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Prior methamphetamine self-administration attenuates the dopaminergic deficits caused by a subsequent methamphetamine exposure

Lisa M. McFadden [a], Paula L. Vieira-Brock [a], Glen R. Hanson [b], Annette E. Fleckenstein [b, *]

[a] Department of Pharmacology and Toxicology, University of Utah, Salt Lake City, UT 84112, United States
[b] School of Dentistry, University of Utah, Salt Lake City, UT 84112, United States

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ABSTRACT

Others and we have reported that prior methamphetamine (METH) exposure attenuates the persistent striatal dopaminergic deficits caused by a subsequent high-dose "binge" METH exposure. The current study investigated intermediate neurochemical changes that may contribute to, or serve to predict, this resistance. Rats self-administered METH or saline for 7 d. On the following day (specifically, 16 h after the conclusion of the final METH self-administration session), rats received a binge exposure of METH or saline (so as to assess the impact of prior METH self-administration), or were sacrificed without a subsequent METH exposure (i.e., to assess the status of the rats at what would have been the initiation of the binge METH treatment). Results revealed that METH self-administration *per se* decreased striatal dopamine (DA) transporter (DAT) function and DA content, as assessed 16 h after the last self-administration session. Exposure to a binge METH treatment beginning at this 16-h time point decreased DAT function and DA content as assessed 1 h after the binge METH exposure: this effect on DA content (but not DAT function) was attenuated if rats previously self-administered METH. In contrast, 24 h after the binge METH treatment prior METH self-administration: 1) attenuated deficits in DA content, DAT function and vesicular monoamine transporter-2 function; and 2) prevented increases in glial fibrillary acidic protein and DAT complex immunoreactivity. These data suggest that changes 24 h, but not 1 h, after binge METH exposure are predictive of tolerance against the persistence of neurotoxic changes following binge METH exposures.

1. Introduction

Methamphetamine (METH) is a widely abused psychostimulant that can cause persistent alterations in monoaminergic neuronal function. Preclinical studies demonstrate that exposure to high-dose METH, administered to mimic some aspects of human "binge" usage, causes persistent dopaminergic deficits. METH-induced alterations in dopamine (DA) content, DA transporter (DAT) function, vesicular monoamine transporter-2 (VMAT2) function/distribution, and tyrosine hydroxylase (TH) activity 1–24 h post-treatment may contribute to these long-term deficits (Brennan et al., 2010; Chu et al., 2010; Eyerman and Yamamoto, 2007; Hadlock et al., 2009, 2010; Hotchkiss and Gibb, 1980; McFadden et al., 2012a). Increases in extracellular glutamate, reactivity species and oxidative stress formation, and glial activation are also thought to contribute to the dopaminergic deficits following binge METH exposure (for reviews see Krasnova and Cadet, 2009; Tata and Yamamoto, 2007).

Researchers have noted that administration of escalating doses or intake of METH models human patterns of METH use (Fischman and Schuster, 1974; Schmidt et al., 1985). Further, preclinical studies indicate that pretreatment with escalating doses of METH attenuates the deficits induced by a large neurotoxic binge exposure to METH (Schmidt et al., 1985), including persistent losses of DA and serotonin (5HT) content (Cadet et al., 2009; Hodges et al., 2011; Johnson-Davis et al., 2003; McFadden et al., 2012a,b; Segal et al., 2003), DAT function/immunoreactivity/binding (Belcher et al.,

Abbreviations: DOPAC, 3,4-dihydroxyphenylacetic acid; DA, dopamine; DAT, dopamine transporter; GFAP, glial fibrillary acidic protein; METH, methamphetamine; 5HT, serotonin; TH, tyrosine hydroxylase; VMAT2, vesicular monoamine transporter-2.

* Corresponding author. Tel.: +1 801 585 7474; fax: +1 801 585 5111.
E-mail address: fleckenstein@hsc.utah.edu (A.E. Fleckenstein).

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2008; Krasnova et al., 2011; McFadden et al., 2012a,b; Segal et al., 2003), VMAT2 function (McFadden et al., 2011), and glial activation (McFadden et al., 2012a; Thomas et al., 2004). These studies are important since most individuals who abuse METH receive multiple exposures to the drug. However, relatively little is known of the mechanisms underlying the "neuroprotection" afforded by prior METH exposure.

Similar to the escalating-dose studies described above, our laboratory reported that prior METH self-administration reduced the persistent neurotoxic decreases in striatal DA content and DAT function caused by a subsequent binge METH exposure (McFadden et al., 2012a). These findings are of potential clinical relevance in that the resistance to binge-induced dopaminergic deficits caused by the repeated METH exposures may provide a model to explain why human METH abusers generally do not display dopaminergic deficits as great in magnitude as those resulting pre-clinically from a binge METH treatment, even after using large quantities of METH (McFadden et al., 2012a,b). Further, preclinical studies have suggested that contingent drug administration may lead to distinct neurochemical changes compared to non-contingent administration (Frankel et al., 2011; Hanson et al., 2012, 2013; Lominac et al., 2012; Stefanski et al., 2004).

Given the importance noted above of alterations 1–24 h post-METH treatment, the current study focused on such changes that may contribute to and/or predict this resistance to METH-induced deficits. Results revealed that METH self-administration *per se* decreased striatal DAT function and DA content, as assessed 16 h after the last self-administration session. Exposure to a binge METH treatment beginning at this 16-h time point decreased DAT function and DA content as assessed 1 h after the binge METH exposure: this effect on DA content, but not DAT function, was attenuated if rats were exposed previously to METH self-administration. In contrast, prior METH self-administration attenuated deficits in DA content, as well as DAT function and VMAT2 function as assessed 24 h after the binge treatment. Further, prior METH self-administration prevented increases in glial fibrillary acidic protein (GFAP) and DAT complex immunoreactivity compared to saline self-administrating/binge METH exposed rats at this 24 h time point. These findings suggest that striatal changes occurring 24 h after the binge exposure to METH are predictive of persistent deficits induced by a binge exposure to METH as previously reported (McFadden et al., 2012a).

