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Research report

Long-term effects of a high-dose methamphetamine regimen on subsequent methamphetamine-induced dopamine release in vivo

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Abstract

Rats were treated with a high-dose methamphetamine (METH) regimen (40 mg/kg/injection, four times at 2-h intervals) or a saline regimen (four injections at 2-h intervals). Temperature related measures taken during the high-dose METH treatment were maximum core temperature and minimum chamber temperature. Fourteen rats (METH $N=7$; Saline $N=7$) were implanted with in-vivo dialysis probes 4–7 weeks post-regimen (average=6 weeks). The next day, they received a challenge dose of METH (4.0 mg/kg) and dopamine release was measured. Results showed a significant decrease in challenge-induced dopamine release in rats previously treated with the high-dose METH regimen. These findings demonstrate a functional deficit in the dopamine system 6 weeks after high-dose METH treatment. Temperature-related measures taken during the high-dose regimen were not correlated with METH-induced dopamine release 6 weeks later. An additional group of rats were sacrificed 6 weeks after the high-dose regimen (METH $N=12$; Saline $N=10$), and their brains was analyzed for dopamine and serotonin concentrations. Tissue concentrations of dopamine were significantly depleted in striatum and nucleus accumbens/olfactory tubercle, but not septum, hypothalamus, or ventral mid-brain 6 weeks after the high-dose regimen. Tissue concentrations of serotonin were also significantly depleted in striatum, nucleus accumbens/olfactory tubercle, hippocampus, somatosensory cortex, but not septum, hypothalamus or ventral mid-brain. Significant correlations between the temperature-related measures and post-mortem neurotransmitter tissue concentrations were region and transmitter dependent.

Theme: Disorders of the nervous system

Topic: Neurotoxicity

Keywords: Methamphetamine; High-dose regimen; In vivo dopamine release; Neurotoxicity; Temperature

1. Introduction

High-dose methamphetamine (METH) treatment causes damage to dopamine and serotonin terminals in the brains of laboratory animals. Tissue levels of dopamine [14,20] and serotonin [14] are decreased; the capacity of nerve terminals to take up dopamine [20] and serotonin [14] is reduced; immunohistochemical markers indicate damage to serotonin nerve terminals [1], and the dopamine and serotonin synthetic enzymes tyrosine hydroxylase (TH) and tryptophan hydroxylase (TPH) show decreased activity [10]. Recent reports have demonstrated decreases in dopamine transporter in non-human primates [19] as well as humans [12] after METH exposure. Ernst et al. [6] reported decreases in the density of neurons of the basal ganglia.

Several studies have investigated the relationship between high-dose METH pre-treatment and dopamine release in-vivo in response to a subsequent METH or amphetamine challenge. Robinson et al. [16] found no significant change in 1.5 mg/kg D-amphetamine-induced dopamine release 1 week after a high-dose METH regimen (15 mg/kg, five times, at 6-h intervals). Cass et al. [5] demonstrated a significant decrease in D-amphetamine-induced dopamine release (1.5 mg/kg, IP) 1 week after a high-dose METH regimen (5 mg/kg, four times, at 2-h intervals). Wallace et al. [21] reported significant attenuations of METH-induced dopamine release after a 7.5 mg/

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kg dose but not after a 1.0 mg/kg dose in animals pre-treated with a high-dose METH regimen (10 mg/kg, four times, at 2-h intervals). In addition, Cass and Manning [4] reported a decreased D-amphetamine-induced dopamine release at 1 but not 6 months after a high-dose METH regimen (5 mg/kg, four times, at 2-h intervals). Amphetamine was administered through the perfusate in the report of Cass and Manning [4].

The purpose of the present study was to further investigate the relationship between high-dose METH pre-treatment and subsequent stimulated dopamine release. Rats were treated with a high-dose METH regimen (40 mg/kg, four times, at 2-h intervals) or saline. After a period of 6 weeks, challenge-induced dopamine release was measured in vivo (4.0 mg/kg METH, I.P.). It was hypothesized that rats pre-treated with a high-dose METH regimen would release less dopamine in response to a subsequent METH challenge in vivo, compared to rats pre-treated with saline.

