Short Communication

Pharmacology

Pharmacology 2003;69:180–182
DOI: 10.1159/000073661

Received: July 15, 2003
Accepted: July 15, 2003

Methylenedioxymethamphetamine (MDMA, 'Ecstasy') Serves as a Robust Positive Reinforcer in a Rat Runway Procedure

Gudrun Wakonigg Katja Sturm Alois Saria Gerald Zernig

Division of Neurochemistry, Department of Psychiatry, University of Innsbruck, Innsbruck, Austria

Key Words

3,4-Methylenedioxymethamphetamine · Ecstasy · Runway · Sprague-Dawley rats · Long-Evans rats · Reinforcement

Abstract

Although 'ecstasy' (3,4-methylenedioxymethamphetamine, MDMA) is, after marijuana, the second most prevalent illegal drug of abuse in European adolescents, animal experimental evidence of MDMA's reinforcing effect has remained scarce, particularly in the rodent model, raising questions about the robustness of MDMA's reinforcing effect under controlled laboratory conditions. In the present rat runway study, Sprague-Dawley and Long-Evans rats were given the opportunity to run for intravenous injections of saline or MDMA (1 mg/kg). MDMA significantly decreased runtimes in both rat strains. Thus, MDMA's positive reinforcing effect can be demonstrated not only across rat strains but also across operant conditioning paradigms. These findings should reassure the drug abuse research community that the investigation of MDMA's reinforcing effect in the inexpensive and widely used rodent model is indeed feasible.

Introduction

In contrast to the well-documented high abuse liability and toxicity of 'ecstasy' (3,4-methylenedioxymethamphetamine, MDMA) in humans [1–4], animal experimental evidence of MDMA's reinforcing effect has remained very limited. In the rodent model in particular, only two independent laboratories have been successful so far in demonstrating a reinforcing effect of MDMA in rats in lever-press-based operant procedures [3, 5]. Reports on MDMA reinforcement in lever-press-based operant conditioning experiments in nonhuman primates (baboons, rhesus monkeys) are not too numerous either [6–8]. As one of the two independent laboratories that published reports on MDMA reinforcement in rats, we have been approached by several colleagues who tried, in vain, to replicate our results obtained in a lever-press-based operant conditioning paradigm [3]. This was a matter of concern for us. We therefore investigated MDMA reinforcement in rats in another operant conditioning paradigm, a runway procedure originally adapted by Ettenberg and colleagues for the testing of drugs of abuse [9] and successfully used by us for the thorough investigation of the mechanisms underlying the steepness of dose-effect relationships in drug reinforcement [10]. Since our original investigation of MDMA [3], we have also switched from using Long-Evans to Sprague-Dawley rats, as the latter strain is less expensive and easier to handle. Thus, the present study was designed to evaluate MDMA's reinforcing effect not only across operant conditioning paradigms (i.e. Skinner box vs. rat runway), but also across rat strains. Worthy of notice, MDMA reinforcement has also been inferred on the basis of MDMA-conditioned place preference [11–13] or enhancement of intracranial self-stimulation by MDMA [14]. However, neither of these two experimental approaches is considered a bona fide operant conditioning paradigm [15–17].

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Dr. Gerald Zernig
Division of Neurochemistry, Department of Psychiatry
Anichstrasse 35, AT–6020 Innsbruck (Austria)
Tel. +43 699 1714 1714, Fax +43 512 504 5866
E-Mail gerald.zernig@uibk.ac.at

Materials and Methods

Subjects

Male Sprague-Dawley rats (300–350 g) were obtained from the Research Institute of Laboratory Animal Breeding (Himberg, Austria), male Long-Evans rats (250–300 g) from the Charles River Company (Sulzfeld, Germany). Rats were group-housed at a constant room temperature of 24° C with free access to tap water. Each rat was fed 3.5 g pelleted chow (Tagger, Innsbruck, Austria) in the morning and approximately 12 g in the evening. Under these feeding conditions, weight increases remained within the standard range provided by the breeder. All animals had been handled for at least 1 week and exposed to the runway for at least 15 min before they were tested during the light phase of a 12-hour light-dark cycle (lights on at 07.00 h). Rats were implanted with self-made jugular vein catheters under deep isoflurane anaesthesia as described in detail previously [10] and tested in the runway 2 days after surgery. The experiment was approved by the Ethics Committee of the Austrian Federal Ministry of Science and performed in accordance with the Guide for the Care and Use of Laboratory Animals (www.nap.edu/readingroom/books/labrats).

