

Research report

Acute and long-term consequences of single MDMA administration in relation to individual anxiety levels in the rat

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Abstract

Our previous work has shown that normal male Wistar rats can differ systematically in their behavioral response to the elevated plus-maze (EPM), where animals with high (HA) or low anxiety (LA) levels can be identified based on the percentage of time spent in the open arms. These animals also differ in other behavioral tests (e.g. active avoidance), and in their serotonin levels in the ventral striatum. Here, we tested whether such HA and LA rats might respond differently to the amphetamine analogue 3,4-methylenedioxymethamphetamine (MDMA, “ecstasy”). This drug can affect psychomotor activation and anxiety; effects which are probably due to its pronounced serotonergic and dopaminergic impacts in the rat brain. Based on a routine screening procedure in the plus-maze, male Wistar rats were divided into HA and LA sub-groups, in which rectal temperature was measured. Thirty minutes after the i.p. injection of MDMA (7.5 or 15 mg/kg) or vehicle, they were again tested in the plus-maze. During the next 3 weeks, the animals underwent further behavioral tests (plus-maze, open field, active avoidance, forced swimming) to test for possible long-term consequences of MDMA. Rectal temperature was found to be higher in LA than HA rats and was especially increased with the higher dose of MDMA (15 mg/kg). In the acute plus-maze test, the lower dose of MDMA led to an anxiogenic-like profile, whereas the higher dose led to an anxiolytic-like profile, both in HA and LA rats. Possible long-term consequences of MDMA were only tested with 7.5 mg/kg MDMA, since the 15 mg/kg dose led to a high level of lethality. The analysis of open field, plus-maze (performed after 9–12 days), and forced swimming behavior (performed after 20–21 days) did not provide indications for lasting effects of MDMA. In contrast, active avoidance learning was impaired in LA- but not HA-rats treated with MDMA. A single injection of MDMA does not only have acute effects on anxiety and psychomotor activation, but can also have some prolonged or delayed task-dependent behavioral consequences. The detection of such sequels can require that individual differences are taken into account and here, determining anxiety levels in the EPM seems to serve as a useful approach.

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1. Introduction

3,4-Methylenedioxymethamphetamine (MDMA, “ecstasy”), a synthetic amphetamine analogue, is a psychoactive drug that has increasingly become popular for recreational use over the past two decades. In humans, MDMA acutely leads to a number of psychological changes including euphoria, feelings of intimacy, increased arousal, and others. Furthermore, it is known that MDMA can have acute adverse effects, including hypertension, hyperthermia, and gait instability. With, or after, repeated intake other adverse symptoms may appear, including anxiety, depression,

impulsiveness, hostility, and deficits of memory and attention [35,54]. These long-term consequences are thought to be due to the known neurotoxic properties of MDMA, especially on serotonin (5-HT) in the forebrain [35]. Furthermore, part of the consequences of ecstasy use may be determined by premorbid differences between ecstasy users and non-users, including the level of personality and central serotonin function [10,35].

It is extremely difficult to adequately address such possible premorbid issues in clinical studies, whereas they can more easily be investigated in animal models. Here, MDMA has already been studied in great detail, especially in rodents and primates. This work has shown that MDMA has prominent and characteristic effects on brain and behavior which are partly similar to those of other amphetamines. Thus, it is known that acute MDMA increases sympathetic impact,

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leads to a so-called 5-HT-syndrome, and has psychostimulatory and reinforcing effects, which are probably due to its pronounced stimulatory actions on 5-HT and dopamine in the brain [4,13,25,56]. Furthermore, it has repeatedly been shown that MDMA can be neurotoxic when given in high or repeated doses, and here forebrain 5-HT systems seem to be especially at risk [50]. Behaviorally, long-term changes have also been observed in animal models including changes in anxiety, social behavior, learning and memory [4,14,17,28,32,37].

Apart from this wealth of behavioral and physiological evidence, individual factors have not yet received substantial evidence in the animal literature. Comparing data from studies, where different rat strains were used, have provided indirect evidence indicating that the effects of MDMA on anxiety may differ between strains with high (HA) or low anxiety (LA) [16]. Systematic differences in brain physiology and behavior cannot only be observed between but also within rat strains, and such differences can affect the responsiveness to drug challenges [6,8,9,41,52]. With respect to MDMA, our recent work on normal male Wistar rats may especially be relevant: we have shown that rats, which were screened for high or low levels of anxiety in the elevated plus-maze (EPM) also differed in another test of anxiety (object burying) and in active avoidance learning, but not in the forced swimming model of depression [18]. Physiologically, they were found to differ with respect to ventral striatal 5-HT levels [48], and the expression of striatal interleukin-2 mRNA [42].

MDMA is known to act largely by central 5-HT (and dopamine), for example, via the nucleus accumbens of the ventral striatum [56]. Therefore, one might expect that the acute and/or long-term outcome of MDMA might differ between animals with high or low anxiety levels. As a first approach to examine such possible relationships, we performed the present experiment, using normal male Wistar rats. We screened these rats for their anxiety levels in the EPM according to our routine procedures [18,48] and then treated them once with systemic MDMA (7.5 or 15 mg/kg). The outcome of MDMA was tested acutely (EPM, body temperature), and during the following 3 weeks (EPM, open field, active avoidance, forced swimming). We asked whether MDMA has dose-dependent acute or later effects, and whether these might differ between rats with high or low basal anxiety levels.

