

A role for the mesolimbic dopamine system in the psychostimulant actions of MDMA *

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Abstract. Methylenedioxymethamphetamine (MDMA) is a phenylethylamine with a chemical structure that resembles both the amphetamines and mescaline and has both stimulant and perception altering properties. The stimulant properties of MDMA were assessed in photocell cages designed to measure locomotor activity in rats. MDMA, over a range of doses (2.5–10.0 mg/kg, SC) produced locomotor hyperactivity which lasted up to 4 h. Further studies examined the role of the mesolimbic dopamine system in the hyperactivity induced by MDMA. 6-Hydroxydopamine lesions of the Nucleus accumbens attenuated the locomotor response produced by MDMA. The well characterized attenuation of the locomotor response produced by amphetamine was also demonstrated in the same rats. The present study demonstrates similarities in the stimulant properties of MDMA and amphetamine, and also suggests that as with amphetamine, the locomotor activation associated with MDMA may involve the presynaptic release of dopamine in the region of the Nucleus accumbens. However, MDMA may have a more unusual pharmacological profile because of its longer duration of action, neurotoxic potential, and differences in the qualitative aspects of its psychoactive effects.

Key words: Methylenedioxymethamphetamine – MDMA – Nucleus accumbens – Locomotor activity – Mesolimbic dopamine – 6-Hydroxydopamine

Chemically MDMA resembles both mescaline and amphetamine (Shulgin and Nichols 1978; Nichols et al. 1986) and produces effects similar to both of these compounds. MDMA produces altered states of consciousness (Shulgin and Nichols 1978), which serve to promote introspective thought and facilitate interpersonal communication (Nichols et al. 1986). This effect has prompted psychotherapists to view MDMA as an important tool by which to aid the psychotherapeutic process (Grinspoon and Bakalar 1986). However, MDMA also has sympathomimetic activity (Shulgin and Nichols 1978; Grinspoon and Bakalar 1986; Barnes 1988) and concern over the potential to induce arrhythmias in individuals with underlying disease has been reported (Dowling et al. 1987). Additionally, long-term de-

pletions of serotonergic markers following single and multiple injections of MDMA in experimental animals indicate a neurotoxic effect (Stone et al. 1986; Mokler et al. 1987; Schmidt 1987a).

It is now well documented that MDMA is a recreationally used drug with significant potential for abuse (Beck and Morgan 1986; Peroutka 1987). Investigations utilizing animal models which purportedly assess a drug's potential abuse liability have also yielded positive results. For example, baboons trained to self-administer cocaine also self-administer MDMA at rates above those maintained by saline (Lamb and Griffiths 1987; see also Beardsley et al. 1986). Additionally, MDMA has been found to produce lowering of the threshold for rewarding brain stimulation (Hubner et al. 1988).

Investigations into the mechanism by which MDMA produces its effects have implicated both dopamine and serotonin. Evidence from biochemical studies indicates that MDMA releases serotonin and to a lesser extent dopamine (Nichols et al. 1982; Johnson et al. 1986; Schmidt et al. 1987). Behavioral studies using the drug-discrimination procedure have demonstrated that MDMA will generalize to *D*-amphetamine, suggesting that MDMA has dopaminergic actions in common with this psychomotor stimulant drug. Other studies, however, have reported that MDMA will generalize to the serotonin uptake inhibitor fenfluramine (Schechter 1986) as well as the direct serotonergic agonist mescaline (Callahan and Appel 1987). Further, several authors have concluded that MDMA has behavioral effects that are distinctly different from related drugs which share discriminative stimulus effects with both stimulants and hallucinogens (Glennon et al. 1988; Oberlander and Nichols 1988).

An important characteristic of dopaminergic drugs is their ability to influence motor activity. Classic stimulants like amphetamine are known to produce a hyperactivity, which is thought to be mediated by mesolimbic dopamine transmission (Kelly et al. 1975; Joyce and Koob 1981). These studies showed that the ability of amphetamine to increase locomotor activity was significantly reduced in rats with 6-hydroxydopamine (6-OHDA) lesions of the Nucleus accumbens (N. Acc.). The same lesions have also been reported to block the intravenous self-administration of cocaine (Roberts et al. 1980; Pettit et al. 1984). Because of the structural similarity of amphetamine and MDMA, human reports of sympathomimetic side effects and references to increases in locomotor activity in mice (Braun et al. 1980;

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Glennon et al. 1988) the psychostimulant effects of MDMA were examined in rats. In the present investigation a dose-effect function for MDMA-induced locomotor activity was performed, and in addition, the role of the mesolimbic dopamine system, known to be critical for locomotion stimulated by amphetamine, was investigated in rats treated with MDMA.

