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Human Methamphetamine Pharmacokinetics Simulated in the Rat: Behavioral and Neurochemical Effects of a 72-h Binge

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Bingeing is one pattern of high-dose methamphetamine (METH) abuse, which involves continuous drug taking over several days and can result in psychotic behaviors for which the brain pathology remains poorly defined. A corresponding animal model of this type of METH exposure may provide novel insights into the neurochemical and behavioral sequelae associated with this condition. Accordingly, to simulate the pharmacokinetic profile of a human METH binge exposure in rats, we used a computer-controlled, intravenous METH procedure (dynamic infusion, DI) to overcome species differences in METH pharmacokinetics and to replicate the human 12-h plasma METH half-life. Animals were treated over 13 weeks with escalating METH doses, using DI, and then exposed to a binge in which drug was administered every 3 h for 72 h. Throughout the binge, behavioral effects included unabated intense oral stereotypies in the absence of locomotion and in the absence of sleep. Decrements in regional brain dopamine, norepinephrine, and serotonin levels, measured at 1 and 10 h after the last injection of the binge, had, with the exception of caudate–putamen dopamine and frontal cortex serotonin, recovered by 48 h. At 10 h after the last injection of the binge, [^{3}H]ligand binding to dopamine and vesicular monoamine transporters in caudate–putamen were reduced by 35 and 13%, respectively. In a separate METH binge-treated cohort, post-binge behavioral alterations were apparent in an attenuated locomotor response to a METH challenge infusion at 24 h after the last injection of the binge. Collectively, the changes we characterized during and after a METH binge suggest that for human beings under similar exposure conditions, multiple time-dependent neurochemical deficits contribute to their behavioral profiles.
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INTRODUCTION

The continuing spread of methamphetamine (METH) abuse has stimulated research primarily aimed at understanding the consequences of prolonged exposure to this drug. Converging evidence from clinical and preclinical studies indicates that chronic, high doses of METH can lead to neurotoxicity and cognitive impairments, which have been characterized at various times after discontinuation of drug administration (see Davidson *et al*, 2001; Guilarte *et al*, 2003; Cadet *et al*, 2003; McCann and Ricaurte, 2004 for recent reviews). In addition to these enduring consequences of METH abuse, there is also considerable interest in the neurochemical and behavioral effects occurring during METH intoxication; in particular, the state of paranoid psychosis that can develop with higher doses of the drug. This pathological state seems to be related to the amount and duration of drug use and is most frequently induced during the drug exposure associated with 'binge' or 'run' patterns of METH administration. It has been suggested that this METH-induced paranoid state is responsible for the reckless, often aggressive antisocial behavior associated with high-dose intoxication (Jaffe, 1995; Buffenstein *et al*, 1999). One theme that has consistently appeared in the clinical literature regarding psychotogenic METH treatment is the prolonged, continuous high-dose exposure, such as is observed with binge patterns of abuse (Kramer, 1972; Davis and Schlemmer, 1980; Sherer, 1988; Sherer *et al*, 1988; Satel *et al*, 1991; Brady *et al*, 1991; Gawin and Khalsa, 1996). Also associated with this condition are sleep deprivation and disruption of circadian cyclicity that have been argued by some to have important functions in the induction of psychosis (Kramer, 1972; Gawin, 1991). As a first step toward a better understanding of the behavioral and neurochemical alterations associated with human binge METH abuse patterns, we here characterize an animal model that incorporates METH pharmacokinetics that would be expected with a binge pattern of METH abuse. Our longer-term goal is to incorporate multiple binges into this study paradigm for modeling METH pharmacokinetics

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associated with the onset of stimulant-induced psychosis. Clinical evidence suggests that the appearance of this condition is increasingly more likely with repeated, high-dose binges (Angrist, 1994a, b; Gawin and Khalsa, 1996).

In our earlier studies, we had been using an escalating dose (ED)-multiple binge rodent model, which we have argued more closely simulates in rats the drug exposure associated with METH abuse (Kuczenski and Segal, 1997; Segal and Kuczenski, 1997a, b). Most abusers who have achieved the binge stage of drug taking initially began with very low doses of the drug and have had a long history of progressively increasing or escalating their doses. Dose escalation allows tolerance to develop to the sympathomimetic properties of the drug (Angrist, 1994b; Fischman *et al*, 1985; Fischman and Schuster, 1974), and tolerance to these undesirable effects enables the use of higher doses to achieve desired effects (Angrist, 1987, 1994b; Gawin and Khalsa, 1996). However, modeling high dose-psychotogenic stimulant exposure is particularly difficult in rodents (eg, repeated subcutaneous injections), in part because of the profound species differences in pharmacokinetics between human beings and rats. The plasma METH half-life is 10–12 h in human beings (Cook *et al*, 1992, 1993) *vs* 60–70 min in rats (Melega *et al*, 1995; Rivière *et al*, 1999). Thus, repeated administration of METH to rats using more traditional treatment approaches results in significant fluctuations in drug levels, including prolonged periods in the absence of drug (Cho *et al*, 2001). In contrast, high-dose METH abusers are virtually continuously exposed to the drug, and micromolar plasma concentrations are maintained throughout a 2–3 day binge. As different dynamic changes in neurotransmitter function and behavioral adaptation occur depending on the amount of time neurons are exposed to METH (eg, Pickel and Chan, 1995; Frey *et al*, 1997; Kuczenski and Segal, 1999a, b; McCann and Ricaurte, 2004; Ben Shahar *et al*, 2004; Davidson *et al*, 2005; Samaha *et al*, 2002, 2004; Ferrario *et al*, 2008), both the duration and the temporal pattern of drug exposure become critical parameters for developing accurate models of METH binge abuse.

In an attempt to overcome this pharmacokinetic obstacle, we have developed and described (Segal and Kuczenski, 2006) a computer-controlled, intravenous drug delivery methodology, dynamic infusion (DI), which allows us to precisely reproduce in rats a plasma METH profile that approximates human METH pharmacokinetics. Briefly, METH bolus injections are each followed by mini-pulses of METH to effect a protracted decline in plasma METH levels that correspond to the human 10–12 h plasma half-life. This report documents in rodents neurochemical and behavioral characteristics associated with a DI 72-h METH binge that simulates a binge pattern of human METH abuse after a prolonged DI ED pretreatment.

