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ScienceDirect
Pharmacology, Biochemistry and Behavior 88 (2008) 318–331
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

MDMA (*N*-methyl-3,4-methylenedioxyamphetamine) and its stereoisomers: Similarities and differences in behavioral effects in an automated activity apparatus in mice

Richard Young *, Richard A. Glennon

Department of Medicinal Chemistry, School of Pharmacy, Box 980540, Virginia Commonwealth University, Richmond, Virginia 23298, United States

Received 1 May 2007; received in revised form 28 August 2007; accepted 6 September 2007
Available online 14 September 2007

Abstract

Racemic MDMA (0.3–30 mg/kg), S(+)-MDMA (0.3–30 mg/kg), R(−)-MDMA (0.3–50 mg/kg) and saline vehicle (10 ml/kg) were comprehensively evaluated in fully automated and computer-integrated activity chambers, which were designed for mice, and provided a detailed analysis of the frequency, location, and/or duration of 18 different activities. The results indicated that MDMA and its isomers produced stimulation of motor actions, with S(+)-MDMA and (±)-MDMA usually being more potent than R(−)-MDMA in measures such as movement (time, distance, velocity), margin distance, rotation (clockwise and counterclockwise), and retraced activities. Interestingly, racemic MDMA appeared to exert a greater than expected potency and/or an enhanced effect on measures such as movement episodes, center actions (entries and distance), clockwise rotations, and jumps; actions that might be explained by additive or synergistic (i.e. potentiation) effects of the stereoisomers. In other measures, the enantiomers displayed different effects: S(+)-MDMA produced a preference to induce counterclockwise (versus clockwise) rotations, and each isomer exerted a different profile of effect on vertical activities and jumps. Furthermore, each isomer of MDMA appeared to attenuate the effect of its opposite enantiomer on some behaviors; antagonism effects that were surmised from a lack of expected activities by racemic MDMA. S(+)-MDMA (but not R(−)-MDMA), for example, produced an increase in vertical entries (rearing) and a preference to increase counterclockwise (versus clockwise) rotations; (±)-MDMA also should have induced such effects but did not. Apparently, R(−)-MDMA, when combined with S(+)-MDMA to form (±)-MDMA, prevented the appearance of those increases (from control) in activities. Similarly, R(−)-MDMA (but not S(+)-MDMA) produced increases in episodes (i.e. jumps) and vertical distance that racemic MDMA also should have, but were not, exhibited. Evidently, the presence of S(+)-MDMA in the racemic mixture inhibited the appearance of those increases (from control) in behavior. Taken together, the various and complex effects of MDMA and its stereoisomers are noted and a strategy is suggested for future studies that stresses the importance of steric effects and interplay, probable interaction(s) with various neurotransmitters, and interaction(s) with the particular behavioral or biological event (or action) being measured.

Keywords: Behavior; Designer drug; Drug abuse; Ecstasy; Enantiomers; MDMA; Optical isomers

MDMA (*N*-methyl-3,4-methylenedioxyamphetamine, 3,4-methylenedioxymethamphetamine) is commonly known as "Ecstasy, XTC, X or Adam". In humans, MDMA reportedly produces psychological and physiological effects that include a sense of euphoria, greater tolerance of the views and feelings of other people, and increases in wakefulness, endurance, well being, sexual arousal, and social abilities (e.g., Cohen, 1995; Peroutka et al., 1988; Siegel, 1986). Although the exact mechanism(s) of action of MDMA is not known with certainty, its effects are thought to be the result of biochemical interactions that influence, to various degrees, the brain monoamine neurotransmitters serotonin (5-HT), dopamine (DA), and norepinephrine (NE) (e.g., Baumann et al., 2007; Liechti et al., 2000; Liechti and Vollenweider, 2001; Nader et al., 1989; Stuerenberg et al., 2002).

In animals, the administration of MDMA is reported to produce minimal or significant alterations in motor activity.

* Corresponding author. Department of Medicinal Chemistry, Box 540 School of Pharmacy 410 North 12th Street Virginia Commonwealth University Richmond, Virginia 23298-6540, United States. Tel.: +1 804 828 7403; fax: +1 804 828 7625.
E-mail address: ryoung@vcu.edu (R. Young).

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doi:10.1016/j.pbb.2007.09.002

Studies in non-human primates, for example, have reported that MDMA exerts a negligible impact on general activity (Crean et al., 2006; Taffe et al., 2006). On the other hand, studies in rodents have shown (with some exceptions) that MDMA increases motor activity and decreases rearing behavior. Moreover, a few studies have reported that the heightened activity occurred along the margins of an enclosed arena with an associated decreased number of entries into the center area (Bhattacharya et al., 1998; Compan et al., 2003; Daws et al., 2000; Fantegrossi et al., 2003, 2005; Fletcher et al., 2002; Fone et al., 2002; Glennon et al., 1988; Gold et al., 1988, 1989; Gold and Koob, 1989; Gurtman et al., 2002; Kehne et al., 1996; Maldonado and Navarro, 2000; Marston et al., 1999; McNamara et al., 1995; Modi et al., 2006; O'Loinsigh et al., 2001; Powell et al., 2004; Scearce-Levie et al., 1999; Spanos and Yamamoto 1989; Yeh and Hsu 1991; for exceptions, see Bexis and Docherty 2006, Bhattacharya et al., 1998, and Matthews et al., 1989). It is also noted that the chemical structure of MDMA contains a chiral center and, thus, exists as a pair of optical isomers: S(+)-MDMA and R(−)-MDMA. As such, it is reasonable to assume that one of the two isomers might be more potent, or more responsible, for producing certain pharmacological action(s), relative to the racemate. In rodent studies, S(+)-MDMA increases (in rats and mice) motor activity and decreases (in rats and mice) or increases (in rats) rearing activity (Bankson and Cunningham 2002; Bubar et al., 2004; Callaway et al., 1990, 1991, 1992; Callaway and Geyer 1992; Herin et al., 2005; McCreary et al., 1999; Rempel et al., 1993; Russell and Laverty 2001). In comparison, R(−)-MDMA is reported to produce less stimulation of motor activity (Fantegrossi et al., 2003, 2005; Glennon et al., 1988; Paulus and Geyer 1992). Lastly, the relative potencies of (±)-MDMA and its enantiomers to alter motor behavior have been compared and the results indicate that S(+)-MDMA is slightly more potent, slightly less potent, or equipotent to racemic MDMA; R(−)-MDMA is reported to be notably less potent than (±)-MDMA or S(+)-MDMA (Bengel et al., 1998; Fantegrossi et al., 2003, 2005; Glennon et al., 1988; Paulus and Geyer 1992).

An enclosed chamber that is equipped with photo-beams is one of the most widely used devices to investigate the relatively spontaneous behavioral reaction of an animal to a drug. In general, the number of photo-beam breaks usually scores activity. It has been argued, however, that animals show a behavioral repertoire in an arena that is infinitely richer than simply an overall tally of breaks of photo-beams (e.g., Van Abeelen, 1963). A rodent's set of activities may include, for example, rearing, rotations, jumps, center region entries, margin area activity, and repetitive actions (e.g. stereotypy). In most of the aforementioned studies that examined the effects of MDMA (and/or its enantiomers) on behavior, only total photo-beam breaks for a test session were recorded and, thus, many different kinds of action may (or may not) have been compiled into a single score. A typical study consisted of an animal placed in an unfamiliar (or familiar) enclosure, surrounded by one set (for floor-plane movement) or two sets (for floor-plane movement and rearing activity) of photo-beams. The effect of MDMA on behavior consisted of the number of photo-beam breaks that occurred during a test period. Thus, MDMA and its stereoisomers have not been compared, in detail, with an instrument that dissociates their potential effects on various components of an animal's repertoire of behavior. In the present study, the behavioral effects of these agents were evaluated more extensively in a fully automated and computer-integrated apparatus that was specifically designed for mice and that incorporated eighteen measures of activity.

1. Materials and methods

1.1. Animals

The subjects used in these experiments were male ICR mice (Harlan Sprague Dawley Inc., Indianapolis, IN, USA), weighing 27 to 34 g at the time of testing. Animals were housed in groups of 5 in solid-bottomed plastic cages (38 × 22 × 15 cm) in a temperature (~22 °C)- and humidity (~50%)-controlled room. A standard 12:12 h light:dark cycle (lights on at 0700) was used and food and water were available *ad lib*. The experiments were conducted according to the standards set by the Institutional Animal Care and Use Committee (IACUC) of Virginia Commonwealth University and the NIH Guide for Care and Use of Laboratory Animals.