Materials and methods

Subjects

Male, albino Wistar rats (220–270 g at start of studies, Charles River, Kingston, NY) were housed in groups of three with free access to food and water and maintained in a temperature controlled environment under a normal 12 h light cycle. All experiments were conducted during the light phase of this cycle. Before behavioral testing, rats were briefly handled by the experimenter (5 min).

Behavioral apparatus

Locomotor activity was measured in a bank of 16 wire cages 20 cm × 25 cm × 36 cm, each with two horizontal infrared beams across the long axis 2 cm above the floor. Total photocell beam interruptions and crossovers were recorded by a computer every 10 min.

Drugs

±3,4-Methylenedioxymethamphetamine HCl (National Institute on Drug Abuse), *d*-amphetamine sulfate, and methysergide maleate were mixed in saline and injected subcutaneously (SC) at the back of the neck, in a volume of 1 ml/kg body weight. 6-Hydroxydopamine (6-OHDA) HCl and apomorphine HCl were dissolved in saline containing ascorbic acid (0.1 mg/ml).

Surgery

Rats were divided into two groups. One group received intracranial, bilateral injections of 6-OHDA (8 µg/2 µl, expressed as the free base) dissolved in saline containing ascorbic acid (0.1 mg/ml; lesion group). The other group received bilateral injections of saline-ascorbic acid vehicle alone (sham group). Rats were anesthetized with pentobarbital (50 mg/kg, IP) and secured in a Kopf stereotaxic instrument with the toothbar 5 mm above the interaural line. Injections were made into the Nucleus accumbens (N. Acc.) through a 30 gauge cannula at a rate of 1 µl per 3 min at coordinates: AP + 3.2 (from bregma), L ± 1.7, DV – 7.8 (from skull surface).

Biochemistry

Following completion of behavioral testing all 6-OHDA and representative vehicle sham subjects were decapitated and the brains dissected on ice. The N. Acc. was removed from coronal slices and stored at –70° C until assayed for dopamine (DA) and dihydroxyphenylacetic acid (DOPAC) using electrochemical detection following separation by high pressure liquid chromatography (Felice et al. 1978). A lesion was defined as complete if ≥ 75% dopamine was determined to be depleted from the N. Acc. compared to mean sham group values.

Data analysis

For the dose-effect study photocell beam interruptions were analyzed by a two-way analysis of variance (ANOVA) with repeated measures on time. Following a significant main effect of drug, a Newman-Keuls individual means comparison was performed. The lesion studies were analyzed using two-factor ANOVAs (surgery, time) with repeated measures on the second factor, time. Biochemical results were analyzed using Student's *t*-test.

Behavioral testing

Experiment 1: Dose-response. Each rat was habituated to the photocell cages overnight, and prior to drug injection the rats were habituated again to the photocell cages for at least 90 min. Following drug administration, activity was measured for 120 min. Rats were randomly divided into four groups and injected with one of the following: MDMA 0, 2.5, 5 or 10 mg/kg SC. Two separate replications were performed to yield *N* = 8 rats/group.

Experiments 2–4: Nucleus accumbens 6-hydroxydopamine lesions. Three separate experiments were conducted. Each rat was habituated to the photocell cages overnight, and prior to drug injection the rats were habituated again to the photocell cages for at least 90 min. In experiment 2, at 14–15 days after surgery, 34 rats (sham: *N* = 17, lesion: *N* = 17) were injected with MDMA 10 mg/kg and placed in the photocell cages. Locomotor activity was recorded for 4 h (see Table 1). Four to 5 days later, all rats were injected with apomorphine 0.1 mg/kg SC and locomotor activity measured for 60 min.

In experiments 3 and 4, rats were allowed to recover from surgery for 8–9 days and were injected with saline SC on day 9–10. Locomotor activity in these experiments

Table 1. Surgery refers to 6-OHDA or sham lesions of the Nucleus accumbens. MDMA = methylenedioxymethamphetamine, sal = saline, AMPH = amphetamine, MSG = methysergide. Because the surgery for all animals in each experiment took 2 days to perform, subsequent counting uses 2-day specifications

Experiment 2					
Day 0					14/15
Surgery	RECOVERY				MDMA 10 mg/kg
Experiment 3					
Day 0		9/10	10/11	13/14	14/15
Surgery	RECOVERY	sal	MDMA 5 mg/kg	AMPH 0.5 mg/kg	sal
Experiment 4					
Day 0		9/10	10/11	13/14	16/17
Surgery	RECOVERY	sal	AMPH 0.5 mg/kg	MDMA 5 mg/kg	MDMA 5 mg/kg MSG 2.5 mg/kg

was measured for 120 min. In experiment 3, rats (sham: $N=8$, lesion: $N=8$) were injected on the following day with MDMA 5 mg/kg, 3 days later with AMPH 0.5 mg/kg and the next day with saline. In experiment 4, rats (sham: $N=8$, lesion: $N=8$) were injected with AMPH 0.5 mg/kg on day 10–11 and MDMA 5 mg/kg 3 days later. On day 16–17 these rats received two injections, a serotonin antagonist, methysergide 2.5 mg/kg and MDMA 5 mg/kg (refer to Table 1).