MATERIALS AND METHODS

Subjects

Male Sprague–Dawley rats weighing 275–300 g were obtained from Harlan Labs (Gilroy, CA) and were housed for at least two weeks before surgery in groups of two or three animals, in wire mesh cages, with *ad libitum* access to food and water. The room was temperature (20°C) and humidity (55 ± 5%) controlled and maintained on a reversed 12 h dark (7:00 a.m. to 7:00 p.m.) and 12 h light cycle to allow for the start of treatment during the normal active phase of the awake/sleep cycle of the rat (Devoto *et al*, 2004). During the dark period, all facilities were illuminated with red light to facilitate observation of the animals. A total of 72 animals were catheterized for intravenous drug administration, and were randomly divided into two groups: METH-treated ($n = 47$) and saline controls ($n = 25$). These studies adhered to animal welfare guidelines (National Research Council, A Guide for the Care and Use of Laboratory Animals, 1996).

Drugs

METH hydrochloride (Sigma Chemical Co., St Louis, MO) was dissolved in 0.9% saline and administered intravenously. During the initial 10 days of drug administration, the drug solution also contained 3.75 mg/day timentin. Control animals received a comparable vehicle administration.

Surgery

After 2 weeks of acclimation, animals were implanted with intravenous catheters under Halothane anesthesia. Catheters were constructed by fitting a 13-cm length of silastic tubing to a guide cannula, bent at a right angle. The guide cannula was embedded in dental cement and attached to a 1 inch. circle of marlex mesh and mounted on the back of the animal. The silastic tubing was passed subcutaneously from the back of the rat to the right external jugular vein. A Tygon cap was inserted over the guide cannula to maintain a closed system. Animals were singly housed after surgery, and on a daily basis between surgery and experimental testing, the catheter was flushed with sterile saline (0.1 ml) containing 3.0 USP units heparin and 3.75 mg timentin.

Apparatus

Behavior was monitored in custom-designed activity chambers (Segal and Kuczenski, 1987). Each of the chambers (30 × 20 × 38 cm) was located in a sound-attenuated cabinet maintained on a reversed 12-h dark/12-h light cycle with constant temperature (20°C) and humidity (55 ± 5%). Movements of the animal between quadrants (ie, crossovers) and rearings against the wall, as well as eating and drinking and other vertical and horizontal movements, were monitored continuously by computer. In addition, the behavior in all experimental chambers was concurrently and continuously digitally recorded using micro-pinhole cameras equipped with wide-angle lenses and mounted on the door of each chamber. Video images were collected through GV-800 BNC Capture cards and Geovision software, then stored on DVD media for subsequent evaluation. Representative animals were selected from each experimental group to reflect the full range and pattern of locomotor activation associated with the drug response; subsequently, raters who were unaware of the specific experimental conditions rated the recordings on the basis of behavior ethograms and rating procedures established

earlier (Segal and Kuczenski, 1987). Specific behaviors were rated as the percentage of the observation interval during which the animal displayed that behavior. The appearance of any novel behavior patterns, undetectable by our automated methods, was also noted by the rater.

General Procedures

About 2 weeks after surgery, animals were placed in individual experimental chambers in which they remained for the duration of the study; tubing from a PHM-100 syringe pump (Med Associates Inc) was attached to the animal's catheter through a liquid swivel and a commercially available cannula connector (Plastics One). Each morning throughout the study, at the beginning of the dark phase (7 a.m.), the behavioral chambers were serviced and the animals were weighed and examined for health. After a 3-day acclimation period, during which animals received daily DIs of saline (see below), drug administration was initiated. In addition, body temperature was evaluated in a subgroup of animals ($n = 10$) using an ear probe (Braun Thermoscan Plus) from the day the animals were introduced to the behavioral chambers until Day 79 (when animals received $3 \times$ 0.5 mg/kg at 4 h intervals), at which time their behavior had become too aggressive, including attacking the experimenter, and the evaluations were terminated. Temperature during predrug acclimation was $37.0 \pm 0.1^{\circ}$C. Temperatures remained relatively constant for about 25 days in the treatment regimen, then gradually declined until the last measurement day to $36.2 \pm 0.1^{\circ}$C. The mild hypothermia is consistent with earlier reports that the direction of stimulant-induced changes in core temperature is dependent on ambient temperature (eg, see Yehuda and Wurtman, 1972; Bowyer *et al*, 1992; Malberg and Seiden, 1998; Myles *et al*, 2008). Although we did not monitor temperature at later time points during the drug treatment, examination of video recordings of the animals revealed no evidence of substantial hyperthermia (eg, excessive salivation), including during the binge. Although not well documented, salivation associated with METH-induced hyperthermia has been described and characterized (Myles *et al*, 2008; Myles and Sabol, 2008), and, in our experience, is always present during hyperthermia.

Drug Administration

Remote drug or saline administration was initiated at 10:00 a.m. according to the following pattern: drug delivery involved an initial intravenous infusion of METH (0.125 mg/kg, administered in 0.105 ml) over a 10-s interval. The 10-s interval was chosen on the basis of recent studies, suggesting that many intravenous METH abusers typically inject drug within this time range (Samaha *et al*, 2004). An 80 dB, 2.2 kHz tone-cue was presented to each animal for 5 s before and during the 10-s drug injection. The audible tone was presented to provide rats with a drug-predictive cue shown in recent studies to be a potentially significant factor in stimulant-induced neuronal alterations (Ghitza *et al*, 2003).

As rat plasma METH concentrations decline much more rapidly than in human beings, additional METH administration after each dose was required to simulate human METH pharmacokinetics. To accomplish this, subsequent to each bolus infusion, METH was delivered according to a computer-controlled program in the form of short-duration injections or mini-pulses [each 0.28 μl (1.3 μg/kg for a 0.5 mg/kg dose) over a 16-ms duration], to generate a plasma profile of the drug corresponding to a 12-h plasma half-life (see Segal and Kuczenski, 2006 for a more detailed discussion).

ED DI Treatment Protocol

After acclimation to the behavioral chambers, animals received either single DI of 0.125 mg/kg METH or comparable volumes of saline daily for eight consecutive days. Subsequently, until Day 68, the METH dose was progressively increased (through 0.15, 0.175, 0.225, 0.30, and 0.35 mg/kg) until each injection delivered 0.5 mg/kg METH. From Day 69 to 92, the number of 0.5 mg/kg injections was then progressively increased to four per day (at 3 h intervals during the 12-h awake cycle). From Day 93 to 95, plasma METH concentrations were allowed to decline according to a 12-h plasma half-life. We made no effort to adjust METH concentrations to account for changes in pharmacokinetics (ie, metabolic tolerance) because most evidence suggests that repeated administration of METH in human beings is not associated with changes in drug disposition or metabolism (Anggard *et al*, 1973; Perez-Reyes *et al*, 1991; Cook *et al*, 1992).

