Published in final edited form as:

Drug Alcohol Depend. 2016 February 1; 159: 181–189. doi:10.1016/j.drugalcdep.2015.12.008.

Delayed emergence of methamphetamine's enhanced cardiovascular effects in nonhuman primates during protracted methamphetamine abstinence

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Abstract

Background—Methamphetamine abuse is linked with brain abnormalities, but its peripheral effects constitute an integral aspect of long-term methamphetamine use.

Methods—Eight male rhesus monkeys with long histories of intravenous methamphetamine self-administration were evaluated 1 day, and 1, 4, 12, 26, and 52 weeks after their last methamphetamine self-administration session. On test days, isoflurane-anesthetized animals received a 0.35 mg/kg IV methamphetamine challenge. A control group consisted of 10 age and gender matched drug naïve monkeys. Cardiovascular responses to methamphetamine were followed for 2.5 h. Echocardiograms were acquired at 3 and 12 months of abstinence and in the control animals.

Results—No pre-methamphetamine baseline differences existed among 7 physiological measures across all conditions and controls. As expected, methamphetamine increased heart rate and blood pressure in controls. However, immediately following the self-administration period, the blood pressure response to methamphetamine challenge was reduced when compared to control monkeys. The peak and 150-min average heart rate increases, as well as peak blood pressure increases following methamphetamine were significantly elevated between weeks 12 to 26 of abstinence. These data indicate the development of tolerance followed by sensitization to methamphetamine cardiovascular effects. Echocardiography demonstrated decreased left

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ventricular ejection fraction and cardiac output at 3 months of abstinence. Importantly, both cardiovascular sensitization and cardiotoxicity appeared to be reversible as they returned toward control group levels after 1 year of abstinence.

Conclusions—Enhanced cardiovascular effects may occur after prolonged abstinence in addicts relapsing to methamphetamine and may underlie clinically reported acute cardiotoxic events.

Keywords

Methamphetamine; abstinence; blood pressure; heart rate; rhesus monkey

1. INTRODUCTION

Methamphetamine is an approved pharmacotherapy for attention deficit hyperactivity disorder (ADHD) and obesity, yet its clinical utility is limited due to high levels of abuse. Several methamphetamine epidemics have occurred since the first widespread abuse was reported during the post-World War II era, and its abuse remains problematic in Asia, Australia, and North America (Gawin and Ellinwood, 1988; Krasnova and Cadet, 2009, Lineberry and Bostwick, 2006; Seiden and Ricaurte, 1987; Ujike and Sato, 2004). As an abused stimulant, methamphetamine offers the promise of euphoria, feelings of being energized, positive mood, and enhanced sexual pleasure. These psychopharmacological actions are accompanied by stimulation of the cardiovascular system, principally elevating blood pressure and accelerating heart rate in humans (Kirkpatrick et al., 2012; Martin et al., 1971) and animals (Schindler et al., 1992; Varner et al., 2002). Such pressor and tachycardic effects can place the methamphetamine user at acute cardiotoxic risk (Kaye et al., 2007).

In the context of chronic methamphetamine use, the characteristic psychopathology includes psychosis, mood and anxiety disorders, and cognitive deficits (Darke et al., 2008). Limited preclinical evidence demonstrates enhanced chronotropic and pressor responses to methamphetamine in rodents following repeated dosing (Fukunaga et al., 1987; Varner et al., 2002; Yoshida et al., 1993), suggesting that sensitized processes may underlie the cardiac arrhythmias and sudden cardiac death in humans following re-exposure to methamphetamine, even after low doses (Kaye et al., 2007). However, tolerance to the cardiovascular effects of methamphetamine following chronic treatment also was reported (Perez-Reyes et al., 1991).

Less prominent is the available clinical evidence suggesting that the risk of cardiovascular complications can become chronic in nature, often persisting beyond the period of active methamphetamine use into the timeframe comprising acute and protracted abstinence. During these phases of abstinence the compounding of acute and long-term cardiotoxic effects can enhance the risk of cardiac pathology and includes fatal outcomes (Cruickshank and Dyer, 2009; Kaye et al., 2007). Abstinence from methamphetamine is formidable as the user may be confronted with depression, sleeplessness, anxiety, and fatigue, subjective effects that are in stark contrast to its initially pleasurable effects (London et al., 2004). Other obstacles to remaining abstinent include the psychological manifestations of drug craving, drug-conditioned cues, and stress (Sinha et al., 1999) leading to direct re-exposure to the drug (Jaffe et al., 1989).

We employed a longitudinal, within-subjects design not available in human studies to evaluate the cardiovascular consequences of long-term methamphetamine use and abstinence in a rhesus monkey self-administration model. Tolerance to methamphetamine's cardiovascular effects were assessed immediately following long-term methamphetamine drug exposure. Cardiovascular effects of methamphetamine were then assessed periodically throughout 1 year of abstinence in a cohort of rhesus monkeys who averaged 5.5 ± 0.7 years of intravenous methamphetamine self-administration. Dependent variables included heart rate, blood pressure, plasma methamphetamine concentrations, and intermittent echocardiograms. The efficacy of a methamphetamine challenge on the cardiovascular system during protracted abstinence revealed a dynamic cycle of tolerance, enhanced effects, and normalization that were not attributable to drug pharmacokinetic changes.

