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Memory deficit and reduced anxiety in young adult rats given repeated intermittent MDMA treatment during the periadolescent period

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Abstract

3,4-Methylenedioxymethamphetamine (MDMA, or "Ecstasy") is a popular recreational drug among adolescents that is often taken primarily on weekends. The goals of this study were to develop a model of the typical intermittent pattern of human MDMA use in periadolescent rats and to determine the behavioral consequences of MDMA exposure in this model. Male Sprague–Dawley rats received s.c. injections of 10 mg/kg of MDMA or saline twice daily with an interdose interval of 4 h. Treatments were given every fifth day from postnatal day (PD) 35 to PD 60. Beginning at PD 65, the animals were tested for open-field activity, object recognition memory, and anxiety-related behaviors in the elevated plus-maze. Brain tissues were collected at PD 70 for determination of radiolabeled paroxetine binding to the serotonin transporter (SERT) in the neocortex and hippocampus. Repeated MDMA administration led to a reduced rate of weight gain that was evident by PD 50. There was no treatment effect on ambulatory behavior in the open-field. However, the MDMA group displayed an impairment of object recognition memory and reduced anxiety as indicated by a twofold increase in open-arm duration in the elevated plus-maze. Only modest decreases in SERT binding were observed, although there was a significant negative correlation between hippocampal SERT levels and open-arm duration within the MDMA group. These findings demonstrate that intermittent MDMA exposure during the adolescent period of development can influence subsequent cognitive and affective functioning in the absence of severe serotonergic damage.

Keywords: Ecstasy; Adolescence; Behavior; Serotonin

1. Introduction

The number of new teenaged Ecstasy users increased at a high rate during the mid-to late-1990s (Substance Abuse and Mental Health Services Administration, 2003). The principal ingredient of most Ecstasy tablets is 3,4-methylenedioxymethamphetamine (MDMA), although other psychoactive substances may be present as well. According to both self-report and laboratory data, Ecstasy produces a number of subjective effects such as mild euphoria, enhanced sensory perception, increased energy, feelings of well-being and self-confidence, and a desire to be with and interact with other people. Users often take the drug only on weekends while attending dances or other social events.

Numerous studies in laboratory animals have shown that MDMA can lead to persistent changes in the serotonergic system. Forebrain target areas such as the neocortex, hippocampus, and striatum exhibit reductions in a variety of different serotonergic markers, including tryptophan hydroxylase activity, serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) concentrations, 5-HT transporter (SERT) binding, and the density of 5-HT-immunoreactive fibers (Lyles and Cadet, 2003). These findings have been interpreted by most investigators to reflect a pruning of serotonergic fibers in the affected areas. Possible MDMA neurotoxicity in recreational users has been a controversial issue; however, clinical studies have found evidence for

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decreased SERT binding capacity, reduced 5-HIAA levels in cerebrospinal fluid, and blunted hormonal responses to serotonergic challenge drugs in MDMA users compared to non-users (Green et al., 2003).

The behavioral consequences of MDMA exposure have also been studied extensively. Heavy users reportedly suffer from cognitive deficits, depressed mood, and other behavioral disturbances (Parrott, 2001). The relationship between these behavioral changes and possible drug-induced serotonergic damage is not yet clear. Interestingly, initial studies of laboratory animals were unable to identify functional alterations following MDMA treatment. For example, Slikker et al. (1989) examined a variety of behavioral endpoints following MDMA administration to rats and nonhuman primates. No changes were found in schedule-controlled behavior, nociception, and maze learning ability in rats, or spontaneous behaviors in rhesus monkeys. Subsequent studies by Seiden et al. (1993) and Ricaurte et al. (1993) found no effects of neurotoxic doses of MDMA on food or water intake, open-field activity, operant behavior, or performance on several learning and memory tasks. These negative findings were interpreted to mean either that the drug-induced depletions of 5-HT were not severe enough to produce behavioral alterations (Seiden et al., 1993; Slikker et al., 1989), or that the particular task under investigation (spatial short-term memory in this case) was not susceptible to disruption by selective serotonergic damage (Ricaurte et al., 1993).