Binge Treatment

On Day 96, the 'binge' was commenced with 0.5 mg/kg METH administered at 3-h intervals over a 72-h period. Predicted plasma METH concentrations during the binge are modeled in Figure 1. At 1 and 10 h after the last binge injection, groups of animals, including groups of saline-treated controls, were euthanized by decapitation to measure plasma levels of METH for further pharmacokinetic validation of the model. These time points provided those data with a minimal number of animals to estimate plasma METH half-life. In addition, the 1-h time point provided a window corresponding to near-maximum drug levels and drug-induced stimulation. Brain analyses of those animals provided early post-binge assessment of regional levels of neurotransmitters and [^{3}H]ligand binding to the dopamine transporter (DAT) and the vesicular monoamine transporter ($VMAT_2$). Separate groups of animals were also euthanized at 48 h after the last binge injection to assess the relative recovery of neurotransmitter levels across those multiple brain regions.

METH Challenge

Six hours after the last binge injection, METH infusion was terminated in one group of METH-treated animals to allow plasma levels of the drug to decline according to a normal 1 h rat METH plasma half-life. Eighteen hours later, these animals along with a saline-treated control group (no METH exposure) received a single DI of 0.5 mg/kg METH. Twenty-four hours later, these animals were euthanized for regional analyses of brain neurotransmitter levels. This METH challenge at 24 h after the last injection of the binge

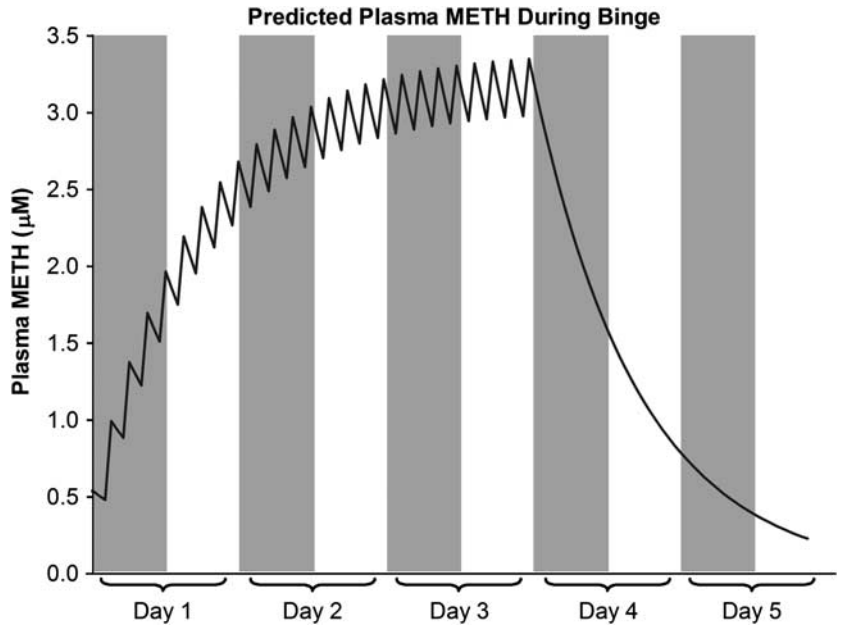

Figure 1 Predicted plasma METH concentrations during a 72-h binge using DIs of 0.5 mg/kg METH for each injection at 3 h intervals. During the initial 72 h of the binge, animals would receive a cumulative dosage of 101 mg/kg METH. Shading represents the dark (active) phase of the 12-h light/12 h dark cycle.

was intended to assess the status of those neural systems involved in the production of stimulant-induced locomotion and stereotypy after complete washout of residual drug.

Animal Morbidity and Mortality

Animals weighed ~350 g on introduction to the chambers, and continued to gain weight until Day 69 (414 ± 8 g); they lost about 20 g between Day 69 and the day preceding the binge (Day 95) and lost 40 g during the binge. Thirteen animals from the METH-treated group did not complete the study. Nine animals died at various times during the escalating METH exposure from Day 46 (0.225 mg/kg, 1 × per day) to Day 85 (0.5 mg/kg 3 × per day). Examination of videotapes revealed no seizures or convulsions, but rather a progressive decline in activity and responsiveness. In all cases, these animals exhibited a similar profile consisting of precipitous weight loss (in excess of 20 g from the earlier day's weighing), and symptoms of severe dehydration. They did not appear hyperthermic, but rather mildly hypothermic insofar as they were invariably cool to the touch. These animals received an intraperitoneal injection of 10 ml of sterile lactated Ringer's solution followed by 10 ml subcutaneously at the first sign of dehydration, and then daily until no longer required. If amelioration was evident, the animal remained in the study. Nine animals ultimately died, two were removed, then recovered (not included in the data analyses), one animal lost catheter function, and one animal was removed because of intense self-directed oral behaviors leading to bloody paws. No mortality was associated with the subsequent METH binge or challenge DI administrations.

Plasma METH and Amphetamine

For pharmacokinetic analyses, separate groups of animals ($n = 8$ per time point) were killed by decapitation at 1 and 10 h after the last injection of the binge, and trunk blood was collected in EDTA tubes. Plasma was isolated and immediately frozen on dry ice. The concentrations of METH and AMPH were determined by NMS Laboratories, Willow Grove, PA.

Neurochemistry

Materials. [^{3}H]WIN 35 428, 87.0 Ci/mmol, was obtained from Perkin Elmer Life Sciences Inc (Boston, MA) and [^{3}H]-dihydrotetrabenazine ([^{3}H]TBzOH), 20 Ci/mmol, from American Radiolabeled Chemicals Inc; cocaine hydrochloride and tetrabenazine from Sigma-Aldrich (St Louis, MO). All other chemicals were obtained from Fisher Scientific (Pittsburgh, PA).