2. METHODS

2.1 Subjects

This study was approved by the NIDA-IRP Animal Care and Use Committee. Rhesus macaques (*Macaca mulatta*) were individually housed and maintained on a standard diet of monkey biscuits, fresh fruit, and vegetables with water available *ad libitum*. Monkeys participated in a daily environmental enrichment program after their study sessions with continual visual, auditory, and olfactory contact with other cohorts. There were two cohorts of subjects: a methamphetamine self-administration group (n=8) and a control group (n=10). The groups were matched for age: controls, 10.6 ± 0.2 yr (9M, 1F) and methamphetamine-experienced population, 9.5 ± 0.8 yr (8 M).

The self-administration group had 3.6 to 8.6 years (5.5 ± 0.7 yr) of methamphetamine exposure (Table 1). During the self-administration studies, monkeys were anesthetized with ketamine (10 mg/kg IM) every other week to inspect the catheter exit site or check catheter patency. Catheters were replaced as necessary. In contrast, the drug-naïve controls were only exposed to pharmacological agents as part of their regular physical examinations. Details of the self-administration training and equipment can be found elsewhere (Schindler et al., 2011). Daily sessions included a 3-hr self-administration component during which the monkeys could self-administer up to 4 mg/kg methamphetamine. During the years that each monkey performed methamphetamine self-administration, test compounds were occasionally substituted for methamphetamine and various pharmacological pretreatments were given (see Schindler et al., 2010, 2011). Several test compounds, whose names cannot be revealed due to confidentiality agreements with pharmaceutical companies, were administered blind by investigators as potential therapeutics, and most were tested at multiple doses.

2.2 Study time frame

The experimental rhesus monkey group had extensive histories of methamphetamine self-administration, ranging from 3.6 to 8.6 years, and our goal was to evaluate this cohort during 1 year of abstinence with non-invasive MRI imaging techniques (Yang et al., 2015). Initiation of the study began within 24 to 28 h of the last session of methamphetamine self-administration (Day 1) and continued for 1 year. Following Day 1, experiments were

performed at 1, 4, 12, 26, and 52 weeks after the final self-administration sessions. After 12 weeks of abstinence, 3 subjects were euthanized in order to harvest brain tissue. Consequently, at weeks 26 and 52, only 5 subjects comprised the methamphetamine group. Each animal in the drug-naïve control group was tested once with methamphetamine.

2.3 Experimental procedures

Animals were initially anesthetized with ketamine (10 mg/kg IM) and intubated, after which two venous lines were placed in the right and left saphenous veins. A continuous infusion of propofol (0.4 – 0.6 mg/kg/min) was started to maintain anesthesia while the animal was transported to the imaging suite at which time the propofol infusion was stopped. The monkey was moved to the scanner bed, connected to an isoflurane delivery system, and prepared for imaging. Isoflurane maintenance concentrations ranged between 1.4 and 2.4% for the self-administration group (Table 2) and were 2.3% for the controls. Eyes were coated with sterile ocular lubricant. Surface coils were placed on each side of the head and secured to the head holder to minimize motion. Body temperature was maintained with a heating pad placed on the chest (37°C). Systolic, diastolic, and mean arterial blood pressure were determined indirectly using a human neonatal cuff positioned on the upper left arm, and heart rate and blood oxygen level (determined by pulse oximetry) were monitored continuously using an Invivo monitor (model 3150M, Invivo Corp., Orlando, FL). Respiration rate and expired end tidal carbon dioxide were measured by sampling the air at the end of endotracheal tube using a SurgiVet capnograph (model 9400, Smiths Medical PM, Inc., Norwell, MA). Animals breathed spontaneously and approximate inspiratory air volumes were recorded from the anesthesia delivery system (Hallowell EMC, Pittsfield, MA). Supplemental fluids were delivered via one of the intravenous lines. At the conclusion of the experiment the endotracheal tube and intravenous lines were removed, the animals were returned to their home cages, and observations were made until an upright positive supporting posture could be maintained with minimal ataxia.

2.4 Methamphetamine challenge and cardiovascular physiology

This report presents the cardiovascular and respiratory responses acquired throughout a 50-min fMRI study that also incorporated a methamphetamine challenge and an additional 110 min of monitoring. The challenge dose of methamphetamine served as a probe to assess changes in response to this stimulant occurring over time. The 50-min pharmacological MRI segment had a timeline consisting of a 5-min baseline period (0 – 5 min), a bolus saline injection (1.0 ml/kg IV) followed by 5-min of observations, a bolus methamphetamine injection (0.35 mg/kg IV) given at the 10 min mark, and a 40-min post-methamphetamine observation period. The 0.35 mg/kg dose was chosen to be within the upper portion of the methamphetamine dose-effect function, and to still allow for observance of reduced and enhanced effects on cardiovascular function (Schindler et al., 1992). Dose selection was ultimately based on preliminary dose-ranging studies in non-participating rhesus monkeys; 0.35 mg/kg is comparable to the daily amount received during self-administration sessions. Autonomic cardiovascular and respiratory effects were measured during the 50-min epoch at 2- or 5-min intervals. At the conclusion of the imaging, autonomic parameters were continually acquired at 10-min intervals for the next 110 min at which point the session was stopped. This provided a 2.5-h time course following methamphetamine administration; a

direct comparison of saline and methamphetamine was limited to the initial 5 min after injection due to the experimental design (see Results).