1.2. Apparatus

Tests of motor activity (Truscan© for mice, Coulbourn Instruments, Allentown, PA) were conducted in three chambers (model E63-10; 26 cm × 26 cm × 39 cm). The walls of a chamber were transparent and surrounded by two rings of infrared photo detectors (model E63-12): one located ~1.75 cm above the floor of the drop-pan to measure floor-plane activities and one located ~6.5 cm above the floor of the drop-pan to measure vertical (i.e. rearing) activities. Each ring contained an array of 16 × 16 infrared detectors (spaced 1.524 cm (0.6 in) apart), which were interfaced to a computer for the continuous recording of coordinates of a mouse's location. In this system, coordinates of an animal's body center were determined by scans of the infrared beams, which located the weighted shadow-center of the mouse (scan rate = 1 kHz). The locations of new coordinates were analyzed with "run-time, date stamps." The activity monitor automatically recorded the following behavioral measures: (a) *Movement Time* (s) — the sum of elapsed time (i.e. out of 1800 s) of all movements in the floor plane; (b) *Movement Distance* (cm) — the sum of all vectored coordinate changes in the floor plane; (c) *Velocity* (cm/min) — average speed of all floor plane coordinate-change defined movements; (d) *Movement Episodes* (total movements in the floor plane) — when a mouse is placed in an environment, its movements alternate between progressions and stops (i.e. movement episodes). In the current test system, a movement episode by a mouse is defined as a series of successive coordinate changes for at least one sample interval with no stop or "rest"; rest occurs when an animal has the same coordinates for more than one sample interval; (e) *Margin distance traveled* (cm) — the total distance traveled within a 2.5-beam margin-of-space that is toward the interior walls; (f) *Margin time* (s) — the total time

spent within a 2.5-beam margin-of-space that is toward the interior walls; (g) *Center arena entries* — the number of times the animal enters the center of the arena, which is defined as the region that is more than 2.5-beam spaces away from the interior walls of the arena; (h) *Center distance* (cm) and (i) *Center time* (s) — the total amount of distance (cm) traveled and time (sec) spent in the center of the arena, respectively; (j) *Clockwise* (CW) *center point rotation counts* — tallied when the animal completes a clockwise turn in 4 sequential (i.e. radial contiguous) quadrants about the center (see above) of the arena; (k) *Counterclockwise* (CCW) *center point rotation counts* — recorded when the animal completes a counterclockwise turn in 4 sequential (i.e. radial contiguous) quadrants about the center of the arena; (l) *Retraced local movements*, termed "Stereotypy-2 Moves"(STPY-2 Moves) by the software program of the apparatus — defined as coordinate changes by a mouse that are less than ± 1.499 beam spaces in the floor plane arena and then back to the original point that do not exceed 2 s apart. The term *local* refers to the fact that an animal's movement does not produce a change in location that is far from a starting point. Such movement by a mouse could be indicative of "exploratory behavior" or considered an aspect of, but neither synonymous with nor interpreted as, traditionally defined stereotypy (e.g., head weaving or bobbing, sniffing, ear scratching, biting, and/or chewing). Three such retraced movements must be made before a (m) *Retraced local movement episode* is recorded. When it occurs, the qualifying 3 movements are included in the total number of moves. When the mouse moves outside of the region of qualified coordinates, or fails to move back to the starting point for 2 s, the episode breaks, and the animal's position at that time, becomes the new starting point and (n) *Retraced local movement time* (s) — the total amount of time the animal is engaged in the behavior; (o) *Vertical plane entries* (rearing) — the number of times the animal enters the vertical plane (i.e. activation of upper, or 2nd, ring of infrared photo detectors); (p) *Vertical time* (s) and (q) *Vertical distance traveled* (cm) — the total amount of time (s) spent, and distance (cm) traveled, in the vertical plane, respectively, and (r) *Jumps* — the total number of time-contiguous 0–0 coordinate sets (i.e. the animal is not on the floor plane) which occur under 2 s.

1.3. Procedure

Animals were naïve to the test room (22 °C, 50% humidity) and transported from the vivarium to the test site for a 60- to 90-min acclimation period prior to the start of the experiment. The mice were tested between 0930 and 1730 h over a three-day period. A saline control group ($n=8$/day; $N=24$) was used each day. Animals were assigned to treatment groups ($n=8$/group) according to a table of random numbers (Winer, 1962). Drug doses were as follows: (±)-MDMA (0.3, 1, 3, 10, and 30 mg/kg), S(+)-MDMA (0.3, 1, 3, 10, and 30 mg/kg), and R(−)-MDMA (0.3, 1, 3, 10, 17, 30, and 50 mg/kg). The mice were injected with either saline or a dose of drug and immediately placed into the test cage and left undisturbed for 30 min. The latter time period was chosen on the basis of analyses of pilot data of the effects (studied over 90 min) of saline, 3 mg/kg, and 10 mg/kg of MDMA, that revealed mice treated with a) saline exhibited most of their activity in the chamber within 20 to 30 min and b) MDMA produced pronounced and significant effects on components of behavior (see measures above) within 10–30 min. Thus, a total recording time of 30 min was chosen for each animal.

1.4. Data presentation and statistics

The dose-effect functions for the drugs (i.e. 18 dose groups) on each activity measure were analyzed by one-way analysis of variance (statistically significant F value set at $p \leq 0.05$) and followed, when appropriate, by Newman–Keuls multiple comparison post-hoc tests ($p \leq 0.05$) to determine statistical significance between dose response groups.

1.5. Drugs

(±)-, S(+)-, and R(−)-MDMA HCl (*N*-methyl-1-(-3,4-methylenedioxyphenyl)-2-aminopropane hydrochloride) were obtained as gifts from the National Institute on Drug Abuse (NIDA). Doses of each drug refer to the weight of the salt. Each agent was dissolved in 0.9% saline, prepared fresh daily, and administered intraperitoneally in a 10 ml/kg injection volume.

2. Results

The mean response of saline vehicle and each dose of (±)-, S(+)-, and R(−)-MDMA on each measure of activity is displayed in Figs. 1–9. The dose-response functions of the drugs (i.e. 18 dose groups) on each measure were analyzed by one-way analysis of variance (ANOVA; significant F value set at $p \leq 0.05$) and followed by Newman–Keuls multiple comparison post-hoc tests ($p \leq 0.05$) to determine statistical significance between (selected) dose response groups.

2.1. Movement time

Racemic MDMA, S(+)-MDMA, and R(−)-MDMA produced statistically significant (F (17,142) = 7.98, $p < 0.0001$) and dose related increases in the animals' movement time (Fig. 1). Newman–Keuls multiple comparison post-hoc tests revealed that mean movement time by the groups of mice that received 10 mg/kg and 30 mg/kg of (±)-MDMA, 1 mg/kg, 3 mg/kg, 10 mg/kg and 30 mg/kg of S(+)-MDMA, or 10 mg/kg, 17 mg/kg, 30 mg/kg, and 50 mg/kg of R(−)-MDMA was significantly greater than that of the saline vehicle control group (S on *x*-axis of Fig. 1, top graph) of mice. In addition, (±)-MDMA or S(+)-MDMA (at 30 mg/kg) produced a maximal effect on movement time that was notably (but not statistically) higher than that of R(−)-MDMA (at 30 mg/kg or 50 mg/kg).

2.2. Movement distance

(±)-MDMA, S(+)-MDMA, and R(−)-MDMA produced statistically significant (F (17,142) = 12.73, $p < 0.0001$) and dose related increases in mean movement distance (Fig. 1). Newman–Keuls multiple comparison post-hoc tests revealed

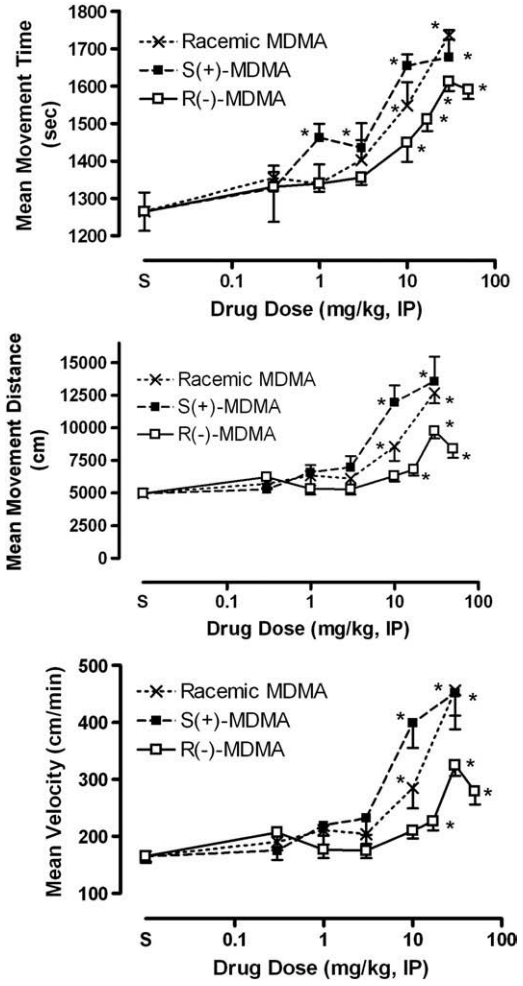

Fig. 1. Results of (±)-MDMA (X symbol), S(+)-MDMA (closed square), and R(−)-MDMA (open square) on movement time (top), movement distance (middle), velocity (bottom) in mice. Ordinate: mean (with ±S.E.M.) values were obtained after the intraperitoneal administration of 10 ml/kg of 0.9% saline (i.e. S; $N=24$) or doses of each compound ($n=8$ mice at each point). Abscissa: drug doses plotted on a logarithmic scale. An asterisk (*) denotes a dose that produced a statistically significant difference ($p \leq 0.05$) in a measure as compared with saline vehicle (S).

that the distance covered by the groups of mice that received 10 mg/kg and 30 mg/kg of (±)-MDMA or S(+)-MDMA was significantly greater than that of the saline vehicle group (S on abscissa of Fig. 1, middle graph) of mice. In comparison, R(−)-MDMA (at 17 mg/kg, 30 mg/kg, and 50 mg/kg) also produced statistically significant increases (from saline control) in mean movement distance. In addition, (±)-MDMA or S(+)-MDMA (at 30 mg/kg) produced a maximal effect on movement distance that was statistically ($p<0.05$) greater than the peak increase in traversed distance that occurred at 30 mg/kg of R(−)-MDMA.