[^{3}H]WIN 35 428 binding to the DAT was measured in rat striatal homogenates after the earlier reported methods with modifications (Madras *et al*, 1989; Villemagne *et al*, 1998). Briefly, frozen striatal tissue (30–40 mg wet weight) was homogenized with Tissue-Tearor (Biospec Products Inc, Bartlesville, OK), setting 5, 15 s in cold (0–4°C)-binding buffer (20 mM sodium phosphate buffer, pH 7.4; 0.32 M sucrose; 1 : 100 w/v). The suspension was centrifuged at 40 000 × *g* for 20 min at 4°C. The supernatant was discarded and the pellet was resuspended and centrifuged again. This washing procedure was repeated twice. The resulting pellet was suspended in binding buffer to obtain final concentrations of 25–30 mg ww/ml. The [^{3}H]WIN 35 428 binding experiment was performed in a total volume of 0.5 ml. Each sample (100 μl of stock tissue suspension) was assayed in triplicate for total and duplicate for non-specific binding. The [^{3}H]WIN 35 428 concentration was 5 nM and cocaine hydrochloride at 30 μM was used for determination of non-specific binding. Samples were incubated for 90 min at 0–4°C (ice-bath). To terminate the incubation, ice-cold-binding buffer was added (1 ml) and the suspension rapidly

filtered through Whatman GF/C glass fiber filters (presoaked for 2 h in binding buffer containing 0.1% polyethyleneimine) using a Millipore cell harvester. Filters were washed with ice-cold-binding buffer (3×5 ml) and then placed in 10 ml of Ecoscint A (National Diagnostics, Atlanta, GA) overnight before liquid scintillation counting. Non-specific binding represented $<10\%$ of the total binding and maximum [^{3}H]WIN 35 428 DAT binding in the striatal tissue suspension comprised of $<2\%$ the total radioactivity introduced into the assay. Specific [^{3}H]WIN 35 428 binding was expressed in fmol/mg protein.

[^{3}H]TBzOH binding to the $VMAT_2$ was measured using modifications of earlier reported methods (Scherman *et al*, 1986; Wilson *et al*, 1996b; Hogan *et al*, 2000; Segal *et al*, 2005). Briefly, striatal tissue homogenates (100 μl) in the phosphate/sucrose-binding buffer, prepared as described above for [^{3}H]WIN 35 428 binding assay, were incubated in a total volume of 0.5 ml. [^{3}H]TBzOH was used at a final concentration of 2 nM, and non-specific binding was determined in the presence of 10 μM tetrabenazine. Samples in triplicate for total and duplicate for non-specific binding were incubated for 2 h at room temperature. The incubation was terminated by the addition of ice-cold-binding buffer (1 ml) and suspension was filtered through Whatman GF/C glass fiber filters (presoaked for 2 h in binding buffer containing 0.1% polyethyleneimine) using Millipore cell harvester. Filters were washed with ice-cold-binding buffer (3×5 ml). Filters were placed in 10 ml of Ecoscint A overnight before liquid scintillation counting. Non-specific binding represented $<11\%$ of the total binding and maximum [^{3}H]TBzOH $VMAT_2$ binding in the striatal tissue suspension comprised of $<4\%$ the total radioactivity introduced into the assay. Specific [^{3}H]TBzOH binding was expressed in fmol/mg protein. Although recent PET imaging data in human beings (Boileau *et al*, 2008) suggest that the tracer dose of [^{13}C]TBzOH may be affected by cytosolic DA content, *in vivo*, significant competition between residual dopamine in the pellet and TBZ binding, *in vitro*, is highly unlikely. Dopamine levels were not measured in our washed pellet; however, results of earlier *in vitro* competition-binding studies of 4 nM [^{3}H] TBZ with norepinephrine (NE) showed an NE IC_{50} value of 12.5 μM (Scherman *et al*, 1983). The magnitude of effect likely extends to DA, as shown by subsequent studies (Partilla *et al*, 2006) in which an IC_{50} of >100 μM was reported in DA—2 nM [^{3}H] TBZ competition studies (note: 2 nM [^{3}H]TBZ was used in our *in vitro* studies). In addition, 2 nM [^{3}H]TBZ concentration was used in VMAT-binding studies of human control and Parkinsonian striatal homogenates without any washing; the authors referenced their earlier work showing that 'at concentrations lower than 100 μM, endogenous monoamines did not displace [^{3}H]TBZOH from its binding site' (Scherman *et al*, 1989).

Protein concentrations in rat striatal tissue homogenates were determined by the modified Lowry method (Peterson, 1977).

For analysis of biogenic amines, brains were hand dissected and brain samples were frozen in liquid nitrogen, then stored at -80°C until assayed for neurochemical levels. Regional brain levels of NE, DA, and serotonin (5HT) were assessed using methods modified from Schmidt *et al* (1990). Briefly, tissue samples were sonicated in 0.5 ml ice-cold 0.1 N perchloric acid, then centrifuged for 15 min at 10 000 r.p.m., and an aliquot of the supernatant was assayed using HPLC with electrochemical detection as described earlier (Kuczenski *et al*, 1995). The HPLC-EC consisted of a 100×4.6 mm ODS-C18 3 μ column (Regis) maintained at 40°C. Mobile phase (0.05 M citric acid, 7% methanol, 0.1 mM Na_2EDTA, and 0.2 mM octane sulfonate, adjusted to pH 4.0–4.5) was delivered at 0.6–0.8 ml/min by a Waters model 510 pump. Amines were detected with a Waters 460 detector with a glassy carbon electrode maintained at + 0.65 V relative to a Ag/AgCl reference electrode. Values are presented as pmol/mg protein.

Data analysis. Behavioral and neurochemical data were statistically analyzed using repeated measures ANOVA and *t*-tests with Bonferroni corrections for specific group/time comparisons. As the response to saline infusion did not exhibit significant changes across days, an average saline response was included in all figures for ease and clarity of presentation.

RESULTS

Behavioral Characteristics

The behavioral activation associated with the DI of escalating METH doses for this dose range was, with the exception of relatively prolonged duration, typical of effects observed with low to moderate doses of the drug in our earlier ED-METH studies. For example, the initial injection of 0.125 mg/kg resulted in a significant increase in locomotor activity over a 4-h interval after drug administration compared with the earlier day's saline administration (saline: 116 ± 13 crossovers; METH: 210 ± 18; $p < 0.0001$; $t = 5.846$, d.f. $= 19$). Increasing the dose of METH resulted in progressively increasing locomotor activation, both in terms of magnitude and duration. After the highest dose, 0.5 mg/kg, locomotor activation was evident for about 12 h, throughout the dark (active) phase, and into the initial hours of the light (inactive) phase. At these doses, locomotor activation occurred in the absence of periods of continuous focused stereotypies, though occasional episodes of repetitive behaviors such as downward-directed focused sniffing and nose poking were observed at the intermediate doses in some animals. During multiple administrations of 0.5 mg/kg (Days 68–92), more substantial focused stereotypies in the form of repetitive head movements and oral behaviors did become apparent. On the basis of a plasma METH half-life of 12 h, multiple daily infusions of the drug during this time period apparently resulted in sufficient METH accumulation to support focused stereotypies. This suggestion is supported by the quantitative and qualitative features of the temporal behavioral profile of the binge. The first 0.5 mg/kg binge injection was characterized by pronounced locomotor activation, comparable to the responses during the single daily administrations. With successive administrations during the binge, locomotor activation was almost completely replaced by continuous oral stereotypies, during which the animals typically remained within one position in the chamber and engaged in continuous licking and biting at the cage bars. This behavioral pattern persisted throughout