2.5 Plasma assays of methamphetamine and amphetamine

Blood samples were collected to quantify methamphetamine and amphetamine plasma concentrations before and after the acute methamphetamine challenge. A baseline sample was collected immediately following anesthesia induction, which occurred within 24 to 28 h after the last methamphetamine self-administration session for the Day 1 study. Subsequent sampling occurred at 5, 15, 30, 60 and 180 min after the methamphetamine challenge dose. Ten mL blood was taken for the first sample, and 5 mL for the remaining samples for a total of 35 mL.

Based upon the method of Scheidweiler and Huestis (2006), 750 μ L plasma specimen volumes were subjected to solid phase extraction with CleanScreen mixed mode cation exchange columns (part#ZSDAU020, United Chemical Technologies, Bristol, PA). Specimens were derivatized with heptafluorobutyric acid anhydride followed by neutralization via liquid-liquid extraction. Methamphetamine and amphetamine concentrations were determined by gas chromatography - mass spectrometry with electron impact ionization selected ion monitoring using an HP - 5ms column (30 m $\times$ 0.32 mm $\times$ 0.25 μ m). Extraction efficiency was >95% for methamphetamine and amphetamine with linear ranges of 1–250 μ g/L for both analytes. Inter-assay imprecision ranged between 3.6–4.6 and 3.2–4.6% coefficient of variation for methamphetamine and amphetamine, respectively (n = 32). Inter-assay accuracy (bias) was 101.7–104.5% and 99.4–102.8% of target concentrations for methamphetamine and amphetamine, respectively (n = 32).

2.6 Echocardiogram

Anesthesia was induced by ketamine (10 mg/kg IM) and maintained with 2% isoflurane via a facemask during the echocardiogram (Echo) examination. Monkeys were placed in a supine position, the chest shaved, and ECG leads were set and monitored with the standard lead II settings. Using an echo probe (12MHz, HP SONOS 5500), 2D images of left ventricle (LV), basal aorta, and left atrium were recorded at parasternal long axis, short axis and four chamber views. M-mode tracings of LV and basal aorta were recorded at parasternal long axis. A pulse doppler recorded the mitral valve blood flow velocity and tissue doppler at intra-ventricular septum. LV end-systolic volume (ESV) and end-diastolic volume (EDV) were calculated by the modified Simpson's method. Ejection fraction (EF) was then derived as $EF = (EDV - ESV) / EDV \times 100$. All measurements were made by a single observer blind to the identity of the tracings, and values were averaged over 3–5 consecutive cardiac cycles. The entire procedure took ~30–40 min. Reproducibility and variability of the measurements did not exceed 5%. Data acquisition in the methamphetamine-experienced monkeys was limited to 4 animals at 3 months of abstinence, and 5 animals at 12 months of abstinence. There were 10 control animals.

2.7 Anesthetic levels during the MRI session

At the onset of the study, our approach was to maintain the anesthetic level as low as possible in order to maximize the signal to noise ratio of the BOLD MRI signal. Initial

anesthesia values for Day 1 through Week 4 averaged 1.4 to 1.5% isoflurane for the methamphetamine group. However, beginning at Week 12, it became necessary to increase isoflurane levels to a mean of 1.7% because the stimulant effects of the methamphetamine challenge dose began to emerge in some subjects. Primary signs were movement of fingers, eye blinks, or sudden increases in breathing rate. At Week 52 the mean concentration of isoflurane was $2.4 \pm 0.1\%$, which was nearly equivalent to the control group ($2.3 \pm 0.1\%$). Control studies were initiated after most self-administration experiments were completed. Knowledge from the methamphetamine self-administration group (see Table 2, Week 26) guided our selection of initially using 2.2% isoflurane for the controls and making minor adjustments in concentration as required. Isoflurane concentrations employed from Day 1 to Week 12 were significantly lower than the control group (*t*-tests, all $P < 0.001$; all $P_c < 0.01$). In the face of markedly different anesthesia levels, homeostatic mechanisms provided stable physiology within the methamphetamine self-administration cohort.

