



Published in final edited form as:

*Neuropsychopharmacology*. 2009 October ; 34(11): 2430–2441. doi:10.1038/npp.2009.73.

## HUMAN METHAMPHETAMINE PHARMACOKINETICS SIMULATED IN THE RAT: BEHAVIORAL AND NEUROCHEMICAL EFFECTS OF A 72- HOUR BINGE

Ronald Kuczenski<sup>1</sup>, David S. Segal<sup>1</sup>, William P. Melega<sup>2</sup>, Goran Lacan<sup>2</sup>, and Stanley J. McCunney<sup>1</sup>

<sup>1</sup>Department of Psychiatry, University of California, San Diego, School of Medicine, La Jolla, California

<sup>2</sup>Department of Molecular and Medical Pharmacology, David Geffen School of Medicine at University of California, Los Angeles, California

### Abstract

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) 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 dynamic infusion, and then exposed to a binge in which drug was administered every 3 h for 72h. 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, [<sup>3</sup>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 24h after the last injection of the binge. Collectively, the changes we characterized during and following a METH binge suggest that for humans under similar exposure conditions, multiple time-dependent neurochemical deficits contribute to their behavioral profiles.

Users may view, print, copy, and download text and data-mine the content in such documents, for the purposes of academic research, subject always to the full Conditions of use:[http://www.nature.com/authors/editorial\\_policies/license.html#terms](http://www.nature.com/authors/editorial_policies/license.html#terms)

Corresponding Author: Ronald Kuczenski, Ph.D., Department of Psychiatry, School of Medicine, University of California, San Diego, La Jolla, CA 92093-0603, Telephone: 858-534-4107, FAX: 858-534-9917, rkuczenski@ucsd.edu.

#### DISCLOSURE/CONFLICTS OF INTEREST

All authors have no financial interests or potential conflicts of interest to declare.

## Keywords

Methamphetamine; binge; dopamine; norepinephrine; serotonin; stereotypy; locomotion

---

## 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 appears 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; Ling et al, 2005). 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 play important roles 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 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 prior 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; Segal and Kuczenski, 1997b). 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

(e.g., repeated subcutaneous injections), in part because of the profound species differences in pharmacokinetics between humans and rats. The plasma METH half-life is 10 – 12 h in humans (Cook et al, 1992; Cook et al, 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. Because different dynamic changes in neurotransmitter function and behavioral adaptation occur depending on the amount of time neurons are exposed to METH (e.g., (Pickel and Chan, 1995; Frey et al, 1997; Kuczenski and Segal, 1999a; Kuczenski and Segal, 1999b; McCann and Ricaurte, 2004; Ben Shahar et al, 2004; Davidson et al, 2005; (Samaha et al, 2002; Samaha et al, 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 corresponds to the human 10–12 h plasma half-life. The present 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 following a prolonged DI escalating dose pretreatment.

## MATERIALS AND METHODS

### Subjects

Male Sprague-Dawley rats weighing 275 – 300g were obtained from Harlan Labs (Gilroy, CA) and were housed for at least two weeks prior to surgery in groups of 2 or 3 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 am to 7:00 pm), 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 2 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

Methamphetamine 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 two weeks of acclimation, animals were implanted with i.v. 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 one 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 (30×20×38cm) was located in a sound-attenuated cabinet maintained on a reversed 12-hr dark/12-hr light cycle with constant temperature (20°C) and humidity (55 ± 5%). Movements of the animal between quadrants (i.e., 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 via 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 previously (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 two weeks after surgery, animals were placed in individual experimental chambers where 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 via a liquid swivel and a commercially available cannula connector (Plastics One). Each morning throughout the study, at the beginning of the dark phase (7 am), the behavioral chambers were serviced and the animals were weighed and examined for health. Following a 3 day acclimation period, during which animals received daily dynamic infusions 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× 0.5 mg/kg, at 4 hr 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 ± 0.1°C. Temperatures remained relatively constant for about

25 days into the treatment regimen, then gradually declined until the last measurement day to  $36.2 \pm 0.1^\circ\text{C}$ . The mild hypothermia is consistent with previous reports that the direction of stimulant-induced changes in core temperature is dependent on ambient temperature (see, for example, (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 (e.g., 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 i.v. 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 i.v. 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 prior to, 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).

Since rat plasma METH concentrations decline much more rapidly than in humans, additional METH administration following 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  $\mu\text{l}$  (1.3  $\mu\text{g}/\text{kg}$  for a 0.5 mg/kg dose) over a 16 msec duration], in order to generate a plasma profile of the drug corresponding to a 12-hr plasma half-life (see (Segal and Kuczenski, 2006) for a more detailed discussion).

### Escalating Dose Dynamic Infusion Treatment Protocol

Following acclimation to the behavioral chambers, animals received either single dynamic infusion of 0.125 mg/kg METH or comparable volumes of saline daily for 8 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 Day 92, the number of 0.5 mg/kg injections was then progressively increased to 4/day (at 3 h intervals during the 12 h awake cycle). From Day 93 – Day 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 (i.e, metabolic tolerance) because most evidence suggests that repeated administration of METH in humans 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 following 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 for 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 [<sup>3</sup>H]ligand binding to the dopamine transporter (DAT) and the vesicular monoamine transporter (VMAT<sub>2</sub>). 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 following 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 hrs after the last injection of the binge was intended to assess the status of those neural systems involved in the production of stimulant-induced locomotion and stereotypy following complete wash-out of residual drug.

### **Animal Morbidity, Mortality**

Animals weighed approximately 350 g on introduction to the chambers, and continued to gain weight until day 69 ( $414 \pm 8$  g); they lost about 20 grams 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, 1X per day) to Day 85 (0.5 mg/kg 3X 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 previous 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 10cc of sterile lactated Ringer's solution followed by 10cc 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, 2 were removed, then recovered (not included in the data analyses), 1 animal lost catheter function and 1 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 (AMPH)**

For pharmacokinetic analyses, separate groups of animals (n = 8 per time point) were sacrificed 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**— $[^3\text{H}]$ WIN 35,428, 87.0 Ci/mmol, was obtained from Perkin Elmer Life Sciences Inc. (Boston, MA) and  $[^3\text{H}]$ -Dihydrotrabenzazine ( $[^3\text{H}]$ 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).

