Behavioral Neuroscience
2010, Vol. 124, No. 5, 662–676

0735-7044/10/$12.00 DOI: 10.1037/a0020827

Behavioral Profiles in Rats Distinguish Among "Ecstasy," Methamphetamine and 2,5-Dimethoxy-4-Iodoamphetamine: Mixed Effects for "Ecstasy" Analogues

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3,4-methylenedioxymethamphetamine (MDMA; "ecstasy") is a psychoactive drug structurally related to other phenylisopropylamines acting as stimulants or hallucinogens in humans. Although MDMA has a pharmacological identity of its own, the distinction of its acute effects from those of stimulants or even hallucinogens is controversial. In this work, dose-response curves (0.25, 0.5, 1, 3, 5, and 10 mg/kg) representing the acute in vivo effects of MDMA were compared with those of a structurally related stimulant (methamphetamine, MA) and a hallucinogenic analogue (2,5-dimethoxy-4-iodoamphetamine, DOI) in a set of behavioral protocols in rats, including spontaneous psychomotor activity, anxiolytic/anxiogenic-like effects and active avoidance conditioning responses. The behavioral profiles obtained allowed us to differentiate among racemic MDMA, MA, and DOI at different dose ranges. In addition, the evaluation of four MDMA analogues (1, 5, and 10 mg/kg) comprising two well-known MDMA analogues (MDA [3,4-methylenedioxyamphetamine] and MDE (*N*-ethyl-MDA, believed to substitute for MDMA) and two other structural analogues (MDOH [*N*-hydroxy-MDA] and MMDA-2 [2-methoxy-4,5-methylenedioxyamphetamine]) showed that none of these exactly resembles MDMA in their pharmacological profiles, highlighting the unique character of this prototypical entactogen. In fact, their effects exhibited similarities with the behavioral profiles of either MA or DOI, as well as novel profiles in specific behavioral paradigms.

Keywords: 3,4-methylenedioxymethamphetamine (MDMA), methamphetamine (MA), 2,5-dimethoxy-4-iodoamphetamine (DOI), MDMA analogues, rat behavior

MDMA (3,4-methylenedioxymethamphetamine), also known as "ADAM," "ecstasy" or "XTC," is the prototypical entactogen and it has been used as a recreational drug for decades (Leung & Cottler, 2008). This drug engenders in humans a controllable altered state of consciousness described as a feeling of heightened self-acceptance and openness for communication and empathy with other persons, without impairing cognitive or orientation capabilities and decreasing fear responses (Nichols, 1986; Greer & Tolbert, 1990; Green, Mechan, Elliott, O′Shea, & Colado, 2003). Because of its unique pharmacological effects which are different from classical stimulant and/or hallucinogenic effects, evidence has accumulated regarding its potential applications in psychotherapy (Grinspoon & Bakalar, 1986; Greer & Tolbert, 1990, 1998; Parrott, 2007; Sessa & Nutt, 2007; Johansen & Krebs, 2009) and as an adjunct in the treatment of neuropsychiatric disorders with a high rate of therapeutic failure (Riedlinger & Riedlinger, 1994; Doblin, 2002; Check, 2004; Bouso, Doblin, Farré, Alcázar, and Gómez-Jarabo, 2008; Riedlinger, 1985; Shulgin, 1990; Morton, 2005). On the other hand, due to its status as the "most popular street drug" and because of current legal restrictions (ACMD, 2009), most of the efforts in characterizing MDMA have focused on its detrimental effects (Morton, 2005; El-Mallakh & Abraham, 2007; Karlsen, Spigset, & Slørdal, 2008; Rogers et al., 2009; Wu et al., 2009), which are usually induced in animal models after chronic administration regimens (Green et al., 2003). The latter certainly do not mimic MDMA use by humans (Parrott, 2001), and are associated with essentially different molecular events at both serotonergic and dopaminergic neurotransmission systems compared to those induced by acute administration of MDMA (Gudelsky & Yamamoto, 2008).

Behavioral paradigms in rodents (e.g., locomotion, rearing, grooming, head shakes, anxiolytic-anxiogenic responses, active avoidance conditioning, and drug discrimination) have been used

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This work was supported by FONDECYT Grant 1085051 and ICM Grant P05-001-F.

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traditionally for the pharmacological evaluation of psychoactive phenylalkylamines. In rats, acute doses of MDMA enhance locomotion in a dose-dependent manner, together with a decrease in the number of head shakes and rearing behavior (Green et al., 2003). Methamphetamine (MA) elicits similar effects to MDMA in psychomotor models, which is associated with the widespread belief that MDMA is an amphetamine-like stimulant (Hall, Stanis, Marquez-Avila, and Gulley, 2008). Available data suggest that MDMA may also impair passive avoidance learning in rats (Marston, Marston, Reid, Lawrence, Olvermann, & Butcher, 1999; Moyano, Frechilla, & Del Rio, 2004), although this effect seems to be particularly strong during the perinatal period (Skelton et al., 2008; Vorhees et al., 2009). Similarly, MA has been shown to impair working memory (Nagai et al., 2007). On the other hand, the most characteristic behavioral effect of the structurally related serotonergic hallucinogen 2,5-dimethoxy-4-iodoamphetamine (DOI) in rodents is the induction of head shakes (Schreiber et al., 1995; Dursun & Handley, 1996), reflecting the high affinity of DOI for serotonergic 5-HT_{2A} receptors (Nelson, Lucaites, Wainscott, & Glennon, 1999). Low doses of DOI also decrease locomotion (Krebs-Thomson, Paulus, and Geyer, 1998) and induce an anxiolytic-like response in rats (Onaivi, Bishop-Robinson, Darmani, and Sanders-Bush, 1995). Drug discrimination experiments have shown that rats trained to discriminate MDMA from saline completely generalize the MDMA cue to amphetamine, but only partially to LSD and DOM (2,5-dimethoxy-4-methylamphetamine, an analogue of DOI), and at disruptive doses of the latter drugs. Conversely, MDMA weakly substituted for amphetamine in not more than 25% of the animals tested (Oberlender & Nichols, 1988). Other studies on the spatial patterns of rat locomotion activity using the behavioral pattern monitor (BPM) model show a highly characteristic behavior structure in animals treated with MDMA when compared with other psychoactive substances (Paulus & Geyer, 1991). The hyperlocomotion after administration of MA was shown later to be different, but the pattern elicited by DOI, although with considerably reduced locomotion, is similar to that observed with MDMA (Paulus & Geyer, 1992). Thus, taken individually, these more complex analyses of rat behavior, while revealing some differences in the actions of these drugs, do not provide clear-cut criteria to distinguish among them. Unfortunately, in spite of the fact that the available behavioral data may reveal the unusual pharmacology of MDMA in different behavioral paradigms, the acute effects of this drug are often misrepresented as those of a hallucinogen or a stimulant. This is presumably based on models addressing a single behavioral outcome which do not consider the simultaneous occurrence of other behavioral effects. Therefore, we hypothesize that the comparison of the profiles obtained by use of a panel of different behavioral paradigms will reveal distinctive identifying patterns elicited by the acute administration of subtoxic doses of MDMA or the structurally related MA (stimulant) and DOI (hallucinogen). The application of these patterns for the behavioral characterization of MDMA analogues will enable each analogue to be assigned to a specific place among the psychotropic amphetamines.

In the present work, we used a sequence of behavioral paradigms in rats, where in a first step different spontaneous behaviors (motor activity, locomotion, rearing, grooming, head shakes) were measured at six different acute doses of MDMA and compared with the same doses of structurally related amphetamine derivatives representing the stimulant (MA) and hallucinogen (DOI) classes. In a second step, the three drugs were evaluated in the elevated plus-maze. Finally, an active avoidance model was used to characterize the influence of each drug on the acquisition ability of the animal. Using the pharmacological data obtained, behavioral profiles were constructed for each prototypical drug. We further applied these models to evaluate the effects elicited by MDA (3,4-methylenedioxyamphetamine) and MDE (*N*-ethyl-MDA), two psychoactive drugs that are claimed to resemble MDMA in humans and are believed to share many of its pharmacological properties. Two other MDMA analogues, MDOH (*N*-hydroxy-MDA) and MMDA-2 (2-methoxy-4,5-methylenedioxyamphetamine) for which little is known about their behavioral effects in humans and rodents, were also evaluated. The analysis included all the behaviors studied, with special focus on those which, taken together, were able to differentiate between MDMA, MA, and DOI. All six drugs were tested as racemic mixtures, considering that these are the forms commonly used in clinical and recreational settings.