2.8 Data Analysis

Since the evaluation of physiological changes during a 1 year period of methamphetamine abstinence focused on time-dependent relationships, some of which could be anticipated and others could not, the Bonferroni-Holm step down (sequential rejection method) procedure was employed in which pairwise comparisons are prioritized based on their level of significance to identify significant differences regardless of their timing. This method also corrects for multiple comparisons. Effects within the methamphetamine self-administration group were compared to the control group. Baseline changes were evaluated using two-tailed tests. The responsiveness to the methamphetamine challenge was anticipated to be dynamic in nature with tolerance being present closest to the end of methamphetamine self-administration, and sensitization or reverse tolerance (if it developed) was expected to emerge at later times. Because the direction of change for tolerance and sensitization are opposite of each other, one-tailed tests were used. Plasma methamphetamine concentrations and echo data were likewise analyzed with the sequential rejection procedure using two-tailed *t*-tests. $P < 0.05$ was accepted to consider statistically significant difference between the effects, and P_c represents corrected probability values.

3. RESULTS

3.1 Baseline physiology parameters

Throughout the 1-year course of methamphetamine abstinence, baseline physiological parameters acquired under isoflurane anesthesia for the self-administration experienced group remained statistically unchanged from those of the control group (Table 2), despite the necessity to modify the anesthetic conditions as described above. To normalize the data and to facilitate analyses over time, individual time courses were adjusted to 0 immediately prior to starting the methamphetamine challenge. Most comparative analyses utilized change-based normalized data. The single exception in the analysis was characterization of the effects of methamphetamine relative to a saline injection in the control group.

3.2 Cardiovascular effects of methamphetamine in controls

Because the present study was designed as a brain imaging study of methamphetamine effects utilizing 2.5-h time-action curves, there is no appropriate time-matched saline control (see Methods). The only available direct comparisons of methamphetamine to saline relative to a baseline condition were based on either 5-min averages (weighted average of the 2- and 5-min measurements) or peak effects within the matched 5-min periods following each injection. Relative to the average 5-min post-saline baseline, 0.35 mg/kg methamphetamine significantly increased systolic, diastolic, and mean arterial pressures (all $P < 0.0005$; one-tailed paired t -tests, 9 df) (Fig. 1b), but failed to elevate mean heart rate to reach a statistically significant level. However, the peak increase in heart rate produced by methamphetamine was significant ($P = 0.049$). This analysis did underestimate the chronotropic effect because the modest increase was distinctly sustained for nearly 1 hour (Fig. 1a) and its duration exceeded that of the pressor response that declined toward baseline by 40 – 50 min (Fig. 1a). Among the saline – baseline comparisons, the only significant finding was that systolic pressure averaged over 5 min (83 ± 9 mm Hg) was lower than the baseline value (87 ± 9 mm Hg) ($P = 0.01$). Collectively these pressor and rate increases served as the basis for comparing the change in the methamphetamine response across the abstinence period in the methamphetamine group.

3.3 Cardiovascular changes during abstinence following chronic methamphetamine self-administration

Diastolic blood pressure responses to a methamphetamine challenge during abstinence following extensive drug exposure revealed dynamic changes within the cardiovascular system over the course of the year. On Day 1 of abstinence, tolerance to the methamphetamine-induced increase in diastolic pressure (a 56% reduction relative to controls) was evident during the initial 20 min after the challenge dose of methamphetamine ($t_{(16)} = 2.99$; $P = 0.0045$; $P_c < 0.05$) (Fig. 2a and 2b). Tolerance dissipated over time and by Week 12 the 20-min change in response to methamphetamine exceeded the control value, but not to a significant level (Fig 2b). However, it was clear from the full 2.5-h time course that the diastolic response was prolonged. A comparison of the mean 2.5-h diastolic pressure increases demonstrated that the responsiveness of the methamphetamine group (13 ± 3 mm Hg) significantly exceeded that of the controls (4 ± 3 mm Hg) ($t_{(16)} = -3.10$; $P = 0.0035$; $P_c < 0.05$) (Fig. 2c).

Tolerance to the increase in systolic blood pressure was more prolonged than for diastolic pressure. Following the methamphetamine challenge during early abstinence, peak increases on Day 1 [14 ± 2 mm Hg ($t_{(16)} = 6.64$; $P < 0.005$; $P_c < 0.01$)] and Week 1 [20 ± 4 mm Hg ($t_{(16)} = 3.45$; $P = 0.0015$; $P_c < 0.01$)] were reduced in amplitude relative to the control group (37 ± 2 mmHg). Additionally, mean systolic pressure elevation averaged over the first hour also exhibited tolerance on Day 1 [4 ± 2 ($t_{(16)} = 3.44$; $P = 0.0015$; $P_c < 0.01$;)] and Week 1 [4 ± 3 mm Hg ($t_{(16)} = 3.02$; $P = 0.004$; $P_c < 0.05$)] when compared to the corresponding control value of 14 ± 2 mm Hg. Both of the previously diminished mean changes as well as the 2.5-h changes continually increased over the course of the 52 weeks, with all means exceeding control values after 1 year; however, none attained significance.