Recent findings have prompted a re-evaluation of these earlier perspectives on the inter-relationships between MDMA, 5-HT, and behavioral function. It is now apparent that MDMA administration to adult rats can alter specific behavioral domains involving working memory and anxiety-related responses (Gurtman et al., 2002; Mechan et al., 2002; Morley et al., 2001). The nature and extent of these effects depend on several factors, including the treatment regimen, ambient temperature during drug administration, elapsed time between treatment and testing, and strain (e.g., see Green and McGregor, 2002). Furthermore, although drug-induced hyperthermia was once thought to be critical for both the neurotoxic and long-lasting behavioral effects of MDMA, there is now evidence that many of these effects can occur in the absence of elevated body temperature (McGregor et al., 2003b).

Despite the important findings resulting from previous studies, these studies suffer from two significant limitations. First, treatments have usually been administered to adult animals, even though Ecstasy use commonly begins during adolescence. Pharmacological agents often exert different effects in adolescents than in adults (Spear, 2000), thus emphasizing the need to evaluate MDMA action during this important stage of development. Second, because most investigators have focused on the relationship between MDMA neurotoxicity and behavioral deficits, they have used typical neurotoxic dosing regimens in which the drug is administered multiple times over a concentrated time period on 1 day or for two consecutive days (although see O'Shea et al., 1998). Such treatment regimens do not adequately model the common pattern of teenage Ecstasy usage involving intermittent use over longer time periods (Yacoubian, 2002). Therefore, the objectives of the present investigation were to model the typical "weekend" or intermittent MDMA human use pattern in adolescent rats and to assess the behavioral and neurotoxic effects of MDMA exposure in this model.

2. Methods

2.1. Animals and drug administration

Male Sprague–Dawley rats were obtained from Charles River Laboratories (Wilmington, MA). The animals were at PD 26 on the day of arrival from the vendor, and they were allowed to acclimate to the laboratory for 9 days before the beginning of drug dosing. The animals were housed in pairs in plastic tubs (44.5×23.5×20.0 cm) at a room temperature of approximately 22 °C on a reversed 12-h light/dark cycle. Drug dosing and behavioral testing occurred during the dark phase of the cycle. Each subject was habituated to the experimenters by gentle handling for approximately 1 min each day for 3 days prior to the beginning of drug administration. The animals received tap water and Formulab 5008 rodent diet (PMI Feeds, St. Louis, MO) ad lib throughout the study. Animal care was in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996) and the experimental protocol was approved by the University of Massachusetts-Amherst Institutional Animal Care and Use Committee.

(±) MDMA–HCl was purchased from Sigma (St. Louis, MO). MDMA solutions were prepared daily in sterile 0.9% NaCl at a concentration of 10 mg/ml and administered in a volume of 1 ml/kg of body weight. On every fifth day from postnatal day (PD) 35 to PD 60, the animals (N=8 per group) received s.c. injections of either 10 mg/kg of MDMA (calculated as the salt) or saline vehicle, twice daily, with an interdose interval of 4 h. Doses were administered at approximately 09:00 and 13:00. The ages at dosing were selected to encompass the periadolescent stage of development in rats (Spear, 2000). Although human Ecstasy users typically take MDMA orally, a subcutaneous route of administration was chosen for ease of drug delivery and because oral and subcutaneous administration of MDMA to rats produces equivalent serotonin depletions (Finnegan et al., 1988). The present dose can be compared to human adolescent recreational doses by applying the principles of interspecies scaling (Chappell and Mordenti, 1991) and solving for the formula:

$$\mathrm{Human}_{\mathrm{DOSE}} = [\mathrm{Animal}_{\mathrm{DOSE}} * (\mathrm{Human}_{\mathrm{Weight}}/\mathrm{Animal}_{\mathrm{Weight}}) \wedge \text{Scaling Coefficient}]/\mathrm{Human}_{\mathrm{Weight}}$$