Materials and Methods

Animals

A total of 251 adult male Sprague–Dawley rats, weighing 200–230 g, were housed eight per cage in a temperature-controlled vivarium, under a 12:12 hour light–dark cycle (lights on from 0800 to 2000 hr) with free access to standard rodent pellet diet and tap water. Behavioral observations took place in a soundproof room at the same time of the day to reduce the confounding influence of diurnal variation on spontaneous behavior. Each animal was tested only once, and the minimum number of animals and duration of observations required to obtain consistent data were employed. Experimental protocols were conducted in accordance with international standards of animal welfare and following the Guide for Care and Use of Laboratory Animals and they were approved by the Faculty of Medicine Ethics Committee (University of Chile).

Drugs

Racemic MDMA, MA, DOI, MDA, MDE, MDOH, and MMDA-2 (see Figure 1) were synthesized as described by Shulgin and Shulgin (1991), and prepared as water-soluble hydrochlorides. All drugs were freshly dissolved in saline (0.9% NaCl) and administered intraperitoneally (i.p.) in a volume of 1 ml/kg body weight. MDMA, MA, and DOI were administered at 0.25, 0.5, 1, 3, 5, and 10 mg/kg, whereas MDA, MDE, MDOH, and MMDA-2 were administered at 1, 5, and 10 mg/kg (i.p.), 30 min before the behavioral tests. Each experimental group consisted of 8–11 animals. Saline was used as control treatment.

Spontaneous Motor Activity

Each rat was placed individually in a Plexiglas cage (30 × 30 × 30 cm). The floor of the cage was an activity platform (Lafayette Instrument Co., Lafayette, IN) connected to an amplifier and an electromechanical counter. Spontaneous motor activity was monitored automatically and recorded every 5 min for 30 min. Simul-

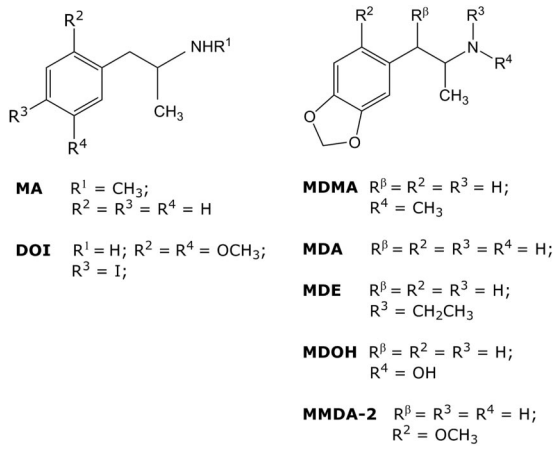

Figure 1. Chemical structures of psychotropic amphetamines, representing examples of a psychostimulant (MA), a hallucinogen (DOI), and MDMA and its studied analogues (MDA, MDE, MDOH, and MMDA-2).

taneously, the number of times each animal reared, the number of head shakes and the time (in seconds) spent in grooming behavior were recorded manually every 5 min for 30 min. One head shake was scored when the animal exhibited a rapid up- and down and/or rotating motion of the head, sometimes affecting the trunk as in "wet-dog shakes." One spontaneous grooming episode was scored as the time spent by the animal in wiping and/or licking different body parts. A rearing episode was scored each time the animal stood on its hind legs. To avoid the influence of disturbing noises, the platform was placed in a soundproof chamber and observations were recorded in real time using a digital camera connected to a PC.

Elevated Plus-Maze

This test has been widely validated to measure anxiety in rodents (Pellow & File, 1986). The apparatus consisted of two black Plexiglas open arms (50 × 10 cm each), two closed arms (50 × 10 × 20 cm each) and a central platform (10 × 10 cm). The maze was elevated 70 cm above the floor. Each animal was placed at the center of the maze, facing one of the closed arms. During a test period of 5 min, an observer recorded: (a) the number of open-arm entries; (b) the number of closed-arm entries; (c) the time spent in open arms; and (d) the time spent in closed arms. Arm entries were counted when the animal placed all four paws in an arm. Because illumination seems to play a crucial role in the plus-maze behavior of rats (Mora, Dussaubat, & Díaz-Véliz, 1996), the test was conducted under low artificial illumination conditions (approximately 10 lux). After the test, the maze was carefully cleaned with a wet tissue paper (70% ethanol solution). The results were expressed as percentages of open-arm entries and of time spent in open arms, with regard to the total number of arm entries and the total time spent in both open and closed arms, respectively. Since, in this test, anxiety is reflected in the unconditioned aversion to heights and open spaces, percentage of entries and time spent in open arms provide measures of fear-induced inhibition of exploratory activity. This ratio is increased by anxiolytic and reduced by anxiogenic compounds (Pellow, Chopin, File, & Briley, 1985).

Active Avoidance Conditioning

Each rat was individually placed into a two-way shuttle box (Lafayette Instrument Co., Lafayette, IN) composed of two stainless steel modular testing units. Each unit was equipped with an 18-bar insulated shock grid floor, two 28 V DC lights and a tone generator (Mallory Sonalert 2800 Hz, Lafayette Instrument Co., Lafayette, IN). Electric shocks were delivered to the grid floor by a Master shock supply (Lafayette Instrument Co., Lafayette, IN). The rats were trained over 50 trials, after a 5-min period of habituation. Each trial consisted of the presentation of a tone that after 5 s was overlapped with a 0.20 mA footshock until the animal escaped to the opposite chamber (maximum shock duration of 10 s). Between trials, the animal was allowed to rest for at least 15 s. A conditioned avoidance response (CAR) was defined as a crossing to the opposite chamber within the first 5 s (tone alone). If the rat did not escape by crossing to the opposite chamber during the footshock, this was considered as an escape failure.

Statistical Analysis

Data are presented as mean ± SEM and they were analyzed by one-way analysis of variance (ANOVA), followed by post hoc Dunnett's test or the Newman–Keuls Multiple Comparison test when appropriate, using GraphPad Prism software. A probability level of 0.05 or less was accepted as significant.

Results

Behavioral Profiles of MDMA, MA, and DOI

The overall effects of the i.p. administration of MDMA, MA, and DOI on rat spontaneous motor activity, during a 30-min period of observation, are summarized in Table 1 and Figures 2 and 3.

Total Motor Activity

One-way ANOVA revealed significant effects of drug administration on total motor activity [$F(18, 190) = 8.62$, $p < .0001$]. Subsequent Dunnett's comparison test demonstrated that MDMA (5 and 10 mg/kg) significantly increased motor activity compared with controls ($p < .05$). MA, at doses of 3 and 5 mg/kg ($p < .05$ and $p < .01$, respectively), also increased motor activity, but did not at 10 mg/kg. On the other hand, DOI only increased motor activity at 3 mg/kg ($p < .01$) but significantly decreased it after the highest dose (10 mg/kg; $p < .05$). At 10 mg/kg significant differences among the three drugs were evident ($p < .01$; see Table 1).

Rearing Behavior

ANOVA indicated significant effects of drug treatment on rearing behavior [$F(18, 190) = 7.37$, $p < .0001$]. While MDMA was unable to induce significant changes in this behavior, DOI significantly reduced it at almost every dose ($p < .01$), and MA did so only at the highest dose ($p < .05$). Rearing activity was very similar for MDMA and MA, but significantly different for DOI ($p < .001$; see Table 1).