2.3. Velocity

Racemic MDMA, S(+)-MDMA, and R(−)-MDMA produced statistically significant (F (17,142)=12.83, $p<0.0001$) and dose related increases in the animals' travel speed (Fig. 1). Newman–Keuls multiple comparison post-hoc tests revealed that the velocity of the groups of mice that received 10 mg/kg and 30 mg/kg of (±)-MDMA or S(+)-MDMA was significantly faster than that of the control group (S on x-axis of Fig. 1, bottom graph) of mice. R(−)-MDMA (17 mg/kg, 30 mg/kg, and 50 mg/kg) also produced statistically significant increases (from saline control) in velocity. In addition, the maximal increase in velocity of (±)-MDMA or S(+)-MDMA (which occurred at 30 mg/kg) was significantly ($p<0.05$) greater than the peak increase in velocity that occurred at 30 mg/kg of R(−)-MDMA.

2.4. Movement episodes

(±)-MDMA, S(+)-MDMA, and R(−)-MDMA produced a statistically significant (F (17,142)=23.27, $p<0.0001$) effect on movement episodes (Fig. 2). Newman–Keuls multiple comparison post-hoc tests revealed that the mean number of episodes completed by the groups of mice that received 10 mg/kg or 30 mg/kg of (±)-MDMA or S(+)-MDMA was significantly less than that of the saline vehicle group (S on abscissa of Fig. 2) of mice. In comparison, R(−)-MDMA produced a statistically significant but biphasic dose effect function on this measure. That is, 1 mg/kg of R(−)-MDMA produced a significant increase (and 3 mg/kg produced a near statistically significant increase) in episodes from control level whereas doses at, or higher than, 10 mg/kg of R(−)-MDMA occasioned significant decreases.

2.5. Margin distance and margin time

(±)-MDMA, S(+)-MDMA, and R(−)-MDMA produced statistically significant (F (17,142)=11.30, $p<0.0001$) and dose related increases in the amount of *distance* covered by the animals in the margins of the apparatus (Fig. 3). Newman–Keuls multiple comparison post-hoc tests revealed that mean margin distance by the groups of mice that received (±)-MDMA (10 mg/kg and 30 mg/kg), S(+)-MDMA (3 mg/kg, 10 mg/kg, and 30 mg/kg) or R(−)-MDMA (17 mg/kg, 30 mg/kg, and 50 mg/kg) was significantly greater than that of the saline vehicle group (S on x-axis of Fig. 3) of mice. In contrast, (±)-

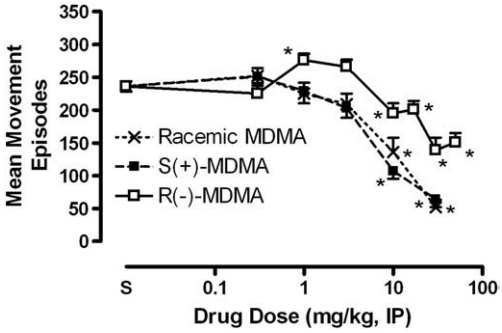

Fig. 2. Results of (±)-MDMA (X symbol), S(+)-MDMA (closed square), and R(−)-MDMA (open square) on movement episodes in mice. Ordinate: Mean (with ±S.E.M.) episodes were obtained after the intraperitoneal administration of 10 ml/kg of 0.9% saline (i.e. S; $N=24$) or doses of each compound ($n=8$ mice at each point). Abscissa: drug doses plotted on a logarithmic scale. See Fig. 1 for further details.

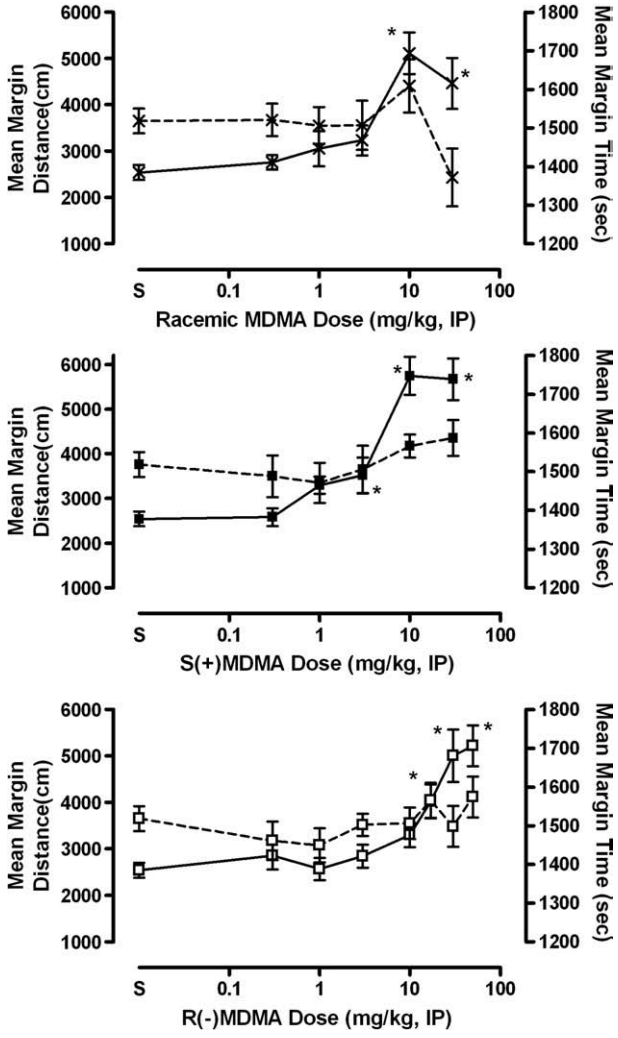

Fig. 3. Results of (±)-MDMA (top), S(+)-MDMA (middle), and R(−)-MDMA (bottom) on margin distance (left ordinate — solid line) and margin time (right ordinate — broken line) in mice. Mean (with ± S.E.M.) values were obtained after the intraperitoneal administration of 10 ml/kg of 0.9% saline (i.e. S; $N=24$) or doses of each compound ($n=8$ mice at each point). Abscissa: drug doses plotted on a logarithmic scale. See Fig. 1 for further details.

MDMA, S(+)-MDMA, and R(−)-MDMA did not produce statistically significant (F (17,142) = 1.16, $p>0.05$) changes (from saline control) in the amount of *time* the animals spent in the margins (Fig. 3).

2.6. Center entries

The ANOVA indicated that at least one of the drugs produced a statistically significant (F (17,142) = 2.32, $p<0.0005$) effect on the number of entries into the center area (Fig. 4). Newman–Keuls multiple comparison post-hoc test revealed, however, that only mean center entries by the group of mice that received 30 mg/kg of (±)-MDMA was significantly greater than that of the saline vehicle control group (S on abscissa of Fig. 4, upper left graph) of mice. In contrast, S(+)-MDMA and R(−)-MDMA did not produce statistically significant changes (from saline control) in the number of entries into the center area.

2.7. Center distance and center time

The ANOVA revealed that at least one of the drugs produced a statistically significant (F (17,142) = 1.76, $p<0.05$) increase in *distance* covered in the center area (Fig. 4). Newman–Keuls multiple comparison post-hoc test revealed, however, that only the center distance of the group of mice that received 30 mg/kg of (±)-MDMA was significantly greater than that of the saline vehicle control group (S on *x*-axis of Fig. 4, upper right graph) of mice. In contrast, S(+)-MDMA and R(−)-MDMA did not produce statistically significant differences (from saline vehicle control) in the distance tracked in the center area. Lastly, racemic MDMA, S(+)-MDMA, and R(−)-MDMA did not produce a statistically significant (F (17,142) = 1.16, $p>0.05$) change (from control) in the amount of *time* the animals spent in the center area (Fig. 4).