$[^3\text{H}]$ WIN 35,428 binding to the dopamine transporter (DAT) was measured in rat striatal homogenates following the previously 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 sec in cold (0–4°C) binding buffer (20 mM sodium phosphate buffer, pH7.4; 0.32M 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  $[^3\text{H}]$ WIN 35,428 binding experiment was performed in a total volume of 0.5ml. Each sample (100µl of stock tissue suspension) was assayed in triplicate for total and duplicate for nonspecific binding. The  $[^3\text{H}]$ WIN 35,428 concentration was 5 nM and cocaine hydrochloride at 30µM 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 2h in binding buffer containing 0.1% polyethyleneimine) using a Millipore cell harvester. Filters were washed with ice-cold binding buffer (3 × 5ml) and then placed in 10 ml of Ecoscint A (National Diagnostics, Atlanta, GA, USA) overnight before liquid scintillation counting. Non-specific binding represented <10% of the total binding and maximum  $[^3\text{H}]$ WIN 35,428 DAT binding in the striatal tissue suspension comprised <2% of the total radioactivity introduced into the assay. Specific  $[^3\text{H}]$ WIN 35,428 binding was expressed in fmol/mg protein.

$[^3\text{H}]$ TBzOH binding to the vesicular monoamine transporter (VMAT<sub>2</sub>) was measured using modifications of previously reported methods (Scherman et al, 1986; Wilson et al, 1996b; Hogan et al, 2000; Segal et al, 2005). Briefly, striatal tissue homogenates (100µl) in the phosphate/sucrose binding buffer, prepared as described above for  $[^3\text{H}]$ WIN 35,428 binding assay, were incubated in a total volume of 0.5 ml.  $[^3\text{H}]$ TBzOH was used at a final concentration 2nM, and non-specific binding was determined in the presence of 10µM tetrabenazine. Samples in triplicate for total and duplicate for non-specific binding were incubated for 2h at room temperature. The incubation was terminated by the addition of ice-cold binding buffer (1ml) and suspension was filtered through Whatman GF/C glass fiber filters (presoaked for 2h in binding buffer containing 0.1% polyethyleneimine) using Millipore cell harvester. Filters were washed with ice-cold binding buffer (3 × 5ml). 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  $[^3\text{H}]$ TBzOH VMAT<sub>2</sub>

binding in the striatal tissue suspension comprised < 4% of the total radioactivity introduced into the assay. Specific [<sup>3</sup>H]TBzOH binding was expressed in fmol/mg protein. Although recent PET imaging data in humans (Boileau et al, 2008) suggest that the tracer dose of [<sup>13</sup>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 prior in vitro competition binding studies of 4 nM [<sup>3</sup>H] TBZ with norepinephrine showed a NE IC<sub>50</sub> value of 12.5uM (Scherman et al, 1983). That magnitude of effect likely extends to DA, as shown by subsequent studies (Partilla et al, 2006) in which an IC<sub>50</sub> of >100 uM was reported in DA - 2 nM [<sup>3</sup>H] TBZ competition studies (note: 2 nM [<sup>3</sup>H]TBZ was used in our in vitro studies). Also, 2 nM [<sup>3</sup>H]TBZ concentration was used in VMAT binding studies of human control and Parkinsonian striatal homogenates without any washing; the authors referenced their prior work showing that “at concentrations lower than 100 uM, endogenous monoamines did not displace [<sup>3</sup>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 -80C until assayed for neurochemical levels. Regional brain levels of norepinephrine (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 rpm, and an aliquot of the supernatant was assayed using HPLC with electrochemical detection as previously described (Kuczenski et al., 1995). The HPLC-EC consisted of a 100 × 4.6 mm ODS-C18 3μ column (Regis) maintained at 40°C. Mobile phase (0.05 M citric acid, 7% methanol, 0.1 mM Na<sub>2</sub>EDTA 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. Because 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 prior ED-METH studies. For example, the initial injection of 0.125 mg/kg resulted in a significant increase in locomotor activity over a 4-hr interval following drug administration compared to the previous day's saline administration (Saline: 116 ± 13 Crossovers; METH: 210 ± 18; p < 0.0001; t = 5.846, df =



19). Increasing the dose of METH resulted in progressively increasing locomotor activation, both in terms of magnitude and duration. Following 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. Based on 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 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 sub-group of animals along with saline-pretreated controls received a dynamic infusion 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 to saline-pretreated controls. The decrement did not appear to be related to the differential appearance of stereotyped behaviors in the METH pretreated group, since 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 following the 72-h binge**—Plasma METH levels measured at 1 h after the last binge injection (2.9  $\mu\text{M}$ ; 440 ng/ml; summarized in Table 1) were comparable to values estimated from our pharmacokinetic modeling (Figure 1; 3.2  $\mu\text{M}$ ) and declined over the subsequent 9 h to 1.7  $\mu\text{M}$  (250 ng/ml), reflecting a plasma half-life of 11.7 h. Plasma levels of AMPH, a metabolite of METH, were about 40% of METH levels.

**Striatal DAT and VMAT<sub>2</sub> following the 72-hr binge**—We used [<sup>3</sup>H]WIN 35,428 and [<sup>3</sup>H]TBzOH binding to provide measures, respectively, of DAT and VMAT<sub>2</sub> levels in striatum 10 hrs following the last injection of the 72-h binge (Table 2). We found a small decrease in [<sup>3</sup>H]TBzOH binding (13%) 10 h after the last binge injection, but the decrement

did not achieve statistical significance ( $p = .061$ ). In contrast, we observed a substantial (35%) decrement in [ $^3\text{H}$ ]WIN 35,428 binding to DAT ( $p < 0.01$ ).

**Regional neurotransmitter levels following the 72-hr 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 norepinephrine 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; e.g., 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).

## DISCUSSION

We designed the dynamic infusion 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, since qualitatively different behavioral and/or neurochemical consequences may sometimes occur depending on whether the stimulant is self- or experimenter-administered (see, for example (Stefanski et al, 1999; Jacobs et al, 2003), although see (Winsauer et al, 2003; Kiyatkin and Brown, 2004; Stuber et al, 2005))

Previously, 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 ( $\sim 3 \mu\text{M}$  at 1 h post-binge), as well as the plasma METH half-life ( $\sim 12$  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 prior 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  $\sim 15\%$  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 appears necessary for accurate interpretation of behavioral changes associated with repeated METH administration since our previous 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 prior 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 (e.g., 4 injections at 2h 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 following 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 due to the DI –dependent 12-h plasma half-life of the drug likely contributed to the gradual appearance and intensification of stereotyped behaviors since 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 (e.g., Days 82 – 92, 4 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 previously argued (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 previously.

