Published in final edited form as:

Drug Alcohol Depend. 2012 November 1; 126(1-2): 87–94. doi:10.1016/j.drugalcdep.2012.04.020.

Prior Methamphetamine Self-Administration Attenuates Serotonergic Deficits Induced by Subsequent High-Dose Methamphetamine Administrations

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Abstract

Background—Pre-clinical studies indicate that high-dose, non-contingent methamphetamine (METH) administration both rapidly and persistently decreases serotonergic neuronal function. Despite research indicating the hippocampus plays an important role in METH abuse and is affected by METH use, effects of METH self-administration on hippocampal serotonergic neurons are not well understood, and were thus an important focus of the current study. Because humans often administer METH in a binge-like pattern, effects of prior METH self-administration on a subsequent “binge-like” METH treatment were also examined.

Methods—Rats were treated as described above, and sacrificed 1 or 8 d after self-administration or 1 h or 7 d after the final binge METH or saline exposure. Hippocampal serotonin (5-hydroxytryptamine; 5HT) content and transporter (SERT) function were assessed.

Results—METH self-administration *per se* had no persistent effect on hippocampal 5HT content or SERT function. However, this treatment attenuated the persistent, but not acute, hippocampal serotonergic deficits caused by a subsequent repeated, high-dose, non-contingent METH treatment administered 1 d the last self-administration session. No attenuation in persistent deficits were seen when the high-dose administration of METH occurred 15 d after the last self-administration session.

Conclusions—The present findings demonstrate that METH self-administration alters serotonergic neurons so as to engender “tolerance” to the persistent serotonergic deficits caused by a subsequent METH exposure. However, this “tolerance” does not persist. These data provide a foundation to investigate complex questions including how the response of serotonergic neurons to METH may contribute to contingent-related disorders such as dependence and relapse.

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Author Disclosures

Conflict of Interest

All authors declare that they have no conflicts of interest.

Contributors

Authors McFadden, Fleckenstein, and Hanson designed the study. Authors McFadden, Hunt, Allen, Muehle, Vieira-Brock, and Nielsen conducted the experiments and undertook the statistical analysis. Authors McFadden and Fleckenstein wrote the manuscript. All authors contributed to and have approved the final manuscript.

Keywords

Methamphetamine; Self-administration; Serotonin; Hippocampus

1. Introduction

Methamphetamine (METH) is a highly addictive substance that is abused worldwide. Abusers often display structural and neurochemical changes within the brain and higher rates of psychiatric disorders and cognitive deficits (for review, see Barr et al., 2006; Chang et al., 2007; but see also Hart et al., 2012). For example, positron emission tomography has indicated decreased serotonin (5-hydroxytryptamine; 5HT) transporter (SERT) densities in several brain regions in abstinent METH users (Sekine et al., 2006). Additionally, increased depression scores and impaired memory performance on a word recall test have been correlated with reduced hippocampal volumes in METH abusers (Thompson et al., 2004). Reductions in gray matter volumes of both the amygdala and hippocampus have been reported in subjects with METH-induced psychosis (Orikabe et al., 2011). Further, functional magnetic resonance imaging has indicated decreased hippocampal activation in METH abusers during an empathy task (Kim et al., 2010). These latter findings emphasize the need for study of the neurochemical changes in the hippocampus following METH exposure.

Preclinical data indicate that hippocampal serotonergic neurons are vulnerable to non-contingent high-dose METH administration. For example, METH, given in a “binge-like” pattern (e.g., 3 - 6 injections, 7.5 - 50 mg/kg/injection, 2 - 8-h intervals), decrease rat hippocampal SERT function, [¹²⁵I]RTI55 binding to SERT and 5HT content as assessed 72 h to 3 months after the METH injections (Farfel et al., 1995; Haughey et al., 2000; Johnson-Davis et al., 2003; Richards et al., 1993; Schroder et al., 2003). Further, tryptophan hydroxylase (TPH) activity is decreased 30 d (Hotchkiss et al., 1980), and 5HT content is decreased 35 d, following a binge METH exposure (Herring et al., 2010). These changes are not unique to the hippocampus, as persistent decreases in 5HT content, SERT function and TPH activity have also been reported in the striatum (Bowyer et al., 2008; Danaceau et al., 2007; Haughey et al., 2000; Herring et al. 2010; Hotchkiss et al., 1980; Stephans et al., 1996). Additionally, serotonergic axonal degeneration has been reported using immunocytochemistry in animals that receive large METH doses (Axt et al., 1991).

