Methamphetamine self-administration causes persistent striatal dopaminergic alterations and mitigates the deficits caused by a subsequent methamphetamine exposure


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Text Pages: 34
Tables: 0
Figures: 7
References: 39
Abstract: 237
Introduction: 647
Discussion: 1440

Abbreviations: METH, methamphetamine; DA, dopamine; DAT, dopamine transporter; VMAT-2, vesicular monoamine-2 transporter; FR, fixed ratio; LP, low-pressing; HP, high-pressing;
GFAP, glial fibrillary acidic protein; TH, tyrosine hydroxylase

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Abstract

Preclinical studies have demonstrated that repeated methamphetamine (METH) injections (referred to herein as a “binge” treatment) cause persistent dopaminergic deficits. A few studies have also examined the persistent neurochemical impact of METH self-administration in rats, but with variable results. These latter studies are important because: 1) of their relevance to the study of METH abuse; and 2) the effects of non-contingent METH treatment do not necessarily predict effects of contingent exposure. Accordingly, the present study investigated the impact of METH self-administration on dopaminergic neuronal function. Results revealed that self-administration of METH, given according to a regimen that produces brain METH levels comparable to those reported post-mortem in human METH abusers (0.06 mg/infusion, 8-h sessions for 7 d), decreased striatal dopamine (DA) transporter (DAT) uptake and/or immunoreactivity as assessed 8 or 30 d after the last self-administration session. Increasing the METH dose per infusion did not exacerbate these deficits. These deficits were similar in magnitude to decreases in DAT densities reported in imaging studies of abstinent METH abusers. Importantly, METH self-administration mitigated the persistent deficits in dopaminergic neuronal function, as well as the increases in glial fibrillary acidic protein immunoreactivity, caused by a subsequent “binge” METH exposure. This protection was independent of alterations in METH pharmacokinetics, but may have been attributable (at least in part) to a pretreatment-induced attenuation of binge-induced hyperthermia. Taken together, these results may provide insight into the neurochemical deficits reported in human METH abusers.
Introduction

Methamphetamine (METH) is a widely abused psychostimulant that can cause persistent alterations in monoaminergic neuronal function. For example, imaging studies indicate that METH abusers display a 15 – 25% decrease in caudate dopamine (DA) transporter (DAT) density (Chang et al, 2007; Volkow et al., 2001) that can persist years (McCann et al., 1998), as well as variable effects on caudate vesicular monoamine transporter-2 (VMAT-2) density (Boileau et al., 2008; Johanson et al., 2006). Postmortem studies of METH abusers also indicate variable changes in caudate VMAT-2 immunoreactivity or binding, as well as decreased tyrosine hydroxylase (TH) and DAT immunoreactivity (Kitamura et al., 2007; Wilson et al., 1996). However, the magnitude of alterations in DAT in the post-mortem studies is generally greater than in the imaging studies cited above, perhaps owing to the presence of METH in the brains of the former. Alternatively, abusers assessed postmortem may have been exposed to greater METH levels than those assessed in imaging studies (Melega et al., 2007). Differences in the amount or presence of METH in the brain may contribute to differences in findings between post-mortem studies and imaging studies.

As in humans, METH can cause persistent dopaminergic deficits in rodent models. For example, administration of a “binge” METH regimen (e.g., 4 - 6 injections, 7.5 - 15 mg/kg/injection, 2 - 6-h intervals): 1) decreases DAT and VMAT-2 binding, immunoreactivity, and function; and 2) decreases DA content and TH immunoreactivity (Brown et al., 2000; Cappon et al., 2000; Eyerman & Yamamoto, 2007). Binge METH treatment increases glial fibrillary acidic protein (GFAP) expression as well (Hadlock et al., 2010).

Importantly, although the binge model has provided insight into both METH-induced dopaminergic deficits and DA-related degenerative disorders, its associated neurochemical
alterations are frequently greater in magnitude than those reported in human imaging studies (see Brennan et al. 2010 & Chang et al., 2007). These and other data demonstrate the importance of evaluating the impact of METH self-administration (vs. binge administration) in that the effects of non-contingent METH treatment do not necessarily predict effects of contingent exposure (Brennan et al., 2010; Frankel et al., 2011). Accordingly, the present study investigated the impact of METH self-administration on dopaminergic neuronal function.

Of relevance are preclinical findings that pretreatment with intermittent low-dose or multiple escalating-dose METH injections attenuates the dopaminergic deficits caused by a subsequent binge treatment (McFadden et al. 2011, and references therein). Similarly, a binge pretreatment protects against both acute and persistent dopaminergic deficits caused by a second binge treatment (Hanson et al., 2009; Thomas & Kuhn, 2005). These studies are important, since most individuals who abuse METH receive multiple exposures to the drug. However, little is known regarding mechanisms underlying these “neuroprotective” changes.

