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Behavioral and neurochemical effects of repeated MDMA administration during late adolescence in the rat

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Abstract

Adolescents and young adults disproportionately abuse 3,4-methylenedioxymethamphetamine (MDMA; ‘Ecstasy’); however, since most MDMA research has concentrated on adults, the effects of MDMA on the developing brain remain obscure. Therefore, we evaluated place conditioning to MDMA (or saline) during late adolescence and assessed anxiety-like behavior and monoamine levels during abstinence. Rats were conditioned to associate 5 or 10 mg/kg MDMA or saline with contextual cues over 4 twice-daily sessions. Five days after conditioning, anxiety-like behavior was examined with the open field test and brain tissue was collected to assess serotonin (5-hydroxytryptamine, 5-HT) and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) in the dorsal raphe, amygdala, and hippocampus by high-pressure liquid chromatography (HPLC). In a separate group of rats, anxiety-like and avoidant behaviors were measured using the light–dark box test under similar experimental conditions. MDMA conditioning caused a place aversion at 10, but not at 5, mg/kg, as well as increased anxiety-like behavior in the open field and avoidant behavior in light–dark box test at the same dose. Additionally, 10 mg/kg MDMA decreased 5-HT in the dorsal raphe, increased 5-HT and 5-HIAA in the amygdala, and did not alter levels in the hippocampus. Overall, we show that repeated high (10 mg/kg), but not low (5 mg/kg), dose MDMA during late adolescence in rats increases anxiety-like and avoidant behaviors, accompanied by region-specific alterations in 5-HT levels during abstinence. These results suggest that MDMA causes a region-specific dysregulation of the serotonin system during adolescence that may contribute to maladaptive behavior.

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Keywords

Amygdala; Anxiety; Conditioned place preference; Dorsal raphe; MDMA; Serotonin

1. Introduction

3,4-Methylenedioxymethamphetamine (MDMA), also known as ‘Ecstasy’, is one of the most commonly abused drugs among adolescents and young adults (Johnston et al., 2010). Specifically, MDMA use is disproportionately greater during late adolescence and young adulthood (i.e. 18 to 25year olds) and declines with age (SAMHSA, 2002). Although young adults constitute the largest proportion of Ecstasy users, most human studies and the majority of preclinical animal experiments only assess adults. As a result, the effects of MDMA on the developing brain and the subsequent behavioral effects are poorly understood.

The rewarding properties of MDMA are known to differ between adolescent and adult animals. For example, in adult rodents, a range of MDMA doses have positive associative effects demonstrated by a conditioned place preference (Meyer et al., 2002; Robledo et al., 2007; Salzmann et al., 2003), a behavioral paradigm that associates contextual cues with a drug to assess its rewarding or aversive effects (Bardo and Bevins, 2000). In adolescent rodents, only a few studies have examined the rewarding effects of MDMA (with place conditioning), and positive associations were only seen at a single, low dose (2.5 mg/kg) in rats (Catlow et al., 2010; Llorente-Berzal et al., 2013). In mice, MDMA causes place preference at high doses (5–20 mg/kg) in adults and adolescents (Daza-Losada et al., 2007; Ribeiro Do Couto et al., 2011, 2012); however, mice show a differential regulation after MDMA where a dopaminergic mechanism plays a larger role that may account for the place conditioning at high doses (Kindlundh-Hogberg et al., 2007). Importantly, other doses that produce rewarding effects in adults do not have an effect in younger rats (Catlow et al., 2010; Llorente-Berzal et al., 2013; Meyer et al., 2002; Robledo et al., 2007; Salzmann et al., 2003), suggesting that the developing brain is unique in its response to MDMA (Broening et al., 1994).

In humans, acute MDMA is anxiolytic and reinforcing (Liechti et al., 2000; Tancer and Johanson, 2003), however, repeated MDMA use increases symptoms of anxiety (MacInnes et al., 2001; Parrott et al., 2000; Verkes et al., 2001). Similarly, in animal models, repeated administration of MDMA causes anxiety-like behaviors, specifically; both adult and adolescent rodents show subsequent anxiogenic behavior following conditioned place preference training (Faria et al., 2006; Fone et al., 2002; Gurtman et al., 2002; Lin et al., 1999). However, the mechanism through which MDMA increases anxiety is still unclear. In the amygdala, a brain region with a well-established role in emotional regulation and anxiety behavior, both activation of the amygdala (measured by increased c-fos expression) (Navarro et al., 2004) and depletion of serotonin (5-hydroxytryptamine, 5-HT) (Gurtman et al., 2002) occur after MDMA treatment. These studies suggest that effects of MDMA on the 5-HT system in the amygdala may contribute to heightened anxiety. Therefore, it is important to model MDMA-induced anxiety-like behavior to determine the neuronal

underpinnings, particularly in the amygdala, that are responsible for driving maladaptive behaviors.