Of relevance, pretreatment with escalating-dose administrations of METH attenuated the decreased hippocampal 5HT content caused by a binge METH treatment, although this effect appears dependent upon the length of time between the binge exposure and monoamine assessment. For example, such pretreatment attenuates hippocampal 5HT deficits as assessed 7 d (Johnson-Davis et al., 2003), but not 1 d (Cadet et al., 2009; Graham et al., 2008), after a binge METH treatment. Further, escalating-dose pretreatment attenuated the decreases in hippocampal [¹²⁵I]RTI-55 binding caused by a binge METH treatment as assessed 7 d later (Belcher et al., 2008). Finally, pretreatment with escalating doses of METH affords protection against subsequent binge METH-induced serotonergic deficits when the binge is administered within 1 week, but not 14 or 31 d, after the final escalating-dose exposure (Danaceau et al., 2007).

The hippocampus is important for METH reinforcement. For example, when METH is administered directly into the dorsal hippocampus, rats self-administer the drug and develop conditioned place preference for the location where it was administered (Ricoy et al., 2009). Further, METH self-administration disrupts object-in-place recognition, which is thought to rely upon perirhinal cortex-prefrontal cortex-hippocampal circuitry (Reichel et al., 2012).

Despite research indicating that the hippocampus plays an important role in METH abuse and is affected by METH use, effects of METH self-administration on hippocampal serotonergic neurons are not well understood.

Human METH addiction is characterized by escalating drug use and the loss of the ability to control drug intake sometimes resulting in high-dose exposure to the drug of abuse (Koob and Volkow, 2010). Previous research utilizing this self-administration model has demonstrated that 7 d of METH self-administration (0.12 mg/infusion; McFadden et al., 2012) lead to: 1) an escalation in daily drug intake; 2) brain METH concentrations 1 h after the last self-administration session that are comparable to those found in human postmortem studies (Kalasinsky et al., 2001); 3) persistent decreases in dopamine transporter immunoreactivity within the striatum which were similar in magnitude to those observed using PET imaging of human METH abusers (Chang et al., 2007); and 4) an attenuation of the striatal dopaminergic deficits induced by a subsequent binge METH treatment.

Because of the importance of serotonergic neurons and the hippocampus, the present study evaluated SERT function and 5HT content after METH self-administration. It was hypothesized that METH self-administration would lead to resistance to serotonergic deficits. To test this, a high-dose METH regimen was administered after METH self-administration. Results revealed that prior METH self-administration did not cause persistent (herein defined as 7 - 8 d following last METH exposure) serotonergic deficits *per se*. However, such treatment attenuated the persistent, but not acute, serotonergic deficits induced by a subsequent binge METH treatment given 1 d after the start of the last self-administration session. The attenuation of persistent deficits was not seen when the binge exposure occurred 15 d after the last self-administration session. These data may provide important insight into the neurochemical deficits reported in human METH abusers.

2. Methods

2.1 Animals

Male Sprague-Dawley rats (275-300 g; Charles River Laboratories, Portage, MI) were housed four rats/cage. Following surgery, each rat was individually housed in a transparent plastic cage. Water was available in their home cage *ad libitum*. During food training, rats were food restricted such that no rat dropped below 90% of their body weight at the time the experiment was initiated. Rats were maintained under the same 14:10 h light/dark cycle in the animal facility and in the operant chambers. Animals were sacrificed by decapitation. All experiments were approved by the University of Utah Institutional Animal Care and Use Committee, in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Lever pressing behavior, core body temperatures and striatal dopaminergic assessments from rats utilized to generate Figures 1 and 2 were published previously (McFadden et al., 2012; Figures 6 and 7).