Table 1
Effects of MDMA, MA, and DOI on Spontaneous Motor Behavior

	Motor activity (counts/30 min)	Rearing behavior (number/30 min)	Head shakes (number/30 min)
Saline	944.8 ± 67.2	40.5 ± 3.1	4.9 ± 0.7
MDMA (mg/kg)			
0.25	1147.1 ± 124.7	51.6 ± 4.4	6.0 ± 1.1
0.5	1022.9 ± 97.1	41.3 ± 6.7	5.9 ± 1.4
1.0	1404.6 ± 176.6	48.5 ± 5.5	4.3 ± 0.6
3.0	735.6 ± 115.1	43.3 ± 5.7	2.5 ± 0.5
5.0	1655.3 ± 175.3*	48.8 ± 9.8	1.8 ± 0.8
10.0	2148.2 ± 159.6*	41.8 ± 8.7	0.0 ± 0.0*
MA (mg/kg)			
0.25	968.1 ± 100.8	39.7 ± 4.8	2.2 ± 0.6
0.5	844.0 ± 137.5	42.1 ± 7.6	1.9 ± 0.9
1.0	1234.9 ± 145.6	51.8 ± 9.0	1.7 ± 0.7*
3.0	1876.0 ± 145.8*	56.4 ± 11.9	1.3 ± 0.4*
5.0	2924.2 ± 526.8*	33.0 ± 7.3	0.4 ± 0.2*
10.0	1213.5 ± 239.8	18,1 ± 6.1*	0.0 ± 0.0*
DOI (mg/kg)			
0.25	721.5 ± 52.8	15.4 ± 3.3	1.1 ± 0.5
0.5	935.5 ± 118.2	9.5 ± 1.0*	7.3 ± 1.6
1.0	1399.4 ± 200.2	13.3 ± 3.0*	16.3 ± 3.1*
3.0	2101.9 ± 410.5*	7.8 ± 2.2*	21.8 ± 7.6*
5.0	1473.3 ± 164.0	12.0 ± 3.6*	16.0 ± 4.3*
10.0	470.7 ± 99.4*	9.7 ± 3.7*	25.1 ± 7.6*

Note. Data are presented as mean ± SEM for 11 rats/dose (* $p < .05$). Doses were injected intraperitoneally in a total volume of 1 ml/kg.

Head Shakes

ANOVA results were also significant for head shakes [$F(18, 190) = 7.86$, $p < .0001$]. The number of head shakes was severely diminished by MA in doses ranging from 1 to 10 mg/kg ($p < .01$), while only the highest dose of MDMA (10 mg/kg) was able to decrease this behavior. In contrast, DOI doses higher than 0.5 mg/kg significantly increased head shakes. In this behavior, MDMA did not differ significantly from MA, but both were significantly different from DOI ($p < .01$; see Table 1).

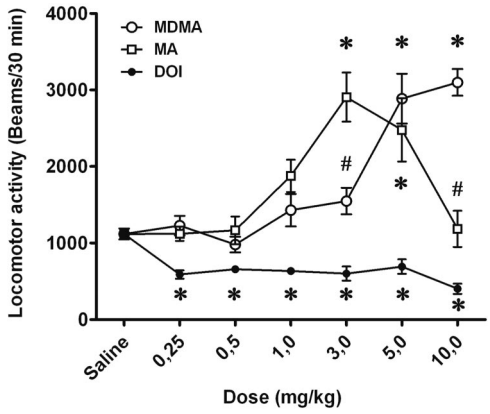

Figure 2. Locomotion dose-response curves for MDMA (○), MA (□), and DOI (●). Significance symbols indicate differences referred to * controls or # between drugs ($p < .05$). Each point represents a mean ± SEM ($n = 11$). Saline was used as control. Doses were injected i.p. in a total volume of 1 ml/kg.

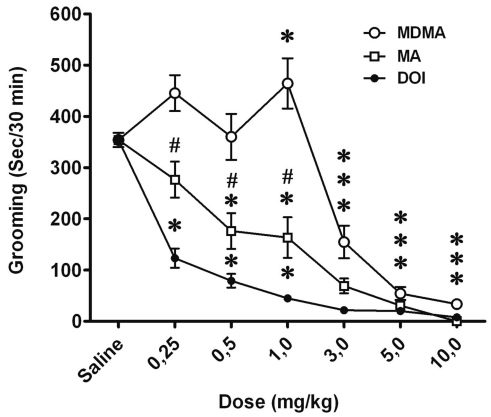

Figure 3. Grooming dose-response curves for MDMA (○), MA (□), and DOI (●). Significance symbols indicate differences referred to * controls or # between drugs ($p < 0.05$). Each point represents a mean ± SEM ($n = 11$). Saline (■) was used as control. Doses were injected i.p. in a total volume of 1 ml/kg.

Locomotor Activity

The significant effects of drug treatment on locomotion are shown in Figure 2, [$F(18, 190) = 7.37$, $p < .0001$]. All doses of DOI induced significant reduction of this behavior ($p < .01$). Whereas MDMA increased locomotion at doses of 5.0 and 10.0 mg/kg ($p < .01$), MA only enhanced this behavior after the administration of 3.0 and 5.0 mg/kg ($p < .01$). When the profiles of the three drugs were compared, significant differences among them were demonstrated at doses of 3 and 10 mg/kg ($p < .01$).

Grooming Behavior

One way ANOVA for grooming behavior also showed significant changes [$F(18, 190) = 37.23$, $p < .0001$; see Figure 3]. Post hoc comparisons indicated that MDMA (1.0 mg/kg) induced a significant increase in grooming ($p < .05$), but a progressive and significant decline of this behavior was observed after higher doses as compared with saline ($p < .01$). On the other hand, both MA and DOI decreased the time spent in grooming behavior after almost every dose ($p < .01$). When the effects of MA and DOI were compared, DOI proved to be a significantly more potent grooming inhibitor than MA. In the dose range from 0.25 to 1 mg/kg all three drugs induced significantly different effects ($p < .001$).

Elevated Plus-Maze

One-way ANOVA revealed significant effects of drug treatment on the percentage of entries into the open arms of the elevated plus-maze [$F(18, 190) = 3.42$, $p < .0001$], and on the percentage of time spent in the open arms [$F(18, 190) = 4.49$, $p < .0001$]. As shown in Table 2, DOI significantly increased plus-maze exploration ($p < .05$), with an enhancement in the percentage of entries into and time spent in the open arms at doses ranging from 1 to 10

Table 2
Effects of MDMA, MA, and DOI in the Elevated Plus Maze

	Plus maze total entries	% entries to open arms	% time spent in open arms
Saline	7.5 ± 1.3	31.5 ± 6.2	15.9 ± 4.0
MDMA (mg/kg)			
0.25	7.9 ± 1.0	28.3 ± 4.3	15.6 ± 3.8
0.5	6.9 ± 1.3	21.0 ± 6.6	11.7 ± 4.9
1.0	8.6 ± 1.6	33.6 ± 6.4	20.3 ± 6.2
3.0	9.5 ± 1.7	30.9 ± 5.9	15.0 ± 4.4
5.0	9.2 ± 1.6	41.1 ± 7.9	27.5 ± 7.1
10.0	9.7 ± 1.8	53.4 ± 8.8*	49.1 ± 10.2*
MA (mg/kg)			
0.25	9.5 ± 1.5	22.8 ± 5.5	14.4 ± 4.0
0.5	12.1 ± 1.7	36.3 ± 6.1	28.3 ± 6.7
1.0	11.3 ± 1.1	39.3 ± 4.2	24.0 ± 5.2
3.0	8.7 ± 1.6	43.0 ± 7.8	27.2 ± 6.9
5.0	7.9 ± 1.5	45.7 ± 8.6	28.6 ± 9.9
10.0	5.9 ± 1.1	46.4 ± 10.2	34.5 ± 11.1
DOI (mg/kg)			
0.25	9.3 ± 1.0	25.4 ± 3.8	15.9 ± 4.2
0.5	10.5 ± 1.2	38.5 ± 6.1	31.1 ± 6.9
1.0	8.9 ± 1.6	53.5 ± 4.6*	39.3 ± 7.9*
3.0	6.4 ± 1.3	55.9 ± 12.1*	53.6 ± 13.0*
5.0	7.7 ± 1.6	67.6 ± 8.7*	64.9 ± 9.8*
10.0	5.5 ± 1.3	58.2 ± 10.6*	53.5 ± 12.5*

Note. Data are presented as mean ± SEM for 11 rats/dose (* $p < .05$). Doses were injected intraperitoneally in a total volume of 1 ml/kg.

mg/kg ($p < .01$), whereas only the highest dose of MDMA induced a similar effect ($p < .05$). MA was unable to induce any significant effect on this behavior. Plus-maze total entries were not significant different [$F(18, 190) = 1,44$].

Active Avoidance Conditioning

As shown in Figure 4, one-way ANOVA revealed significant effects on the acquisition ability of the rat [$F(18, 190) = 12.50\ p < .0001$]. The post hoc test indicated that the conditioning performance was seriously impaired after the administration of all doses of DOI except the lowest ($p < .01$). The administration of MA was unable to induce any significant change in this behavior. In contrast, MDMA (5 and 10 mg/kg) induced a significant improvement in the capacity of pairing both stimuli ($p < .05$ and $p < .01$ for each dose, respectively). At these two doses, the profiles exhibited by the three drugs were significantly different ($p < .01$).

