Development and Characterization of a Novel Animal Model of Intermittent MDMA ("Ecstasy") Exposure during Adolescence

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Adult animals treated with high doses of MDMA ("ecstasy") either on a single day or for several consecutive days show numerous behavioral changes as well as persistent reductions in brain serotonin (5-HT) concentrations and 5-HT transporter (SERT) protein expression. However, such dosing regimens do not adequately mimic the intermittent use patterns commonly seen in adolescent recreational ecstasy users. We have developed and characterized a rat model of intermittent adolescent MDMA exposure that simulates many of the features of human weekend use. Animals treated with our dosing regimen experience only small increases in core body temperature, and their plasma MDMA levels compare favorably with the levels reported for heavy ecstasy users under naturalistic conditions when species differences in drug clearance rates are taken into account. Intermittent adolescent MDMA exposure causes later deficits in object-recognition memory, increased impulsivity in the elevated plus-maze, and reduced sensitivity to a 5-HT 1A agonist challenge. SERT-immunoreactive fiber density is significantly reduced in the hippocampus but not the neocortex, suggesting that the hippocampus may be particularly vulnerable to moderate MDMA exposure during adolescence. Finally, adolescent MDMA-treated animals are protected (i.e., show tolerance) against the neurotoxic and depressant effects of a subsequent MDMA "binge" challenge.

We believe that the present animal model has important clinical relevance based on the similarities between the model and the reported effects of regular ecstasy use.

Key words: MDMA; ecstasy; rat; adolescence; neurotoxicity; behavior; tolerance; animal model

Introduction

Recreational drug use typically begins during adolescence.1,2 Theories attempting to explain this phenomenon have focused on the neurodevelopmental events taking place during adolescence, as well as certain key behavioral characteristics of adolescence such as heightened risk taking and novelty/sensation seeking.3-6 The heightened vulnerability of adolescents to develop patterns of drug use and, in some cases, drug dependence has led to a number of research groups to develop animal models of both adolescent drug exposure (i.e., cellular and molecular effects of drug administration during adolescence) and adolescent drug-seeking behavior.7-10

3, 4-Methylenedioxymethamphetamine (MDMA), or "ecstasy" as it is commonly known on the street, is a popular recreational drug among adolescents and young adults. For
example, 6.5% of 12th graders reported having used ecstasy at least once according to the 2006 Monitoring the Future survey,\textsuperscript{11} and ecstasy use among college students has also been well documented.\textsuperscript{12} Patterns of use vary widely, ranging from occasional use to regular weekend use, and further upward to frequent and even binge use. Numerous studies have found associations between heavy ecstasy use and various adverse consequences including depressed mood, heightened anxiety, cognitive deficits, and reduced serotonin transporter (SERT) binding potential in several cortical and subcortical areas.\textsuperscript{13–15} The changes in SERT binding could simply reflect a biochemical downregulation of SERT protein expression, but it is also possible that the reduction in this biomarker represents a degeneration of distal serotonergic fibers and terminals (see later section entitled Serotonergic Neurotoxicity).

As with studies of any drug of abuse, studies of ecstasy in humans suffer from numerous problems and confounding variables, including unknown MDMA content of tablets being consumed, wide intra-individual variation in consumption patterns, and polysubstance use.\textsuperscript{16} Such problems highlight the importance of animal models of MDMA exposure that simulate at least some of the features of typical human ecstasy-use patterns. One approach used by several research groups has been to determine the efficacy of MDMA in self-administration paradigms and to investigate the neurochemical and behavioral consequences of self-administered MDMA.\textsuperscript{17–20} However, much more common have been studies in which multiple high doses of MDMA are given on a single day or spread over 2–4 days in order to determine the neurotoxic effects of such dosing regimens.\textsuperscript{13} Although the clinical relevance of high-dose MDMA treatment paradigms has been questioned by some,\textsuperscript{21,22} it might be argued that these paradigms model the binges that occur in many heavy ecstasy users.\textsuperscript{23,24} Nevertheless, the fact remains that most recreational ecstasy use occurs intermittently and at somewhat lower levels than reflected by high-dose MDMA neurotoxicity studies.