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 hrs: 1<sup>st</sup> injection of binge:  $360 \pm 58$ ; post-binge challenge:  $40 \pm 10$ ;  $P < 0.01$ ). This attenuation appeared to be a direct consequence of the binge pretreatment, since these same animals exhibited a robust

locomotor response to the first injection of the binge. In addition, the attenuated response did not appear 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,” following cessation of drug intake in humans (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 and sucrose reward (Vacca et al, 2007). The decreased locomotor response we observed to the METH challenge during withdrawal following 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 play important roles 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-hrs following 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 appear to be more complex and may involve elements of both adaptation and “neurotoxicity.” First, in contrast to nucleus accumbens, there appeared 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<sub>2</sub> and DAT. There is ample evidence from studies in both humans 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<sub>2</sub>, 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 escalating dose pretreatment, we have consistently observed a 10 – 15% decrement in [<sup>3</sup>H]TBzOH binding (Segal et al, 2003; Segal et al, 2005), similar to our present results (Table 2). Since it has been argued that VMAT<sub>2</sub> levels are not readily subject to regulation (Vander Borgh 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<sub>2</sub> likely reflects changes in DA terminal integrity. In an earlier study (Segal et al, 2005), the decrease in VMAT<sub>2</sub> persisted for at least 30 days. In contrast to these relatively small but long-lasting changes in VMAT<sub>2</sub>, DA and DAT levels exhibit substantially larger decreases (30–35% in the present study; Table 2, Table3), 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 to the VMAT<sub>2</sub>, 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 greater than 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 amphetamine-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 following 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 (e.g., 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 following cessation of drug exposure suggests that those neuronal systems were not irreversibly damaged, i.e., 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 previously shown 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 play a role 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 humans, further studies using our experimental approach with multiple binges, may provide more meaningful and translationally relevant data in identifying neurobiological mechanisms underlying that psychopathology.

## ACKNOWLEDGMENTS

This research was supported by NIH Grant DA-01568.

## REFERENCES

- Angrist, B. Clinical effects of central nervous system stimulants: A selective update. In: Engel, J.; Oreland, L., editors. *Brain Reward Systems and Abuse*. New York: Raven Press; 1987. p. 109-127.
- Angrist, B. Amphetamine psychosis: clinical variations of the syndrome. In: Cho, AK.; Segal, DS., editors. *Amphetamine and its analogues*. San Diego: Academic Press; 1994a. p. 387-414.
- Angrist B. Psychosis-inducing effects of cocaine may show sensitization more than other effects. *Neuropsychopharmacol.* 1994b; 10:197S.
- Anggard E, Jonsson LE, Hogmark AL, Gunne LM. Amphetamine metabolism in amphetamine psychosis. *Clin.Pharmacol.Ther.* 1973; 14:870–880. [PubMed: 4729903]
- Ben Shahar O, Ahmed SH, Koob GF, Etenberg A. The transition from controlled to compulsive drug use is associated with a loss of sensitization. *Brain Res.* 2004; 995:46–54. [PubMed: 14644470]
- Boileau I, Rusjan P, Houle S, Wilkins D, Tong J, Selby P, Guttman M, Saint-Cyr JA, Wilson AA, Kish SJ. Increased vesicular monoamine transporter binding during early abstinence in human methamphetamine users: Is VMAT2 a stable dopamine neuron biomarker? *J Neurosci.* 2008; 28:9850–9856. [PubMed: 18815269]
- Bowyer JF, Tank AW, Newport GD, Slikker W Jr, Ali SF, Holson RR. The influence of environmental temperature on the transient effects of methamphetamine on dopamine levels in rat striatum. *J.Pharmacol.Exp.Ther.* 1992; 260:817–824. [PubMed: 1346646]
- Brady KT, Lydiard RB, Malcom R, Ballenger JC. Cocaine-induced psychosis. *J.Clinical Psychiatry.* 1991; 52:509–512. [PubMed: 1752853]
- Buffenstein A, Heaster J, Ko P. Chronic psychotic illness from methamphetamine. *Amer.J.Psychiatry.* 1999; 156:662. [PubMed: 10200757]
- Cadet JL, Jayanthi S, Deng XL. Speed kills: cellular and molecular bases of methamphetamine-induced nerve terminal degeneration and neuronal apoptosis. *FASEB Journal.* 2003; 17:1775–1788. [PubMed: 14519657]
- Cass WA, Manning MW. Recovery of presynaptic dopaminergic functioning in rats treated with neurotoxic doses of methamphetamine. *J.Neurosci.* 1999; 19:7653–7660. [PubMed: 10460271]
- Cook CE, Jeffcoat AR, Hill JM, Pugh DE, Patetta PK, Sadler BM, White WR, Perez-Reyes M. Pharmacokinetics of methamphetamine self-administered to human subjects by smoking S-(+)-methamphetamine hydrochloride. *Drug Metab Dispos.* 1993; 21:717–723. [PubMed: 8104133]
- Cook CE, Jeffcoat AR, Sadler BM, Hill JM, Voyksner RD, Pugh DE, White WR, Perez-Reyes M. Pharmacokinetics of oral methamphetamine and effects of repeated daily dosing in humans. *Drug Metab Dispos.* 1992; 20:856–862. [PubMed: 1362938]
- Costall, B.; Naylor, RJ. Mesolimbic and extrapyramidal sites for the mediation of stereotyped behavior patterns and hyperactivity by amphetamine and apomorphine in the rat. In: Ellinwood, EH., Jr; Kilbey, MJ., editors. *Cocaine and other stimulants*. New York: Plenum Press; 1977. p. 47-76.
- Davidson C, Gow AJ, Lee TH, Ellinwood EH Jr. Methamphetamine neurotoxicity: necrotic and apoptotic mechanisms and relevance to human abuse and treatment. *Brain Res.Revs.* 2001; 36:1–22. [PubMed: 11516769]