2. Materials and methods

2.1. Animals

Sixty-five male Wistar rats (Harlan Winkelmann, Borcheln, Germany) were housed in groups of five rats in acrylic cages (35 cm × 56 cm × 19 cm) in an animal room (22–24 °C) under a 12 h/12 h light/dark cycle (lights on at 07:00 h) with food and water provided ad libitum. The

mean body weight was 271.9 ± 1.0 g (mean $\pm$ S.E.M.) at the beginning of the experiment. Each animal was handled for 5 min on 4 consecutive days prior to the experiment.

2.2. Body temperature

Rectal body temperature was taken pre-drug and 35 min thereafter. The measurement was performed in the awake, hand-restrained animal using a Testo 110 thermometer (Testo GmbH & Co., Lenzkirch, Germany) with a metal probe (4 mm in diameter) which was inserted by 2.5 cm according to [39].

2.3. Drug treatment

D,L-MDMA-HCl (Lipomed, Switzerland) was dissolved in saline and was administered i.p. in doses of either 7.5 or 15 mg/kg. Immediately after the first temperature test, drug or vehicle were injected (HA animals: vehicle $n = 11$, 7.5 mg/kg MDMA $n = 15$, 15 mg/kg MDMA $n = 7$; LA animals: vehicle $n = 13$, 7.5 mg/kg MDMA $n = 9$, 15 mg/kg MDMA $n = 10$). After drug administration, the animals were kept singly for 30 min in a new animal cage without bedding.

2.4. Behavioral tests

2.4.1. Elevated plus-maze

This test was applied 1 day before, and 30 min and 11 days after drug treatment. The apparatus was made of plastic and consisted of two opposed open arms (50 cm × 10 cm), two opposed enclosed arms with no roof (50 cm × 10 cm × 40 cm), and an open square (10 cm × 10 cm) in the center. The maze was elevated 50 cm above the floor and was monitored by a video camera from above. The open arms were surrounded by a small plastic rim (2 mm high, 3 mm wide) to reduce the likelihood that animals fell from these arms. In addition, a 10-cm thick foam mattress was placed under the EPM to avert injuries in case of falls. If an animal fell from the maze, it was immediately placed back to the position from which it had fallen.

Behavioral tests were conducted under red light (28 lx in the center). Each test lasted 5 min. The animals were placed into the center of the EPM, facing one of the open arms. The following measures were analyzed from videotapes by trained observers: (a) the number of entries into open and closed arms, (b) the time spent on center, open, or enclosed arms, and (c) open arm latency, that is, the time from placing the rat into the EPM until it entered one of the open arms. An entry into any of the compartments was defined as all four paws being placed on the arm. Furthermore, within-arm activity was measured in the test performed acutely after MDMA administration, that is, the number of times were counted which an animal crossed a virtual line which divided an arm into a proximal and a distal half.

Based on behavior in the pre-drug EPM test, the rats were ranked using the relative time spent in the open arms (expressed as percentage of total observation time; according to [18,48]) and were assigned to sub-groups with either HA ($n = 33$); high anxiety or LA levels ($n = 32$); low anxiety.

2.4.2. Open field

Open field activity was measured 2 days before, and on days 9–10 after drug treatment under conditions of red light (28 lx in the center). The animals were placed into an acrylic box (41 cm $\times$ 41 cm $\times$ 41 cm) which was monitored by an automated activity monitoring system (Tru ScanTM, Photo-beam Sensor-E63-22, Coulbourn Instruments, USA). One tier of infrared beams was mounted horizontally 3 cm, and a second tier was mounted 15.5 cm above the floor. The following measures were taken: (1) rearing number, (2) locomotion: the horizontal distance moved in cm, (3) center time: defined as the animal's center of gravity being within the center area of the open field (27 cm $\times$ 27 cm), (4) center entry: the number of entries into the center area of the open field. Each test lasted 10 min.

2.4.3. Two-way active avoidance

Active avoidance behavior was tested 15 days after drug treatment. This test was carried out in the observation room under 900 lx illumination, using a two-way shuttle box (33 cm $\times$ 66 cm $\times$ 39 cm). The floor was made of 2 mm diameter stainless steel rods spaced 1.5 cm apart. The box was divided into two equal compartments by a 5-cm high plexiglas barrier. Each compartment could be electrified separately through a shock scrambler (521/C, Camden Instruments). A speaker was mounted in the center on the top of the box for delivery of auditory stimuli. The animals were placed into the shuttle box and allowed to explore the entire apparatus for 2 min. Then, they received 20 shuttle trials. Each trial began with a 115 dB tone (3 s) which was followed by a 0.3-mA scrambled foot shock. If the animal crossed the barrier during the tone, the stimulus was terminated and no shock was delivered (active avoidance response). If the animal crossed the barrier during shock delivery, an escape response was measured. If the rat failed to cross, the shock was terminated after 15 s (escape failure). After 42–60 s, the next trial was initiated. The latency to avoid or escape, and the number of avoidances, escapes, and failures were recorded by trained observers.

