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RAPID COMMUNICATION

MDMA Produces a Conditioned Place Preference and Elicits Ejaculation in Male Rats: A Modulatory Role for the Endogenous Opioids

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BILSKY, E. J., C. L. HUBBELL, J. D. DELCONTE AND L. D. REID. *MDMA produces a conditioned place preference and elicits ejaculation in male rats: A modulatory role for the endogenous opioids.* PHARMACOL BIOCHEM BEHAV **40**(2) 443–447, 1991.—Methylenedioxymethamphetamine (MDMA) can produce a conditioned place preference (CPP) among rats. The ability of MDMA to produce a CPP was assessed while some rats were under the influence of naltrexone, 56 mg/kg, given 4 h before conditioning. Naltrexone attenuated MDMA's ability to produce a CPP without completely blocking MDMA's effects. Having noticed previously the production of seminal plugs by rats receiving MDMA, the presence of seminal plugs was recorded across the 8 days of conditioning. Roughly half of the rats receiving 6.3 mg/kg of MDMA left plugs during the conditioning period, while over two-thirds of those receiving a combination of MDMA and naltrexone left plugs. A second study, assessing further doses of MDMA, tabulated the drug's effects on the production of seminal plugs across 3 h. Besides eliciting ejaculation, MDMA also led to increased urination and defecation and a loss of body weight. These results support suggestions that the endogenous opioids modulate the reinforcing properties of stimulant drugs and affect male sexuality.

Methylenedioxymethamphetamine MDMA Naltrexone Male rat Seminal plugs
Conditioned place preference Affect Reward Urination Defecation

STUDIES with methylenedioxymethamphetamine (MDMA) are of interest from a number of points of view. People use the drug recreationally (23,24). People report that MDMA produces a number of apparently desirable effects, including an occasional report of enhanced sexuality (11,24). Furthermore, MDMA is typical of a class of drugs having considerable potential to become a focus of an addiction.

Monkeys (6) and baboons (19) will self-administer MDMA, indicating that MDMA has considerable liability for addiction. Studies with rats indicate that the effects of MDMA are capable of producing a conditioned place preference (CPP) (7, 9, 26) and lowering the threshold for reinforcing intracranial stimulation ICS (17), thereby further supporting the idea that MDMA is apt to be addictive. Studies with rats also indicate that MDMA is neurotoxic to serotonergic neurons (5,28).

Although the neurochemical mechanisms responsible for MDMA's reinforcing and neurotoxic effects are unclear, increased dopaminergic activity following MDMA's administration may be critical for MDMA's reinforcing effects (9,10). Since the endogenous opioid systems can modulate dopaminergic transmission (16,22), perhaps naltrexone, a long-acting opioid antagonist, might affect MDMA's reinforcing properties, a hypothesis previously tested using ICS procedures [reviewed in (4)].

EXPERIMENT 1

Previously, we assessed the effects of a 5-HT_3 antagonist on MDMA's ability to establish a CPP. The antagonist, MDL 72222, which attenuates the release of dopamine (12, 14, 18), blocked MDMA's ability to establish a CPP (9). Here, we assessed the effects of naltrexone on MDMA's ability to establish a CPP using methods very similar to those used to assess the 5-HT_3 antagonist (9) and to those of another study which assessed naltrexone's effects on morphine's ability to establish a CPP (8). While studying MDMA (9), we noticed, but did not report, the presence of seminal plugs in the place where rats were conditioned with MDMA. In this study, we systematically recorded the presence of seminal plugs during conditioning and testing.