Peak increases in mean pressure to the methamphetamine challenge exhibited tolerance on Day 1 [10 ± 3 ($t_{(16)} = 4.65$; $P < 0.0005$; $P_c < 0.01$)] and Week 1 [17 ± 3 mm Hg ($t_{(16)} = 2.96$; $P = 0.0045$; $P_c < 0.05$)] of abstinence compared to controls [31 ± 3 mm Hg]. Also, the mean pressure elevation measured over 1 h was significantly diminished on abstinence Day 1 [4 ± 2 mm Hg] vs. controls [11 ± 2 mm Hg ($t_{(16)} = 2.93$; $P = 0.005$; $P_c < 0.05$)]. Similar to the systolic and diastolic pressures, the magnitude of the mean pressor response to methamphetamine recovered from its depressed levels in early abstinence and increased particularly during the final 40 weeks of the study. Still, sensitization was not present, as the Week 12 difference in mean pressure increase over 2.5 h between the self-administration group, 11 ± 2 , and controls, 5 ± 1 mm Hg, ($t_{(16)} = -2.58$; $P < 0.01$) lost significance when corrected for multiple comparisons ($P_c < 0.1$).

Sensitization did emerge for methamphetamine-induced tachycardia, although it developed at a slower rate than for diastolic pressure, appearing at Week 26 of abstinence for the former in contrast to Week 12 for the latter. Over the entire 2.5-h time course, the methamphetamine-experienced group manifested a 22 ± 3 beats/min heart rate increase relative to controls of 7 ± 3 beats/min ($t_{(13)} = -2.98$; $P < 0.0055$; $P_c < 0.05$) (Fig. 3b). This increase in heart rate was attributable to a sustained tachycardic response that was undiminished even at the end of the observation period (Fig. 3a). By Week 52 the response to the methamphetamine challenge approached control group values (Fig. 3b). Unlike the pressor effects, the reduction in heart rate during early abstinence (Day 1 and Weeks 1 and 4) lacked statistical significance (Fig. 3a and 3b).

3.4 Plasma methamphetamine concentration

The pharmacokinetic time course established for methamphetamine in the control group subsequent to IV administration showed that maximum plasma methamphetamine concentrations at 5 min (time of the first blood collection) were 193 ± 19 $\mu\text{g/L}$ ($n = 8$) and decreased to 49 ± 4 $\mu\text{g/L}$ after 3 h (Fig. 1c). The amphetamine metabolite first appeared 15 min after methamphetamine injection (3 ± 1 $\mu\text{g/L}$) and continued increasing to a final value of 11 ± 1 $\mu\text{g/L}$ at 3 h (Fig. 1c). At Day 1 of abstinence, initiated 24 to 28 h after the final methamphetamine self-administration session, the baseline (0 min) plasma samples were positive for amphetamine, 22 ± 6 $\mu\text{g/L}$, when compared to none detected in controls (Mann-Whitney Rank Sum Test, $T=65$, $P=0.002$). Thereafter, all other baseline values were negative for amphetamine. Plasma methamphetamine comparisons made throughout the 1 year of abstinence demonstrated a transient change during early abstinence based on 180-min AUCs. At Week 1, plasma $\text{AUC}_{0 \text{ to last}}$ was significantly lower when compared to controls based on a two-tailed t -test ($t_{(16)} = 3.138$, $P = 0.006$; $P_c = 0.0083$) (Fig. 4). Thereafter, the AUC values increased approaching control levels at Week 26. At Week 52 the absolute mean value in the abstinence group exceeded that of the controls, but not to a significant degree. The coefficient of variation was quite stable for controls (19%) and the methamphetamine groups (19 to 26%) through Week 26, but increased at 1 year.

3.5 Echocardiogram

Echocardiogram data indicated changes in cardiac function. Left ventricular ejection fraction was significantly lower in the 12-week abstinent methamphetamine group compared to

controls ($t_{(10)} = 6.843$, $P = 0.001$; $P_c < 0.01$) (Fig. 5). Importantly, at Week 52 the left ventricular ejection fraction was significantly higher than at Week 12 indicating recovery within the methamphetamine group ($t_{(6)} = -4.386$, $P = 0.005$; $P_c < 0.05$). Additionally, cardiac output in the methamphetamine group, which was significantly lower than controls at Week 12 ($t_{(10)} = 3.169$, $P = 0.01$; $P_c < 0.05$), appeared to be recovering at 1 year (Fig. 5). In addition to functional changes, there were structural improvements in the heart from 12 weeks to 1 year (e.g., left ventricle posterior wall thickness, ESV, EDV and left atrium diameter), but none were significant for the 12-week measurements. At one year, all of these parameters returned to control values. Basal heart rate did not differ between the groups.

4. DISCUSSION

The purpose of this study was to evaluate the effects of methamphetamine during 1 yr of abstinence from chronic methamphetamine exposure. Control monkeys, age-matched to their cohort with long histories of methamphetamine exposure, showed significant increases in blood pressure and a small non-significant increase in heart rate following their first and only exposure to an IV methamphetamine challenge. These effects were similar to previous results reported for methamphetamine in animals (Schindler et al., 1992; Varner et al., 2002). The use of anesthesia most likely blunted the cardiovascular response to methamphetamine, as has been reported for the psychomotor stimulant cocaine (Schindler, 1996; Tella et al., 1990; Wilkerson, 1988). Here monkeys exposed to methamphetamine for years showed a reduced cardiovascular response to a methamphetamine challenge relative to age-matched control monkeys. This effect persisted for up to 1 week following the end of self-administration and most likely reflects tolerance to the cardiovascular effects of acute methamphetamine exposure that developed during the prolonged self-administration period. Tolerance to the cardiovascular effects of self-administered cocaine is also observed in the absence of anesthesia (Ambrosio et al., 1996, Tella et al., 1999) and this tolerance is likely reflected under anesthetic conditions as seen here.