2.8. Clockwise (CW) center point rotations

Racemic MDMA, S(+)-MDMA, and R(−)-MDMA produced statistically significant (F (17,142) = 1.95, $p<0.05$) increases in the animals' clockwise rotations (Fig. 5, top graph). Newman–Keuls multiple comparison post-hoc tests revealed that the mean number of CW rotations by the groups of mice that received 30 mg/kg of (±)-MDMA, 3 mg/kg and 10 mg/kg of S(+)-MDMA, or 30 mg/kg of R(−)-MDMA was significantly greater than that of the saline vehicle control group (S on abscissa of Fig. 5, top graph) of mice. In addition, racemic MDMA (at 30 mg/kg) produced a maximal increase in CW rotations that was notably (but not statistically) greater than that of each isomer of MDMA.

2.9. Counterclockwise (CCW) center point rotation

Racemic MDMA, S(+)-MDMA, and R(−)-MDMA produced statistically significant (F (17,142) = 6.50, $p<0.0001$) increases in the animals' counterclockwise rotations (Fig. 5, bottom graph). Newman–Keuls multiple comparison tests revealed that the mean number of CCW rotations by the groups of mice that received 30 mg/kg of (±)-MDMA, 10 mg/kg and 30 mg/kg of S(+)-MDMA, or 30 mg/kg of R(−)-MDMA was significantly greater than that of the saline vehicle control group (S on *x*-axis of Fig. 5, bottom graph) of mice.

2.9.1. Rotation preference

In Fig. 5, the administration of S(+)-MDMA appeared to produce markedly more counterclockwise versus clockwise rotations. To investigate this issue further, the data in Fig. 5 (i.e. data from measures in Sections *2.8* and *2.9* above) were re-graphed (Fig. 6) and re-evaluated to determine a possible preference for direction of turning rotation for each agent. A two way (dose groups × rotation) ANOVA with repeated measures on one factor (direction of rotation) indicated a statistically significant effect for dose groups (F (17,142) = 9.17, $p<0.01$), rotation (F (1,142) = 4.51, $p<0.05$), and dose groups × rotation interaction (F (17,142) = 2.37, $p<0.005$). Newman–Keuls multiple comparison tests for rotation within dose groups revealed that

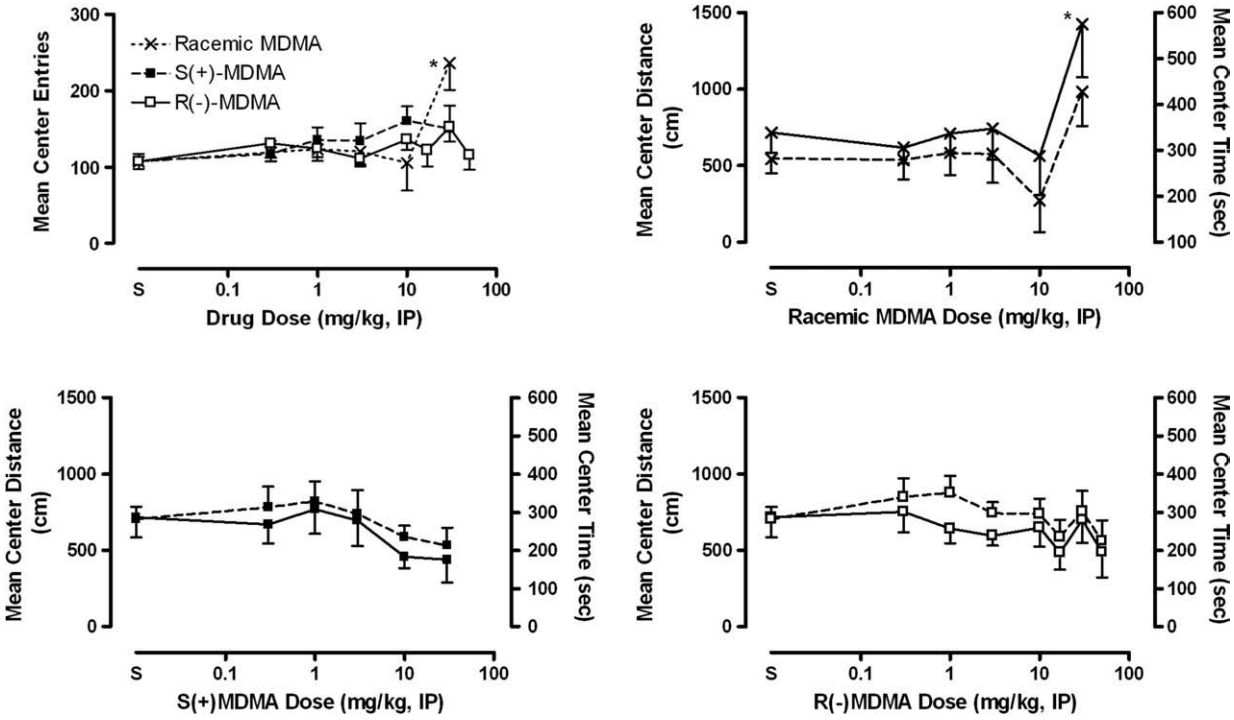

Fig. 4. Results of (±)-MDMA (X symbol), S(+)-MDMA (closed square), and R(−)-MDMA (open square) on center entries (upper left). Results of (±)-MDMA (upper right), S(+)-MDMA (bottom left), and R(−)-MDMA (bottom right) on center distance (left ordinate — solid line) and center time (right ordinate — broken line) in mice. Mean (with ± S.E.M.) values were obtained after the intraperitoneal administration of 10 ml/kg of 0.9% saline (i.e. S; $N=24$) or doses of each compound ($n=8$ mice at each point). Abscissa: drug doses plotted on a logarithmic scale. See Fig. 1 for further details.

only S(+)-MDMA, at 30 mg/kg, produced a statistically greater number of counterclockwise versus clockwise rotations. Thus, S(+)-MDMA exerted a dose related and significant tendency to induce counterclockwise rotations.

2.10. Retraced movements, retraced episodes and retraced time

Racemic MDMA, S(+)-MDMA, and R(−)-MDMA exerted a significant (F (17,142)=3.46, $p<0.0001$) effect on retraced local *movements* (Fig. 7). Newman–Keuls multiple comparison tests revealed that retraced movements by groups of mice that received (±)-MDMA (30 mg/kg), S(+)-MDMA (10 mg/kg and 30 mg/kg), and R(−)-MDMA (30 mg/kg) were significantly less than that of the saline vehicle control group (S on abscissa of Fig. 7, upper right and bottom graphs) of mice. Similarly, (±)-MDMA, S(+)-MDMA, and R(−)-MDMA induced a significant (F (17,142)=5.13, $p<0.0001$) effect on retraced local movement *episodes*. Newman–Keuls multiple comparison tests revealed that retraced episodes by groups of mice that received (±)-MDMA (30 mg/kg), S(+)-MDMA (10 mg/kg and 30 mg/kg), and R(−)-MDMA (30 mg/kg) were significantly less than that of the saline vehicle control group (S on x-axis of Fig. 7, upper left graph) of mice. Lastly, (±)-MDMA, S(+)-MDMA, and R(−)-MDMA produced a significant (F (17,142)=3.80, $p<0.0001$), effect on retraced local movement *time*. Newman–Keuls multiple comparison tests revealed that retraced time by groups of mice that received (±)-MDMA (30 mg/kg), S(+)-MDMA (10 mg/kg and 30 mg/kg), and R(−)-MDMA (30 mg/kg) were significantly less than that of the saline vehicle control group (S on x-axis of Fig. 7, upper right and bottom graphs) of mice.

2.11. Vertical entries (rearing)

The ANOVA indicated that at least one of the drugs produced a statistically significant (F (17,142)=4.12, $p<0.0001$) effect on vertical entries. Newman–Keuls multiple comparison tests revealed that (±)-MDMA produced significant decreases (from saline vehicle control) in rearing at 10 mg/kg and 30 mg/kg (Fig. 8, top graph). In comparison, S(+)-MDMA produced a statistically significant and biphasic dose effect function on this measure. That is, 1 mg/kg of S(+)-MDMA produced a significant increase in vertical entries compared to saline vehicle (S on abscissa of Fig. 8, top graph) whereas higher doses reduced rearing such that a significant decrease occurred at 30 mg/kg. In contrast, R(−)-MDMA did not produce significant alterations (from control) in the number of entries (Fig. 8, top graph) into the vertical plane.

