ASHDIN PUBLISHING

*Research Article*

# Adolescent MDMA Exposure Diminishes the Physiological, Behavioral, and Neurotoxic Consequences of a Subsequent Methamphetamine Binge

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**Abstract** Previous research showed that repeated adolescent ±3,4-methylenedioxymethamphetamine (MDMA) treatments diminished the temperature dysregulation, hypoactivity/"hangover" effect, and serotonin transporter reductions caused by a subsequent MDMA binge. This study evaluated whether MDMA would confer cross-tolerance against a methamphetamine (METH) binge. Rats received MDMA (10 mg/kg × 2) every fifth day from postnatal day (PD) 35 to PD 60 followed by a low or high METH binge (4 or 8 mg/kg × 4) on PD 67. Adolescent MDMA preexposure diminished, but did not prevent, the METH-induced increase in core body temperature. Adolescent MDMA exposure conferred resistance to the METH-induced hypoactivity in rearing behavior on PD 68. The high-dose METH binge caused reductions in [$^3$H]citalopram binding to the serotonin transporter in the frontal cortex, parietal cortex, and hippocampus which were attenuated by adolescent MDMA. Similarly, adolescent MDMA blunted the METH binge-induced decrease in striatal [$^3$H]WIN35,428 binding to the dopamine transporter. Together, these findings are supportive of a preconditioning effect between the two different substituted amphetamines.

**Keywords** activity; dopamine transporter; ecstasy; hangover; hyperthermia; preconditioning; rat; serotonin transporter; temperature; weight

## 1. Introduction

Amphetamine-type stimulants, including methamphetamine, amphetamine, and ecstasy (3,4-methylenedioxymethamphetamine; MDMA), constitute the second most widely used illicit drug class worldwide [71]. Recent evidence indicates that over one out of every fourteen high-school seniors (7.2%) in the U.S. have tried ecstasy, with an even higher percentage (11.9%) in the western states [32]. Over one-third of this demographic reported that ecstasy was readily available. Wastewater is one of the matrices that creative analytical chemists have examined to corroborate self-reports of substituted amphetamine use [2,38], and indeed quantities of MDMA in wastewater exhibited a pronounced spike that corresponded with a large music festival in eastern Spain [7]. Ecstasy use, at least in the U.S. and Australia, has expanded beyond its dance party roots to be used on other occasions and by other populations [38,43]. Finally, rodent studies have demonstrated that adolescent exposure to MDMA has adverse consequences for both neurochemistry and behavior [49,53,57].

Most ecstasy users are polydrug users [48], and one of the illicit substances sometimes taken with ecstasy is methamphetamine (METH). Methamphetamine is a particularly toxic substituted amphetamine that has been implicated in numerous Emergency Department visits [26, 63] as well as a relatively high degree of mortality [37, 64]. Ecstasy-METH co-use has been reported in a number of studies [8,12,34,39,41], and this drug combination has been associated with greater harm than the use of either drug alone [8,34,41]. Finally, hair analysis has shown that some ecstasy users are inadvertently being exposed to METH, which may be either an additive to their ecstasy tablets or, in some cases, the principal psychoactive ingredient instead of MDMA [33].

Several studies have investigated the behavioral and neurochemical effects of coadministering MDMA and METH to rats (see, e.g., [13,14]), but less is known about the influence of one of these compounds on subsequent sensitivity to the other. One possible outcome of the latter arrangement is preconditioning. Preconditioning is a fundamental phenomenon in which there is an adaptive response to a low-level insult that protects against a larger subsequent insult. The original discovery noted that brief and intermittent episodes of ischemia protect the myocardium from a later sustained ischemia [50]. Preconditioning, also known by an alternative terminology as hormesis [42], has also been demonstrated in the brain following preclinical models of stroke [35]. Preconditioning studies involving substituted amphetamines have focused

**Table 1:** Comparison of preconditioning paradigms involving substituted amphetamines. Interval is the period between the end of the preconditioning paradigm and the drug challenge.