METHOD

Subjects

The subjects were 36 male Sprague-Dawley rats (Taconic Farms, Germantown, NY). At the start of these procedures, rats weighed between 375 and 475 g. They were housed individually

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TABLE 1

GROUP ASSIGNMENTS AND SCHEDULES OF DRUG ADMINISTRATION FOR THE 1ST ASSESSMENT ARE DEPICTED

Group (N)	Putative	Alternate
Saline Control (6)	SAL/SAL	SAL/SAL
NTX Control (6)	NTX/SAL	NTX/SAL
MDMA (12)	SAL/MDMA	SAL/SAL
MDMA/NTX (12)	NTX/MDMA	NTX/SAL

Putative refers to the injections each group received prior to being placed on their putative side. Alternate refers to the injections administered prior to being placed on the other side. The label to the left of the slash is the type of injection administered first. Labels correspond to the following injections: SAL = saline; MDMA = MDMA at a dose of 6.3 mg/kg; NTX = naltrexone HCl at a dose of 56 mg/kg. N refers to the number of subjects in each group.

in standard hanging metal cages in a windowless colony room. The colony was maintained at 22°C with 12 h of artificial light a day (lights on at 0700 h). Food and water were always available in the rats' home cages.

Drugs

(±)-3,4-Methylenedioxymethamphetamine (MDMA) and naltrexone HCl were dissolved in physiological saline and administered in doses of 6.3 and 56.0 mg/kg, respectively. The dose of MDMA reliably produces a CPP in our apparatus (7), while the dose of naltrexone blocks morphine's ability to establish a CPP (8). All injections were administered subcutaneously (SC) in a volume of 1 ml/kg. Naltrexone or its placebo was injected 4 h before conditioning while MDMA or its placebo was injected 10 min before conditioning. Injection times were based upon previous research (7,8).

Apparatus

The apparatus, described in detail elsewhere (25), was 12 nearly identical alleys, each housed in a sound-attenuating chamber. Each alley was divided into two equal halves having distinct visual (solid grey or black and white striped sides) and textural cues (flooring made of steel rods running either parallel or perpendicular to the length of the alley). A wooden barrier, with sides painted to match the respective halves of the alley, was used to separate the distinct environments. An alley tilted slightly when a rat moved to either side, completing a circuit monitored by a personal computer. Each side of the alley had an adjustable light overhead. The amount of reflected light on each side of the alley was adjusted so that the side of putative conditioning was slightly brighter than the alternate side.

Procedure

The procedures spanned a 3-week schedule of habituation, conditioning and testing with procedures occurring between 0900 and 1300 h. Across Days 1–5, rats were habituated to the general procedures. Rats were weighed daily, as they were on almost every day of the procedures, and placed into a cart (12 cages/cart, 1 rat/cage). The cart was then moved into an adjacent room containing the apparatus and each rat was handled briefly before being returned to its home cage.

On Days 6 and 7, each rat was placed into its respective alley and allowed access to either side for 30 min. Time spent on the side of putative conditioning was recorded on Day 7 and served as a baseline measure. Rats were subsequently assigned to groups so that each group was roughly equal in terms of baseline scores and were equal in number of rats assigned the grey or striped side as place of putative conditioning. A treatment was then randomly assigned to each group. On Days 8 and 9, rats were not weighed or given special treatment.

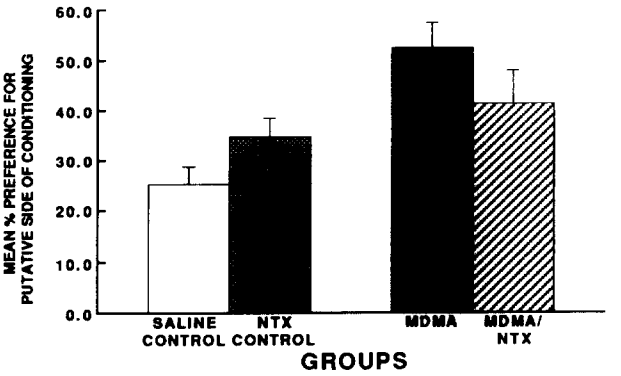

FIG. 1. Test scores are depicted as mean percent of the total test time (30 min) spent on side of putative conditioning for each group. Groups are labelled according to the designations used in Table 1, e.g., MDMA/NTX refers to the group receiving conditioning with 6.3 mg/kg MDMA in combination with a 56.0 mg/kg dose of naltrexone. Bars represent standard errors of the mean.