2.2 Drugs

Racemic-METH hydrochloride (Research Triangle Institute; Research Triangle Park, NC) was dissolved in 0.9% sterile saline. Doses are expressed as free base. Rats were anesthetized using ketamine (90 mg/kg; Hospira Inc., Lake Forest, IL, USA) and xylazine (7 mg/kg; Sigma-Aldrich, St. Louis, MO, USA). The antibiotic cefazolin (10 mg/ml; Schein Pharmaceutical, Florham Park, NJ, USA) was dissolved in heparinized saline (63.33 U/ml; Sigma, St. Louis, MO, USA). Flunixin meglumine (1.1 mg/kg; MWI Veterinary Supply, Meridian, ID, USA) was used for post-surgery analgesia.

2.3 Food Training and Surgery

Food training and self-administration occurred in an operant chamber (30.5 cm × 25.5 cm × 30.5 cm; Coulbourn Instruments, Whitehall, PA USA) as described in McFadden et al., 2011. Prior to surgery, each rat was trained to press for a 45-mg food pellet during four overnight 14-h sessions. Following food training, rats were anesthetized and an indwelling catheter was implanted. The catheter was constructed as described previously (Frankel et al. 2008; 2010). The outlet of the catheter was implanted subcutaneously in the back and the free end of the Silastic tubing was inserted 25 mm into the right jugular vein and secured to the surrounding tissue with sutures. Each rat received flunixin meglumine on the day of the surgery and the day following the surgery. Immediately following surgery and daily thereafter, each rat was infused with 0.1 ml of cefazolin followed by 0.05 ml of heparinized saline and heparinized glycerol through the catheter. Catheter patency was confirmed by infusing 0.03 ml (20 mg/ml) of xylazine.

2.4 Self-Administration and METH Challenge

Rats underwent 7 d of self-administration (8 h/session; FR1; 0.12 mg/infusion METH or saline) during the light cycle in a room maintained at 29±1 °C to promote lever pressing (Cornish et al., 2008). For each active lever press, an infusion pump connected to a liquid swivel (Coulbourn Instruments) suspended outside the operant chamber delivered 10 µl of METH or saline per infusion over a 5-s duration through a polyethylene tube located within a spring leash (Coulbourn Instruments) tethered to the rat. During this period, both levers were retracted. Following the infusion, the levers remained retracted for an additional 20 s. The active lever was counterbalanced within each group. Pressing the inactive lever resulted in no programmed consequences although it was recorded. Rectal temperatures were measured using a digital thermometer (Physiotemp Instruments, Clifton, NJ) approximately 30 min after the end of each session. Animals were sacrificed 1 or 8 d after the start of the last self-administration session or received a binge of METH or saline as described below.

Twenty-four h after the start of the last self-administration session, rats were challenged with 4 injections of METH (7.5 mg/kg/injection; 2-h interval) or saline (1 ml/kg/injection). This time point was chosen based on research utilizing escalating doses of METH (see Danaceau et al., 2007; O'Neil et al., 2006). In the initial binge experiment, all animals received drug in an ambient temperature of 22-23 °C. In subsequent studies, METH self-administering rats challenged with METH (METH/METH) were maintained in a warm environment (25 °C) to promote hyperthermia. Previous research in our laboratory has shown that this ambient temperature permits binge METH-induced that would have otherwise been attenuated by prior exposure to METH during development (see McFadden et al., 2011). Animals were sacrificed 1 h or 7 d after the binge exposure. Another group of rats received a binge of METH or saline 15 d following the final self-administration session and were sacrificed 7 d later. Rectal temperatures were measured using a digital thermometer approximately 30 and 90 min after each injection.