| Preconditioning regimen | | | | | Challenge regimen | | Dependent measures |
|---|---|---|---|---|---|---|---|
| Citation | Species | Sex | Weight or age | Drug | Interval | Drug | |
| [66] | Rat | Male | 200–250 g | ↑ METH × 3 d | 2 d | 90 mg/kg METH | $D_2$ receptors[P], substance P[P], PK[P] |
| [65] | Rat | Male | 200–250 g | ↑ METH × 3 d | 2 d | 90 mg/kg METH | DA[P], 5-HT[P], MA enzymes[P] |
| [10] | Rat | Male | 350–400 g | ↑ METH for 2 w | 1 d | 30 mg/kg METH | DA[P], 5-HT, NE |
| [11] | Rat | Male | 330–370 g | ↑ METH for 2 w | 3 d | 40 mg/kg METH | Striatal gene expression[P] |
| [9] | Rat | Male | 330–370 g | ↑ METH for 2 w | 3 d | 40 mg/kg METH | Striatal gene expression[P] |
| [28] | Rat | Male | 350–400 g | ↑ METH over 2 w | 3 d | 30 mg/kg METH | DA[P], 5-HT[P] |
| [36] | Rat | Male | 350–400 g | ↑ METH for 2 w | 3 d | 30 mg/kg METH | DA[P], transcription factors[P] |
| [24] | Rat | Male | 180–220 g | ↑ METH × 3 d | 70 h | 46 mg/kg METH | Tryptophan hydroxylase[P], PK[P] |
| [22] | Rat | Male | 60 d | ↑ METH for 2 w | 2–24 h | 30 mg/kg METH | DA[P], 5-HT, temp[P] |
| [30] | Rat | Male | 300–325 g | ↑ METH × 3 d | 66 h | 40 mg/kg METH | DA[P], 5-HT[P], temp, PK |
| [31] | Rat | Male | 290–310 g | ↑ METH × 3 d | 66 h | 32 mg/kg METH | DA[P], VMAT2[P], PK |
| [17] | Rat | Male | 200–225 g | ↑ METH × 3 d | 1–31 d | 62.5 mg/kg METH | DA[P], 5-HT[P], PK |
| [67] | Rat | Male | 325–350 g | ↑ METH for 2 w | 1 d | 24 mg/kg METH | DAT[P], VMAT2, stereotypy[P] |
| [51] | Rat | Male | 325–350 g | ↑ METH for 2 w | 1 d | 24 mg/kg METH | DAT[P], PK, temp[P] |
| [45] | Rat | Male | 275–300 g | SA METH × 7 d | 1 d | 30 mg/kg METH | DAT[P], GFAP[P], temp[P] |
| [46] | Rat | Male | 275–300 g | SA METH × 7 d | 1 or 15 d | 30 mg/kg METH | 5-HT[P], SERT function[P] |
| [47] | Rat | Male | 275–300 g | SA METH × 7 d | 1 or 15 d | 30 mg/kg METH | NE, 5-HT[P] |
| [1] | Rat | Male | 200–260 g | 4 mg/kg METH × 10 d | 7 d | 12–20 mg/kg METH | DA, 5-HT[P], stereotypy[P] |
| [69] | Rat | Male | 175–300 g | 2 mg/kg METH × 7 d | 7 d | 22.5 mg/kg METH | DA, 5-HT[P], glutamate[P] |
| [62] | Rat | Male | 40 d | 15 mg/kg METH × 12 d | 7 d | 40 mg/kg METH | DA uptake[P], DAT[P], PK |
| [70] | Mouse | Female | 20–25 g | 8 mg/kg/d METH | 72 h | 20 mg/kg METH | DA[P], microglial activation[P] |
| [19] | Mouse | NA | Embryonic | 0–3 mM METH for 24 h | None | 100 $\mu$M 6-OHDA | DA uptake, cell viability[P] |
| [3] | Rat | Male | 250–275 g | 10 mg/kg MDMA × 4 d | 1–4 d | 40 mg/kg MDMA | SERT[P], 5-HT[P], temp, PK |
| [3] | Rat | Male | 250–275 g | 10 mg/kg MDMA × 4 d | 1 d | 40 mg/kg METH | 5-HT, DA[P] |
| [44] | Rat | Male | 175–200 g | 3 mg/kg MDMA × 4 d | 1–7 d | 12.5 mg/kg MDMA | SERT[P], IL-1$\beta$[P], temp |
| [54] | Rat | Male | 60 d | 20 mg/kg MDMA × 6 d | 7 d | 10–20 mg/kg MDMA | SERT[P], 5-HT[P], temp |
| [56] | Rat | Female | 35 d | 20 mg/kg MDMA × 6 d | 7 d | 40 mg/kg MDMA | SERT[P], temp[P] |
| [58] | Rat | Male | 35 d | 20 mg/kg MDMA × 6 d | 7 d | 20–40 mg/kg MDMA | SERT[P], 5-HT$_{1A}$ agonist resp[P] |

[P]: preconditioning effect; 5-HT: serotonin; 6-OHDA: 6-hydroxydopamine; DA: dopamine; DAT: dopamine transporter; GFAP: glial fibrillary acidic protein; IL: interleukin; MA: monoamine; METH: methamphetamine; MDMA: ±3,4-methylenedioxymethamphetamine; NA: not applicable (tissue culture); NE: norepinephrine; PK: pharmacokinetics; resp: responsiveness; SA: self-administered; SERT: serotonin transporter; temp: core temperature; VMAT2: vesicular monoamine transporter 2.