The time points for drug administration following surgery were chosen because maximum depletions have been measured 1 week post-lesion with 6-OHDA (Onn et al. 1986). Furthermore, similar timepoints have been found to result in significant decreases in amphetamine-induced locomotion without affecting heroin stimulated locomotion (Vaccarino et al. 1986).

Results

Dose response

Experiment 1. Doses of 2.5, 5.0 and 10.0 mg/kg of MDMA significantly increased locomotor activity for up to 2 h [Fig. 1, $F(3,28)=8.72$, at all three doses compared to saline $P<0.05$, Newman-Keuls test]. Rats in the two highest dose groups failed to show an increase in locomotion for the first 10 min and subsequently showed a sustained increase. The effects on crossovers were not qualitatively different from beam interruptions and therefore are not reported.

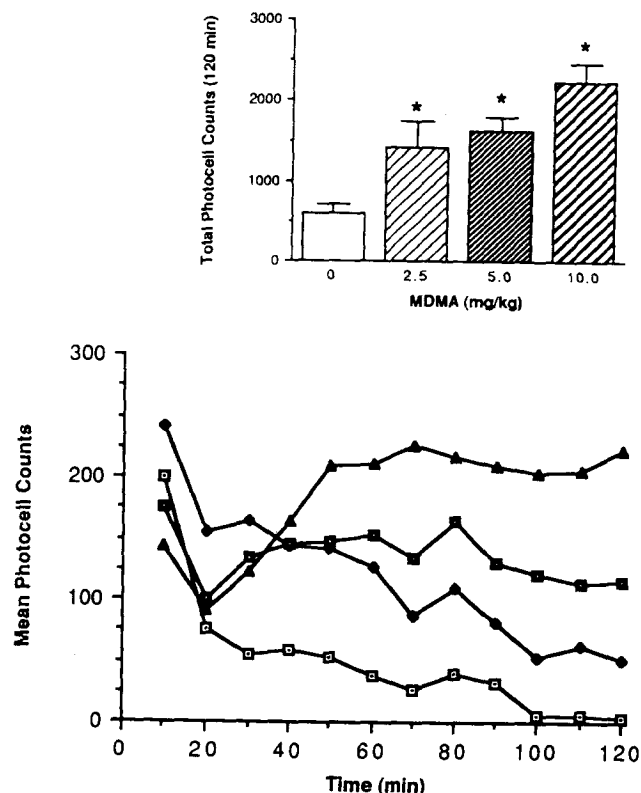


Fig. 1. Effects of MDMA on locomotor activity in rats. The effects of selected doses of MDMA on photocell interruptions per 10 min are shown as group means. Inset represents mean $\pm$ SEM for the total activity over the 120 min experimental session. $N=8$ rats/group. * $P<0.05$. —□— Saline; —♦— 2.5; —■— 5.0; —▲— 10.0

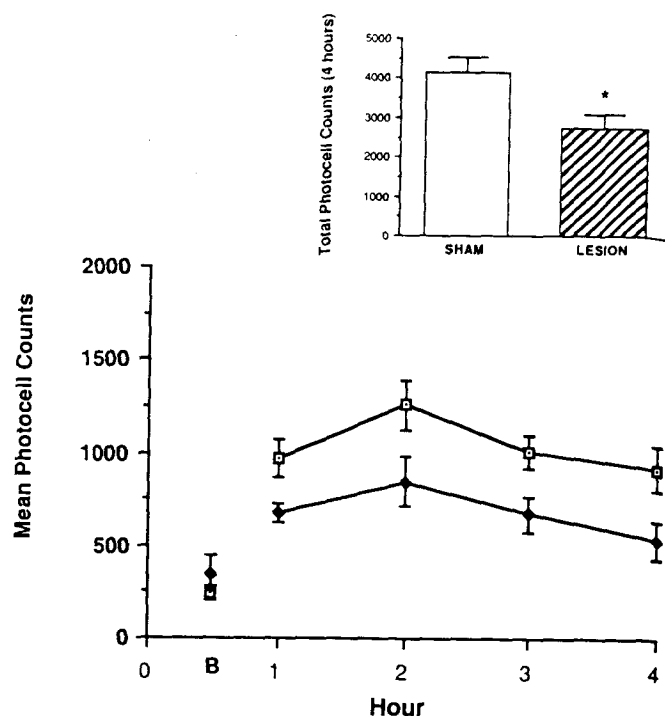


Fig. 2. Time course effects of MDMA on locomotor activity in rats with 6-OHDA or sham lesions of the nucleus accumbens. The effect of MDMA 10 mg/kg on photocell interruptions was measured for 4 h 14/15 days post-surgery. Photocell interruptions per 60 min are shown as group means. Note B = baseline which indicates levels of activity in photocell cages for 60 min prior to drug injection. Inset represents mean $\pm$ SEM for the total photocell interruptions during the 4 h test session. Sham group: $N=17$, Lesion group: $N=12$, * $P<0.05$. —□— sham; —♦— lesion

