Repeated Adolescent 3,4-Methylenedioxymethamphetamine (MDMA) Exposure in Rats Attenuates the Effects of a Subsequent Challenge with MDMA or a 5-Hydroxytryptamine<sub>1A</sub> Receptor Agonist

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ABSTRACT

Adolescent users of 3,4-methylenedioxymethamphetamine (MDMA, Ecstasy) may escalate their dose because of the development of tolerance. We examined the influence of intermittent adolescent MDMA exposure on the behavioral, physiological, and neurochemical responses to a subsequent MDMA "binge" or to a 5-hydroxytryptamine<sub>1A</sub> (5-HT<sub>1A</sub>) receptor challenge. Male Sprague-Dawley rats were given MDMA (10 mg/kg b.i.d.) or saline every 5th day on postnatal days (PDs) 35 to 60. One week later on PD 67, animals were challenged with either multiple doses of MDMA (four 5 or 10 mg/kg doses) or a single dose of the 5-HT<sub>1A</sub> agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) (0.1 or 0.5 mg/kg). Adolescent MDMA exposure partially attenuated the hyperthermic effects of the PD 67 MDMA challenge, completely blocked the locomotor hypoactivity otherwise observed on the day after the challenge, and also prevented MDMA-induced serotonin neurotoxicity assessed on PD 74 by measuring regional [3H]citalopram binding to the serotonin transporter (SERT). Adolescent MDMA-treated animals also showed a partial attenuation of the serotonin syndrome but not the hypothermic response to the high dose of 8-OH-DPAT. However, there was no effect of MDMA administration on regional [3H]N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridinyl)cyclohexanecarboxamide trihydrochloride (WAY-100635) binding to 5-HT<sub>1A</sub> receptors in the brain or spinal cord. These results suggest that chronic, intermittent MDMA exposure during adolescence induces neuroadaptive changes that can protect against the adverse consequences of a subsequent dose escalation. On the other hand, the same exposure pattern appears to produce a partial 5-HT<sub>1A</sub> receptor desensitization, which may negatively influence the therapeutic responses of chronic MDMA users treated with serotonergic agents for various affective or anxiety disorders.

The entactogen 3,4-methylenedioxymethamphetamine (MDMA, Ecstasy) produces several subjective and somatic effects, including increased emotional closeness, elation, sensory pleasure, empathy, temperature dysregulation, jaw clenching, muscle cramping, and nausea (Parrott, 2002; Green et al., 2003). The short-term affective responses quickly subside with regular use, which may contribute to dose escalation and binging in some individuals (Parrott, 2005). Indications of tolerance to MDMA have also been reported in a number of preclinical studies. For example, MDMA self-administration was found to decrease over time in rhesus monkeys, suggesting a reduction in the reinforcing activity of the drug (Faintegrossi et al., 2004). The ability of MDMA to impair schedule-controlled behavior also seems to change with repeated exposure (Li et al., 1989; LeSage et al., 1993). Most strikingly, monkeys were less sensitive to MDMA-induced disruption of cognitive performance 18 months after chronic MDMA treatment (Frederick and Paule, 1997). Likewise, the dose of MDMA necessary to attenuate consummatory behavior increased after daily exposure (Zacny et al., 1990). On the other hand, the physiological and behavioral responses to repeated MDMA exposure may be either diminished or enhanced, depending on the test measure and the treatment regimen. Thus, a neurotoxic dosing regimen of MDMA over a single day reduced the serotonin (5-HT)-releasing, temperature, and behavioral effects to a subsequent MDMA challenge given 1 week later (Shankaran and Gudelsky, 1999). In contrast, Dafters (1995) determined that daily MDMA exposure in rats led to an enhance-

ABBREVIATIONS: MDMA, (±)-3,4-methylenedioxymethamphetamine (Ecstasy); 5-HT, 5-hydroxytryptamine (serotonin); PD, postnatal day; SERT, serotonin transporter; 8-OH-DPAT, 8-hydroxy-2-(di-n-propylamino)tetralin; AUC, area under the curve; WAY-100635, [3H]N-[2-[4-(2-methoxyphenyl)-1-piperazinyl][ethyl]-N-(2-pyridinyl)cyclohexanecarboxamide trihydrochloride; ANOVA, analysis of variance.
ment of the hyperthermic and locomotor responses. Likewise, MDMA administration every other day for approximately 3 weeks elevated autonomic activity (i.e., salivation) and 5-HT syndrome behaviors evoked by the treatment (Spanos and Yamamoto, 1989). All of the aforementioned investigations, which identified either tolerance or reverse tolerance (i.e., sensitization), were conducted in reproductively mature subjects.

Although adolescents and young adults frequently use Ecstasy (Pope et al., 2001, Lieb et al., 2002; Degenhardt et al., 2004; Jacobsen et al., 2004), only recently have results with controlled animal studies begun to characterize the physiological, neurochemical, and behavioral consequences of MDMA exposure during this important developmental period (Spear, 2000). Initial work by Broening et al. (1995) found that both the thermal and neurotoxic effects of MDMA in rats increase with age from infancy through adolescence to adulthood. Although subsequent studies have confirmed that younger animals are less sensitive to MDMA neurotoxicity than adults (Kelly et al., 2002; Meyer et al., 2004), adolescent exposure to this compound has been found to cause measurable decreases in 5-HT levels as well as serotonin transporter (SERT) binding (Fone et al., 2002; Morley-Fletcher et al., 2002; Bull et al., 2003, 2004; Piper and Meyer, 2004; Piper et al., 2005). Treatment with MDMA during adolescence has also been shown to produce a variety of functional consequences. Short-term treatment regimens resulted in a later decrease in social interaction but an increase in cocaine reward measured using place conditioning (Horan et al., 2000; Bull et al., 2004). Finally, our group has shown that intermittent MDMA administration throughout adolescence in rats can elicit tolerance to the temperature dysregulation and 5-HT syndrome responses to the drug (Piper et al., 2005) and can also affect performance on later tests of working memory and anxiety (Piper and Meyer, 2004).