In response to the need for new animal models that possess greater clinical relevance, some investigators have begun to study the behavioral, neurochemical, and neurotoxic consequences of MDMA exposure during adolescence at doses that do not engender large deficits in serotonergic markers.\textsuperscript{25–28} Our laboratory has developed one such model and has proceeded to characterize this model with respect to MDMA pharmacokinetics, physiological responses during the dosing period, and the effects of adolescent MDMA exposure on subsequent behavioral tests and pharmacologic challenges. Below we describe the development of our model, the details of the dosing regimen, and the characteristics of the model as mentioned. Wherever possible, we compare the features of the model to aspects of recreational ecstasy use so that the strengths and limitations of the model can be properly evaluated.

### Development of the Model

In developing our animal model, we considered the following key issues: (1) selection of species and sex, (2) developmental time frame, (3) MDMA dose, (4) route of administration, and (5) pattern and frequency of dosing. The first issue was species selection. For many reasons, nonhuman primates are the first choice for animal models of drug exposure. Indeed, in collaboration with Dr. Craig Ferris, we have used neuroimaging methods to examine the acute and short-term effects of MDMA administration on marmoset monkeys,\textsuperscript{29,30} and we are currently engaged in a longitudinal study of repeated MDMA exposure during adolescence in this species. However, primate studies are extremely costly, are usually limited by small sample sizes, and can be quite lengthy when developmental phenomena are being investigated. Turning to rodents, then, we decided on rats as the species of choice given the extensive
prior literature on the effects of MDMA (and other substituted amphetamines) on rats and the important fact that rats, unlike mice, are similar to nonhuman primates (and humans) in showing primarily serotonergic rather than dopaminergic deficits when exposed to high doses of MDMA.13 The selection of subject sex was also relevant, given the growing evidence for sex differences in MDMA responsiveness in both clinical and preclinical studies.31–33 Because of the much greater background information on MDMA effects in male compared to female rats, we chose to develop our model using male animals. However, we have recently done some work with females as well, the results of which will be reported elsewhere.

The second issue concerned the time frame of drug administration. Our choice was to treat the animals with MDMA beginning in the periadolescent period of development and continuing into young adulthood. As discussed by Spear,6,34 adolescence in rats is often considered to subsume the period between postnatal day (PD) 28 and PD 42; however, some features of adolescence in males continue out to PD 55. Although a small percentage of ecstasy users begins taking the drug in their early teenage years, onset of use typically occurs later in adolescence. Basing our decision on these considerations, we chose a dosing period of PD 35 through PD 60.