Effects of 6-OHDA lesions of the Nucleus accumbens

Experiment 2. As above, MDMA 10 mg/kg produced a long-lasting increase in locomotor activity in sham operated rats measured over 4 h [$F(1,27)=7.75$, $P<0.05$; Fig. 2]. The mean $\pm$ SEM total number of photocell counts per 60 min for these rats was h 1 = 971.7 ± 99.4 , h 2 = 1256.8 ± 135.5 , h 3 = 1008.5 ± 86.2 , h 4 = 917.9 ± 121.1 compared to a mean $\pm$ SEM of 591.1 ± 123.4 per 120 min of the saline group in experiment 1 (Fig. 1) and means of 503.8 ± 97.2 and 471.9 ± 133.3 per 120 min, respectively, for the sham-saline groups in experiment 3 (Fig. 3) and 4 (Fig. 4). This indicates a marked stimulant effect, the duration of which exceeds 4 h. In addition, the locomotor stimulant effect of MDMA was significantly attenuated following 6-OHDA lesions of the N. Acc. (Fig. 2). The mean $\pm$ SEM total number of photocell counts per 60 min for rats with 6-OHDA lesions was h 1 = 673.3 ± 48.5 , h 2 = 845.5 ± 136.2 , h 3 = 668.9 ± 101.5 and h 4 = 536.9 ± 97.6 . An apomorphine 0.1 mg/kg challenge administered 4–5 days after administration of MDMA resulted in significant hyperactivity in the rats with 6-OHDA lesions compared with sham-operated rats [sham group: $381.1 \pm 55.1/60$ min; lesion group: $843.4 \pm 185.3/60$ min; $F(1,27)=7.6$, $P<0.05$].

Experiment 3. In this experiment all rats received an injection of saline 9–10 days post surgery. The significant difference between sham-operated rats and those with 6-OHDA lesions following saline injection was attributed to a reduced

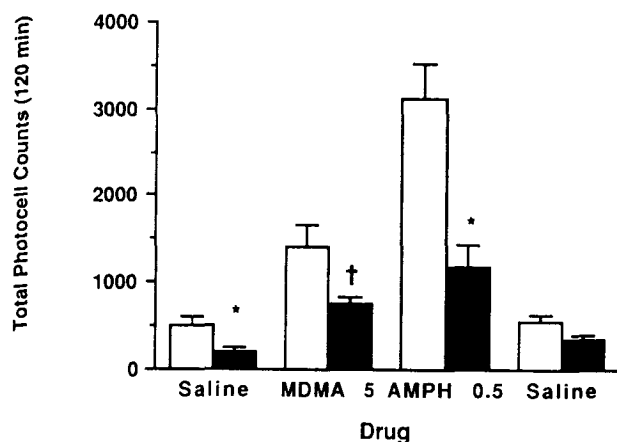


Fig. 3. The effects of MDMA 5 mg/kg (10/11 days post-surgery) or amphetamine (AMPH) 0.5 mg/kg (13/14 days post-surgery) on locomotor activity in rats with 6-OHDA or sham lesions of the nucleus accumbens. The total photobeam interruptions for the 120 min test session are shown as group means $\pm$ SEM. Sham group: $N=8$, Lesion group: $N=8$, $^{\dagger}P=0.055$, $^*P<0.05$. □ sham; ■ lesion

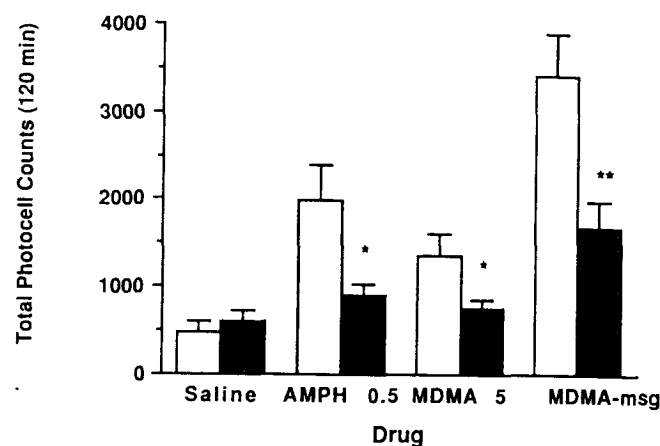


Fig. 4. The effects of AMPH 0.5 mg/kg (10/11 days post-surgery), MDMA 5 mg/kg (13/14 days post-surgery) and MDMA 5 mg/kg plus methysergide 2.5 mg/kg (16/17 days post-surgery) on locomotor activity in rats with 6-OHDA or sham lesions of the nucleus accumbens. The total photobeam interruptions for the 120 min test session are shown as group means $\pm$ SEM. Sham group: $N=8$, Lesion group: $N=6$, $^*P<0.05$, ** significant interaction. □ sham; ■ lesion