As noted above, recent studies have focused on effects of METH self-administration in the rodent model. Some (Schwendt et al., 2009; Krasnova et al., 2010), but not all investigators (Brennan et al., 2010; Shepard et al., 2006; Stefanski et al., 2002), reported persistent dopaminergic deficits caused by self-administration. For example, Schwendt et al. (2009) reported relatively small “neuroadaptations” that were specific to the DAT. In contrast, Krasnova et al. (2010) reported larger deficits in several monoaminergic markers, including the DAT. Given these disparate findings, and again noting that the effects of non-contingent METH treatment do not necessarily predict effects of contingent exposure (Brennan et al., 2010; Frankel et al., 2011), one purpose of the present study was to further investigate the neurochemical impact of METH self-administration. A second purpose was to investigate if repeated self-exposure to METH, like effects observed after intermittent low-dose or multiple
escalating-dose injections, influences the expression of dopaminergic deficits caused by a subsequent binge METH treatment. Lastly, one purpose of this study was to investigate possible mechanisms underlying the changes in dopaminergic deficits following a subsequent binge treatment of METH. The results of the current study may provide important insight into the neurochemical deficits reported in human METH abusers.
Methods

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 Institutional Animal Care and Use Committee, in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Drugs (±)-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. Equithesin (3 ml/kg) or ketamine (90 mg/kg; Hospira Inc., Lake Forest, IL, USA) and xylazine (7 mg/kg; Sigma-Aldrich, St. Louis, MO, USA) was 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.

Apparatus Food training and self-administration occurred in an operant chamber (30.5 cm x 25.5 cm x 30.5 cm; Coulbourn Instruments, Whitehall, PA USA) located within a sound-attenuating cubical (79 cm x 53 cm x 53 cm; Coulbourn Instruments). Each chamber was equipped with a food pellet hopper, two retractable levers, and house light (Coulbourn Instruments).
Food Training Prior to surgery, each rat was trained to press for a 45-mg food pellet during four overnight 14-h sessions. During the first 2 h of each session, a food pellet was dispensed every 90 s or after an active lever press, whichever came first. Each time the pellet was dispensed, the levers were retracted for 20 s. For the remaining 12 h, food pellets were dispensed based on a FR1 schedule of reinforcement. Upon each active lever press, both levers were retracted for 20 s and a food pellet was dispensed. Inactive lever presses were recorded, but had no programmable consequence. On each day the active lever was counterbalanced among all the rats such that half of the rats had the right lever as the active lever and the other half had the left lever as active. For each rat, the active lever was changed from day to day such that each lever was reinforced for 2 d of training.

Catheters & Surgery The catheter was constructed in the laboratory as previously described in Frankel et al. (2011). Rats were anesthetized and an indwelling catheter was implanted. 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 (s.c.) 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 the antibiotic cefazolin followed by 0.05 ml of heparinized saline and heparinized glycerol through the catheter. Catheter patency was checked by infusing 0.03 ml (20 mg/ml) of xylazine.

Self-Administration Rats underwent 7 d of self-administration (8 h/session; FR1) 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 (Coulbourn Instruments) connected to a liquid swivel (Coulbourn Instruments) suspended outside the operant chamber delivered 10 µl of METH (0.06, 0.12, or 0.24 mg/infusion) 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, but remained the same for each rat from day to day. 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 and 30 and 90 after each METH injection.

**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 NaH₂PO₄, and 12.7 mM Na₂HPO₄). The left striatum was quickly frozen on dry ice.

**Plasmalemmal & Vesicular [³H]DA Uptake Assays** [³H]DA uptake assays were conducted according to Johnson-Davis et al. (2004). For plasmalemmal uptake of [³H]DA, striatal synaptosomes were prepared accordingly and resuspended in ice-cold Krebs’ buffer (126 mM NaCl, 4.8 mM KCl, 1.3 mM CaCl₂, 16 mM sodium phosphate, 1.4 mM MgSO₄, 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 [7,8-³H]DA (0.5 nM final concentration, Perkin Elmer, 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 [³H]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\(^{\circ}\)C) in the presence of [7,8-\(^{3}\)H]DA (30 nM final concentration, Perkin Elmer, Boston, MA). Nonspecific values were found by measuring vesicular [\(^{3}\)H]DA uptake at 4\(^{\circ}\)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.