particularly on the drugs' neurotoxic and behavioral effects. In humans, nonhuman primates, and rats, METH neurotoxicity is manifested by long-lasting reductions in dopamine (DA) and dopaminergic markers, whereas MDMA neurotoxicity in these species is more typically associated with the serotonergic system [25]. Although MDMA-related reductions in serotonin (5-HT) and other serotonergic markers have traditionally been interpreted as damage to serotonergic fibers and nerve terminals [23], there is increasing evidence for an alternate explanation of biochemical downregulation [4,5,16]. Table 1 lists the many preconditioning studies conducted with METH and a handful with MDMA. Repeated exposure to a low dose of a substituted amphetamine diminishes the neurotoxic effects that result from later exposure to a high dose of the same drug [1,9,22,28,30,31,45,46,47,54,56, 58,62,65,66,70]. Fewer studies have evaluated whether preconditioning can diminish the neurotoxic effects of

a different substance [3,19,68]. Therefore, the goal of this investigation was to determine whether intermittent adolescent exposure to MDMA reduces the physiological, behavioral, and neurochemical sequelae of a later METH binge. Based on earlier work by Zacny et al. [73] showing that repeated MDMA administration led to cross-tolerance to the effects of METH in a milk-drinking test, it was predicted that MDMA would exert a preconditioning effect on the behavioral and/or neurotoxic effects of the METH treatment.

## 2. Method

### 2.1. Animals

Male Sprague-Dawley rats ($N = 76$) arrived between postnatal day (PD) 25 and PD 28 from Charles River Laboratories (Wilmington, MA, USA) where they were pair-housed in plastic tubs ($44.5 \times 23.5 \times 20.0$ cm) and

given food and water *ad libitum*. The room was set on a reverse 12-hour light/dark cycle with lights on at 8:00 PM. All drug administrations and behavioral and physiological assessments were conducted during the lights-off phase. The room was set at a stable temperature of $23 \pm 1 \,°C$. Animals were provided 7–10 d to adjust to the surrounding environment including becoming accustomed to human handling. Animals were treated in accordance with guidelines specified by the University of Massachusetts, Amherst Institutional Animal Care, and Use Committee and the National Research Council Guide for the Care and Use of Laboratory Animals.

## 2.2. Drug treatments

Adolescent MDMA pretreatment began on PD 35 and continued every fifth day up to and including PD 60 (six dosing days total). Animals received either two injections of ±MDMA-HCl (RTI, Research Triangle Park, NC, USA) (10 mg/kg/injection dissolved in 0.9% NaCl, $N = 40$) or two saline injections ($N = 36$) with an interdose interval of 4 h. Additional information regarding the rationale for this dosing regimen is available elsewhere [49,57].

Seven days after the adolescent pretreatment ceased (PD 67 or during young-adulthood), the animals received a binge treatment administered as four injections of either a low (4 mg/kg) or high (8 mg/kg) dose of D-methamphetamine HCl (Sigma-Aldrich, St. Louis, MO, USA) or saline vehicle, with an interdose interval of 2 h. The METH dosing regimen was selected based on prior investigations [67,69]. This resulted in the formation of six Adolescent/Binge conditions ($N = 10$–14/group). All injections were administered subcutaneously.

## 2.3. Neurobehavioral and physiological measures

### 2.3.1. Body weight

Body weight was obtained 1 hour prior to the first dose on all dosing days. Further, on PD 35, PD 45, and PD 60, weights were also recorded 2 h following the final dose to determine the percent weight change.

### 2.3.2. Temperature

Core body temperature was obtained via a rectal probe (RET-2) connected to a Thermalert TH-5 digital thermometer (Physitemp, Clifton, NJ, USA) on the challenge day (PD 67). Temperature was taken at time 0, immediately before the first dose, and at half-hour intervals until 3 h after last METH dose. If an animal's core temperature exceeded $40.5 \,°C$, it was iced until its temperature fell below that threshold. Care was taken not to over-ice the animals. A subset of animals was also videotaped (Casio Exilim EX-Z70, 7.2 MP) to document the acute behavioral response to METH.

## 2.4. Motor-activity (hangover)

Animals were placed into an activity chamber (ENV-510 MED Associates, St. Albans, VT, USA) (27.5 × 27.5 × 20.5 cm) lighted with a 28-V bulb for 20 min at 24 h after the METH Binge (PD 68). Three photobeam sets were used to quantify horizontal activity and rearing behavior, two on the floor level and one raised 13.5 cm above ground level. Additional information on the apparatus including a rationale for this as an index of the after-effects (i.e., hangover) of substituted amphetamine exposure is available elsewhere [58].

## 2.5. Neurochemistry

Animals were anesthetized with $CO_2$ and sacrificed by decapitation on PD 74. The brain was extracted, immersed in cold 0.9% NaCl, and then placed in an acrylic brain block over ice. The frontal cortex, parietal cortex, and the striatum were collected, and the hippocampus was isolated by free-hand dissection. Each brain area was placed in a separate microcentrifuge tube on dry ice before storage at $-70 \,°C$ until quantification of cortical and hippocampal serotonin transporter (SERT) or striatal dopamine transporter (DAT) levels by means of radioligand binding assays.