Using an animal dose of 10 mg/kg, a human adolescent weight of 60 kg, a rat weight at the midpoint of dosing of 0.23 kg, and an allometric scaling coefficient of 0.66 (Chiou et al., 1998), the human equivalent dose is 6.56 mg/kg. The amount of MDMA in an Ecstasy tablet varies considerably but the average is roughly 150 mg (Dance Safe, 2004). Thus, a dose of 6.5 mg/kg for a 60 kg user would result from the consumption of two to three Ecstasy tablets, each containing about 150 mg of MDMA. On this basis, a 10 mg/kg dose given to rats during the periadolescent period may be considered to represent a moderate human recreational dose. As mentioned earlier, young recreational users typically take the drug on weekends. However, because of the shorter life span of rats compared to humans, we administered MDMA with a 5-day instead of a 7-day interval between doses. Finally, because users often take Ecstasy repeatedly over several hours to extend the drug's subjective effects (MacInnes et al., 2001), we chose a 4-h inter-dose interval to model this self-administration pattern. Body weights were monitored throughout drug dosing and at PD 65.

2.2. Behavioral measures

2.2.1. Open-field activity

All behavioral testing was conducted in a separate room from the animal colony. Locomotor activity (ambulation) was determined on PD 65, 5 days after the last MDMA treatment. Each animal was tested for 10 min in a wooden open-field apparatus measuring 60×60×30 cm. The apparatus was painted flat black with the floor divided by a 3×3 grid into nine identical squares (eight peripheral and one central), each 20×20 cm. A Panasonic Digital 5000 VHS camera mounted to the ceiling of the testing room was used to record the animals' behavior for later measurement of the number of grid crossings per minute. For all behavioral analyses, the videotapes were played on a Panasonic AG 1950 Proline freeze-frame VHS player that displays time to the nearest 0.1 s. Behaviors were scored manually by a trained observer who was blinded to the treatment status of the animals. In this and all subsequent tests, the apparatus was cleaned with a 10% ethanol solution between animals.

2.2.2. Object-recognition test

The object-recognition test of working memory was developed by Ennaceur and Delacour (1988), and behavior in this test has been shown to be a valid measure of memory (Ennaceur and Meliani, 1992). The object-recognition test was conducted on PD 67. On the day prior to the test, the animals were habituated to the presence of objects in the open-field. Two identical objects were placed in the northwest and southwest corners of the open-field 10 cm from the walls. The animal was placed into the open-field facing the wall opposite the objects and after 3 min, one of the familiar objects was removed and replaced with a novel one. The habituation session videotape was not scored but was briefly viewed to verify that all animals inspected the objects. The same procedures were followed on the day of the test except that a 15-min inter-trial interval was interposed between the 3-min sample trial and the 3-min test trial. Previous research with adult rats found that prior MDMA administration influenced behavior in this task when a 15-min interval was used (Morley et al., 2001). Animals were returned to their home cage during the inter-trial interval. A white coffee cup and a plastic pipette container weighted with gravel served as experimental objects on the test day. The identity of the sample objects (coffee cup or container) and position of the novel object (northwest or southwest corner) were counterbalanced across experimental groups. Three copies of the objects were available so that for the test trial, the "familiar" object was replaced with a duplicate in order to avoid the possibility of scent cues left on the object by the animal during the sampling period.

Behaviors were videotaped as described above. The frequency and duration (to the nearest 0.1 s) of exploration of each object was recorded for determination of the discrimination ratio. Exploration was defined as occurring when the nose of the animal was within 2 cm of the object. The discrimination ratio is the duration of exploration of the novel object divided by the total exploration duration of both objects during the test phase (Morley et al., 2001). A discrimination ratio equal to 0.5 indicates chance behavior, with scores above 0.5 indicating preference for the novel object and therefore memory of the familiar object.

2.2.3. Elevated plus-maze

On PD 69, each animal was placed on the elevated plus-maze for 10 min. This task provides a measure of anxiety-like behavior (Pellow et al., 1985). The maze was constructed of gray plastic and consisted of two open arms (10×50 cm) and two enclosed arms (10×50 cm) with 50 cm side-walls and a 10×10 cm center platform. The maze was elevated 50 cm above the floor. Testing was conducted under white florescent lighting with a white noise generator turned on to obscure any background sounds. Number of entries and duration of time spent in the open and closed arms were determined from videotapes. Arm entries were coded when all four of the animal's paws were within the arm.