**Western Blotting** Western blotting was conducted according to Hadlock et al. (2009). Equal quantities of protein (8 µg) were loaded into each well of a 4 to 12% NuPAGE Novex Bis-Tris Midi gradient gel (Invitrogen, Carlsbad, CA) and electrophoresed using a 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 DAT antibody (a generous gift from Dr. Roxanne Vaughan, University of North Dakota; Freed et al., 1995), GFAP (556329; BD Biosciences, San Jose, CA), or TH (MAB418; Millipore, Billerica, MA). The polyvinylidene difluoride membrane was then washed five times in Tris-buffered saline with Tween (250 mM NaCl, 50 mM Tris, pH 7.4, and 0.05% Tween 20). 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. Immunoreactivity was normalized from arbitrary units to percent of saline control.
DA 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 15-20% 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 X 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, 0.035% sodium octylsulfate, and 15-20% methanol (pH= 2.8, flow rate=0.75 ml/min) was used. DA was detected using an amperometric electrochemical detector with the working electrode potential set at +0.73 V relative to an Ag+/AgCl reference electrode.

METH Concentrations  Brain METH concentrations were measured by liquid chromatography-tandem mass spectrometry (LC-MS-MS) as previously described (Truong et al., 2005). The whole brains (except for the striatum, hippocampus and frontal cortex) were weighed and homogenized separately in 10 ml water. A Vibra Cell homogenizer (Sonics, Newton, CT) was used for the homogenization. A 0.5 ml volume of the homogenate was used for the analysis. An Agilent Liquid Chromatograph (Agilent, Santa Clara, CA) coupled to a ThermoQuest Finnigan TSQ 7000 tandem mass spectrometer (Therm Electron Corporation, Waltham, MA) was used for the analysis. Electrospray ionization was employed. The limit of quantitation was 1 ng/ml in the homogenates.

Statistical Analysis  All samples within a given experiment were processed concurrently. Statistical analysis was conducted in SAS 9.2 (Cary, NC, USA). Statistical analyses among groups were conducted using a t-test, one-way analysis of variance (ANOVA; neurochemical data/ self-administration temperature data) or repeated measures ANOVA (lever pressing/binge temperature data) followed by Newman-Keuls posthoc analyses. Violations of the sphericity assumption resulted in the use of a Huynh-Feldt correction. Neurochemical analyses were
normalized to the percent of saline self-administering rats. The data represent means ±
standard error of the mean (S.E.M.) of 3-21 rats/group.
Results

For analysis of data presented in Figure 1, rats self-administering METH (0.06 mg/infusion) were divided into low- and high-pressing rats (Figure 1A) based largely on the criteria set by Brennan et al. (2010). Specifically, rats were considered high-pressers (HP) 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. Rats that did not achieve these criteria were considered low-pressers (LP). Rats that met HP criteria discriminated between the active and inactive levers at a ratio as much as 9:1 beginning at d 4 and also pressed the active lever significantly more than the saline self-administering animals beginning on d 4. Of note, due to a low number of rats meeting the LP criteria in this and other experiments (e.g., presented in Figures 2 – 6), only data from HP rats are provided.

Results presented in Figure 1B revealed that 7 d of METH self-administration (0.06 mg/infusion; 8-h sessions) decreased DAT function in HP rats compared to saline-administering controls, with no differences between LP and saline-administering rats (F(2,32)=7.87, p < 0.01), as assessed 8 d after the final self-administration session. Of note, the HP rats did not escalate the number of daily active lever presses over the course of the 7 d METH exposure (F(6,114)=0.83, ns). This regimen likewise decreased in DAT immunoreactivity in HP but not LP rats (Saline: 100 ± 2.63%; LP METH: 85.88 ± 12.14%; HP METH: 79.54 ± 4.05%; F(2,31)=3.91, p<0.05). In contrast, METH self-administration was without effect on striatal GFAP immunoreactivity (Saline: 100 ± 25.7%; LP METH: 128.7 ± 45.2%; HP METH: 88.8 ± 16.9%; F(2,31)=0.45, ns), TH immunoreactivity (Saline: 100.0 ± 5.46%; LP METH: 100.21 ± 11.95%; HP METH: 94.26 ± 3.98%; F(2,9)=0.41, ns), or DA content (Saline: 224.3 ± 20.3 pg/µg; LP METH: 187.9 ± 32.8 pg/µg; HP METH: 235.9 ± 11.9 pg/µg; F(2,22)=0.88, ns). The total quantities of METH received over the entire 7 d of self-administration in LP and HP rats were
2.39 ± 0.27 mg and 14.07 ± 1.16 mg, respectively (t(23)=4.33, p<0.05). METH self-administration also increased core body temperatures (Saline: 37.68 ± 0.07°C; LP METH: 38.08 ± 0.12°C; HP METH: 38.6 ± 0.05°C; F(2,32)=50.1, p < 0.05).

In a separate experiment, the impact of this METH regimen (7 d of METH self-administration (0.06 mg/infusion)) on VMAT-2 activity was assessed. Results revealed that METH self-administration did not alter cytoplasmic VMAT-2 activity in HP rats as assessed 8 d after the last self-administration session (t(23)=1.12, ns; Figure 1C). In this experiment, all rats met the HP criteria. The rats received a total of 12.37 ± 1.53 mg of METH over the entire 7-d course of self-administration, and had core body temperatures of 38.39 ± 0.08°C vs. 37.61 ± 0.06°C in saline controls (t(23)=7.76, p < 0.05).