Conditioning began on Day 10. Rats were weighed and given their assigned injections (Table 1; putative) before being placed into their side of putative conditioning for 30 min. These procedures were repeated on Days 11 and 12. On Day 13, rats received injections (Table 1; alternate) and were placed into the alternate side of the alley. Three days of no special handling followed. The procedure of 3 days of putative conditioning and 1 day of alternate conditioning was repeated again (Days 17–20) followed by a test (30 min) for place preference on Day 21. Subsequent to each individual rat's conditioning and testing sessions, the area under the alley was scanned for any plugs.

RESULTS AND DISCUSSION

Results of the preference testing are summarized in Fig. 1. A 4 by 2 analysis of variance (ANOVA) for repeated measures having factors of Groups and Tests (Baseline versus Test 1), respectively, yielded $F(3,32) = 3.48$, $p = 0.027$, for the interaction term. Scores of groups did not differ reliably at Baseline ($p > 0.9$) and were as expected (approximately a 43% preference for putative side). A Student's t-test, for independent measures, between the saline and naltrexone control groups' scores at Test yielded a $t(10) = 1.85$, $p = 0.094$. Regardless of whether the two control groups are kept separate or combined, a one-way ANOVA at Test reveals statistically reliable results, $F(3,32) = 3.49$, $p = 0.027$ and $F(2,33) = 4.83$, $p = 0.014$, respectively.

Analyses of the Test scores indicates that MDMA produces a CPP compared to the combined control group, $t(22) = 3.95$, $p < 0.001$, or to each group separately, $ps < 0.035$. Naltrexone seemed to attenuate MDMA's ability to establish a CPP. The scores of the MDMA/NTX group are not reliably different from either of the control groups' scores, $ps > 0.12$ or from the MDMA group, $p > 0.19$.

Observations of seminal plugs are presented in Table 2. On

TABLE 2

PRESENCE OF SEMINAL PLUGS FOR EACH GROUP ON EACH OF THE 6 DAYS OF PUTATIVE CONDITIONING

	Day					
Group	10	11	12	17	18	19
Saline Control	0/6	0/6	0/6	0/6	0/6	0/6
NTX Control	1/6	1/6	0/6	0/6	0/6	1/6
MDMA	7/12	9/12	6/12	4/12	6/12	2/12
MDMA/NTX	12/12	11/12	11/12	9/12	11/12	8/12

A score of 7/12 for the group getting only MDMA on Day 10 indicates that of the 12 rats receiving MDMA, 7 of those rats left seminal plugs during the 30-min session. Seminal plugs were not observed for any rat on Baseline, alternate days of conditioning or Test day.

putative days of conditioning, rats conditioned with MDMA generally left plugs but on alternate days (placebo) and at testing they left none. Chi-Square tests across groups on each of the putative days of conditioning all yielded reliable p-values, $ps<0.009$. Rats receiving saline never left seminal plugs while rats receiving only naltrexone rarely left a plug.

These data confirm that MDMA produces a CPP (7, 9, 26) and lead to the suggestion that naltrexone modulates the reinforcing properties of stimulant drugs, an observation similar to that of others (3,4). MDMA also apparently elicits ejaculation in the rat. The observations of this experiment indicate that tolerance may develop to MDMA's ability to elicit seminal plugs, and that naltrexone seems to facilitate the elicitation of plugs and prevent the development of tolerance. Naltrexone's effects, in combination with MDMA, discount the possibility that the MDMA group was depleted of their seminal reserve unless naltrexone was having an effect on seminal production.

EXPERIMENT 2

In Experiment 1, the presence of seminal plugs was recorded across a limited time. Naltrexone may have only facilitated or delayed the period between MDMA injection and ejaculation. The MDMA-treated rats may have ejaculated in the 10-min period prior to placement in the CPP alley or in the period after they were returned to their home cages. Consequently, we performed the following experiment to gather germane information and extend our observations.