2.2.4. Serotonin transporter binding

On PD 70, animals were lightly anesthetized by CO_2 inhalation for approximately 1 min and then decapitated. The hippocampus and parietal cortex were quickly dissected, frozen on dry ice, and stored at −70 °C for later determination of SERT. Tissue samples were homogenized in 40 vol. of ice-cold buffer (pH=7.4) containing 10 mM sodium phosphate, 120 mM sodium chloride, and 5 mM potassium chloride, and then centrifuged at 20,000×*g* for 20 min at 3 °C. The supernatant was decanted and the pellet was resuspended in buffer followed by another centrifuga-

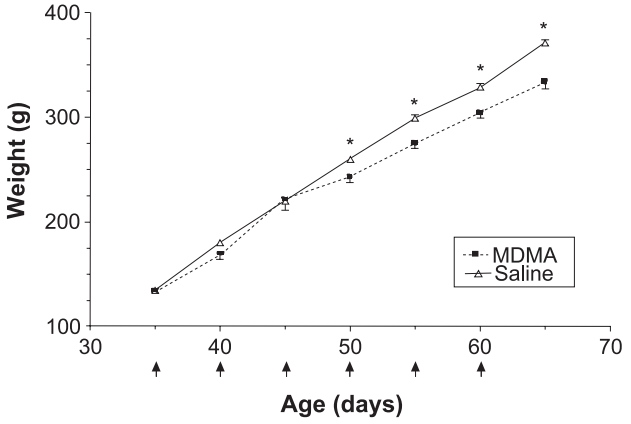

Fig. 1. Body weight as a function of age in rats receiving 10 mg/kg MDMA or saline (n=8 per group). Arrows indicate days of dosing (*p<0.007, Bonferroni correction for multiple t-tests).

tion. This was repeated once more, after which 100 μl of each washed membrane fraction was incubated in the presence of 1.0 nM [^{3}H]paroxetine (21.5 Ci/mM, New England Nuclear) to label SERT binding sites. This is a near-saturating concentration, as the K_d for paroxetine binding to rat SERT is approximately 0.15 nM (Habert et al., 1985). Incubations were carried out in triplicate for 1 h at room temperature in a total volume of 0.5 ml. Specific binding was defined by means of 10 μM unlabeled imipramine. The incubation was terminated by rapid filtration through Whatman GF/B filters that were presoaked in 0.05% polyethyleneimine. Filters were washed twice with 5 ml ice-cold buffer, placed in 5 ml of scintillation cocktail (Scintisafe 30%, Fisher Scientific, Fair Lawn, NJ), and counted the following day using a Packard 1900CA liquid scintillation analyzer. The Bradford dye-binding method with bovine gamma globulin as the standard was used to measure protein concentrations (Bradford, 1976).

2.2.5. Statistical analyses

Data were entered into Systat version 10.2 (Systat Software, Point Richmond, CA) for statistical analyses. Differences in elevated plus-maze behavior, object-recognition performance, and SERT binding as a function of adolescent drug treatment were assessed with a two-sample t-test using the pooled variance. A mixed-design ANOVA (Treatment×Age) was performed on the body weight data followed by Bonferroni-corrected t-tests at each age. A mixed-design ANOVA (Treatment×Time) was conducted on the frequency of grid crossings/2-min block in the open-field test. The relationship between SERT binding and behavioral measures was determined with Pearson product-moment correlation coefficients. A p value less than 0.05 was considered statistically significant except for the body weight data, in which a p value of less than 0.007 was used (Bonferroni correction for multiple t-tests). The effect size of statistically significant group differences was also expressed in terms of Cohen's d where a value of 0.2 is considered a small difference, 0.5 a medium difference, and 0.8 or greater a large difference (Cohen, 1988). Inter-rater reliability for two coders for two test sessions selected at random, calculated as frequency of agreements/(frequency of agreements+disagreements), was 91.3% for object exploration and 100% for plus-maze open-arm entries.

3. Results

3.1. Body weight

The effects of MDMA exposure on body weight are depicted in Fig. 1. A mixed-design (Treatment X Age) ANOVA revealed a significant effect of adolescent MDMA treatment [F (1,14)=11.34, p<0.01], an age effect [F (6,84)=800.8, p<0.001], and a significant Drug×Age interaction [F (6,84)=6.46, p<0.001]. MDMA significantly reduced the rate of growth by the fourth day of dosing. On PD 65, 5 days after the last MDMA dose, there was a 37.6 g group difference in mean body weight [t (14)=4.76, p<0.001, d=2.43].