2. Materials and methods

2.1. Subjects

Male, Sprague–Dawley (Harlan) rats, 325 g at the start of the experiment, were maintained on a 14-h light (8:00 am–10:00 pm)/10-h dark (10:00 pm–8:00 am) cycle. The rats were individually housed in hanging metal cages, and they had free access to food and water. Animals in this experiment were used in accordance with the National Institutes of Health's Guidelines for the care and use of laboratory animals. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Mississippi.

2.2. Methamphetamine treatment and temperature monitoring

2.2.1. Surgery

For the measurement of core temperature radio transmitters (Mini-mitter, model #VM-FH disc) or dummy transmitters, were implanted into the abdomen of all rats. Animals were anesthetized with xylazine (7 mg/kg) and ketamine (100 mg/kg), or sodium pentobarbital (50 mg/kg) with supplemental injections of ketamine (10 mg) as needed.

2.2.2. METH treatment

Rats were treated with either four injections of (+)methamphetamine hydrochloride (40 mg/kg/injection, Sigma) or saline administered at 2-h intervals, IP. Dose calculations were made based on the salt. All METH treatments occurred in four computer-controlled environmental chambers (custom constructed). The chambers were modified refrigerator units, with heating elements installed; they were interfaced with a 486 microcomputer. Core temperature was controlled by changing the ambient temperature of the chamber [11,17]. Each time the animal surpassed 39.5°C (hyperthermic threshold), the cooling unit was activated. The cooling unit remained active until the rat's core temperature returned to 39.5°C. If the animal's core temperature remained below the hyperthermic threshold, the chamber was maintained at 24°C by activating heating or cooling units as needed. The rat was housed in a small Plexiglas compartment, placed within the modified cooling/heating chamber. The dimensions of the animal compartment were 20 cm (W)×16.5 cm (L)× 15 cm (H). The animals received four METH injections, and remained in the temperature chambers for up to 24-h.

Temperature-related measures taken during the 24-h METH treatment period were: maximum core body temperature (single maximum temperature during the 24-h period) and minimum chamber temperature (single minimum temperature during the 24-h period). This second measure indicated the extent to which chamber cooling was required to counteract hyperthermia for each rat.

2.3. In vivo dialysis

2.3.1. Dialysis probes

Concentric dialysis probes were constructed of fused silica tubing (75 μm i.d.×150 μm o.d. in-flow and out-flow tubing), and regenerated cellulose dialysis fiber (Spectrum Medical, 216 μm o.d., 200 μm i.d.). In-flow tubing and out-flow tubing were covered with a stainless steel sleeve (23 gauge); the exposed dialysis surface was 4 mm.

2.3.2. Surgery

Rats were permanently implanted with dialysis probes into the striatum. Stereotaxic coordinates were AP 0.3 mm posterior to Bregma; ML 3.1 mm to the right of mid-line; DV 7.0 mm below the surface of the skull [13]. Anesthetic was sodium pentobarbital (Butler) (50 mg/kg) with supplemental injections of ketamine (Ketaset) (10 mg) as needed.

2.3.3. Dialysis system

A 1-ml glass air-tight syringe was mounted on a Harvard Apparatus microliter syringe pump (model 2274); the rats were perfused with artificial cerebral spinal fluid (aCSF) (145 mM NaCl, 2.7 mM KCl, 1.2 mM $CaCl_2$, 1.0 mM $MgCl_2$, 2.0 mM Na_2HPO_4, pH 7.4) at a flow-rate of 1.1 μl/min. The in-flow and out-flow tubes of the probes were connected via plastic tubing (BAS MF-5164) to a dual channel liquid swivel (Insteck 375/D/22QE). The out-flow of the liquid swivel was connected directly to the port of a pneumatically operated HPLC injection valve. Injections were automatically made onto the analytical column every 30 min (10 μl loop size) during the experimental session under the control of a Shimadzu integrator (CR5A) and programmer (PRG-102A). Dead

volume from rat to injection port was 11.7 μl, representing a 12 min. delay from rat to injector. A Plexiglass cylinder, 41 cm high and 24 cm in diameter was used to test the rats. A balance arm was attached to hold the commutator in the center of the chamber and to compensate for the rats' vertical movements.