2. Methods

2.1. Animals

Male Sprague–Dawley rats (275–300 g; Charles River Laboratories, Portage, MI) were housed four rats/cage (35 × 30 × 16 cm). Following surgery, each rat was individually housed in a transparent plastic cage (45 × 23 × 21 cm). 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 starting body weight. Rats were maintained under the same 14:10 h light/dark cycle in the animal facility and in the operant chambers. All experiments were approved by the University of Utah's Institutional Animal Care and Use Committee, in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Drugs

Racemic-METH hydrochloride (Research Triangle Institute; Research Triangle Park, NC) was dissolved in 0.9% sterile saline, with the dose described as the free-base form. Ketamine (90 mg/kg; Hospira Inc., Lake Forest, IL, USA) and xylazine (7 mg/kg; Sigma–Aldrich, St. Louis, MO, USA) were used to anesthetize animals. 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. (2012a). 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., 2011). Each rat received flunixin meglumine on the day of and 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. Catheter patency was confirmed by infusing 0.03 mL (20 mg/mL) of xylazine. The animals' food was reduced to 25 g the day before self-administration began. On all following days, animals were allowed to free feed.

2.4. Self-administration and METH challenge

Fig. 1 illustrates the time course of experiments. All rats underwent 7 d of self-administration (8 h/session; FR1; 0.12 mg/infusion METH or saline; with associated lever pressing presented as Supplemental Figure 1) 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) 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. METH self-administering rats were only included in the analysis 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 ≥2:1. These criteria were largely based upon those of Brennan et al. (2010). METH intake and body weight changes were similar between experiments and therefore representative pressing behavior and weights are illustrated in Fig. 2A and B. Animals were sacrificed 16 h after the end of the last self-administration session or received a binge of METH or saline as described below (Fig. 1).

Sixteen h after the end 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 followed by a binge exposure (see Danaceau et al., 2007; O'Neil et al., 2006) and to replicate previous methods (McFadden et al., 2012a,b). Previous studies (McFadden et al., 2012a,b) have demonstrated that hyperthermia is attenuated during the binge exposure to METH in animals that had previously self-administered METH. To promote hyperthermia, METH self-administering rats challenged with METH (METH/METH) were maintained in a warm environment (25 °C). Rats that self-administered saline prior to the binge of METH (Saline/METH) were maintained in 22.5 °C environment to promote similar hyperthermia between METH/METH and Saline/METH groups. Previous research in our laboratory has shown that this higher ambient temperature permits binge METH-induced neurotoxicity that would have otherwise been attenuated by prior exposure to METH during development (see McFadden et al., 2011). Representative temperature data during the binge exposure to METH are provided in Fig. 2C. Animals were sacrificed 1 h or 24 h after the binge exposure (Fig. 1).

2.5. Tissue preparation

Tissue preparation was conducted as previously described (Hanson et al., 2009). Following decapitation, the striata were quickly dissected out and the right striatum was homogenized in ice-cold sucrose buffer (0.32 M sucrose, 3.8 mM NaH_2PO_4, and 12.7 mM Na_2HPO_4). The left striatum was quickly frozen on dry ice.

2.6. Plasmalemmal & vesicular [^{3}H]DA uptake assays

[^{3}H]DA uptake assays were conducted according to Johnson-Davis et al. (2004). For plasmalemmal uptake of [^{3}H]DA, striatal synaptosomes were prepared accordingly and resuspended in ice-cold Krebs' buffer (126 nM NaCl, 4.8 mM KCl, 1.3 mM $CaCl_2$, 16 mM sodium phosphate, 1.4 mM $MgSO_4$, 11 mM dextrose, 1 mM ascorbic acid, pH 7.4). Assay tubes containing 1.5 mg striatal tissue and 1 μM pargyline were incubated (3 min, 37 °C) with [^{3}H]DA (0.5 nM final concentration, Perkin Elmer,

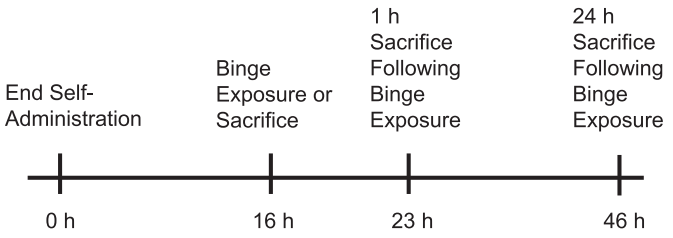

Fig. 1. Sacrifice times of rats in the manuscript. Animals were allowed to self-administer METH or Saline for 7 d. Sixteen hours after the last self-administration session rats were sacrificed or received a binge exposure to METH or Saline. Rats were sacrificed 1 h or 24 h after the binge exposure.