2.12. Vertical time and vertical distance

Racemic MDMA, S(+)-MDMA, and R(−)-MDMA produced a statistically significant effect on vertical *time* (F (17,142)=5.39, $p<0.0001$) and vertical *distance* (F (17,142)=3.18, $p<0.0001$). Newman–Keuls multiple comparison tests revealed that (±)-MDMA produced statistically significant and dose related

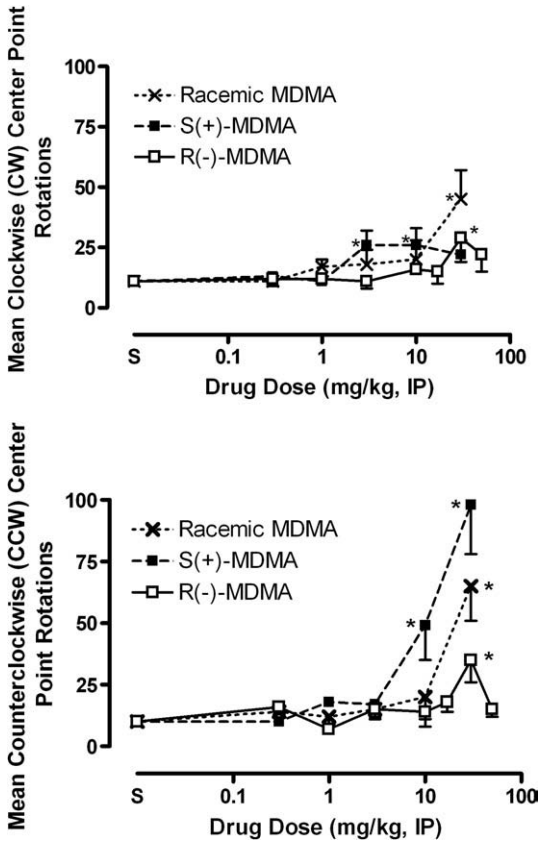

Fig. 5. Results of (±)-MDMA (X symbol), S(+)-MDMA (closed square), and R(−)-MDMA (open square) on clockwise rotations (top) and counterclockwise rotations (bottom) in mice. Ordinate: Mean (with ± S.E.M.) values were obtained after the intraperitoneal administration of 10 ml/kg of 0.9% saline (i.e. S; $N=24$) or doses of each compound ($n=8$ mice at each point). Abscissa: drug doses plotted on a logarithmic scale. See Fig. 1 for further details.

reductions in vertical distance and vertical time; each measure was significantly decreased at 10 mg/kg and 30 mg/kg from the saline vehicle group (S on *x*-axis of Fig. 8, middle and bottom graphs) of mice. In comparison, S(+)-MDMA produced a statistically significant and biphasic dose effect function on vertical time. That is, 1 mg/kg of S(+)-MDMA produced a significant increase in vertical time compared to saline vehicle (S on *x*-axis of Fig. 8, middle graph) whereas higher doses reduced rearing time such that a significant decrease occurred at 30 mg/kg. S(+)-MDMA also produced statistically significant and dose related reductions in vertical distance; a significant decrease occurred at 30 mg/kg versus saline vehicle (S on *x*-axis of Fig. 8, bottom graph). Lastly, R(−)-MDMA produced a statistically significant increase in vertical distance and vertical time; each measure was significantly increased at 1 mg/kg versus saline vehicle (S on *x*-axis of Fig. 8, middle and bottom graphs) but no statistically significant change (from saline vehicle) at doses between 3 mg/kg and 50 mg/kg.

2.13. Jumps

Racemic MDMA, S(+)-MDMA, and R(−)-MDMA produced a statistically significant ($F(17,142)=3.86$, $p<0.0001$) effect on jumps (Fig. 9). Newman–Keuls multiple comparison post-hoc tests revealed that the mean number of jumps exhibited by the groups of mice that received 10 mg/kg and 30 mg/kg of (±)-MDMA or 30 mg/kg of S(+)-MDMA was significantly less than that of the saline group (S on abscissa of Fig. 9) of mice. In contrast, R(−)-MDMA produced statistically significant increases in jumps at doses of 1 and 3 mg/kg but no statistically significant change (from saline vehicle) at doses between 10 mg/kg and 50 mg/kg.

2.14. Summary of results

Table 1 provides a summary of the effects and/or potency relationships (where applicable) of (±)-, S(+)-, and R(−)-

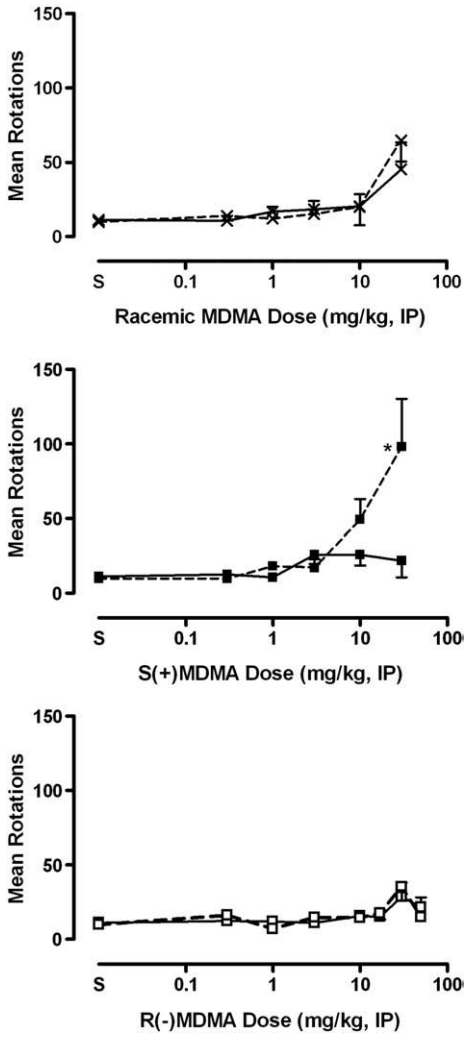

Fig. 6. Results of (±)-MDMA (top), S(+)-MDMA (middle), and R(−)-MDMA (bottom) on clockwise (solid line) and counterclockwise (broken line) rotations in mice. Mean (with ± S.E.M.) rotations were obtained after the intraperitoneal administration of 10 ml/kg of 0.9% saline (i.e. S; $N=24$) or doses of each compound ($n=8$ mice at each point). Abscissa: drug doses plotted on a logarithmic scale. An asterisk (*) denotes a dose that produced a statistically significant difference (Newman-Keuls, $p \leq 0.05$) between counterclockwise rotations as compared to clockwise rotations.

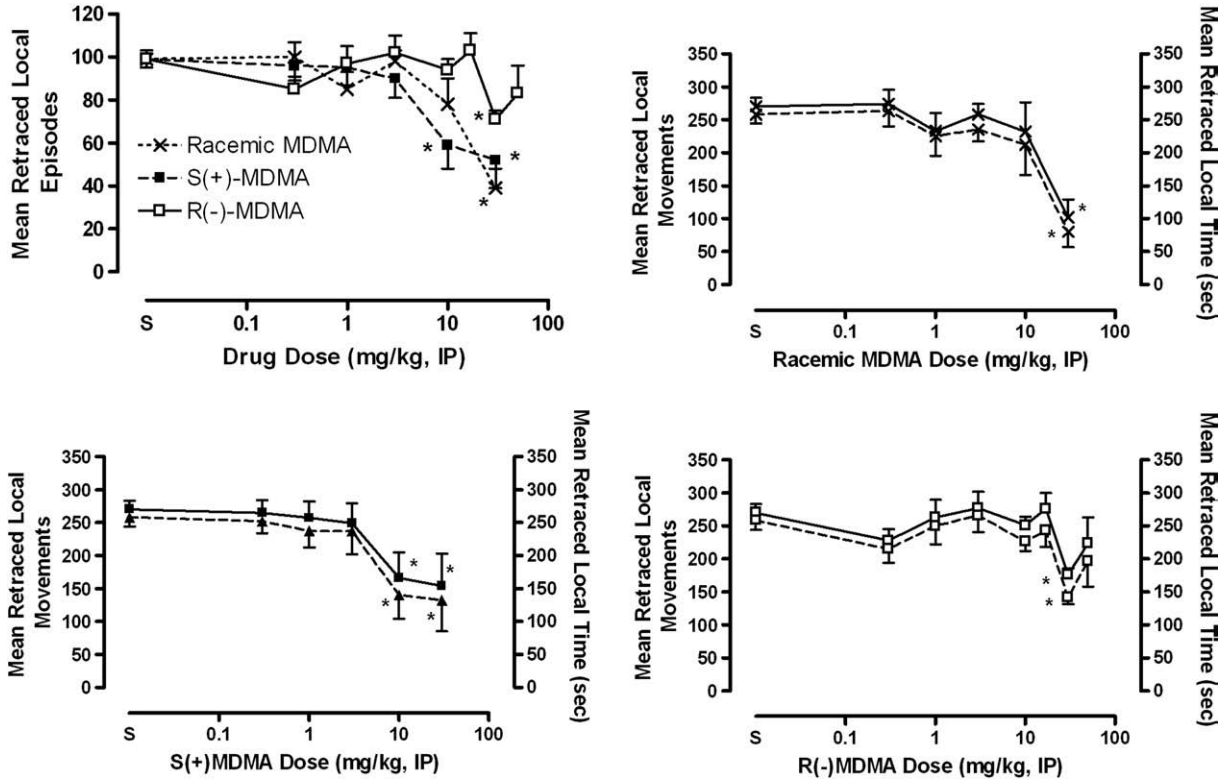

Fig. 7. Results of (±)-MDMA (X symbol), S(+)-MDMA (closed square), and R(−)-MDMA (open square) on retraced episodes (upper left). Results of (±)-MDMA (upper right), S(+)-MDMA (bottom left), and R(−)-MDMA (bottom right) on retraced movements (left ordinate — solid line) and retraced time (right ordinate — broken line) in mice. Mean (with ± S.E.M.) values were obtained after the intraperitoneal administration of 10 ml/kg of 0.9% saline (i.e. S; $N=24$) or doses of each compound ($n=8$ mice at each point). Abscissa: drug doses plotted on a logarithmic scale. See Fig. 1 for further details.