Behavioral Effects of MDA and MDE

The overall psychomotor effects of the i.p. administration of MDA are summarized in Figure 5. One-way ANOVA revealed significant effects of the administration of MDA on total motor activity [$F(3, 36) = 11.63$, $p < .0001$], number of rearings, [$F(3, 36) = 11.28$, $p < .0001$], grooming behavior, [$F(3, 36) = 48.89$, $p < .0001$], and number of head shakes [$F(3, 36) = 15.44$, $p < .0001$]. Subsequent Dunnett's test demonstrated that the drug induced a significant increase in total motor activity and locomotor activity [$F(3, 36) = 12.06$, $p < .0001$], only at the highest dose (10 mg/kg; see Figure 6). The number of rearings was significantly diminished at 5 and 10 mg/kg. The other motor behaviors (number of head shakes and time spent in grooming), were significantly depressed at all doses. In the elevated plus-maze (see Figure 7), ANOVA revealed significant effects of MDA on the percentage of entries into the open arms, [$F(3, 36) = 8.46$, $p < .0002$]; and on the percentage of time spent in the open arms [$F(3, 36) = 7.06$, $p < .001$]. Post hoc analysis showed a significant increase of the percentage of entries and of time spent in the open arms at 5 and 10 mg/kg. Plus-maze total entries were not significantly different [$F(3, 36) = 2,845$]. The effect of MDA on avoidance conditioning was also significant [$F(3, 36) = 20.11$, $p < .0001$], and the post hoc analysis demonstrated a biphasic effect: the lowest dose (1 mg/kg) significantly reduced the conditioned avoidance response, whereas the highest dose (10 mg/kg) enhanced the response (see Figure 8). When compared with the three reference drugs, MDA resembled the effects of MDMA in motor activity, locomotion, head shakes, and avoidance acquisition. In contrast, its effects on rearing and grooming, as well as in the elevated plus-maze exploration, bring MDA closer to the behavioral profile of DOI (see Table 3).

Figure 5 shows the overall effects of MDE on rat behavior. ANOVA revealed statistically reliable effects for total activity [$F(3, 38) = 6.68$, $p < .001$], number of rearings, [$F(3, 38) = 8.13$, $p < .0003$], grooming behavior, [$F(3, 38) = 32.08$, $p < .0001$], and number of head shakes [$F(3, 38) = 26.87$, $p < .0001$]. Subsequent Dunnett's test demonstrated that MDE induced a significant increase in total motor activity at the lower doses (1 and 5 mg/kg), and in locomotor activity [$F(3, 38) = 18.64$, $p < .0001$], at the higher doses (5 and 10 mg/kg; see Figure 6). The number of rearings was significantly increased only at 5 mg/kg. In an interesting finding, the number of head shakes showed an inverse dose-response relationship, with significant differences among the three doses considered ($p < .05$). The time spent in grooming was significantly less at all doses. In the elevated plus-maze (see Figure 7), ANOVA revealed significant effects of MDE on the percentage of entries into the open arms [$F(3, 38) = 8.11$, $p < .0003$], and on the percentage of time spent in the open arms [$F(3, 38) = 4.06$, $p < .05$], but Dunnett's post hoc analysis showed that only the

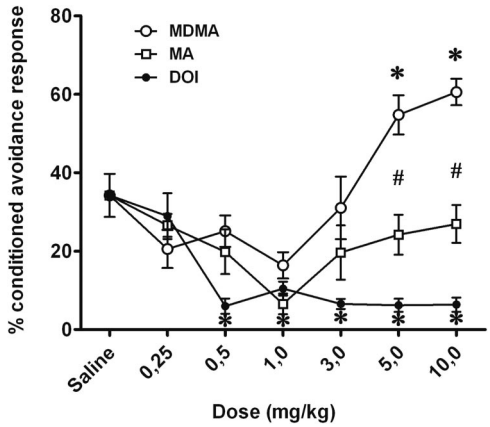

Figure 4. Dose-response curves for MDMA (○), MA (□), and DOI (●) after 50 trials of active avoidance conditioning. Significance symbols indicate differences referred to * controls or # between drugs ($p < .05$). Each point represents a mean ± SEM ($n = 11$). Saline was used as control. Doses were injected i.p. in a total volume of 1 ml/kg.

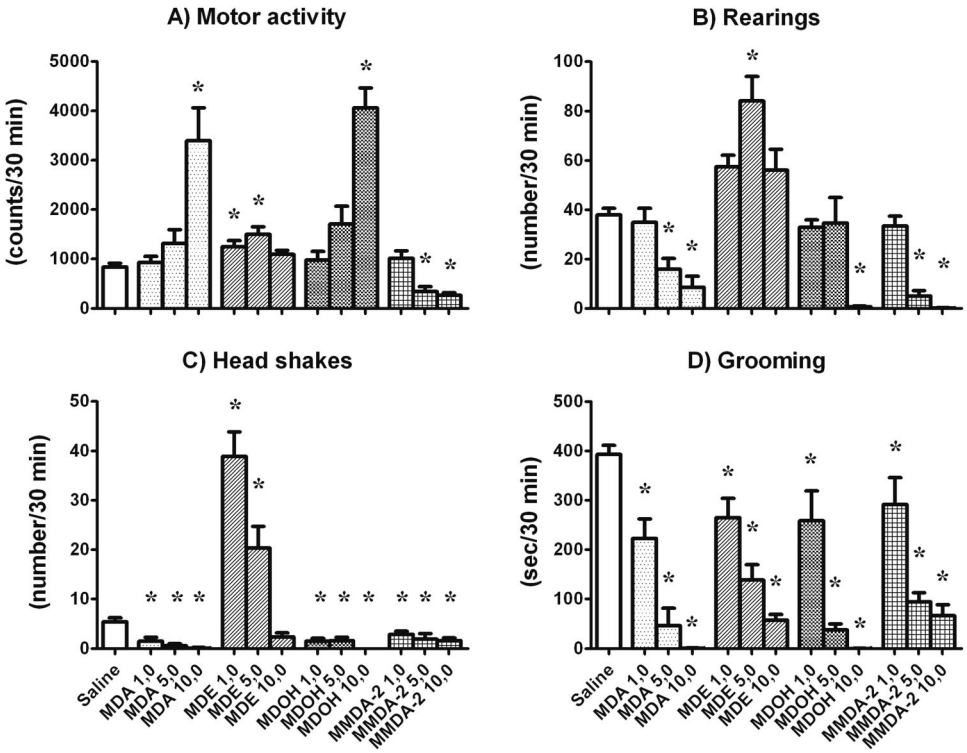

Figure 5. Spontaneous motor behavior for MDMA analogues. * Significant difference referred to controls ($p < .05$). Each point represents a mean ± SEM ($n = 11$). Saline was used as control. Doses (1, 5, and 10 mg/kg) were injected i.p. in a total volume of 1 ml/kg.

highest dose (10 mg/kg) significantly enhanced both variables. Plus-maze total entries were not significantly different [$F(3, 38) =$ 0,499]. The effect of MDE on avoidance conditioning was also significant [$F(3, 38) = 13.06$, $p < .0001$], and the post hoc analysis demonstrated again a biphasic effect, where the lower dose (1 mg/kg) significantly diminished acquisition, whereas the higher dose (10 mg/kg) enhanced it (see Figure 8). Therefore, MDE's effects cannot be easily matched with those elicited by MDMA, MA, or DOI. In fact, MDE resembles MDMA in its effects on locomotion, elevated plus-maze exploration and avoidance acquisition, and the effects of MA on motor activity and grooming. However, its profiles on rearing and head shake behaviors do not fit those of any of the prototypical drugs.