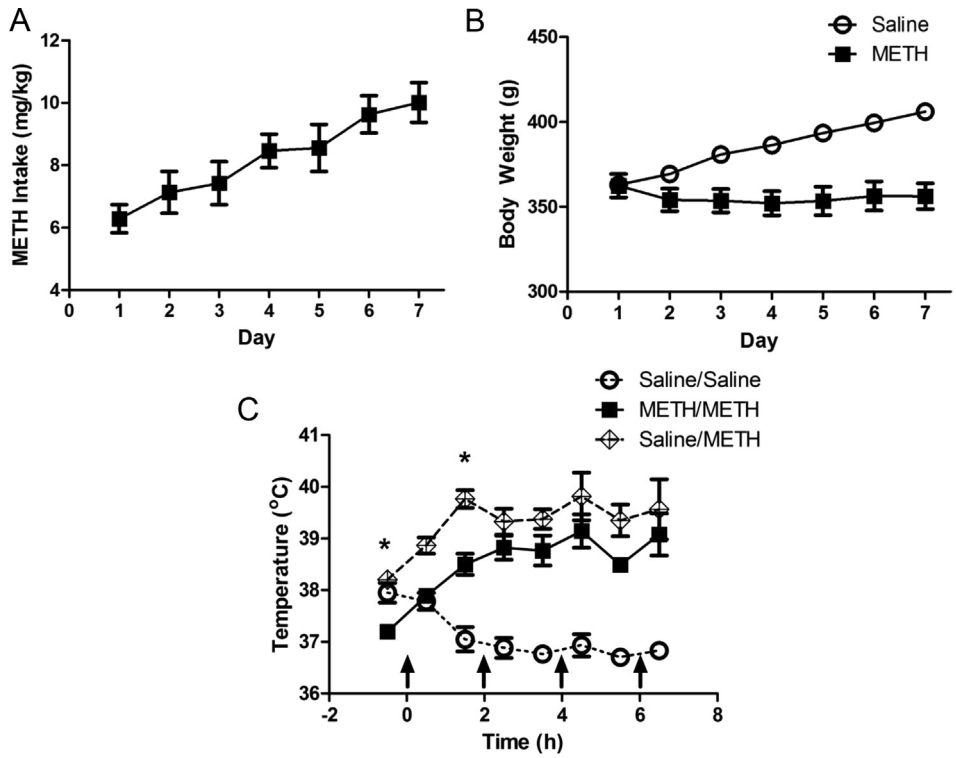

Fig. 2. Representative METH intake and body weight during self-administration, and hyperthermia during the binge exposure to METH. Rats were allowed to self-administer METH (0.12 mg/infusion; normalized to mg/kg) for 7 d (8 h/d; Panel A). Body weight was recorded daily (Panel B). Sixteen h after the last self-administration session rats received a binge exposure to METH (7.5 mg/kg) or saline (1 mL/kg) and hyperthermia was recorded 30 min and 90 min following each injection (as indicated by the arrows; Panel C). $^{*}p < 0.05$ METH/METH compared to Saline/METH.

Boston, MA). Nonspecific values were ascertained in the presence of 10 μM cocaine. Samples were filtered using a filtering manifold (Brandel, Inc., Gaithersburg, MD) through Whatman GF/B filters (Whatman International LTD, Maidstone, England) soaked previously in 0.05% polyethylenimine and washed three times with 3 mL of ice-cold 0.32 M sucrose.

For vesicular [^{3}H]DA uptake, synaptic vesicles were isolated according to Johnson-Davis et al. (2004) and resuspended in assay buffer (25 mM HEPES, 100 mM potassium tartrate, 1.7 mM ascorbic acid, 0.05 mM EGTA, 0.1 mM EDTA and 1.8 mM ATP-Mg^{2+}; pH 7.5). Vesicles were incubated (3 min, 30 °C) in the presence of [^{3}H]DA (30 nM final concentration, Perkin Elmer, Boston, MA). Nonspecific values were found by measuring vesicular [^{3}H]DA uptake at 4 °C in the absence of ATP. Samples were filtered through GF/F filters (Whatman International LTD, Maidstone, England) previously soaked in 0.5% polyethylenimine and washed 3 times with cold wash buffer. The radioactivity trapped in filters was counted using a liquid scintillation counter. Protein concentrations were determined using the Bradford Protein Assay.

2.7. Western blotting

Western blotting was conducted according to Hadlock et al. (2010). Equal quantities of protein (8 μg) were loaded into each well of a 4–12% NuPAGE Novex Bis–Tris Midi gradient gel (Invitrogen, Carlsbad, CA) and electrophoresed using an XCell4 Surelock Midi-cell (Invitrogen). Membranes were blocked for 30 min with Starting Block Blocking Buffer (Pierce Chemical, Rockford, IL) and incubated for 1 h at room temperature with a D2 antibody (SC-7522; Santa Cruz Biotechnology, Santa Cruz, CA), DAT antibody (a generous gift from Dr. Roxanne Vaughan, University of North Dakota; Freed et al., 1995), or GFAP (556329; BD Biosciences, San Jose, CA). The polyvinylidene difluoride membrane was then washed five times in Tris-buffered saline with Tween (250 mM NaCl, 50 mM Tris, and 0.05% Tween 20, pH 7.4). The membranes were then incubated for 1 h with a horseradish peroxidase-conjugated secondary antibody (BioSource International, Camarillo, CA). After five washes in Tris-buffered saline with Tween, the bands were visualized using Western Lightning Chemiluminescence Reagents Plus (PerkinElmer Life and Analytical Sciences) and were quantified by densitometry using a FluorChem SP Imaging System (Alpha Innotech, San Leandro, CA). Protein concentrations were determined using the Bradford Protein Assay.

2.8. DA and 3,4-dihydroxyphenylacetic acid (DOPAC) content

The anterior portion of the left striatum was sonicated for 3–5 s in 1 mL tissue buffer (0.1 M phosphate/citrate buffer, pH = 2.5, containing 10% methanol) and were prepared according to Haughey et al. (2000). Fifty μL was injected onto a partisphere C-18 reverse-phase analytical column (5-μm spheres; 250 × 4.6 mm; Whatman, Clifton, NJ, USA). For vesicular DA content, vesicles were prepared as described in Chu et al. (2010), resuspended in tissue buffer and 100 μL was injected. Mobile phase consisting of 0.05 M sodium phosphate, 0.03 M citrate buffer, 0.1 M EDTA, 0.035% sodium octylsulfate, and 15% methanol (pH = 2.8, flow rate = 0.7 mL/min) was used. DA and DOPAC was detected using an amperometric electrochemical detector with the working electrode potential set at +0.70 V relative to an Ag^{+}/AgCl reference electrode. Protein concentrations were determined using the Bradford Protein Assay for tissue content. Vesicular content was normalized to wet weights.