MDMA on the eighteen measures of activity that were recorded and quantified.

3. Discussion

When a rodent is placed in an enclosed environment its movements usually alternate between episodes of progressions and stops. The animal's forward progression carries it from one location to the next whereas a stop may involve an investigation of a particular site(s) (Berlyne, 1960). An animal treated with a drug that stimulates motor activity typically displays increased movement time, distance, and velocity that is accompanied by decreased movement episodes because the drug increases the duration of motor activity (from one location to other locations) with concomitant decreases in the number of stops (e.g., Berlyne, 1960; Young and Johnson, 1991). The current results indicated that the administration of (±)-MDMA and its enantiomers to mice stimulated their movement time, distance, and velocity with the following approximate order of potency: S(+)-MDMA ≥ (±)-MDMAN>R(−)-MDMA. Fig. 1 shows that the dose effect function of S(+)-MDMA was (slightly) to the left of that of racemic MDMA and the dose response of R(−)-MDMA was to the right of that of racemic MDMA; furthermore, R(−)-MDMA (at the doses tested) did not produce as pronounced a maximal effect as S(+)- or (±)-MDMA. The latter results are consistent with previous findings that these agents demonstrated increased motor activity, stereoselective potency that favored S(+)-MDMA over R(−)-MDMA, and a close relationship between the dose response functions of S(+)-MDMA and (±)-MDMA (e.g., Bankson and Cunningham, 2002; Fantegrossi et al., 2003, 2005; Glennon et al., 1988; Gold et al., 1988, 1989; Gold and Koob, 1989; Paulus and Geyer, 1992). Fig. 2 reveals that the administration of (±)-, S(+)-, and R(−)-MDMA at, or higher than, doses of 10 mg/kg significantly reduced movement episodes, which is consistent with the conclusion that the agents can function as stimulants of behavior. However, (±)-MDMA and S(+)-MDMA produced dose response functions that are nearly identical. In comparison, R(−)-MDMA produced a dose response effect that was shifted to the right and biphasic such that 1 mg/kg produced a significant increase (and 3 mg/kg produced a near statistically significant increase) and higher doses produced significant decreases in episodes; the increments in episodes are likely related to the animals' increased jumps at those doses of R(−)-MDMA (see below). Consequently, when the enantiomers of MDMA are combined to form (±)-MDMA a) the increases in the number of episodes produced by R(−)-MDMA are probably suppressed by S(+)-MDMA, an action that is concluded from the stereochemical consideration that doses between 2 mg/kg and 6 mg/kg of (±)-MDMA should have produced noticeable increases (from control) in movement episodes but did not and b) the racemic mixture seems to exert more potency than expected in the reduction of movement episodes.

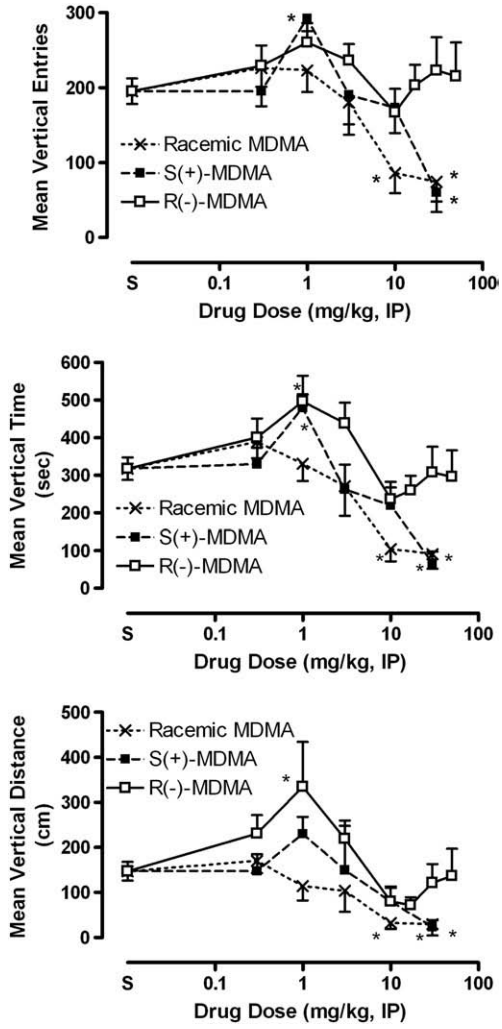

Fig. 8. Results of (±)-MDMA (X symbol), S(+)-MDMA (closed square), and R(−)-MDMA (open square) on vertical entries (top), vertical time (middle), and vertical distance (bottom) in mice. Ordinate: Mean (with ± S.E.M.) values were obtained after the intraperitoneal administration of 10 ml/kg of 0.9% saline (i.e. S; $N=24$) or doses of each compound ($n=8$ mice at each point). Abscissa: drug doses plotted on a logarithmic scale. See Fig. 1 for further details.

A rodent's motor activity also can be scored according to its location, with the most frequently used major regions being the peripheral and central areas of an apparatus. Normally, rodents tend to move around and spend a considerable amount of time in the perimeter of an arena (i.e. thigmotaxis) but make very few entries, and spend very little time, in the center of an apparatus. In fact, an animal's occupancy of the peripheral areas, either in corners or near walls, has been promoted as an index of "timidity" (Walsh and Cummins, 1976) or "anxiety" (Simon et al., 1994; Treit and Fundytus, 1988). In rats, (±)-MDMA increased motor activity along the periphery, and decreased entries into the center area (Gold et al., 1988, 1989; Gold and Koob, 1989; Paulus and Geyer, 1992). In comparison, S(+)-MDMA reportedly increased activities in peripheral and central zones (Bankson and Cunningham 2002; Bubar et al., 2004; Herin et al., 2005; McCreary et al., 1999), or decreased actions in central territory (Callaway et al., 1992; Paulus and Geyer, 1992). Lastly, R(−)-MDMA increased motor activity along the periphery (Paulus and Geyer, 1992).

In contrast to the numerous studies in rats of the effect(s) of MDMA and its isomers on spatial location of activity, a search of the literature did not reveal any studies that performed the same type of analyses of these drugs in mice. Indeed, studies have simply reported that (±)-MDMA, S(+)-MDMA, and R(−)-MDMA stimulated motor behavior (Fantegrossi et al., 2003; Glennon et al., 1988; Powell et al., 2004). The results of the current study (Fig. 3) revealed that MDMA and its isomers significantly increased distance traveled along the margins and that this effect was stereoselective with the following order of potency: S(+)-MDMA (3–30 mg/kg) > (±)-MDMA (10 and 30 mg/kg) > R(−)-MDMA (17–50 mg/kg). An evaluation of the animals' time spent in the margin areas of the arena, however, revealed that the agents did not produce a significant difference from that of saline vehicle and, thus, the drug-treated animals did not exhibit increased thigmotaxis, an action that may have been interpreted as an increase in "timidity" or "anxiety". Instead, analyses indicated that although mice treated with MDMA (or its isomers) traversed more territory (i.e. distance) in the periphery they accomplished the deed without an increase in their (margin) time of stay (Fig. 3). The data can be explained by a quickened pace of the mice (see *Velocity* in Results section) in the margin areas and is consistent with the characterization of MDMA and its isomers as being, at least in part, stimulants of motor action.

An evaluation of the effects of (±)-MDMA and its enantiomers on entries, distance covered, and time spent in the center area indicated that only (±)-MDMA (and only at a 30 mg/kg dose) produced a statistically significant increase in the number of entries and amount of distance covered in this area, but had no effect on the amount of time the mice spent in the center area (Fig. 4). In comparison, neither isomer (at any dose tested) significantly increased or decreased (from control values) any of the three measures of activity in the center area (Fig. 4). The effects produced by racemic MDMA, albeit at one dose, were not expected because neither enantiomer exerted a comparably significant effect(s). It appears that the central

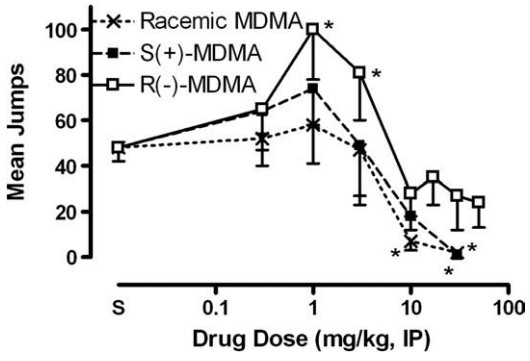

Fig. 9. Results of (±)-MDMA (X symbol), S(+)-MDMA (closed square), and R(−)-MDMA (open square) on jumps in mice. Ordinate: mean (with ± S.E.M.) jumps were obtained after the intraperitoneal administration of 10 ml/kg of 0.9% saline (i.e. S; $N=24$) or doses of each compound ($n=8$ mice at each point). Abscissa: drug doses plotted on a logarithmic scale. See Fig. 1 for further details.