2.4.4. Forced swim

This test was carried out on days 20–21 after drug treatment in a clear glass tank (25 cm $\times$ 25 cm $\times$ 60 cm) containing tap water (height: 39 cm; temperature: 26 °C). The apparatus was cleaned thoroughly, and water was changed from rat to rat. Two swimming sessions were conducted and videotaped: 15 min on the first day and 5 min on the day thereafter. After each test, the rats were dried and kept under a heating bulb for 30 min before being returned to their home cages. The following parameters were measured from videotapes: (1)

struggling: strong movements of the limbs occurring during diving, breaking the water surface, or scratching the walls, and (2) immobility, which occurred when the rats remained motionless, or floating, including small limb movements to keep their heads above the water [1].

2.5. Data analysis

Statistical testing was performed using either unpaired *t*-tests (HA versus LA rats), or ANOVA with treatments (vehicle, MDMA) and sub-groups (LH, HA) as factors. In case of open field behavior and active avoidance, the time points of testing were added as an additional factor (ANOVA for repeated measures). Whenever indicated by the ANOVA, least-significant (LSD) tests were used as post hoc tests. The level of significance was defined as $P < 0.05$.

3. Results

3.1. Pre-drug behavior in the EPM

As outlined above, animals were assigned to HA and LA sub-groups based on pre-drug behavior in the EPM (percentage of open arm time). These sub-groups had the following profiles in the EPM (Table 1): on average, HA rats spent about 82 s on the open arms compared to 188 s in LA rats. HA spent most of the time in the closed arms (172 s compared to 80 s in LA rats), and they also spent more time in the center (40 s compared to 26 s in LA rats). The latency to the first entry into an open arm was slightly higher in HA rats, whereas the overall number of arm entries (open, closed) did not differ between the two sub-groups.

3.2. Body temperature

Pre-drug body temperature (Fig. 1) was higher in LA than in HA rats ($P < 0.01$). After injection, temperature was increased irrespective of group (factor time: $P < 0.001$), but was increased most strongly after 15 mg/kg than after vehicle, or 7.5 mg/kg of MDMA (factor treatment: $P < 0.001$;

Table 1
Plus-maze behavior before drug administration

	HA	LA
Open arm percentage	27.3 $\pm$ 1.7***	63.4 $\pm$ 3.0
Open arm time (s)	81.7 $\pm$ 5.2***	188.4 $\pm$ 8.9
Closed arm time (s)	172.2 $\pm$ 8.2***	79.5 $\pm$ 8.2
Center time (s)	39.7 $\pm$ 5.7*	26.0 $\pm$ 2.3
Open arm latency (s)	10.0 $\pm$ 2.3	5.2 $\pm$ 1.1
Arm entries (number)	13.3 $\pm$ 0.7	14.0 $\pm$ 0.6

Means $\pm$ S.E.M. are shown.

* Difference between HA and LA rats according to two-tailed *t*-test ($P < 0.05$).

*** Difference between HA and LA rats according to two-tailed *t*-test ($P < 0.001$).

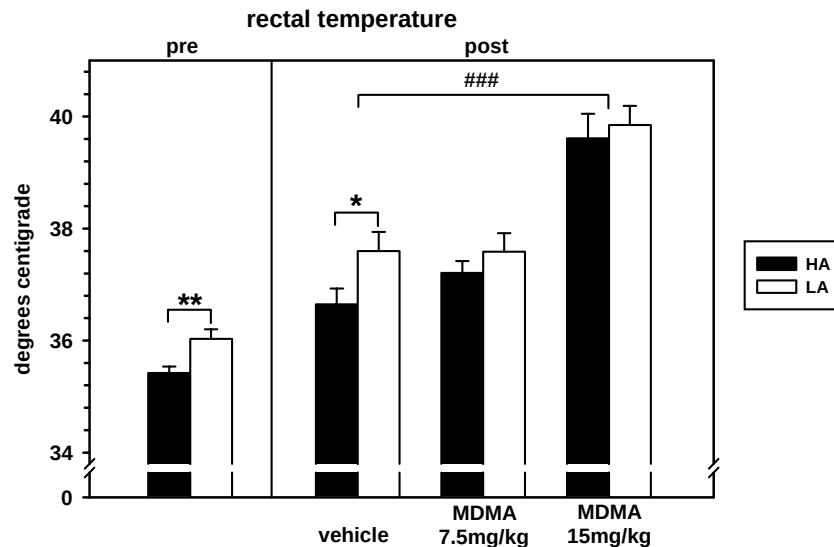


Fig. 1. Rectal body temperature in degrees centigrade (mean $\pm$ S.E.M.) before (pre) and 35 min after (post) i.p. injection of saline or MDMA (7.5 or 15 mg/kg) in rats with high (HA; full bars) or low levels of anxiety (LA; open bars). Asterisks indicate differences between HA and LA rats (* P < 0.05, ** P < 0.01), or between treatment groups (### P < 0.001).

interaction between sub-groups and time: P < 0.001; LSD tests: P < 0.001). Post-injection LA rats had significantly higher body temperature levels than HA rats when administered saline (P < 0.05) but not following MDMA.