Determination of dose was the third key issue in developing our model. A wide range of doses has been used in preclinical MDMA studies, ranging from low doses in the 1–5 mg/kg range typical of self-administration to up to 40 mg/kg in acute behavioral studies (as a single or cumulative dose) or even higher in many neurotoxicity studies. Moreover, several different approaches could be used to ascertain what doses within this range are appropriate for modeling recreational ecstasy use. The simplest solution is to dose animals at the same drug-to-body-weight ratio seen in ecstasy users. There are at least two problems with this approach: first, that ecstasy tablets vary widely in their size and purity,35 thereby complicating the determination of human dosing levels; and second, that small animals are often less sensitive than humans to the same drug dose, largely on account of more rapid drug metabolism and clearance. One proposed solution to the problem of equating doses between species (especially between humans and laboratory animals) is to perform interspecies dose scaling using allometric equations that account for differences in body mass or surface area.36 Alternatively, one might attempt to equate maximum plasma drug concentrations ($C_{\text{max}}$) between human drug users and the model species, to equate the area under the plasma concentration–time curve (an index of total drug exposure produced by each dose), or to equate as much as possible behavioral and physiological outcomes due to drug dosing. In modeling recreational ecstasy use, it could also be considered appropriate to simulate the widespread practices (at least in regular users) of “stacking” (taking multiple doses at once in order to increase the desired effect and/or overcome tolerance from prior use) and “boosting” (taking supplemental doses over time in order to maintain the drug effect).37 In our own case, a consideration of the existing MDMA literature along with interspecies dose-scaling calculations led us to select a dose for each treatment of 10 mg/kg of (+)-MDMA HCl (given as the weight of the salt). We chose to use the racemic mixture because that is the form of the drug available to ecstasy users on the street. To calculate the human equivalency of this dose, we used an allometric scaling coefficient of 0.66,38 a human adolescent weight of 60 kg, and a rat weight at the midpoint of adolescent dosing (see below) of 0.23 kg, which yielded a value of about 6.5 mg/kg (see Piper and Meyer39). As noted, the MDMA content of ecstasy tablets varies considerably, but based on chemical analyses of samples obtained by the organization DanceSafe,40 it is not unreasonable to assume that a high-purity tablet contains roughly 150 mg of MDMA. Thus, the 6.5 mg/kg human-equivalent dose corresponds
to the stacking of 2–3 ecstasy tablets (each containing 150 mg of MDMA) by a 60-kg adolescent. We additionally chose to give the animals a second dose of 10 mg/kg MDMA 4 h after the first dose on each treatment day. The purpose of this additional dose is to model ecstasy boosting that occurs in order to prolong the positive effects of the drug. Although interspecies dosing scaling seemingly suggests that the individual 10 mg/kg doses of MDMA are not unreasonable when compared to the doses taken by many ecstasy users, it has become increasingly evident that such scaling methods have limitations when applied to MDMA. Consequently, we now believe that our dose selection is better justified by pharmacokinetic considerations and by functional outcomes as well (see below).

The final issues in model development were route of administration and the pattern and frequency of dosing. Ecstasy is almost always taken orally, and some animal studies have used this route of administration. On the other hand, there is good evidence that, at least from the perspective of neurotoxicity, the subcutaneous (s.c.) injection route yields results similar to those observed after oral administration. Therefore, for the sake of convenience and safety we chose to dose the animals by s.c. injection. With respect to the pattern of dosing, our general aim was to simulate weekend ecstasy use. However, because of the much greater rate of development in rats and the fact that we had chosen a restricted dosing period of PD 35 to PD 60, we decided that a rat “week” in our model would consist of 5 days. Thus, the animals were given two doses of MDMA (10 mg/kg/dose) every 5th day for a total of just 6 treatment days: PD 35, PD 40, PD 45, PD 50, PD 55, and PD 60. The first dose is delivered in the morning, and the second is given 4 h later in the afternoon. The drug is dissolved in physiological saline solution and is administered at a volume of 1 mL/kg. Control rats receive vehicle only at the same dosing intervals.

**Pharmacokinetics**

In both humans and laboratory animals such as rats, MDMA undergoes a complex process of biotransformation resulting in a variety of metabolites, some of which may be responsible for the drug’s neurotoxic effects. One of the major bioactive (though not necessarily neurotoxic) metabolites is the N-demethylated compound 3,4-methylenedioxyamphetamine, or MDA. Using high-performance liquid chromatography with fluorescence detection, we carried out a pharmacokinetic analysis of plasma MDMA and MDA concentrations after a single 10 mg/kg s.c. injection in previously drug-naive rats at either PD 35 or PD 60. Animals at both ages were tested to determine whether there were developmental changes in the time course of MDMA or MDA plasma concentrations between the first and last days of dosing in our intermittent-exposure model. Figure 1 shows the data resulting from this study, and Table 1 presents several standard pharmacokinetic parameters that were obtained from the dataset using the software program PKCALC. Statistical analysis of the PKCALC results yielded no significant differences in the disposition of plasma MDMA between PD 35 and PD 60, but a lower $C_{\text{max}}$ for MDA at the later age. This finding suggests that N-demethylation may be a more important metabolic pathway in adolescent compared to early adult rats.