2.5 Synaptosomal [³H]5HT uptake

[³H]5HT uptake was determined using a rat hippocampal synaptosomal preparation as previously described (Hadlock et al., 2011). In brief, synaptosomes were prepared by homogenizing freshly dissected hippocampal tissue in ice-cold 0.32 M sucrose pH 7.4, and centrifuged (800 × g, 12 min; 4 °C). A small section of the left anterior hippocampus was quickly frozen on dry ice and retained for determining 5HT content. The supernatants were centrifuged (22,000 × g, 15 min; 4 °C) and the resulting pellets were resuspended in ice-cold assay buffer (in mM: 126 NaCl, 4.8 KCl, 1.3 CaCl₂, 16 sodium phosphate, 1.4 MgSO₄, 11 glucose and 1 ascorbic acid; pH 7.4) and 1 µM pargyline. Samples were incubated for 10 min at 37 °C and the assays initiated by the addition of [³H]5HT (5 nM final concentration).

Following incubation for 3 min, samples were placed on ice to stop the reaction. Samples were then filtered through GF/B filters (Whatman, Florham Park, NJ) soaked previously in 0.05% polyethylenimine. Filters were rapidly washed three times with 3 ml of ice-cold 0.32M sucrose buffer using a filtering manifold (Brandel, Gaithersburg, MD). Nonspecific values were determined in the presence of 10 μ M fluoxetine. Radioactivity trapped in filters was counted using a liquid scintillation counter. Protein concentrations were determined using the Bio-Rad Protein Assay (Bio-Rad Laboratories Inc., Hercules, CA).

2.6 5HT and 5-hydroxyindoleacetic acid (5HIAA) Content

The anterior portion of the left hippocampus or striatum was sonicated for 3-5 s in 1 ml tissue buffer (0.1 M phosphate/citrate buffer, pH=2.5, containing 10% methanol) according to Haughey et al., (2000). Fifty μ l were injected onto a partisphere C-18 reverse-phase analytical column (5- μ m spheres; 250 $\times$ 4.6 mm; Whatman, Clifton, NJ, USA). Mobile phase consisting of 0.05 M sodium phosphate, 0.03 M citrate buffer, 0.1 M EDTA, 0.030-0.035% sodium octylsulfate, and 20-25% methanol (pH=2.85, flow rate=0.75 ml/min) was used. Serotonin was detected using an amperometric electrochemical detector with the working electrode potential set at +0.70 V relative to an Ag⁺/AgCl reference electrode.

2.7 Statistical Analysis

Statistical analysis was conducted in SAS 9.2 (Cary, NC, USA). Statistical analyses among groups were conducted using a t-test, analysis of variance (ANOVA) or repeated measures ANOVA followed by Newman-Keuls posthoc analyses. Violations of the sphericity assumption resulted in the use of a Huynh-Feldt correction. The data represent means $\pm$ standard error of the mean (S.E.M.) of 5-14 rats/group.

3. Results

The pattern of METH self-administration was consistent with that described in McFadden et al. (2012). Only animals that met the criteria largely based on Brennen et al. (2010) were included. Specifically, rats were included if they: 1) pressed an average of more than 10 active lever presses per d; and 2) the ratio of active/inactive lever presses was $\geq$ 2:1. Discrimination between the active and inactive lever exceeded 5:1 in METH self-administering animals by the last day of self-administration. Further, an escalation in daily METH intake occurred in all METH self-administration experiments similar to that described in McFadden et al. (2012; see Figures 4A and 6A therein). Of note, in the studies described below, some saline self-administering/METH challenged rats succumbed to METH treatment. However, there were no fatalities among METH self-administering/METH challenged rats in any of the studies described below.