3.3. Lethality

None of the animals treated with vehicle or the lower dose of MDMA died during the course of the experiment. In contrast, 14 out of 17 animals treated with 15 mg/kg of MDMA died within 2–8 h after injection, that is, they could only be tested in the acute EPM test immediately following injection. The remaining 3 animals survived until the end of the experiment, but were not considered for further data evaluation except the acute EPM test.

3.4. Post-drug behavior

3.4.1. EPM—acute test

MDMA affected acute EPM behavior in a dose-dependent way (Table 2): with the 15 mg dose, open arm time and percentage (P < 0.001), open arm entries (P = 0.001), and activity within the open arms (P < 0.001) were increased, whereas center time (P = 0.009) and closed arm time (P < 0.001) were decreased compared to vehicle. With the 7.5 mg/kg dose, open arm latency (P < 0.001) was increased, and activity within the closed arms (P < 0.001) and total arm activity (P < 0.001) were increased compared to vehicle controls. There were no overall differences between HA and LA rats (factor sub-groups), nor significant interactions between treatments and sub-groups (P -values > 0.10).

Table 2
Plus-maze behavior 30 min after injection

	Vehicle		7.5 mg/kg MDMA		15 mg/kg MDMA	
	HA	LA	HA	LA	HA	LA
Open arm percentage	19.5 $\pm$ 4.7	37.6 $\pm$ 5.9	13.3 $\pm$ 6.8	20.2 $\pm$ 7.3	68.9 $\pm$ 8.3***	68.8 $\pm$ 9.6***
Open arm time (s)	58.5 $\pm$ 14.0	112.5 $\pm$ 17.6	39.1 $\pm$ 19.9	59.4 $\pm$ 21.3	203.3 $\pm$ 25.6***	197.1 $\pm$ 27.2***
Closed arm time (s)	212.1 $\pm$ 18.4	149.9 $\pm$ 19.0	233.3 $\pm$ 23.3	204.8 $\pm$ 24.5	56.9 $\pm$ 21.5***	68.9 $\pm$ 27.3***
Center time (s)	27.4 $\pm$ 6.8	34.7 $\pm$ 4.9	18.9 $\pm$ 4.2	27.6 $\pm$ 3.6	19.4 $\pm$ 4.7**	14.8 $\pm$ 3.6**
Open arm latency (s)	69.7 $\pm$ 36.5	11.8 $\pm$ 9.4	203.4 $\pm$ 36.5***	121.9 $\pm$ 45.1***	12.6 $\pm$ 3.3	46.0 $\pm$ 28.5
Arm entries (number)	10.5 $\pm$ 1.1	15.7 $\pm$ 0.9	15.4 $\pm$ 2.2	14.0 $\pm$ 2.6	18.0 $\pm$ 3.2	15.8 $\pm$ 2.5
Open arm entries (number)	3.8 $\pm$ 0.8	8.0 $\pm$ 0.8	3.8 $\pm$ 1.9	4.6 $\pm$ 1.6	13.4 $\pm$ 3.5**	10.9 $\pm$ 2.2**
Closed arm entries (number)	7.1 $\pm$ 0.8	8.4 $\pm$ 0.7	11.5 $\pm$ 1.9	9.6 $\pm$ 1.8	4.7 $\pm$ 1.8	4.8 $\pm$ 1.4
Open arm activity (number)	5.5 $\pm$ 1.2	12.0 $\pm$ 1.9	5.2 $\pm$ 2.6	3.6 $\pm$ 1.2	23.1 $\pm$ 5.6***	18.7 $\pm$ 4.1***
Closed arm activity (number)	14.8 $\pm$ 1.4	15.8 $\pm$ 1.3	33.5 $\pm$ 4.0***	27.9 $\pm$ 4.5***	9.4 $\pm$ 3.9	8.8 $\pm$ 2.4
Total arm activity (number)	20.4 $\pm$ 1.7	27.8 $\pm$ 1.5	38.7 $\pm$ 3.1***	31.4 $\pm$ 4.1***	32.6 $\pm$ 5.2	27.5 $\pm$ 4.0

Means $\pm$ S.E.M. are shown.

** Difference between a given MDMA treatment and vehicle according to ANOVA followed by post hoc LSD tests (P < 0.01).

*** Difference between a given MDMA treatment and vehicle according to ANOVA followed by post hoc LSD tests (P < 0.001).

It should be noted, however, that differences between vehicle-treated HA and LA rats were observable during this test, since HA rats spent more time in the closed arms (*t*-tests: $P = 0.030$), and less time in the open arms ($P = 0.029$). Furthermore, the number of total arm entries was lower in HA than LA rats ($P < 0.001$). This difference was due to open but not closed arm entries, since only the number of open arm entries was lower in HA than in LA rats ($P = 0.002$). Activity within the arms was higher in LA than HA rats treated with vehicle ($P = 0.004$). Again, this difference was observed only in the open ($P = 0.012$), but not in the closed arms.