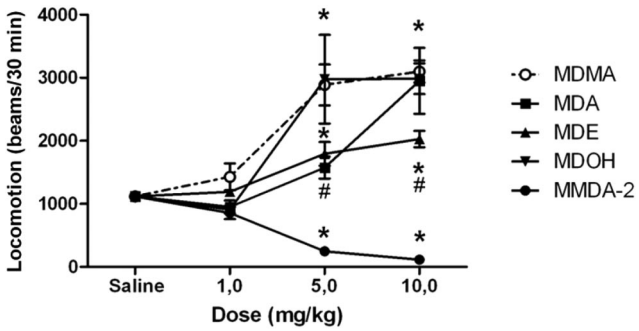

Figure 6. Dose-response curves for MDA (■), MDE (▲), MDOH (▼), and MMDA-2 (●) compared with the dose-response curve of prototypical drug MDMA. Significance symbols indicate differences referred to * controls or # between drugs ($p < .05$). Each point represents a mean ± SEM ($n \geq 8$). Saline was used as control. Doses were injected i.p. in a total volume of 1 ml/kg.

Behavioral Effects of MDOH and MMDA-2

The overall psychomotor effects of MDOH are shown in Figure 5. One-way ANOVA revealed significant effects of MDOH administration on total motor activity [$F(3, 38) = 29.97$, $p <$ .0001], number of rearings, [$F(3, 38) = 10.51$, $p < .0001$], number of head shakes [$F(3, 38) = 14.11$, $p < .0001$], and grooming behavior, [$F(3, 38) = 36.07$, $p < .0001$; see Figure 5]. Subsequent Dunnett's test demonstrated that MDOH induced a significant increase in total motor activity only at the highest dose (10 mg/kg), and locomotor activity [$F(3, 38) = 11.38$, $p < .0001$] at the two higher doses (5 and 10 mg/kg; see Figure 6). The number of rearings was significantly diminished only at 10 mg/kg. The other motor behaviors (number of head shakes and time spent in grooming), were significantly depressed at all doses. In the elevated plus-maze (see Figure 7), ANOVA revealed significant effects of MDOH on the percentage of entries into the open arms [$F(3, 36) =$ 6.08, $p < .005$], and on the percentage of time spent in the open arms [$F(3, 36) = 5.35$, $p < .005$]. Post hoc analysis showed a significant increase of the percentage of entries and of time spent in the open arms at 5 and 10 mg/kg. Plus-maze total entries were not significantly different [$F(3, 36) =$ 2,675]. The effect of MDOH on avoidance conditioning was also significant [$F(3, 36) = 6.39$,

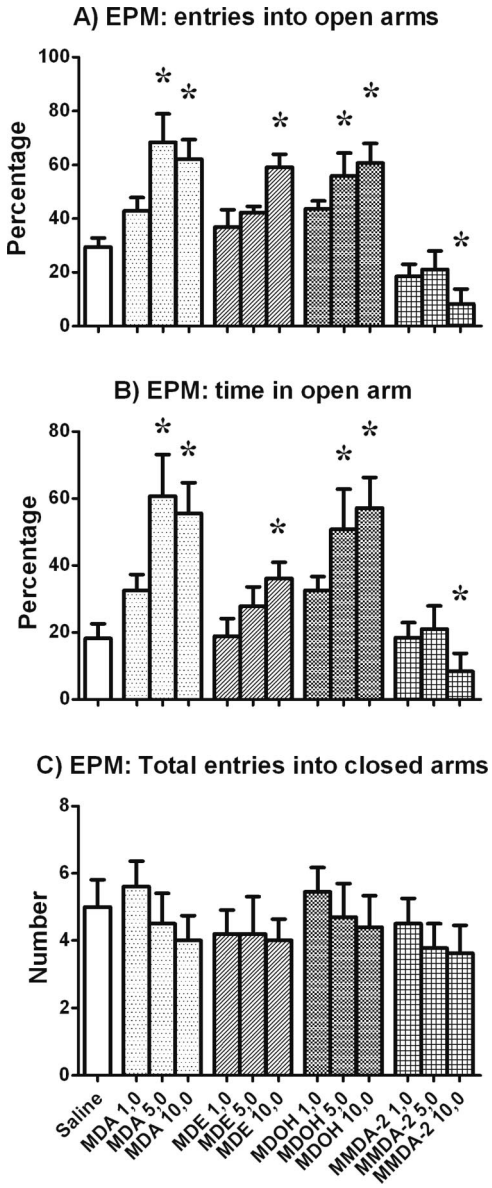

Figure 7. Effects of MDMA analogues in the elevated plus-maze. * Significant differences referred to controls ($p < .05$). Each point represents a mean ± SEM ($n \geq 8$). Saline was used as control. Doses (1, 5, and 10 mg/kg) were injected i.p. in a total volume of 1 ml/kg.

$p < .005$], and the post hoc analysis demonstrated that the lowest dose (1 mg/kg) significantly reduced the conditioned avoidance response, whereas the higher doses (5 and 10 mg/kg) were ineffective (see Figure 8). When compared with the three reference drugs, MDOH resembled the effects of MDMA in motor activity, locomotion, and head shakes. Rearing behavior and the elevated plus-maze exploration bring MDOH closer to the behavioral profile of DOI while the effects of MDOH on avoidance acquisition were similar to the MA profile.

The overall psychomotor effects of MMDA-2 administration are summarized in Figure 5. One-way ANOVA revealed significant effects of the administration of MMDA-2 on total motor activity [$F(3, 38) = 29.97$, $p < .0001$], number of rearings, [$F(3, 38) = 10.51$, $p < .0001$], number of head shakes [$F(3, 38) = 14.11$, $p < .0001$], and grooming behavior, [$F(3, 38) = 36.07$, $p < .0001$]. Subsequent Dunnett's test demonstrated that the drug induced a significant decrease in total motor activity, locomotor activity [$F(3, 38) = 11,38$, $p < .0001$; see Figure 6] and rearing behavior at the two higher doses (5 and 10 mg/kg). Grooming behavior was diminished at all doses administrated. The analysis of head shakes demonstrated a biphasic effect: the lower doses (1 and 5 mg/kg) significantly reduced this response, whereas the highest dose (10 mg/kg) enhanced the number of head shakes. In the elevated plus-maze (see Figure 7), ANOVA revealed significant effects of MMDA-2 on the percentage of entries into the open arms, [$F(3, 36) = 6.08$, $p < .005$]; and on the percentage of time spent in the open arms [$F(3, 36) = 5.35$, $p < .005$]. Post hoc analysis showed a significant decrease of the percentage of entries and of time spent in the open arms at 10 mg/kg. Plus-maze total entries were not significantly different, $F(3, 36) = 1,669$. The effect of MMDA-2 on acquisition was also significant [$F(3, 36) = 6.39$, $p < .005$], and the post hoc analysis demonstrated a significant reduction of conditioned avoidance response with all doses tested (see Figure 8). MMDA-2 resembled the effects of DOI in motor activity, locomotion, rearings, and avoidance acquisition. In contrast, its effects on grooming behavior fit well with the MA profile. However, head shakes as well as the elevated plus-maze exploration do not correspond to any of the prototype drugs.

Discussion

The main purpose of this work was to test if the profiles of the behavioral effects of the acute administration of racemic MDMA based on a sequence of psychomotor behaviors in the rat can be distinguished from those of MA and DOI on single behaviors and/or between different specific behaviors. The distinctive identifying patterns elicited can be used for a more accurate placement of MDMA analogues among the psychotropic amphetamines.

In agreement with our hypothesis, the dose-response curves obtained (0.25–10 mg/kg) showed qualitative differences between MDMA, MA, and DOI. Indeed, at doses higher than 1 mg/kg,

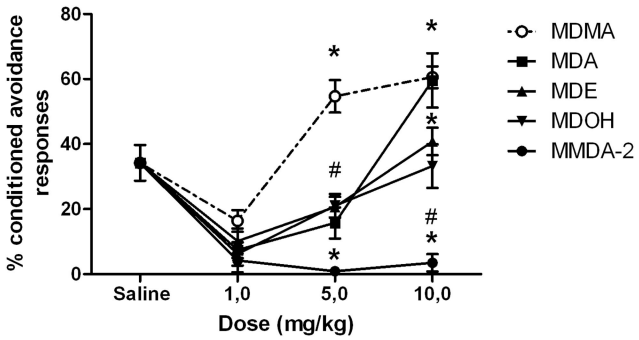

Figure 8. Dose-response curves for MDA (■), MDE (▲), MDOH (▼), and MMDA-2 (●) compared with the dose-response curve of prototypical drug MDMA. Significance symbols indicate differences referred to * controls or # between drugs ($p < .05$). Each point represents a mean ± SEM ($n \geq 8$). Saline was used as control. Doses were injected i.p. in a total volume of 1 ml/kg.