In order to determine if the alterations in DAT activity persist, rats were allowed to self-administer METH as described for Figure 1, and then were sacrificed 30 d later. Results presented in Figure 2 demonstrate that the decreases in DAT activity in HP rats persisted for 30 d (t(10)=2.29, p < 0.05). In contrast, METH self-administration (0.06 mg/infusion) did not alter striatal DAT immunoreactivity (Saline: 100 ± 2.62%; METH HP: 89.93 ± 5.32%; t(10)=1.05, ns), DA content (Saline: 248.9 ± 6.52 pg/µg, HP: 223.8 ± 13.2 pg/µg; t(10)=1.049, ns), TH immunoreactivity (Saline: 100 ± 6.33%; HP: 89.22 ± 5.46%; t(10)=1.05, ns), or GFAP immunoreactivity (Saline: 100 ± 21.55%; HP: 138.2 ± 20.24%; t(10)=1.01, ns) as assessed 30 d after the final drug exposure. Rats in this experiment received a total of 11.45 ± 1.83 mg of METH over the entire 7-d course of self-administration, and attained core body temperatures of 38.27 ± 0.09°C vs. 37.65 ± 0.09°C for saline controls (t(10)=3.66, p < 0.05).

To better characterize our self-administration paradigm, we examined the effects of increasing the dose/infusion of METH. Results presented in Figure 3A demonstrate that increasing the
dose of METH received per infusion from 0.06 mg to 0.12 mg/10 µl infusion led to a greater escalation in daily METH intake (F(6,60)=3.02, p<0.05; Figure 3A). This resulted in greater total METH intake over the course of the 7 d of self-administration (0.06 mg/infusion HP: 12.26 ± 1.65 mg; 0.12 mg/infusion HP: 22.77 ± 0.70 mg; t(10)=4.29, p<0.05). However, both METH self-administration groups had similar increased average end-of-session body temperatures compared to saline control rats (Saline: 37.69 ± 0.09ºC; 0.06 mg/infusion HP: 38.37 ± 0.14ºC; 0.12 mg/infusion HP: 38.48 ± 0.07ºC; F(2,13)=22.11, p<0.05). A separate group of rats was sacrificed 1 h after the last session, and brain METH levels were assessed. Doubling the METH infusion dose nearly doubled METH levels within the brain (t(9)=2.09, p = 0.07; Figure 3B).

Results presented in Figure 4 confirm that, as in Figure 3A, increasing the dose of METH received per infusion to 0.12 mg/infusion led to an escalation in daily active lever presses (F(6,30)=8.65, p<0.05; Figure 4A). However, this did not enhance the magnitude of decrease in DAT uptake (t(10)=3.36, p<0.05; Figure 4B) compared to using 0.06 mg/infusion as assessed 8 d after the last 8-h session (see Figure 1B). This METH regimen increased core body temperature (METH: 38.48 ± 0.07ºC; Saline: 37.69 ± 0.08 ºC; t(10)=6.92, p<0.05), and rats infused a total of 21.24 ± 0.83 mg of METH over the 7-d course of treatment. This paradigm decreased DAT immunoreactivity (Saline: 100 ± 1.70%, METH HP: 82.8 ± 5.92%; t(8)=2.79, p < 0.05), but did not alter DA content (Saline: 356.58 ± 14.76 pg/µg, HP: 379.13 ± 30.19 pg/µg; t(10)=0.6418, ns), TH immunoreactivity (Saline: 100 ± 11.43%; HP: 99.65 ± 7.75%; t(8)=0.03, ns), or GFAP immunoreactivity (Saline: 100 ± 28.59%; HP:76.95 ± 9.51%; t(8)=0.76, ns) as assessed 8 d after the final METH exposure.

Figure 5A demonstrates that increasing the infusion dose of METH still further to 0.24 mg/infusion did not result in escalation in daily METH active lever pressing (F(6,48)=0.72, ns). Although increasing the dose resulted in greater total METH intake compared to the 0.12
mg/infusion group due to the greater amount of METH/infusion (0.24 mg/infusion Total METH: 32.56 ± 1.89 mg) and increased core body temperature (Saline: 37.80 ± 0.07°C; METH: 38.64 ± 0.07°C; t(16)=8.45, p < 0.05), decreases in DAT monomer immunoreactivity were similar to those resulting from lower infusion doses (Saline: 100.00 ± 7.19%; METH: 71.19 ± 9.28%; t(15)=2.40, p<0.05; Figure 5B; compare with Figures 1B and 3B). DAT uptake was not assessed in this experiment. This regimen did not alter GFAP (Saline: 100.0 ± 15.03%; METH: 90.1 ± 12.9%; t(16)=0.50, ns) or TH immunoreactivity (Saline: 100.0±5.11; METH: 95.85±5.49%; t(16)=0.59, ns). However, this regimen decreased striatal DA content (Saline: 227.74 ± 5.62 pg/µg protein; METH: 165.00 ± 17.00 pg/µg protein; t(16)=3.50, p < 0.05).