The present study was designed to extend our previous work involving an intermittent adolescent dosing regimen in rats (Piper and Meyer, 2004). In the first experiment, we examined the influence of this treatment regimen on the behavioral, physiological, and neurochemical effects of a subsequent MDMA “binge” administered in young adulthood. Of particular interest was the question of whether the adolescent preexposure would increase or decrease the animals’ vulnerability to the neurotoxic effects of the binge treatment. The second experiment assessed whether adolescent MDMA administration would alter 5-HT1A-mediated functions, based on the involvement of this receptor subtype in temperature regulation and the serotonin syndrome (Aguirre et al., 1998; Granoff and Ashby, 2001; Hedlund et al., 2004).

Materials and Methods

Experiment 1: MDMA Binge

Animals and Drug Treatments. Male Sprague-Dawley rats (n = 52) were obtained from Charles River Laboratories (Wilmington, MA) and acclimated to the laboratory for approximately 2 weeks before the beginning of experimentation. Animals were pair-housed in plastic tubs (44.5 x 23.5 x 20.0 cm) at a temperature of 23 ± 1°C on a reverse 12-h light/12-h dark cycle (lights off at 8:00 AM) with drug treatments administered during the dark phase of the cycle.

Animals received (±)-MDMA-HCl (two 10 mg/kg s.c. doses; dose calculated based on the weight of the salt; RTI, Research Triangle Park, NC) or saline every 5th day from PD 35 to PD 60, with each dose separated by 4 h. All drugs were administered in the home cage with cage mates receiving the same treatments. The rationale for this dosing paradigm is found in Piper and Meyer (2004). The dosing ages were selected to include the periadolescent developmental period (Smith, 2002). Seven days after the last dose (i.e., on PD 67), animals from each adolescent treatment group received either 5 or 10 mg/kg MDMA hourly for 4 h or saline only at the same intervals. The treatment regimen on this day was selected to model a human MDMA binge (Parrott, 2005). The combination of adolescent and binge conditions resulted in six groups: saline/saline, saline/low MDMA (four 5 mg/kg doses), saline/high MDMA (four 10 mg/kg doses), MDMA/saline, MDMA/low MDMA, and MDMA/high MDMA (n = 8–10/group). Animal care was in accordance with the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, 1996). The experimental protocol for this study was approved by the University of Massachusetts–Amherst Institutional Animal Care and Use Committee.

Physiological and Behavioral Measures During Dosing. Core body temperature was recorded on PDs 35, 45, 60, and 67 with a rectal probe (RET-2; Physiotemp Instruments, Clifton, NJ) connected to a digital thermometer (TH-5; Physiotemp). If core temperature became excessive (≥40.5°C) during or after drug dosing, then the rat was cooled by wrapping it in ice-cold towels, usually for less than 10 min, to minimize the potential for lethality. Care was taken to prevent overheating during the binge so that all rats (cooled and noncooled) could be included in the subsequent data analyses. Temperature measurements conducted between PD 35 and PD 60 were regularly obtained for 300 min after the first dose because core temperature is not appreciably elevated beyond this interval at these ages (Piper et al., 2005). Body weight was obtained approximately 1 h before and again 2 h after the last MDMA dose on PDs 35, 45, 60, and 67 to calculate the percent change in weight ([postdosing weight − predosing weight]/predosing weight) caused by the treatment regimen on those days. As MDMA can elicit ejaculation in adult male rats (Bilsky et al., 1991), the pelvic area was inspected every 30 min during dosing on PD 67 to ascertain the presence or absence of seminal discharge. In addition, the head-weaving and forepaw-treading components of the 5-HT syndrome were also obtained from videotaped home cage behavior every 30 min for 5 h during dosing according to the procedures described in Spanos and Yamamoto (1989).

Motor Activity. Animals were placed into one of four ENV-510 activity chambers (Med Associates Inc., St. Albans, VT) with internal dimensions of 27.5 x 27.5 x 20.5 cm. Each chamber was illuminated by a 28-V bulb (Med Associates model ENV-221 CL) and had fans running during testing to limit background noise. Two sets of photobeam strips were located at floor level to determine horizontal activity, and a third set was elevated 13.5 cm above the floor to record vertical activity. Dependent measures were the distance traveled as well as rearing frequency and duration. This test was conducted for 10 min, 1 day after the drug treatments on PD 67. Human Ecstasy users report that they experience “mid-week blues” characterized by loss of energy and fatigue after MDMA use (Parrott, 2002). This test was designed to ascertain whether depressed activity soon after MDMA exposure can also be demonstrated experimentally.

[3H]Citalopram Binding. Animals were lightly anesthetized via CO2 inhalation and decapitated on PD 74. The brain was rapidly removed, immersed in ice-cold 0.9% NaCl, and placed into a chilled acrylic brain block. A 2-mm thick slice beginning 5 mm from the anterior pole was obtained, and the cerebral cortex was separated from the underlying striatum. The hippocampus was dissected free-hand from the remaining sample. Brain tissue was frozen on dry ice and stored at −70°C until the binding assay was conducted. Washed membrane fractions were assayed in triplicate for SERT binding using a 1.0 nM concentration of [3H]citalopram (81.2–84.2 Ci/mmol; New England Nuclear, Boston, MA) according to the procedures.
described in Piper et al. (2005). This concentration of citalopram is roughly equal to the apparent \( K_D \) for \([\text{H}]\text{citalopram} \) binding to rat brain SERT under the present assay conditions (B. J. Piper and J. S. Meyer, unpublished observations). A higher, saturating concentration of the radioligand was not used to avoid excessive nonspecific binding.

