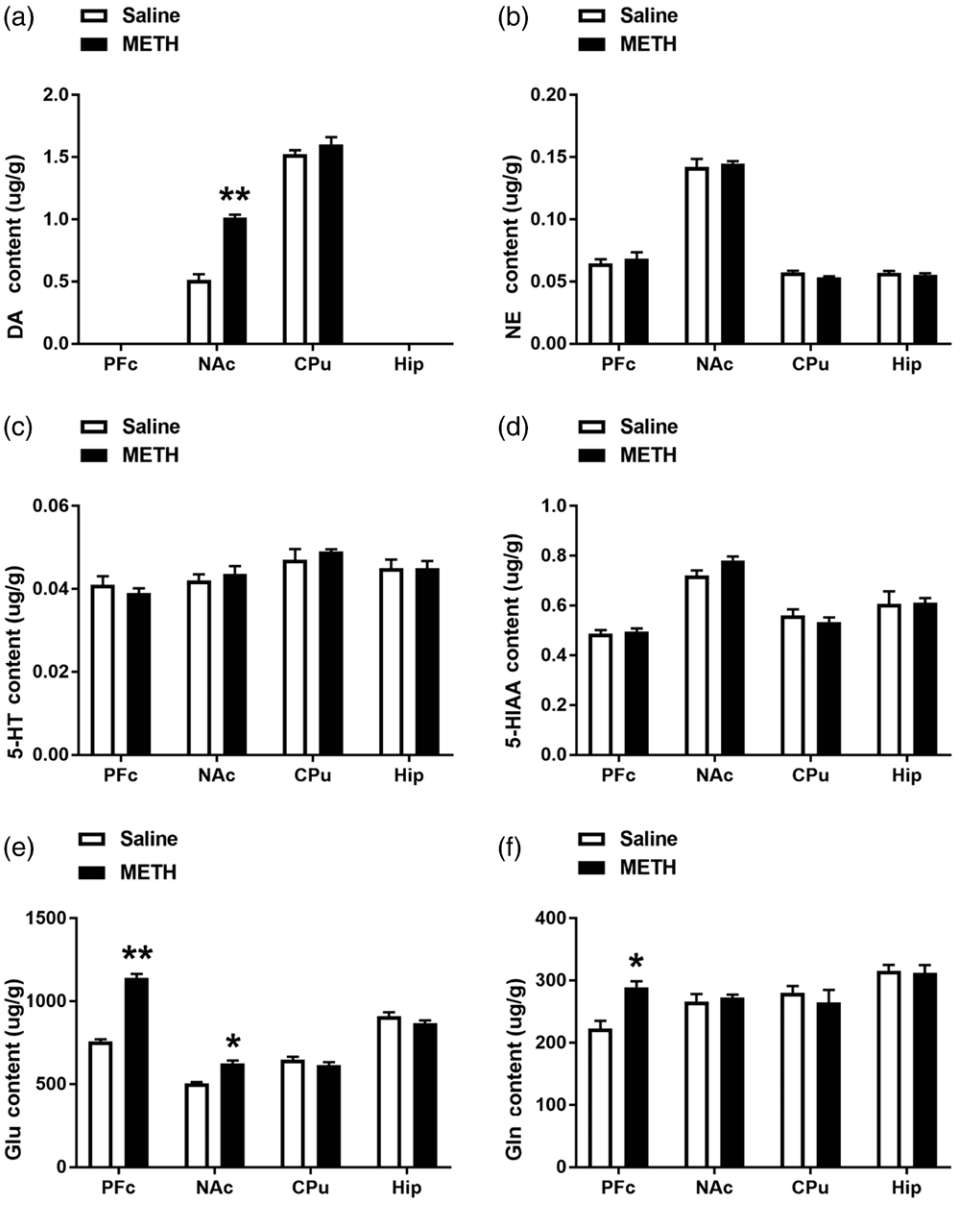
The results of ANOVAs on the content of DA revealed significant main effects of drug treatment [$F(1, 8) = 47.306$; $P < 0.001$] and brain region [$F(1, 8) = 360.000$; $P < 0.001$], and the interaction [$F(1, 8) = 24.806$; $P < 0.001$] was significant. Post hoc analyses showed that the METH group had a significant increase of DA in NAc (Fig. 3a, compared with the saline group, $^{**}P < 0.01$), and there was no difference in the content of DA in CPu between the two groups (Fig. 3a, $P > 0.05$). In addition, DA was not detected in PFc and Hip (Fig. 3a), probably because its content failed to reach the quantitative lower limit. The results of ANOVAs on the content of NE revealed an insignificant main effect of drug treatment [$F(1, 16) = 0.005$; $P = 0.944$], a significant main effect of brain region [$F(3, 16) = 327.968$; $P < 0.001$] and an insignificant interaction [$F(3, 16) = 0.594$; $P = 0.628$]. The results of ANOVAs on the content of 5-HT revealed an insignificant main effect of drug treatment [$F(1, 16) = 0.106$; $P = 0.749$], a significant main effect of brain region [$F(3, 16) = 7.017$; $P = 0.003$] and an insignificant interaction [$F(3, 16) = 0.515$; $P = 0.678$]. The results of ANOVAs on the content of 5-HIAA revealed an insignificant main effect of drug treatment [$F(1, 16) = 0.432$; $P = 0.520$], a significant main effect of brain region [$F(3, 16) = 41.046$; $P < 0.001$] and an insignificant interaction [*F*