Historically the scientific literature has focused on MDMA-induced neurotoxicity (or neurodegeneration of 5HT-containing neurons), although a recent paradigm shift in research strategies favors MDMA-induced neuromodulation of the 5-HT system (see review by (Biezonski and Meyer, 2011)). Acute MDMA induces 5-HT release from the dorsal raphe nucleus (Bradberry et al., 1990), known to be a major source of 5-HT for the forebrain (Azmitia and Segal, 1978; Jacobs and Azmitia, 1992; Koch and Galloway, 1997), although the subsequent effects of various repeated (experimenter-administered) dosing regimens have produced differential alterations of the 5-HT system. A commonly used neurotoxic dosing regimen (4 injections per day for 1–2 days) depletes monoamine neurotransmitter levels in adult rats, specifically 5-HT and its metabolite, 5-hydroxyindoleacetic acid (5-HIAA), as well as 5-HT markers such as 5-HT transporters (SERT) and receptors, with varying degrees of recovery (Gurtman et al., 2002; McNamara et al., 1995; Perrine et al., 2010). These ‘neurotoxic’ effects are observed in a number of 5-HT projection fields although neuroanatomical specificity is seen with greater depletion in cortical and limbic areas and less in thalamic structures (Battaglia et al., 1991). In comparison, studies utilizing daily repeated or intermittent treatment regimens, that may have greater face validity to modeling human use patterns, report limited or no decreases in monoamine levels (Clemens et al., 2007; Piper et al., 2010). Few studies have examined the effects of MDMA on monoamines in adolescent rodents, although it appears that young animals are less sensitive to MDMA neurotoxicity than adults (Kelly et al., 2002; Meyer et al., 2004; Piper, 2007). Importantly, rats in early adolescence do not show serotonin depletion after repeated administration (Fone et al., 2002), although the effects of MDMA during late adolescence have yet to be examined.

As most humans use MDMA during young adulthood (18–25 years of age) (SAMHSA, 2002), this study was designed to assess the effects of MDMA in rats during what is termed middle to late adolescence (~PND 44–54) by previously established age ranges (Adriani et al., 2002; Kolyaduke and Hughes, 2013; Morley-Fletcher et al., 2002). Specifically, the present study sought to investigate the effect of low (5 mg/kg) and high (10 mg/kg) doses of MDMA on place conditioning during late adolescence and the subsequent effects on anxiety and brain 5-HT levels five days after cessation of treatment.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (Charles River Laboratories; Portage, MI) weighing 139 ± 2 g (~postnatal day 38–41) upon arrival were allowed to acclimate to the facility for 4–5 days. The rats were group housed in standard polycarbonate micro-isolator rat cages in a humidity and climate-controlled vivarium (AAALAC accredited) on a 12 h light–dark cycle (on at 7 AM). Rats had access ad libitum to food and water (standard rat chow) and were handled and weighed daily throughout the experiment. The Wayne State University Institutional Animal Care and Use Committee approved all experiments with animals.

2.2. Drugs

$\pm$ 3,4-Methylenedioxymethamphetamine hydrochloride (NIDA Drug Supply Program, Bethesda, MD) was dissolved in sterile saline (0.9% NaCl; Progressive Medical International, Vista, CA) and injected intra-peritoneally at 0 (saline-control), 5, or 10 mg/kg. Concentrations were adjusted such that the volume of all injections was equal to 1 ml solution per kg animal weight (~0.2 ml/injection). MDMA or saline-control was given once daily for four days as part of the place conditioning paradigm or in the home cage in a separate group of animals used for light–dark box testing. MDMA was administered and rats were tested during the day (i.e. during their normal sleep period) to model human use patterns, where MDMA is typically used by humans during the night and into their normal sleep period). Potential disruptions in the rats' sleep pattern due to the psychostimulant were meant to mimic effects in humans.