The frontal cortex, parietal cortex, and hippocampus were homogenized with ice-cold assay buffer containing 10 mM sodium phosphate, 120 mM sodium chloride, and 5 mM potassium chloride (pH = 7.4) using a Polytron and then centrifuged at $20,000 \times g$ for 20 min at $4 \,°C$. The procedure was performed three times to obtain a washed membrane preparation for the SERT binding assay. After the third Polytron cycle, the samples had a 20-minute incubation period at $30 \,°C$ to remove any endogenous 5-HT still bound to the transporter. Each brain area was then incubated for 1 hour at room temperature in triplicate using a 1 nM concentration of [³H]citalopram (81.2 Ci/mmol, PerkinElmer, Waltham, MA, USA) in assay buffer to quantify SERT levels, while another set of triplicates containing fluoxetine (30 μM; Sigma-Aldrich) was prepared to determine nonspecific binding. After 1-hour incubation, the samples underwent vacuum filtration with Whatman GF/B filters soaked in cold assay buffer containing 0.05% polyethyleneimine to reduce nonspecific binding. Scintillation fluid (Scintisafe, Fisher Scientific, Pittsburgh, PA, USA) was added to each filter, and samples were vortexed and counted in a liquid scintillation counter 24 h after the assay was complete.

Similarly, the striatum was assayed to quantify DAT via radioligand binding. Identical procedures as with [³H]citalopram binding were taken, except the homogenization and wash buffer contained 20 mM phosphate buffer and 0.32 M sucrose (pH = 7.4), cocaine (30 μM) was used for nonspecific binding, [³H]WIN 35,428 (85.9 Ci/mmol, PerkinElmer) was used as a DAT specific ligand (5 nM final

concentration), no warming step was necessary, and the incubation was increased to 90 min in an ice bath in a 4 °C cold room.

## 2.6. Statistical analysis

All data were entered and analyzed with Systat 13.0 (San Jose, CA, USA). Results are expressed as the mean ±SEM. Despite efforts to prevent hyperthermia, six animals receiving the high METH dose died (four pretreated with MDMA) and were not included in subsequent analyses. Area under the curve (AUC) for temperature analysis on the binge day was determined with GraphPad Prism (San Diego, CA, USA) software with 0 °C as the baseline. A 2 (adolescent) by 3 (binge) factor analysis of variance was used to determine an overall effect of the two treatment variables (adolescent pretreatment and binge dose). ANOVAs were followed by planned comparisons ($t$-tests) to determine statistically significant differences between selected groups. Correlations were obtained between the AUC and neurochemical endpoints to quantify whether temperature dysregulation contributed to subsequent neurotoxicity. A $P \leq .05$ was considered significant although statistics that met more conservative alphas were noted.

## 3. Results

On the first day of the intermittent treatments (PD 35), predosing weight was equivalent in the saline ($129.0 \pm 1.8$ g) and MDMA ($128.5 \pm 1.5$) groups. In contrast, the MDMA ($317.6 \pm 2.9$) group weighed 5.9% less than the Saline ($337.5 \pm 4.0$) group on the last (PD 60) intermittent day ($t(74) = 4.08$, $P < .0005$). There were also significant differences in the percent change in body weight on the first (PD 35: Saline = $+3.5 \pm 0.4\%$, MDMA = $-6.4 \pm 0.3\%$, $t(74) = 20.23$, $P < .0005$), third (PD 45: Saline = $+3.7 \pm 0.4$, MDMA = $-7.3 \pm 0.2$, $t(74) = 25.29$, $P < .0005$), and last (PD 60: Saline = $+3.5 \pm 0.2$, MDMA = $-6.8 \pm 0.3$, $t(74) = 26.00$, $P < .0005$) intermittent dosing days.