METHOD

Twenty-four rats, similar to those of Experiment 1, were allowed 4 days to acclimate to the laboratory before the start of the formal procedures. Then, rats were handled and weighed daily across 4 days. On the following day, each rat was weighed, injected with saline and returned to its home cage. Each rat was then monitored hourly across the next 3 h on the following measures; body weight, number and total weight of seminal plugs, amount of urination and defecation, and intake of food and water. On the following day, rats were randomly assigned treatments and given an injection of saline or one of three doses of MDMA (2.0, 6.3 or 20.0 mg/kg, SC), and the procedures of the previous day repeated.

RESULTS AND DISCUSSION

A summary of the data following drug administrations is displayed in Table 3. In general, there were neither differences between groups on placebo day nor were there differences between the control group's placebo and drug scores for any measure. MDMA dose-relatedly led to the production of seminal plugs across the 3-h observation period, $\chi^2(3)=14.4$, $p=0.0024$. The majority of the plugs appeared within the 1st h. A Kruskal-Wallis test revealed reliable differences between groups in the total weight of the seminal ejaculate, $H(3)=12.8$, $p<0.01$. An ANOVA using total scores across the 3 h, revealed that MDMA increased rats' level of urination and defecation ($ps<0.005$) with the majority occuring in the 1st h. Intake of food and water were not reliably affected by MDMA's administration. MDMA-treated rats lost considerable weight across the 3-h period. Though not quantified, rats receiving a 20.0 mg/kg dose of MDMA also exhibited marked salivation.

GENERAL DISCUSSION

Informal observations indicate that MDMA elicits salivation and considerable loss of muscle tone with stereotypic circling in

TABLE 3

SUMMARY OF MEASURES ACROSS A 3-HOUR PERIOD FOLLOWING MDMA ADMINISTRATION

Group	δBW	Plugs	WP	Urine	Boli
Saline	−4.3 (0.45)	0/6	0.0 (0.0)	0.82 (0.36)	1.42 (0.30)
MDMA 2.0	−7.0 (0.60)*	3/6	10.0 (5.0)	1.87 (0.41)	2.12 (0.34)
MDMA 6.3	−12.5 (0.85)*	5/6	50.0 (20.0)	5.30 (0.58)*	3.55 (0.43)*
MDMA 20.0	−15.2 (0.72)*	6/6	35.0 (10.0)	6.55 (0.81)*	2.70 (0.41)*

The following measures are displayed; δBW (change in body weight), Plugs (number of rats, out of 6, which left seminal plugs), WP (weight of plugs), Urine (weight of urine) and Boli (weight of fecal boli). All scores reflect means across the 3-h period with weights being given in grams except for weight of seminal plugs which is in milligrams. Standard errors of the mean are displayed in parentheses. Means that are reliably different from the saline group's (t-test, $p<0.05$) are marked with an asterisk.

the rat. Formal observations indicate that MDMA elicits increased urination and defecation. Although not apparent in these procedures, MDMA probably reduces intake of food and water (7). In part, the combined effects of increased urination and defecation, without apparent replacement, lead to rapid loss of body weight. MDMA also increases body temperature (27) and elicits seminal plugs.

MDMA produced a CPP that was sensitive to naltrexone's effects. It is likely that MDMA causes the release of dopamine (15), which is apparently critical to its reinforcing effects (9). Since opioidergic systems can modulate the release of dopamine (16,22), there are extant mechanisms by which naltrexone might modulate MDMA's reinforcement.

The effects of MDMA in laboratory animals are often similar to effects observed in people. MDMA, for example, is self-administered by both people and laboratory primates (6, 19, 24). Furthermore, there is a report that MDMA produces a feeling of urgency to urinate among people [reviewed in (13)] and it increased urination among these rats (Experiment 2). There are, however, no reports of MDMA eliciting spontaneous ejaculation in men and, in fact, MDMA might inhibit the ability to produce an erection and delay ejaculation in men (11).