3.2. Open-field activity

With respect to motor activity in the open-field, there was a main effect of time [F (9,126)=9.50, p<0.001] such that activity was greater in the first minute than at all later time points. There were no significant differences in total activity (defined as the number of grid crossings), activity at each time point, or the frequency of entry into the center of the open-field as a function of adolescent drug treatment (data not shown).

3.3. Object-recognition test

Object exploration behaviors during the sample and test trials of the working memory test are shown in Table 1. There were no group differences in the duration or frequency of object exploration during the sample trial.

Table 1
Mean (S.E.M.) behavior on the object-recognition test in MDMA- and saline-treated rats

	Saline	MDMA
Sample trial object exploration		
Duration (s)	25.6 (3.1)	23.5 (1.4)
Frequency	16.1 (1.3)	16.8 (1.2)
Test trial object exploration		
Familiar object:		
Duration (s)	11.8 (2.0)	14.9 (1.4)
Frequency	8.3 (1.0)	9.8 (1.1)
Novel object:		
Duration (s)	24.7 (3.5)	20.0 (3.1)
Frequency	10.9 (1.5)	9.4 (1.4)
Discrimination ratio	0.68 (0.03)	0.56 (0.04)*

* p<0.05 compared to the saline group.

Table 2
Mean (S.E.M.) behavior on the elevated plus-maze in MDMA- and saline-treated rats

	Saline	MDMA	*p*-value
Total arm entries	19.1 (3.0)	27.4 (3.0)	0.074
Open-arm entries	3.1 (1.4)	7.9 (1.8)	0.058
Open/Total (%)	2.8 (5.2)	27.3 (5.3)	0.071
Open-arm duration (s)	41.4 (18.7)	108.9 (20.3)	<0.05
Closed-arm duration (s)	507.4 (30.5)	400.5 (30.3)	<0.05

During the test trial, however, the MDMA-exposed animals showed a significantly reduced discrimination ratio compared to the saline-treated controls [t (14)=2.16, p<0.05, d=1.09]. Indeed, the mean discrimination ratio of the MDMA group was near the chance level of 0.5.

3.4. Elevated plus-maze

Table 2 shows the frequency and duration of arm entries during the 10-min elevated plus-maze test. Prior MDMA exposure led to over a twofold increase in open-arm duration [t (14)=2.41, p<0.05, d=1.21] as well as a corresponding decrease in time spent in the closed arms [t (14)=2.49, p<0.05, d=1.24]. There were also nonsignificant trends towards a greater number of total arm entries and a higher percentage of open-arm entries in the MDMA group.

3.5. Serotonin transporter binding

SERT binding in the hippocampus and neocortex on PD 70 as a function of treatment condition is presented in Fig. 2. The hippocampal sample from one of the MDMA-treated animals was lost and is therefore not included in the data. The results from the remaining animals showed that hippocampal SERT levels in the MDMA group were decreased by 22.5% compared to the controls, although large individual differences in the control group precluded statistical significance. There was also a 25.2% reduction in cortical SERT binding in the MDMA-treated animals

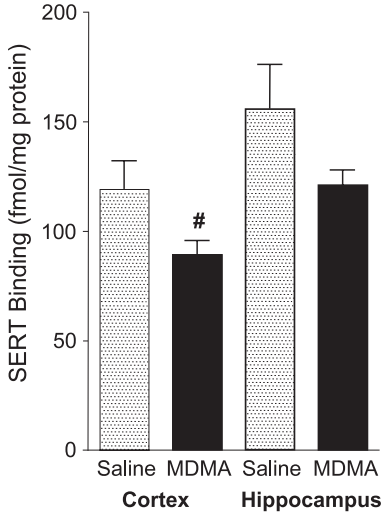

Fig. 2. Serotonin transporter (SERT) density in the cortex and hippocampus in rats on PD 70 that received either 10 mg/kg MDMA or saline treatment on PD 35–60 (n=8 per group, except for the loss of one hippocampal sample from the MDMA group). There was a trend towards a group difference in the cortex ($^{\#}p$=0.06).