- Davidson C, Lee TH, Ellinwood EH. Acute and chronic continuous methamphetamine have different long-term behavioral and neurochemical consequences. *Neurochemistry International*. 2005; 46:189–203. [PubMed: 15670635]
- Davis, JM.; Schlemmer, RF, Jr. The Amphetamine Psychosis. In: Caldwell, J., editor. *Amphetamines and Related Stimulants: Chemical, Biological, Clinical and Social Aspects*. Boca Raton, FL: CRC Press; 1980. p. 161-173.
- Devoto P, Flore G, Pira L, Longu G, Gessa GL. Alpha<sub>2</sub>-adrenoceptor mediated co-release of dopamine and noradrenaline from noradrenergic neurons' in the cerebral cortex. *J.Neurochem*. 2004; 88:1003–1009. [PubMed: 14756822]
- Dickson PR, Lang CG, Hinton SC, Kelley AE. Oral stereotypy induced by amphetamine microinjection into striatum: An anatomical mapping study. *Neurosci*. 1994; 61:81–91.
- Ferrario CR, Shoui M, Samaha A-N, Watson CJ, Kennedy RT, Robinson TE. The rate of intravenous cocaine administration alters c-fos mRNA expression and the temporal dynamics of dopamine, but not glutamate, overflow in the striatum. *Brain Res*. 2008; 1209:151–156. [PubMed: 18396266]
- Fischman MW, Schuster CR. Tolerance development to chronic methamphetamine intoxication in the rhesus monkey. *Pharmac.Biochem.Behav*. 1974; 2:503–508.
- Fischman MW, Schuster CR, Javaid J, Hatano Y, Davis J. Acute tolerance development to the cardiovascular and subjective effects of cocaine. *J.Pharmacol.Exp.Ther*. 1985; 235:677–682. [PubMed: 4078729]
- Frey KA, Kilbourn MR, Robinson TE. Reduced striatal vesicular monoamine transporters after neurotoxic but not after behaviorally-sensitizing doses of methamphetamine. *Eur.J.Pharmacol*. 1997; 334:273–279. [PubMed: 9369358]
- Friedman SD, Castañeda E, Hodge GK. Long-term monoamine depletion, differential recovery, and subtle behavioral impairment following methamphetamine-induced neurotoxicity. *Pharmacol Biochem Behav*. 1998; 61:35–44. [PubMed: 9715805]
- Gawin FH. Cocaine addiction: Psychology and neurophysiology. *Science*. 1991; 251:1580–1586. [PubMed: 2011738]
- Gawin, FH.; Khalsa, ME. Sensitization and "street" stimulant addiction. In: Majewska, MD., editor. *Neurotoxicity and neuropathology associated with stimulant abuse*. NIDA Research Monograph Series. Washington, DC: U.S. Government Printing Office; 1996. p. 224-250.
- Gawin FH, Kleber HD. Abstinence symptomatology and psychiatric diagnosis in cocaine abusers. *Arch.Gen.Psychiatry*. 1986; 43:107–113. [PubMed: 3947206]
- Ghitza UE, Fabbriatore AT, Prokopenko V, Pawlak AP, West MO. Persistent cue-evoked activity of accumbens neurons after prolonged abstinence from self-administered cocaine. *J.Neurosci*. 2003; 23:7239–7245. [PubMed: 12917356]
- Guilarte TR. Is methamphetamine abuse a risk factor in parkinsonism? *Neurotoxicology*. 2001; 22:725–731. [PubMed: 11829406]
- Guilarte TR, Nihei MK, McGlothlan JL, Howard AS. Methamphetamine-induced deficits of brain monoaminergic neuronal markers: distal axotomy or neuronal plasticity. *Neurosci*. 2003; 122:499–513.
- Harvey DC, Lacan G, Tanious SP, Melega WP. Recovery from methamphetamine induced long-term nigrostriatal dopaminergic deficits without substantia nigra cell loss. *Brain Res*. 2000; 871:259–270. [PubMed: 10899292]
- Hogan KA, Staal RGW, Sonsalla PK. Analysis of VMAT2 binding after methamphetamine or MPTP treatment: Disparity between homogenates and vesicle preparations. *J.Neurochem*. 2000; 74:2217–2220. [PubMed: 10800969]
- Jacobs EH, Smit AB, De Vries TJ, Schoffelmeer ANM. Neuroadaptive effects of active versus passive drug administration in addiction research. *Trends Pharmacol.Sci*. 2003; 24:566–573. [PubMed: 14607079]
- Jaffe, JH. Amphetamine (or amphetaminelike)-related disorders. In: Kaplan, J., editor. *Comprehensive Textbook of Psychiatry*. Baltimore: Williams and Wilkins; 1995. p. 791-799.
- Johnson RG, Carty SE, Hayflick S, Scarpa A. Mechanism of accumulation of tyramine, metaraminol, and isoproterenol in isolated chromaffin granules. *Biochem Pharmacol*. 1982; 31:815–823. [PubMed: 7082350]

- Jones AW, Holmgren A, Kugelberg FC. Driving under the influence of central stimulant amines: age and gender differences in concentrations of amphetamine, methamphetamine, and ecstasy in blood. *J Stud Alcohol Drugs*. 2008; 69:202–208. [PubMed: 18299760]
- Kalasinsky KS, Bosy TZ, Schmunk GA, Reiber G, Anthony RM, Furukawa Y, Guttman M, Kish SJ. Regional distribution of methamphetamine in autopsied brain of chronic human methamphetamine users. *Forensic Sci.Int*. 2001; 116:163–169. [PubMed: 11182268]
- Kampman KM, Volpicelli JR, Alterman AI, Cornish J, O'Brien CP. Amantadine in the treatment of cocaine-dependent patients with severe withdrawal symptoms. *Am J Psychiatry*. 2000; 157:2052–2054. [PubMed: 11097979]
- Kelly PH, Iversen SD. Selective 6OHDA-induced destruction of mesolimbic dopamine neurons: Abolition of psychostimulant-induced locomotor activities in rats. *Eur.J.Pharmacol*. 1976; 40:45–56. [PubMed: 1033072]
- Kelly PH, Seviour P, Iversen SD. Amphetamine and apomorphine responses in the rat following 6-OHDA lesions of the nucleus accumbens septi and corpus striatum. *Brain Res*. 1975; 94:507–522. [PubMed: 1171714]
- Kilbourn MR, Frey KA, Vander Borgh T, Sherman PS. Effects of dopaminergic drug treatments on in vivo radioligand binding to brain vesicular monoamine transporters. *Nucl.Med.Biol*. 1996; 23:467–471. [PubMed: 8832701]
- Kish SJ, Fitzmaurice PS, Boileau I, Schmunk GA, Ang LC, Furukawa Y, Chang LJ, Wickham DJ, Sherwin A, Tong J. Brain serotonin transporter in human methamphetamine users. *Psychopharmacol*. 2008
- Kiyatkin EA, Brown PL. Brain temperature fluctuations during passive vs. active cocaine administration: clues for understanding the pharmacological determination of drug-taking behavior. *Brain Res*. 2004; 1005:101–116. [PubMed: 15044070]
- Kramer, JC. Introduction to Amphetamine Abuse. In: Ellinwood, EH., Jr; Cohen, S., editors. *Current Concepts on Amphetamine Abuse*. Washington, D.C: U.S. Gov. Printing Office; 1972. p. 177-184.
- Kuczenski R, Segal DS. An escalating dose-high dose binge pattern of amphetamine administration results in differential changes in the extracellular dopamine response profiles in caudate-putamen and nucleus accumbens. *J.Neurosci*. 1997; 17:4441–4447. [PubMed: 9151761]
- Kuczenski R, Segal DS. Dynamic changes in sensitivity occur during the acute response to cocaine and methylphenidate. *Psychopharmacol*. 1999a; 147:96–103.
- Kuczenski R, Segal DS. Sensitization of amphetamine-induced stereotyped behaviors during the acute response. *J.Pharmacol.Exp.Ther*. 1999b; 288:699–709. [PubMed: 9918578]
- Kuczenski R, Segal DS, Cho AK, Melega WP. Hippocampus norepinephrine, caudate dopamine and serotonin, and behavioral responses to the stereoisomers of amphetamine and methamphetamine. *J.Neurosci*. 1995; 15:1308–1317. [PubMed: 7869099]
- Ling W, Jaffe JH, Rawson R. Amphetamine (or Amphetamine-like)-related disorders. 2005
- Liu YJ, Peter D, Merickel A, Krantz D, Finn JP, Edwards RH. A molecular analysis of vesicular amine transport. *Behavioural Brain Research*. 1995; 73:51–58. [PubMed: 8788477]
- Madras BK, Spealman RD, Fahey MA, Neumeyer JL, Saha JK, Milius RA. Cocaine receptors labeled by [<sup>3</sup>H]2β-carbomethoxy-3β-(4-fluorophenyl)tropane. *Mol.Pharmacol*. 1989; 36:518–524. [PubMed: 2811854]
- Malberg JE, Seiden LS. Small changes in ambient temperature cause large changes in 3,4-methylenedioxymethamphetamine (MDMA)-induced serotonin neurotoxicity and core body temperature in the rat. *J.Neurosci*. 1998; 18:5086–5094. [PubMed: 9634574]
- McCann UD, Ricaurte GA. Amphetamine neurotoxicity: accomplishments and remaining challenges. *Neurosci Biobehav Rev*. 2004; 27:821–826. [PubMed: 15019431]
- Melega WP, Cho AK, Harvey DC, Lacan G. Methamphetamine blood concentrations in human abusers: Application to pharmacokinetic modeling. *Synapse*. 2007; 61:216–220. [PubMed: 17230548]
- Melega WP, Raleigh MJ, Stout DB, Lacan G, Huang SC, Phelps ME. Recovery of striatal dopamine function after acute amphetamine- and methamphetamine-induced neurotoxicity in the vervet monkey. *Brain Res*. 1997; 766:113–120. [PubMed: 9359594]