2.9. Statistical analysis

Statistical analysis was conducted in GraphPad Prism (5.1; La Jolla, CA, USA). Statistical analyses among groups were conducted using a t-test or one-way analysis of variance (ANOVA) followed by Newman–Keuls posthoc analyses. The data represent means ± standard error of the mean (S.E.M.) of 5–11 rats/group.

3. Results

METH self-administration decreased DAT function and striatal DA content compared to saline self-administration when assessed 16 h after the last session (Fig. 3). METH self-administration decreased DAT ($t(13) = 3.63$, $p < 0.05$; Fig. 3A) function, but VMAT2 function was not altered in either the cytoplasmic ($t(10) = 1.43$, ns; Saline: 110.20 ± 8.03 fmol DA/μg protein; METH: 93.33 ± 7.73 fmol DA/μg protein) or membrane-associated fractions ($t(10) = 0.68$, ns; Saline: 13.50 ± 1.15 fmol DA/μg protein; METH: 12.50 ± 0.88 fmol DA/μg protein). A trend towards a decrease in dopamine D2 receptor immunoreactivity was observed in the METH self-administering group compared to the saline controls ($t(12) = 1.84$, $p = 0.09$; Fig. 3B). A slight but significant decrease in DA ($t(13) = 2.41$, $p < 0.05$; Fig. 3C), as well as an increase in DOPAC ($t(13) = 2.56$, $p < 0.05$; Fig. 3D) content was observed in METH self-administering compared to saline self-administering rats.

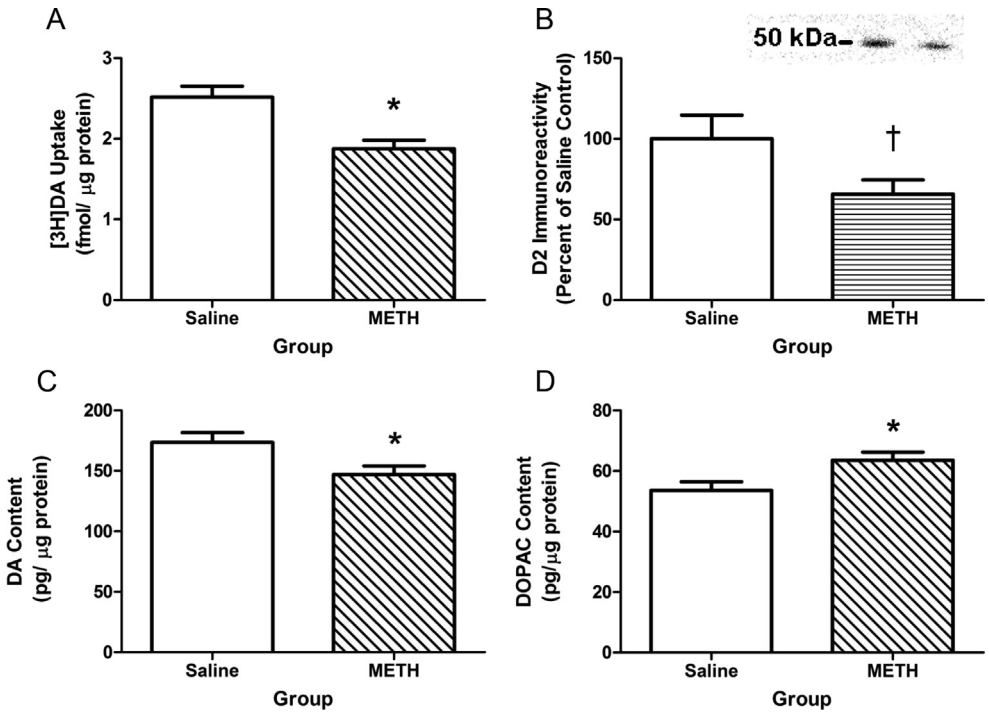

Fig. 3. Dopaminergic changes in the striatum 16 h following the last self-administration session. Rats were allowed to self-administer METH (0.12 mg/infusion) or saline (10 µL/infusion) for 7 d (8 h/d) and were sacrificed 16 h after the last session. METH self-administration resulted in a reduction in DAT function (Panel A) and a trend toward a reduction in D2 receptor immunoreactivity (Panel B). Striatal DA content was reduced in METH self-administering rats and DOPAC content was increased compared to saline self-administering rats (Panels C & D). Insert: Representative D2 immunoreactivity of Saline (left) and METH (right) self-administering rats depicted in Panel B. $^{*}p < 0.05$ compared to saline self-administrating rats. †$p < 0.10$ compared to saline self-administering rats.

Prior METH self-administration attenuated the decrease in DA content, but not striatal DAT function, as assessed 1 h following the binge of METH (Fig. 4). Specifically, the binge METH exposure similarly decreased DAT function in both binge METH groups ($F(2,24) = 52.75$, $p < 0.05$; Fig. 4A). Prior METH self-administration attenuated the decrease in striatal DA content ($F(2,24) = 18.00$, $p < 0.05$; Fig. 4B). Of note, prior METH self-administration did not alter binge METH-induced decreases in striatal DOPAC concentrations compared to the Saline/METH group ($F(2,24) = 47.09$, $p < 0.05$; Fig. 4C).