Furthermore, several symptoms (not quantified) were observed in MDMA-treated animals, including stereotypic head movements, some seizures, sprayed hind legs, ataxia, and placing deficits, especially with the higher dose. The placing deficits were probably the reason for an increased rate of falls from the open arms of the EPM: 10 out of 17 animals treated with 15 mg/kg MDMA fell between one and six times (five HA and five LA rats), whereas 5 out of 24 animals treated with 7.5 mg/kg MDMA fell between one and two times (two HA and three LA rats), and 2 out of 24 vehicle-treated animals fell once (two LA rats). In order to measure how falling from an open arm might affect subsequent open arm avoidance, the time to re-enter the respective arm was measured. This analysis showed that the two vehicle-treated rats did not re-enter the open arm thereafter, whereas all animals treated with 7.5 mg/kg re-entered the open arm within 14–41 s. Two animals treated with 15 mg/kg did not re-enter the arm from which they fell, whereas the other animals re-entered it within 15–50 s.

In order to exclude falling as a possible confounding factor for EPM behavior, data analysis was repeated after exclusion of all animals which had fallen from the maze. The ANOVA analysis (Table 3) of the remaining animals yielded the following patterns: with 15 mg/kg MDMA, open arm time and percentage ($P < 0.001$), and open arm activity ($P = 0.004$) were increased, whereas closed arm time

($P < 0.001$) and activity ($P = 0.039$) were decreased as compared to vehicle. With 7.5 mg/kg, open arm time and percentage ($P < 0.001$), open arm entries ($P = 0.015$), and activity within the open arms ($P = 0.002$) were reduced, and open arm latency ($P < 0.001$), closed arm time ($P < 0.001$), closed arm entries ($P = 0.023$), activity within closed arms ($P < 0.001$), and total arm activity ($P < 0.001$) were increased.

3.4.2. EPM test—12 days after treatment

In this test, there were no longer significant differences between rats which had received vehicle or 7.5 mg/kg MDMA 12 days before (Table 4; factor treatment: P -values > 0.05). There were overall differences between HA and LA rats (factor sub-groups), since HA rats showed less open arm time and percentage ($P = 0.006$), more closed arm time ($P = 0.004$), and a higher latency to enter the open arms ($P = 0.031$). There were no significant interactions between treatments and sub-group (P -values > 0.10).

3.4.3. Open field test

Locomotor activity, center time, and rearing behavior (days 9 and 10 after treatment; Table 5) did not differ between vehicle- and MDMA-treated rats (P -values > 0.10), and there were no differences in locomotion or rearing, between HA and LA rats (P -values < 0.10), whereas center time was lower in HA than LA rats ($P = 0.019$). There were no interactions between treatments, sub-groups and days of testing (P -values > 0.10).

3.4.4. Two-way active avoidance

There was no general difference of avoidance learning (Fig. 2) between vehicle- and MDMA-treated rats (factor treatment: $P = 0.245$), nor between HA- and LA-treated rats (factor sub-groups: $P = 0.584$). However, there was a significant interaction between treatments and sub-groups ($P = 0.007$), since the number of avoidances in vehicle-treated HA rats was lower than in vehicle-treated LA rats ($P =$

Table 3
Re-analysis of plus-maze behavior 30 min after injection

	Vehicle ($n = 22$)	7.5 mg/kg MDMA ($n = 19$)	15 mg/kg MDMA ($n = 7$)
Open arm percentage	28.9 $\pm$ 4.5	5.8 $\pm$ 3.2***	64.1 $\pm$ 12.0***
Open arm time (s)	86.6 $\pm$ 13.6	17.5 $\pm$ 9.6***	192.2 $\pm$ 36.1***
Closed arm time (s)	181.1 $\pm$ 15.7	256.0 $\pm$ 12.6**	83.8 $\pm$ 34.7***
Center time (s)	30.6 $\pm$ 4.4	20.0 $\pm$ 3.3	14.8 $\pm$ 4.3
Open arm latency (s)	41.7 $\pm$ 19.6	213.7 $\pm$ 30.1***	57.0 $\pm$ 40.9
Arm entries (number)	13.0 $\pm$ 0.9	13.9 $\pm$ 1.8	13.6 $\pm$ 2.0
Open arm entries (number)	5.9 $\pm$ 0.7	2.2 $\pm$ 1.1*	9.3 $\pm$ 2.0
Closed arm entries (number)	7.7 $\pm$ 0.6	11.7 $\pm$ 1.6*	4.4 $\pm$ 1.0
Open arm activity (number)	8.9 $\pm$ 1.4	1.9 $\pm$ 1.1**	17.4 $\pm$ 4.1**
Closed arm activity (number)	15.2 $\pm$ 1.0	35.4 $\pm$ 3.1***	8.3 $\pm$ 2.0*
Total arm activity (number)	24.1 $\pm$ 1.5	37.3 $\pm$ 2.8***	25.7 $\pm$ 4.1

Behavioral data are given after excluding those animals which had fallen at least once from the plus-maze during this test. Means $\pm$ S.E.M. are shown.

* Different from vehicle according to LSD tests performed post hoc to ANOVAs ($P < 0.05$).