Not surprisingly, there are major differences in MDMA pharmacokinetics in rats compared to humans. For example, in one study in which human subjects were given a single oral 100-mg dose of MDMA, the mean $t_{1/2}$ was 9 h, the $C_{\text{max}}$ was 222 ng/mL, and the $t_{\text{max}}$ was 2.3 h. Indeed, the much slower elimination of MDMA in humans is one rationale for administering higher doses in rats and mice. On the other hand, the $C_{\text{max}}$ value obtained in this study is only about 10–13% of the value we obtained in our animals. Thus, it seems clear that the peak plasma MDMA concentration produced...
by each dose of MDMA in our animal model is much lower than the peak concentration likely to be found in a recreational user taking a single low dose of ecstasy. However, as discussed earlier, many users take multiple tablets, and these tablets frequently contain much more than 100 mg of MDMA. For these reasons it is instructive to consider the results of a recent study by Irvine and coworkers,49 who determined plasma MDMA concentrations in blood samples collected after a late-night dance party in South Australia at which the partygoers reported consuming between 1–7 ecstasy tablets each. Whereas the mean plasma MDMA concentration across all subjects was 310 ng/mL, there was a small subgroup (presumably representing high-dose users) who exhibited MDMA concentrations greater than 750 ng/mL. Because the blood samples were obtained during the morning after the dance, which was hours after drug consumption, the reported values almost certainly do not represent the maximum circulating levels attained by the subjects. The results of the study by Irvine et al. emphasize the importance of considering real-world ecstasy consumption versus drug administration under controlled laboratory settings. These findings further indicate that peak plasma MDMA concentrations in the range of 1700 to 1900 ng/mL are not unreasonable in a species (i.e., rats) with relatively rapid MDMA metabolism and clearance compared to humans. Nevertheless, from a purely pharmacokinetic perspective, we recognize that a treatment regimen that produces such concentrations is modeling a higher dosing pattern than occurs in most novice or occasional users of ecstasy.

### Physiological Responses during Dosing

MDMA is well known to act as an appetite suppressant and to alter core body temperature. Thermic responses are complex and
depend in part on ambient temperature, with hyperthermia occurring when the animals are in a sufficiently warm environment, but hypothermia occurring instead when the animals are in a cooler environment. For this reason, we maintain our colony rooms at a minimum temperature of 22–23°C, which in our hands yields an overall hyperthermic response in most MDMA-treated animals.

To determine the thermic effects of MDMA across the adolescent treatment period, we measured core temperature responses to MDMA or saline on PD 35, 45, and 60. Small (around 0.5°C) elevations in temperature were observed in the MDMA-treated rats on PD 35 and 45. This mild hyperthermia peaked at 1–2 h after the first dose of MDMA and did not seem to occur again after the second dose. The hyperthermic response to MDMA was slightly higher on PD 60, with a peak increase of about 1°C. It is important to note that the hyperthermic effects produced by MDMA in our adolescent dosing regimen are much less severe than the effects seen in adult rats given a typical neurotoxic dose of the drug. On the other hand, our results compare favorably with the 0.6°C increase in core temperature found in human subjects ingesting 2 mg/kg MDMA in a warm environment.

We also examined the acute and chronic effects of MDMA on body weight over the course of drug administration. Comparing the body weights of the drug- versus saline-treated rats on each dosing day, we found that MDMA administration significantly reduced the rate of weight gain over time, an effect that was already evident by the second dosing day (PD 40) and that continued across the entire treatment period. In addition, we ascertained the acute effects of drug treatment on body weight by measuring weight 1 h before drug administration and again 2 h after the second MDMA dose on PD 35, 45, and 60. We found that, in contrast to control rats that showed no significant change in body weight over the same 7-h interval, MDMA treatment produced an acute weight loss averaging 6–8% of pre-treatment weight. Reductions in body weight after acute administration of MDMA are likely due to the combined effects of suppressed food intake and increased locomotor activity, defecation, urination, and evaporative water loss related to heightened respiratory rate.