2.3. Place conditioning

Testing took place in a custom-made, Plexiglas chamber (62.2 × 31.8 × 35.6 cm; Formtech Plastics, Oak Park, MI) consisting of two equal sized (30.5×31.8×35.6cm) but distinct compartments separated by a black divider that acted as a wall creating the two compartments or a black divider with a small doorway that allowed access between the two compartments. One compartment had white walls with black circles (5.1cm diameter) and a textured floor, and the other compartment had white walls with black vertical stripes (2.5 cm wide) and a smooth floor. As part of an unbiased design, half the animals were conditioned in the vertical compartment and half in the circle compartment. If an initial side bias was >80% the rat was excluded from the study, however no rats in this study were excluded for this reason. During the pre-test on day 1 the animals were allowed to freely explore the chamber for 20 min and the time spent on each side of the chamber was recorded in a drug-free state. The compartment paired with MDMA (0, 5, or 10 mg/kg) was counterbalanced within each group of animals for the subsequent four conditioning days (days 2–5) and a design with two sessions per day (AM and PM) was used. During the AM session of conditioning, saline was injected and the rats were confined to the saline-paired compartment for 30 min. During the PM session of the conditioning, 0, 5, or 10mg/kg MDMA was injected and rats were placed in the treatment-paired compartment (i.e., the other side of the two compartment chamber) for 30 min. The test for place preference occurred (on day 6) one day following the last conditioning day, and testing took place during the time period between the two conditioning sessions to control for time of day. On the test day, animals were given free access to both compartments of the chamber for 20 min in a drug-free state, and the time spent on each side was recorded. This day also served as the first day of the five-day drug free period (days 6–10) before open field activity was tested (on day 10). Since control animals received saline on both sides of the chamber during conditioning and because an unbiased design was used, the absolute value of the time spent in one chamber on the test day was used to determine place preference or aversion (Harris and Aston-Jones, 2003; LaPlant et al., 2010). Behaviors were recorded by a digital video camera located above the chamber and measured by EthoVision XT version 6 (Noldus Information Technology, Leesburg, VA). An entry into one compartment was defined when the center point of the rat entered the side. Note that according to Baumann and Rothman (2009) plasma levels of MDMA return to zero in less than 12h after an IP injection of 10

mg/kg MDMA (the highest dose used in this study); therefore, given that the time between MDMA injection in the afternoon and saline injection the following morning was 15 h, drug carry-over effects are not a concern.

2.4. Open field test

The open field test was used to measure anxiety-like behavior and locomotor activity five days after cessation of MDMA administration (on day 10). The animals were placed in a custom-made open field chamber ($62.2 \times 62.2 \times 35.6$ cm; Formtech Plastics, Oak Park, MI) with a smooth black floor and black walls for 5 min and their behavior was recorded by a digital video camera located above the chamber. Using EthoVision software an artificial zone consisting of the inner 40% of the open field was termed the 'center square' and the time spent as well as number of entries into this area were assessed as a measure of thigmotaxis (i.e. wall hugging) and anxiety-like behavior (Prut & Belzung, 2003). Locomotor behavior was measured as distance traveled during the test.

2.5. Light–dark box test

In a separate group of animals the light–dark box test was used to measure avoidant and anxiety-like behavior five days after cessation of repeated (4 day) MDMA or saline administration. The light–dark box used here is a custom-made testing chamber made of Plexiglas with a matte black floor (Formtech Plastics, Oak Park, MI). It is divided into two sides by a black wall with an arched opening (11.5 cm wide $\times$ 11.5 cm high) to allow the rat to cross between sides. The testing chamber contained a "light" side (inner dimensions: 40.0 cm length $\times$ 26.8 cm width $\times$ 32.8 cm height) with an open ceiling and white walls and a "dark" side (inner dimensions: 27.2 cm length $\times$ 26.8 cm width $\times$ 32.8 cm height) with a closed ceiling (lid) and black walls. The animals were placed in the dark portion of the box and allowed to freely explore both sides of the box for 5 min. Typical endpoint behaviors for light–dark box testing were used, including latency to first enter into the light side, number of transitions, and time spent in the light side (Hascoet and Bourin, 1998). Behaviors were recorded by a digital video camera and measured by EthoVision XT version 8 (Noldus Information Technology, Leesburg, VA). An independent rater, blind to the treatment conditions, verified automated tracking manually.