** Different from vehicle according to LSD tests performed post hoc to ANOVAs ($P < 0.01$).

*** Different from vehicle according to LSD tests performed post hoc to ANOVAs ($P < 0.001$).

Table 4
Plus-maze behavior on day 12 after drug administration

	Vehicle		7.5 mg/kg MDMA	
	HA	LA	HA	LA
Open arm percentage	14.8 ± 4.5**	22.6 ± 5.7	8.9 ± 2.5**	31.0 ± 8.0
Open arm time (s)	44.5 ± 13.5**	67.8 ± 16.9	26.6 ± 7.4**	92.9 ± 23.9
Closed arm time (s)	224.8 ± 16.9**	188.1 ± 17.3	227.9 ± 12.4**	159.2 ± 24.3
Center time (s)	28.4 ± 6.9	41.9 ± 6.6	43.4 ± 7.3	42.3 ± 4.7
Open arm latency (s)	69.9 ± 36.8*	1.9 ± 0.5	116.8 ± 34.6*	57.3 ± 22.6
Arm entries (number)	12.7 ± 1.2	14.2 ± 1.1	11.3 ± 1.1	11.7 ± 0.6

Means ± S.E.M. are shown.

* Difference between HA and LA rats irrespective of treatment according to LSD tests performed post hoc to ANOVAs ($P < 0.05$).

** Difference between HA and LA rats irrespective of treatment according to LSD tests performed post hoc to ANOVAs ($P < 0.01$).

Table 5
Open field behavior 9 and 10 days after drug administration

	Vehicle				7.5 mg/kg MDMA			
	Day 9		Day 10		Day 9		Day 10	
	HA	LA	HA	LA	HA	LA	HA	LA
Locomotion (cm)	506.9 ± 44.0	533.2 ± 28.7	520.8 ± 36.6	570.5 ± 25.2	581.7 ± 33.6	540.0 ± 44.3	543.9 ± 26.5	534.9 ± 46.2
Center time (s)	244.5 ± 33.1*	356.2 ± 33.6	289.5 ± 28.1*	395.2 ± 32.0	334.8 ± 24.3*	337.2 ± 36.5	325.6 ± 28.8*	371.0 ± 34.5
Rearings (no)	20.8 ± 3.4	25.8 ± 2.0	26.1 ± 1.8	30.5 ± 2.0	27.9 ± 3.2	24.8 ± 3.0	27.9 ± 2.8	27.8 ± 4.2

Means ± S.E.M. are shown.

* Difference between HA and LA rats irrespective of treatment according to LSD tests performed post hoc to ANOVAs ($P < 0.05$).

0.018). Furthermore, MDMA-treated LA rats showed less avoidances than vehicle-treated LA rats ($P = 0.009$).

3.4.5. Forced swim test

Immobility in the first forced swim test did not differ between vehicle- and MDMA-treated animals (ANOVA; factor treatment: $P = 0.162$), nor between LA and HA rats (factor sub-group: $P = 0.283$). Furthermore, there were no

significant interactions between treatments and sub-groups ($P = 0.846$). During the second test, immobility time was slightly, but not significantly, higher in vehicle- than MDMA-treated rats (factor treatment: $P = 0.055$). Again, there were no differences between HA and LA rats (factor sub-group: $P = 0.391$), nor interactions between treatments and sub-groups ($P = 0.650$; results not shown in detail).

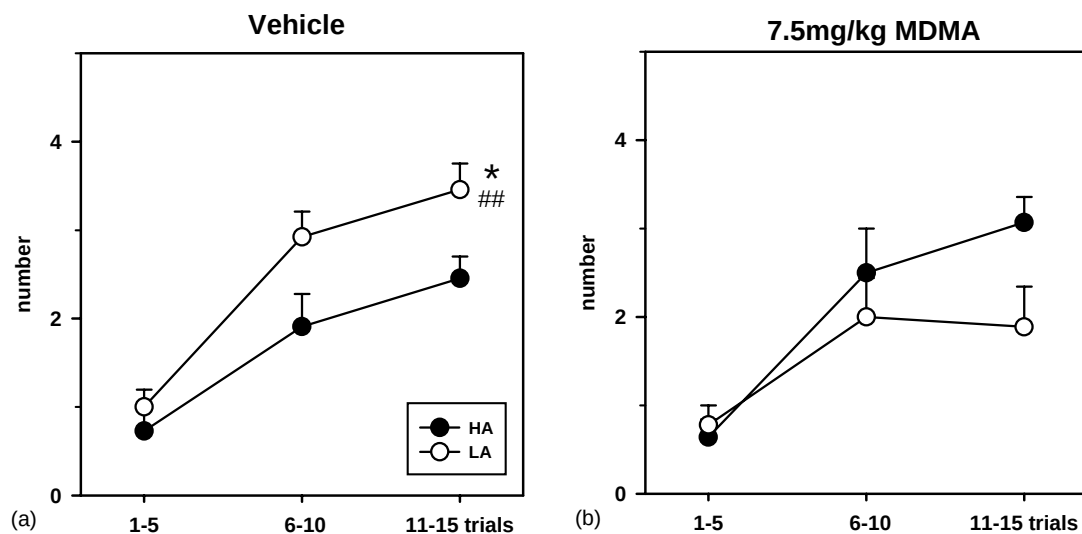


Fig. 2. The number of avoidances (mean ± S.E.M.) during three consecutive blocks of five trials each. Fifteen days before, the animals had received either an i.p. injection of vehicle (left) or 7.5 mg/kg MDMA. Asterisk (*) indicates a difference ($P < 0.05$) between HA (full circles) and LA rats (open circles), and ## indicates a difference ($P < 0.01$) between vehicle- vs. MDMA-treated LA rats (based on ANOVA and post hoc LSD tests).