Serotonergic Neurotoxicity

In the MDMA literature, the term “serotonergic neurotoxicity” has two different though related meanings. The broad meaning of the term consists of the notion that MDMA, particularly at high doses, can cause long-lasting reductions in specific serotonergic markers such as levels of serotonin (5-HT) and its principal metabolite 5-hydroxyindoleacetic acid (5-HIAA), tryptophan hydroxylase activity, and SERT binding in forebrain terminal areas like the hippocampus, neocortex, and striatum. There is a general consensus among researchers that this form of serotonergic neurotoxicity does occur in rats and in nonhuman primates in response to high-dose MDMA treatment. Neuroimaging of regional SERT binding potential has shown that heavy ecstasy users may also exhibit some degree of serotonergic neurotoxicity, although the results of these studies have been mixed.

In its narrower form, the term “serotonergic neurotoxicity” refers to the idea that high doses of MDMA (either over a short time period as in most animal studies or as a consequence of the cumulative exposure seen in some ecstasy users) cause physical damage to serotonergic nerve fibers and terminals. This putative process is commonly referred to as a “distal axotomy,” or, more colloquially, as a “pruning” of the serotonergic fibers. Neurotoxic doses of MDMA stimulate the formation of reactive oxygen species in the same forebrain areas that exhibit serotonergic deficits, and this increase in oxidative stress is a plausible mechanism by which the drug could damage serotonergic nerve fibers and terminals. On the other hand, several (though not all) studies have found
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no evidence for MDMA-induced activation of astroglial and microglial responses (e.g., upregulation of glial fibrillary astrocytic protein, or GFAP), which have become accepted biomarkers of neurotoxicity. These and other findings continue to raise doubts among some investigators as to whether MDMA “serotonergic neurotoxicity” involves distal axotomy or alternatively a long-lasting downregulation of 5-HT synthesis and SERT expression by the serotonergic neurons.

In our view, there is at present no definitive evidence on which to base a firm conclusion regarding the distal axotomy question. Consequently we have chosen to use the term “serotonergic neurotoxicity” in the broad sense without necessarily implying the presence of fiber degeneration. Moreover, we have most commonly used SERT protein expression as our indicator of neurotoxicity. In two studies, we measured the effects of intermittent MDMA exposure on SERT expression in the hippocampus, parietal cortex, and (in one study) the striatum by means of standard radioligand binding assays. These assays were performed on tissue samples collected at 1 week or 10 days after the last adolescent dose (i.e., at PD 67 or PD 70). When both studies are considered, the reductions in SERT binding averaged 22–30% in the hippocampus, 16–25% in the cortex, and 20% in the striatum. Only the decreases of 25–30% reached or approached statistical significance. Overall, these results indicate that the intermittent MDMA treatment produces a relatively modest degree of serotonergic neurotoxicity compared to that seen with high-dose binge treatments, which can cause 60–80% decreases in 5-HT levels and SERT binding.