Table 3
Comparison of Behavioral Profiles of MDMA Analogues

	MDMA analogue (1–10 mg/kg)			
Behavior	MDA	MDE	MDOH	MMDA-2
Motor activity*	MDMA	MA	MDMA	DOI
Locomotion*	MDMA	MDMA	MDMA	DOI
Rearings**	DOI	—#	MDMA-MA	DOI
Head shakes**	MDMA-MA	—#	MDMA-MA	MDMA-MA
Elevated plus-maze**	DOI	MDMA-MA	DOI	—#
Active avoidance*	MDMA	MDMA	MA?	DOI

* Discriminative behaviors between MDMA, MA, and DOI. ** Discriminative behaviors between DOI and MDMA-MA. # Analogue profile does not match any of the reference drugs (MDMA, MA, and DOI).

some behaviors (total motor activity, locomotion, and acquisition ability) were found to exhibit dose-response curves reflecting differential pharmacological profiles. In addition, grooming behavior at doses lower than 1 mg/kg was also able to distinguish among MDMA, MA, and DOI. Other behaviors evaluated (head shakes, rearing, and plus-maze exploration) seem to differentiate DOI from both MDMA and MA, suggesting that these behaviors comprise a separate group which might be influenced by MDMA and MA in a similar manner.

The generation of behavioral profiles elicited by MDMA in animals through an appropriate choice of behavioral paradigms has been proposed as a reliable strategy to characterize the complex pharmacology of this drug (Piper, 2007). Nevertheless, integrated analyses using different behavioral approaches to compare the acute effects of MDMA with stimulants and/or hallucinogens are rather uncommon, and do not provide enough evidence to dispute the logic of recent papers where MDMA is lumped together with amphetamine and MA (Elliott & Beveridge, 2005; Cadet, Krasnova, Jayanthi, & Lyles, 2007; Berman, O′Neill, Fears, Bartzokis, and London, 2008). Moreover, publications addressing the construction of comparative behavioral profiles for MDMA and structurally related compounds are scanty. Gold, Koob, and Geyer (1988) proposed that MDMA and its analogue MDE induce psychomotor effects in rats compatible with a combination of stimulant- and hallucinogen-like properties, as hypothesized earlier (Beck & Morgan, 1986). This assumption was later shown not to be consistent with the actual effects induced by MDMA and MDE in humans (Shulgin & Shulgin, 1991). Some years later, Hegadoren, Martin-Iverson, and Baker (1995) published for the first time a comparative characterization of spontaneous psychomotor behaviors in the rat induced by MDMA and some related analogues at a single equimolar dose of MDMA, PMA, and amphetamine, as well as MDA and MDE. Here, the choice of the reference drugs was not appropriate, as PMA was considered to be a hallucinogen and the characterization of MDA and MDE was confusing. On the other hand, as LSD (lysergic acid diethylamide) is viewed as a characteristic hallucinogen, the psychotropic effects of synthetic hallucinogenic drugs based on the phenylethylamine moiety (all conceptually derived from the naturally occurring mescaline) were considered as "ergoline-like" molecules for a long time (Nichols, 2004). Nevertheless, psychotropic phenylalkylamines also possess pharmacological features of their own, reflected not only by different modes of binding at 5-$HT_{2A/2C}$ receptors (and their lack of affinity for other major receptor subtypes) but also at a behavioral level (Monte et al., 1998; Chambers et al., 2003; Nichols, 2004). Therefore, for the purpose of our work, the choice of reference drugs to compare them with MDMA was made most carefully, considering undoubted structurally related representatives of the stimulant and hallucinogenic amphetamine derivatives (MA and DOI, respectively) which could allow pharmacological comparisons on a more rational basis.

Aside from the evidence that MA, d-amphetamine and MDMA enhance locomotion in rats (comprehensively summarized in Green et al., 2003 and Hall et al., 2008) and hallucinogenic 5-HT_{2A} agonists like DOI diminish it (Krebs & Geyer, 1994), our analysis of locomotion profiles indicates that MDMA, MA, and DOI elicit different behavioral patterns. Indeed, MDMA enhanced locomotion increasingly up to 10 mg/kg. In contrast, MA exhibited a maximum effect at 3 mg/kg and fell to control values at 10 mg/kg. As already reported, the MA pattern seems to be a consequence of reaching "stereotypy-inducing" doses reported for MA in which the animal remains in a focused stereotypy state where its psychomotor skills are altered (Segal & Kuczenski, 1997). In this regard, it should be noted that local perseverative movements compatible with stereotypes (Randrup & Munkvad, 1967) in treated rodents were only observed during our experiments with doses of 5 and 10 mg/kg of MA. On the other hand, previous evidence obtained using the Behavioral Pattern Monitor (BPM) showed that drugs such as d-amphetamine and MDMA exhibited marked differences in the structure of their behavioral activity patterns in the rat (Paulus & Geyer, 1991). In that work, alterations of rat locomotion in the presence of different doses of a number of psychoactive substances were analyzed using both spatial (d) and temporal (α) scaling exponents along a d-α plane. Here, MDMA and d-amphetamine were shown to alter α and d differently in a dose-dependent manner. In this regard, it should be noted that although our locomotion data were not obtained using the BPM, the corresponding dose-response curves for MDMA and MA (which might induce similar effects in the BPM as d-amphetamine) essentially agree with the observation that MDMA and MA generate different profiles in locomotion activity. Aside from this evidence, as the unique locomotion activity pattern induced by MDMA in rodents seems to be strongly dependent on the differential activation of central D_1, D_2, and D_3 receptors (Starr & Starr, 1986; Risbrough et al., 2006), one could speculate that the differences observed between MDMA and MA might be related to a differential capability of each drug to activate central dopaminergic receptors and/or to bind to monoamine transporters. The

latter might be expressed as different availability ratios for dopamine and serotonin, as already reported for MDMA (Baumann, Clark, & Rothman, 2008), an effect that seems to be dependent on the ability of the drug to activate postsynaptic 5-$HT_{1B/1D}$ and presynaptic 5-HT_{2B} receptors (Bankson & Cunningham, 2001; Doly et al., 2008) when administered acutely. Low doses of MDMA might favor 5-HT_1 receptor activation, whereas high doses of the drug might promote 5-HT_{2A} receptor activation (McCreary, Bankson, & Cunningham, 1999). In addition, high doses of MDMA might enhance dopamine release by direct activation of 5-HT_{2A} receptors located at dopaminergic neurons which in turn might enhance locomotion (Herin, Liu, Ullrich, Rice, & Cunningham, 2005).

Locomotion activity in rodents has been proposed for a long time as a reliable pharmacological parameter for the behavioral characterization of hallucinogens. Adams and Geyer (1985) demonstrated that whereas high doses of LSD (30–80 μg/kg) depress locomotion in a specific time-scale window, low doses (10 μg/kg) enhance this behavior. Moreover, effects may vary depending on whether the animal is free or forced to explore, reinforcing the idea that locomotion alteration induced by LSD is an indirect manifestation of a potentiated responsiveness to threatening stimuli in the animal (probably reflecting a diminished capability to manage stress) which might be correlated with at least some of the effects induced by LSD in humans. Similar effects have been reported for different types of hallucinogens, including those based on the phenylethylamine moiety (Geyer et al., 1979). Certainly, our results for DOI do not seem to be consistent with the expected effects considering the structural similarity between DOI and its previously tested congeners. Instead, they extend the observation that a low dose of DOI (0.25 mg/kg) induces a significant decrease in locomotion in the rat by activating serotonergic 5-HT_{2A} receptors (Krebs-Thomson et al., 1998), an effect that can be reversed in amount and distance moved by subthalamic nucleus deep brain stimulation (Hameleers, Blockland, Steinbusch, Visser-Vandewalle, & Temel, 2007). In an interesting finding, the behavioral profile of DOI in mice resembles that of LSD in rats, where it produces an inverted U-shaped dose-response function. Moreover, the effects of DOI on locomotion in mice are proposed to be mediated in part by 5-HT_{2C} receptors, which might exert opposing effects to 5-HT_{2A} receptors in controlling locomotion activity (Halberstadt et al., 2009). Taking into account that 5-HT_{2C} receptors are present in rats as well as in mice, one might speculate about the possibility that the functional regulatory link between dopaminergic neurotransmission and 5-HT_{2C} receptors in rats (but not in mice) is qualitatively similar to that between DA and 5-HT_{2A} receptors.