Tolerance to the effects of methamphetamine is also supported by the finding that the lower isoflurane concentrations maintaining anesthesia in the methamphetamine group during early abstinence were insufficient as abstinence progressed. Initially lower anesthetic concentrations of isoflurane were utilized to reduce fMRI signal loss. This effect was persistent and was still significant at 12 weeks following self-administration. As the abstinence period lengthened, higher isoflurane concentrations were required in the methamphetamine-experienced monkeys to prevent effects of methamphetamine from overcoming the level of anesthesia. The control studies were conducted at an isoflurane concentration (2.3%) commensurate with the concentration necessary to maintain anesthesia later in abstinence (2.4%). At this time point, the tolerance to the cardiovascular effects of methamphetamine was no longer evident. In fact, enhanced effects were starting to be observed.

Within the first 12 weeks following the end of methamphetamine exposure, the cardiovascular responses to methamphetamine returned toward the control response. At Week 12 following the end of self-administration, however, evidence of an enhanced cardiovascular response to methamphetamine began to appear. At this time there was an

enhanced blood pressure response and later at Week 26 there was an enhanced tachycardia. This enhanced response may represent sensitization to the cardiovascular effects of methamphetamine. Sensitization is often seen in the behavioral effects of psychomotor stimulants with intermittent, but not continuous, administration of the drug (Robinson and Becker, 1986). Sensitization has also been observed to the cardiovascular effects of cocaine with daily experimenter administration (Tella et al., 1991). Once established, sensitization can be long lasting (Robinson and Becker, 1986). However, it would seem unlikely that sensitization to methamphetamine developed during the abstinence period in the current study, where methamphetamine was administered infrequently and while animals were anesthetized. It would seem more likely that if sensitization developed, it would do so during the methamphetamine self-administration period. However, immediately after self-administration termination, reduced cardiovascular effects were observed, most likely reflecting tolerance to methamphetamine. Dalia et al. (1998) showed that both tolerance and sensitization could be observed to the locomotor effects of cocaine in rats, with tolerance predominating immediately following continuous treatment with cocaine. However, as the tolerance effect waned, the sensitization effect appeared. It is possible that tolerance to the cardiovascular effects of methamphetamine was apparent immediately following the self-administration period, but as tolerance was lost, sensitization to the cardiovascular effects became apparent.

Neither the reduced (tolerance) nor the enhanced (sensitization) cardiovascular effects of methamphetamine could be attributed to changes in pharmacokinetics. It is unlikely that the metabolite amphetamine contributes to the acute actions of methamphetamine because it accumulates slowly in plasma in controls while the concurrent time-action curves for mean arterial pressure and heart rate peak sooner and are in decline or at baseline when amphetamine concentrations are increasing (Fig. 1). While methamphetamine concentrations were significantly reduced in monkeys 1 week following self-administration in comparison to control animals, that reduction did not track the peak reduction in blood pressure, which occurred 1 day following self-administration termination. Likewise at Weeks 12 and 26, methamphetamine concentrations were no different from controls, while blood pressure and heart rate were increased above control levels. Thus, neither the nadir of the reduced effects nor the peak of the enhanced effects occurred at a time of significant changes in blood concentrations, indicating that these effects were not the result of pharmacokinetic changes.

With the appearance of enhanced blood pressure effects of methamphetamine, other changes in cardiovascular function were also noted. Ejection fraction and cardiac output were reduced at 12 weeks following the last self-administration session in the absence of acute methamphetamine. Both of these parameters appeared to return to normal by 1 year following the last methamphetamine exposure. Whether the parameters were reduced immediately after the last self-administration session is not known. However, the suppression of cardiac function at the time that the effects of methamphetamine were enhanced suggests that the cardiovascular system would be less able to respond to the stimulant effects of methamphetamine, and thus more adverse effects could be expected following methamphetamine use.