2.3.4. HPLC system

HPLC instrumentation included an ESA HPLC pump (model #580); SSI pulse dampener (model #LP-21); Rheodyne injection valve (model #7010) and pneumatic actuator (model #5701); Keystone spherisorb 100×3 mm column (3 μm particle size); ESA Coulochem II coulometric detector (model #5200), with applied potentials of −100 mV (E1) and +270 mV (E2); and a Shimadzu integrator (model #C-R5A). Mobile phase consisted of 14.2 g/l monochloroacetic acid, 0.745 g/l EDTA, 0.4 g/l octyl sodium sulfate, 5.25 g/l NaOH, 1.4% tetrahydrofuran, 1.0% acetonitrile.

2.4. Post-mortem tissue dissection and neurochemical analysis

2.4.1. Tissue dissection

Rats were sacrificed by decapitation and the following sections were rapidly dissected over ice: somatosensory cortex, hippocampus, striatum, nucleus accumbens/olfactory tubercle, septum, hypothalamus, and ventral mid-brain [7].

2.4.2. Neurochemical analysis

HPLC instrumentation included an ESA HPLC pump (model #580); SSI pulse dampener (model #LP-21); Rheodyne injection valve (model #7010); Adsorbosphere Catecholamine 100×4.6 mm column (C_{18}, 3 μm particle size); ESA Coulochem II coulometric detector (model #5200), with applied potentials of −100 mV (E1) and +330 mV (E2); EZ-Chrome, Chromatography Data System, version 6.7. Mobile phase consisted of 14.2 g/l monochloroacetic acid, 0.745 g/l EDTA, 0.15 g/l octyl sodium sulfate, 5.25 g/l NaOH, 2.5% tetrahydrofuran, 2.5% acetonitrile.

2.5. Procedure

2.5.1. In vivo dialysis experiment

Temperature sensitive transmitters were implanted into the abdomen 1 week after arrival into the colony. A period of 2–4 weeks later, rats were treated with a high dose METH regimen ($N=7$, 40 mg/kg, four times, at 2-h intervals) or saline ($N=7$, four times, at 2-h intervals). In vivo dialysis experiments were then conducted 4–7 weeks later. METH pre-treated rats had an average post-regimen interval of 5.3 weeks (37 days) and saline pre-treated rats had an average post-regimen interval of 6.1 weeks (42.7 days) prior to in vivo dialysis probe implantation. Dialysis probes were implanted into the striatum on the right side of all rats. Immediately after surgery, the rats were connected to the dialysis system and were perfused with aCSF overnight. A period of 16–24 h after surgery, baseline dialysis samples were collected in the awake rat, and analyzed for dopamine concentrations. After baseline values were established, a challenge METH injection (4.0 mg/kg) was administered. Data were collected for an additional 3 h. See Table 1 for summary of experimental parameters.

2.5.2. Post-mortem tissue assay experiment

A separate group of rats was treated with either a high-dose METH regimen ($N=12$, 40 mg/kg, four times, at 2-h intervals) or saline ($N=10$, four times, at 2-h intervals) under the conditions described above. A period of six weeks later, they were sacrificed; their brains were removed for post-mortem monoamine analysis.

2.6. Data analysis

For the in vivo dialysis data, dopamine values were expressed as pg/10 μl dialysate (volume of sample loop). Probe recoveries were measured prior to use and ranged

Table 1
Summary of experimental parameters and results for the in vivo dialysis experiment (mean±S.E.M.)

Pre-treatment	METH-dialysis (40 mg/kg/injection, four times, 2-h intervals)	Saline-dialysis (four injections, four times, 2-h intervals)
Dialysis probe recovery — in vitro (%).	14.5±0.7	13.6±0.4
Interval from high-dose METH or Saline regimen to Dialysis test (days).	37.4±3.7	42.7±2.1
Interval from probe insertion to challenge METH treatment (h).	20.2±0.9	20.9±0.8
Dopamine release, 30 min post challenge (pg/10 μl).	39.3±6.4	71.0±8.2

from 11.8 to 17.4% (average of 13.3% for saline and 14.5% for METH pre-treated rats). Dopamine in vivo values were not corrected for in vitro probe recoveries. In vivo dialysis data were analyzed using a 2-factor ANOVA. Factors were treatment (METH vs. saline) and time (baseline and post-treatment up to 120 min). Post hoc analysis was performed using *t*-tests. Post-mortem tissue concentrations were analyzed using *t*-tests. Correlations between METH-induced dopamine release and the two temperature-related measures were performed. Correlations were also performed between post-mortem tissue concentrations and the two temperature-related measures for brain regions showing significant depletions. Correlational analysis was performed on data from rats which received the high-dose METH treatment only (data from saline-treated rats were not included).