Measurements of tissue 5-HT levels and SERT expression are relatively simple, quick, and convenient, but they do not enable researchers to determine whether serotonergic fiber density has been altered. However, this goal can be accomplished by selectively staining those fibers with an antibody against either 5-HT or SERT. We further argue that it is important to use appropriate amplification methods when conducting 5-HT or SERT immunohistochemical studies in MDMA-treated animals to help guard against a failure to stain fibers that are still present, but have reduced antigen expression due to the drug’s neurotoxic effects. To determine the effects of our adolescent MDMA treatment regimen on forebrain serotonergic fiber innervation, we measured the density of SERT-immunoreactive fibers in brain sections obtained from rats 2 weeks after the last day of MDMA or vehicle dosing (PD 74). Forty-μm sagittal sections were stained using a Calbiochem (EMD Chemicals, Gibbstown, NJ, USA) anti-SERT polyclonal antibody (1:1000 dilution) and the Vector Elite kit (Vector Laboratories, Burlingame, CA, USA) with diaminobenzidine as the chromogen. Stained slide-mounted sections were then subjected to a silver-gold intensification procedure, cover-slipped, and finally examined under a light microscope. Brain areas chosen for study were as follows: frontal cortex, primary visual cortex, primary somatosensory cortex, hippocampal CA1 stratum oriens, CA1 stratum lacunosum-moleculare, dorsal caudate-putamen, and medial forebrain bundle (MFB). A selected portion of each area on each tissue section was digitized using Scion Image v. 1.62 (Scion Corporation, Frederick, MD, USA), and then serotonergic fiber density (i.e., percentage of area covered by positively-stained fibers) was determined by gray-level thresholding. As shown in Table 2 and Figure 2, there were highly significant decreases in SERT-immunoreactive fiber density in both regions of hippocampal CA1 and a smaller but still significant decrease in the dorsal caudate-putamen. No treatment-related differences were found in any cortical area or in the MFB. These results suggest that intermittent adolescent MDMA damages serotonergic fibers in the hippocampus but not the neocortex, which contrasts with the previously mentioned membrane binding data that showed measurable decreases in SERT binding in the cortex after the same treatment regimen.
Table 2. Effects of Intermittent MDMA Administration on Regional SERT-Immunoreactive Fiber Density

<table>
<thead>
<tr>
<th></th>
<th>FC</th>
<th>SSC</th>
<th>VC</th>
<th>CA1-SO</th>
<th>CA1-SLM</th>
<th>CPu</th>
<th>MFB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100.0 ± 2.2</td>
<td>100.0 ± 3.6</td>
<td>100.0 ± 5.1</td>
<td>100.0 ± 2.5</td>
<td>100.0 ± 3.3</td>
<td>100.0 ± 2.6</td>
<td>100.0 ± 1.7</td>
</tr>
<tr>
<td>MDMA</td>
<td>95.6 ± 4.1</td>
<td>102.5 ± 2.7</td>
<td>101.8 ± 7.4</td>
<td>78.3 ± 2.5**</td>
<td>81.5 ± 2.0**</td>
<td>93.5 ± 1.6*</td>
<td>100.2 ± 1.7</td>
</tr>
</tbody>
</table>

Data shown represent % control (mean ± SEM, 8 animals per group) for each brain area.

Mean control data (proportion of pixels positively-stained for SERT): FC (frontal cortex) = 0.411; SSC (primary somatosensory cortex) = 0.365; VC (primary visual cortex) = 0.336; CA1-SO (hippocampal CA1 stratum oriens) = 0.443; CA1-SLM (hippocampal CA1 stratum lacunosum moleculare) = 0.459; CPu (dorsal caudate-putamen) = 0.382; MFB (medial forebrain bundle) = 0.468.

*P < 0.05; **P < 0.001 compared to controls.

Perhaps the decreased cortical binding represents reduced SERT expression rather than fiber loss. Although other explanations cannot be ruled out at present, the immunohistochemical results combined with the binding data raise the possibility that the same MDMA dosing regimen could produce different kinds of serotonergic neurotoxicity in different brain areas, with some areas suffering distal axotomy but other areas undergoing a milder insult consisting of a temporary downregulation in serotonergic function, but no loss of fibers. Whether or not this hypothesis is ultimately confirmed, the present findings point to the hippocampus as a brain area that is particularly vulnerable to the neurotoxic effects of intermittent adolescent MDMA treatment.