[t (14)=2.02, p=0.06]. Finally, we performed Pearson product-moment correlations to determine whether any behavioral endpoints were related to SERT levels in either the hippocampus or cortex. Within the MDMA group, there was a strong negative correlation between hippocampal SERT binding and duration of open-arm exploration in the elevated plus-maze [r (5)=−0.78, p<0.05]. In contrast, this relationship was not present for the saline control group [r (6)=−0.28, NS] (Fig. 3).

4. Discussion

The MDMA dosing regimen in the present study was designed to be more clinically relevant for typical human recreational use patterns than most previous studies that

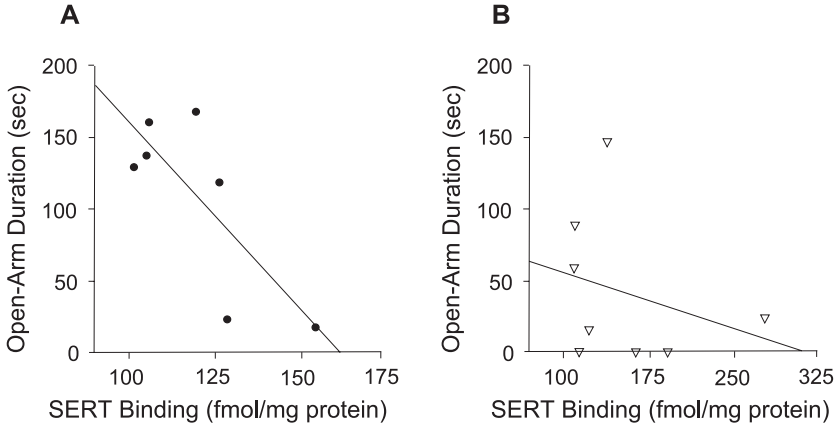

Fig. 3. Scatterplots of the relationship between serotonin transporter (SERT) density in the hippocampus and duration of exploration of the open-arms during a 10-min elevated plus-maze test in MDMA-treated (A) and saline control (B) rats. A significant correlation was found only for the MDMA group [r (5)=−0.78, p<0.05].

have focused on the drug's neurotoxic effects in adult animals. To that end, MDMA was administered in an intermittent dosing schedule over a prolonged time period, beginning in the periadolescent period. We found that repeated treatment during this period produced an anorectic effect, which is consistent with previously published studies in adult and neonatal animals (Frith et al., 1987; Meyer and Ali, 2002) as well as in human users (Parrott et al., 2002). Beginning 1 week after the last MDMA dose, animals completed the novel object-recognition test of working memory and the elevated plus-maze test of anxiety. Prior drug exposure reduced the discrimination ratio in the object-recognition test, indicating an impairment of non-spatial working memory. Importantly, general motor activity in the open-field and object exploration during the sample phase of object recognition testing did not differ between groups. This suggests that the reduction in the discrimination ratio was caused by an effect of MDMA on retention and was not the result of alterations in motor, sensory, attentional, or motivational (e.g., neophobia) factors. The MDMA animals also showed a large increase in open-arm exploration in the elevated plus-maze. Together, these findings indicate that periadolescent MDMA exposure impaired working memory and decreased anxiety-like behavior when testing was performed in young adulthood, shortly after the end of the drug treatment period.

In contrast to early research that did not identify any long-term functional changes following MDMA exposure (Ricaurte et al., 1993; Seiden et al., 1993; Slikker et al., 1989; although see Robinson et al., 1993), more recent studies have determined that MDMA treatment can disrupt later cognitive performance (Broening et al., 2001; Marston et al., 1999; McGregor et al., 2003b; Morley et al., 2001; Sprague et al., 2003) and alter anxiety-related behaviors (Mechan et al., 2002; Morley et al., 2001). The age at which the drug is administered appears to be an important variable in determining the likelihood that functional deficits will be observed. Exposure to MDMA on PD 11–20 but not on PD 1–10 impaired later performance on both the Cincinnati water maze and the Morris water maze (Broening et al., 2001). Marston et al. (1999) found that MDMA treatment decreased acquisition of a delayed non-match-to-position task. The subject ages were not provided in this report but the body weight range (150–180 g) indicates that these rats were not fully mature. Young adult rats exposed to MDMA and housed in an elevated ambient temperature (28 °C) to potentiate serotonin neurotoxicity were found to exhibit reductions in novel object recognition performance (Morley et al., 2001). Thus, the present results add to the growing literature showing learning or memory deficits in animals given MDMA under varying conditions of age, treatment regimen, and environmental conditions.