The Bonferroni correction was applied to all *t*-tests and correlational analyses with a family-wise error level of alpha=0.05 (*P*-values for individual comparisons differed for each analysis, depending on the number of comparisons made).

3. Results

3.1. In vivo dialysis experiment

Rats were pre-treated with a high-dose METH regimen (40 mg/kg, four times, at 2-h intervals) or saline. A period of 6 weeks later, they were administered a METH challenge injection (4 mg/kg).

3.1.1. Dopamine

In vivo measurements of extra-cellular dopamine after the challenge METH dose demonstrated a significant effect of time [$F(4,48)=100.718$, $P=0.0001$] and a significant interaction effect [$F(4,48)=11.681$, $P=0.0001$]. Post hoc analysis indicated that the METH pre-treated group was different from the saline pre-treated group 30 min after the challenge injection (see Fig. 1). There was no difference between METH and saline-pre-treated rats in baseline dopamine concentrations (4.26 pg/10 μl dialysate, saline-pre-treated; 4.56 pg/10 μl dialysate, METH-pre-treated).

3.2. Post-mortem experiment

In a separate group of rats, the effects of the high-dose METH regimen on post-mortem tissue concentrations were evaluated, 6 weeks after treatment.

3.2.1. Dopamine

Significant dopamine depletions were found in striatum [$t(20)=9.42$, $P<0.0001$] and nucleus accumbens/olfactory tubercle [$t(20)=3.24$, $P=0.0041$]. Dopamine was not significantly changed in septum, hypothalamus or ventral mid-brain (see Fig. 2).

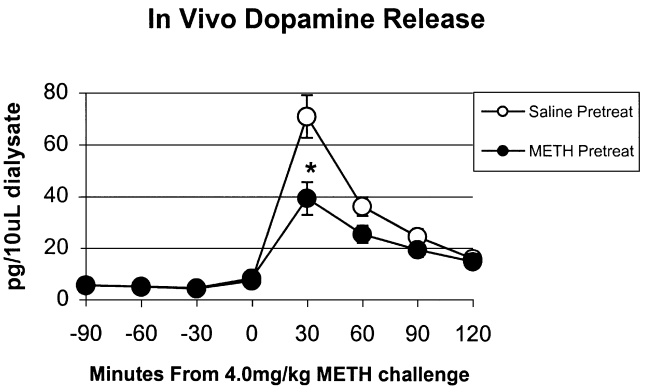

Fig. 1. Dopamine release in response to a 4.0 mg/kg METH challenge injection. A period of six weeks (average) prior to the in vivo dialysis test, rats were treated with a high-dose METH regimen (40 mg/kg/injection, four times, at 2-h intervals) or saline. *Significantly different from saline pre-treated control group.

3.2.2. Serotonin

Significant serotonin depletions were seen in striatum [$t(20)=6.37$, $P<0.0001$], somatosensory cortex [$t(20)=7.30$, $P<0.0001$], hippocampus [$t(20)=9.65$, $P<0.0001$], and nucleus accumbens/olfactory tubercle [$t(20)=6.58$, $P<0.0001$]. Serotonin was not significantly depleted in septum, hypothalamus, or ventral mid-brain (see Fig. 3).

3.3. High-dose METH treatment and temperature

The mortality rate due to the high-dose METH regimen was 23%. The number of rats requiring cooling was: 4/7 (dialysis experiment) and 11/12 (post-mortem tissue experiment). Although a large dose of METH (40 mg/kg) was administered four times at 2-h intervals, several rats (4/19) did not attain sufficient hyperthermia to require cooling. The resistance of these rats to hyperthermia may be related to individual differences in response to METH treatment, or to factors not yet identified (e.g., humidity).

Two different temperature related variables were evaluated: minimum chamber temperature and maximum core body temperature. These two measures showed a significant negative correlation with each other (see Table 2).