**Experiment 2: 8-Hydroxy-2-(di-n-propylamino)tetrain Challenge**

Rats (\( n = 48 \)) in the 5-HT\(_{1A} \) experiment received the same regimen of adolescent MDMA or saline at the same ages and intervals as in the prior experiment. However, on PD 67 the animals were randomly assigned to groups (\( n = 8/\text{group} \)) that received either 0.1 or 0.5 mg/kg of the 5-HT\(_{1A} \) agonist 8-hydroxy-2-(di-n-propylamino)tetrain (8-OH-DPAT; Sigma Chemical, St. Louis, MO) or saline (s.c.). Animals were placed into clear glass chambers and videotaped for coding of headwaving, forepaw-treading, and low body posture components of the serotonin syndrome. Further details about the testing environment are found elsewhere (Piper and Meyer, 2006). Core body temperature was also obtained immediately before the injection and at 15, 30, 45, 60, 75, 90, and 100 min thereafter.

\([\text{H}]\text{N-[2-[4-(2-methoxyphenyl)-1-piperazinyl] ethyl}]-N-(2-pyridinyl)cyclohexanecarboxamide Trihydrochloride Binding.\)

On PD 68, the saline/saline and MDMA/saline groups were sacrificed, and the frontal cortex, hippocampus, brainstem, and lumbar spinal cord were dissected and frozen on dry ice. 5-HT\(_{1A} \) receptor density was determined by measuring the binding of 0.5 nM \([\text{H}]\text{N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}]-N-(2-pyridinyl)cyclohexanecarboxamide trihydrochloride (WAY-100635; 83.0 Ci/mmol; Amersham Biosciences Inc., Piscataway, NJ) to washed membrane fractions according to previously published methods (Gozlan et al., 1995; Khawaja et al., 1995). The chosen concentration of WAY-100635 was approximately 4 to 5 times the apparent \( K_D \) for binding of this compound to the rat 5-HT\(_{1A} \) receptor (Gozlan et al., 1995; Khawaja et al., 1995). Incubations were carried out in triplicate for 1 h at room temperature in a buffer containing 10 mM sodium phosphate, 120 mM sodium chloride, and 5 mM potassium chloride (pH = 7.40). Nonspecific binding was defined with 0.3 \( \mu \)M 8-OH-DPAT.

**Statistical Analysis**

Statistical analysis was performed with Systat, version 10.2 (Systat Software, Inc., Point Richmond, CA). Results were expressed as means ± S.E.M., and a \( p \) value of <0.05 was considered statistically significant. The total thermal response on PDs 35, 45, 60, and 67 was quantified by determining the area under the curve (AUC) of core body temperature measurements using the linear trapezoid rule with the data expressed as degrees Celsius × hour as described elsewhere (Piper et al., 2005). Ratio level measures in the binge experiment (i.e., AUC, percent change in body weight, latency until ejaculation, and SERT binding) were analyzed with a 2 (adolescent treatment: saline versus MDMA) × 3 (binge treatment: saline, low MDMA, and high MDMA) factor ANOVA. Likewise, the challenge dose (saline, low 8-OH-DPAT, and high 8-OH-DPAT) was a between-groups variable for the 5-HT\(_{1A} \) experiment. Serotonin syndrome scores were initially analyzed using an adolescent treatment × challenge dose × time mixed ANOVA. Significant interactions were then further examined using simple effects ANOVAs for each challenge dose. On rare occasions for which outliers occurred, defined in Systat on the basis of extreme Studentized residuals, they were removed. Associations among variables were determined with Pearson product-moment correlations, followed by Fisher \( t \) to \( z \) transformations to determine statistical significance. Nominal level variables (i.e., the presence or absence of seminal discharge and the number of animals requiring cooling on PD 67 after an MDMA binge) were analyzed with a \( \chi^2 \) test.

**Results**

**Experiment I: MDMA Binge**

**Core Temperature and Body Weight Changes during Dosing.** MDMA elicited core temperature dysregulation during adolescence on PDs 35, 45, and 60, as revealed by mixed time × treatment ANOVAs that were conducted on the data from each age. On PD 35, there were significant main effects of time \([F(7,350) = 7.25, p < 0.001]\) and treatment \([F(1,50) = 4.20, p < 0.05]\) and a time × treatment interaction \([F(7,350) = 9.09, p < 0.001]\). The temperature response to MDMA at this age was bidirectional with an initial hypothermia followed by a small, but significant, elevation in temperature relative to the vehicle treatment (Fig. 1A). In contrast, the temperature change after MDMA at PD 45 was unidirectional (Fig. 1B). A mixed ANOVA on the PD 45 temperature data showed main effects of time \([F(7,350) = 8.41, p < 0.001]\) and treatment \([F(1,50) = 11.08, p < 0.01]\) and a time × treatment interaction \([F(7,350) = 5.10, p < 0.001]\). Core temperature was significantly elevated by MDMA from 60 until 240 min after the first drug administration. The temperature response on PD 60 was similar to that on PD 45 but with a more intense and prolonged hyperthermia (Fig. 1C). The mixed ANOVA revealed the usual time \([F(7,336) = 8.98, p < 0.001]\) and treatment main effects \([F(1,48) = 23.46, p < 0.001]\) and a time × treatment interaction \([F(7,336) = 13.85, p < 0.001]\). Overall, increasing age was associated with greater MDMA-induced elevations in core temperature, which can also be seen by comparing the temperature AUC at each age (Fig. 1D). A mixed (age × treatment) ANOVA on the AUC at PDs 35, 45, and 60 documented the main effects of age \([F(2,100) = 52.32, p < 0.001]\) and treatment \([F(1,50) = 19.39, p < 0.01]\) and an age × treatment interaction \([F(2,100) = 5.18, p < 0.01]\).