2.6. Tissue preparation

In order to ensure that changes in 5-HT and 5-HIAA were not due to the novelty of behavioral testing, animals were rapidly decapitated without anesthesia using a small rodent guillotine 6 h following open field testing (on day 10). Brains were quickly removed and placed into a chilled brain matrix (Zinc Instruments, Pittsburgh, PA). Coronal slices (2mm) containing the dorsal raphe, amygdala complex, and hippocampus were sectioned and placed flat on a block of solid CO₂. Once frozen, the slice was moved to a chilled glass surface and a tissue biopsy-punch (1.5 mm diameter; Miltex Inc., York, PA) was used to extract a tissue sample of the region of interest based on a rat brain atlas (see Fig. 5) (Paxinos and Watson, 2007). The samples were then stored untreated in micro-centrifuge tubes at -80°C until analysis of 5-HT and 5-HIAA levels.

2.7. High-pressure liquid chromatography (HPLC) analysis

Frozen tissues were weighed (~2–3 mg/tissue) and then sonically disrupted in 0.16N HClO₄. Insoluble protein was removed by centrifugation at 14,000 ×g for 5 min at 22 °C. The resulting supernatant was collected and diluted 1:4 in fluorescence dilution solution (0.01 M HCl, 0.5 mM EDTA, and 1 mg/ml ascorbic acid). 5-HT and 5-HIAA were detected in the diluted supernatant using a Shimadzu Prominence HPLC system with fluorescence detection (Shimadzu Scientific Instruments, Columbia, MD). Separation was achieved with a C₁₈ column (5 µM particle size, 300 Å pore size; Phenomenex, Inc., Torrance, CA) and detection by fluorescence at 285 nm excitation and 340 nm emission wavelengths. Mobile phase consisted of 25 mM sodium acetate and 20% methanol, adjusted to pH 5.1 with acetic acid. Concentrations of 5-HT and 5-HIAA were quantified by interpolating peak areas relative to those generated by a range of standards that were run in parallel to unknowns and calculated as ng of 5-HT or 5-HIAA to mg of wet tissue weight.

2.8. Statistical analyses

The data were analyzed by comparing means among groups using one-way analysis of variance (ANOVA), except for the body weight data, which were collected daily and analyzed by two-way repeated measures ANOVA. In the event of a significant ANOVA ($p < 0.05$) subsequent pairwise comparisons were made using Bonferroni's or Tukey's multiple comparisons (*post hoc*) test. Data were analyzed and graphs were created using Prism 5 (GraphPad Software, La Jolla, CA).

3. Results

3.1. Weight data

For rats in the place conditioning and open field experiments ($n = 29$, 8–10 in each group) an interaction between days (i.e. age) and treatment (0, 5 or 10mg/kg MDMA) was observed ($F_{18,234} = 2.50$, $p < 0.001$) which was driven by a main effect of days ($F_{9,234} = 1608$, $p < 0.001$); however, no treatment effect was seen ($F_{2,234} = 0.72$, $p > 0.05$) (Fig. 1). In the light–dark box experiment ($n = 24$) we saw similar results, with an overall interaction ($F_{16,168} = 3.806$, $p < 0.001$) and a main effect of days ($F_{8,168} = 1063$, $p < 0.001$), although no treatment effect was observed ($F_{2,21} = 1.54$, $p > 0.05$). Bonferroni's *post-hoc* tests showed no significant difference among the 3 treatment groups on any given day for animals in either experiment.

3.2. Place conditioning

An unbiased design was employed for place conditioning as there was no difference in percent of time spent on the vertical ($49.9\% \pm 0.02$) vs. circle side ($51.1\% \pm 0.02$) for all rats ($t_{28} = 0.48$, $p > 0.05$). Additionally, a one-way ANOVA showed no significant difference for time spent in the vertical side between groups ($F_{2,25} = 0.23$, $p > 0.05$).

Fig. 2 shows the average time spent in the MDMA-paired compartment during the test day for place preference. Rats that had been conditioned to 0 (saline) or 5 mg/kg MDMA during late adolescence did not show a preference for the paired chamber, while rats conditioned to the 10 mg/kg dose spent significantly less time spent in the paired chamber indicating a

place aversion developed during MDMA conditioning. A one-way ANOVA showed a significant difference among groups ($F_{2,24} = 4.02, p < 0.05$), and Tukey's *post hoc* tests revealed a significant decrease in the 10 mg/kg MDMA group compared to saline-controls ($p < 0.05$). An additional analysis assessing time spent in the MDMA paired chamber (Post-Pre-test) showed a significant difference among groups ($F_{2,24} = 3.708, p < 0.05$), and Tukey's *post hoc* tests showed a significant decrease in the 10 mg/kg MDMA group compared to saline-controls ($p < 0.05$).