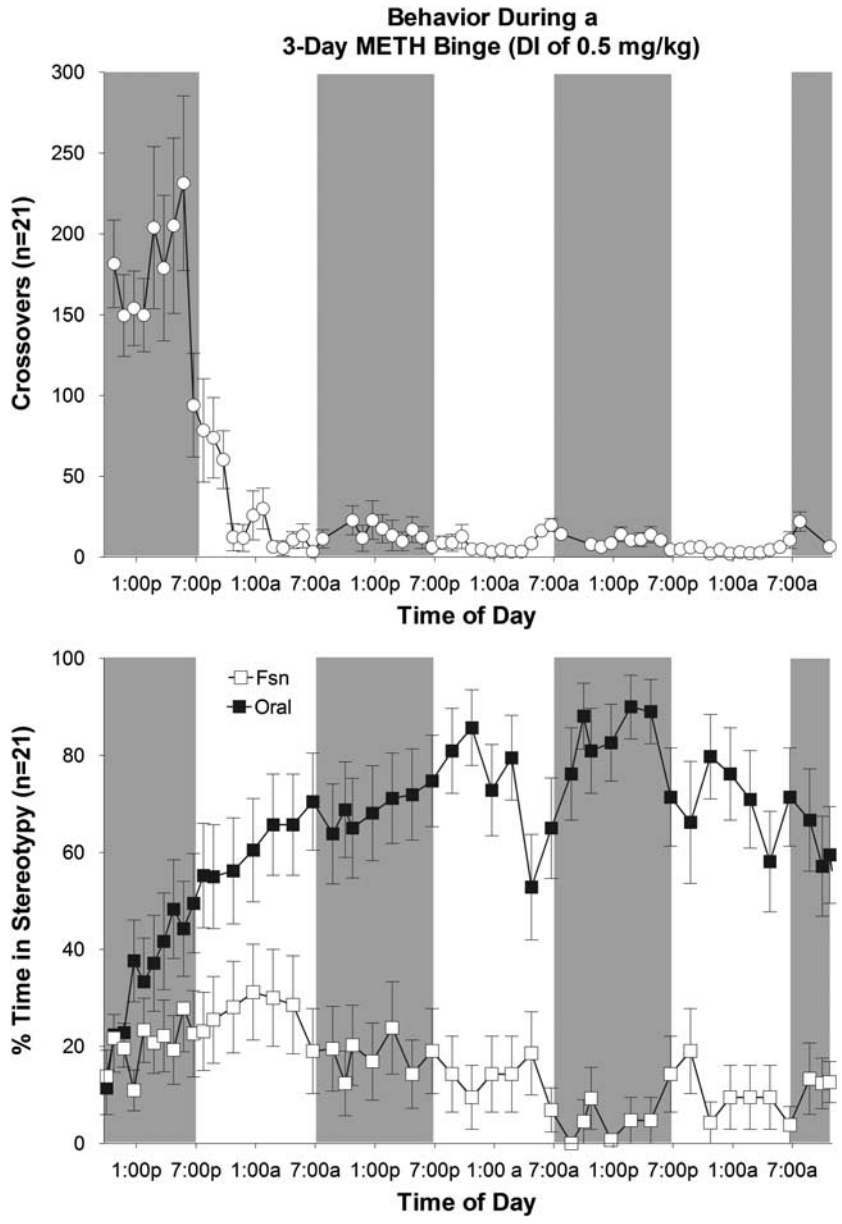

Figure 2 Behavioral response during the 72-h DI-METH binge. Upper: locomotor response (open circles), $n = 21$; values are presented as mean crossover ± SEM. Lower: stereotypy response; each value represents the percent of time (mean ± SEM) during the indicated interval during which the animals exhibited stereotypies (oral, ■; focused sniffing, □). Shading represents the dark (active) phase of the 12-h light/12 h dark cycle.

the remainder of the binge (Figure 2) and was associated with disruption of the animals' circadian cycles insofar as they did not sleep throughout the entire 72 h binge.

Twenty-four hours after the last binge injection when METH elimination was essentially complete, a subgroup of animals along with saline-pretreated controls received a DI of 0.5 mg/kg; their behavioral responses during the initial 3 h of the drug infusion are summarized in Figure 3. Throughout the entire behavioral observation period, chronic METH-exposed animals exhibited a profound decrease in the magnitude of locomotor activation compared with saline-pretreated controls. The decrement did not seem to be related to the differential appearance of stereotyped behaviors in the METH-pretreated group, as the total frequency of stereotyped behaviors was equivalent for the two groups (Figure 3). The stereotyped behaviors were not intense and were episodic rather than continuous in both groups. However, the qualitative features of the stereotypies differed substantially. Drug-naïve animals engaged in downward-directed focused sniffing, including frequent nose poking between the bars of the floor of the chamber, whereas METH-pretreated animals exhibited mild oral behaviors, predominantly self-directed or directed at the food grid.

Plasma METH and AMPH After the 72-h Binge

Plasma METH levels measured at 1 h after the last binge injection (2.9 μM, 440 ng/ml, summarized in Table 1) were comparable to values estimated from our pharmacokinetic modeling (Figure 1, 3.2 μM) and declined over the subsequent 9 h to 1.7 μM (250 ng/ml), reflecting a plasma