3.3.1. Temperature and in vivo release

There were no significant correlations between METH-induced dopamine release and the two temperature-related measures (see Table 3).

3.3.2. Temperature and post-mortem tissue concentration

Correlational analysis was performed between the two temperature measures and post-mortem neurotransmitter concentrations in brain regions showing significant depletions. Striatal dopamine concentrations were significantly correlated with minimum chamber temperature (positive correlation) and maximum core temperature (negative correlation), while nucleus accumbens/olfactory tubercle

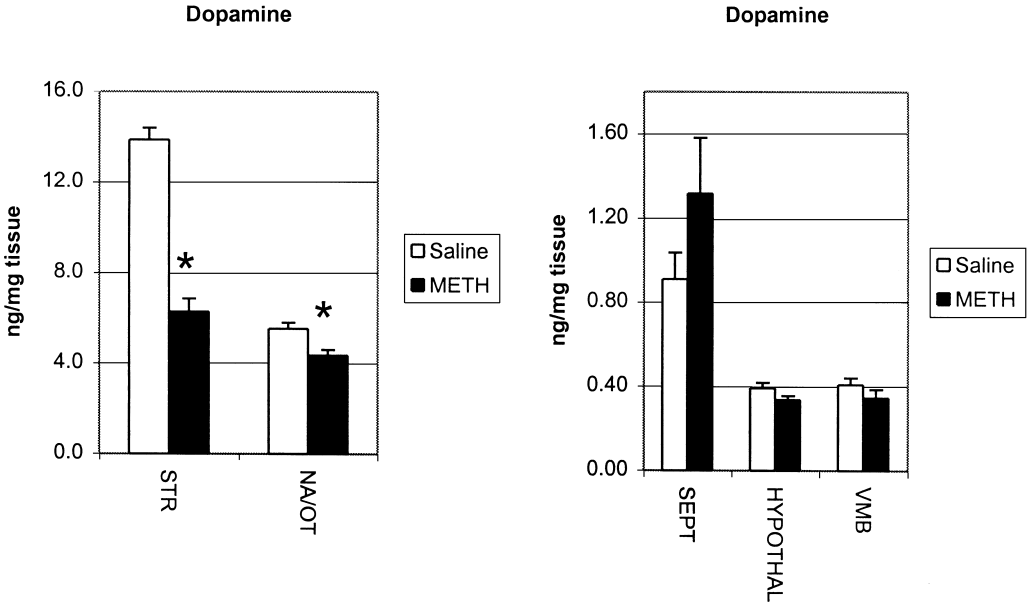

Fig. 2. Dopamine tissue concentrations (post-mortem) 6 weeks after treatment with a high-dose METH regimen (40 mg/kg/injection, four times, at 2-h intervals) or saline. *Significantly different from saline treated control group. STR, striatum; NA/OT, nucleus accumbens/olfactory tubercle; SEPT, septum; HYPOTHAL, hypothalamus; VMB, ventral mid-brain.

dopamine was not correlated with either of the temperature measures. Significant correlations were found between minimum chamber temperature and serotonin in nucleus accumbens/olfactory tubercle, striatum, somatosensory cortex, and hippocampus. Maximum core temperature showed significant correlations with nucleus accumbens/olfactory tubercle and striatal serotonin (but not with hippocampal and somatosensory cortex serotonin) (see Tables 4 and 5). Fig. 4 demonstrates the relationship between neurotransmitter concentrations in striatum and maximum core temperature.