Behavioural Procedure

We used a straight wooden runway (Sambax, Thaur, Austria; www.sambax.com) as described in detail previously [10] which is a slight modification of the runway designed by Ettenberg et al. [9]. In the runway procedure, the time a rat takes to traverse an alley in order to reach a goal area in which it is presented with a stimulus is thought to be inversely proportional to the reinforcing strength of the stimulus. In other words, the faster the rat runs to obtain an i.v. injection of a drug of abuse the stronger the reinforcing effect of this drug is thought to be. The operant, runtime, is defined as the time span between the opening of the start area sliding door and the crossing of the photobeam in front of the goal area.

The experiment- and drug-naive animals, 12 Sprague-Dawley and 11 Long-Evans rats, were either given the opportunity to run for 1 mg/kg i.v. MDMA or for i.v. saline (operant level). All rats were tested for 5 consecutive runs (inter-run interval, 90 min) on a single experimental day. The drug or saline was delivered over 2–6 s through a polyethylene tubing (about 40 cm in length). The injection was initiated immediately after the rat had reached the goal area. The rat was allowed to experience the drug effect for 5 min within the confines of the runway, i.e. within the goal area and the alley. Rats of the same group cage were always run in the same order. If a rat did not traverse the alley within 60 s (experimenter-imposed cut off), it was gently moved into the goal area by the experimenter. To emphasize, all experiments were conducted under experimenter-blind conditions.

Drug

MDMA (3,4-methylenedioxymethamphetamine) HCl was generously provided by the National Institute on Drug Abuse (Bethesda, Md., USA). It was diluted in saline and injected at a volume of 1 ml/kg. The MDMA dose is given in grams pure base/kg.

Statistical Analysis

Raw runtime data, which displayed non-Gaussian distribution [10], were log-transformed for analysis by two-factor ANOVA. Significance was assumed at a value of $p < 0.05$. In order to conform to research field convention, runtimes are shown as means ± SEM.