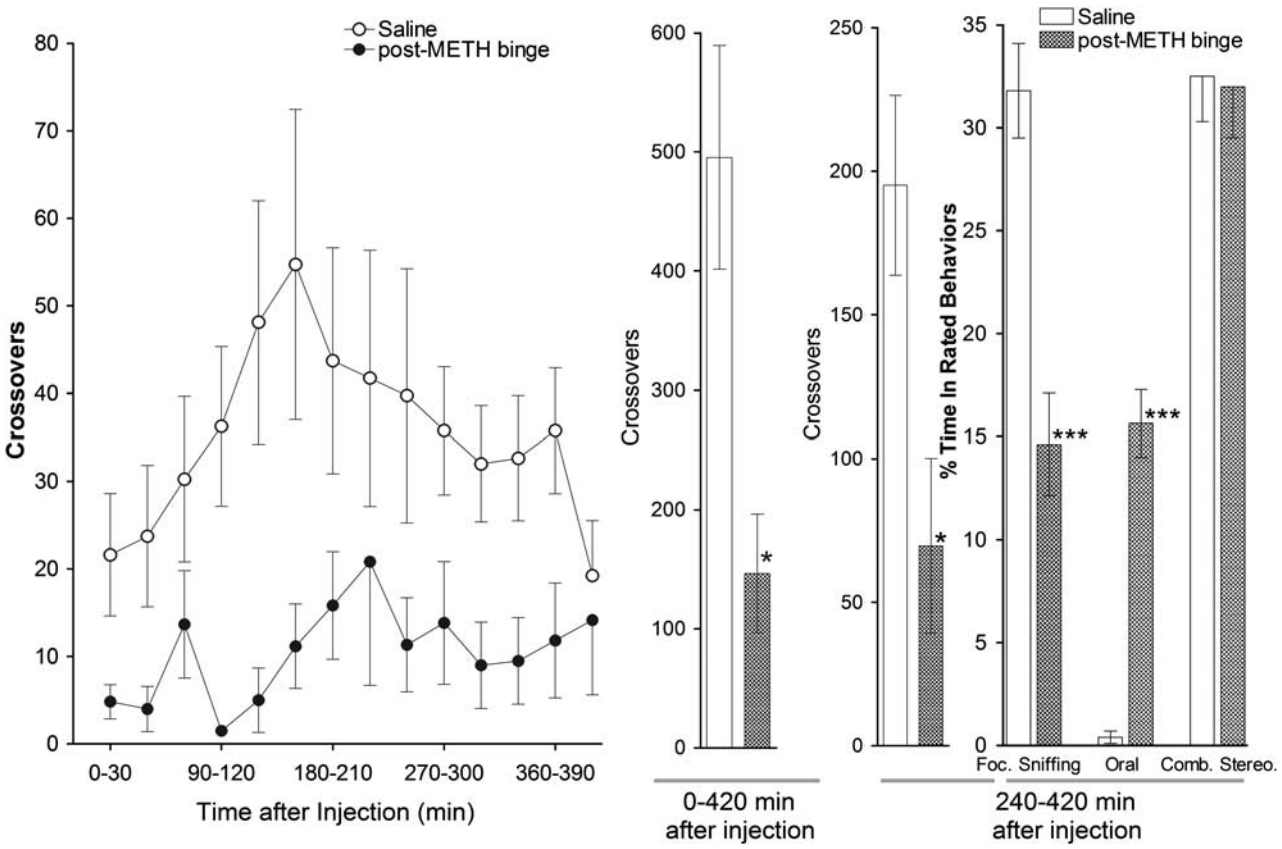

Figure 3 Behavioral response to the DI of METH (0.5 mg/kg). Groups of animals pretreated with saline or the ED-binge protocol received a DI of 0.5 mg/kg METH at 18 h after the last METH binge injection. Left: temporal profile of the locomotor response to the METH DI. Values represent the means ± SEM for each 30 min interval. Histograms represent the cumulative response during the indicated interval. $^{*}P<0.05$, $^{***}P<0.001$ compared with the corresponding acute response in saline-pretreated animals.

Table 1 Plasma Levels of METH and AMPH during the Binge ($n=8$ for Each Time Point)

Time after last (25th) injection of 72 h binge	1 h	10 h
METH (μM)	2.9 ± 0.4	1.7 ± 0.2
AMPH (μM)	1.2 ± 0.2	0.7 ± 0.1

Table 2 Caudate–Putamen [^{3}H]WIN 35 428 and [^{3}H]TBzOH Binding (fmoles/mg Protein) at 10 h after the 72 h Binge ($n=9$ for Each Group)

	[^{3}H]WIN35,428	[^{3}H]TBzOH
Control	153 ± 18	210 ± 11
Binge	100 ± 6[a]	182 ± 8

[a] $P<0.01$ compared with controls.

half-life of 11.7 h. Plasma levels of AMPH, a metabolite of METH, were about 40% of METH levels.

Striatal DAT and $VMAT_2$ After the 72-h Binge

We used [^{3}H]WIN 35 428 and [^{3}H]TBzOH binding to provide measures, respectively, of DAT and $VMAT_2$ levels in striatum 10 h after the last injection of the 72-h binge (Table 2). We found a small decrease in [^{3}H]TBzOH binding (13%) 10 h after the last binge injection, but the decrement did not achieve statistical significance ($p=0.061$). In contrast, we observed a substantial (35%) decrement in [^{3}H]WIN 35 428 binding to DAT ($p<0.01$).

Regional Neurotransmitter Levels After the 72-h Binge

We also assessed regional brain levels of the biogenic amines as a function of time after the last METH injection of the binge, and the results are summarized in Table 3. Regional NE levels were profoundly depleted (by 24–58%) in multiple brain regions during the binge, but were fully recovered at 48 h after the last METH binge injection. Serotonin levels did not exhibit consistent regional patterns; for example, transient decreases in caudate–putamen and nucleus accumbens, a relatively persistent decrease in prefrontal cortex, and no significant changes in hippocampus.

The striatum and nucleus accumbens exhibited decrements (30–40%) in tissue dopamine levels 1-h after the last injection of the binge. The decrease in striatum persisted for 48 h, whereas the decrement in nucleus accumbens was no longer significant by this time point. Prefrontal cortex and hippocampus dopamine levels were not affected by the binge (Table 3).

Table 3 Regional Levels of Biogenic Amines after a 72-h Binge (nmoles/mg Tissue)

Time after last (25th) injection	Control	1 h	10 h	48 h
Dopamine				
Caudate–putamen	129.9 ± 6.6	89.8 ± 6.1***	88.0 ± 6.6**	81.9 ± 8.6**
Nucleus accumbens	33.6 ± 1.9	20.5 ± 2.4**	25.5 ± 3.3*	29.9 ± 6.0^{+}
Prefrontal cortex	20.7 ± 1.3	21.0 ± 2.0	>22.8 ± 1.5	22.4 ± 0.9
Hippocampus	0.24 ± 0.05	0.25 ± 0.08	0.22 ± 0.02	0.20 ± 0.05
Serotonin				
Caudate–putamen	3.51 ± 0.23	2.61 ± 0.26	2.25 ± 0.30*	3.84 ± 0.38^{+}
Nucleus accumbens	5.47 ± 0.25	3.22 ± 0.32***	3.79 ± 0.34**	4.44 ± 0.38
Prefrontal cortex	3.46 ± 0.13	1.82 ± 0.24***	2.10 ± 0.15***	2.48 ± 0.22*
Hippocampus	2.14 ± 0.15	1.78 ± 0.16	1.80 ± 0.17	1.88 ± 0.18
Norepinephrine				
Nucleus accumbens	1.69 ± 0.08	1.30 ± 0.10*	1.47 ± 0.11	1.51 ± 0.15^{+}
Prefrontal cortex	1.94 ± 0.03	0.96 ± 0.10***	1.01 ± 0.07***	2.11 ± 0.12^{+++}
Hippocampus	3.54 ± 0.13	1.59 ± 0.22***	1.50 ± 0.12***	2.82 ± 0.28^{++}