To further investigate the attenuation in DA content, vesicular content was determined in animals sacrificed 1 h following the binge exposure to METH. Prior METH self-administration did not alter the binge METH-induced decreases in cytosolic ($F(2,20) = 21.41$, $p < 0.05$; Fig. 5A) or membrane-associated ($F(2,20) = 8.99$, $p < 0.05$; Fig. 5B) vesicular DA content. Similarly, prior METH self-administration did not attenuate the binge METH-induced decreases in cytosolic VMAT2 immunoreactivity ($F(2,19) = 7.06$, $p < 0.05$; Fig. 5C). As in other experiments described in this manuscript and of interest because of its role in METH-induced toxicity, binge METH-induced hyperthermia was attenuated in the METH/METH group 1 h following the first injection of METH as assessed in the METH/METH group compared to the Saline/METH group in these animals ($F(14,140) = 7.13$, $p < 0.05$; Fig. 2B).

In contrast to effects at 1-h, prior METH self-administration attenuated all of the binge METH-induced alterations investigated as assessed 24 h after treatment. In particular, prior METH self-administration attenuated decreases in DAT function ($F(2,16) = 17.49$, $p < 0.05$; Fig. 6A), DA content ($F(2,17) = 77.77$, $p < 0.05$; Fig. 6B) and DOPAC content ($F(2,18) = 32.92$, $p < 0.05$; Fig. 6C) compared to the Saline/METH group. Further, prior METH self-administration attenuated binge METH-induced decreases in cytosolic VMAT2 function ($F(2,16) = 28.96$, $p < 0.05$; Fig. 7A), membrane-associated VMAT2 function ($F(2,18) = 30.50$, $p < 0.05$; Fig. 7B) and membrane-associated VMAT2 DA content ($F(2,21) = 71.09$, $p < 0.05$; Saline/Saline: 104.5 ± 2.62 pg DA/mg wet weight; METH/METH: 55.98 ± 2.51 pg DA/mg wet weight; Saline/METH 44.53 ± 4.39 pg DA/mg wet weight). Prior METH self-administration also attenuated binge METH-induced increases in GFAP ($F(2,18) = 3.55$, $p = 0.05$; Fig. 8A) and DAT complex immunoreactivity ($F(2,18) = 6.92$, $p < 0.05$; Fig. 8B) at this 24-h time point. Consistent with DA uptake, prior METH self-administration also attenuated decreases in DAT monomer immunoreactivity ($F(2,18) = 28.28$, $p < 0.05$; Saline/Saline: 197.20 ± 54.41 arbitrary units; METH/METH: 117.70 ± 38.71 arbitrary units; Saline/METH: 44.56 ± 12.40 arbitrary units).

4. Discussion

Previous studies have demonstrated that prior METH self-administration attenuates the persistent dopaminergic deficits caused by a subsequent binge METH exposure (McFadden et al., 2012a). We have reported that this protective effect was not likely due to alterations in the pharmacokinetics of METH caused by prior METH self-administration (McFadden et al., 2012a). Further, the attenuation of binge METH-induced hyperthermia afforded by prior METH self-administration likely contributes to protection. Still, additional mechanisms underlying this phenomenon have not been elucidated. The present study focused attention on dopaminergic deficits occurring prior to and 1–24 h after a binge METH treatment, as these have been suggested to be important contributors to and/or predictors of METH-induced persistent dopaminergic deficits (McFadden et al., 2012a).

Striatal dopaminergic changes induced by METH self-administration were assessed 16 h after the last self-

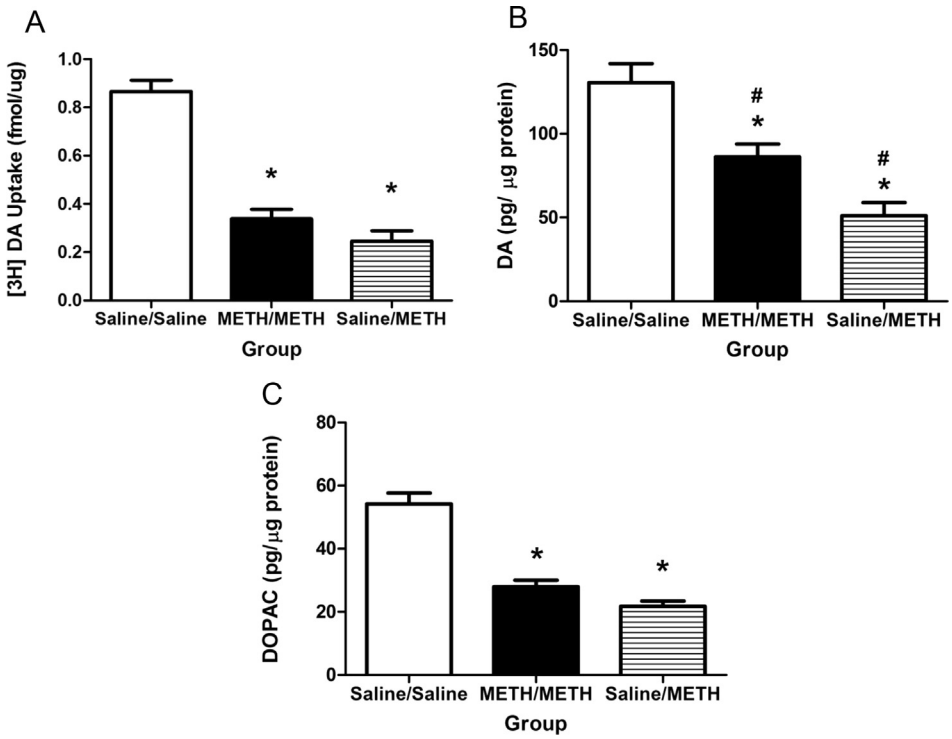

Fig. 4. Prior METH self-administration attenuated decreases in DA content but not DAT function 1 h after binge METH exposure. Rats were allowed to self-administer METH (0.12 mg/infusion) or saline (10 µL/infusion) for 7 d (8 h/d), received a binge exposure to METH (7.5 mg/kg/injection; 4 injections) 16 h after the last session, and were sacrificed 1 h following the last injection. Binge exposure to METH similarly reduced DAT function in the METH/METH and Saline/METH groups (Panel A). Prior METH exposure attenuated reductions in striatal DA content (Panel B) following the binge exposure, but did not attenuate decreases in DOPAC content (Panel C). *$p < 0.05$ compared to Saline/Saline. #$p < 0.05$ compared to all other groups.