response to the injection procedure in the lesion-operated group [Fig. 3, $F(1,12)=6.3$, $P<0.05$]. It should be noted that the means for the two groups were almost identical (sham = 576 ± 84 , lesion = 523 ± 55) for the 90 min habituation period preceding the saline injection. Rats were injected with MDMA 5 mg/kg on the next day and as in experiment 2 the locomotor hyperactivity produced by MDMA was attenuated in the group with 6-OHDA lesions [Fig. 3, $F(1,12)=4.5$, $P=0.055$]. When the rats were injected with AMPH 0.5 mg/kg 3 days later the sham-operated group showed a large increase in locomotor activity; this effect was also significantly reduced in the rats with lesions [Fig. 3, $F(1,12)=13.1$, $P<0.05$]. Moreover, the hyperactivity seen in the sham-operated rats was somewhat greater than that usually observed following this dose of AMPH. The day after the AMPH injections, all the rats were reinjected with saline. At this time there was no significant difference between the sham and lesion operated rats [Fig. 3, $F(1,12)=2.59$, $P>0.05$].

Experiment 4. In this experiment there was no effect of saline injection in the sham versus lesion groups of rats 9–10 days post-surgery [Fig. 4, $F(1,13)<1.0$]. Rats were injected with AMPH 0.5 mg/kg on the next day and as in previous experiments the locomotor hyperactivity produced by AMPH was attenuated in the group with 6-OHDA lesions [Fig. 4, $F(1,14)=7.03$, $P<0.05$]. The mean $\pm$ SEM per 120 min for the sham and lesion groups was 1995.9 ± 389.3 and 906 ± 132 , respectively. In experiment 3 the means for these groups were 3111.9 ± 421.8 and 1176.5 ± 248.1 . When the rats were injected with MDMA 5 mg/kg, 3 days later the sham-operated group showed a large increase in locomotor activity; this effect was also significantly reduced in the rats with lesions [Fig. 4 $F(1,14)=5.11$, $P<0.05$]. The mean $\pm$ SEM for the sham and lesion groups were 1368 ± 249.5 and 754.1 ± 107.4 , respectively. These values were not different from those recorded in experiment 3 (sham: 1401.3 ± 257.8 , lesion: 745.2 ± 81.7); therefore a cross sensitization from AMPH to MDMA was not evident. On the next day locomotor activity was measured following

Table 2. Dopamine and DOPAC (mean $\pm$ SEM) levels in the nucleus accumbens (ng/mg protein). DA = dopamine; DOPAC = dihydroxyphenylacetic acid. 1 ng DA = 5.27 pmole. 1 ng DOPAC = 5.95 pmole. $^*P<0.05$, Student's *t*-test. † DOPAC values were measured in only 3 rats/group for experiment 2

Experiment	Number of subjects	Dopamine	% Depletion	DOPAC	% Depletion
Sham					
2	8	61.0 $\pm$ 2.6		36.9 $\pm$ 6.3 †	
3	4	59.9 $\pm$ 7.8		34.9 $\pm$ 8.8	
4	5	70.0 $\pm$ 4.7		30.9 $\pm$ 4.5	
Total	17	63.4 $\pm$ 2.6		33.7 $\pm$ 2.6	
Lesion					
2	12	5.2 $\pm$ 1.4 *	91.4	6.9 $\pm$ 0.4 * †	81.3
3	5	2.4 $\pm$ 2.1 *	96.1	1.3 $\pm$ 0.9 *	96.2
4	5	6.6 $\pm$ 2.0 *	90.5	4.3 $\pm$ 0.9 *	86.3
Total	24	5.1 $\pm$ 1.0 *	92.0	3.8 $\pm$ 0.7 *	88.7

injections of a serotonin antagonist and MDMA, and methysergide potentiated the effects of MDMA. A two-factor repeated measures ANOVA revealed a significant main effect of surgery [$F(1,14)=8.2$, $P<0.05$], a significant difference between MDMA 5 mg/kg alone and MDMA 5 mg/kg with methysergide 2.5 mg/kg [$F(1,1)=72.5$, $P<0.05$] and a significant interaction effect [$F(1,1)=9.96$, $P<0.05$]. However, a log transformation of the data eliminated the significant interaction which suggests that the interaction effect was due to scaling differences. Thus, the response of both the sham rats and the rats with lesions was increased by the serotonin antagonist.