Fig. 2



Effects of methamphetamine (METH) on place conditioning. The results revealed that the conditioned place preference (CPP) scores of the METH group were significantly higher than those of the saline group on day 10, indicating that METH (1.0 mg/kg) can induce obvious CPP in mice. Data was represented by mean ± SEM. $^{**}P < 0.01$ compared with the saline group.

Fig. 3



Effect of methamphetamine (METH) on transmitters in relevant brain regions. All mice were euthanized immediately after the behavioral test on day 10. Their PFc, NAc, CPu and Hip were dissected respectively. The content of DA, NE, 5-HT, 5-HIAA, Glu and Gln detected in different brain regions were respectively showed as following: (a and b) DA and NE, the METH group showed a significant increase of DA in NAc. There was no difference in the content of NE between the METH group and the Saline group in different brain regions. (c and d) 5-HT and 5-HIAA, there was no difference in the content of 5-HT and 5-HIAA between the METH group and the Saline group in different brain regions. (e and f) Glu and Gln, in METH group, the content of Glu increased significantly in the PFc and NAc, and the content of Gln increased significantly in the PFc. Data was represented by mean ± SEM. $^{*}P<0.05$ and $^{**}P<0.01$ compared with the saline group respectively. 5-HT, 5-hydroxytryptamine; 5-HIAA, 5-hydroxyindole acetic acid; CPu, caudate-putamen; DA, dopamine; Glu, glutamic acid;Gln, glutamine; NAc, nucleus accumbens, NE, norepinephrine; PFc, prefrontal cortex.

(3, 16) = 1.085; $P = 0.384$]. Post hoc analyses showed that there was no obvious difference in the content of NE, 5-HT and 5-HIAA between the METH group and the saline group in four brain regions (Fig. 3b–d, $P > 0.05$). As to the content of Glu, the results of ANOVAs revealed significant main effects of drug treatment [$F(1, 16) = 72.130$; $P < 0.001$] and brain region [$F(3, 16) = 225.154$; $P < 0.001$] and the interaction [$F(3, 16) = 62.518$; $P < 0.001$] was also significant. Post hoc analyses indicated that the METH group showed a significant increase of Glu in PFc and NAc (Fig. 3e, compared with the saline group, $^{**}P < 0.01$ and $^{*}P < 0.05$, respectively), and there was no difference

in the content of Glu between the METH group and the saline group in CPu and Hip (Fig. 3e, $P > 0.05$). With regard to the content of Gln, the results of ANOVAs revealed an insignificant main effect of drug treatment [F (1, 16) = 2.394; $P = 0.141$], a significant main effect of brain region [F (3, 16) = 8.543; $P < 0.001$] and a significant interaction [F (3, 16) = 4.552; $P = 0.017$]. Post hoc analyses indicated that the METH group showed a significant increase of Gln in PFc (Fig. 3f, compared with the saline group, $^*P < 0.05$), and there was no difference in the content of Gln between the METH group and the saline group in NAc, CPu and Hip (Fig. 3f, $P > 0.05$).