administration session to determine if preexisting dopaminergic changes may have contributed to differences following the binge exposure (Fig. 3). Consistent with previous reports using escalating METH intake and measuring short-term effects, the current study found no changes in VMAT2 function (Laćan et al., 2013). Slight decreases in DA content also occurred in METH self-administering rats that were associated with increases in DOPAC content. In contrast to these previously mentioned effects, DAT function was decreased in METH self-administering rats when animals were sacrificed at this time point. Inhibiting the DAT with selective drugs such as amfonelic acid attenuates persistent DA depletions and inhibition of TH activity caused by binge METH treatments (Marek et al., 1990; Schmidt and Gibb, 1985). Thus, the decreased DAT function caused by METH self-administration present at the time of the initiation of the binge METH exposure may have acted similarly to amfonelic acid and thereby contribute to tolerance against METH-induced dopaminergic deficits. Alternatively, altering DAT densities or function has been shown to affect DA release after administration of the METH-related stimulant, amphetamine (Calipari et al., 2013; Siciliano et al., 2014). Reduction of DAT function in the current study may have reduced the impact of METH during the binge exposure and thus contributed to reductions in its neurotoxic effects.

The observed changes in dopaminergic markers at 1 h following the binge METH exposure suggest that the acute pharmacological effects of METH on DAT and VMAT2 are unaltered by prior METH exposure (Figs. 4 and 5). Similar decreases in DAT function and decreases in vesicular content 1 h following the binge exposure to METH suggest that the ability of METH to inhibit DAT function and redistribute DA from the vesicles to the cytosol are unaffected by prior METH self-administration. Previously our laboratory has reported similar brain METH content in the METH/METH and Saline/METH groups, further suggesting that many of the acute effects of the binge METH exposure are unaltered (McFadden et al., 2012a).

Of interest, DA content was approximately 25% higher in the METH/METH group compared to the Saline/METH group 1 h following the binge exposure (Fig. 4B). Prior exposure to METH may have attenuated METH's ability to decrease TH activity (Schmidt et al., 1985). A one week escalating-dose pretreatment paradigm attenuated decreases in TH activity 18 h following the binge exposure and was associated with increases in DA content (Schmidt et al., 1985). A similar phenomenon could also occur when METH is self-administered prior to the binge exposure, such that TH activity is not inhibited as greatly in the METH/METH group compared to the Saline/METH group.

No differences in vesicular DA content were found in the METH/METH group in comparison to the Saline/METH group 1 h following the binge exposure to METH (Fig. 5A and B), which suggests that the 25% increase in total DA content may not reflect DA sequestered in vesicles, but rather in the cytosol of the dopaminergic neurons. Previous work suggests decreasing DA sequestered by vesicles increases METH-induced toxicity (Guillot et al., 2008; Thomas et al., 2008). Inhibited VMAT2 function either by prior treatment with reserpine or through genetic manipulation leads to increased markers of striatal METH-induced toxicity including greater loss in DA content, and DAT and TH immunoreactivity, and increases in GFAP immunoreactivity and oxidative stress (Guillot et al., 2008; Thomas et al., 2008). However, these effects lasted for at least 2 d following the METH exposure (Guillot et al., 2008; Thomas et al., 2008). In the current study, many of these markers showed signs of recovery at 24 h following the binge METH exposure in the METH/METH group which may have minimized the potentially toxic effects of the unsequestered DA.