Head shake responses in rats, which are mediated by activation of serotonergic 5-HT_{2A} receptors located in the medial prefrontal cortex (Willins & Meltzer, 1997), are known to be enhanced by classical hallucinogens such as DOI, an effect that is consequently blocked after co-administration of a 5-HT_{2A} receptor antagonist (Schreiber et al., 1995; Dursun & Handley, 1996) reflecting the high affinity of DOI for this receptor subtype (Nelson, Lucaites, Wainscott, and Glennon, 1999). Under our experimental conditions, acute administration of MDMA totally abolished head shake behavior at 10 mg/kg, probably reflecting its very low affinity for 5-HT_{2A} receptors, which might not be relevant to elicit behavioral effects in the rat after single acute exposure to a nontoxic dose (Green et al., 2003). This evidence contrasts with the enhancing effects on head shake behavior mediated by DOI after intermittent intake of MDMA in rats, highlighting the differences between acute and nonacute MDMA exposure (Biezonski, Courtemanche, Hong, Piper, and Meyer, 2009). On the other hand, at 10 mg/kg MA also abolished this behavior, whereas DOI significantly increased it. Therefore, the DOI profile in the dose range 1 – 10 mg/kg allows us to distinguish it from the corresponding MDMA and MA profiles, not supporting the notion that MDMA might be hallucinogenic (Check, 2004).

With regard to grooming, our results clearly show that this behavior allows us to differentiate among MDMA, MA and DOI in the dose range 0.25–1 mg/kg. One could propose that the differences observed here might to some extent reflect differences in the way in which each drug might alter dopaminergic and/or serotonergic neurotransmission (Green et al., 2003; Nichols, 2004; Sulser, Sonders, Poulsen, & Galli, 2005) which seems to be manifest only at doses under 1 mg/kg.

MA and DOI may induce anxiety-like states, psychotic states or even panic attacks in humans (Geyer & Vollenweider, 2008; Cruickshank & Dyer, 2009; Sareen, Elliot, Green, & Moran, 2006). In addition, transient related symptoms like aggression, anger or even depression have been reported after taking MDMA, especially in polydrug users (Bond, Verheyden, Wingrove, & Curran, 2004; Guillot, 2007), probably reflecting a link between MDMA toxicity after chronic drug intake and the occurrence of neuropsychiatric disorders (Cadet et al., 2007). Unfortunately, these reports do not properly consider the differences between acute and chronic effects. Our results showed that the acute administration of MDMA, MA or DOI does not induce anxiogenic-like effects in the elevated plus-maze over the whole dose range considered (0.25–10 mg/kg). In contrast, several publications report anxiogenic effects for MDMA in rats and mice in the open field model, but only at doses of 10 mg/kg or higher, and under high light levels (Gold, Koob, & Geyer, 1988; Powell et al., 2004). This discrepancy can be explained by considering that we evaluated plus-maze exploration under low light conditions which are designed to primarily measure anxiolytic-like drug effects (Mora et al., 1996). In this regard, it should be mentioned that such discrepancies may be induced not only by different test situations but also by differences between different models used to test anxiolytic/anxiogenic effects (Gold et al., 1988; Morley & McGregor, 2000), as well as by the basal anxiety state of the animal at the beginning of the experiment, or even the animal strain used (Green & McGregor, 2002). The latter might explain the apparent contradiction between the entactogenic effects elicited by MDMA in humans and the reports of anxiogenic activity of the drug in rats (Navarro, Rivera, Maldonado, Cavas, and De la Calle, 2004). Our results support those of previous publications showing that the acute administration of MDMA in mice at doses as low as 1 mg/kg elicited anxiolytic-like effects in the plus-maze (Navarro & Maldonado, 2002), and are also in agreement with a recent proposal regarding its usefulness in the treatment of anxiety in humans after nonchronic exposure (Johansen & Krebs, 2009).

On the other hand, DOI (which consistently diminished locomotion in a dose-independent manner) was shown to elicit anxiolytic-like effects in this model, supporting previous reports of the acute effects of this drug (Onaivi, Bishoprobinson, Darmani, and Sanders-Bush, 1995; Dhonnchadha, Hascoët, Jolliet, and

Bourin, 2003; Massé, Hascoët, Dailly, and Bourin, 2006; Ripoll, Hascoët, and Bourin, 2006). DOI might achieve these effects because of its high affinity for 5-HT_{2A} receptors, as proposed earlier (Green et al., 2003; Bourin & Dhonnchadha, 2005).

With regard to MA, this drug is reported to induce anxiety in humans (Rawson, González, and Brethen, 2002) depending on the dose used (Cruickshank & Dyer, 2009). In contrast, our results extend other recent evidence indicating that MA does not exhibit convincing anxiogenic properties in the Y maze at 1 and 2 mg/kg in hooded rats (Herbert & Hughes, 2009). Therefore, one may propose that in rats there is a nondetrimental dose range for MA as seems to be the case in humans.

The therapeutic use of MDMA has been limited mainly because of growing evidence supporting the notion that this drug may severely impair cognitive function in humans (Zakzanis & Campbell, 2006; Kalechstein, De la Garza, Mahoney, Fantegrossi, and Newton, 2007). Such impairment is usually seen in rats after chronic treatment with the drug (Galizio, McKinney, Cerutti, & Pitts, 2009), although there is also evidence of its occurrence regardless of the dose regimen (Skelton et al., 2008). In contrast, our results indicate a significant enhancement of acquisition induced by MDMA at the 5–10 mg/kg dose level. One might explain the acquisition results as a direct consequence of hyperlocomotion, as already reported for MDMA in mice at 10 and 30 mg/kg, which led some data to be disregarded for this reason (Trigo, Cabrero-Castel, Berrendero, Maldonado, and Robledo, 2008). In order to avoid this pitfall, we considered only those responses where a moving animal escaped from one chamber to the other changing its direction of movement upon receiving the nonconditioned stimulus. Our results also support other evidence indicating associative learning enhancement in rabbits (Romano & Harvey, 1994) and acquisition enhancement induced by acute administration of MDMA in the operant responding paradigm at 3.2 and 5.6 mg/kg in rats (Byrne, Baker, & Polling, 2000). In contrast, the consistent impairment of acquisition induced by DOI agrees with the classical descriptions of the distinctive effects of hallucinogens in humans (Nichols, 2004). MA did not elicit any changes in avoidance conditioning at the 5–10 mg/kg drug level, extending previous results in the same experimental model (Bustamante, Díaz-Véliz, Paeile, Zapata-Torres, and Cassels, 2004), and allowing us to differentiate among the three reference drugs at the doses of 5 and 10 mg/kg using this behavioral paradigm.

MDA and MDE are popular MDMA analogues which are reported to exert MDMA-like effects in humans (Green et al., 2003; Shulgin & Shulgin, 1991). Moreover, some polydrug users view MDE as an acceptable MDMA substitute (Gouzoulis-Mayfrank & Hermle, 1998; Gouzoulis-Mayfrank, 2001). Nevertheless, its pharmacological properties seem to be much more complex, including stimulant- and hallucinogenic-like effects (Hermle, Spitzer, Borchardt, Kovar, and Gouzoulis, 1993; Shulgin & Shulgin, 1991). In agreement with these descriptions, our results indicate that MDA and MDE only exhibited similar effects to MDMA in two specific behavioral paradigms (locomotion and active avoidance response). In other models, differences between MDA and MDE were evident: in rearing behavior MDA seemed to resemble DOI, whereas MDE did not fit with any of the reference drugs; in head shake behavior MDA was similar to MDMA, and again, MDE showed a pattern of its own. In the elevated plus-maze the effects induced by MDA were similar to the corresponding DOI profile and MDE was similar to MDMA (see Table 3).