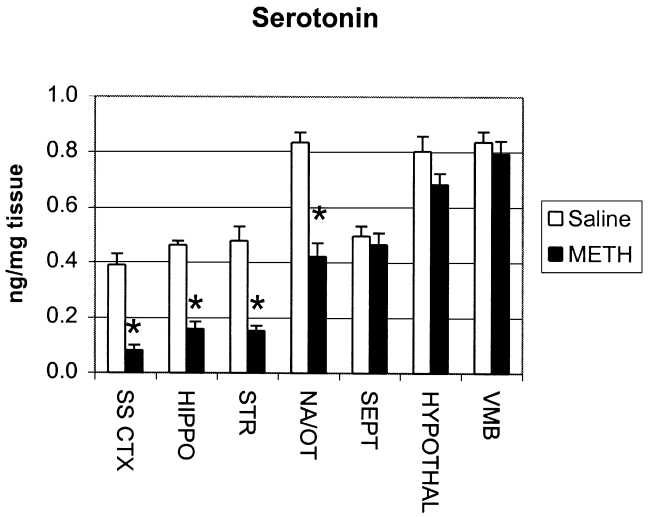

Fig. 3. Serotonin tissue concentrations (post-mortem) 6 weeks after treatment with a high-dose METH regimen (40 mg/kg/injection, four times, at 2-h intervals) or saline. *Significantly different from saline treated control group. SS. CTX, somatosensory cortex; HIPPO, hippocampus; STR, striatum; NA/OT, nucleus accumbens/olfactory tubercle; SEPT, septum; HYPOTHAL, Hypothalamus; VMB, ventral mid-brain.

Table 2
Temperature related measures during high-dose METH treatment: Mean (±S.E.M.) and simple correlations[a]

	Minimum chamber temperature (°C)[b]	Maximum core temperature (°C)[c]	Correlation between minimum chamber temperature and maximum core temperature
Mean ±S.E.M.	17.44 ±1.01	39.7 ±0.26	$r=-0.8078$ $P=0.000$*

[a] All rats in this analysis received the high-dose METH regimen (40 mg/kg/injection, four times, at 2-h intervals). Data from the seven dialysis rats and the 12 tissue assay rats are combined. *Significant correlation.
[b] Lowest chamber temperature recorded over the 24-h treatment period.
[c] Highest core body temperature recorded over the 24-h treatment period.

Table 3
Simple correlations between temperature-related measures (at time of high-dose METH regimen) and METH-induced dopamine release (4 mg/kg, 6 weeks after high-dose treatment)[a]

	Minimum chamber temperature (°C)	Maximum core temperature (°C)
Dopamine release	$r=0.0445$	$r=-0.3085$

[a] Analysis included only rats which received the high-dose METH regimen, $N=7$ (40 mg/kg/injection, four times, at 2-h intervals). No significant correlations were found. See Table 2 for description of column headings.

Table 4
Simple correlations between temperature measures and post-mortem *dopamine* concentrations after high-dose METH treatment ($N=12$)

	Minimum chamber temperature (°C)	Maximum core temperature (°C)
NA/OT	$r=0.6881$ $P=0.013$	$r=-0.6468$ $P=0.023$
STR	$r=0.8549$ $P=0.000$*	$r=-0.8139$ $P=0.001$*

*Significant correlation (using a Bonferroni correction). NA/OT=nucleus accumbens/olfactory tubercle, STR=striatum. See Table 2 for description of column headings.

4. Discussion

Rats treated with 40 mg/kg METH, four times, at 2-h intervals showed significant reductions in moderate-dose (4.0 mg/kg) METH-induced dopamine release 6 weeks after the high-dose regimen.

Several studies have demonstrated that challenge-induced dopamine release is attenuated after high-dose METH pre-treatment. Seven days after a high-dose METH regimen, dopamine release induced by 1.5 mg/kg D-amphetamine [16] or 1.0 mg/kg METH [21] was not different from saline pre-treated rats. In contrast, Cass et al. [5] reported that 1.5 mg/kg D-amphetamine resulted in an attenuation of dopamine release after prior high-dose pre-treatment. Within these low-dose challenge studies there are conflicting findings; however, Cass et al. [5] suggested that this difference may be due to different strains of rats used (see Table 6). In a higher challenge dose range (7.5 mg/kg) a high-dose METH regimen resulted in a significant attenuation of challenge-induced dopamine release [21].

Using longer post-METH intervals, high-dose METH pre-treatment resulted in a significant attenuation of dopamine release after 1 but not 6 or 12 months (challenge dose of 100 μM D-amphetamine, through the probe, Fischer-344 strain [4]). We extend these findings by demonstrating an attenuation of METH-induced dopamine release 6 weeks after a high-dose regimen (challenge dose of 4.0 mg/kg, I.P., Sprague–Dawley strain).