METH self-administration did not cause persistent serotonergic deficits as assessed 8 d after the last session. Specifically, this treatment (described in Methods) did not alter hippocampal [3H]5HT uptake (Saline: 1.53 $\pm$ 0.10 fmol/ μ g protein; METH: 1.54 $\pm$ 0.07 fmol/ μ g protein; $t(12)$ =0.73, ns), 5HT content (Saline: 4.99 $\pm$ 0.49 pg/ μ g protein; METH: 5.67 $\pm$ 0.38 pg/ μ g protein; $t(12)$ =1.10, ns) or 5HIAA content (Saline: 8.51 $\pm$ 0.67 pg/ μ g protein; METH: 8.60 $\pm$ 0.54 pg/ μ g protein; $t(12)$ =0.11). Core body temperatures were 38.0 $\pm$ 0.1 $^{\circ}$ C in saline self-administering and 38.5 $\pm$ 0.1 $^{\circ}$ C in METH self-administering rats ($t(12)$ =4.32, p <0.05). In this study, rats self-administered a total of 26.33 $\pm$ 1.49 mg of METH over the course of the 7 d of self-administration.

A separate group of animals were allowed to self-administer for 7 days and were sacrificed 1 d after the start of the last self-administration session. No differences were found in hippocampal [3H]5HT uptake ($t(12)$ =0.62, ns; Saline: 1.26 $\pm$ 0.04 fmol/ μ g protein; METH:

1.31±0.08 fmol/μg protein), 5HT hippocampal tissue content ($t(13)=0.56$, ns; Saline: 5.06±0.21 pg/μg protein; 5.33±0.47 pg/μg protein), or 5HIAA content ($t(13)=0.43$, ns; Saline: 7.05±0.35 pg/μg protein; METH: 6.75±0.61 pg/μg protein). These METH self-administering animals consumed a total of 22.33±1.82 mg METH over the course of self-administration.

Results presented in Figure 1 indicate that 7 d of METH self-administration attenuated the persistent hippocampal serotonergic deficits caused by a subsequent binge METH treatment administered 1 d after the start of the last self-administration session, as assessed 7 d later. METH self-administration attenuated deficits in [3H]5HT uptake ($F(2,29)=3.59$, $p<0.05$; Figure 1A), 5HT content ($F(2,29)=15.88$, $p<0.05$; Figure 1B), and 5HIAA content ($F(2,29)=9.14$, $p<0.05$; Saline/Saline: 5.27±0.15 pg/μg protein; METH/METH: 4.43±0.32 pg/μg protein; Saline/METH: 3.03±0.29 pg/μg protein) relative to Saline/METH controls. No attempt was made to maintain equal hyperthermia during the binge, which resulted in the METH/METH group maintaining a cooler body temperatures during the first 2.5 h following METH administration compared to the Saline/METH group ($F(16,224)=7.70$, $p<0.05$; reprinted with permission from McFadden et al., 2012, Figure 6G). Animals self-administered 20.99±1.33 mg METH over the course of self-administration.

In subsequent experiments, an attempt to maintain equal hyperthermia in the METH/METH and Saline/METH groups was done as described in the Methods section. Results presented in Figure 2 indicate that, as in Figure 1, 7d of METH self-administration attenuated the persistent hippocampal SERT deficits caused by a subsequent binge METH treatment, as assessed 7 d later. In particular, METH self-administration attenuated deficits in [3H]5HT uptake ($F(2,16)=14.00$, $p<0.05$; Figure 2A) and 5HIAA content ($F(2,14)=9.73$, $p<0.05$; Saline/Saline: 9.23±1.02 pg/μg protein; METH/METH: 5.57±0.71 pg/μg protein; Saline/METH: 3.66±0.95 pg/μg protein) relative to Saline/METH controls. Resembling effects in Fig. 1B, prior METH self-administration trended toward attenuating binge-induced deficits in 5HT content (although the attenuation was not statistically significant; $F(2,14)=4.13$, $p<0.05$; Figure 2B). Results presented in Fig. 2C indicate that despite the warmer ambient environment, prior METH self-administration attenuated the increase in core body temperatures caused by the subsequent binge METH treatment as assessed during the first 2.5 h after treatment ($F(2,16)=103.3$, $p<0.05$; Figure 2C; reprinted with permission from McFadden et al., 2012, Figure 7A). In this study, rats self-administered 21.68±1.86 mg METH over the course of self-administration.