To better model the human condition wherein drug escalation increases over time (Cho, 1990; Kitamura et al., 2006), the 0.12 mg/infusion dose of METH was selected for further study as it caused an escalation in daily intake from D1 to D7 (Figures 3A, 4A, and 6A). Results presented in Figure 6B demonstrate that 7 d of METH self-administration (0.12 mg/infusion, 8-h sessions) attenuated the decrease in DAT uptake caused by a subsequent binge METH treatment (4 injections, 7.5 mg/kg/injection 2-h intervals, initiated 24 h after the beginning of the final METH self-administration session), as assessed 7 d after the binge exposure (F(2,29)=15.45, p<0.05). METH self-administration also attenuated the binge-induced decrease DAT monomer immunoreactivity (Figure 6C; F(2,29)=8.06, p < 0.05), increase in DAT complex formation (Figure 6D; F(2,29)=12.97, p < 0.05), increase in GFAP immunoreactivity (Figure 6E; F(2,29)=3.82, p<0.05), and decrease in DA content (F(2,28)=4.859, p<0.05; Figure 6F) in these animals. Decreased striatal TH immunoreactivity in the saline self-administering and METH challenged rats was also found, but did not reach significance (Saline/Saline: 100.00 ± 6.51%; METH/METH: 100.50 ± 5.85%; Saline/METH: 82.88 ± 5.58%; F(2,29)=2.80, p=0.08).
Of note, results presented in Figure 6G demonstrate that METH self-administration attenuated the hyperthermic response to a subsequent binge METH treatment, as assessed during the first 2.5 h of the binge regimen (F(16,224)=7.70, p<0.05). In this experiment, no attempt was made to manipulate core body temperature. However, because it is well established that attenuation of hyperthermia can attenuate the monoaminergic deficits caused by a binge METH treatment (see Discussion below), an additional experiment was conducted wherein rats were placed in a warm environment during the binge treatment in order to promote METH-induced hyperthermia. In this experiment, lever pressing behavior was similar to that presented in Figure 6A, with rats pressing for a total of 21.68 ± 1.86 mg of METH over the 7 d of treatment. Results revealed that despite treatment in the warm environment (i.e., a common practice used to promote METH-induced hyperthermia (see, for example Hadlock et al., 2010; McFadden et al., 2011) METH self-administration still attenuated the hyperthermic response caused by the binge treatment during the first 2.5 h of the binge (Figure 7A; F(14,112)=14.44, p<0.05). As in Figure 6A, prior exposure to METH via self-administration attenuated the deficits in DAT uptake caused by the binge treatment (Figure 7B; F(2,16)=11.15, p<0.05), and attenuated DA depletions in the striatum (F(2, 16)=16.04, p<0.05; Saline/Saline: 150.10 ± 9.28 pg/µg; METH/METH: 104.60 ± 17.97 pg/µg; Saline/METH: 39.90 ± 14.36 pg/µg).

Brain concentrations of METH were assessed in saline and METH (0.12 mg/infusion) self-administering animals challenged with a binge of METH and sacrificed 1 h after the last injection. Prior self-administration of METH lead to no significant changes concentrations of METH compared to rats who self-administered saline prior to the binge (Saline/METH: 7.77 ± 0.62 ng/mg wet weight; METH/METH: 9.09 ± 0.97 ng/mg wet weight; t(18)=1.08, ns). Further, no significant differences were seen in brain AMPH concentrations (Saline/METH: 2.21 ± 0.18 ng/mg wet weight; METH/METH: 2.59 ± 0.15 ng/mg wet weight; t(18)=1.63, ns). METH self-administering animals pressed for a total of 21.78 ± 1.24 mg METH during the 7 d of self-
administration and had pressing behavior similar to Figure 5A. Although, METH self-administering rats were kept in a warm environment during the binge of METH, similar attenuation in hyperthermia were seen in the animals that self-administered METH compared to saline prior to the METH binge at the 30 min, 150 min, and 270 min time points (F(14,168)=11.79, p<0.05).
Discussion

Previous studies indicate that METH self-administration in rats provides a clinically relevant exposure pattern of human METH abuse (Yahyavi-Firouz-Abadi et al., 2009). Results of the current study extend these findings by revealing that METH self-administration decreases striatal DAT immunoreactivity and/or function. Specifically, this study expands upon the current preclinical METH self-administration literature by being the first to demonstrate that: 1) changes in DAT function persist for at least 30 d; 2) increasing the amount of METH per infusion from 0.06 mg - 0.24 mg/infusion did not lead to greater deficits in DAT immunoreactivity and/or function; 3) the deficits in dopaminergic parameters and the concentrations of METH within the brain 1 h following self-administration of 0.06 – 0.12 mg/infusion are comparable to those of human METH abusers; and 4) the self-administration of METH attenuates the dopaminergic deficits induced by a subsequent binge METH treatment. Attenuation of hyperthermia, but not alterations in pharmacokinetics, likely contributes this latter phenomenon.