4. Discussion

The present results show that MDMA not only has acute and dose-dependent effects in the EPM, but that a single injection of MDMA (7.5 mg/kg) can have relatively lasting effects on behavior. These later effects seem to depend (A) on the kind of test paradigm used, and (B) on subject-dependent factors, namely individual levels of anxiety.

4.1. Body temperature

Rectal temperature was measured to gauge acute physiological effects of MDMA treatment. Unexpectedly, we found that LA rats had higher rectal temperature levels than HA rats when tested before drug treatment, or after i.p. saline. An examination of the literature shows that individual and breeding-dependent differences in body temperature have been observed before in rats [21,23,41]. Interestingly, it was found that male Wistar rats bred for high or low levels of anxiety did not differ in their basal body temperature, but that temperature increased more strongly in rats with low anxiety when these were stressed, like by EPM exposure or social defeat [23]. These results from genetically selected rats bear strong resemblance to the present data, since we also observed higher body temperature in (outbred) rats with low anxiety. Furthermore, our temperature method, which requires handling of the awake animals and insertion of a rectal probe, probably also had stressor-like properties which can lead to a rise in body temperature [33]. Therefore, the present findings may actually reflect differential physiological responses to a stressor, rather than basal differences.

MDMA treatment led to an increase in body temperature which has repeatedly been observed before [15]. Compared to vehicle, this increase was observed only with 15 mg/kg of MDMA. The lack of effect with the 7.5 mg/kg dose was probably due to the fact that the injection procedure as such led to an increase of temperature, since body temperature was also increased after vehicle treatment (as compared to the preceding measurement); an increase, which might have concealed the possible effect of the lower dose of MDMA. In contrast to the baseline measurement and that after vehicle treatment, there were no substantial temperature differences between HA and LA rats after either dose of MDMA, indicating that the acute actions of MDMA outweighed the individual differences.

4.2. Lethality

The single dose of 15, but not 7.5 mg/kg of MDMA, led to a high level of lethality in the present study. This high lethality could be due to the Wistar strain used, since comparable doses have often been used in Dark agouti, Lister-hooded, Long-Evans, and Sprague-Dawley rats without reports of lethality [2,5,22,28,39,45,51,57], and “only” 20% lethality was reported with even 40 mg/kg in Holtzman rats [27]. Furthermore, there are also studies in Wistar rats without

reports of lethality with repeated doses of 5 or 10 mg/kg [36,43], or a single dose of 15 mg/kg [7]. It can, therefore, be assumed that strain and dose were not the critical factors for the present lethality. It is known that amphetamines like MDMA can compromise thermoregulatory ability leading to lethal hyperthermia and heatstroke [3,15]. This may have also been the case in the present experiment, especially since the higher dose was the one which led to pronounced increases of body temperature. Furthermore, an interaction with ambient temperature has to be considered, since the likelihood of lethality seems to increase with increasing ambient temperature [15,27]. Possibly, a temperature level of 24 °C as used here is already critical. Furthermore, environmental humidity which impedes heat loss from the body seems to contribute to lethality after MDMA [15]. Finally, since animals were housed in groups, the phenomenon of “aggregate toxicity” may have played a role [12]. These methodological factors deserve special attention in future experiments to reduce lethality to a minimum.

4.3. Elevated plus-maze

When tested 30 min after injection, MDMA led to dose-dependent effects in EPM behavior: with 7.5 mg/kg, anxiogenic-like and psychostimulatory effects were obtained, since this dose led to increased latencies to enter the open arms, less time spent in these arms, and more total arm entries. The anxiogenic profile became even more pronounced when excluding those animals which had fallen from the EPM. In contrast, the 15 mg/kg dose appeared to be anxiolytic since it was followed by increased open arm time. This pattern was still observable when excluding rats which had fallen from the EPM, indicating that falling acted only as a minor confound on drug-induced behavior. Nevertheless, this confound should be excluded in the future, for example, by using higher rims around the open arms.

Interestingly, the effects with the higher dose were observed although behavioral performance was severely affected by the drug. Thus, most of the animals treated with 15 mg/kg continued to ambulate within the open arms despite ataxia, placing deficits and eventually dropping from the open arms. Furthermore, when placing them back to the position from which they had dropped, they continued to ambulate on the open arms. This persistent ambulation together with repeated head waving indicates that the animals behaved in a stereotypic way. These stereotypies, however, were linked to the environment and to anxiety, since the animals persisted to ambulate within the open rather than in the closed arms. These results are well in accord with the current literature, since dose-dependent MDMA effects on anxiety have been observed before in several test paradigms [17,36,38], including anxiogenic effects with lower and anxiolytic effects with higher doses [26].