- Melega WP, Williams AE, Schmitz DA, DiStefano EW, Cho AK. Pharmacokinetic and pharmacodynamic analysis of the actions of D-amphetamine and D-methamphetamine on the dopamine terminal. *J.Pharmacol.Exp.Ther.* 1995; 274:90–96. [PubMed: 7616454]
- Myles BJ, Jarrett LA, Broom SL, Speaker HA, Sabol KE. The effects of methamphetamine on core body temperature in the rat-PART 1: chronic treatment and ambient temperature. *Psychopharmacol.* 2008; 198:301–311.
- Myles BJ, Sabol KE. The effects of methamphetamine on core body temperature in the rat--Part 2: an escalating regimen. *Psychopharmacol.* 2008; 198:313–322.
- Naudon L, Raisman-Vozari R, Edwards RH, Leroux-Nicollet I, Peter D, Liu Y, Costentin J. Reserpine affects differentially the density of the vesicular monoamine transporter and dihydrotetrabenazine binding sites. *Eur.J.Neurosci.* 1996; 8:842–846. [PubMed: 9081637]
- Partilla JS, Dempsey AG, Nagpal AS, Blough BE, Baumann MH, Rothman RB. Interaction of amphetamines and related compounds at the vesicular monoamine transporter. *J Pharmacol Exp Ther.* 2006; 319:237–246. [PubMed: 16835371]
- Paulson PE, Camp DM, Robinson TE. Time course of transient behavioral depression and persistent behavioral sensitization in relation to regional brain monoamine concentrations during amphetamine withdrawal in rats. *Psychopharmacol.* 1991; 103:480–492.
- Perez-Reyes M, White WR, McDonald SA, Hicks RE, Jeffcoat AR, Hill JM, Cook CE. Clinical effects of daily methamphetamine administration. *Clin.Neuropharmacol.* 1991; 14:352–358. [PubMed: 1913701]
- Peterson GL. A simplification of the protein assay method of Lowry et al. which is more generally applicable. *Analyt.Biochem.* 1977; 83:346–356. [PubMed: 603028]
- Phillips JH. Dynamic aspects of chromaffin granule structure. *Neurosci.* 1982; 7:1595–1609.
- Pickel VM, Chan J. Use of quantitative ultrastructural immunoperoxidase labeling for analysis of catecholamine neurotoxicity and plasticity. *Neurochem.Int.* 1995; 26:125–134. [PubMed: 7599533]
- Rivière GJ, Byrnes KA, Gentry WB, Owens SM. Spontaneous locomotor activity and pharmacokinetics of intravenous methamphetamine and its metabolite amphetamine in the rat. *J.Pharmacol.Exp.Ther.* 1999; 291:1220–1226. [PubMed: 10565845]
- Samaha AN, Li YL, Robinson TE. The rate of intravenous cocaine administration determines susceptibility to sensitization. *J Neurosci.* 2002; 22:3244–3250. [PubMed: 11943825]
- Samaha A-N, Mallet N, Ferguson SM, Gonon F, Robinson TE. The rate of cocaine administration alters gene regulation and behavioral plasticity: implications for addiction. *J.Neurosci.* 2004; 24:6362–6370. [PubMed: 15254092]
- Satel SL, Southwick SM, Gawin FH. Clinical features of cocaine-induced paranoia. *Am.J.Psychiatry.* 1991; 148:495–498. [PubMed: 2006696]
- Scherman D, Boschi G, Rips R, Henry J-P. The regionalization of [<sup>3</sup>H]dihydrotetrabenazine binding sites in the mouse brain and its relationship to the distribution of monamines and their metabolites. *Brain Res.* 1986; 370:176–181. [PubMed: 2423178]
- Scherman D, Jaudon P, Henry JP. Characterization of the monoamine carrier of chromaffin granule membrane by binding of [2-3H]dihydrotetrabenazine. *Proc Natl Acad Sci U S A.* 1983; 80:584–588. [PubMed: 6572908]
- Scherman D, Desnos C, Darchen F, Pollak P, Javoy-Agid F, Agid Y. Striatal dopamine deficiency in Parkinson's disease: role of aging. *Ann Neurol.* 1989; 26:551–557. [PubMed: 2817829]
- Segal DS, Kuczenski R. Individual differences in responsiveness to single and repeated amphetamine administration: behavioral characteristics and neurochemical correlates. *J.Pharmacol.Exp.Ther.* 1987; 242:917–926. [PubMed: 3656119]
- Segal DS, Kuczenski R. An escalating dose "binge" model of amphetamine psychosis: behavioral and neurochemical characteristics. *J.Neurosci.* 1997a; 17:2551–2566. [PubMed: 9065515]
- Segal DS, Kuczenski R. Repeated binge exposure to amphetamine and methamphetamine: behavioral and neurochemical characterization. *J.Pharmacol.Exp.Ther.* 1997b; 282:561–573. [PubMed: 9262316]