These results suggested that the neurotransmitters of DA, Glu and Gln may work together and play important roles in METH-induced CPP in relevant brain regions, especially in PFc and NAc.

Discussion

CPP is one of the most popular procedures to evaluate the reward effect of addictive drugs [23,33], whose secondary craving properties (conditioned reward effects) could be developed when paired with a primary reinforcer [34]. For CPP, in the context of Pavlovian learning, the drug (i.e. the unconditioned stimulus) is expected to elicit a hedonic feeling of pleasure. The drug is paired with a distinct contextual environment in the CPP chamber (e.g. wall colors and floor texture), which, following conditioning, becomes a conditional stimulus. After conditioning, in the absence of the drug, re-exposure to the drug-paired chamber may evoke hedonic feelings of pleasure (i.e. conditioned response) and spontaneous behavior, and increase time spent in the drug-paired chamber [24]. In other words, the animal seeks out or prefers the drug-paired context during the CPP test because this behavioral response has produced a rewarding outcome. This is a valid interpretation and supported by neurobiologic responses related to reward encoding that occurs during the conditioning sessions [23,34]. In the present study, METH was paired with the white compartment during the conditioning phase. In post-test, the METH group showed an obvious preference for the white compartment (Fig. 2). In other words, METH (1.0 mg/kg) can induce obvious CPP in mice, which was consistent with our previous studies [27,28].

As the most abundant catecholamine neurotransmitter in the brain, DA is involved in the regulation of various physiologic functions of the central nervous system. Pharmacologic studies have shown that METH can lead to the increase of DA content in the synaptic space [2,6]. In our study, the METH group showed a significant increase in the content of DA in NAc (Fig. 3a), and the animals in this group showed significant CPP (a preference for METH-paired compartment). Our results were similar to those of Sun *et al*'s studies, for their results showed that the mice with morphine-induced CPP had significantly higher DA content in NAc than the mice treated with saline [35]. The realization of the correlation between elevated DA and behavior is important in identifying the drug-induced reward effect. The previous study also suggested that a reduction in CPP in the magnitude shows a reduction in DA release in NAc in cocaine-induced CPP [36]. Therefore, it can be concluded that NAc plays important role in the drug-induced reward effect, in which the change of DA was involved. In addition, it is also well known that NE is an indirect product of DA acting as an important neurotransmitter in the brain. Studies have suggested that many neurotransmitters in the brain, such as NE, may have an antireward effect [37]. In the present study, no significant change in NE was observed in all nuclei in the METH group, and the saline CPP group showed a similar level of NE (Fig. 3b).

5-HT, another important neurotransmitter in psychopharmacologic actions in the brain, performs its physiologic functions by binding to a specific receptor, and its levels will usually change significantly when psychiatric disorders, such as depression and anxiety occur [38]. However, this is not always the case. The previous study suggested that METH primarily interacts with the dopaminergic system but scarcely interacts with the serotonergic system, and METH was considered to be a more potent releaser of DA than 5-HT (whereas MDMA was a more potent releaser of 5-HT than DA) [12]. For example, a previous study showed that the content of 5-HT did not change significantly in rats that received an intravenous injection of 0.3 mg/kg (+)-amphetamine at 0 h and another injection of 1 mg/kg 60 min later [39]; some amphetamine-related hallucinogens, such as 2,5-dimethoxy-4-methylamphetamine, also failed to increase extracellular levels of 5-HT in NAc in rats [12]. These results were consistent with our results – there was no difference in the content of 5-HT in different brain regions between the METH group and the saline group (Fig. 3c). Interestingly, previous studies indicated that 5-HT did not appear to be directly involved in drug addiction, but contributed to addiction by regulating the release of DA [40]. For example, it was found that injection of 5-HT3 receptor agonist into NAc could increase the content of DA in NAc, whereas 5-HT3 receptor antagonist could inhibit the increase of DA in NAc [41]; M100907, a potent selective 5-HT2A receptor antagonist, could also inhibit the increase of psychomotor activity induced by METH [42]. It is well known that, apart from DAT, the presynaptic plasma membrane SERT is another main METH binding site, but further research is needed to determine whether SERT are involved in METH addiction. In addition, we examined the levels of 5-HIAA, a main metabolite of 5-HT, in different brain regions in the METH group and the saline group, and no significant difference was found (Fig. 3d), which was consistent with the results from Lu *et al*.'s studies [43].