Interestingly, our sub-groups of HA and LA rats treated with MDMA did not differ in acute EPM behavior, whereas the vehicle-treated sub-groups did (like in open

arm percentage). Possibly, the dose-dependent anxiogenic and anxiolytic drug effects acutely blunted the endogenous differences between HA and LA rats. In the future, an intermediate dosage of MDMA should also be tested assuming that with such an intermediate dose the direction or magnitude of effect (anxiolytic, anxiogenic) might depend on individual levels of emotionality.

During the re-test performed 12 days later, differences between vehicle- and MDMA-treatment (7.5 mg/kg) could no longer be observed, for example, in open arm time, indicating that the drug experience in the EPM (and possible changes induced by MDMA treatment) did not have lasting effects on behavior in this test. In contrast, general differences between HA and LA rats could again be observed in this test, indicating that our measure of anxiety is actually measuring a behavioral trait [18].

4.4. Open field

The open field test performed 9 and 10 days after treatment did not yield differences in psychomotor activity between animals previously treated with vehicle or MDMA, since these had comparable levels of locomotion and rearing. In other studies, decreased locomotion [55], trends for more locomotion and rearings [32], or no effects were observed [30]. The inconsistencies between studies are probably due to several methodological differences, including dosages and behavioral testing. The present study indicates that a single dose of MDMA as used here does not necessarily lead to long-term changes in these open field measures. Nevertheless, the open field test provided evidence for differences between HA and LA rats, since HA rats in the vehicle group showed less center time than LA rats. Avoiding the center of an open field also serves as a measure of anxiety [53]; thus, less center time in HA rats is plausible. This result shows that differences between HA and LA rats are not restricted to the EPM, but are also observable in other tests where anxiety plays a role [18].

4.5. Forced swim test

Similar to other work, we used immobility time as the critical measure in the forced swim test [1]. When analyzing immobility, we did not obtain evidence for differences between HA and LA rats, neither in animals which had received vehicle nor MDMA 3 weeks before. These data support the conclusion that differences between HA and LA rats which can be measured in EPM, or active avoidance behavior, are not necessarily paralleled by differences in forced swim performance [18]. This lack of relationship between the two tests seems to contrast findings in selectively bred Wistar lines, where rats with high anxiety show more immobility time [24]. Interestingly, using the anxiolytic diazepam these authors came to the conclusion that anxiety and forced swim immobility seem to be regulated by different (but yet unknown) mechanisms which may not be effective in the

same way when using unbred Wistar rats, as in the present case.

4.6. Active avoidance

This test showed differences between HA and LA rats and interactions with MDMA treatment. First of all, HA rats pre-treated with vehicle acquired the appropriate avoidance response less efficiently than LA rats, since their numbers of avoidances were lower. This finding adds further evidence to previous data obtained by others and us [18,24], showing that the active avoidance paradigm is especially sensitive to detect differences in Wistar rats with high or low anxiety. These results are unlikely due to differences in spinal pain reactivity since we recently found that HA and LA rats do not differ in the tail-flinch test [49]. An alternative explanation could be that HA rats are more emotional in response to shock than LA rats which might impair acquisition of avoidance learning in the shuttle box [11].

Furthermore, one should point out that the avoidance test did not yield differences between MDMA and vehicle pre-treatment when analyzing data irrespective of the HA and LA criteria, since similar rates of overall avoidances were observed after either treatment. This finding shows that moderate treatment effects can remain undetected when individual differences are not taken into account. However, when analyzing avoidance in relation to HA and LA criteria, MDMA effects were found, since the number of avoidances in LA rats pre-treated with MDMA was lower than that of LA rats treated with vehicle. This adds further support to previous findings showing that MDMA can impair performance in tests of learning and memory [28,34,37,46] and supports other work showing that even single injections of MDMA can have relatively lasting behavioral effects [32]. Importantly, our results indicate that such deficits may depend on trait variables, here individual levels of anxiety [16]. We have previously shown that HA and LA rats differ in their levels of 5-HT in the ventral striatum [48]. It is known that MDMA acts critically via 5-HT mechanisms, and here, the nucleus accumbens which is part of the ventral striatum appears to play a role [56]. Since the nucleus accumbens also seems to be involved in aversive learning and anxiety [19,29,44,47], including an involvement of local 5-HT [40], one could at least speculate that the differential patterns of behavioral long-term consequences of MDMA were due to physiological differences in the nucleus accumbens of HA and LA rats. It is known that repeated MDMA treatment leads to neuronal damage, especially of 5-HT and dopamine neurons in the forebrain, including the nucleus accumbens [50]. With single injections, 5-HT damage may also occur, but has not consistently been observed [5,7,20,31,51,57]. These factors have to be controlled in future studies with HA and LA rats either by neurochemical analysis of possible transmitter depletions in this and other brain areas, or by administering MDMA locally to the ventral striatum.

Together, the present experiment supports and extends our previous work [18,48] by showing that individual differences of EPM behavior in male Wistar rats are paralleled by physiological differences (here body temperature) and reactivity in other behavioral tests (e.g. active avoidance). Furthermore, a single MDMA treatment can have relatively lasting and task-dependent behavioral effects which are dependent on individual anxiety levels.

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