Memory and Anxiety/Impulsivity

As mentioned in the Introduction, regular ecstasy users commonly display memory deficits and various mood changes, including heightened anxiety. Thus, one of our early aims was to determine the effects of MDMA on memory and anxiety in our animal model. For assessment of memory, we chose the object-recognition test developed originally by Ennaceur and Delacour. To test changes in anxiety, we selected the elevated plus-maze. On the basis of the literature in humans, we hypothesized that MDMA-treated rats would show impaired memory performance and increased anxiety compared to vehicle-treated controls. The results did indeed show poorer performance of the MDMA group.
in the object-recognition test as indicated by a significantly lower discrimination ratio.\textsuperscript{39} Surprisingly, however, we also found that those same drug-exposed animals spent more time than the controls in the open arms of the plus-maze. Such a finding is generally thought to indicate decreased anxiety, and that is how we initially interpreted the results. However, some investigators have viewed increased time spent in and/or entries into the open arms of the plus-maze as reflecting behavioral disinhibition or impulsivity.\textsuperscript{66,67} Consistent with this notion, and particularly relevant to the present results, Harro\textsuperscript{68} has suggested that instances of apparent anxiolysis associated with partial 5-HT depletion (such as that produced by MDMA) may actually represent enhanced impulsivity. If this alternate interpretation is applied, then the behavior of our MDMA-treated rats is similar to the elevated impulsivity reported to occur in ecstasy users.\textsuperscript{69–71}

**Responses to Pharmacologic Challenges**

The final series of studies to be described here involve the influence of adolescent MDMA on responses to subsequent pharmacologic challenges. In these studies, we sought to determine how prior MDMA exposure would affect (1) susceptibility of animals to the neurotoxic effects of an MDMA binge, and (2) functional responses to serotonergic agonist administration. In the first experiment, adolescent MDMA- and vehicle-treated rats were subjected to a binge regimen of MDMA (5 or 10 mg/kg \(\times\) 4, given s.c. at hourly intervals) or saline at 1 week after the last adolescent dose (PD 67). Imposing a drug binge under these conditions can be thought of as simulating a sudden dose escalation by a regular recreational ecstasy user. Among the key experimental endpoints were core body temperature during the binge, activity on the day after the binge, and regional SERT binding in brain samples obtained 1 week after the binge. The results were quite dramatic. Compared to drug-naïve animals, the MDMA-pretreated group showed a partial tolerance to the hyperthermic effects of the binge, but more strikingly they were completely protected from the marked hypoactivity normally observed after the binge (the ecstasy “hangover”) and from the neurotoxic effects of the binge as indicated by a lack of binge-related change in SERT binding.\textsuperscript{51} To our knowledge, these are the first results showing that an intermittent MDMA dosing regimen that is not highly neurotoxic itself is protective against the adverse consequences of a high-dose MDMA treatment. However, it is interesting to note that a similar kind of tolerance was reported by Riddle and coworkers using methamphetamine instead of MDMA.\textsuperscript{72}

The ability of prior MDMA exposure to protect against a subsequent binge treatment is of great interest for two reasons: First, although there is no information concerning whether the same phenomenon occurs in human ecstasy users, tolerance to the subjective effects of the drug has been reported in chronic users.\textsuperscript{73} The present results, therefore, suggest that future studies should be done using our adolescent dosing paradigm to determine whether tolerance to the behavioral effects of MDMA occurs in the pre-exposed animals. Second, it is important to determine the mechanism underlying MDMA-induced neuroprotection. Preliminary follow-up studies have found some evidence for accelerated MDMA clearance in MDMA pretreated rats; however, this change does not appear to be of sufficient magnitude to account for the complete neuroprotective effect observed in the pretreated group. On the other hand, prior MDMA exposure significantly reduced the amount of lipid peroxidation produced by a subsequent MDMA challenge (A.J. Oliver, B.J. Piper, and J.S. Meyer, unpublished observations). On the basis of that finding, we are currently testing the hypothesis that our intermittent adolescent MDMA treatment regimen upregulates one or more endogenous...
TABLE 3. Comparison of the Adolescent Rat MDMA Model with Human Ecstasy Users