Compared with controls: *$P<0.05$; **$P<0.01$; ***$P<0.001$. Compared with 1-h group: $^{+}P<0.05$; $^{++}P<0.01$; $^{+++}P<0.001$.

DISCUSSION

We designed the DI methodology to more closely approximate in rats the pharmacokinetic temporal profile of prolonged human METH exposure, in terms of magnitude and duration, and to include the progressive increase in plasma concentrations associated with successive drug administrations. The resultant behavioral and neurochemical effects should be interpreted predominantly as a function of exposure to the METH and AMPH plasma concentrations that were achieved with passive drug administration throughout this study, as qualitatively different behavioral and/or neurochemical consequences may sometimes occur depending on whether the stimulant is self- or experimenter-administered (eg, see Stefanski *et al*, 1999; Jacobs *et al*, 2003, although see Winsauer *et al*, 2003; Kiyatkin and Brown, 2004; Stuber *et al*, 2005).

Earlier, we described the similarities between the DI-generated pharmacokinetic profile in the rat and a human METH exposure profile (Segal and Kuczenski, 2006). The present results (Table 1) revealed that both plasma METH concentrations (~3 μM at 1 h post-binge), as well as the plasma METH half-life (~12 h), were consistent with the predictions from our model and both values were within the ranges reported for METH abusers (Wilson *et al*, 1996a; Melega *et al*, 2007; Jones *et al*, 2008). Concentrations of the METH metabolite, AMPH, were somewhat higher than those we had anticipated from our earlier single DI METH study (Segal and Kuczenski, 2006) and from human studies. For comparison, data from human METH abusers have shown AMPH levels to be ~15% of METH concentrations, although values as high as 40% have also been reported (Wilson *et al*, 1996a; Kalasinsky *et al*, 2001; Melega *et al*, 2007). Irrespective of the absolute AMPH concentration, quantification of both plasma METH and AMPH levels seems necessary for accurate interpretation of behavioral changes associated with repeated METH administration, as our earlier results indicated that METH and AMPH were equipotent in locomotor and stereotypy activation, and in the striatal dopamine response (Melega *et al*, 1995; Kuczenski *et al*, 1995).

It should be apparent from our earlier data on single DI plasma METH kinetics (Segal and Kuczenski, 2006), the predicted METH levels during the 3-day binge, and the plasma METH levels measured at 1 and 10 h after the last binge injection (Figure 1) that the DI methodology results in a dynamic plasma METH concentration profile substantially different from more traditional attempts to mimic high-dose binge METH exposure, such as an osmotic minipump-based technique or an acute neurotoxic binge (eg, four injections at 2 h intervals). These pharmacokinetic differences are reflected in their respective behavioral profiles. For example, an acute neurotoxic binge using traditional drug administration protocols results in behavioral activation, which is maintained for 10–12 h corresponding to the persistence of METH in the brain and is then followed by sleep. In contrast, our DI regimen replicates the rise and fall of plasma METH that would be expected from successive METH 'hits' occurring during a human binge and results in behavioral activation, which is maintained for 72–84 h, and includes several days of sleep disruption. We recognize that alternative METH administration regimens may be relevant to other aspects of METH abuse and dependence. However, we propose that the DI represents a more accurate plasma METH profile that effectively minimizes additional/extraneous, and perhaps irrelevant, neurobiological adaptations that may be associated with other exposure profiles.

The behavioral response during the METH binge was marked by continuous, intense activation throughout the 72-h period of drug administration (Figure 2). The activation was evident as increased locomotion after the initial 12 h of drug injections, but locomotion was subsequently replaced by focused stereotypies consisting

almost exclusively of oral behaviors directed at the bars of the floor and at the food grid. The progressive accumulation of METH because of the DI-dependent 12-h plasma half-life of the drug likely contributed to the gradual appearance and intensification of stereotyped behaviors, as oral stereotypy in the absence of locomotion was not the predominant response when the same dose of drug had been administered at longer intervals before the binge (eg, Days 82–92, four injections at 4-h intervals; data not shown). During the binge, the stereotypies were unvaried and fixed; the animals rarely moved from an animal-specific, highly restricted site, as was evident in the almost complete absence of locomotion during the approximately final 60 h of the 72-h binge. Further, there was no evidence for behavioral tachyphylaxis/tolerance at any point during the entire binge. As we have argued earlier (Segal *et al*, 2005), this high state of behavioral activation, including the complete absence of episodes of sleep throughout this period, contrasts with the distinct behavioral fluctuations, which occur with the METH pharmacokinetics associated with the successive subcutaneous injections in 'binge' protocols we and others have used earlier.

When binge-treated animals (0.5 mg/kg doses) were subsequently challenged with a single injection of the same METH dose 24 h after the last binge injection, the overall locomotor response (Figure 3) was markedly attenuated (crossovers, 0–3 h: first injection of binge: 360 ± 58; post-binge challenge: 40 ± 10; $P < 0.01$). This attenuation seemed to be a direct consequence of the binge pretreatment, as these same animals exhibited a robust locomotor response to the first injection of the binge. In addition, the attenuated response did not seem to be due to the predominance of focused stereotypies selective to the binge-pretreated group (Figure 3). This diminished locomotor responsivity may be analogous to the psychostimulant withdrawal syndrome, or 'crash,' after cessation of drug intake in human beings (Gawin and Kleber, 1986; Srisurapanont *et al*, 1999; Kampman *et al*, 2000). Anhedonia is a symptom frequently linked to stimulant withdrawal (Gawin and Kleber, 1986), and preclinical studies suggest that stimulant withdrawal is associated with an attenuated mesoaccumbens dopamine response to both amphetamine (AMPH) and sucrose reward (Vacca *et al*, 2007). The decreased locomotor response to the METH challenge we observed during withdrawal after the binge may similarly be mediated by diminished accumbens dopamine responsivity.