As reported by Brennan et al. (2010) and Frankel et al. (2011), the present study provides evidence that the effects of non-contingent METH treatment do not necessarily predict effects of contingent exposure. For example, the magnitudes of the deficits in DAT induced by METH self-administration were less than that often reported after a binge METH treatment (Baucum et al., 2004; Eyerman & Yamamoto, 2007; Hadlock et al., 2010). Similarly, the decreases in DA content caused by self-administration were less than those caused by a binge treatment (Brennan et al., 2010; Cadet et al., 2009; Johnson-Davis et al., 2004), and were only evident after administration of the 0.24 mg/infusion dose. Further, and in contrast to effects of binge METH-treatment (Hadlock et al., 2010), no increases in GFAP immunoreactivity, a marker of astrocytic activation and neuronal damage, were found following METH self-administration.
This is consistent with previous reports that indicate self-administration produces adaptations that differ from effects of binge METH treatment (Brennan et al., 2010; Schwendt et al., 2009).

Of note, increasing the dose of METH per infusion did not linearly increase the magnitude of DAT-associated deficits. Specifically, all three doses tested (e.g., 0.06, 0.12 and 0.24 mg/infusion) similarly affected DAT function and/or immunoreactivity, as well as METH-induced hyperthermia. Since hyperthermia has been shown to contribute to the persistent effects of METH on the DAT (Bowyer, 1995) as discussed below, the similarity in core body temperature may contribute to the lack of dose-dependency in DAT function across the dosing range tested. Alternatively and as discussed below, the self-administration regimen per se may have engendered a tolerance phenomenon (defined herein as the ability to protect against a subsequent METH challenge) such that the last treatment session could not effect larger alterations in the dopaminergic parameters under study.

The findings of the current study are generally consistent with the neuroadaptations reported in Schwendt et al. (2009) after METH self-administration. In particular, METH exposure persistently decreased DAT, without altering GFAP, TH or VMAT-2 immunoreactivity in the striatum. Similar to the findings of Schwendt et al. (2009) and Brennan et al. (2010), no significant decreases in DA content were seen 8 d after self-administration in animals when 0.12 or 0.06 mg/infusion was administered. In contrast, decreases in DA content were observed after self-administration of the higher dose (0.24 mg/infusion) of METH. The present findings also stand in contrast to previous self-administration studies that did not demonstrate changes in DAT protein or mRNA (Shepard et al., 2006; Stefanski et al., 2002) or that reported large dopaminergic deficits resembling those following “binge” METH treatment (Krasnova et al., 2010). Differences in the dosing and duration of the self-administration sessions may contribute
to these differences (e.g., Shepard et al., 2006 and Stefanski et al., 2002 utilized lower doses, and Krasnova et al., 2010 used 15-h self-administration sessions).

While the effects of METH self-administration demonstrated in this report differ from those described pre-clinically after a binge-like treatment, some consequences are very similar to those reported in METH abusers. As one example, METH concentrations within the brain of rats self-administering 0.12 mg/infusion were similar to 8 of 14 subjects reported by Kalasinsky et al. (2001), while METH concentrations following the ‘binge’ of METH resulted in higher concentrations than 12 of 14 subjects. This result must be interpreted cautiously as metabolism differences exist between the species. Further, there is a large amount of variability in total METH intake in both humans and rats, and duration of METH use in human abusers that may lead to differences between the two species.

Other similarities between the present findings and reports involving imaging of abstinent METH abusers are the magnitude of changes in DAT within the striatum. In both self-administration and imaging studies of abstinent human METH abusers, decreases in DAT densities by approximately 15-28% occurred (Chang et al., 2007). Similar to human imaging studies of DAT densities, the magnitude of the changes DAT uptake decreased during protracted abstinence (Volkow et al., 2001). However, these changes were less than those reported in post-mortem studies, perhaps due to the absence of METH in the body at the time of the assays. In agreement with human imaging studies or postmortem transporter binding, small, but non-significant, decreases in VMAT uptake were also observed (Johanson et al., 2006; Wilson et al., 1996; but see Boileau et al. 2008).