MDMA's unique effect with respect to elicitation of seminal plugs in rats may be due to differences in the absorption, disposition and metabolism of the drug between rats and people. Another possibility could be the route of administration of MDMA, since people administer the drug orally while rats are usually given SC or intraperitoneally administered injections. In an effort to clarify our findings, we administered a dose of 20 mg/kg MDMA orally to a group of rats. Four of the six rats receiving MDMA elicited plugs while none of the six controls elicited plugs. A Chi-Square test approached standards of reliability, $\chi^2(1)=3.38$, $p=0.07$. These data suggest that the route of administration is not a salient factor.

The effects inherent to MDMA's CPP are probably separable from MDMA's elicitation of seminal plugs. The administration of a 5-HT_3 antagonist blocked MDMA's ability to establish a CPP, but did not block MDMA's ability to elicit plugs [(9) and unpublished observations]. Naltrexone attenuated MDMA's ability to establish a CPP but enhanced MDMA's elicitation of seminal plugs. It has been demonstrated that the period following ejaculation is sufficient to condition a CPP among rats (1) and, on the surface, it seems reasonable to conclude that the process associated with leaving seminal plugs might be associated with MDMA's positivity. The data collected with different pharmacological agents, however, indicate that the positivity and sexual response are separable.

Despite the considerable number of studies, the exact effects of opioids on sexual behavior are unclear. Opioid antagonists have been shown to both facilitate and inhibit sexual performance (2,20). Naltrexone, for example, can induce spontaneous erections in men (21) while naloxone can increase the percentage of rats ejaculating (20). There are both peripheral and central opioidergic mechanisms that might facilitate MDMA's elicitation of seminal plugs. Perhaps, the endogenous opioids are part of a system that modulates sexuality rather than being part of the critical circuitry.