3.3. Open field test

Anxiety-like behavior was assessed with the open field test five days after the cessation of MDMA treatment. Fig. 3a shows that rats treated with 10 mg/kg of MDMA four days in a row during late adolescence and observed five days later displayed greater thigmotaxis behavior by spending less time in the center square of an open field compared to control animals. A one-way ANOVA showed a significant difference among groups ($F_{2,23} = 3.58, p < 0.05$), and Tukey's *post hoc* tests revealed a difference between the 10mg/kg MDMA group and the saline-controls ($p < 0.05$). Fig. 3b shows that no difference among groups exists in distance traveled during the open field test ($F_{2,23} = 3.27, p > 0.05$).

3.4. Light–dark box

In a separate group of rats ($n = 24$) avoidant and anxiety-like behavior was tested five days after MDMA using the light–dark box to validate the open field results. Fig. 4 shows that rats treated with MDMA for four days show increased latency to enter the light side ($F_{2,20} = 4.00, p < 0.05$; Fig. 4a) and decreased number of transitions ($F_{2,21} = 5.13, p < 0.05$; Fig. 3b); Tukey's *post-hoc* tests revealed a difference between 10 mg/kg MDMA and saline groups for both measures. Overall time spent in the light compartment was unchanged between the groups ($F_{2,21} = 0.96, p > 0.05$; Fig. 4c).

3.5. 5-HT and 5-HIAA analysis by HPLC

Whole tissue levels of 5-HT and its metabolite 5-HIAA were measured in select brain regions by HPLC five days after the cessation of MDMA or saline treatment (Fig. 5). In the dorsal raphe, a significant difference among groups was observed for 5-HT ($F_{2,21} = 6.36, p < 0.01$), and Tukey's *post hoc* tests showed a difference between the 10 mg/kg MDMA group and the 5 mg/kg MDMA or the saline-control groups ($p < 0.05$; Fig. 5a) with 10 mg/kg causing a significant decrease compared to other groups. No differences in 5-HIAA levels in the dorsal raphe were seen among groups ($F_{2,21} = 0.13, p > 0.05$; Fig. 5a). In the amygdala, analysis showed a significant difference among groups for 5-HT ($F_{2,23} = 9.64, p < 0.001$) and 5-HIAA ($F_{2,23} = 9.36, p < 0.01$), and Tukey's *post hoc* tests revealed increases in the 10 mg/kg MDMA group when compared to 5 mg/kg MDMA or saline controls for 5-HT or 5-HIAA ($p < 0.01$; Fig. 5c). In the hippocampus, no differences in 5-HT ($F_{2,23} = 2.45, p > 0.05$; Fig. 5e) or 5-HIAA ($F_{2,23} = 0.189, p > 0.05$; Fig. 5e) were observed. The data in Fig. 5 are presented as a percentage of control values, and raw tissue levels (mean $\pm$ SEM; ng/mg) for the control groups were 3.51 ± 0.11 for 5-HT and 0.49 ± 0.04 for 5-HIAA in the dorsal raphe, 1.73 ± 0.10 for 5-HT and 0.14 ± 0.01 for 5-HIAA in the amygdala, and 4.76 ± 0.06 for 5-HT and 0.34 ± 0.08 for 5-HIAA in the hippocampus.

4. Discussion

The present study demonstrates that repeated administration of 10 mg/kg MDMA during late adolescence causes place aversion, increased anxiety-like behavior in the open field, avoidant behaviors in the light–dark box, and differentially alters regional 5-HT and 5-HIAA levels in the adolescent brain. In contrast, the 5mg/kg dose had no effect on place conditioning, anxiety behavior, or 5-HT levels compared to control treated adolescent rats.