Table 5
Simple correlations between temperature measures and post-mortem serotonin concentrations after high-dose METH treatment ($N=12$)

	Minimum chamber temperature (°C)	Maximum core temperature (°C)
NA/OT	$r=0.7889$ $P=0.002$*	$r=-0.8363$ $P=0.001$*
STR	$r=0.8544$ $P=0.000$*	$r=-0.8901$ $P=0.000$*
SS.CTX	$r=0.8614$ $P=0.000$*	$r=-0.6968$ $P=0.012$
HIPPO	$r=0.7794$ $P=0.003$*	$r=-0.6675$ $P=0.018$

*Significant correlation (using a Bonferroni correction). NA/OT=nucleus accumbens/olfactory tubercle, STR=striatum, SS.CTX=somatosensory cortex, and HIPPO=hippocampus. See Table 2 for description of column headings.

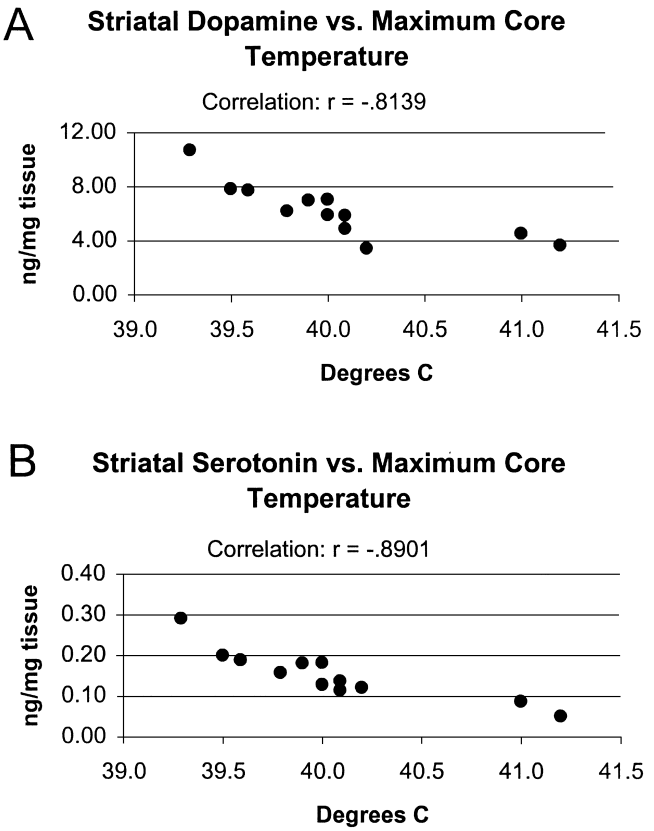

Fig. 4. Scatter plots demonstrating the relationships between striatal dopamine and maximum core temperature (A) and striatal serotonin and maximum core temperature (B).

In the previous and current in vivo dialysis experiments a wide range of parameters were used (see Table 6). In spite of these differences, a differentiation between low-dose and high-dose challenge effects on dopamine release following a high-dose METH regimen is suggested: attenuation after high- but not low-dose challenge. Although toxic dose and strain differences exist across studies, Wallace et al. [21] demonstrated a differential pattern of induced release after low and high challenge doses of METH within one study. This distinction between low- and high-dose challenge is similar to that obtained after pre-treatment with the vesicular reuptake inhibitor, reserpine (see Sabol and Seiden [18] for discussion).

In a separate group of rats, post-mortem dopamine depletions were found in striatum and nucleus accumbens/olfactory tubercle, but not hypothalamus, ventral midbrain, or septum, 6 weeks after the high-dose regimen (40 mg/kg, four times, at 2-h intervals). Serotonin depletions were found in striatum, nucleus accumbens/olfactory