To investigate if alterations in the acute binge-induced effects of METH are associated with the attenuation of persistent deficits seen in Figure 2, animals were sacrificed 1 h following the last injection of the binge-exposure. Results presented in Figure 3 indicate that prior METH self-administration did not attenuate the rapid decreases in [3H]5HT uptake ($F(2,22)=17.31$, $p<0.05$; Figure 3A), 5HT content ($F(2,22)=68.94$, $p<0.05$; Figure 3B), or 5HIAA content (Saline/Saline: 12.71±1.34 pg/μg protein; METH/METH: 4.55±0.32 pg/μg protein; Saline/METH: 3.84±0.18 pg/μg protein; $F(2,22)=38.32$, $p<0.05$) caused by a subsequent binge METH treatment, as assessed 1 h after the binge exposure. Similarly and despite efforts to maintain hyperthermia, the METH/METH group had attenuated the hyperthermia ($F(14,154)=12.09$, $p<0.05$; Figure 3C), as assessed 30 min following the first and third injection of METH. Rats self-administered a total of 21.61±1.23 mg METH during the 7 d of self-administration.

Results presented in Figure 4 indicate that prior self-administration did not afford protection against persistent deficits when the binge of METH was given 15 d after the last self-administration session. Binge METH-induced decreases in [3H]5HT uptake ($F(2,19)=11.39$, $p<0.05$, Figure 4A), hippocampal 5HT content ($F(2,20)=16.47$, $p<0.05$; Figure 4B) and

hippocampal 5HIAA content ($F(2,20)=9.88$, $p<0.05$; Saline/Saline: 5.29 ± 0.23 pg/ μ g protein; METH/METH: 3.19 ± 0.36 pg/ μ g protein; Saline/METH: 2.89 ± 0.47 pg/ μ g protein) were similar between METH/METH and Saline/METH groups. Further, similar hyperthermia was achieved between the METH/METH and Saline/METH groups during the binge of METH ($F(14,140)=6.02$, $p<0.05$; Figure 4C). Animals self-administered 20.57 ± 0.83 mg METH.

Noteworthy, the impact of METH self-administration on 5HT systems was not unique to the hippocampus. Prior METH self-administration also attenuated the persistent ($F(2,15)=50.76$, $p<0.05$; Figure 5A), but not the acute deficits ($F(2,21)=110.70$, $p<0.05$; Figure 5B) in striatal 5HT content caused by a subsequent binge treatment when administered 1 d after the start of the last self-administration session and were treated as in Figures 2 and 3. No attenuation were seen in depletions of striatal 5HT content when the binge was given 15 d after the last self-administration session ($F(2,20)=13.72$, $p<0.05$; Figure 5C). Of note, 5HT content within the striatum did not significantly differ in METH and saline self-administering rats 1 d after the start of the last self-administration session ($t(13)=1.30$, ns; Saline: 7.17 ± 0.37 pg/ μ g protein; METH: 6.52 ± 0.32 pg/ μ g protein).

4. Discussion

These findings are among the first to characterize the effects METH self-administration on SERT function in the hippocampus. Consistent with human postmortem studies measuring SERT immunoreactivity (Kish et al., 2009), the present study demonstrates that METH self-administration *per se* did not lead to persistent changes in SERT function in the hippocampus 1 or 8 d after the last self-administration session. In contrast, others have found that METH self-administration causes slight, but significant, decreases in SERT immunoreactivity in the hippocampus (Reichel et al., 2012). Differences in the dosing paradigms (e.g., length of METH access) may contribute to the discrepancies.

Results also reveal that although METH self-administration paradigm *per se* did not cause apparent serotonergic deficits, it did alter the impact of a subsequent METH binge exposure 1 d after the last self-administration session in 22–23°C and 25 °C rooms. In particular, prior METH self-administration attenuated the persistent SERT deficits resulting from an experimenter-administered METH as assessed 7-d later in the hippocampus and 5HT content in the striatum. This effect of self-administration is reminiscent of effects of escalating-dose regimens of experimenter-administered METH. For example, escalating-dose administration of METH prior to a binge treatment attenuated the METH-induced decreases in SERT as assessed by [125 I]RTI-55 binding and decreases in 5HT tissue content in both the hippocampus and striatum 7 d after the binge METH treatment (Belcher et al., 2008; Johnson-Davis et al., 2003). This protection is also similar to the effect of prior METH self-administration on binge treatment-induced alterations in dopaminergic neuronal function in that the former attenuates the persistent dopaminergic deficits caused by the binge treatment (McFadden et al., 2012).