Table 1
Summary of the effect(s)[a] and/or potency relationships of (±)-, S(+)-, and R(−)-MDMA on eighteen measures of activity in mice

	(±)-MDMA	S(+)-MDMA	R(−)-MDMA	Potencies or effect
Activity measure				
Movement time	↑	↑	↑[b]	S(+)≥(±)>R(−)
Movement distance	↑	↑	↑[b]	S(+)≥(±)>R(−)
Velocity	↑	↑	↑[b]	S(+)≥(±)>R(−)
Movement episodes	↓	↓	∩	(±)=S(+)∉R(−)
Margin distance	↑	↑	↑	S(+)>(±)>R(−)
Margin time	↔	↔	↔	No change
Center entries	↑[c]	↔	↔	(±)-MDMA only
Center distance	↑[c]	↔	↔	(±)-MDMA only
Center time	↔	↔	↔	No change
Clockwise rotations	↑	↑[d]	↑[d]	S(+)>(±)>R(−)
Counterclockwise rotations	↑	↑[e]	↑[b]	S(+)>(±)>R(−)
Retraced episodes	↓	↓	↓	S(+)>(±)≥R(−)
Retraced movements	↓	↓	↓	S(+)>(±)≥R(−)
Retraced time	↓	↓	↓	S(+)>(±)≥R(−)
Vertical entries (rearing)	↓	∩	↔	(±)∉S(+)∉R(−)
Vertical time	↓	∩	↑[f]	(±)∉S(+)∉R(−)
Vertical distance	↓	↓	↑[f]	(±)>S(+)∉R(−)
Jumps	↓	↓	↑[f]	(±)≥S(+)∉R(−)

[a] A (↑) or (↓) indicates that the agent produced a significant increase or decrease in the measure respectively. A (∩) indicates that an agent produced a biphasic effect (low dose(s) *increased* and higher dose(s) *decreased* activity). A (∉) indicates the occurrence of a different profile of dose effects between agents. A (↔) indicates dose effects of an agent that were not statistically different from control level.

[b] Maximal effect of R(−)-MDMA was lower than those of (±)-or S(+)-MDMA.

[c] One dose (30 mg/kg) of racemic MDMA produced a significant increase in activity.

[d] Maximal effect of S(+)-or R(−)-MDMA was lower than that of (±)-MDMA.

[e] S(+)-MDMA induced significantly more counterclockwise than clockwise rotations.

[f] R(−)MDMA produced a biphasic effect (low dose(s) *increased* and higher doses had *no effect* on measure).

region actions produced by (±)-MDMA probably originated by an addition or potentiation of the effects of the individual isomers (e.g., Bondareva et al., 2005). The latter idea should be evaluated more directly, however, by an assessment of the center area effects of racemic MDMA and its stereoisomers at doses that are chosen on an arithmetic scale (e.g., 30 mg/kg of (±)-MDMA and 15 mg/kg of each isomer). Lastly, the present center area results in mice – an increase by (±)-MDMA and the lack of effect by S(+)-MDMA (from 0.3 mg/kg to 30 mg/kg) – are in contrast to findings in rats that reported a decrease by (±)-MDMA and an increase or decrease by S(+)-MDMA (e.g., Bubar et al., 2004; Callaway et al., 1992; Herin et al., 2005; McCreary et al., 1999; Paulus and Geyer, 1992). Thus, a species difference may account for the variations in effects on center region measures exerted by these agents.

Previous studies in rats indicated that (±)-MDMA and its isomers produced, to various extents, turning behavior (Hiramatsu et al., 1989; O'Loinsigh et al., 2001; for exception see Matthews et al., 1989) or characteristics of the serotonin syndrome: lateral head weaving, flat body posture, forepaw treading, and piloerection (e.g., Fone et al., 2002; Marston et al., 1999; Spanos and Yamamoto, 1989; for exception see Matthews et al., 1989). Unfortunately, most of those forms of behavior cannot be adequately accounted for by the current, or any, fully automated activity device. However, certain repetitive behaviors such as turning and retraced movements (including retraced episodes and time) are measures that the present apparatus can identify and record for analyses (see Materials and methods section).

A rodent's turning rotations, as well as the direction of turning rotations, have been used as a component(s) of stereotypy or an indicator(s) of asymmetric brain function although the exact cause(s) of turning behavior is unclear (e.g., Kolb & Whishaw, 1985; Miklyaeva et al., 1995). For example, animals with unilateral damage to the substantia nigra area of the brain, with consequent anterograde degeneration of dopaminergic axons in the caudate nucleus, produce asymmetric patterns of movement that are expressed by rotation away from or toward the side of the lesion (Ungerstedt, 1970, 1971a, b). In fact, the latter procedure is used to characterize drug action on the dopamine neurotransmitter system in terms of a direct vs. indirect receptor action of an agent. Thus, damage to the right-side substantia nigra leads to circling to the left after administration of the dopamine receptor agonist apomorphine and to the right after injection of the dopamine releasing agent amphetamine. It is assumed that the effect of apomorphine is due to a direct action on dopamine (DA) receptors, and, hence, a greater effect on the denervated side. On the other hand, amphetamine produces circling to the right because it can only release dopamine from the intact dopamine terminals in the left caudate. Recent studies indicate that (±)-MDMA, S(+)-MDMA, and R(−)-MDMA induced ipsilateral rotation in unilateral 6-hydroxydopamine lesioned rats, which suggests a prominent role for the release of dopamine at the doses employed (Lebsanft et al., 2003, 2005). In the present study, the administration of (±)-, S(+)-, and R(−)-MDMA to mice (without brain injury, lesion, or neurotransmitter depletion) produced statistically significant increases in rotations (Fig. 5), differences in potencies and, in the case of S(+)-MDMA, a preference to induce counterclockwise (versus clockwise) rotations (Fig. 6). Specifically, (±)-MDMA and its isomers increased both clockwise and counterclockwise rotations with the following stereoselective order of potency: S(+)-MDMA>(±)-MDMA>R(−)-MDMA (Fig. 5). In the case of clockwise rotations, however, each isomer produced a maximal effect that was notably (but not statistically) less than that produced by the racemic mixture. The effect of racemic MDMA on clockwise rotations, therefore, is probably not the result of a completely stereoselective effect because S(+)MDMA did not produce a comparable (maximal) effect to (±)-MDMA, even at twice (i.e. 30 mg/kg of S(+)MDMA) the contribution it made at the 30 mg/kg dose of racemic MDMA (Fig. 5). It seems likely that the enhanced response of racemic MDMA resulted from the (partial) stereoselective action of S(+)-MDMA, which was

augmented by the effect of R(−)-MDMA. The latter notion could be judged more precisely, however, by an evaluation of the clockwise rotation effects of (±)-, S(+)-, and R(−)-MDMA at doses that are chosen on an arithmetic basis (e.g., 30 mg/kg of (±)-MDMA and 15 mg/kg of each isomer). In the case of counterclockwise rotations, the stereoselective effect of S(+)-MDMA appeared to account for the effect of racemic MDMA (Fig. 5). Lastly, the data presented in Fig. 5 seemed to indicate that S(+)MDMA induced considerably more counterclockwise versus clockwise rotations and, therefore, data for the agents were re-graphed and re-analyzed to determine a possible preference for direction of turning rotation. Fig. 6 indicates that (±)-MDMA and R(−)-MDMA did not exhibit a preference for direction of rotation but that S(+)-MDMA, between 10 mg/kg and 30 mg/kg, exerted a marked tendency to induce counterclockwise (versus clockwise) rotations. At this time, the reason(s) for this is not clear but the data do raise questions as to whether a) the S(+)-MDMA rotation preference effect is peculiar to mice, b) S(+)-MDMA exerts a more prominent and/or more potent effect on one side of the brain and c) R(−)-MDMA may function as an antagonist of that particular effect of S(+)-MDMA. The latter idea is derived from the stereochemical view that racemic MDMA (a 50% mixture of each isomer), between projected doses of 20 mg/kg and 60 mg/kg, should have exhibited some evidence of a preference to produce counterclockwise (versus clockwise) rotations. However, (±)-MDMA (up to 30 mg/kg) did not exhibit a preference to induce either type of rotation. This suggests that R(−)-MDMA, when combined with S(+)-MDMA to create (±)-MDMA, may exert a dampening effect on the exaggerated number of counterclockwise rotations induced by S(+)-MDMA. In any case, future biochemical and behavioral studies should investigate all of these issues.