Biochemistry

The results of biochemical analyses for rats from experiments 2–4 are presented in Table 2. Biochemical analyses of 6-OHDA-injected animals for all studies revealed a 92%

depletion of dopamine [$t(39)=23.4$] and an 88.7% depletion of DOPAC [$t(25)=12.0$] recorded as ng/mg protein in the N. Acc. compared to control rats with sham lesions. In experiment 2, 5 of 17 lesions were determined to be incomplete based on the biochemical results ($<75\%$ depletion of DA) and in experiment 3, 2 of 8 rats were determined to have incomplete lesions. These subjects were then excluded from the behavioral analyses. No rats were eliminated from experiment 4. In both experiments 3 and 4 the biochemical data for one rat was not available due to a problem in the tissue preparation. These rats were maintained in the behavioral data because their data was consistent with that of rats with acceptable lesions. In summary, in these studies only 7 of a total of 33 lesions resulted in incomplete depletions when compared to sham operated animals.

Discussion

MDMA produced significant increases in locomotor activity that were sustained for at least 2 h following injection of all doses tested. Although a minimal effective dose was not determined in this experiment, in other work doses below 1.0 mg/kg did not significantly increase locomotion (Gold et al. 1988). Similar increases in locomotion were observed in these photocell cages following doses of amphetamine up to 1.0 mg/kg. However, other studies have shown the duration of action of amphetamine at these doses was between 120 and 180 min and the amount of locomotion frequently returned to baseline levels by 180 min (see Swerdlow et al. 1986). In contrast, MDMA seemed to produce a more long-lasting activation of the behavior of the rats. The level of activity peaked at approximately 50 min and remained there through the end of the experimental session at the highest dose tested. Previous reports have shown that the stimulant effects of MDMA last at least 5 h (Gold et al. 1988).

Such sustained and relatively stable increases in motor activity could be interpreted as a drug-induced decrease in habituation to the test apparatus despite the extensive pre-exposure habituation. The rats were habituated to the photocell cages overnight and then again for 90 min preceding the MDMA testing sessions. However, curve fitting analysis revealed that both the saline-treated rats and those rats treated with the lowest dose of MDMA exhibited levels of activity which decayed exponentially (sal: $R=0.92$, MDMA 2.5 mg/kg: $R=0.96$) with roughly the same fall off (see File 1981 for a discussion of habituation). Therefore, at least the lowest dose of MDMA tested produced a global increase in activity in the absence of an effect on habituation.

The stimulant properties of MDMA in humans have been reported to be more mild than those of amphetamine. However, sympathomimetic activation, jaw clenching, teeth grinding, and heightened alertness are frequently experienced by users (Shulgin and Nichols 1978; Barnes 1988; Peroutka et al. 1988). The basis for such a potent stimulation of locomotor activity in rats but more mild psychostimulant effects in humans is still unclear but may simply depend on differences in the measured responses. Perhaps the euphoria and enhanced sociability experienced by humans masks the classic stimulant cues. A distinct hangover has been described to follow MDMA use which includes drowsiness, muscle aches and depression (Barnes 1988).

Such symptoms may reflect an underlying stimulant action. Interestingly, Oberlender and Nichols (1988) describe a complex discriminative stimulus effect of MDMA in rats, one component of which is amphetamine-like. Using a drug discrimination procedure these authors failed to observe complete substitution for amphetamine and suggest that only relatively large doses of amphetamine provide effects that are shared by MDMA.

Because of the similarities of MDMA and amphetamine hyperactivity and the known role of the mesolimbic dopamine system in the motor actions of amphetamine, the effects of MDMA in rats with destruction of presynaptic terminals in the mesolimbic dopamine system were examined. Although depletion of dopamine within the N. Acc. by 6-OHDA does not disrupt the locomotor activating properties of caffeine, scopolamine (Joyce and Koob 1981) or heroin (Vaccarino et al. 1986), such lesions attenuate the locomotion produced by indirect sympathomimetics (Kelly et al. 1975; Kelly and Iversen 1976; Joyce and Koob 1981). The results from these and similar studies have supported the hypothesis that psychomotor stimulant drugs (like amphetamine and cocaine) produce increases in locomotion through the release of dopamine from the mesolimbic dopamine-containing terminals of the N. Acc. Intact dopamine function in this region has also been implicated as an important substrate for the reinforcing properties of indirect sympathomimetic drugs measured in a self-administration paradigm (Pettit et al. 1984).