Importantly, prior METH self-administration attenuates the persistent dopaminergic deficits caused by a subsequent binge METH exposure. This protection resembles effects of escalating-
dose and other pretreatment exposures (Cadet et al., 2009; Johnson-Davis et al., 2004; Stephans & Yamamoto, 1996; Thomas & Kuhn, 2005). In particular, METH self-administration attenuated the decreases in DAT activity, DA content, and TH immunoreactivity caused by a subsequent binge METH regimen. METH self-administration also attenuated the increase in DAT complex formation; a phenomenon that our laboratory has demonstrated is an indicator of, and perhaps contributor to, the persistent DA deficits caused by the binge treatment (Hadlock et al., 2009, 2010). Further, GFAP immunoreactivity caused by a binge METH treatment was attenuated by prior METH self-administration. These novel 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 do not display greater dopaminergic deficits, even after using large quantities of METH.

Previous studies involving escalating-dose treatments have suggested the role of hyperthermia in attenuating the persistent monoaminergic deficits caused by a subsequent binge METH treatment (Johnson-Davis et al., 2004; O’Neil et al., 2006). This is likely because increases in core body temperatures such as those facilitated by METH can exacerbate the formation of reactive oxygen and nitrogen species that enhances toxicity (Bowyer, 1995; Krasnova & Cadet, 2009; Yamamoto et al., 2010). Similarly, the present study permits speculation that the attenuation of hyperthermia afforded by prior METH self-administration contributed to its ability to attenuate deficits caused by a subsequent binge treatment.

In contrast to previous studies involving escalating-dose pretreatment (Schmidt et al., 1985), alterations in pharmacokinetics did not appear to underlie the attenuated METH-induced dopaminergic deficits caused by the subsequent binge exposure. Future research will examine other possible mechanisms underlying this apparent neuroprotection; these mechanisms potentially include changes in glutamate or DA release, and/or DA receptor activation as each
has been implicated in the persistent neurotoxic effects of METH (for review, see Fleckenstein et al., 2007; Krasnova & Cadet, 2009; O’Neil et al., 2006; Yamamoto et al., 2010).

In summary, the present report demonstrates that METH self-administration (0.12 mg/infusion) led to an escalation of pressing and brain METH levels similar to human users (Cho, 1990; Kalasinsky et al., 2001). This resulted in persistent changes in DAT within the striatum that were comparable in magnitude to imaging studies of human METH abusers, but smaller than the changes following binge exposures to METH in rats. The findings of the first experiment suggest that these changes involved DAT, but neither VMAT-2 function nor GFAP and TH immunoreactivity, thus suggesting that changes may represent neuroadaptations involving dopaminergic neurons rather than a loss of dopaminergic nerve terminals. In addition, these changes in DAT were persistent and did not appear dose-dependent as increasing the amount of METH/infusion resulted in similar magnitude of DAT alterations. Lastly, a novel finding of the present study is that the self-administration of METH attenuated many of the persistent dopaminergic deficits caused by subsequent investigator-administered high-doses of METH. This protection was independent of alterations in METH pharmacokinetics, but likely attributable (at least in part) to an attenuation of METH-induced hyperthermia. These data permit speculation that the lack of dose-dependent response was due to a “tolerance” phenomenon engendered by the self-administration paradigm. Overall, METH self-administration in rats may provide insight into the persistent dopaminergic alterations associated with METH abuse.
Author Contributions

Participated in research design: McFadden, Hanson and Fleckenstein.
Conducted experiments: McFadden, Hadlock, Stout, Allen, Vieira-Brock, Ellis, Hoonakker, Andrenyak, and Nielsen.
Performed data analysis: McFadden and Wilkins.
Wrote or contributed to the writing of the manuscript: McFadden, Hanson, and Fleckenstein.
References


Footnotes

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Legends for Figures

Figure 1: METH self-administration leads to persistent changes in DAT function. A: Rats self-administered METH (0.06 mg/infusion; n=25) or saline (10 µl/infusion; n=10) for 7 d (8 h/d). Rats were treated as in panel A, sacrificed 8 d after the final self-administration session, and DAT function was assessed (Panel B). Rats were treated as in panel A, sacrificed 8 d after the final self-administration session, and VMAT2 function was assessed (Panel C). Mean saline values were 1.60 fmol/µg protein (Panel B) and 80.7 fmol/µg protein (Panel C). Values represent the mean of 10 saline, 4 LP METH, and 21 HP METH (Panel B), or 11 saline and 14 HP METH animals (Panel C). *p<0.05 vs. saline self-administering rats.

Figure 2: METH self-administration leads to changes in DAT function assessed 30 d after the last self-administration session. Rats self-administered METH (0.06 mg/infusion) or saline (10 µl/infusion; n=10) for 7 d (8 h/d). Mean saline values were 0.96 fmol/µg protein. Values represent the mean of 3 saline and 9 HP METH animals. *p<0.05 vs. saline self-administering rats.