As an important excitatory neurotransmitter in the brain, Glu is involved in the behavioral response to the drug craving associated with motivational and neutral cues

[44,45]. Although the mesocorticolimbic DA system is a prominent focus on the reward processing and drug addiction, a growing study has emerged indicating an important role for Glu in mediating the adaptive processes underlying psychostimulant addictions [8,26]. It appears that DA has a primary role in the beginning of the addictive cycle, whereas Glu is a greater factor in the later parts of the cycle (reinstatement and relapse) [46]. Addiction memories formed over the course of repeated drug use and withdrawal can become powerful stimuli that trigger craving and relapse [26]. The glutamatergic projections from PFc to NAc play a crucial role in retrieving and integrating drug-associated memories, and are essential for reinstituting biologic reward behavior, especially relapse [46–48]. In the present study, Glu increased significantly in PFc and NAc in the METH group (Fig. 3e). This result was consistent with a previous study, in which the level of Glu significantly increased in PFc and NAc during METH-induced reinstatement [49]. These results furtherly confirmed that drug-seeking behaviors and relapse require Glu release from PFc to NAc. Additionally, it is noted that DA transmission can be regulated by glutamatergic afferents and, conversely, DA can influence Glu transmission via inputs to glutamatergic neurons [46]. This interdependence of Glu and DA transmission is critical for regulating various aspects of reward processing and addictive behaviors. For example, Mark *et al*. found that METH could induce an increase in the activity of glutamatergic neurons in VTA, thereby inducing the increase of DA release in NAc and PFc [50], conversely, METH and cocaine block the reuptake of DA by binding to the DAT and this increases synaptic DA levels, which activates D1 receptors and indirectly enhances Glu transmission [46]. It is known that Gln can be hydrolyzed to Glu under the action of glutaminase, which is the main biosynthetic pathway of Glu in the brain. In the present study, Gln was also detected in different brain regions. Our results showed that the content of Gln also increased significantly in the PFc (Fig. 3f), indicating a similar trend to Glu. Compared with the saline group, the changes of METH group in Glu content and Gln content in PFc and NAc can be summarized as follows: (1) Gln, the protomer of Glu, increased in PFc only, whereas Glu increased in both PFc and NAc; and (2) Glu increased to a greater degree in PFc than in NAc. The above two points may indicate that Glu is derived from PFc and then released to NAc.

Different experimental designs tend to produce different results, and stimulant-induced neurotransmitters changes may occur at different time for different brain regions. The results about various neurotransmitters in our study may not be entirely consistent with previous studies. A mild dosage (1.0 mg/kg) was used in our study, whereas a high-dosage of METH (10 mg/kg) [13,51] or a METH binge (6 mg/kg, four injections/day) [52] was used in several previous studies, in which high-dosage METH may produce neurotoxicity, leading to the decrease of the content of neurotransmitters in the brain in rodents. In addition, it must be noted that a CPP paradigm was used in the present study, in which brain tissues were collected 48 h after the last injection of METH in post-test. Previous studies demonstrated that DA and 5-HT concentrations were increased in CPu and Hip in rats about 0–2.5 h after the acute effects of METH [12]. In the present study, the content of neurotransmitters (e.g. DA in NAc, Glu in PFc and NAc) increased on day 10 (post-test) when CPP was conducted because CPP is used to measure associations formed between a rewarding stimulus (e.g. drug) and a contextual environment, and re-exposure to cues could evoke neurotransmitters responses. Another study showed that the levels of Glu decreased significantly in PFc and NAc in rats following 10 days of METH intravenous self-administration and 10 extinction sessions [49]; it is worth noting that the levels of Glu increased in PFc and NAc when extinguished rats were re-exposed to cues previously paired with the drug [49], which was consistent with our present result. Taking the above-mentioned factors into consideration helps to better understand the results of the present study.

In conclusion, this study suggested that the neurotransmitters of DA, Glu and Gln may work together and play important roles in METH-induced CPP in relevant brain reward circuits, especially in PFc and NAc. These findings therefore could help to advance the comprehensive understanding of the neurochemic and psychopharmacologic properties of METH in reward effect, which is important for future improvements in the treatment of drug addiction.

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Conflicts of interest

There are no conflicts of interest.

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