The same rhesus monkeys whose cardiovascular changes are reported here were also studied using fMRI-based measurements of the effects of abstinence on brain metabolites (Yang et al., 2015). Those results showed 1 week post-methamphetamine increases in *myo*-inositol, a marker for glial activation observed to be elevated in neuropathology, in the anterior cingulate cortex. These animals also had decreases in indices of brain integrity, specifically in levels of caudate-putamen glutamine and Glx (combined glutamate and glutamine). The changes observed in both areas returned to near baseline levels by 1 year of abstinence. Whether either of these changes in brain metabolites is important to the observed effects on cardiovascular function is unclear, but they do show that long-term methamphetamine exposure alters not only cardiovascular function, but also brain function. Interestingly, both the recovery of changes in the brain and changes on the cardiovascular system appear to follow the same time course. High dose (up to 60 mg/kg/day) exposure to methamphetamine in rhesus monkeys can produce changes in dopamine and serotonin concentrations in the brain (Woolverton et al., 1989). Such changes may affect cardiovascular function and may have occurred in the current monkeys exposed to lower methamphetamine concentrations, albeit over a longer period. However, that high dose exposure regimen also altered the dose-effect function for methamphetamine self-administration (Woolverton et al. 1984). In the current study, the methamphetamine exposure regimen (Table 1) did not change the methamphetamine self-administration dose-effect function when determined over 40 months (Schindler et al., 2011, Figure 1). Whether changes in cardiovascular dose-effect responsiveness occurred is unknown.

There are a number of limitations in this study to consider. In developing this study we took advantage of the fact that a long-term methamphetamine self-administration study was being terminated. With the exception of the acute methamphetamine exposure, no monkeys had identical experience to the self-administration monkeys. We used age-matched monkeys as a control, but we cannot rule out other factors related to the monkey's experience that might influence the results. In addition, the number of monkeys available for study was reduced following Week 12. Three monkeys were euthanized and their brains harvested for neurochemical analysis. While planned prior to the onset of the study, the timing of this subject number reduction was unfortunate as it coincided with the onset of the enhanced cardiovascular effects of methamphetamine. However, close examination of the individual data indicates that the observed enhancement was not due to the simple removal of monkeys that had unusually low cardiovascular responses to methamphetamine. The failure to conduct echocardiograms at earlier time points in the study resulted from logistical issues that unfortunately could not be corrected after study initiation. Nevertheless, this study offered a unique opportunity to explore the cardiovascular effects of methamphetamine during long-term abstinence following self-administration in a non-human primate model.

Our data show that after long-term self-administration of methamphetamine, rhesus monkeys showed a reduced cardiovascular response to methamphetamine challenge in the first week of abstinence. This reduction in the methamphetamine response most likely reflects tolerance to methamphetamine that developed over the self-administration period. After 12 weeks of abstinence, however, the cardiovascular response to methamphetamine was enhanced in monkeys who had previous exposure to methamphetamine through self-

administration. This enhanced response likely reflects sensitization to the cardiovascular effects of methamphetamine that was initially ameliorated by the development of tolerance. In parallel with an enhanced response to methamphetamine, there was evidence of altered cardiovascular function that could decrease the ability of the cardiovascular system to respond to the increases in blood pressure and heart rate following methamphetamine administration. This development could, in turn, increase potential toxicity following methamphetamine administration during this time period. Both the sensitization and the decrease in baseline cardiovascular function seen after 12 weeks appeared to resolve following 1 year of abstinence from methamphetamine.

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Highlights

- The cardiovascular effects of methamphetamine were evaluated in rhesus monkeys.
- The monkeys had long histories of intravenous methamphetamine self-administration.
- The cardiovascular effects were evaluated over 1 year of methamphetamine abstinence.
- Tolerance was observed immediately following the self-administration period.
- Sensitization was observed between weeks 12 to 26 of abstinence.

Fig. 1.

Cardiovascular effects of acute methamphetamine in drug-naïve rhesus monkeys. a) Time course depicting the baseline (BL) period, the saline injection (1.0 ml/kg, IV), the methamphetamine injection (0.35 mg/kg, IV), and post-drug observations for heart rate (HR) and mean arterial pressure (MAP). b) Mean changes in MAP, systolic (SYS) pressure, diastolic (DIA) pressure, and HR based on 5-min intervals following the saline injection (gray arrow) and methamphetamine (black arrow) conditions compared to the BL period. *Significantly greater than saline, $P < 0.0005$. †Significantly less than baseline, $P = 0.01$. c) Plasma concentrations of methamphetamine and its metabolite amphetamine following the IV administration of methamphetamine (black arrow). Values are the means and error bars represent the standard error of the mean (sem).

a **b** **c** **Fig. 2.**

Diastolic pressor response to acute methamphetamine during methamphetamine abstinence. The elevated diastolic pressor response to methamphetamine exhibits early tolerance and delayed sensitization as abstinence progresses. a) Time course of 0.35 mg/kg IV methamphetamine effect on diastolic pressure illustrates how tolerance and sensitization are associated with early and late components of the time course. b) Tolerance to the onset of the pressor response in early abstinence. c) Sensitization is present at Week (Wk) 12 and is attributable to a sustained elevation in diastolic pressure following the peak increase. Values are the mean $\pm$ sem for the change in mm Hg averaged over 20 min or 2.5 h and significant probability values are Bonferroni-Holm corrected (P_c). The animal numbers are: Controls, $n = 10$; Day 1 – Week 12, $n = 8$; and Week 26 and Week 52, $n = 5$.