On PD 67, core temperature of animals that received four saline injections remained steady between 37.5 °C and 38.0 °C (not shown), whereas METH treatment caused a dose-dependent hyperthermia. Adolescent MDMA exposure blunted the elevation in temperature that followed the second, third, and fourth low METH doses (Figure 1(a)). Similarly, MDMA pretreatments diminished the pyrexia induced by the first and second high METH doses (Figure 1(b)). Further analyses were completed on the AUC as an index of the overall 9.5-hour session. An ANOVA revealed main effects of METH ($F(2,64) = 50.70$, $P < .0005$) and MDMA ($F(1,64) = 4.25$, $P < .05$), whereas the interaction was not significant ($F(1,64) = 2.40$, $P = .099$). Figure 1(c) shows that METH caused a dose dependent increase in the AUC but also that the hyperthermic
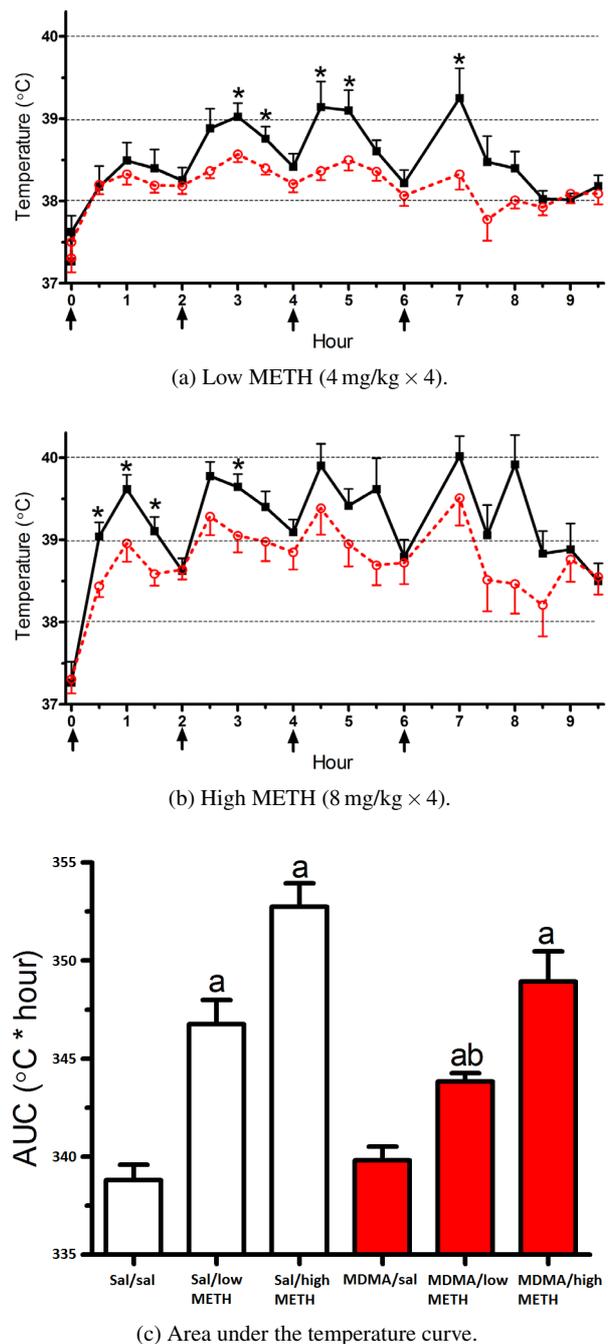


(a) Low METH (4 mg/kg × 4).



(b) High METH (8 mg/kg × 4).



(c) Area under the temperature curve.

**Figure 1:** Core temperature over time in rats that received Low (4 mg/kg × 4; (a)) or High (8 mg/kg × 4; (b)) dose METH (arrows); filled black squares = Saline pretreated, open red circles = MDMA pretreated during adolescence; *$P < .05$ compared to the MDMA pretreated group. (c) Area under the temperature-time curve. Open bars = Saline pretreated, red bars = MDMA pretreated during adolescence. Group sizes for all figures: Saline/Saline, $N = 10$; Saline/Low METH, $N = 12$; Saline/High METH, $N = 12$; MDMA/Saline, $N = 10$; MDMA/Low METH, $N = 12$; MDMA/High METH, $N = 14$. $^{a}P < .0005$ versus Saline/Saline, $^{b}P < .05$ versus Saline/Low METH.

**Table 2:** Motor activity expressed as a percentage (mean plus SEM in parentheses) of the Saline/Saline group on postnatal day (PD) 68 as a function of adolescent $\pm 3,4$-methylenedioxymethamphetamine (MDMA) on PD 35 to 60 and Low $(4\,\mathrm{mg/kg} \times 4)$ or High $(8\,\mathrm{mg/kg} \times 4)$ methamphetamine (METH) on PD 67. Saline/Saline group: distance traveled $= 4{,}242.0 \pm 523.9\,\mathrm{cm}$, rearing frequency $= 61.9 \pm 5.7$, and rearing duration $= 143.2 \pm 16.4\,\mathrm{s}$.

| Adolescent: | Saline | MDMA | Saline | MDMA | Saline | MDMA |
|---|---|---|---|---|---|---|
| Binge: | Saline | Saline | Low METH | Low METH | High METH | High METH |
| Distance traveled (cm) | 100.0 (12.4) | 87.9 (9.0) | 73.9 (7.4) | 74.5 (4.5) | 30.3 (5.7)[a] | 45.4 (6.5)[a] |
| Rearing frequency | 100.0 (9.3) | 91.8 (7.8) | 67.2 (5.7)[a] | 85.4 (6.7)[b] | 32.4 (6.4)[a] | 47.0 (5.7)[a] |
| Rearing duration (s) | 100.0 (11.4) | 93.1 (5.3) | 70.0 (8.1)[a] | 78.8 (8.9) | 28.4 (5.4)[a] | 46.3 (5.7)[a,c] |

[a] $P < .05$ versus Saline/Saline, [b] $P < .05$ versus Saline/Low METH, and [c] $P < .05$ versus Saline/High METH.

response to the low METH binge was diminished by adolescent MDMA. Similarly, the AUC was lower, albeit nonsignificantly, in the adolescent MDMA, relative to saline pretreated, in response to the high METH binge $(t(24) = 1.91, P = .068)$. The acute behavioral responses to METH included several stereotyped behaviors previously reported by other investigators including chewing, self-biting, head bobbing, and backward locomotion.