- Segal DS, Kuczenski R. Human methamphetamine pharmacokinetics simulated in the rat: Single daily intravenous administration reveals elements of sensitization and tolerance. *Neuropsychopharmacol.* 2006; 31:941–955.
- Segal DS, Kuczenski R, O'Neil ML, Melega WP, Cho AK. Escalating dose methamphetamine pretreatment alters the behavioral and neurochemical profiles associated with exposure to a high-dose methamphetamine binge. *Neuropsychopharmacol.* 2003; 28:1730–1740.
- Segal DS, Kuczenski R, O'Neil ML, Melega WP, Cho AK. Prolonged exposure to intravenous methamphetamine: behavioral and neurochemical characterization. *Psychopharmacol.* 2005; 180:501–512.
- Sherer MA. Intravenous cocaine: Psychiatric effects, biological mechanisms. *Biol.Psychiatry.* 1988; 24:865–885. [PubMed: 3233231]
- Sherer MA, Kumor KM, Cone EJ, Jaffe JH. Suspiciousness induced by four-hour intravenous infusions of cocaine. *Arch.Gen.Psychiatry.* 1988; 45:673–677. [PubMed: 3270328]
- Srisurapanont M, Jarusuraisin N, Jittiwutikan J. Amphetamine withdrawal: II. A placebo-controlled, randomised, double-blind study of amineptine treatment. *Aust N Z J Psychiatry.* 1999; 33:94–98. [PubMed: 10197891]
- Stefanski R, Ladenheim B, Lee SH, Cadet JL, Goldberg SR. Neuroadaptations in the dopaminergic system after active self-administration but not after passive administration of methamphetamine. *Eur.J.Pharmacol.* 1999; 371:123–135. [PubMed: 10357249]
- Stuber GD, Roitman MF, Phillips PEM, Carelli RM, Wightman RM. Rapid dopamine signaling in the nucleus accumbens during contingent and noncontingent cocaine administration. *Neuropsychopharmacol.* 2005; 30:853–863.
- Sulzer D, Rayport S. Amphetamine and other psychostimulants reduce pH gradients in midbrain dopaminergic neurons and chromaffin granules: a mechanism of action. *Neuron.* 1990; 5:797–808. [PubMed: 2268433]
- Swerdlow NR, Vaccarino FJ, Amalric M, Koob GF. The neural substrates for the motor-activating properties of psychostimulants: A review of recent findings. *Pharmacol Biochem Behav.* 1986; 25:233–248. [PubMed: 2875470]
- Vacca G, Ahn S, Phillips AG. Effects of short-term abstinence from escalating doses of D-amphetamine on drug and sucrose-evoked dopamine efflux in the rat nucleus accumbens. *Neuropsychopharmacol.* 2007; 32:932–939.
- Vander Borght TM, Kilbourn MR, Desmond T, et al. The vesicular monoamine transporter is not regulated by dopaminergic drug treatments. *Eur.J.Pharmacol.* 1995; 294:577–583. [PubMed: 8750721]
- Villemagne V, Yuan J, Wong DF, Dannals RF, Hatzidimitriou G, Mathews WB, Ravert HT, Musachio J, McCann UD, Ricaurte GA. Brain dopamine neurotoxicity in baboons treated with doses of methamphetamine comparable to those recreationally abused by humans: Evidence from [<sup>11</sup>C]WIN-35,428 positron emission tomography studies and direct *in vitro* determinations. *J.Neurosci.* 1998; 18:419–427. [PubMed: 9412518]
- Whishaw IQ, Fiorino D, Mittleman G, Castañeda E. Do forebrain structures compete for behavioral expression? Evidence from amphetamine-induced behavior, microdialysis, and caudate-accumbens lesions in medial frontal cortex damaged rats. *Brain Res.* 1992; 576:1–11. [PubMed: 1381257]
- Wilson JM, Kalasinsky KS, Levey AI, Bergeron C, Reiber G, Anthony RM, Schmunk GA, Shannak K, Haycock JW, Kish SJ. Striatal dopamine nerve terminal markers in human, chronic methamphetamine users. *Nature Med.* 1996a; 2:699–703. [PubMed: 8640565]
- Wilson JM, Kish SJ. The vesicular monoamine transporter, in contrast to the dopamine transporter, is not altered by chronic cocaine self-administration in the rat. *J.Neurosci.* 1996; 16:3507–3510. [PubMed: 8627383]
- Wilson JM, Levey AI, Bergeron C, Kalasinsky KS, Ang L, Peretti F, Adams VI, Smialek J, Anderson WR, Shannak K, Deck J, Niznik HB, Kish SJ. Striatal dopamine, dopamine transporter, and vesicular monoamine transporter in chronic cocaine users. *Ann.Neurol.* 1996b; 40:428–439. [PubMed: 8797532]

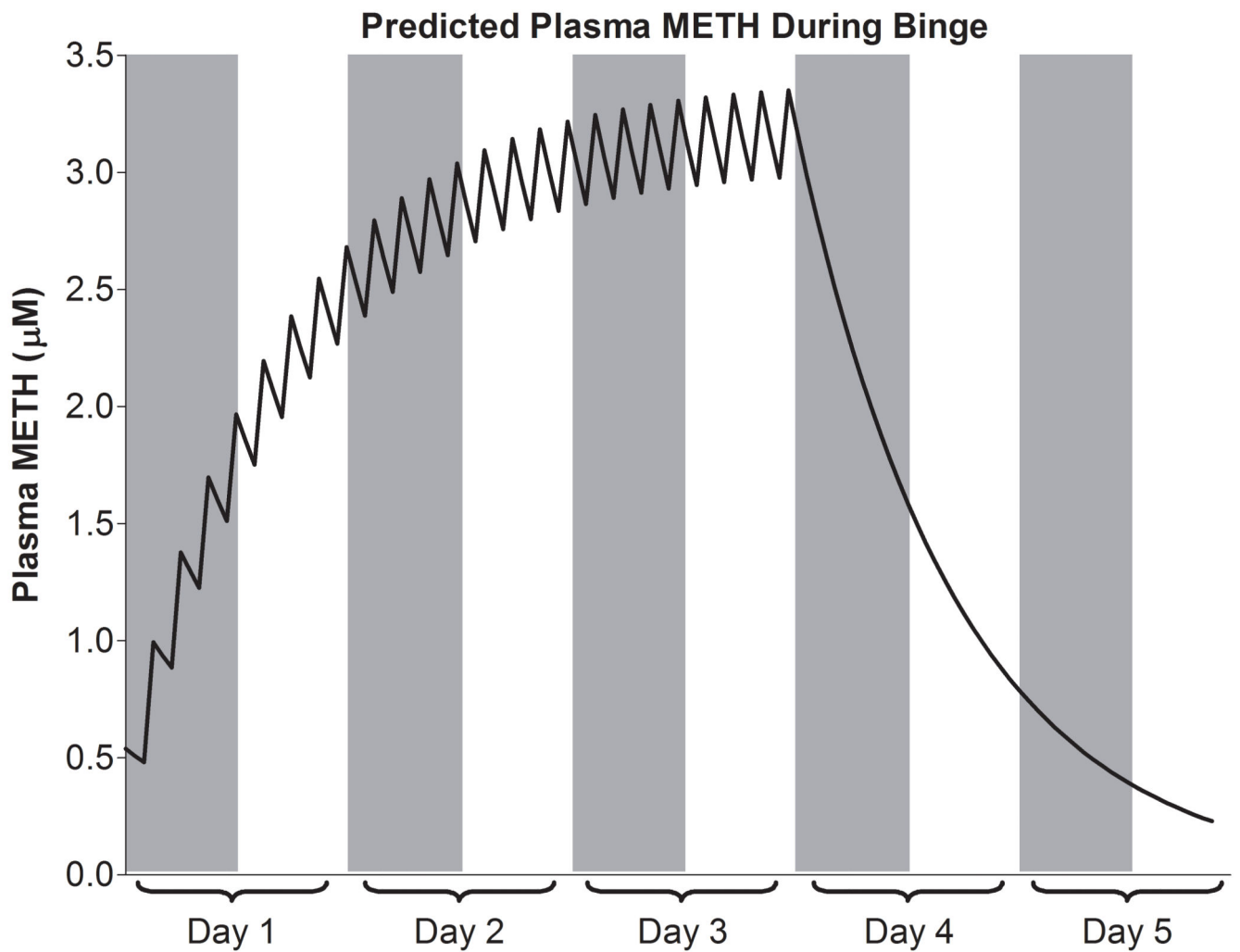
- Winsauer PJ, Moerschbaeher JM, Molina PE, Roussel AM. Contingent and concontingent cocaine administration in rhesus monkeys: a comparison of the effects on the acquisition and performance of response sequences. *Behav.Pharmacol.* 2003; 14:295–306. [PubMed: 12838035]
- Yehuda S, Wurtman RJ. The effects of D-amphetamine and related drugs on colonic temperatures of rats kept at various ambient temperatures. *Life Sci.* 1972; 11:851–859.

