

# MDMA in Adolescent Male Rats

## Decreased Serotonin in the Amygdala and Behavioral Effects in the Elevated Plus-Maze Test

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**ABSTRACT:** Long-term behavioral consequences of the neurotoxicity produced by 3,4-methylenedioxymethamphetamine (MDMA) in the adolescent rat are still mostly unknown. Here, adolescent male rats (postnatal day 45 PND [45]) were exposed to 10 mg/kg of MDMA, intraperitoneally, every 2 h for 6 h. Controls were given 0.9% saline in the same protocol. Ten days after exposure, the behavioral effects of MDMA were assessed in the elevated plus-maze ( $n = 6$  per group). After behavioral testing, animals were sacrificed and the amygdalae were dissected and processed for HPLC determination of dopamine (DA), serotonin (5-HT), and metabolites. Results showed a significant decrease in the 5-HT content ( $P < 0.05$ ), but no significant alterations in DA or its metabolites. Behavioral observation in the elevated plus-maze showed a decreased number of entries in the unprotected arms ( $P < 0.05$ ), which were correlated to the number of entries and time spent in the central platform. Rearing was also decreased ( $P < 0.05$ ). No differences were observed in head dips, grooming, or number of entries in the protected arms of the apparatus. Therefore, we conclude that, as in the adult rat, exposure to MDMA in the adolescent rat is associated to long-term depletion of the 5-HT content and increased anxiety-like behavior.

**KEYWORDS:** MDMA; ecstasy; serotonin; dopamine; amygdala; anxiety

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Ann. N.Y. Acad. Sci. 1074: 643–649 (2006). © 2006 New York Academy of Sciences.  
doi: 10.1196/annals.1369.062

## INTRODUCTION

Exposure to MDMA (3,4-methylenedioxymethamphetamine) in the adult rat is known to produce short- and long-term neurotoxicity mainly affecting the serotonergic system (for revision see Ref. 1). Although short- and long-term cellular, molecular, and behavioral neurotoxic effects of MDMA abuse were extensively studied in the adult, its consequences in the adolescent rat are still mostly unknown.

The amygdala is a limbic structure that integrates positive and aversive emotional information mediating behavioral reactions, and there is a relevant number of studies pointing to its essential role in the acquisition of both emotional and motor-conditioned responses.<sup>2,3</sup> This brain structure has an active role in the conditioning of autonomic fear responses through its projections to the hypothalamus, that in turn project to brain stem areas and spinal premotor neurons.<sup>4,5</sup> The involvement of amygdala in the addictive behavior seems to be fairly specific,<sup>6</sup> comprehending conditioned associations, stress responses, and anxiety behaviors, which are usually related to altered dopamine (DA) and serotonin (5-HT) levels.<sup>7</sup> The elevated plus-maze is a standard test of fear and anxiety, useful in testing the effects of anxiolytic and anxiogenic drugs. Therefore, this article aimed to evaluate the effects of exposure to MDMA in the levels of neurotransmitters, mainly DA and 5-HT, in the amygdala of adolescent rats and to relate these levels with behavioral observations in the elevated plus-maze.

## MATERIALS AND METHODS

Wistar rat litters were culled to eight pups each, with equivalent sex representation, on postnatal day 1 (PND 1). On PND 45, male animals were given 10 mg/kg of MDMA, intraperitoneally, every 2 h for a period of 6 h,<sup>8</sup> or an isovolumetric dose of saline vehicle to control subjects. All procedures used were approved by the Portuguese Agency for Animal Welfare (General Board of Veterinary Medicine).

Ten days later, behavioral effects of MDMA were assessed in the elevated plus-maze ( $n = 6$  per group) in sessions of 5 min, which were recorded and analyzed using the software Observer 4.1 (Noldus Information Technology, Wageningen, Netherlands). After behavioral testing, animals were sacrificed by decapitation, the brains were rapidly removed and dissection of the amygdalae were performed on ice. Tissue samples were frozen by immersion in 4-methylbutane cooled over dry ice and stored at  $-70^{\circ}\text{C}$  until used for neurochemical determinations. Levels of 5-HT and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) and dopamine (DA) and its metabolites, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), were quantified by a modified method<sup>9</sup> of high performance liquid chromatography

combined with electrochemical detection (HPLC/EC) in a system from Gilson Inc. (Middleton, WI). The analytic column was a Supelco Inc. (Bellefonte, PA) Supelcosil LC-18 3  $\mu\text{M}$  (7.5 cm  $\times$  4.6 mm). Concentrations of neurotransmitters were calculated using standard curves. Standards were purchased from Sigma (St. Louis, MO). Final results were expressed in terms of monoamine content per amount of protein. The total content of protein was assayed in duplicate using a colorimetric microassay from Bio-Rad (Munich, Germany) based on the Bradford assay.<sup>10</sup>

Neurochemical determinations and elevated plus-maze data were analyzed using one-way ANOVA (treatment). Significant main effects and interactions were further explored using *post hoc* tests. The statistical level of significance was considered at  $P < 0.05$ . All tests were performed using the software Statistica 5.5A (Statsoft Inc., 2000; Tulsa, OK).