In contrast to the ability of METH self-administration to attenuate persistent serotonergic deficits, the prior experience with METH did not attenuate the acute effects of METH as assessed 1-h after binge METH treatment in the hippocampus and striatum. These findings are similar to reports by Cadet et al. (2009) that escalating-dose METH treatment did not attenuate the decrease in hippocampal and striatal 5HT content 1 d after the binge of METH. The findings of the present study suggest that the attenuation of persistent binge METH-induced deficits afforded prior METH self-administration are not a consequence of attenuating the acute effects of METH on SERT function.

The attenuations of persistent deficits afforded by prior METH self-administration were only significant when the binge of METH occurred 1 d after the start of the last self-administration session. When the binge of METH was administered 15 d after the last self-administration session, prior METH self-administration no longer afforded protection. These findings are consistent with escalating-dose METH pretreatment in that protection by METH pretreatment was only seen when the binge of METH was administered within 7 d of the end of pretreatment but not 14 or 31 d. These findings suggest that the serotonergic system may be vulnerable to deficits when relapse occurs after prolonged abstinence. Future studies will examine if reinstatement of METH self-administration after 15 d of abstinence leads to serotonergic deficits.

Of interest, alterations in hyperthermia may contribute to the attenuations in persistent serotonergic deficits. Reductions in METH-induced hyperthermia and SERT deficits in animals with a history of prior METH self-administration occurred when the binge was administered 1 d after the last session in both the animals maintained in 22–23°C and 25 °C rooms. Similar to other studies, these findings suggest that the attenuation of persistent serotonergic deficits may be due to reduction in hyperthermia (Kiyatkin et al., 2009; Sharma et al., 2007). This suggestion is based, at least in part, on findings by Bowyer and colleagues (2008) that METH-induced 5HT depletions within the striatum occurred only in hyperthermic mice maintained in a warm environment (Bowyer et al., 2008). METH-induced hyperthermia promotes the formation of oxygen radicals and reduces tryptophan hydroxylase activity (Fleckenstein et al., 1997; see also Stone et al., 1989), which may contribute to oxidative damage (Tata et al., 2007; Yamamoto et al., 2010). Additionally, increased temperature has been shown to increase human SERT uptake of [3H]DA in HEK-293 cells, which may further promote oxidative damage (Saldana et al., 2004). Thus, attenuation of binge-induced hyperthermia may have reduced oxygen radical formation thereby diminishing persistent serotonergic deficits when administered within 1 d after the last self-administration session. When the binge of METH was administered 15 d after the last self-administration session, similar hyperthermia was achieved and similar serotonergic deficits occurred. Although alterations in hyperthermia may contribute to these findings, further research is needed to investigate the other mechanisms that may underlie these changes, such as alterations in glutamate regulation, corticosteroids, neurotrophic factors, and microglia activation (for reviews see Cadet and Krasnova, 2009; Fleckenstein et al., 2007; Tata et al., 2007; Yamamoto et al., 2010).

In summary, the results of the current study are the first to demonstrate that the self-administration of METH prior to experimenter-administered binge of METH administered 1 d after the last self-administration session attenuated the METH-induced serotonergic deficits 7 d following the METH binge challenge. The ability of prior self-administration to attenuate these deficits is likely attributable in part to its ability to attenuate binge-induced hyperthermia. In contrast, prior self-administration did not alter the acute effects of the binge METH treatment, suggesting that differences exist in mechanisms underlying the acute and persistent effects of the binge treatment. Further, no protection was afforded by prior METH self-administration when the binge of METH was given 15 d after the last self-administration session. These findings are of importance, since human addicts often escalate METH usage until they lose control of their ability to limit drug intake and resulting in binge-like exposure (Koob and Volkow, 2010). Although these data suggest that recent drug use may temporarily afford protection against METH-induced serotonergic deficits, our findings suggest that this protection is no longer effective if relapse to METH use occurs after prolonged abstinence. Lastly, these data provide a foundation to investigate complex questions including how the response of serotonergic neurons to METH may contribute to contingent-related disorders such as dependence and relapse.