Adolescent MDMA administration caused a reduction in body weight that was already discernible by PD 40 (Fig. 1E). A mixed (age × treatment) ANOVA conducted on the weight data showed the expected age effect \([F(6,288) = 4129.53, p < 0.001]\), a treatment effect \([F(1,48) = 18.67, p < 0.001]\), and an age × treatment interaction \([F(6,288) = 30.51, p < 0.001]\). Figure 1F depicts the acute effects of MDMA on body weight (calculated as percent change from 1-h pretreatment to 2 h post-treatment) on PDs 35, 45, and 60. A mixed (age × treatment) ANOVA carried out on these results revealed main effects of treatment \([F(1,50) = 503.9, p < 0.001]\) and age \([F(2,100) = 11.39, p < 0.001]\) and an age × treatment interaction \([F(2,100) = 6.24, p < 0.01]\). A separate analysis of the data from the saline control group showed that these animals became less responsive over time to the stress of the injections \([F(2,50) = 16.04, p < 0.001]\; PD 35 versus PD 45: \(t(25) = 2.99, p < 0.01\); PD 45 versus PD 60: \(t(25) = 3.59, p < 0.002\)). Likewise, the MDMA-treated group lost less weight on the 6th day of treatment compared with their weight loss on the 3rd day \([F(2,50) = 2.44, p = 0.10]\; PD 45 versus PD 60: \(t(25) = 2.19, p < 0.05\).

**Core Temperature, Body Weight, and Serotonin Syndrome Responses to the Binge.** The core temperature response to the MDMA binge was assessed in three ways. First, the proportion of animals in each group that required intervention to prevent potentially lethal hyperthermia was examined. Icing for body temperature ≥40.5°C was necessary for 31.3% (five of 16) rats in the high-dose MDMA binge
condition, 38.9% (seven of 18) rats in the low-dose MDMA binge condition, and 0% of the saline controls \( \chi^2(2) = 8.12, p < 0.05 \). An additional \( \chi^2 \) test was conducted to determine whether adolescent MDMA experience influenced the need for this intervention. The majority of the MDMA-naive group (55.6% or 10 of 18) required icing compared with only 12.5% (two of 16) that had received prior MDMA exposure during adolescence \( \chi^2(1) = 6.88, p < 0.02 \). Second, core temperature at all intervals was assessed with a 2 (adolescent treatment: saline versus MDMA) × 3 (binge treatment: saline, low MDMA, and high MDMA) × 14 (time) factor mixed ANOVA that showed significant main effects of adolescent treatment \( F(1,14) = 11.85, p < 0.01 \) and time \( F(13,182) = 9.67, p < 0.001 \), and an adolescent treatment × time interaction \( F(13,182) = 2.99, p = 0.001 \). However, in the low-dose MDMA condition (Fig. 2B), there was a main effect of time \( F(13,208) = 12.99, p < 0.001 \), but no significant effect of adolescent treatment or an adolescent treatment × time interaction. Likewise, among the animals that received only the saline vehicle on PD 67 (Fig. 2C), there was only a significant time effect \( F(13,192) = 10.00, p < 0.001 \). Third, the AUC (Fig. 2D) was examined with a 2 (adolescent treatment) × 3 (binge treatment) factor ANOVA that showed main effects of adolescent treatment \( F(1,45) = 11.94, p = 0.001 \) and binge treatment \( F(2,45) = 25.01, p < 0.001 \) but no significant interaction. Summarizing the temperature data, repeated adolescent ex-
posure to MDMA significantly (though not entirely) attenuated the hyperthermic response to the high-dose MDMA binge administered on PD 67 as shown by both the time course results and the AUC values. There was also a slight trend for prior MDMA exposure to reduce the hyperthermic response to the low-dose MDMA binge, but this effect did not attain statistical significance.

Total serotonin syndrome scores and body weight changes on the binge day are presented in Fig. 2, E and F, respectively. A 2 (adolescent treatment) × 3 (binge treatment) factor ANOVA identified a main effect of binge treatment \( [F(2,45) = 13.36, p < 0.001] \) but no adolescent treatment effect or interaction. Post hoc tests showed that serotonin syndrome behaviors were evoked by both the low-dose and high-dose MDMA binge but that the intensity of the syndrome did not differ as a function of either the binge dose or adolescent MDMA exposure. Analysis of body weight measurements before and after the binge showed average reductions of 7 to 9%. A mixed ANOVA revealed a significant main effect for binge treatment \( [F(2,44) = 139.04, p = 0.001] \) but not for adolescent treatment. There was also no significant interaction between these factors.