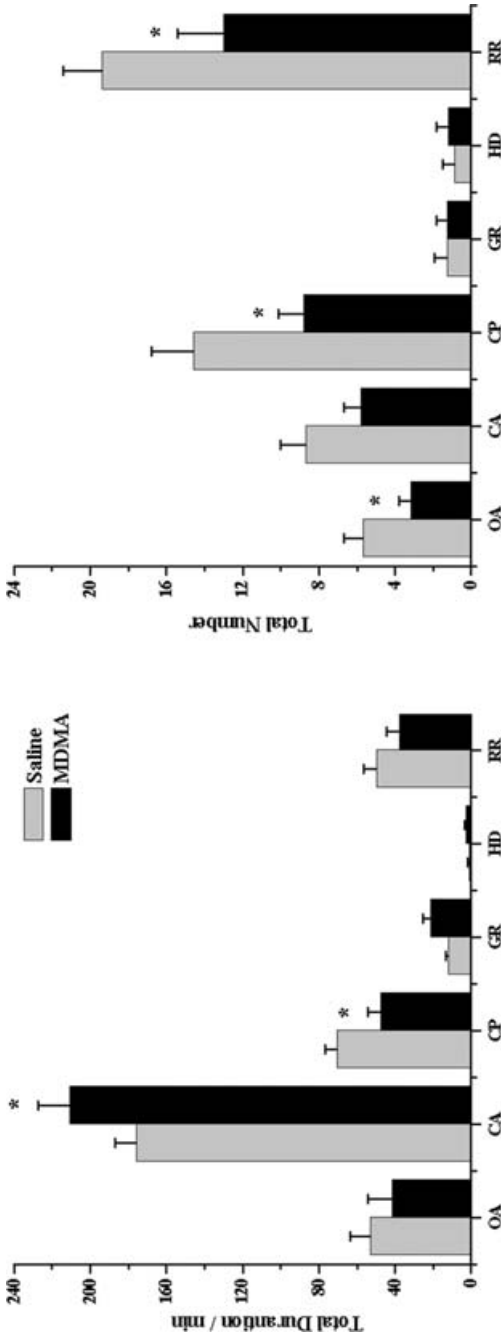
## RESULTS

Analysis of data obtained in the elevated plus-maze, by one-way ANOVA (treatment), followed by *post hoc* analysis using Tukey Honest Significant Difference (HSD), revealed a significantly increased number of entries in the protected arms ( $P < 0.05$ ) and a decreased number of entries and time spent in the central platform ( $P < 0.05$ ). Behavioral analysis showed also a decrease in rearing ( $P < 0.05$ ). No differences were observed in head dips, grooming, or number of entries in the protected arms of the apparatus. Also, there were no differences in the latency of any of the assessed behavioral categories (see FIG. 1).

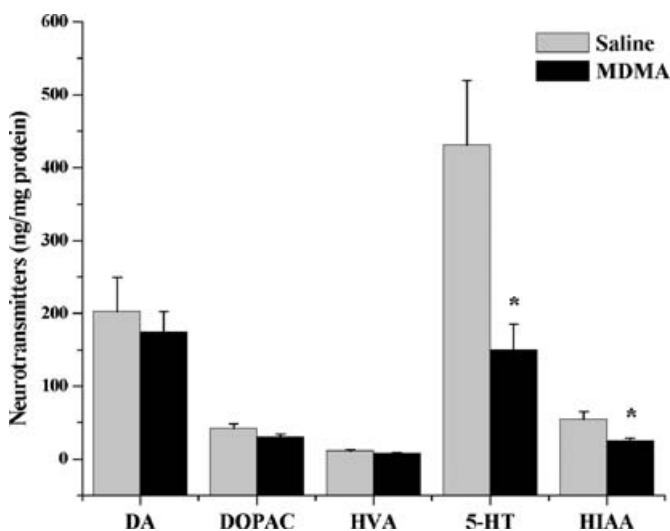
Analysis of neurochemical data showed a significant decrease in the levels of both 5-HT and 5-HIAA ( $P < 0.05$ ) and no differences in the levels of DA and its metabolites (see FIG. 2) or DA turnover.