Acknowledgments

Role of Funding Source

Funding for this study was provided by NIH grants: DA09407, 19447, 12267, 00869, 13389, 0422, 033097, and 00378; the NIH had no further role in study design; in the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the paper for publication.

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Figure 1.

METH self-administration attenuates the persistent effects of a subsequent binge METH treatment when administered in at a similar ambient temperature (22-23°C). Rats self-administered METH (0.12 mg/infusion) or saline (10 ul/infusion) for 7 d (8 h/d). Twenty-four h after the beginning of the final self-administration session, rats received METH (4 × 7.5 mg/kg/injection, s.c., 2-h intervals) or saline (1 ml/kg/injection, s.c., 2-h intervals; Panel C), were sacrificed 7 d later, and SERT function (Panel A) and 5HT tissue content (Panel B) were assessed in the hippocampus. ** $p < 0.05$ differs from all other groups; # $p < 0.05$ METH/METH differs from SAL/METH group

Figure 2.

METH self-administration attenuates the persistent effects of a subsequent binge METH treatment when administered in animals with a previous history of METH self-administration in a warmer environment (25°C). Rats self-administered METH (0.12 mg/infusion) or saline (10 ul/infusion) for 7 d (8 h/d). Twenty-four h after the beginning of the final self-administration session, rats received METH (4 × 7.5 mg/kg/injection, s.c., 2-h intervals) or saline (1 ml/kg/injection, s.c., 2-h intervals; Panel C), were sacrificed 7 d later, and SERT function (Panel A) and 5HT tissue content (Panel B) were assessed in the hippocampus. **p<0.05 differs from all other groups; *p<0.05 differs from SAL/SAL group; #p<0.05 METH/METH differs from SAL/METH group

Figure 3.

METH self-administration does not alter the acute effects of a subsequent binge METH treatment. Rats self-administered METH (0.12 mg/infusion) or saline (10 ul/infusion) for 7 d (8 h/d). Twenty-four h after the beginning of the final self-administration session, rats received METH (4×7.5 mg/kg/injection, s.c., 2-h intervals) or saline (1 ml/kg/injection, s.c., 2-h intervals; Panel C), were sacrificed 1 h after the last injection, and SERT function (Panel A) and 5HT tissue content (Panel B) were assessed in the hippocampus. * $p < 0.05$ differs from SAL/SAL group; # $p < 0.05$ METH/METH differs from SAL/METH group

Figure 4.

METH self-administration does not alter the persistent effects of a subsequent binge of METH when given 15 d after the last self-administration session. Rats self-administered METH (0.12 mg/infusion) or saline (10 μl/infusion) for 7 d (8 h/d). Fifteen days after the final self-administration session, rats received METH (4×7.5 mg/kg/injection, s.c., 2-h intervals) or saline (1 ml/kg/injection, s.c., 2-h intervals; Panel C), were sacrificed 7 d later, and SERT function (Panel A) and 5HT tissue content (Panel B) were assessed in the hippocampus. * $p < 0.05$ differs from SAL/SAL group

Figure 5.

Rats were treated the same as in Figures 2 (Panel A), 3 (Panel B), or 4 (Panel C). Striatal 5HT tissue content was assessed 7 d (Panel A) or 1 h (Panel B) after the binge treatment of METH administered 1 d after the start of the last self-administration session or 7 d (Panel C) after the binge treatment of METH administered 15 d after the last self-administration session. ** $p < 0.05$ differs from all other groups; * $p < 0.05$ differs from SAL/SAL group