With regard to MDA, as this drug exhibits behaviors well allotted between MDMA and DOI, our results do not seem to support the assumed pharmacological similarities between MDA and MDMA in humans (Parker, Marona-Lewicka, Kurrasch, Shulgin, and Nichols, 1998), but they are in agreement with an early report of the effects of MDA on associative learning in the rabbit (Romano, Bormann, & Harvey, 1991) and also with its early proposed hallucinogenic-like properties (Glennon & Young, 1982; Glennon, Young, & Soine, 1984; Nichols, Hoffmann, Oberlender, Jacob, and Shulgin, 1986). The latter might be related to the higher affinity of (−)-MDA for 5-HT_{2A} receptors compared to (+)-MDA or either MDMA enantiomer in mice (Rosencrans & Glennon, 1987). It should be noted that in the present work we only used racemic mixtures. Therefore, it is encouraging that the comparison of different behaviors occurring simultaneously might reflect the complex in vivo profile of MDA as well. Nevertheless, our results with racemic MDA for head shake behavior seem to be in apparent disagreement with previously reported data for S-(+)-MDA in rats (Hiramatsu, Nabeshima, Kameyama, Maeda, and Cho, 1989). This purified MDA isomer was reported to enhance the wet-dog shake behavior in the dose range 5–10 mg/kg during the first 30 min after subcutaneous injection. Despite the link between wet-dog shakes and head shakes (less and more common when simultaneously measured in the same time period, which might be reflected in the control values obtained for further comparisons with treated animals) and the expected differences in pharmacological potency between isolated isomers and racemic mixtures, one possible explanation for the discrepancy might be related to the use of nonparametric statistics for data processing. In contrast, our results were processed using parametric statistics only.

The effects of MDE on head shake behavior deserve a separate comment. In contrast to all expectations derived from the classical descriptions of its effects in humans (Shulgin & Shulgin, 1991), MDE exhibited a new profile in head shake behavior. To the best of our knowledge, this is the first time that an inverse dose-response curve for head shakes is reported for an MDMA analogue or even a structurally related psychotropic substance. Neither MDMA nor DOI have been reported to elicit such a profile in the rat, indicating a separate pharmacological identity for MDE. Further experiments are certainly required to elucidate the meaning of these intriguing effects in terms of the in vivo pharmacology of MDE and its associated molecular mechanism of action. It should be noted that, in spite of the fact that MDE has very low affinity for 5-HT receptors, some users have reported alterations of perception similar to those induced by the hallucinogen DOM. Therefore, a possibly indirect activation of 5-HT_2 receptors remains to be confirmed (Freudenmann & Spitzer, 2004). Nevertheless, the latter is not supported by the fully reciprocal generalization between MDE and MDMA in the drug discrimination task in rats, which excluded any similarity with structurally related stimulants or hallucinogens (Schechter, 1988; Glennon & Misenheimer, 1989; Glennon, Yousif, & Patrick, 1988).

The MDMA analogues MDOH and MMDA-2 belong to the large group of phenylalkylamines described as probably possessing psychoactive properties which might resemble the psychological effects elicited by MDMA in humans (Shulgin & Shulgin, 1991). As a consequence of this assumption, an increasing number

of structurally MDMA-like substances are present in "ecstasy" tablets sold in the informal market (Teng, Wu, Liu, Li, and Chien, 2006). Nevertheless, little has been published regarding key aspects of their mechanism of action at the molecular level or their behavioral pharmacology in animal models. Indeed, the description of their pharmacological effects is restricted to subjective reports of occasional human users or quite generic descriptions (Valter & Arrizabalaga, 1998).

MDOH exhibited similar profiles in locomotion and head-shakes to MDMA, whereas it showed a DOI-like profile in the elevated plus-maze and grooming behavior together with an MA-like acquisition profile (see Table 3). This behavioral evidence is in agreement with some earlier proposals suggesting a complex behavioral spectrum for this compound, including MA- as well as DOI-like effects (Braun, Shulgin, & Braun, 1980). Furthermore, our results also support earlier work indicating that MDOH possesses an even weaker amphetamine-like component than MDMA in drug discrimination studies (Glennon & Misenheimer, 1989), as well as recent data indicating that MDOH has a strongly diminished affinity for monoamine transporters compared to MDMA. Consequently, one might expect that, in spite of the subjective description of its effects in humans, MDOH might be a weak mediator of typical MDMA-like effects (Montgomery et al., 2007).

Although little is known about the behavioral effects of MMDA-2 (Shulgin & Shulgin, 1991), this drug is included in several designer drug directories as a potentially dangerous MDMA analogue which can be distinguished chromatographically from other synthetic phenylalkylamines with hallucinogenic properties (Min et al., 2008). It possesses a metabolic profile of its own, suggesting that different dose ranges compared to MDMA might elicit different pharmacological effects and/or toxicity in humans bearing different CYP2D6 alleles (Ramamoorthy, Tyndale, & Sellers, 2001). Our data show that MMDA-2 exhibited behavioral properties which do not support the impression that this drug might elicit MDMA-like effects (Shulgin & Shulgin, 1991). Rather, our evidence is in agreement with the coexistence of independent mixed DOI-like behavioral properties: First, its DOI-like profiles in locomotion and in the active avoidance conditioning model do not correlate well with its effects on head shake behavior in the dose range considered (see Table 3). Such a profile is not paralleled by any of the reference drugs. Second, its effects on plus-maze exploration seem to be compatible with an anxiogenic-like profile (although a possible confounding influence of a DOI-like hypomotility cannot be discarded). Unfortunately, molecular data regarding specific key targets of MMDA-2 are not currently available for further analysis of our behavioral evidence. In this context, it would be useful to perform binding experiments with MMDA-2 at 5-$HT_{2A/2C}$ receptors in order to elucidate a possible similarity of this compound to the hallucinogen DOI. On the other hand, as already mentioned for MDOH, the quantification of its ability to block monoamine transporters might help to place this analogue more precisely among MDMA, MA, and DOI. In this regard, it should be mentioned that 2C-B (2,5-dimethoxy-4-bromophenylethylamine), a bromo-deiodo phenylethylamine homologue of DOI with very low efficacy at 5-HT_{2A} receptors (Moya et al., 2007), has been shown to be a low-affinity, noncompetitive selective blocker of the serotonin transporter, highlighting its hallucinogenic nature (Montgomery et al., 2007). Therefore, the determination of binding affinities for monoamine transporters and 5-HT_2 receptors might be a first step in the search for molecular correlates of the behavioral data presented here.

Taken together, the behavioral characterization of the MDMA analogues selected by us supports our general hypothesis, highlighting the usefulness of integrated behavioral profiles covering a wide range of spontaneous psychomotor responses in the rat to establish not only similarities but especially differences between them. Paulus and Geyer (1992) evaluated MDMA and a group of MDMA analogues (including MDA and MDE), together with MA and DOI at different doses covering a similar dose range to that of the present study. They demonstrated that MDMA, MDA, MBDB, and MDE elicited three different locomotion dose-response patterns in a stereo-selective manner. These effects were different from those produced by DOI and MA which represented two opposite extremes of locomotion alteration: a reduced amount of motor activity combined with a decrease in its structure (DOI), and an increased amount of activity together with an unaltered structure (MA). As noted by the authors, the differences observed considering a single behavioral paradigm might not provide all the experimental evidence required to distinguish MDMA and MDMA-like analogues from stimulant or hallucinogenic compounds. In this regard, our results with racemic mixtures confirm this proposal, and are in agreement with the prediction that MDMA analogues can alter unconditioned motor behavior in the rat in different ways to MA and DOI. Moreover, they support the existence of these differences in several specific behavioral paradigms.

In conclusion, the main findings of our work can be summarized as follows: first, unlike the responses elicited by a single dose of MDMA, MA, or DOI, the pharmacological profiles of these three structurally related reference drugs differ in specific behavioral paradigms in the rat (i.e., locomotion, grooming behavior and the active avoidance conditioning models), allowing full discrimination among these drugs or to distinguish DOI from MDMA and MA (i.e., head shakes, rearing, elevated plus-maze). Second, the evaluation of two well known MDMA analogues (MDA and MDE) showed mixed behavioral profiles sharing some properties with MDMA, MA, and DOI, indicating that they are not as equivalent to MDMA as suggested by their recreational use. Third, the application of this approach to characterize two further MDMA analogues which are assumed to induce MDMA-like effects (MDOH and MMDA-2) indicated mixed behavioral effects also, highlighting the usefulness of constructing behavioral profiles to avoid misinterpretations of single behavior and/or dose data. Taken together, our results support the notion that the ability to discriminate between different drugs in the rat based on their behavioral profiles and those of appropriate reference drugs can be used advantageously to characterize other psychoactive MDMA analogues.

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Received January 26, 2010
Revision received June 29, 2010
Accepted June 30, 2010 ■