Consistent with a previous report in adolescent rats (Catlow et al., 2010) we did not see place conditioning at the 5mg/kg dose; however, because that study did not include higher doses of MDMA this is the first report to show a conditioned place aversion at 10 mg/kg in rats during late adolescence (Fig. 2). Conversely, studies with adult rats have shown a conditioned place preference at the same dose of MDMA (Marona-Lewicka et al., 1996), which suggests a developmental transition from drug aversion to drug preference between adolescence and adulthood at this dose. Similarly, previous research with other psychostimulants (e.g., cocaine and amphetamine) indicate that rats have substantially different place conditioning scores with the same dose of psychostimulant depending on the stage of adolescence (Badanich et al., 2006; Izenwasser, 2005; Niculescu et al., 2008).

In accordance with the majority of preclinical literature we report an increase in anxiogenic behavior following repeated administration and abstinence of MDMA (Fig. 2a) (Fone et al., 2002; Gurtman et al., 2002; McGregor et al., 2003; Morley et al., 2001). It has been previously suggested that the anxiogenic behavior could be related to the 5-HT depleting effects of MDMA in the amygdala (Gurtman et al., 2002). In contrast, we observed an increase in anxiogenic behavior and an increase in whole tissue 5-HT and its metabolite 5-HIAA in the amygdala (Fig. 5c). Although the increase in amygdalar 5-HT was surprising, as most research reports depletion of 5-HT after MDMA (Faria et al., 2006; Gurtman et al., 2002; McGregor et al., 2003) the fact that increased 5-HT was observed with an anxiogenic effect was less so. Previous reports have shown an anxiogenic effect following an infusion of 5-HT into the amygdala (Higgins et al., 1991; Hodges et al., 1987). Additionally, we observed a decrease in 5-HT in the dorsal raphe nucleus (Fig. 5a), the main source of serotonergic innervation to the forebrain (Azmitia and Segal, 1978; Jacobs and Azmitia, 1992), with no changes in the hippocampus (Fig. 5e). Taken together, our data suggests a more complex mechanism associated with regionally-distinct dysregulation of the 5-HT system to explain how MDMA interacts with the 5-HT system to produce anxiety-like behaviors.

These findings are difficult to interpret in the absence of other neurochemical data, although it is tempting to speculate that the regionally-distinct effects are related and work in concert to underlie the maladaptive anxiety-like and avoidant behaviors caused by MDMA. For example, dysregulation of 5-HT₁ or 5-HT₂ receptor subtypes may contribute to MDMA-induced anxiety-like behavior, as these receptors regulate anxiety in animals and humans and clinically effective anxiolytics include 5HT_{1A} partial agonists (Gordon and Hen, 2004; Heisler et al., 2007; Martin et al., 2002; Weisstaub et al., 2006). One interpretation is that repeated MDMA disrupts 5-HT activation of somatodendritic impulse-modulating 5-HT_{1A} autoreceptors in the dorsal raphe nucleus (Pompeiano, Palacios, & Mengod, 1992) and

function to regulate 5-HT output to areas such as the amygdala (Cerrito and Raiteri, 1979). If reduced dorsal raphe 5-HT levels are accompanied by diminished activation of somatodendritic 5-HT_{1A} autoreceptors, then an increase in 5-HT content, and perhaps function, in projection fields such as the amygdala would be expected (Pineyro and Blier, 1999). Further studies assessing receptor changes after MDMA in this paradigm would help address the relationship of the 5-HT system with the anxiogenic behavior.

The significant increase of 5-HT and 5-HIAA in the amygdala (Fig. 5c) seems to contradict other findings showing MDMA-related decreases in this region (Faria et al., 2006; Gurtman et al., 2002; McGregor et al., 2003). These different outcomes may reflect important experimental differences including the dosing regimen, developmental stage of the animals, and the early time-point in which 5-HT levels were assessed in the present study. For example, the majority of studies showing decreased 5-HT in the amygdala typically observe this after exposure to a neurotoxic dosing regimen and neurochemical assessment of monoamines at least a week after MDMA treatment (Faria et al., 2006; Gurtman et al., 2002; McGregor et al., 2003). Whereas we choose to use a repeated dosing regimen and to assess monoamine levels five days after MDMA exposure in order to examine changes associated with late adolescence rather than adulthood. While neurotoxic dosing regimens have consistently produced depletions of 5-HT in multiple brain regions, the face validity of this administration paradigm relative to human use patterns is debatable. Rather than a neurotoxic (i.e. 5HT-depleting) effect of MDMA, our data suggest a coordinated dysregulation of the 5-HT system, with a decrease of 5-HT in dorsal raphe and an increase in the amygdala.