Author Manuscript

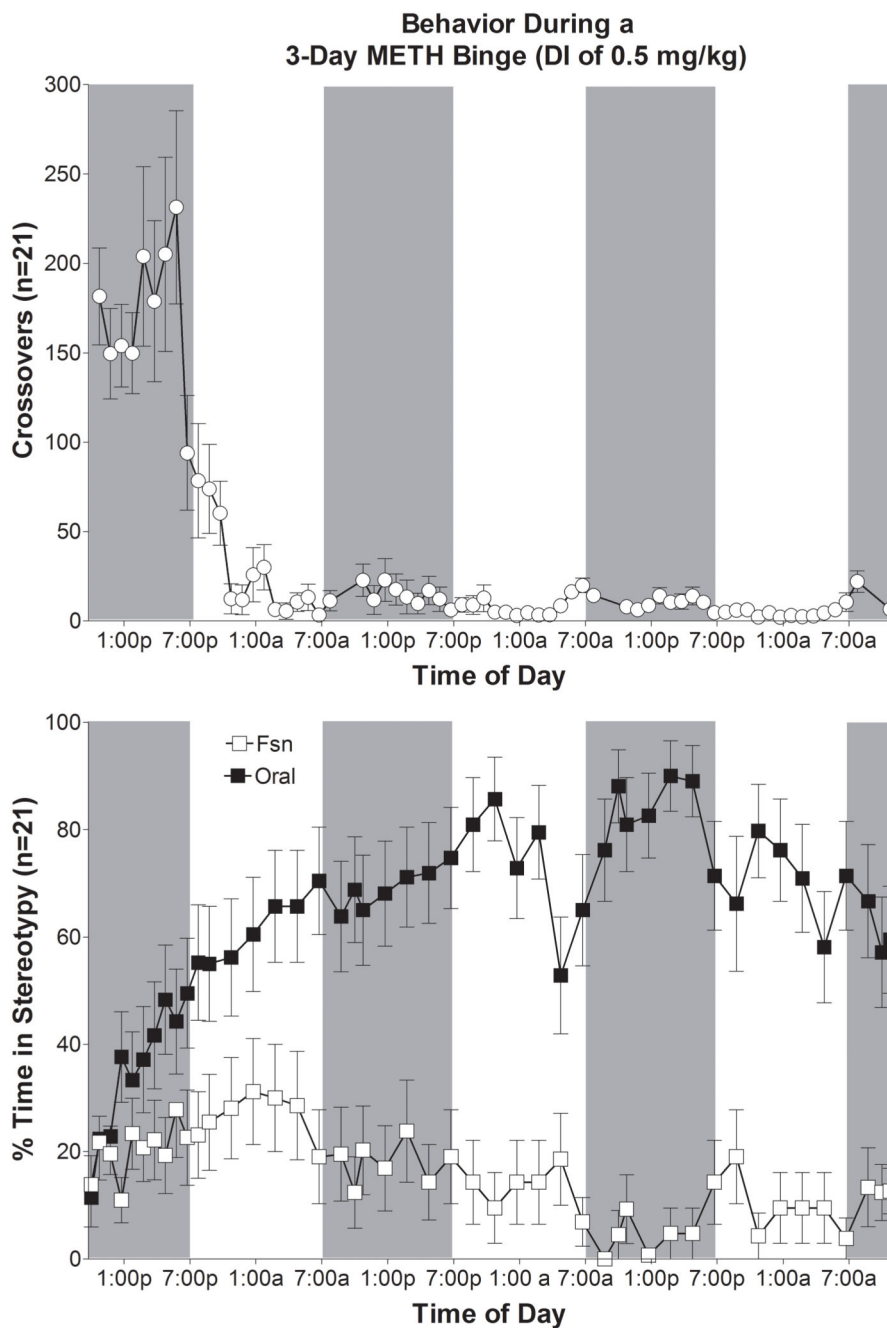
Author Manuscript

Author Manuscript

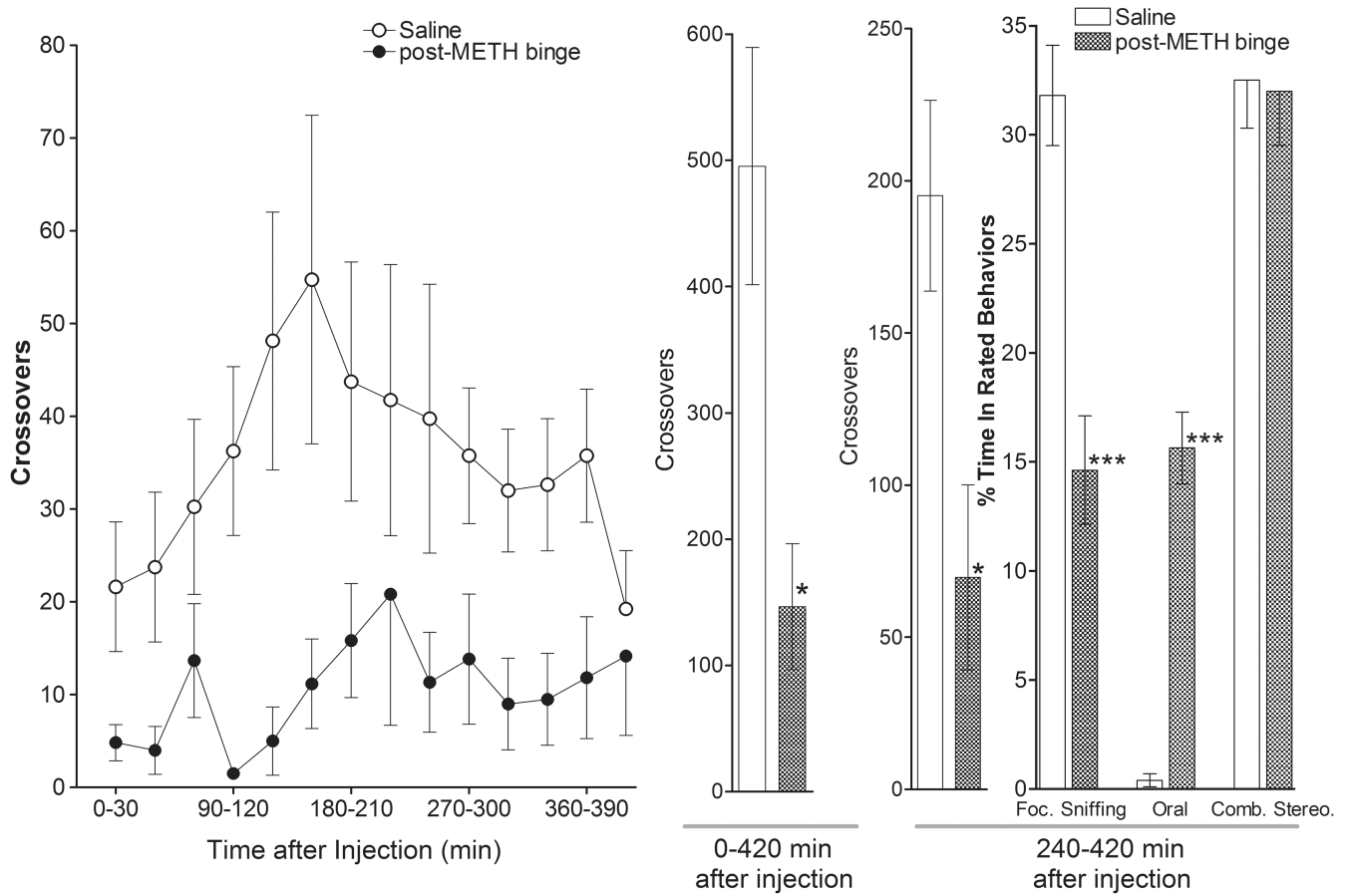
Author Manuscript



**Figure 1.** Predicted plasma METH concentrations during a 72-h binge using dynamic infusions of 0.5 mg/kg METH for each injection at 3 h intervals. During the initial 72 hrs 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.



**Figure 2.** Behavioral response during the 72-hr dynamic infusion-METH binge. . UPPER: Locomotor response (open circles),  $n = 21$ ; Values values are presented as mean crossover  $\pm$  SEM. LOWER : Stereotypy response: Each value represents the percent of time (mean  $\pm$  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.

**Figure 3.**

Behavioral response to the dynamic infusion of METH (0.5 mg/kg). Groups of animals pretreated with saline or the Escalating Dose-Binge protocol received a dynamic infusion of 0.5 mg/kg METH at 18 hrs after the last METH binge injection. **LEFT:** Temporal profile of the locomotor response to the METH dynamic infusion. Values represent the means  $\pm$  SEM for each 30 min interval. Histograms represent the cumulative response during the indicated interval. \* $P < 0.05$ , \*\*\* $P < 0.001$  compared to 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 (25 <sup>th</sup> ) injection of 72 h binge:	1 hr	10 hr
METH ( $\mu\text{M}$ )	$2.9 \pm 0.4$	$1.7 \pm 0.2$
AMPH ( $\mu\text{M}$ )	$1.2 \pm 0.2$	$0.7 \pm 0.1$

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

