Effects of Acute, Subchronic and Intermittent MDMA (‘ECSTASY’) Administration on Agonistic Interactions Between Male Mice

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Recent studies suggest that acute administration of 3,4-methylenedioxymethamphetamine (MDMA), an amphetamine derivative popularly known as “ecstasy,” produces an antiaggressive effect in male mice. However, there is no evidence with respect to the development of tolerance or sensitization after its subchronic or intermittent administration. In this study, we examined the action of low to moderate doses of MDMA (1.25, 2.5 and 5 mg/kg, i.p.), administered acutely, subchronically (for 7 days) or intermittently, on agonistic behavior elicited by isolation in male mice. Individually housed mice were exposed to anosmic “standard opponents” 30 minutes after the drug administration, and the encounters were videotaped and evaluated using an ethologically based analysis. Acute treatment with MDMA provoked a significant reduction of aggressive behaviors, without altering immobility. However, this action was only selective at 1.25 mg/kg. With the intermediate (2.5 mg/kg) and the highest doses (5 mg/kg) of the drug, it was observed a significant decrease of offensive behaviors, accompanied by an increase of exploration from a distance, avoidance/flee and defense/submission behaviors. This ethopharmacological profile could indicate the existence of an anxiogenic-like effect of MDMA. The overall picture of the effects of MDMA was very similar in the acutely, intermittently and daily treated animals. No tolerance or sensitization to the actions of the drug was developed after its repeated or intermittent administration.

Keywords: MDMA, aggression, anxiety, mice

Introduction

Recreational use of MDMA (‘ecstasy’), an amphetamine analog that produces a unique set of effects, has become increasingly popular over the past two decades, being one of the most prevalent illegal drugs of abuse among adolescents [Bhattacharya and Powell, 2001; Rodgers, 2000]. Due to its apparent unique psychological effects, MDMA has been suggested belonging to a new class of compounds termed “entactogens”, differentiating it from classic stimulants and hallucinogens [Liechti et al., 2001]. This term alludes to a peculiar set of psychological effects including euphoria, feelings of intimacy and closeness to others, heightened arousal, self-confidence and increased sensory sensitivity [Morgan, 2000].
In animals, MDMA mainly releases serotonin via interaction with the 5-HT transporter and, to a lesser extent, also dopamine (DA) [Steele, McCann and Ricaurte, 1994]. This release of dopamine is probably due to a direct interaction of MDMA with the DA-carrier, although there is also evidence that the concomitant MDMA-induced release of 5-HT amplifies DA release through activation of postsynaptic 5-HT$_3$ receptors [Gudelsky and Nash, 1996; Koch and Galloway, 1997]. Therefore, MDMA elicits large increases in synaptic concentrations of both dopamine and 5-HT, and the interaction between these neurotransmitters may account for the unique characteristics of the drug [Bankson and Cunningham, 2001].

Laboratory animal research (including nonhuman primates) has shown that repeated doses can lead to serotonergic neurodegeneration [Fischer et al., 1995; Ricaurte et al., 2000]. Although there is controversy as to whether similar damage might occur in the brain following regular use of MDMA in humans, recent studies suggest that MDMA may also be neurotoxic [Bhattacharya and Powell, 2001; Curran, 2000; McCann et al., 2000]. In fact, its possible neurotoxicity in humans led to the assignment of MDMA as a Schedule I compound by the US Drug Enforcement Agency in 1985.

In a previous study, we reported that acute administration of MDMA (5–20 mg/kg, ip) produced in isolated mice a behavioral profile characterized by a reduction of aggression (threat and attack), accompanied by a decrease of social investigation and an increase of exploration from a distance, avoidance/flee and defence/submission behaviors [Navarro and Maldonado, 1999]. This ethopharmacological profile could indicate the existence of an anxiogenic-like effect of the drug. Likewise, these behavioral effects of MDMA in agonistic encounters seem to be very similar to those described with FG 7142, an anxiogenic benzodiazepine receptor ligand [Maldonado and Navarro, 2001].

Although the action of MDMA on aggression has been examined [Miczek and Haney, 1994; Navarro and Maldonado, 1999], there is no studies comparing acute and subchronic effects of this compound using laboratory animal models. Therefore, this study was designed to analyze the effects of subchronic administration of MDMA (1.25, 2.5 and 5 mg/kg) for seven consecutive days on agonistic interactions between male mice, using an ethopharmacological approach. Additionally, we also explore the possible development of sensitization to the effects of MDMA after its intermittent administration to mice. This may be especially relevant since most human use of this recreational drug is not in the form of a single exposure but rather subchronic or (more likely) intermittent exposure. The term “agonistic behavior” comprises all elements of behavior florescent in situations of conflict, including attack, defense and flight. The ethological analysis of these social encounters appears to be an appropriate technique to distinguish between specific and nonspecific drug-induced changes.