The measures of retraced activity used in the present study are expressions of repetitive (explorative or compulsive?) behavior of mice that do not contribute to marked changes of location. The data from control animals indicated that a certain amount of such activity is a normal part of a mouse's repertoire of action(s) (Fig. 7). MDMA and its isomers significantly decreased retraced movements, episodes, and time with the following stereoselective order of potency: S(+)-MDMA (10 mg/kg and 30 mg/kg) > (±)-MDMA (30 mg/kg) ≥ R(−)-MDMA (30 mg/kg). Interestingly, the latter doses of the agents also increased the animals' overall movement time, distance, and velocity (see above and Fig. 1). Those effects are noted because graphic analyses of rodents' movement indicated that the administration of MDMA produced increased activity that occurred in "unusually straight paths" (e.g., Gold et al., 1988, 1989; Gold and Koob, 1989; Paulus and Geyer, 1992; current study: data recorded and stored but not presented). As such, the reduced retraced activity of MDMA was not unexpected.

Another measure of rodent behavior involves the interruption of photo-beams in a vertical plane (i.e. rearing), which is speculated to be an indicator of an animal's "nonspecific excitability level" (Walsh and Cummins, 1976), "exploration" (Barnett and Cowan, 1976), or "level of responsiveness" (Lät and Gollova-Hemon, 1969). Studies in rats have consistently demonstrated that (±)-MDMA decreased rearing (Bhattacharya et al., 1998; Fone et al., 2002; Gold et al., 1988, 1989; Gold and Koob, 1989; Kehne et al., 1996; O'Loinsigh et al., 2001; Rempel et al., 1993). In comparison, S(+)-MDMA decreased (Callaway and Geyer, 1992; Callaway et al., 1990, 1992) or increased (Bankson and Cunningham, 2002; Bubar et al., 2004; Herin et al., 2005; McCreary et al., 1999) rearing events. The effect(s) of R(−)-MDMA on rearing behavior, however, has not been detailed. Previous studies in mice also have consistently demonstrated that racemic MDMA decreased rearing behavior (Fantegrossi et al., 2005; Maldonado and Navarro, 2000; Scearce-Levie et al., 1999) whereas the effect(s) of its enantiomers has not been examined. In the present study, (±)-MDMA and each isomer produced a different profile of effect on rearing (Fig. 8). Specifically, (±)-MDMA significantly decreased the number of entries, distance traversed, and time spent in rearing. In comparison, S(+)-MDMA produced a biphasic effect on the number of entries and time spent in rearing: 1 mg/kg produced statistically significant increases in the latter measures whereas higher doses had no effect on, or significantly decreased, those markers. In contrast, R(−)-MDMA had no effect on the number of entries into the vertical plane but produced, at 1 mg/kg, a significant increase in the amount of distance traversed and time spent in rearing (Fig. 8). The latter effects of R(−)-MDMA are curious because they indicate that this agent might prolong (both in distance and time) a control level of rearing entries. The increases in vertical activities that were produced by the enantiomers should have been mirrored by comparable increases in those measures by racemic MDMA, but such was not the case. It is also curious that the dose effect functions of the isomers did not occur to the left of that of racemic MDMA. On one hand, the dose response effect of one isomer was expected to be more potent than (i.e. to the left of) that of the racemate. On the other hand, the results are perhaps not a surprise given the findings that each of the three agents featured, at some dose(s), different effects on rearing behavior. Indeed, the (unexpected) enhanced potency of (±)-MDMA to decrease markers of vertical activity may be viewed as a distinct pharmacological effect that originated from the diverse and interactive effects of the isomers of MDMA. Overall, the effects of racemic MDMA on rearing behavior reported here are consistent with those of the aforementioned studies. However, the current results also suggest that (±)-MDMA and its isomers, at some dose(s), can exhibit quite different effects on rearing.

Finally, (±)-MDMA and S(+)-MDMA produced effects on jumps that were different from those exerted by R(−)-MDMA. Specifically, (±)-MDMA and S(+)-MDMA produced statistically significant and dose-related decreases in jumps whereas R(−)-MDMA, at 1 mg/kg and 3 mg/kg, produced statistically significant increases in jumps. Higher doses of R(−)-MDMA (up to 50 mg/kg) did not significantly increase or decrease (from control value) the number of jumps (Fig. 9). Thus, R(−)-MDMA exerted an effect on this measure that was not produced by (±)-MDMA or S(+)-MDMA. In addition, the results produced by R(−)-MDMA might explain, at least in part, the increase in movement episodes that were noted in Fig. 2. It is suggested that because a jump is a discreet event or episode (initiated and terminated within 2 s; see apparatus

in Materials and method section) that a) the effect of R(−)-MDMA on jumps, at 1 mg/kg and 3 mg/kg, contributes to the increase in the number of movement episodes by R(−)-MDMA at these doses and b) the increases in the number of jumps that are induced by R(−)-MDMA are likely inhibited by S(+)-MDMA, an effect that is predicated on the steric inference that calculated doses between 2 mg/kg and 6 mg/kg of (±)-MDMA should have produced a noticeable increase (from control) in jumps but did not. Finally, (±)-MDMA produced a dose response function that was slightly to the left of those of S(+)-and R(−)-MDMA and its (unexpected) enhanced potency to affect jumps, like its strengthened potency to reduce vertical activities, may be viewed as a distinct pharmacological effect that resulted from the combination of the particular effects of each isomer of MDMA.

In summary, the effects of MDMA and its optical isomers were evaluated in fully automated and computer integrated activity chambers, which identified and quantified eighteen measures of behavior of mice (see Table 1). In those analyses, one isomer of MDMA was expected to be more potent than its enantiomer and racemic MDMA as an agent that could exert a particular pharmacological action(s). In many cases, the behavioral effects of MDMA were stereoselective, an indication that both optical isomers produced similar actions but that one isomer was more potent than the other. Typically, S(+)MDMA was more potent than R(−)MDMA in numerous measures of activity: movement time, distance, velocity, movement episodes, margin distance, rotations (clockwise and counterclockwise), and retraced activities (movements, episodes, and time). Interestingly, the isomers seemed to produce an additive or synergistic (potentiation) effect to enhance the potency and/or effect of (±)-MDMA on some measures: movement episodes, center actions (entries and distance), clockwise rotations, vertical activities (entries, distance, and time) and jumps. In other measures, however, the enantiomers displayed different (stereospecific) effects: S(+)-MDMA produced a preference to induce counterclockwise (versus clockwise) rotations and each isomer exerted a different profile of effect on vertical activities and jumps. Also important was the observation that one isomer appeared to attenuate the effect of its enantiomer on some behaviors. In particular, R(−)-MDMA appeared to inhibit increases in the number of counterclockwise rotations and vertical entries induced by S(+)-MDMA. Similarly, S(+)-MDMA seemed to attenuate the increases in episodes (i.e. jumps) and vertical distance produced by R(−)-MDMA. The array of effects produced by MDMA and its enantiomers in the current study provide a framework for their evaluation in future studies. First, the three agents should be viewed as separate entities that might exert (stereoselective) similarities and (stereospecific) differences in their effects (for summary of potency/effects, see Table 1). Second, behavioral measures seem to be a critical determinant of the effect(s) and/or potency that the agents display, and divergent results should not be unexpected. In the present data, for example, certain measures of behavior seemed to have more labile baselines for the assessment of MDMA and its isomers and, thus, did not show the same drug sensitivity as other measures (e.g., vertical entries versus center entries). Moreover, the known and numerous neurochemical effects produced by MDMA and its enantiomers might be complicated further by the fact that these agents might interact with components of behavior that are probably not produced by a completely independent mechanism (i.e. neurotransmitter) but rather, by themselves, are the result of an interaction of neurotransmitters to determine a control level of behavioral activity (e.g., Berlyne, 1960; Iversen and Iversen, 1981). Third, each isomer of MDMA may exhibit addition, synergism (potentiation), or antagonism of an effect of its enantiomer and the appearance of those interactions is influenced greatly by the dependent variable. Fourth, the effect and/or potency of racemic MDMA may go beyond, or be more distinctive from, what would be expected of the action(s) of its optical isomers (Table 1). Admittedly, it is unusual for the racemic mixture of a drug to display an effect that is not exhibited by either isomer or to demonstrate potency that is equal to, or (slightly) greater than, the potencies of both of its enantiomers. Nonetheless, it may be the unique interaction(s) of the individual enantiomers of MDMA with components of ongoing behavior that accounts for the seemingly exceptional effect(s) of racemic MDMA. Indeed, the latter conclusion is not inconsistent with a commentary of the human psychopharmacological effects produced by (±)-, S(+)-, and R(−)-MDMA (Shulgin 2001): "*What was unexpected was that neither isomer gave the magic of the racemic MDMA. It was almost as if both the separate pharmacological components needed to be present to experience the unusual properties of the drug*" (italics added). Overall, the ultimate action of MDMA very likely involves complex steric effects and interactions, the interplay of various neurotransmitters, and the particular behavioral or biological event (or action) being measured.

Acknowledgements

This study was supported, in part, by the A.D. Williams Fund of Virginia Commonwealth University and DA 01642.

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