Table 6
In vivo dialysis experimental parameters: comparison among studies

	Robinson et al. [16]	Cass [3]	Cass et al. [5]	Cass and Manning [4]	Wallace et al. [21]	Sabol et al. (this report)
Strain/sex	Holtzman, male	Fischer-344, male	Fischer-344, male	Fischer-344, male	Sprague–Dawley, male	Sprague–Dawley, male
METH regimen	15 mg/kg, five times, 6-h intervals	5 mg/kg, four times, 2-h intervals	5 mg/kg, four times, 2-h intervals	5 mg/kg, four times, 2-h intervals	10 mg/kg, four times, 2-h intervals	40 mg/kg, four times, 2-h intervals
Challenge treatment	1.5 mg/kg D-amph	100 μM D-amph	1.5 mg/kg D-amph	100 μM D-amph	1.0 mg/kg 7.5 mg/kg (+)METH	4.0 mg/kg (+)METH
Route of administration for the challenge treatment	Systemic	through dialysis probe	i.p.	through dialysis probe	i.p.	i.p.
Interval between METH regimen and in vivo challenge	1 week	1 week	1 week	1 week; 1, 6, 12 months	1 week	6 weeks
Probe lowering	Day before dialysis test	Morning of dialysis test	Day before dialysis test	Morning of dialysis test	Morning of dialysis test	Day before dialysis test
Effect of METH regimen on challenge-induced dopamine release	No attenuation	Significant attenuation	Significant attenuation	Significant attenuation at 1 week and 1 month	No attenuation with 1.0 mg/kg challenge	Significant attenuation
				No attenuation at 6 or 12 months	Significant attenuation with 7.5 mg/kg challenge	

tubercle, somatosensory cortex, and hippocampus, but not hypothalamus, ventral mid-brain, or septum. These findings demonstrate that rats treated with a high-dose of METH show significant neurotransmitter depletions at the time corresponding to the attenuated METH-induced dopamine release in vivo.

Two temperature-related measures were assessed during the 24-h METH treatment period: the maximum core body temperature reached by the rats, and the minimum chamber temperature required to counteract hyperthermia. These two measures were negatively correlated with each other (see Table 2). Tables 4 and 5 show the relationships between post-mortem tissue concentrations of dopamine and serotonin with the two temperature-related measures. Striatal dopamine was significantly correlated with both temperatures measures, while nucleus accumbens/olfactory tubercle dopamine was not correlated with either measure. Serotonin post-mortem concentrations were correlated with minimum chamber temperature in nucleus accumbens/olfactory tubercle, striatum, somatosensory cortex, and hippocampus. Only nucleus accumbens/olfactory tubercle and striatum showed correlations between serotonin and maximum core temperature.

We previously reported a lack of correlation between maximum core temperature (during the METH treatment) and dopamine or serotonin post-mortem tissue concentrations [17]. Tissue concentrations were measured 6 weeks after a high-dose METH regimen of 15 mg/kg, four times, at 2-h intervals in our earlier study. In both cases (Sabol et al. [17], and the present report) rats were treated with METH in temperature controlled environmental chambers. If core temperature surpassed 39.5°C, the chamber temperature was lowered until the core temperature returned to 39.5°C. This procedure was intended to prevent lethality, and minimize core temperature variations within the treated groups. The lack of correlation in the previous report [17] may be due to the smaller dose of METH used. Alternatively, other temperature related measures (such as minimum chamber temperature) may be better indicators of neurotransmitter depletions. This is particularly relevant to the procedures used in these studies since maximum core temperature was intentionally limited. The degree of cooling required to counteract hyperthermia for each rat may be a more important variable than maximum core body temperature, and is reflected in the minimum chamber temperature.

There was no relationship between either of the temperature-related measures (at the time of the high-dose

regimen), and challenge-induced dopamine release 6 weeks later. In light of the relationships observed between post-mortem dopamine concentrations and temperature-related measures (Bowyer et al. [2], present results with the same regimen), it is unclear why in vivo release did not show similar relationships. One possible explanation for this outcome is related to procedural issues. The in vivo dialysis procedure has several points at which additional variability is introduced. For example, Holson et al. [8,9] and Robinson and Camp [15] reported time-dependent decreases in dopamine release induced by pharmacological agents after probe insertion. While we attempted to maintain equivalent intervals between probe insertion and challenge injections of METH this parameter varied among the rats. Time between high-dose regimen and in vivo dialysis test, and in vitro probe recoveries, also varied among the rats in the present experiment (see Table 1).

In conclusion, methamphetamine (4.0 mg/kg)-induced dopamine release was reduced after a high-dose methamphetamine regimen. There were corresponding dopamine and serotonin depletions post-mortem in a separate group of rats treated with the same high-dose methamphetamine regimen. These results demonstrate a functional consequence associated with dopamine depletions 6 weeks after initial exposure to a high-dose methamphetamine regimen.

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