Seminal discharge was induced by the MDMA binge, but there were no significant effects of dose or prior adolescent MDMA treatment (Table 1). Seminal plugs were visible in the majority of rats in both the low- and high-dose MDMA binge groups but never after saline. Among animals that ejaculated, the latency until seminal discharge was analyzed with a 2 (adolescent treatment) × 2 (binge treatment: low-dose versus high-dose MDMA) factor ANOVA. There were no significant main effects, but a trend in the adolescent treatment × binge treatment interaction was noted \( [F(1,27) = 3.62, p = 0.07] \). As shown in Table 1, the MDMA-naive group took approximately twice as long to ejaculate as the MDMA-
adolescent treatment main effect \( F(2,45) = 11.17, p < 0.001 \), and adolescent treatment \( \times \) binge treatment interaction \( F(2,45) = 17.30, p < 0.001 \). Similar results were obtained for the cortex: adolescent treatment main effect \( F(1,45) = 11.82, p < 0.002 \), binge treatment main effect \( F(2,45) = 11.17, p < 0.001 \), and adolescent treatment \( \times \) binge treatment interaction \( F(2,45) = 17.30, p < 0.001 \). Similar results were obtained for the striatum: adolescent treatment main effect \( F(1,45) = 11.82, p < 0.002 \), binge treatment main effect \( F(2,45) = 11.17, p < 0.001 \), and adolescent treatment \( \times \) binge treatment interaction \( F(2,45) = 17.30, p < 0.001 \). Similar results were obtained for the cortex: adolescent treatment main effect \( F(1,45) = 11.82, p < 0.002 \), binge treatment main effect \( F(2,45) = 11.17, p < 0.001 \), and adolescent treatment \( \times \) binge treatment interaction \( F(2,45) = 17.30, p < 0.001 \). Similar results were obtained for the striatum: adolescent treatment main effect \( F(1,45) = 11.82, p < 0.002 \), binge treatment main effect \( F(2,45) = 11.17, p < 0.001 \), and adolescent treatment \( \times \) binge treatment interaction \( F(2,45) = 17.30, p < 0.001 \). Similar results were obtained for the cortex: adolescent treatment main effect \( F(1,45) = 11.82, p < 0.002 \), binge treatment main effect \( F(2,45) = 11.17, p < 0.001 \), and adolescent treatment \( \times \) binge treatment interaction \( F(2,45) = 17.30, p < 0.001 \). Similar results were obtained for the striatum: adolescent treatment main effect \( F(1,45) = 11.82, p < 0.002 \), binge treatment main effect \( F(2,45) = 11.17, p < 0.001 \), and adolescent treatment \( \times \) binge treatment interaction \( F(2,45) = 17.30, p < 0.001 \). Similar results were obtained for the cortex: adolescent treatment main effect \( F(1,45) = 11.82, p < 0.002 \), binge treatment main effect \( F(2,45) = 11.17, p < 0.001 \), and adolescent treatment \( \times \) binge treatment interaction \( F(2,45) = 17.30, p < 0.001 \). Similar results were obtained for the striatum: adolescent treatment main effect \( F(1,45) = 11.82, p < 0.002 \), binge treatment main effect \( F(2,45) = 11.17, p < 0.001 \), and adolescent treatment \( \times \) binge treatment interaction \( F(2,45) = 17.30, p < 0.001 \). Similar results were obtained for the cortex: adolescent treatment main effect \( F(1,45) = 11.82, p < 0.002 \), binge treatment main effect \( F(2,45) = 11.17, p < 0.001 \), and adolescent treatment \( \times \) binge treatment interaction \( F(2,45) = 17.30, p < 0.001 \). Similar results were obtained for the striatum: adolescent treatment main effect \( F(1,45) = 11.82, p < 0.002 \), binge treatment main effect \( F(2,45) = 11.17, p < 0.001 \), and adolescent treatment \( \times \) binge treatment interaction \( F(2,45) = 17.30, p < 0.001 \). Similar results were obtained for the cortex: adolescent treatment main effect \( F(1,45) = 11.82, p < 0.002 \), binge treatment main effect \( F(2,45) = 11.17, p < 0.001 \), and adolescent treatment \( \times \) binge treatment interaction \( F(2,45) = 17.30, p < 0.001 \). Similar results were obtained for the striatum: adolescent treatment main effect \( F(1,45) = 11.82, p < 0.002 \), binge treatment main effect \( F(2,45) = 11.17, p < 0.001 \), and adolescent treatment \( \times \) binge treatment interaction \( F(2,45) = 17.30, p < 0.001 \).}

**Motor Activity Postbinge.** In the absence of prior MDMA exposure, binge treatment on PD 67 caused large reductions in motor activity (distance traveled and rearing) on the following day (Fig. 3). Interestingly, intermittent adolescent MDMA exposure completely prevented this postbinge hypoactivity. These conclusions are supported by the results of 2 (adolescent treatment) \( \times \) 3 (binge treatment) factor ANOVAs performed separately on the horizontal (distance traveled) and vertical (rearing) activity data. For distance traveled, we found significant main effects of adolescent treatment \( F(1,45) = 13.78, p < 0.001 \) and binge treatment \( F(2,45) = 3.88, p < 0.05 \) and an adolescent treatment \( \times \) binge treatment interaction \( F(2,45) = 3.45, p < 0.05 \). Analysis of rearing frequency similarly revealed significant main effects of adolescent treatment \( F(1,45) = 20.27, p < 0.001 \) and binge treatment \( F(2,45) = 7.40, p < 0.01 \) and an adolescent treatment \( \times \) binge treatment interaction \( F(2,45) = 7.40, p < 0.01 \). The results for rearing duration indicated the same main effects for adolescent treatment \( F(1,45) = 15.83, p < 0.001 \) and binge treatment \( F(2,45) = 4.71, p < 0.05 \) but no significant interaction.

**[3H]Citalopram Binding.** As shown in Fig. 4, MDMA treatment during adolescence significantly reduced SERT binding by approximately 32% \( p < 0.05 \) in the hippocampus, but it had no effect on binding in the parietal cortex or striatum (adolescent MDMA versus adolescent saline, 0 binge dose). More important, such prior MDMA exposure completely prevented the binge-induced decreases in SERT binding in all brain areas. The binding data were analyzed separately for each brain area by means of 2 \( \times \) 3 factor ANOVAs. One excessively high hippocampal SERT value (Studentized residual = 7.13) in the MDMA/saline group was identified and removed from the analysis. The remaining hippocampal binding data showed significant main effects of adolescent treatment \( F(1,45) = 11.82, p < 0.002 \), binge treatment \( F(2,45) = 11.17, p < 0.001 \), and an adolescent treatment \( \times \) binge treatment interaction \( F(2,45) = 17.30, p < 0.001 \). Similar results were obtained for the cortex: adolescent treatment main effect \( F(1,45) = 28.81, p < 0.001 \), binge treatment main effect \( F(2,45) = 14.82, p < 0.001 \), and adolescent treatment \( \times \) binge treatment interaction \( F(2,45) = 13.48, p < 0.001 \), and for the striatum: adolescent treatment main effect \( F(1,45) = 8.84, p < 0.01 \), binge treatment main effect \( F(2,44) = 9.27, p < 0.001 \), and adolescent treatment \( \times \) binge treatment interaction \( F(2,44) = 7.79, p < 0.002 \).