**Table 2**

Caudate-putamen [<sup>3</sup>H]WIN 35,428 and [<sup>3</sup>H]TBzOH binding (fmoles/mg protein) at 10 h following the 72h Binge (n = 9 for each group)

	[ <sup>3</sup> H]WIN35,428	[ <sup>3</sup> H]TBzOH
Control	153 ± 18	210 ± 11
Binge	100 ± 6 <sup>a</sup>	182 ± 8

<sup>a</sup> p < 0.01 compared to controls.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript



**Table 3**

Regional levels of biogenic amines following a 72-hr binge (nmoles/mg tissue)

Time after last (25 <sup>th</sup> ) injection	Control	1 hr	10 hr	48 hr
Dopamine				
Caudate-putamen	129.9 ± 6.6	89.8 ± 6.1 <sup>***</sup>	88.0 ± 6.6 <sup>**</sup>	81.9 ± 8.6 <sup>**</sup>
Nucleus accumbens	33.6 ± 1.9	20.5 ± 2.4 <sup>**</sup>	25.5 ± 3.3 <sup>*</sup>	29.9 ± 6.0 <sup>+</sup>
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 <sup>*</sup>	3.84 ± 0.38 <sup>+</sup>
Nucleus accumbens	5.47 ± 0.25	3.22 ± 0.32 <sup>***</sup>	3.79 ± 0.34 <sup>**</sup>	4.44 ± 0.38
Prefrontal cortex	3.46 ± 0.13	1.82 ± 0.24 <sup>***</sup>	2.10 ± 0.15 <sup>***</sup>	2.48 ± 0.22 <sup>*</sup>
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 <sup>*</sup>	1.47 ± 0.11	1.51 ± 0.15 <sup>+</sup>
Prefrontal cortex	1.94 ± 0.03	0.96 ± 0.10 <sup>***</sup>	1.01 ± 0.07 <sup>***</sup>	2.11 ± 0.12 <sup>+++</sup>
Hippocampus	3.54 ± 0.13	1.59 ± 0.22 <sup>***</sup>	1.50 ± 0.12 <sup>***</sup>	2.82 ± 0.28 <sup>++</sup>

Compared to controls:

\*  
p < .05\*\*  
p < .01\*\*\*  
p < .001.

Compared to 1-h group:

+  
p < .05++  
p < .01+++  
p < .001.