**Materials and methods**

**Animals**

240 albino male mice of the OF.1 strain (provided by CRIFFA, Barcelona) weighing 25–30 g were used. Animals were housed under standardized lighting conditions (using a 12 h reversed cycle; white lights on: 20:00–8:00), at a constant temperature (21°C) and food and tap water available ad lib, except during behavioral trials. Upon arrival in the laboratory, the subjects were allocated to two different categories. Half were housed individually in transparent plastic cages (24 × 13.5 × 13 cm) as experimental animals. The remainder were
housed in groups of five (in cages of identical dimensions) to be used as “standard opponents” and were rendered temporally anosmic by intranasal lavage with 4% zinc sulfate solution (Sigma Laboratories) on both 1 and 3 days before testing. Fighting in mice, as in most rodents, is closely related to olfaction. We used this type of opponent because it elicits attack but never initiates such behavior [Brain et al., 1981]. Consequently, fighting is always unidirectional, being easily quantified.

All the experimental animals underwent an isolation period of 30 days before the behavioral test (isolation-induced aggression model). Social isolation is an effective form of increasing the level of aggressiveness in different species of animals. This phenomenon is particularly well demonstrated in laboratory mice [Valzelli, 1969; Navarro, 1997].

This experiment was carried out in accordance with the guiding principles for care and use of Laboratory Animals approved by the European Communities Council Directive of November 24, 1986 (86/609/EEC).

Experimental Design

Ten groups of mice were used. Individually housed animals were allocated randomly to one control group receiving vehicle and nine experimental groups (n = 12 each) receiving acute, subchronic or intermittent MDMA injections. The schedules of drug administration consisted of (a) acute treatment: each animal received vehicle for 6 consecutive days and MDMA on day 7; (b) subchronic treatment: each animals received a daily injection of MDMA for seven consecutive days; (c) intermittent treatment: each animal received MDMA on days 1 and 7 and vehicle on days 2 to 6; (d) vehicle: each animal received a daily injection of vehicle for 7 consecutive days (control group).

Drugs

MDMA (Sigma Laboratories) was diluted in physiological saline to provide appropriate doses for injections. Drug was administered either acutely, subchronically or intermittently in three doses: 1.25, 2.5 and 5 mg/kg. The control groups received physiological saline. Drug and vehicle were injected intraperitoneally in a volume of 10 ml/kg. The doses used in this experiment were chosen on the basis of several pilot studies carried out previously in our laboratory and some recently published papers [Navarro and Maldonado, 1999; Maldonado and Navarro, 2001].

Procedure and Behavioral Analysis

Thirty minutes after the last injection, an isolated animal and a “standard opponent” (marked with fur dye for identification) were confronted in a neutral area for ten minutes. This neutral cage consisted of an all glass area, measuring 50 × 26 × 30 cm with a fresh sawdust substrate. Before the encounter, the animals were allowed 1 minute of adaptation to the neutral cage, remaining separated by means of a plastic barrier throughout this time. The social encounters were videotaped using a Sony-V8 camera. All tests were conducted under dim red light between the second and seventh hours of the dark phase of the artificial cycle of the animals. After each encounter, the neutral cage was washed out and the sawdust bedding was replaced.
The tapes were analyzed using a microprocessor and a custom-developed programme [Brain et al., 1989] which facilitated estimation of time allocated to ten broad behavioral categories. The names of the categories and their constituent elements are as follows:
1. Body care (abbreviated groom, self groom, wash, shake, scratch).
2. Digging (dig, kick dig, push dig).
3. Non-social exploration (explore, rear, supported rear, scan).
4. Exploration from a distance (approach, attend, circle, head orient, stretched attention).
5. Social investigation (crawl over, crawl under, follow, groom, head groom, investigate, nose sniff, sniff, push past, walk around).
6. Threat (aggressive groom, sideways offensive, uprigh offensive, tail rattle).
7. Attack (charge, lunge, attack, chase).
8. Avoidance/flee (evade, flinch, retreat, ricochet, wheel, startle, jump, leave, wall, clutch).
10. Immobility (squat, cringe).