The relationships among SERT density and the physiological and behavioral responses to MDMA treatments are shown in Table 2. For the animals that received saline during adolescence, large correlations were obtained between regional [3H]citalopram binding data and at least some indices of acute MDMA response. For example, high positive correlations \( r > 0.70 \) were observed between the body weight

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**TABLE 1**

Seminal discharge (percentage and latency) on PD 67 as a function of adolescent and adult MDMA treatment

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<th>Adolescent Treatment</th>
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<td>Latency***</td>
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N.A., not applicable; *** significant effect of adult treatment \( p < 0.001 \).

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**Fig. 3.** Motor activity on PD 68 in rats as a function of adolescent and adult treatments. A, \( p < 0.05 \) compared with the corresponding group that received MDMA during adolescence; B, \( p < 0.05 \) compared with the saline/saline control group.
change induced by the binge and SERT density in all brain areas (results for the hippocampus are shown in Fig. 5A).

There was also a strong inverse association between tempera-
ture AUC values, a cumulative index of hyperthermia, and SERT density (i.e., greater hyperthermia predicted lower SERT binding; hippocampal data shown in Fig. 5C). In con-
trast, these associations were lacking for the animals that re-
ceived MDMA during adolescence (Table 2; Fig. 5, B and D). Thus, adolescent MDMA induced an uncoupling between acute MDMA responses and neurotoxicity. Likewise, the ex-
tent of locomotor hypoactivity on the day after the binge was
highly predictive of subsequent SERT depletions for animals that
received saline but not MDMA on PDs 35 to 60. Table 2 also shows a substantial degree of consistency between the hyperthermic and weight loss response; that is, animals with the largest AUC lost the most weight after the binge. On the other hand, among the subset of rats that produced a seminal

**TABLE 2**

Correlation matrix showing associations between SERT binding, acute MDMA responses, and next-day motor activity in rats that received saline or MDMA during adolescence and MDMA in adulthood.

Correlation coefficients were derived from pooled data from both adult MDMA treatment groups (four 5 and 10 mg/kg doses). For each pair of correlation coefficients (X/Y), the left value represents $r$ for animals given saline during adolescence, and the right value represents $r$ for animals given MDMA during adolescence. For example, hippocampal SERT binding (parameter A) was highly correlated with distance traveled (parameter G) in the animals that had received saline during adolescence ($r = 0.71$) but not in the animals that had received adolescent MDMA treatment ($r = 0.07$).

<table>
<thead>
<tr>
<th>Experimental Parameter</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>SERT in hippocampus (A)</td>
<td>1.00/1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>SERT in cortex (B)</td>
<td>0.85/0.44$^a$</td>
<td>1.00/1.00</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>SERT in striatum (C)</td>
<td>0.95/0.15$^d$</td>
<td>0.90/0.22$^d$</td>
<td>1.00/1.00</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>AUC on PD 67 (D)</td>
<td>-0.80/-0.25$^d$</td>
<td>-0.90/-0.22$^d$</td>
<td>-0.92/-0.17$^d$</td>
<td>1.00/1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight change on PD 67 (E)</td>
<td>0.93/-0.25$^a$</td>
<td>0.92/-0.06$^d$</td>
<td>0.86/0.06$^d$</td>
<td>-0.85/-0.77$^d$</td>
<td>1.00/1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ejaculation latency (F)</td>
<td>0.07/-0.02</td>
<td>-0.30/0.09</td>
<td>-0.38/0.20</td>
<td>0.33/0.37</td>
<td>-0.25/-0.20</td>
<td>1.00/1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distance traveled (G)</td>
<td>0.71/0.07$^a$</td>
<td>0.73/0.21$^d$</td>
<td>-0.77/0.16$^a$</td>
<td>0.71/0.05$^d$</td>
<td>-0.35/0.05</td>
<td>0.86/0.42$^d$</td>
<td>1.00/1.00</td>
<td></td>
</tr>
<tr>
<td>Rearing duration (H)</td>
<td>0.61/0.12</td>
<td>0.71/0.11$^d$</td>
<td>0.60/-0.14$^d$</td>
<td>-0.71/-0.31</td>
<td>0.07/0.04$^d$</td>
<td>-0.63/-0.02</td>
<td>0.86/0.42$^d$</td>
<td>1.00/1.00</td>
</tr>
</tbody>
</table>

$^a P < 0.05$, $^b P < 0.01$, $^c P < 0.001$ vs. $r = 0.00$, $^d P < 0.05$ compared with the adolescent saline group.
plug, the latency until ejaculation was not associated with other measures of acute MDMA responsiveness.

**Experiment 2: 8-OH-DPAT Challenge**

The effects of intermittent adolescent MDMA treatment on core body temperature and body weight were virtually identical to those obtained in the first experiment (data not shown).

**Serotonin Syndrome and Core Temperature Responses to the 8-OH-DPAT Challenge.** Adolescent exposure to MDMA significantly reduced the serotonin syndrome response (i.e., the sum of the head-weaving, forepaw-treading, and low body posture ratings) response to the high but not the low dose of 8-OH-DPAT. This can be seen in the time course of the total serotonin scores plotted over time (Fig. 6), as well as in the mean scores collapsed across all time points for each treatment group (high-dose 8-OH-DPAT: adolescent saline = 7.0 ± 1.1, adolescent MDMA = 4.0 ± 0.7; low-dose 8-OH-DPAT: adolescent saline = 3.3 ± 0.5, adolescent MDMA = 4.5 ± 1.4; saline: adolescent saline = 1.6 ± 0.5, adolescent MDMA = 2.9 ± 0.8). An initial 2 (adolescent treatment) × 3 (challenge dose) × 6 (time) factorial mixed ANOVA on these data identified a main effect of challenge dose \[F(2,41) = 61.00, p < 0.001\] and time \[F(7,287) = 51.89, p < 0.001\] and a challenge dose × time interaction \[F(14,287) = 21.58, p < 0.001\]. The ANOVA results with AUC as the dependent measure were similar, with an effect of challenge dose \[F(2,41) = 69.42, p < 0.001\] but not of adolescent treatment.