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REFERENCES

1. Agmo, A.; Berenfeld, R. Reinforcing properties of ejaculation in the male rat: Role of opioids and dopamine. Behav. Neurosci. 104(1):177–182; 1990.
2. Agmo, A.; Paredes, R. Opioids and sexual behavior in the male rat. Pharmacol. Biochem. Behav. 30:1021–1034; 1988.
3. Bain, G. T.; Kornetsky, C. Naloxone attenuation of the effect of cocaine on rewarding brain stimulation. Life Sci. 40:1119–1125; 1987.
4. Bain, G. T.; Kornetsky, C. Opioids modification of central reward processes. In: Reid, L. D., ed. Opioids, bulimia and alcohol abuse & alcoholism. New York: Springer-Verlag; 1990:73–87.
5. Battaglia, G.; Zaczek, R.; De Souza, E. B. MDMA effects in brain: Pharmacological profile and evidence of neurotoxicity from neurochemical and autoradiographic studies. In: Peroutka, S. J., ed. Ecstasy: The clinical, pharmacological and neurotoxicological effects of the drug MDMA. Boston: Kluwer; 1990:171–199.
6. Beardsley, P. M.; Balster, R. L.; Harris, L. S. Self-administration of methylenedioxymethamphetamine (MDMA) by rhesus monkeys. Drug Alcohol Depend. 18:149–157; 1986.
7. Bilsky, E. J.; Hui, Y.; Hubbell, C. L.; Reid, L. D. Methylenedioxymethamphetamine's capacity to establish place preferences and modify intake of an alcoholic beverage. Pharmacol. Biochem. Behav. 37:633–638; 1990.
8. Bilsky, E. J.; Marglin, S. H.; Reid, L. D. Using antagonists to assess neurochemical coding of a drug's ability to establish a conditioned place preference. Pharmacol. Biochem. Behav. 37:425–431; 1990.
9. Bilsky, E. J.; Reid, L. D. MDL72222, a serotonin 5-HT3 receptor antagonist, blocks MDMA's ability to establish a conditioned place preference. Pharmacol. Biochem. Behav. 39:509–512; 1991.
10. Bozarth, M. A. Neural basis of psychomotor stimulant and opiate reward: Evidence suggesting the involvement of a common dopaminergic system. Behav. Brain Res. 22:107–116; 1986.
11. Buffum, J.; Moser, C. MDMA and human sexual functioning. J. Psychoactive Drugs 18(4):355–359; 1986.
12. Carboni, E.; Acquas, E.; Frau, R.; DiChiara, G. Differential inhibitory effects of a 5-HT3 antagonist on drug-induced stimulation of dopamine release. Eur. J. Pharmacol. 164:515; 1989.
13. Climko, R. P.; Roehrich, H.; Sweeney, D. R.; Al-Razi, J. Ecstasy: A review of MDMA and MDA. Int. J. Psychiatry Med. 16(4):359–372; 1987.
14. Fozard, J. R. MDL 72222: A potent and highly selective antagonist at neuronal 5-hydroxytryptamine receptors. Naunyn Schmiedebergs Arch. Pharmacol. 326:36–44; 1984.
15. Gibb, J. W.; Stone, D.; Johnson, M.; Hanson, G. R. Neurochemical effects of MDMA. In: Peroutka, S. J., ed. Ecstsay: The clinical, pharmacological and neurotoxicological effects of the drug MDMA. Boston: Kluwer; 1990:171–199.
16. Gysling, K.; Wang, R. Y. Morphine-induced activation of A10 dopamine neurons in the rat. Brain Res. 277:119–127; 1983.
17. Hubner, C. B.; Bird, M.; Rassnick, S.; Kornetsky, C. The threshold lowering effects of MDMA (ecstasy) on brain-stimulation reward. Psychopharmacology (Berlin) 95:49–51; 1988.
18. Imperato, A.; Angelucci, L. 5-HT3 receptors control dopamine release in the nucleus accumbens of freely moving rats. Neurosci. Lett. 101:214; 1989.
19. Lamb, R.; Griffiths, R. Self-injection of d,l-3,4-methylenedioxymethamphetamine (MDMA) in the baboon. Psychopharmacology (Berlin) 91:268–272; 1987.
20. McIntosh, T. K.; Vallano, M. L.; Barfield, R. J. Effects of morphine, β-endorphin and naloxone on catecholamine levels and sexual behavior in the male rat. Pharmacol. Biochem. Behav. 13:435–441; 1980.
21. Mendelson, J.; Ellingboe, J.; Keuhnle, J.; Mello, N. Effects of naltrexone on mood and neuroendocrine function in normal adult males. Psychoneuroendocrinology 3:231–236; 1979.

22. Mitchell, J. B.; Stewart, J. Facilitation of sexual behaviors in the male rat associated with intra-VTA injections of opiates. Pharmacol. Biochem. Behav. 35:643–650; 1990.
23. Peroutka, S. J. Incidence of recreational use of 3,4-methylenedioxymethamphetamine (MDMA; "Ecstasy") on an undergraduate campus. N. Engl. J. Med. 317:1542–1543; 1987.
24. Peroutka, S. J. Recreational use of MDMA. In: Peroutka, S. J., ed. Ecstasy: The clinical, pharmacological and neurotoxicological effects of the drug MDMA. Boston: Kluwer Academic Publishers; 1990:53–62.
25. Reid, L. D.; Marglin, S. H.; Mattie, M. E.; Hubbell, C. L. Measuring morphine's capacity to establish a place preference. Pharmacol. Biochem. Behav. 33:765–775; 1989.
26. Schecter, M. D. Effect of MDMA neurotoxicity upon its conditioned place preference and discrimination. Pharmacol. Biochem. Behav. 38:539–544; 1991.
27. Schmidt, C. J.; Black, C. K.; Abbate, G. M.; Taylor, V. L. Methylenedioxymethamphetamine-induced hyperthermia and neurotoxicity are independently mediated by 5-HT2 receptors. Brain Res. 529: 85–90; 1990.
28. Schmidt, C. J.; Kehne, J. H. Neurotoxicity of MDMA: Neurochemical effects. In: Whitaker-Azmitia, P. M.; Peroutka, S. J., eds. The neuropharmacology of serotonin. New York: The New York Academy of Sciences; 1990:665–681.