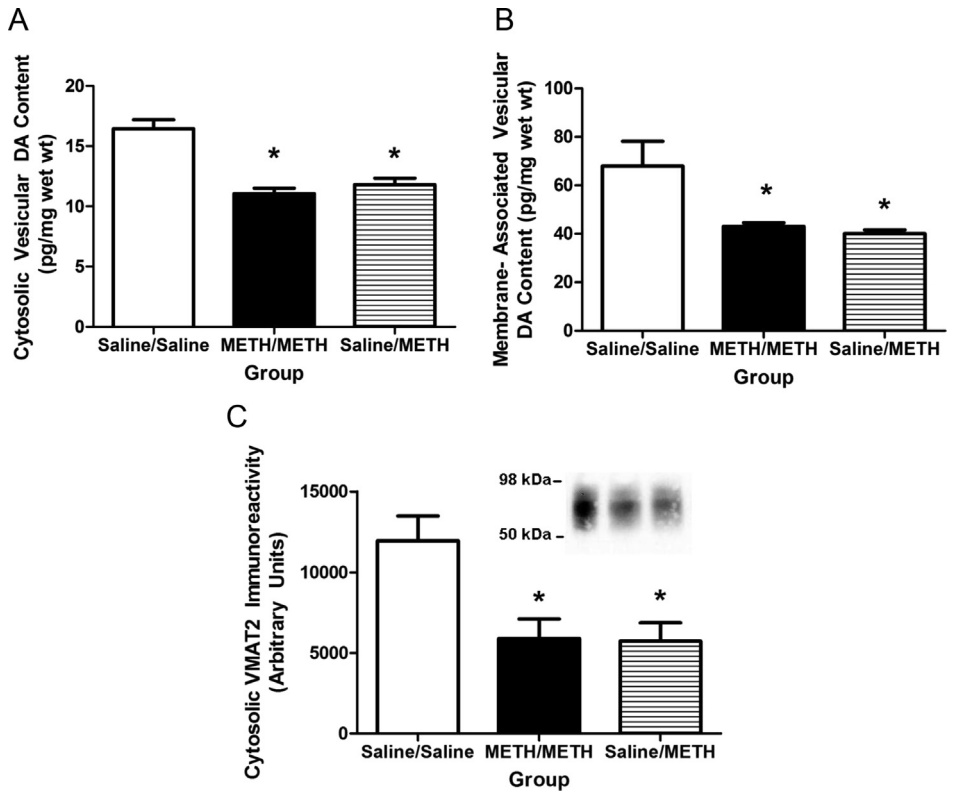

Fig. 5. Binge METH exposure resulted in similar decreases in vesicular DA content and cytosolic VMAT2 immunoreactivity 1 h following binge exposure. Rats were treated as described in Fig. 4. Prior METH self-administration did not alter reductions in cytosolic vesicular DA content (Panel A) or membrane-associated vesicular DA content (Panel B) following the binge exposure to METH. Similar reductions in cytosolic VMAT2 immunoreactivity were observed between the METH/METH and Saline/METH group (Panel C). Insert: Representative VMAT2 immunoreactivity of Saline/Saline, METH/METH and Saline/METH (left to right) shown in Panel C. $^{*}p < 0.05$ compared to Saline/Saline.

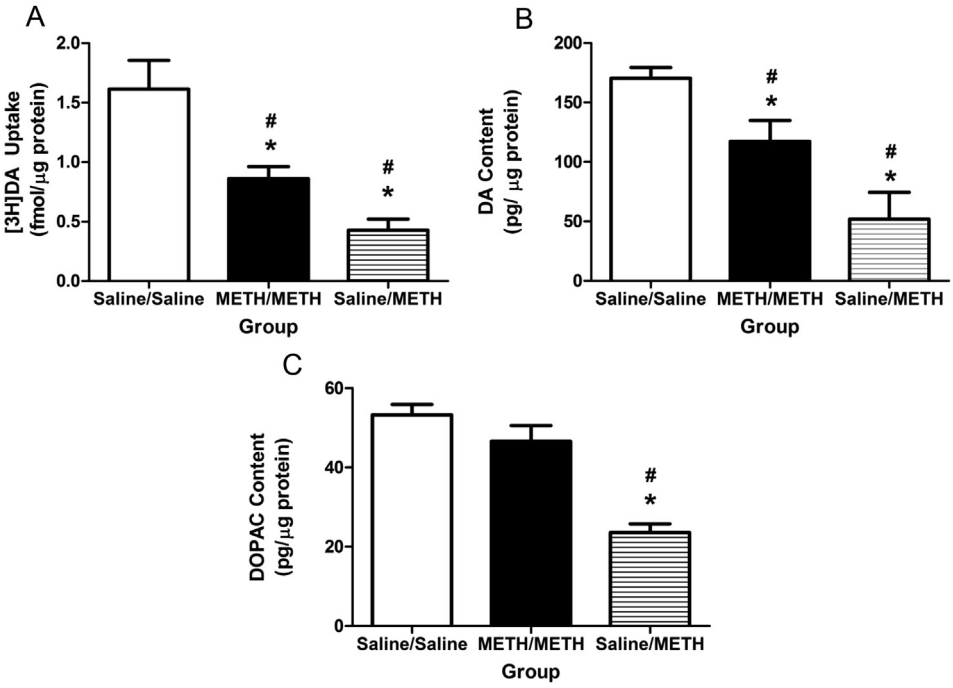

Fig. 6. Prior METH self-administration attenuated dopaminergic deficits 24 h following binge METH exposure. Rats were allowed to self-administer METH or saline for 7 d, received a binge exposure to METH or saline, and were sacrificed 24 h after the last injection. Prior METH self-administration attenuated binge METH-induced deficits in DAT function (Panel A), DA content (Panel B), and DOPAC content (Panel C). $^{*}p < 0.05$ compared to Saline/Saline. $\#p < 0.05$ compared to all other groups.

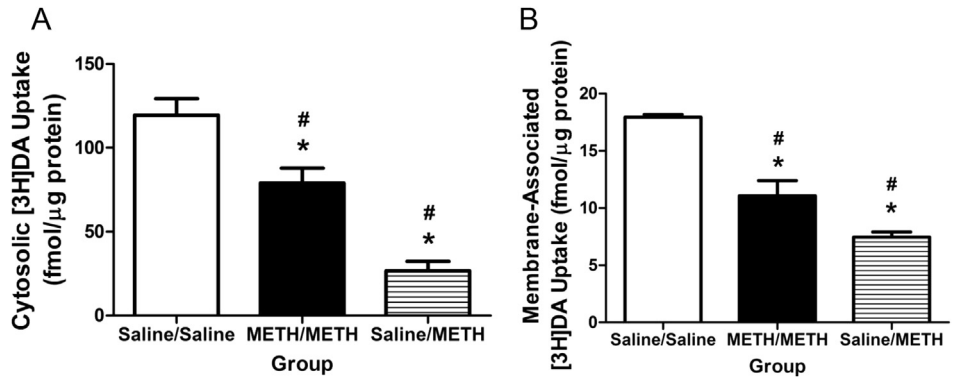

Fig. 7. Prior METH self-administration attenuated VMAT2 functional deficits induced by a binge exposure to METH as assessed 24 h later. Rats were treated as described in Fig. 6. Striatal VMAT2 function was assessed in the cytosolic fraction (Panel A) and membrane-associated fraction (Panel B).