<table>
<thead>
<tr>
<th></th>
<th>Human ecstasy users</th>
<th>Adolescent rat model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose</td>
<td>~ 1–2 mg/kg × ?</td>
<td>10 mg/kg × 2</td>
</tr>
<tr>
<td>Peak plasma MDMA levels</td>
<td>≥ 750 ng/mL in some partygoers</td>
<td>1700–1900 ng/mL from a single 10 mg/kg dose</td>
</tr>
<tr>
<td>Hyperthermia</td>
<td>Mild (0.6°C from a single 2 mg/kg dose)</td>
<td>Mild (0.5–1.0°C from a single 10 mg/kg dose)</td>
</tr>
<tr>
<td>SERT density</td>
<td>Low/moderate ↓ based on neuroimaging studies</td>
<td>Low/moderate ↓ based on membrane-binding studies</td>
</tr>
<tr>
<td>Axonal damage</td>
<td>Unknown</td>
<td>In hippocampus, but not cortex</td>
</tr>
<tr>
<td>5-HT1A receptors</td>
<td>Unknown</td>
<td>↓ sensitivity based on agonist challenge</td>
</tr>
<tr>
<td>5-HT2A receptors</td>
<td>↑ based on neuroimaging</td>
<td>Under investigation</td>
</tr>
<tr>
<td>Memory deficits</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Increased impulsivity</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Tolerance to MDMA</td>
<td>Yes (subjective)</td>
<td>Yes (neuroprotection)</td>
</tr>
</tbody>
</table>

antioxidant systems that protect the brain from the oxidative stress and resulting neurotoxicity of a later MDMA binge.

The second experiment examined the effects of adolescent MDMA exposure on the sensitivity of 5-HT1A receptors to a challenge with the selective agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT). One week after the last adolescent dose (PD 67), pretreated and drug-naïve rats were given a single s.c. injection of either saline or 0.1 or 0.5 mg/kg of 8-OH-DPAT. The MDMA-pretreated animals showed a partial attenuation of the behavioral responses (i.e., the so-called serotonin syndrome consisting of head-weaving, forepaw-treading, and low body posture) to the challenge, but no change in 8-OH-DPAT-induced hypothermia.51 Membrane binding procedures revealed no effects of MDMA on 5-HT1A receptor density in the hippocampus, cortex, brainstem, or spinal cord, although we cannot exclude the possibility of altered receptor expression in other areas. These results demonstrate a partial 5-HT1A receptor desensitization in response to adolescent MDMA administration that could have important consequences for the ability of standard serotonergic agents to treat mood or anxiety disorders that may arise in some ecstasy users. Other serotonergic receptors may also be affected by MDMA, and we are currently examining the functional sensitivity of 5-HT2A receptors using a similar agonist challenge paradigm.

Conclusions

Modeling of human substance use/abuse is generally complicated by the naturally occurring variation in subject age, drug purity (and contaminants), amount consumed per use session, patterns of consumption, routes of administration, polysubstance use and other confounding variables, and co-morbidity with other psychiatric disorders. Consequently, there is no animal model that perfectly simulates human use of any abused substance. Nevertheless, it is incumbent on researchers to evaluate the strengths and limitations of any proposed model by comparing the model to at least some aspects of the clinical literature for the substance under investigation. In the present review, we have described the development of a rat model of intermittent adolescent MDMA exposure, characterized the model along a number of dimensions, and in each case compared our findings with the results obtained from various studies of ecstasy users. As shown in Table 3, many of these comparisons support the clinical relevance of our model, particularly the comparisons that deal with functional outcomes rather than absolute...
dose or peak plasma drug concentrations. Although much more work remains to be done, we would argue that the groundwork has been laid for a new generation of studies that go beyond the traditional binge models of MDMA dosing in an effort to understand the neurobiological and functional consequences of more moderate ecstasy use by teenagers and young adults.

Conflicts of Interest

The authors declare no conflicts of interest.

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