Two earlier investigations reported that MDMA altered behavior in the elevated plus-maze, albeit in opposite directions. One study found evidence for decreased anxiety following MDMA exposure (Mechan et al., 2002), whereas the other study found increased anxiety in the MDMA-treated animals (Morley et al., 2001). Subtle procedural differences in elevated plus-maze testing across laboratories may, in part, account for these discrepant outcomes (Crabbe et al., 1999). Mechan et al. (2002) conducted their testing under white light using a maze with 10 cm side-walls, whereas Morley et al. (2001) tested under red light with the more standard size (50 cm) side-walls. The subject ages also differed in the two published reports. Morley and coworkers administered MDMA to young adult (PD 75–95) rats, whereas the weight of the animals (150 g) in the study of Mechan et al. suggests that these subjects may have been in the periadolescent period of development. Another possible explanation for the discrepant results was offered in a commentary by Green and McGregor (2002). These investigators proposed that strain differences in baseline anxiety may influence the response to MDMA. Wistar rats were used in the study of Morley et al., whereas Mechan et al. used the Dark Agouti strain. Based on open-arm duration, the Wistar control animals showed considerably less anxiety than the Dark Agouti animals, and indeed the Wistars responded to the MDMA treatment with increased anxiety (i.e., reduced open-arm duration) in contrast to anxiolytic effect of MDMA in the Dark Agoutis. The current findings in Sprague–Dawleys are consistent with the Green–McGregor baseline anxiety model, although additional investigation that addresses the role of age, strain, maze construction, illumination, dosing paradigm, and elapsed time since dosing is needed.

MDMA-induced serotonergic neurotoxicity was assessed in the present study by means of single-point determinations of SERT binding using a near-saturating concentration of [^{3}H]paroxetine. Even though we did not perform full saturation analyses, reductions in SERT binding under these conditions are likely to represent changes in B_{max}, not only because of the relatively high radioligand concentration but also because Battaglia et al. (1987) previously showed that administration of a neurotoxic MDMA regimen to adult rats led to significant reductions in B_{max} for [^{3}H]paroxetine binding but no change in K_d. The MDMA treatment regimen used in the present study led to approximately 20–25% reductions in cortical and hippocampal SERT binding. These effects were not statistically significant, although a larger sample size might well have provided enough additional power to reach a minimum level of significance. The modest degree of serotonergic damage suggested by the SERT results was likely due to a combination of the age of the animals, the dosing paradigm, and the ambient temperature. The age at which vulnerability develops to MDMA-induced decreases in [^{3}H]paroxetine binding was recently determined (Kelly et al., 2002). Preweanling rats exhibited a low vulnerability to MDMA, juvenile animals (PD 25–30) showed a moderate vulnerability, and adults (PD 90) were maximally vulnerable. With respect to dose, four treatments given hourly or at 2-h intervals are necessary to obtain severe neurotoxicity in rats

using an individual dose of 10 mg/kg (Green et al., 2003). Finally, animals in the present study were maintained at 22 °C, a relatively low temperature compared to that used by many other labs (e.g., Morley et al., 2001). Ambient temperature is an integral component in the development of neurotoxicity in mature subjects (Malberg and Seiden, 1998). Our findings of behavioral alterations following dosing implies that the practice among rave attendees of going to "chill out" rooms while using Ecstasy may not protect against the development of functional deficits.