As previously mentioned, VMAT2 is thought to play a pivotal role in METH toxicity (Fumagalli et al., 1999; Guillot et al., 2008; Thomas et al., 2008). An important finding of the current study is that prior exposure to METH through self-administration is associated with attenuated deficits in VMAT2 function at 24 h following the binge exposure (Fig. 7). The attenuation in deficits of VMAT2 function may have decreased reactive species formation and METH-induced toxicity. Previous research suggests that protein-bound DA quinones are elevated 3 d and 14 d following binge exposure but not at 1 d following METH in mice (Miyazaki et al., 2006), suggesting that DA quinone formation may increase with time. In the current study, the attenuation of the reductions in VMAT2 function observed in the METH/METH group compared to the Saline/METH group 24 h after the binge exposure may have mitigated the formation of DA quinones by sequestering DA into the vesicles.

METH self-administration prior to binge-METH exposure blocked the formation of DAT complexes as assessed at 24 h and 7 d after the binge exposure (Fig. 8B; McFadden et al., 2012a). This mitigation in the formation of DAT complexes was also associated with an attenuation in binge METH-induced decreases in DAT function (Fig. 6A). Reactive species have been implicated in the formation of DAT complexes, further implicating their role in the toxicity to METH (Baucum et al., 2004; Hadlock et al., 2009). Pre-treatment with D2 receptor antagonists also attenuated DAT complex formation (Hadlock et al., 2009, 2010), as well as the long-term deficits caused by METH. METH self-administration decreases D2 receptor function (Laćan et al., 2013). This decrease in D2 receptors caused by METH self-administration may cause pharmacological effects similar to that of a D2 receptor antagonist and result in a decrease in the formation of DAT complexes. Thus, the reduction in D2 receptors may contribute to the protection afforded by METH self-administration by reducing the formation of DAT complexes and increasing DAT function.

Astrocytes are important regulators glutamate (Cisneros and Ghorpade, 2012; Lau et al., 2000). Of relevance, METH also induces reactive astrogliosis (McFadden et al., 2012a; Hadlock et al., 2010; Thomas et al., 2004; Zhu et al., 2005). Current (Fig. 8A) and previous studies suggest that reactive astrogliosis is significantly increased in the Saline/METH group (McFadden et al., 2012a), and is attenuated by prior METH self-administration. It can thus be speculated that the lack of reactive astrogliosis in the METH/METH group may be an indicator of a reduction in glutamate release caused by the binge exposure of METH.

In conclusion, the current study provides novel insight into the changes associated with tolerance to the neurotoxic effects of METH within the striatum. Effects occurring 24 h after the binge METH exposure appear particularly linked to persistent neurotoxicity, although it is unclear if these effects actually contribute to the persistent dopaminergic deficits or are a result of other changes that may contribute to this toxicity. It is also unclear if the neuroprotection afforded by prior contingent METH administration

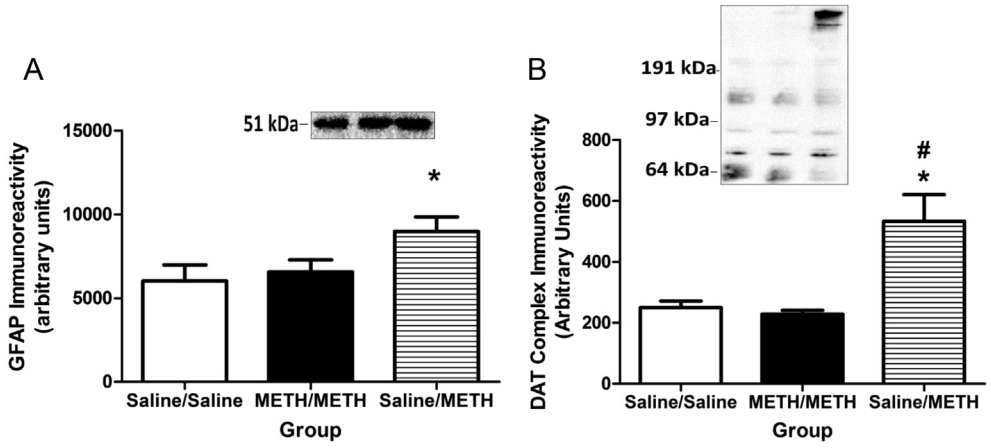

Fig. 8. Other markers of METH-induced toxicity were not elevated in rats that self-administered METH prior to the binge exposure as assessed 24 h later. Rats were treated as described in Fig. 6. Increases in striatal GFAP (Panel A; Insert: Representative GFAP immunoreactivity of Saline/Saline, METH/METH, and Saline/METH [left to right] exposed rats) and DAT complexes immunoreactivity (Panel B; Insert: Representative DAT complex immunoreactivity spanning from above 97 kDa to the top of the gel for Saline/Saline-, METH/METH-, and Saline/METH-exposed rats [left to right]).

differs in magnitude from the effects of prior non-contingent METH exposure. This is an important issue, as that the learned associations between the physiological effects of the drug and the behavioral output may contribute to differences among the various paradigms. Of note, there is precedence for differences between contingent and noncontingent METH exposure. For example, prior METH self-administration increases levels of the neuropeptide, neurotensin, compared to yoked-METH rats (Frankel et al., 2011; Hanson et al., 2012). Given the role of neurotensin as a neuromodulator of DA, differential neuroprotection as a consequence of contingency is indeed possible and an important area for future study. Future studies are needed to elucidate how these dopaminergic changes at 24 h following binge METH exposure are associated with tolerance to the neurotoxic events following binge METH exposure and if these changes are associated with contingency.

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Authorship contributions

Participated in research design: McFadden, Vieira-Brock, Hanson, and Fleckenstein. Conducted experiments: McFadden and Vieira-Brock. Performed data analysis: McFadden. Wrote or contributed to the writing of the manuscript: McFadden, Vieira-Brock, Hanson, and Fleckenstein. All authors contributed to and have approved the final manuscript.

Appendix A. Supplementary material

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.neuropharm.2015.01.013.

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