In the present study, 6-OHDA lesions of the N. Acc. also significantly reduced the amount of locomotor activity produced by MDMA. Depletion in the N. Acc. is used as an index of depletion of the mesolimbic dopamine system but clearly adjacent areas, like the anterior caudate and the olfactory tubercle, may be affected. As discussed above, such lesions do not prevent locomotor activation under all conditions, and hyperactivity is observed following small doses of direct acting dopamine agonists like apomorphine (Kelly et al. 1975). In fact, in the present study apomorphine produced a significant hyperactivity in the rats with lesions when challenged 4-5 days after the MDMA test. Nor does it appear that the reduction in locomotor activity caused by the lesion is due to an increase in stereotyped behavior. During informal observations of these rats every 10 min, rats with lesions were often scored in the locomotor category, suggesting that competing stereotypy did not occur. A comprehensive behavioral analysis of a wide range of MDMA doses in unoperated rats has also been published. In that study also, no classic stimulant-induced stereotypies were observed, but rather a stereotyped locomotor behavior which resulted in large increases in amount of locomotion along a stereotyped path (Gold et al. 1988). Therefore it appears that mesolimbic dopamine is also a critical substrate for MDMA stimulated locomotion. Parallel with the locomotor stimulatory effects may be mechanisms which mediate the reinforcing properties of this drug which are exemplified by the increasing abuse liability of MDMA (Peroutka 1987). Significant abuse potential for MDMA has also been demonstrated by animal self-administration of MDMA (Beardsley 1986; Lamb and Griffiths 1987) and a lowering of self-stimulation thresholds by MDMA (Hubner et al. 1988).

It is possible that the behavioral effects following 6-OHDA lesions are due to non-specific damage in the region of the N. Acc. This is thought to be unlikely because

generally 6-OHDA has proved to be selective for catecholamines without effects on serotonin (Bloom et al. 1969; Sachs and Jonsson 1975). Recently Clarke and colleagues (1988) reported that microinfusions of 6-OHDA into the N. Acc. had little if any effect on regional concentrations of 5-HT except for a small local loss. In the present study the attenuation of MDMA-induced hyperactivity was not considered to be due to such non-specific damage to 5-HT neurons because a decrease in 5-HT neurotransmission would be predicted to potentiate the behavioral effects of MDMA as observed previously when MDMA was administered in conjunction with a 5-HT antagonist (Gold and Koob 1988).

The neurochemical specificity of 6-OHDA is restricted to catecholaminergic neurons but includes both dopamine and norepinephrine. Pretreatment with the norepinephrine uptake inhibitor desmethylimipramine (DMI) has been used to protect the noradrenergic neurons from destruction when 6-OHDA is used as a neurotoxin. This procedure was not deemed necessary in the current experiments for the following reasons. First, the amount of norepinephrine in the N. Acc. is minor relative to dopamine concentrations (Versteeg et al. 1976) and only a few dopamine β -hydroxylase fibers may actually be present in the medial most aspect of the N. Acc. (Swanson and Hartman 1975). Second, dopamine receptor agonists in the N. Acc. induce marked locomotor activity that can be inhibited by low doses of dopamine receptor antagonists. In contrast, norepinephrine blocking agents are ineffective in altering dopamine- or dopamine agonist-induced locomotor activity (Fishman et al. 1983). Similarly, administration of inhibitors of dopamine β -hydroxylase which reduce brain content of norepinephrine failed to alter the locomotor stimulant actions of amphetamine (Thornburg and Moore 1973). Evidence from lesion studies also suggests that the psychomotor stimulant effects of amphetamine depend on dopamine but not norepinephrine in the N. Acc. Whereas, 6-OHDA lesions of the N. Acc. (Kelly et al. 1975) or substantia nigra (Roberts et al. 1975) reduce amphetamine-induced locomotion, selective damage to the dorsal and ventral adrenergic pathways does not (Creese and Iversen 1975; Roberts et al. 1975). Furthermore, animals depleted of norepinephrine by intracerebroventricular xylamine injection were virtually identical to controls with respect to the amount of locomotor activity elicited by amphetamine either 4 or 18 days post-lesion (Geyer et al. 1986).

In studies in which DMI pretreatment has been used, a similar depletion of dopamine in the N. Acc. and blockade of amphetamine induced locomotor activity was observed (Kelly et al. 1975). Although DMI may protect ascending noradrenergic neurons, which pass in close proximity, it may not protect the noradrenergic terminals at the site of injection. Herve et al. (1986) reported that the protective effect of DMI depended on the relative concentration of 6-OHDA and DMI at uptake sites. Where the concentration of 6-OHDA at the injection site (ventral mesencephalic tegmentum) was relatively higher than nearby sites, pretreatment with DMI had little effect on the degeneration of noradrenergic nerve terminals there. Indeed, in some brain regions, DMI has been found to reduce the amount of dopamine depletion produced by 6-OHDA (Tassin et al. 1982).