Figure 3: METH intake and brain levels in self-administering rats. Rats self-administered METH (0.06 or 0.12 mg/infusion; n=8 and 4 respectively) for 7 d (8h/d; Panel A). Rats were sacrificed 1 h after the final self-administration session (n=6 0.06 mg/infusion and n=5 0.12 mg/infusion rats) and brain concentrations of METH were assessed (Panel B). *p<0.05 vs. 0.06 mg METH/infusion.

Figure 4: METH self-administration leads to persistent changes in DAT function. Rats self-administered METH (0.12 mg/infusion; n=6) or saline (10 µl/infusion; n=6) for 7 d (8 h/d) and were sacrificed 8 d after the final self-administration session (Panel A). DAT transporter
function was assessed in these animals (Panel B). Mean saline values were 2.68 fmol/µg.
*p<0.05 vs saline self-administration.

Figure 5: METH self-administration leads to persistent changes in DAT immunoreactivity. Rats self-administered METH (0.24 mg/infusion; n=9) or saline (10 µl/infusion; n=8) for 7 d (8 h/d) and were sacrificed 8 d after the last session (Panel A). DAT immunoreactivity was assessed in these animals (Panel B). Insert: Representative blot of saline self-administering rat (left) and 0.24 mg METH/infusion rat (right) *p<0.05 vs saline self-administration.

Figure 6: METH self-administration mitigates the effects of a subsequent binge METH treatment. Rats self-administered METH (0.12 mg/infusion) or saline (10 µl /infusion) for 7 d (8 h/d; Panel A). Twenty-four h after the beginning of the final self-administration session, rats received METH (4 x 7.5 mg/kg/injection, s.c., 2-h intervals) or saline (1 ml/kg/injection, s.c., 2-h intervals), were sacrificed 7 d later, and DAT function was assessed (Panel B). DAT monomer (Panel C), DAT complex (Panel D), and GFAP (Panel E) immunoreactivity in striatal synaptosomes was assessed in these animals. C-E Inserts: Representative blot of samples from saline self-administering/saline challenged rats (lane 1), METH self-administering/METH challenged (lane 2), and saline self-administering/METH challenged rats (lane 3). DA content was assessed in the anterior portion of the left striatum (Panel F). Temperatures were taken every 30 and 90 min following each injection of METH or saline (Panel G). Average saline values for DAT uptake were 2.85 fmol/µg protein. Average of 7 saline self-administering/saline-challenged, 14 METH self-administering/METH-challenged, and 11 saline self-administering/METH-challenged rats in all panels. *p<0.05 vs. all other groups.

Figure 7: METH self-administration mitigates the effects of a subsequent binge METH treatment when kept in a warm environment to promote hyperthermia. Rats self-administered
METH (0.12 mg/infusion) or saline (10 µl/infusion) for 7 d (8 h/d). Twenty-four h after the beginning of the final self-administration session, rats received METH (4 x 7.5 mg/kg/injection, s.c., 2-h intervals) or saline (1 ml/kg/injection, s.c., 2-h intervals) and core temperatures were assessed 30 and 90 min after each injection (Panel A). Animals were sacrificed 7 d later and DAT function was assessed (Panel B). Average of 8 saline self-administering/saline-challenged, 6 METH self-administering/METH-challenged, and 5 saline self-administering/METH-challenged rats in all panels. *p<0.05 vs. all other groups.
Figure 1.

A. Active Lever Presses vs. Day for Saline, LP METH, and HP METH groups.

B. DAT Uptake (Percent of Saline Control) for Saline, LP METH, and HP METH groups. 

C. VMAT2 Uptake (Percent of Saline Control) for Saline and HP METH groups.
Figure 2.
Figure 3.

A. 

B. 

- METH Intake (mg)
- Day
- 0.06 mg/Infusion
- 0.12 mg/Infusion

- Brain METH (ng/mg)
- Group
- 0.06 mg/Infusion
- 0.12 mg/Infusion
Figure 4.

A. Saline
   0.12 mg/Infusion METH

B. DAT Uptake
   (Percent of Saline Control)

Group
Saline
HP METH

*
Figure 5.
Figure 6.

A. Active Lever Presses over Days for different groups:
- Saline/Saline
- METH/METH
- Saline/METH

B. DAT Uptake (Percent of Saline Control) by Group:
- Saline/Saline
- METH/METH
- Saline/METH

C. DAT Monomer Immunoreactivity (Percent of Saline Control) by Group:
- Saline/Saline
- METH/METH
- Saline/METH

D. DAT Complex Immunoreactivity (Percent of Saline Control) by Group:
- Saline/Saline
- METH/METH
- Saline/METH
Figure 6 (continued).

E. 

F. 

G.
Figure 7.