## DISCUSSION

MDMA in the adult rat is known to be selectively neurotoxic to the serotonergic system causing almost no deleterious effects on the dopaminergic system.<sup>1</sup> Nevertheless, there is evidence for a possible interaction between these two systems concerning the behavioral effects of MDMA,<sup>11,12</sup> which is likely to be expected, since these two systems are intimately connected.<sup>11,13</sup> As previously described for the adult rat in the amygdala<sup>14</sup> and other brain areas,<sup>15,16</sup> also in the adolescent rat, we have found that the amygdalar 5-HT content is markedly decreased 10 days after exposure and therefore, behavioral alterations are likely to appear at this stage. Regardless of the possible relation between 5-HT and DA, in the present work we could not determine any significant differences in the dopaminergic system.



**FIGURE 1.** Behavioral effects of MDMA in the elevated plus-maze, tested 10 days after exposure to MDMA ( $4 \times 10$  mg/kg i.p. every 2 h for 6 h), representing the total number of times that each behavior was repeated and the total time spent in each category. Data expressed as mean values  $\pm$  SEM ( $n = 6$  per group). OA = open arms, CA = closed arms, CP = central platform, GR = grooming, HD = head dips, RR = rearing. \*Significantly different from control ( $P < 0.05$ ).



**FIGURE 2.** Effects of MDMA exposure in the amygdalar levels of monoamines, determined by HPLC/EC. Each column represents mean + SEM for six animals per group. \*Significantly different from control ( $P < 0.05$ ).

Acutely, MDMA is known to be anxiogenic in the elevated plus-maze, reducing the percentage of entries and the time spent in the open arms, as well as all other exploratory behaviors.<sup>8</sup> Albeit, when testing social interaction, acute MDMA causes a marked increase in the displayed interaction,<sup>17</sup> which probably results from the interaction of different neurotransmitter systems. In a previous study, no differences in the time spent in the open arms of the elevated plus-maze were seen in adult rats MDMA pretreated (12.5 mg/kg, i.p.) and tested 8 days after exposure.<sup>18</sup> Likewise, another study, using the same protocol of exposure to MDMA as the present work, also failed to identify long-term (14 days later) behavioral effects in the elevated plus-maze.<sup>8</sup> However, using a similar pretreatment dose, other authors were able to observe increased anxiety in the plus-maze in adult rats tested 9 weeks after exposure to MDMA, which was also associated to 5-HT depletion in the amygdala, hippocampus, and caudate-putamen.<sup>14</sup>

Here, we describe that adolescent rats pretreated with MDMA and tested in the elevated plus-maze 10 days after treatment, presented anxiety-related behavior, spending more time in the closed arms of the apparatus and making a reduced number of entries in the open arms and central platform, evidencing reduced exploration and increased fear. Likewise, these rats also presented reduced rearing behavior, which is considered to reflect emotional behavior components and that may be associated with increased fear. Therefore, it seems that in the adolescent rat the behavioral neurotoxic effects of MDMA are

evidenced earlier than in the adult. On PND 45 gliogenesis, synaptogenesis, and myelination processes are still occurring at a high rate, which increases the risk of suffering a neurotoxic insult.<sup>19</sup>

Although traditionally amygdala-related behavioral alterations are associated to changes in the DA content,<sup>20,21</sup> we did not observe any changes in either DA or its metabolites. Our findings are in agreement with previous results describing increased anxiety in adult rats pretreated with MDMA, which was also associated to serotonin depletion but not to DA changes.<sup>14</sup> However, it is important to remember that MDMA induced sustained hyperthermia (data not shown) through all the period of exposure, which is known to have a significant role in producing 5-HT depletion.<sup>22,23</sup>

In conclusion, we report here that exposure to MDMA in the adolescent rat induced depletion of the amygdalar 5-HT content, increasing anxiety-like behavior measured in the elevated plus-maze; these effects were observed 10 days after exposure and evidence the presence of long-term neurotoxic effects of MDMA in the adolescent male rat.

### ACKNOWLEDGMENTS

This work was supported by project Fundação Calouste Gulbenkian ref. 65710 and Programa de Financiamento Plurianual do IBMC. Teresa Sumavielle was partially granted by Fundação para a Ciência e Tecnologia (FCT) SFRH/BPD/20997/2004.

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