Finally, there is a paucity of data on the actions of MDMA on the noradrenergic system. MDMA has been

found to bind with relatively high affinity to the α_2 adrenoreceptor in frontal cortex tissue (Battaglia et al. 1988) and non-stereoselective inhibition of [3H]NE uptake into hypothalamic synaptosomes has also been reported (Steele et al. 1987). In general the psychoactive properties of MDMA may be explained by alterations in 5-HT neurotransmission both through direct and indirect action at post- and presynaptic-recognition sites and dopamine indirect agonist-like effects of MDMA on presynaptic release of dopamine (Battaglia et al. 1988; Johnson et al. 1986). However it has been speculated that noradrenergic mechanisms in the periphery may contribute to effects on cardiovascular function and cardiotoxicity associated with MDMA use (Battaglia et al. 1988; Steele et al. 1987).

There is significant neuropharmacological evidence to support the hypothesis of a dopaminergic component to the actions of MDMA while its neurotoxic effects appear restricted to the serotonergic neurons (Stone et al. 1986; Battaglia et al. 1987; Commins et al. 1987). In vitro studies have shown an effect of MDMA on dopamine release (Schmidt 1987b) and uptake (Steele et al. 1987). Although MDMA is more potent at releasing serotonin than dopamine in the rat striatum (Schmidt et al. 1986), Oberlander and Nichols (1988) suggest that as the dose increases, a level is probably reached where dopaminergically-mediated behavioral effects become evident. In a recent study using in vivo voltammetry, dopamine release in the nucleus accumbens and caudate were measured in awake-behaving rats following various doses of MDMA (Yamamoto and Spanos 1988). Interestingly, dopamine has also been implicated as a partial mediator of the initial decrease in tryptophan hydroxylase activity caused by MDMA and as an important prerequisite to the development of long-term MDMA-induced serotonin toxicity. (Stone et al. 1988).

The behavioral effects of MDMA may also reflect an interaction between serotonergic and dopaminergic systems. We have previously reported that the 5-HT antagonist methysergide significantly potentiated the hyperactivity produced by MDMA but not that produced by amphetamine (Gold and Koob 1988). This result was interpreted to suggest that 5-HT neurotransmission may inhibit dopamine transmission in MDMA-treated animals. Serotonin antagonism may disinhibit the dopamine system and result in enhanced hyperactivity. Hollister et al. (1976) observed that various manipulations which decreased serotonergic neurotransmission potentiated the locomotor response to amphetamine. Additionally, rats with lesions of the mesolimbic serotonergic pathway were found to be hyper-responsive to amphetamine (Geyer et al. 1976).

In the present set of experiments two instances of drug interactions with MDMA were observed. Rats which experienced MDMA seemed to exhibit an enhanced response to amphetamine; however, cross sensitization was not seen when the order of the drugs was reversed. Secondly, a serotonin antagonist was found to potentiate significantly the hyperactivity produced by MDMA in sham-operated rats and those with 6-OHDA lesions. A single neurochemical mechanism may be responsible for both of these results. As suggested previously (Gold and Koob 1988), methysergide may act to antagonize the serotonin neurotransmission which normally inhibits dopamine function. Consequently, the effect of dopamine-mediated locomotor stimulation is exaggerated. Rats with 6-OHDA lesions have less dopamine and therefore show a more blunted response. In fact the

significant interaction between the potentiation and lesion factors was abolished when a log transformation of the data was performed, suggesting the interaction was due to a difference in scaling and that the influences of methysergide and 6-OHDA lesions are additive rather than multiplicative. As for the cross sensitization seen with amphetamine, it is possible that previous exposure to MDMA may have resulted in neurotoxic damage to some serotonin neurons. Depletions of serotonin and its metabolites have been reported following single injections of MDMA (Stone et al. 1986; Mokler et al. 1987; Schmidt 1987a). A decrease in serotonergic tone would also result in a disinhibition of dopamine neurons and may explain the enhanced amphetamine response. Evidence for such a "functional lesion" has been reported in an operant procedure in which MDMA-induced serotonin depletion was found to potentiate its psychomotor stimulant effects (Li et al. 1986). In the case where amphetamine was given first followed by MDMA no change in responsiveness would then be expected.

Although 6-OHDA lesions to the region of the N. Acc. may not be entirely selective for dopamine, the effects of these lesions on MDMA measured in the current report can be compared with effects of the same lesions on other well-characterized psychostimulants. The stimulation of locomotor activity by MDMA and the importance of mesolimbic dopamine in this response measured in the present study reflect similarities with the prototype phenylethylamine, amphetamine. It is important to note that these parameters are frequently associated with rewarding aspects of drugs and drug abuse. The social facilitatory effect of MDMA and its neurotoxic potential which were not addressed here seem to set this analog apart from other amphetamines. Examination of the individual components of the varied actions of MDMA and the underlying neurochemical mechanisms may aid in our understanding of the complex nature of this drug, a drug which ultimately may become a representative for a novel psychoactive class.

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