The attenuated locomotor response to a METH challenge during withdrawal contrasts sharply with the intense stereotypy profile, which was sustained throughout binge administration of METH, suggesting different adaptive mechanisms in the mesoaccumbens and nigro-striatal DA pathways that have important functions in the locomotor and stereotypy response, respectively (Kelly *et al*, 1975; Kelly and Iversen, 1976; Costall and Naylor, 1977; Swerdlow *et al*, 1986; Whishaw *et al*, 1992; Dickson *et al*, 1994). In the case of the former, our present results revealed a substantial, but transient, reduction in nucleus accumbens DA levels, which was no longer significant by 48-h after the last binge injection (Table 3). This decrease may reflect a temporary depletion of available DA, and may account for the attenuated locomotor response to METH we observed.

The neurochemical effects we observed in caudate–putamen seem to be more complex and may involve elements of both adaptation and 'neurotoxicity.' First, in contrast to nucleus accumbens, there seemed to be no short-term transient decrease in caudate–putamen DA levels. The absence of a tachyphylaxis-like effect in the intense stereotypy, which persisted throughout the binge, would be consistent with the continued maintenance of an available pool of METH-releasable dopamine. Second, also in contrast to nucleus accumbens, caudate–putamen exhibited relatively persistent decreases in DA levels, along with decrements in $VMAT_2$ and DAT. There is ample evidence from studies in both human beings and experimental animals that prolonged high-dose exposure to METH results in decrements of DA terminal markers, including a decrease in DA levels, as well as reductions in the DAT and the $VMAT_2$, but it is not entirely clear to what extent these changes reflect neurotoxicity or neuroadaptations (Wilson *et al*, 1996a; Davidson *et al*, 2001; Guilarte, 2001; Cadet *et al*, 2003). When high-dose METH exposure is preceded by an ED pretreatment, we have consistently observed a 10–15% decrement in [^{3}H]TBzOH binding (Segal *et al*, 2003, 2005), similar to our present results (Table 2). As it has been argued that $VMAT_2$ levels are not readily subject to regulation (Vander Borght *et al*, 1995; Wilson and Kish, 1996; Naudon *et al*, 1996; Kilbourn *et al*, 1996; Frey *et al*, 1997), but rather are related to integrity of DA nerve terminals (Frey *et al*, 1997), we and others have argued that the decrease in $VMAT_2$ likely reflects changes in DA terminal integrity. In an earlier study (Segal *et al*, 2005), the decrease in $VMAT_2$ persisted for at least 30 days. In contrast to these relatively small but long-lasting changes in $VMAT_2$, DA and DAT levels exhibit substantially larger decreases (30–35% in this study; Tables 2 and 3), which, in an earlier study, partially recovered by 30 days (Segal *et al*, 2005). Many prominent indices of DA terminal neurotoxicity display at least some degree of recovery (Bowyer *et al*, 1992; Melega *et al*, 1997; Friedman *et al*, 1998; Cass and Manning, 1999; Harvey *et al*, 2000), which suggests that multiple underlying molecular mechanisms regulate nerve terminal integrity. In this study, the substantially greater decrements in DA and DAT levels compared with the $VMAT_2$, along with the relatively short-term reversal of these dopaminergic parameters, may likewise reflect partial recovery after initial, short-term inhibition or downregulation in response to excessive release of DA (Wilson and Kish, 1996; Guilarte, 2001).

In addition to the transient decrease in accumbens DA levels, the METH binge resulted in substantial depletions of both 5HT and NE. The 5HT changes varied as a function of brain region and included both a transient decrease in caudate–putamen, as well as a more persistent decrease in frontal cortex, but no significant change in hippocampus. It is notable that, in a recent study of the serotonin transporter in abstinent METH abusers, orbitofrontal and occipital cortices were the only regions, which exhibited a significant decrement (Kish *et al*, 2008). Those authors suggested that cortical decrements in 5HT may contribute to decision-making problems in chronic METH abusers. In contrast to 5HT, all brain regions examined exhibited transient decreases in NE levels, ranging from 23% in nucleus accumbens to $>50\%$ in frontal cortex and hippocampus.

The depletion of the biogenic amines by prolonged exposure to METH is not surprising because of the ability of AMPH derivatives to disrupt vesicular transport and storage (Johnson *et al*, 1982; Phillips, 1982; Sulzer and Rayport, 1990; Liu *et al*, 1995). The pronounced effects on NE levels may also be due to decreases in vesicular transport of precursor DA and subsequent reductions in NE synthesis. A number of investigators have reported decreases in regional or whole brain NE after cessation of chronic stimulant administration, and the depletion has been suggested to contribute to the depression associated with the stimulant withdrawal syndrome (Paulson *et al*, 1991). Our data show that binge-like METH exposure results in different region- and time-dependent effects on multiple biogenic amine systems in the early post-binge time period that likely impact behavior. In the absence of functional measures of neurotransmission (eg, extracellular transmitter concentrations) and more detailed studies of neurochemistry–behavior relationships, it would be difficult to speculate regarding the magnitude of the changes we observed and their potential behavioral significance. Nevertheless, the apparent reversibility of most of those decreases after cessation of drug exposure suggests that those neuronal systems were not irreversibly damaged, that is neurodegenerated, but rather were temporarily depleted of their neurotransmitter content.

In addition to alterations in presynaptic components of these biogenic amine systems, it is possible that prolonged exposure to METH results in adaptations at post-synaptic receptor sites. For example, we have shown earlier that prolonged exposure to intravenous METH resulted in transient decreases in striatal D1 and D2 DA receptors (Segal *et al*, 2005). Similar mechanisms may be operative in the present treatment regimen and likely have a function in the behavioral consequences associated with this METH treatment regimen.

In summary, we have characterized neurochemical and behavioral consequences of a novel METH administration regimen coupled with a 72-h binge that simulates in rodents, several important aspects of a prominent pattern of METH abuse. In general, the pattern of changes in biogenic amine markers using this treatment regimen parallels those changes characterized in human post-mortem studies. Insofar as the overall pattern of stimulant exposure, continuous behavioral activation and sleep deprivation during the binge are important in the generation of stimulant-induced psychosis in human beings, further studies using our experimental approach with multiple binges, may provide more meaningful and translationally relevant data in identifying neurobiological mechanisms underlying that psychopathology.

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DISCLOSURE/CONFLICT OF INTEREST

The authors declare no conflict of interest.

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