**[^3H]WAY-100635 Binding.** Results of the 5-HT1A binding assay were analyzed with a 4 (tissue) × 2 (adolescent treatment) factor mixed ANOVA. This test revealed the anticipated main effect of tissue \[F(3,36) = 355.57, p < 0.001\]; however, adolescent MDMA exposure did not alter \[^3H]WAY-100635 binding in any of the areas examined (frontal cortex, hippocampus, brainstem, and lumbar spinal cord) (Fig. 7B).

**Discussion**

The major finding of the first experiment was that intermittent exposure to MDMA during adolescence profoundly attenuated or even prevented some of the physiological, behavioral, and neurotoxic responses to an MDMA binge treatment in adulthood. The development of tolerance occurred in the absence of substantial serotonergic neurotoxicity (from the adolescent exposure) and was most apparent after the...
high-dose binge. In contrast, most measures showed little tolerance to MDMA during the adolescent treatment regimen itself, suggesting that at least some of the underlying neural changes produced by this regimen (see below) require sufficient maturation and/or the appropriate challenge conditions to be manifested.

Measurement of MDMA-induced body temperature and weight changes during the adolescent treatment period confirmed and extended previous findings obtained during this stage of development. Core body temperature was altered by $\pm 0.5^{\circ}{\text{C}}$ on the first day of MDMA administration, but the temperature response subsequently increased to approximately $1.0^{\circ}{\text{C}}$ on PD 60. MDMA is often described as a hyperthermic drug but actually causes poikilothermia. Like alcohol (Myers, 1981), MDMA disrupts the thermoregulatory response to the environment which, depending on ambient temperature, can result in either hyperthermia, hypothermia, or no appreciable change in core temperature (Green et al., 2003). Although statistically significant, the changes observed during adolescence were relatively modest compared with the thermic effects of MDMA in adult rats (Shankaran and Gudelsky, 1999; Piper et al., 2005) (also note the 1-h temperature rise in the adolescent MDMA-treated rats compared with the 1-h rise in the animals given saline during adolescence but challenged with MDMA in adulthood). One unexpected finding was the lack of any apparent temperature response to the second daily dose of MDMA given during the adolescent dosing period. This result is suggestive of a rapid within-session tolerance to MDMA that contrasts with the absence of an observable between-session (i.e., across days) tolerance to the treatment. Interestingly, a prior study of intermittent adolescent MDMA administration using a different dosing regimen (four doses of 5 mg/kg at hourly intervals) also showed evidence of within-session tolerance to the thermic effects of MDMA (Piper et al., 2005). These findings of acute tolerance add a new dimension to the existing reports of chronic MDMA (Ecstasy) tolerance in both human users and animal subjects (Parrott, 2005).

Chronic intermittent MDMA administration attenuated the animals’ rate of weight gain, which is in agreement with previous findings (Piper and Meyer, 2004; Piper et al., 2005).
Less commonly studied is the decrease in body weight on the day of MDMA administration, which was found to be quite substantial in the present study. This decrease in weight presumably results from the combined effects of reduced food consumption and increased locomotor activity, defecation, urination, and evaporative water loss because of heightened respiratory rate (Bilsky et al., 1991; Green et al., 2003). The 6 to 8% weight reductions elicited acutely by MDMA were not modified by repeated exposure during adolescence.

Although all rats that received the MDMA binge in adulthood (PD 67) exhibited hyperthermia, this increase in core temperature was blunted by prior adolescent exposure. These results are consistent with those of Shankaran and Gudelsky (1999), who observed a significant reduction in the hyperthermic response to MDMA in adult Sprague-Dawley rats previously given a neurotoxic regimen (four doses of 10 mg/kg i.p.) of this compound. In contrast, Beveridge et al. (2004) reported no effect of a lower, although still neurotoxic, MDMA treatment (one dose of 12.5 mg/kg i.p.) on the later thermic response to MDMA in adult Dark Agouti rats. Moreover, Dafters (1995) found a sensitization of MDMA-induced hyperthermia in Wistar rats given daily doses of MDMA (7.5 mg/kg s.c.) for 2 weeks. It is likely that the effects of MDMA pretreatment on later sensitivity to this drug are strongly dependent on the dosing regimen and perhaps also on the strain of the animals. Whether developmental stage (e.g., adolescence versus adulthood) is also an important variable remains to be determined. The mechanism by which adolescent MDMA treatment altered subsequent MDMA-induced hyperthermia in the present study is not yet clear, although it could involve changes in any of a number of systems that contribute to MDMA-related temperature dysregulation, including the serotonergic, dopaminergic, and cytokine systems. One particularly intriguing possibility involves changes in the dopamine D1 receptor, which has been strongly implicated in the hyperthermic response to MDMA (Green et al., 2004). The diminished hyperthermia is probably not due to 5-HT neurotoxicity per se, as our adolescent MDMA regimen caused only modest reductions in SERT binding. In particular, hipocampal SERT binding was reduced by approximately 32%, although the cortex and striatum were not significantly affected. We previously reported 20 to 25% reductions in SERT binding in the hippocampus and cortex after the same adolescent treatment regimen used in the present study, although in the former study the SERT reduction reached statistical significance in the cortex instead of the hippocampus (Piper and Meyer, 2004). Most important were the effects of the prior MDMA exposure on the neurotoxic effects of the subsequent binge treatment. One possibility was that the repeated perturbation of the serotonergic system would enhance the vulnerability of the animals to the damaging effects of the MDMA binge; however, the opposite result was obtained: the pre-exposed animals were completely protected against the serotonergic neurotoxicity observed in the drug-naive group. Interestingly, Riddle et al. (2002) reported that intermittent adolescent methamphetamine administration (biweekly over 6 weeks beginning at PD 40) led to similar protection against the dopamine neurotoxicity associated with an adult methamphetamine binge. Moreover, the hyperthermic response to the binge was completely prevented by the previous methamphetamine exposure.