Locomotor activity was evaluated on PD 68, 1 day after binge dosing (Table 2). Overall, the METH binge evoked a dose-dependent reduction in horizontal and vertical motor activity. This was reflected by main effects of the binge for the distance traveled $(F(2,64) = 28.30, P < .0005)$, rearing frequency $(F(2,64) = 34.75, P < .0005)$, and duration $(F(2,64) = 30.90, P < .0005)$. Low METH only decreased rearing behaviors in the saline pretreated group. In addition, adolescent MDMA-treated animals reared more frequently than animals in the saline condition. High METH evoked a generalized decrement in the distance traveled, rearing frequency, and rearing duration. However, adolescent MDMA showed longer rearing times relative to adolescent saline.

Figure 2 shows [3H]citalopram binding to SERT. In the frontal cortex, statistically significant results were obtained for the binge main effect $(F(2,64) = 39.28, P < .0005)$ and the adolescent treatment by binge interaction $(F(2,64) = 13.51, P < .0005)$ but not for the main effect of adolescent treatment $(F(1,64) = 3.60, P = .062)$. The same pattern was observed in the hippocampus, namely, a binge main effect $(F(2,64) = 44.76, P < .0005)$ and an adolescent treatment by binge interaction $(F(2,64) = 11.31, P < .0005)$. In contrast, the parietal cortex showed a main effect of adolescent treatment $(F(1,64) = 7.97, P < .01)$ in addition to a binge main effect $(F(2,64) = 30.97, P < .0005)$ and an adolescent treatment by binge interaction $(F(2,64) = 8.47, P < .001)$. Post hoc testing revealed that adolescent MDMA produced a modest, but statistically significant, reduction in radioligand binding in the frontal cortex (13.1%) and hippocampus (16.9%). The high dose METH binge substantially decreased SERT binding in the frontal cortex (65.4%), parietal cortex (63.1%), and hippocampus (77.0%) in the saline pretreated groups. Adolescent MDMA attenuated this METH-induced

decrement in [3H]citalopram binding in all three forebrain regions.

Figure 3 depicts [3H]WIN35,428 binding to DAT in the striatum. A main effect of the METH binge was observed $(F(2,64) = 22.53, P < .0005)$. Within the adolescent saline group, the low METH binge reduced DAT by 32.2% while the high dose reduced radioligand binding by 79.6%. Adolescent MDMA preexposed animals were less sensitive to the high METH binge.

Finally, correlation analyses were performed to ascertain the possible relationships between drug-induced changes in core body temperature and radioligand binding. These analyses revealed substantial negative correlations of the temperature AUC on the day of the binge with striatal DAT binding $(r(68) = -.54, P < .005)$ and with SERT in the parietal cortex $(r(68) = -.76, P < .005)$, frontal cortex $(r(68) = -.79, P < .0005)$, and hippocampus $(r(68) = -.82, P < .0005)$.

## 4. Discussion

The primary finding of this report is that adolescent MDMA exposure reduced the magnitude of reductions in SERT and DAT binding induced by a subsequent METH binge. These results fit within a preconditioning (aka hormesis) framework (Table 1). Although the majority of preconditioning investigations with amphetamines have employed METH as the low or nontoxic insult [1,10,11,17,22,24,30,31,62,65,66,69,70], there has been some interest in MDMA [3,44]. We previously determined that adolescent MDMA prevents the SERT depletion induced by a MDMA binge in males [58]. The same general pattern was verified with adolescent females [56] and when the intermittent treatment began during young adulthood (PD 60) instead of adolescence [54]. Bhide et al. [3] determined that MDMA exposure prevented the 5-HT, but not DA, depletions caused by a METH binge. One of the differences between the current investigation and the earlier study [3] is that the latter employed older animals with a more compressed (4-day) MDMA exposure period in conjunction with a very brief interval between the preconditioning and the neurotoxic METH administration. Preconditioning is generally known to be most robust at younger ages [35]. The interval between
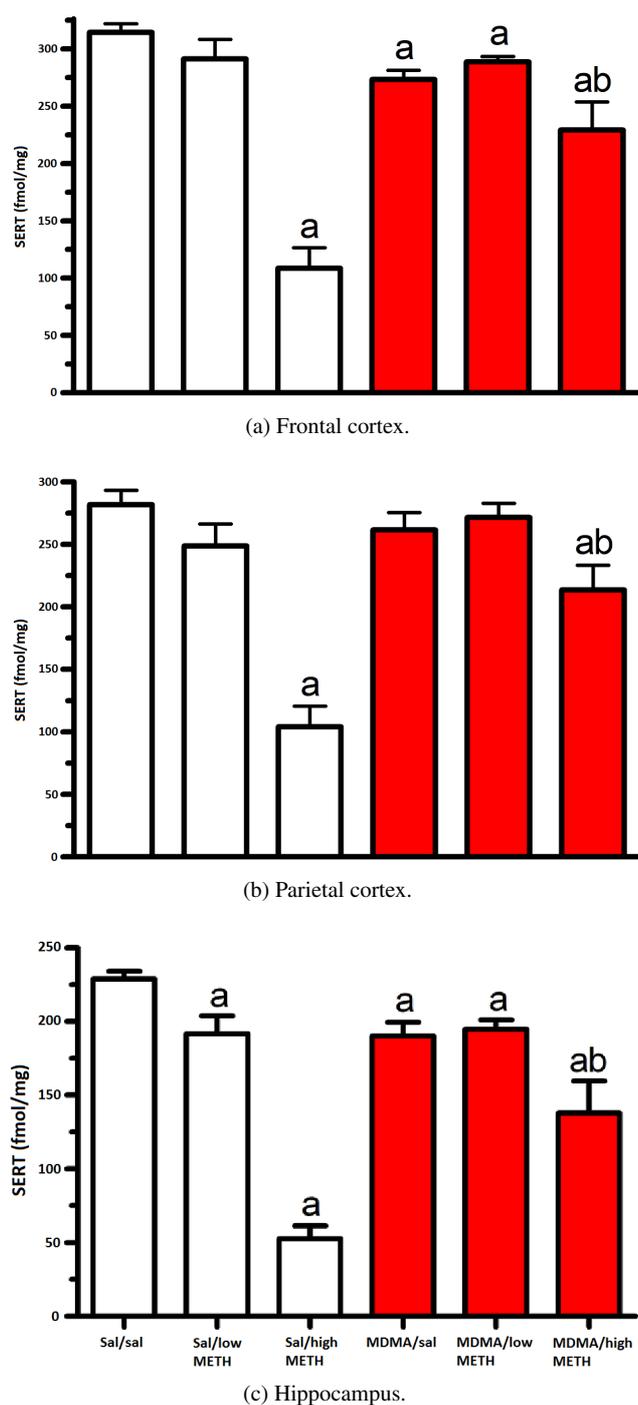
(a) Frontal cortex.