A detailed description of all elements can be found in Brain et al. [1989]. This ethoexperimental procedure allows a complete quantification of the behavioral elements shown by the subject during the agonistic encounters. Only the behavior of the isolated animal was assessed. The analysis was carried out by a trained experimenter, unaware of the treatment of the groups. The intra-observer reliability was approximately of 95% for all the behavioral categories analyzed.

**Statistical Analysis**

Nonparametric Kruskal-Wallis tests were used to assess the variance of the behavioral measures over different treatment groups. Subsequently, appropriate paired comparisons were carried out using Mann-Whitney U-tests. The analysis were performed using nonparametric statistics since the criteria for parametric statistics were not met by the data. The criterion for statistical significance for all the tests was P < .05.

**Results**

Table 1 illustrates the medians of the accumulated times allocated to the broad categories described above. Kruskal-Wallis analysis showed that there was significant variance in the categories of digging, nonsocial exploration, exploration from a distance, threat, attack, defense/submission (all P < .01) and avoidance/flee (P < .05).

Post-hoc Mann-Whitney U-tests revealed that acute treatment with MDMA significantly reduced the time spent in digging (2.5 and 5 mg/kg; P < .01), threat (2.5 and 5 mg/kg; P < .05 and P < .01, respectively) and attack (all doses; P < .01), in comparison with the control group. Nonsocial exploration (all doses; P < .01), exploration from a distance (2.5 and 5 mg/kg; P < .05 and P < .01, respectively), avoidance/flee (5 mg/kg; P < .01) and defense/submission (5 mg/kg; P < .01) were significantly increased after acute MDMA administration, as compared with the control group.

Moreover, animals treated subchronically with MDMA showed a significant increase in digging (1.25 mg/kg) and exploration from a distance behaviors (2.5 mg/kg) as well as a reduction of nonsocial exploration behaviors (2.5 mg/kg), in comparison with mice treated acutely or intermittently with the drug (P < .05).
Table I: Median Values (with ranges) for Times (in seconds) Allocated to Broad Behavioral Categories in Animals Receiving Treatment with MDMA (1.25, 2.5 and 5 mg/kg)

<table>
<thead>
<tr>
<th>Behavioral categories</th>
<th>Vehicle</th>
<th>Acute treatment</th>
<th>Subchronic treatment</th>
<th>Intermittent treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1.25</td>
<td>2.5</td>
<td>5</td>
</tr>
<tr>
<td>Body care</td>
<td></td>
<td>4.75</td>
<td>4.43</td>
<td>3.56</td>
</tr>
<tr>
<td>(0.9–15.1)</td>
<td></td>
<td>(0–5.8)</td>
<td>(0.7–9.3)</td>
<td>(0–18)</td>
</tr>
<tr>
<td>Digging&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td>8.21</td>
<td>3.71</td>
<td>0.87**</td>
</tr>
<tr>
<td>(0.6–31)</td>
<td></td>
<td>(0–29.3)</td>
<td>(0–11.3)</td>
<td>(0–0.8)</td>
</tr>
<tr>
<td>Non social exploration&lt;sup&gt;b&lt;/sup&gt;</td>
<td>342.5</td>
<td>411.6**</td>
<td>408.1**</td>
<td>434.4**</td>
</tr>
<tr>
<td>Explore from a distance&lt;sup&gt;b&lt;/sup&gt;</td>
<td>39.93</td>
<td>44.49</td>
<td>58.44**</td>
<td>105.1**</td>
</tr>
<tr>
<td>(13–98)</td>
<td></td>
<td>(1.7–69)</td>
<td>(6–218)</td>
<td>(0–76.2)</td>
</tr>
<tr>
<td>Threat&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td>63.65</td>
<td>65.06</td>
<td>40.41*</td>
</tr>
<tr>
<td>(30–145)</td>
<td></td>
<td>(0–161)</td>
<td>(0.9–124)</td>
<td>(0–44)</td>
</tr>
<tr>
<td>Attack&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td>61.83</td>
<td>21.71**</td>
<td>4.44**</td>
</tr>
<tr>
<td>(20.2–115)</td>
<td></td>
<td>(0.92–8)</td>
<td>(0–80.1)</td>
<td>(0–6.9)</td>
</tr>
<tr>
<td>Avoidance/Flee&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td>0.21</td>
<td>0.8</td>
<td>3.94**</td>
</tr>
<tr>
<td>(0–2.53)</td>
<td></td>
<td>(0–5.02)</td>
<td>(0–2.88)</td>
<td>(0–8.5)</td>
</tr>
<tr>
<td>Defense/Submission&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>(0–5)</td>
<td></td>
<td>(0–23)</td>
<td>(0–0.73)</td>
<td>(0–24.4)</td>
</tr>
</tbody>
</table>

Kruskal-Wallis test showed significant variance: *<sup>P<.05</sup>; **<sup>P<.01</sup>.