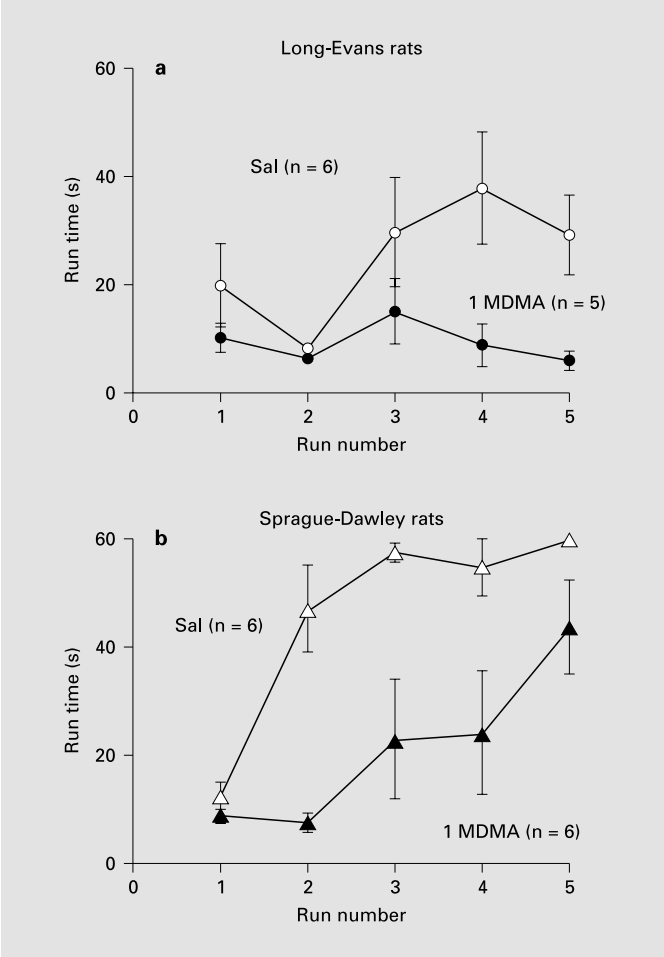

Fig. 1. Reinforcing effect of MDMA in two different rat strains in a runway procedure. Completely drug- and experiment-naive Long-Evans (**a**, circles) and Sprague-Dawley (**b**, triangles) rats were given the opportunity to run for i.v. saline (open symbols) or 1 mg/kg i.v. MDMA (filled symbols) in five consecutive trials spaced 90 min apart. To emphasize, all tests were performed under experimenter-blind conditions. Runtime (shown are means ± SEM; 60 s = experimenter-imposed cutoff) is inversely proportional to the reinforcing effect of the drug stimulus.

Results

Runtimes gradually increased over five consecutive trials if the rats received only i.v. saline after reaching the goal area (operant level; fig. 1, open symbols) as has been demonstrated and discussed in detail previously [10]. The MDMA dose of 1 mg/kg i.v. was selected because MDMA had exerted its maximum reinforcing effect in the lever-press-based operant procedure at 1 mg/kg [3] or at 0.25 mg/kg [5]. In our laboratory, 3.2 mg/kg MDMA had produced grossly observable effects (freezing, head weav-

ing, other stereotypes, piloerection) that would have jeopardized the experimenter-blind design of the present study. Therefore, this dose was not investigated.

In Long-Evans rats, MDMA (1 mg/kg i.v.; fig. 1a) significantly decreased runtimes (runtime of the last run, 6 ± 2 s; n = 5) compared to saline (runtime, 29 ± 8 s; n = 6): treatment $F_{1,9}$ = 9,726; p = 0.012, run number $F_{4,6}$ = 2,957; p = 0.114, run number × treatment $F_{4,6}$ = 1,728; p = 0.261. A Mann-Whitney test comparing only the last, i.e. the fifth run of the two treatment groups, MDMA and saline, confirmed the ANOVA results (p = 0.003).

In Sprague-Dawley rats (fig. 1b) MDMA also significantly decreased runtimes (runtime of the last run, 44 ± 9 s; n = 6) compared to saline (runtime, 60 ± 0 s; n = 6): treatment $F_{1,10}$ = 24,454; p = 0.001, run number $F_{4,7}$ = 11,901; p = 0.003, run number × treatment $F_{4,7}$ = 4,169; p = 0.049. A Mann-Whitney test performed for the last run of the experiment confirmed the results (p = 0.011). Thus, MDMA clearly served as a positive reinforcer in the rat runway procedure in the two different rat strains.

Discussion

MDMA (1 mg/kg i.v.) proved to be a robust positive reinforcer in Long-Evans as well as in Sprague-Dawley rats tested in a runway operant conditioning paradigm. The present study thus extends and corroborates previous lever-press-based operant conditioning rodent work [3, 5], responding to concerns in the drug abuse research community that the MDMA reinforcement in bona fide operant conditioning experiments cannot be shown in rodents. Quite on the contrary, MDMA's reinforcing effect can be demonstrated not only across rat strains [ref. 3 vs. 5; present study] but also across operant conditioning paradigms, i.e. Skinner box [3, 5] vs. runway [present study]. Even the dose at which i.v. MDMA exerted its maximum reinforcing effect was comparable across laboratories and rat strains (0.25 mg/kg [5] vs. 1 mg/kg [3]). Even across operant conditioning paradigms employed in the same laboratory, the dose of i.v. MDMA (i.e. 1 mg/kg) that served as a positive reinforcer was identical [3, present study]. In conclusion, the findings of the present study should reassure the drug abuse research community that the investigation of MDMA's reinforcing effect in the rodent model is indeed feasible and can be used to further investigate MDMA in the inexpensive and widely used rodent model.

Acknowledgments

This study was supported by the Austrian Federal Ministry of Science (research contract GZ 70.071/2-Pr/4/2000) and the Austrian Science Fund (SFB 00206 and project P16394-B05).

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