(b) Parietal cortex.



(c) Hippocampus.

**Figure 2:** [³H]Citalopram binding to the serotonin transporter by adolescent treatment (MDMA or Saline) and METH binge (Low: 4 mg/kg × 4; High: 8 mg/kg × 4; or Saline). $^{a}P < .05$ versus Saline/Saline, $^{b}P < .05$ versus Saline/High METH.
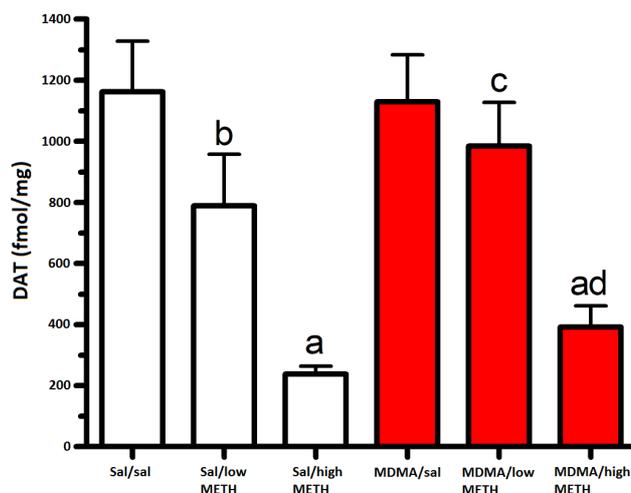


**Figure 3:** [³H]WIN35,428 binding to the striatal dopamine transporter by adolescent treatment (MDMA or Saline) and METH binge (Low: 4 mg/kg × 4; High: 8 mg/kg × 4; or Saline). $^{a}P < .001$ versus Saline/Saline, $^{b}P < .01$ versus Saline/High METH, $^{c}P < .005$ versus MDMA/High METH, and $^{d}P < .05$ versus Saline/High METH.

then abates over 1 month [17, 44, 46]. The size of the challenge dose is also important as preconditioning confers a resistance, but not an immunity, to a later insult [35, 50]. Finally, we note that MDMA itself seems to exert greater effects on DAT expression than on DA levels [6]. Consequently, the choice of neurochemical marker may be important for assessing the influence of preconditioning regimens on monoamine systems.

Several factors have received significant attention regarding the mechanisms underlying substituted amphetamine preconditioning. Evidence has been presented for a variety of potentially relevant cellular/biochemical/molecular changes [9, 11, 19, 21, 29, 36, 44, 60, 61, 70]. But until additional research is conducted, it will remain unclear whether these changes work in concert or whether there are multiple pathways leading to the neuroprotective effects of low-dose MDMA or METH administration.

Other studies have investigated the potential involvement of thermal and pharmacokinetic changes in the preconditioned animals. For example, pretreating adolescent rats with a nonneurotoxic METH regimen not only blocked the reduction of striatal DAT and DA uptake produced by a METH binge in adulthood but also attenuated binge-induced hyperthermia [62]. METH-induced hyperthermia was similarly blunted by MDMA pretreatment in the present study, and indeed there were significant negative correlations between core temperature AUC during the METH binge and subsequent DAT and SERT binding values. These findings support the hypothesis that MDMA-METH preconditioning depends, at least in part, on a blunting of METH-induced

the MDMA and the METH may be less of consideration because amphetamine preconditioning has been reported to be maximal immediately after preconditioning and

hyperthermia. It is important to note, however, that the hyperthermia induced by substituted amphetamines and the subsequent monoamine neurotoxicity are not uniformly coupled processes [30,55]. For example, the cortical SERT depletions, but not the prolonged hyperthermia, following a MDMA binge were mitigated by prior MDMA treatments in adult male rats [44,54]. Further, adolescent females showed an MDMA preconditioning effect to a later MDMA binge dose that did not evoke a hyperthermic response [56]. Overall, although the magnitude of hyperthermia is generally associated with the extent of substituted amphetamine neurotoxicity [40], current evidence is not sufficient to conclude that amphetamine preconditioning is simply secondary to a blunted hyperthermic response to the high-dose challenge.