In conclusion, the present study shows that repeated daily administration of 10, but not 5, mg/kg MDMA during late adolescence caused a significant place aversion. In addition, this dose increased anxiety-like and avoidant behaviors, as well as increased 5-HT and 5-HIAA levels in the amygdala and decreased 5-HT levels in the dorsal raphe. Further studies are needed to define the mechanism by which repeated MDMA leads to regionally-distinct, neuroplastic changes in the 5-HT system that are observed with aversive and anxiety-like behavior.

Abbreviations

MDMA	3,4-methylenedioxymethamphetamine
HPLC	High Pressure Liquid Chromatography
5-hydroxytryptamine	5-HT serotonin
5-HIAA	5-hydroxyindoleacetic acid
SERT	serotonin transporter
PND	postnatal date
IP	Intraperitoneal
ANOVA	analysis of variance

SEM

standard error of the mean

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Fig. 1.

MDMA treatment does not alter weight gain throughout the study. The experimental design is depicted here and included 4–5 days of acclimation (not shown), MDMA or saline administration during conditioned place preference, and open field testing and brain tissue collection 5 days after drug treatment. Adolescent rats of the 3 groups (0-saline, 5, or 10 mg/kg MDMA) were weighed daily throughout the experiment. Data are expressed as grams and graphed as the mean $\pm$ SEM, control ($n = 10$), 5 MDMA ($n = 10$), 10 MDMA ($n = 9$). Repeated-measures ANOVA did not detect group differences, but showed an age (days)-dependent increase during the adolescent period.

Fig. 2.

MDMA causes conditioned place aversion during late adolescence. Rats underwent condition place training for 4 days (2 conditioning sessions per day) with MDMA (5 or 10 mg/kg IP) or saline, with a preference test assessed the following day. Time (s) spent in the paired chamber (paired with 0, 5, or 10 mg/kg MDMA) minus time spent in the unpaired side (paired with saline) was used as a measure of place conditioning; therefore, a result below zero reflects conditioned place aversion (to the MDMA conditioned side). All data are depicted as the mean $\pm$ SEM, control (n = 9), 5 MDMA (n = 10), 10 MDMA (n = 8); * p < 0.05 when compared to the saline-control group.

Fig. 3.

Increased anxiety-like behavior is observed in the open field five days after repeated MDMA administration. Rats were tested in an open field five days after cessation of 0 (saline-control), 5, or 10mg/kg MDMA. Time (s) spent in the center square of the open field (i.e., thigmotaxis) was used as a measure of anxiety-like behavior (a) and distance traveled (cm) during the test was used as a measure of locomotor activity (b). All data are depicted as the mean $\pm$ SEM, control (n= 8), 5 MDMA (n= 10), 10 MDMA (n= 8); * $p < 0.05$ when compared to the saline-control group.

Fig. 4.

Avoidant and anxiety-like behaviors are increased in the light–dark box five days after repeated administration of MDMA. As an additional measure of anxiety-like behavior, light–dark box activity was tested in a separate group of rats five days after MDMA (or saline). Rats received MDMA (or saline) in the same pattern as the rats in the place conditioning experiment, although they did not undergo place preference training or testing. Repeated administration of 10mg/kg MDMA significantly decreased latency to enter the light compartment of the chamber (a) and decreased number of transitions between the light and dark compartments (b), although there was no difference in percent of time spent in the light compartment (c). All data are depicted as the mean $\pm$ SEM, control (n = 8), 5 MDMA (n = 8), 10 MDMA (n = 8); * p < 0.05 when compared to the saline-control group.

Fig. 5.

Dysregulation of serotonin levels in dorsal raphe and amygdala five days after repeated MDMA administration. Brain tissue from adolescent rats was collected for HPLC analysis of 5-HT and its metabolite 5-HIAA five days after MDMA or saline (0mg/kg MDMA) administration. Repeated administration of 10mg/kg MDMA significantly decreased 5-HT but not 5-HIAA in the dorsal raphe nucleus (panels a & b), increased 5-HT and 5-HIAA in the amygdala (panels c & d), and no effect in the hippocampus (panels e & f) five days after MDMA administration. All data are presented as a percent of control within a given brain region, and are depicted as the mean $\pm$ SEM of 8 to 10 rats per group, and $*p < 0.05$ and $**p < 0.01$ when comparing the 10 mg/kg MDMA with the saline-control or 5 mg/kg MDMA group.

Coronal images modified from Paxinos and Watson (2007).