A number of potential mechanisms could underlie the absence of serotonergic neurotoxicity in the binge-treated animals that had received prior MDMA exposure during adolescence. One obvious factor is the reduced thermal effect of the MDMA binge in this group. Indeed, the acute elevation in core temperature associated with high doses of MDMA is often thought to be integral to the drug's neurotoxic effects (Green et al., 2003). For example, Malberg and Seiden (1998) found that increasing the ambient temperature within a certain range enhanced both the hyperthermic response and the neurotoxic effects of MDMA in rats. In addition, Broening et al. (1995) identified a significant relationship between the degree of hyperthermia and 5-HT depletions in both adolescent (PD 40) and young adult (PD 70) rats given MDMA. Nevertheless, there is growing evidence that serotonergic neurotoxicity to MDMA does not always parallel changes in core body temperature (McGregor et al., 2003; Meyer et al., 2004). In the present study, the hyperthermic response to MDMA was only partially attenuated in the pre-exposed animals, yet the neurotoxic effect was completely prevented. Moreover, the correlational analyses showed that SERT reductions were strongly associated with core body temperature for the MDMA-naive animals but not for those that received MDMA during adolescence. The mechanisms underlying this uncoupling (which was also evident for the weight change produced by the binge treatment) have yet to be determined. However, taken together, the data suggest that additional factors beyond a blunted hyperthermic response are involved in the protective effect of adolescent MDMA administration. One such factor could be an alteration in MDMA pharmacokinetics, particularly a reduction in the
rate of formation of bioactive metabolites hypothesized to be responsible for the drug's neurotoxic action (Green et al., 2003). Yet another possibility is that the repeated adolescent treatment could have enhanced the activity of antioxidant systems capable of scavenging free radicals generated during the binge treatment. In this regard, it is interesting to note that Dietrich et al. (2005) recently found that chronic cocaine administration to rats led to increased activity of two antioxidant enzymes, glutathione peroxidase and superoxide dismutase.

In the second experiment, we assessed the effects of repeated adolescent MDMA exposure on the behavioral and thermic responses to a challenge with the 5-HT1A receptor agonist 8-OH-DPAT. The results showed a blunting of the serotonin syndrome response to the higher dose of 8-OH-DPAT but no alteration in the hypothermia produced by the drug challenge. Several previous studies have examined the influence of neurotoxic MDMA treatment regimens in adult rats on the behavioral and thermic effects of a similar 8-OH-DPAT challenge. For example, Granoff and Ashby (2001) reported a selective reduction in 8-OH-DPAT-induced reciprocal forepaw treading after MDMA administration. Prior MDMA treatment produced either no change (McNamara et al., 1995; Granoff and Ashby, 2001; Mechan et al., 2001) or an enhancement (Aguirre et al., 1998) of the hypothermic response to 8-OH-DPAT. It is noteworthy that there was no tendency at all for 5-HT1A receptor binding to be altered in either the brain or spinal cord by the present adolescent MDMA treatment regimen, whereas a neurotoxic dose of MDMA given to adult rats has been found to increase 5-HT1A receptor density in the frontal cortex and hypothalamus and to decrease the density of these receptors in the hippocampus and brainstem (Aguirre et al., 1997, 1998).

There are at least two plausible hypotheses to explain how either adolescent or adult MDMA administration could produce differential effects on the behavioral versus the thermic effects of 8-OH-DPAT. First, MDMA may alter the sensitivity of 5-HT1A receptors in some areas of the brain and spinal cord (i.e., those areas responsible for 8-OH-DPAT elicitation of the serotonin syndrome) but not others (i.e., areas involved in 8-OH-DPAT-induced hypothermia). Investigators have traditionally believed that the serotonin syndrome is triggered at the level of the spinal cord or brainstem (Sternbach, 1991); however, recent results of Osei-Owusu et al. (2005) suggest that at least some components of this syndrome, specifically hind limb abduction and slow body posture, are mediated by 5-HT1A receptors in the paraventricular nucleus of the hypothalamus. The location of the receptors involved in the hypothermic response to 8-OH-DPAT is controversial, with some investigators favoring a presynaptic localization (i.e., in the raphe nuclei) and others favoring a postsynaptic localization, possibly in the hypothalamus (see references cited in Aguirre et al., 1998). The second hypothesis pertains to the fact that 8-OH-DPAT has significant agonist activity at 5-HT7 receptors in addition to 5-HT1A receptors and that 5-HT7 receptors may be the most important for eliciting hypothermia within the dose range of 8-OH-DPAT used in the present study (Hedlund et al., 2004). These findings raise the possibility that intermittent adolescent MDMA treatment may reduce 5-HT1A receptor sensitivity but not the sensitivity of 5-HT7 receptors. In summary, the ability to accurately extrapolate from controlled animal studies to the human situation to understand the risks of regular MDMA use is a difficult but important process that is dependent upon many factors including the drug regimen. In the present study, we found that repeated MDMA exposure during adolescence, which by itself produced relatively little neurotoxicity, diminished the hyperthermia, and protected against SERT depletions subsequent to an MDMA binge. Furthermore, adolescent MDMA impaired the behavioral response to a 5-HT1A agonist. Future researchers should further investigate dosing paradigms that emulate the escalating pattern of self-administration seen in some Ecstasy users and should aim to determine the pharmacodynamic and/or pharmacokinetic changes responsible for the development of tolerance to the physiological, behavioral, and neurotoxic effects of exposure either to MDMA or to more selective serotonergic agents.

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References


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