The absorption, distribution, metabolism, and elimination of amphetamines after preexposure have been frequently assessed. In one early study, the quantity of METH in the forebrain of METH preexposed rats was half the level in METH-naïve animals [65]. A follow-up report determined that brain levels of METH were again decreased, but plasma METH levels were doubled, a finding which indicates that METH pretreatments caused pronounced alterations in METH distribution [24]. In contrast, three other reports by this research team did not show any appreciable effects of METH preexposure on the subsequent levels of METH in the brain [17,45,62]. One study even reported group differences that were in the opposite of the previously identified direction [31]. O'Neil et al. evaluated a greater number of time points than other investigators and noted that prior exposure to an escalating METH regimen had no effect on METH or amphetamine levels resulting from a METH binge [51]. Overall, pharmacokinetic tolerance does not appear to be integral for METH or MDMA [3] preconditioning.

Previous animal studies have explored the adverse psychobiological effects the morning after alcohol [59] or other hypnotics [27], but, to our knowledge, there are no similar preclinical investigations with METH. Residual after-effects of controlled oral METH administration to human volunteers include "heavy limbs" and feeling "miserable" [15]. Drug abusers reported feeling less energetic and alert 24 h after intranasal METH [52]. Therefore, motor activity was evaluated 1 day after the METH binge as an animal model of the more overt symptoms of this METH hangover. Testing revealed the anticipated dose-dependent decreases in locomotor activity (see [58] for similar findings with MDMA). Interestingly, prior adolescent MDMA limited the METH binge-induced reductions in vertical activity. This finding is concordant with prior results that intermittent MDMA prevented a subsequent MDMA binge-induced hangover [54,58].

Two caveats of this dataset as well as the broader amphetamine preconditioning area are noteworthy. First,

the vast preponderance of studies (Table 1) have been conducted in one species (rat) and with a single sex (males), and this report is no exception. Although we have recently examined preconditioning in adolescent female rats [56], additional study with other species such as nonhuman primates is warranted. In this regard, the reported tolerance to MDMA-induced acute behavioral disruption in rhesus monkeys [20] forms an important foundation for further study. A careful examination of a drug abuser's lifetime pattern of drug use (e.g., a slow increase versus a rapid escalation in dosing) may account for an appreciable portion of the variance in radioligand binding in positron emission tomography studies of METH and ecstasy users. A more detailed understanding of preconditioning in a wider range of species and in both sexes might optimize the development of better therapeutic approaches against stimulant addiction and overdoses.

Second, despite efforts to limit hyperthermia, some lethality following high-dose METH (17.6%) was observed. Increased mortality among METH users is well known [72], although this effect originates from a variety of factors including suicide, accidents, and cardiovascular disease [37]. Similarly, Emergency Department visits among METH users occur for a variety of reasons including psychosis, skin infections, and dental disorders [26]. The $LD_{50}$ for METH in adult, male Sprague-Dawley rats was reported to be 50 mg/kg [18]. Substituted amphetamine binges typically employ repeated administration of moderate doses with 1–2 h between each dose with a total daily dose as high, or even higher (Table 1), than was employed in this report. Therefore, it is rather surprising that, with rare exception [30], the preponderance of investigations in Table 1 make no reference to lethality in their subjects. Based on our experience with amphetamines, we suspect that malignant hyperthermia/rhabdomyolysis results in some degree of lethality for other investigators as well, particularly in the ones that make no reference to mitigating hyperthermia. On rare occasion, we and others [22] have found that rodents receiving amphetamines will asphyxiate on the pine shaving bedding, which may be at least partially analogous to the psychotic events that occur in some human high-dose METH users. As noted above, preconditioning regimens have frequently [17,22,30,31,51,56,58,62,67], although not uniformly [3,44,54,55], been associated with blunted temperature dysregulation to a subsequent substituted amphetamine challenge. Additional study is warranted to determine if amphetamine preexposure also attenuates other adverse outcomes of psychostimulants including the diverse stereotypies.

In conclusion, adolescent intermittent MDMA exposure reduced the hyperthermic, locomotor, and neurotoxic (measured by SERT and DAT radioligand binding) effects of a subsequent high-dose METH binge. Interpretation of these

and other findings from a preconditioning perspective may provide a more nuanced perspective on the neurobiological changes that occur after repeated exposure to substituted amphetamines.

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