Although the present SERT reductions were small, the primary objective of this experiment was to assess functional alterations following a moderate dose of MDMA delivered intermittently. Previous investigations have thoroughly characterized the deleterious effects of MDMA on the serotonergic system using higher drug doses (Green et al., 2003). For example, Seiden et al. (1993) administered 40 mg/kg, every 12 h, for 4 days. The short-duration and high level of this neurotoxic dose does not simulate either the episodic pattern or the typical dose range of human Ecstasy use. Intermittent administration of a relatively moderate dose was chosen to model the human situation more accurately. Applying the principles of interspecies scaling suggests that the present 10 mg/kg dose given to rats during the periadolescent period may approximate the use of two to three 150-mg MDMA tablets by a human adolescent. Allometric scaling may be preferable to equating MDMA doses on an equal mg/kg basis across species. However, interspecies scaling is subject to limitations including large variability across drugs in the allometric exponent (Chiou et al., 1998). The scaling approach also encounters difficulties making predictions for drugs with biologically active metabolites (Caccia et al., 1982). Interspecies scaling, as with any mathematical model, provides a general approximation of the human dosage and must be viewed as a tentative estimate pending further empirical validation with MDMA in several species.

Interestingly, we found a significant association between anxiety behavior and hippocampal SERT density within the MDMA group. Animals with lower levels of hippocampal SERT binding showed less avoidance of the open arms of the elevated plus-maze (that is, less anxiety). Although the involvement of the hippocampus in spatial memory is particularly well established, this brain area has also been implicated in the regulation of anxiety. For example, Kjelstrup et al. (2002) recently found that hippocampal lesions increased exploration of the open arms of the elevated plus-maze. In another study, intracerebroventricular administration of the anxiogenic neuropeptide corticotropin-releasing factor (CRF) stimulated 5-HT release in the ventral hippocampus and reduced open arm entries (Kagamiishi et al., 2003). Moreover, these effects of CRF were attenuated by pretreatment with the 5-HT_{1A} receptor agonist 8-OH-DPAT. In contrast to the association with anxiety-like behavior, we did not find any relationship between SERT levels and performance in the object-recognition test. Previous studies with MDMA have noted that the extent of indoleamine depletions and cognitive deficits are not necessarily related. For example, Broening et al. (2001) found no relationship between MDMA-related learning deficits and regional 5-HT concentrations in rats treated on postnatal days 11–20. In addition, McGregor et al. (2003b) reported similar reductions in 5-HT when MDMA was administered at an ambient temperature of either 16 and 28 °C, but only the 28 °C group exhibited an impairment in working memory. Changes in learning and memory might not be the direct outcome of reductions in 5-HT or SERT, but may be more closely related instead either to the aberrant axon reinnervation patterns observed following neurotoxic doses of MDMA (Fischer et al., 1995; Meyer et al., 2004) or to alterations in 5-HT receptor functioning (Bull et al., 2004; McGregor et al., 2003a). Alternatively, some of the behavioral effects produced by MDMA treatment might be related to neurochemical or neuroanatomical changes that have not yet been identified.

Adolescence is a very active, and possibly vulnerable, neurodevelopmental stage (Spear, 2000). The serotonergic system undergoes substantial reorganization during this period. For example, Chen et al. (1997) reported that 5-HT levels in the rat hippocampus were sixfold higher at puberty (PD 45) relative to both juvenile and adult ages. These dynamic changes may contribute to a heightened sensitivity to develop altered behavior following adolescent MDMA exposure. Indeed, the enduring functional effects of MDMA exposure during this developmental period are now beginning to be described. Early adolescent (PD 28–29) MDMA treatment increased the conditioned place preference response to cocaine and decreased social interaction in rats (Bull et al., 2004; Fone et al., 2002). In contrast, adolescent mice treated for 2 days with MDMA showed an increase in social interaction (Morley-Fletcher et al., 2002). Clearly, the behavioral toxicity of adolescent MDMA exposure requires further characterization.

In conclusion, the present experiment assessed the neurobehavioral effects of a moderate MDMA dose with intermittent exposure during the periadolescent period. This dosing paradigm of MDMA was anorectic and attenuated the rate of weight gain. When tested during young-adulthood, animals exposed to MDMA showed a deficit in object recognition memory and decreased anxiety-like behavior. Serotonergic neurotoxicity in these animals was minimal as shown by the small reductions in SERT density in the cortex and hippocampus. Therefore, repeated exposure to MDMA during adolescence can alter subsequent cognitive and affective function in the absence of severe damage to the serotonergic system.

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