Fig. 3. Acute effects of methamphetamine on heart rate during methamphetamine abstinence. Sensitization to the heart rate stimulating action of methamphetamine, 0.35 mg/kg IV, occurred 6 months after abstinence from methamphetamine self-administration. a) Time course of the tachycardic response in early and protracted abstinence. b) Longitudinal changes in tachycardia following methamphetamine administration illustrate initially reduced responsiveness, although not significant, and subsequent sensitization when compared to the control group. Values are the mean $\pm$ sem for the change in beats/min averaged over 2.5 h, and the significant probability value is Bonferroni-Holm corrected (P_c). Animal numbers are the same as for Figure 2.

Fig. 4. Plasma methamphetamine concentrations during 1 year of abstinence from methamphetamine self-administration. Values represent the mean AUC $\pm$ sem encompassing the 180-min period following 0.35 mg/kg IV methamphetamine, and the significant probability value is Bonferroni-Holm corrected (P_c). Numbers inside the bars are the number of samples analyzed.

Fig. 5.

Comparison echocardiogram parameters of methamphetamine-exposed animals after 12 and 52 weeks of abstinence with drug-naïve control animals. Echocardiogram analysis.

Decreases in ejection fraction (a) and cardiac output (b) at Week 12 of abstinence are suggestive of decreased cardiac function, which has normalized 9 months later. The effect on aortic diameter (c), though not significant, may afford possible mechanistic insight into the observations shown in (a) and (b). Values are the means $\pm$ sem, and significant probability values are Bonferroni-Holm corrected (P_c). Numbers inside the bars are the number of animals analyzed.

Intake histories for the methamphetamine (METH) hydrochloride and age of rhesus monkeys in self-administration (SA) cohort. Ages are rounded to the nearest half-year and SA length was rounded to the nearest month.

Table 1

Subject	Age METH SA Onset: years	Years METH SA	Maintenance Dose: µg/kg/inj	Daily Intake: mg/kg	Lifetime Intake: mg
Sv	4	8.6	5	0.73	1760
Ch	3.5	3.8	5	0.40	404
Go	4	5.8	10	0.56	564
Gz	4	3.6	5	0.40	222
Ha	4.5	7.8	10	1.20	2454
Sc	3.5	3.8	10	0.82	748
Dj	4.5	5.9	10	0.54	521
Ji	4	4.7	10	0.70	710
Avg ± sem	4.0 ± 0.1	5.5 ± 0.7	9 ± 1	0.67 ± 0.08	751 ± 257
Range	3.5 – 4.5	3.6 – 8.6	5 – 10	0.40–1.20	404–2454

Table 2

Cardiovascular and respiratory baseline values for the control and methamphetamine self-administration groups. Among the physiological parameters there were no differences between the controls and any time point during abstinence from methamphetamine self-administration. This result differs from the isoflurane concentrations used to maintain anesthesia, in which the concentrations administered through Week 12 were significantly less than that required for the control group. Data are presented as mean $\pm$ sem.

	Control	Day 1	Week 1	Week 4	Week 12	Week 26	Week 52
Heart rate: bpm	115 $\pm$ 3	108 $\pm$ 3	113 $\pm$ 4	107 $\pm$ 7	107 $\pm$ 6	107 $\pm$ 6	102 $\pm$ 6
MAP: mm Hg	56 $\pm$ 5	51 $\pm$ 3	56 $\pm$ 4	52 $\pm$ 3	44 $\pm$ 2	48 $\pm$ 4	48 $\pm$ 2
Systolic BP: mm Hg	87 $\pm$ 9	76 $\pm$ 3	87 $\pm$ 4	85 $\pm$ 4	72 $\pm$ 3	78 $\pm$ 6	86 $\pm$ 11
Diastolic BP: mm Hg	41 $\pm$ 3	38 $\pm$ 3	40 $\pm$ 4	36 $\pm$ 3	30 $\pm$ 3	35 $\pm$ 4	36 $\pm$ 2
RR: breaths/min	23 $\pm$ 2	19 $\pm$ 2	18 $\pm$ 2	19 $\pm$ 2	18 $\pm$ 2	19 $\pm$ 3	18 $\pm$ 2
PO2 saturation: %	96 $\pm$ 1	97 $\pm$ 1	96 $\pm$ 1	94 $\pm$ 1	96 $\pm$ 1	94 $\pm$ 1	95 $\pm$ 1
ET CO2: mm Hg	41 $\pm$ 2	43 $\pm$ 2	43 $\pm$ 2	42 $\pm$ 2	42 $\pm$ 3	46 $\pm$ 3	46 $\pm$ 2
Isoflurane: %	2.3 $\pm$ 0.1	1.4 $\pm$ 0.1 [†]	1.5 $\pm$.04 [†]	1.4 $\pm$.04 [†]	1.7 $\pm$ 0.1 [†]	2.2 $\pm$ 0.2	2.4 $\pm$ 0.1
n	10	8	8	8	8	5	5

Abbreviations: MAP – mean arterial pressure; P – oxygen saturation pressure; RR – respiration rate; ET – end tidal.

[†] = P < 0.01 corrected.

Statistical comparisons between the control and methamphetamine self-administration groups were based on family-wise *t*-tests using a Bonferroni-Holm correction.