Differs from controls on Mann-Whitney U-test, *<sup>P<.05</sup>; **<sup>P<.01</sup>.
The median values for the behavioral category of immobility were zero for all groups in the three conditions of treatment examined.

Discussion

The present study was designed to investigate the acute, subchronic and intermittent effects of MDMA on agonistic interactions between male mice using a low to moderate dose range (1.25, 2.5 and 5 mg/kg), similar to those typically used by humans (1–4 mg/kg) [Boot et al., 2000; Topp et al., 1999; Vollenweider et al., 1998].

Acute treatment with MDMA produced a significant decrease of offensive behaviors (threat and attack) in isolated male mice, without a concomitant increase of immobility. This reduction of aggression was especially marked with the intermediate (2.5 mg/kg) and the highest doses of MDMA used (5 mg/kg), as compared with the control group. However, it is interesting to note that the lowest dose used (1.25 mg/kg) also provoked a significant decrease of attack behaviors, being this effect specific since other behavioral categories such as avoidance/flee, defense/submission, digging or exploration from a distance were not significantly affected by the drug (see Table I).

In contrast, the antiaggressive effects observed after administration of 2.5 and 5 mg/kg of MDMA were clearly unselective. Thus, avoidance/flee and defense/submission behaviors were significantly enhanced after treatment with the drug (5 mg/kg). Furthermore, the increase of exploration from a distance (2.5 and 5 mg/kg). is also consistent with an increase of anxiety, whereas the increase of the time spent in nonsocial exploration behaviors might reflect attempts to escape from the test arena. Overall, this ethopharmacological profile suggests the existence of an anxiogenic-like activity of MDMA at these doses in mice. These results are in concordance with two previous studies carried out in our laboratory using higher doses of MDMA [Navarro and Maldonado, 1999; Maldonado and Navarro, 2001]. Likewise, the results of the present experiment are in line with recent investigations in which an anxiogenic-like action has been described using animal models of anxiety such as the elevated plus-maze [Bathacharya et al., 1998; Lin et al., 1999] and the light/dark box tests [Maldonado and Navarro, 2000]. Nevertheless, Morley and McGregor [2000] have communicated recently that MDMA has both anxiogenic and anxiolytic effects depending upon the test situation employed.

Although the precise mechanism by which MDMA may provoke this anxiogenic-like effect remains unknown, perhaps it could reflect an increased synaptic 5-HT acting at 5-HT$_{2A/2C}$ receptors. In fact, 5-HT$_{2A/2C}$ receptor agonists such as mCPP are markedly anxiogenic and rodents and humans [Broocks et al., 1997; Meert et al., 1997]. On the other hand, MDMA might exert its anxiogenic-like effect acting on central nucleus of the amygdala and medial prefrontal cortex, two regions traditionally associated with fear and anxiety, which express significant amounts of c-fos following MDMA administration [Stephenson et al., 1999].

The overall picture of the effects of MDMA was very similar in the acutely, intermittently and daily treated animals. Thus, when a schedule of intermittent administration was used no sensitization to the acute effects of the drug was evident. In addition, with repeated treatment, no tolerance to the anti-aggressive effects of MDMA developed. In this sense, as Table I indicates, no significant differences in the categories of attack and threat were found when subchronically and acutely treated groups were compared. This absence of tolerance to
the antiaggressive effects of MDMA contrasts to the tolerance described after subchronic administration of amphetamine [Moro et al., 1997], a compound structurally similar to MDMA.

Likewise, no tolerance or sensitization to the anxiogenic-like effects of MDMA was observed in our study. In fact, the significant increases of the time spent by mice in avoidance/flee, defense/submission, exploration from a distance and nonsocial exploration behaviors after acute MDMA administration were maintained with the drug was injected intermittently or daily (for seven days). This lack of tolerance to the anxiogenic-like activity of MDMA (8 mg/kg) has been also described in mice tested in the elevated plus-maze [Maldonado et al., 2000].

In conclusion, at a low dose (1.25 mg/kg) MDMA selectively reduces offensive behavior while higher doses (2.5 and 5 mg/kg) produce behavioral changes indicative of increased anxiety. Moreover, the schedule of administration used (subchronic and intermittent) has little influence on these behavioral changes indicating that neither tolerance nor sensitization develops to the acute antiaggressive (low dose) or anxiogenic (high dose) effects of this drug.

REFERENCES


