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ORIGINAL INVESTIGATION

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Relevance of MDMA ("ecstasy")-induced neurotoxicity to long-lasting psychomotor stimulation in mice

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Abstract *Rationale:* Although many studies have focused on the mechanisms underlying MDMA-induced neurotoxicity, little is known about the subsequent long-term response to psychostimulants following exposure to a neurotoxic dose of MDMA. *Objectives:* We investigated the effect of pre-exposure to neurotoxic and non-neurotoxic doses of MDMA on the response of mice to the psychomotor stimulating effects of MDMA and cocaine. *Methods:* To investigate MDMA-induced neurotoxicity, male Swiss Webster mice were subjected to three regimens of MDMA: i) 40 mg/kg×2, ii) 30 mg/kg×2, and iii) 15 mg/kg×2 for 2 days. On day 5 following the last exposure to MDMA, the levels of dopaminergic and serotonergic markers were determined. For the behavioral experiments, mice received either a single injection of 10 mg/kg MDMA [MDMA(L)] or one of the following doses of MDMA: 30 mg/kg×2 or 15 mg/kg×2 for 2 days [MDMA (H)]. A third group received saline as a control. On day 5 after the last pretreatment injection, the first MDMA (10 mg/kg) challenge was given, and on day 12, cocaine (20 mg/kg) was administered. Subsequently, mice were re-challenged with MDMA on days 35, 50 and 80, after which locomotor activity was monitored by infrared beam-interrupts. On day 83, mice were killed to detect the levels of dopaminergic and serotonergic markers. *Results:* MDMA-induced mortality and depletion of dopaminergic and serotonergic markers were dose-dependent. MDMA (H) mice endured a sensitized response to MDMA challenge from days 5 through 80, e.g. a persistent 3-fold increase in locomotor activity compared to the response of mice that were not pretreated with a neurotoxic dose of MDMA. The depletion of DAT and 5-HTT binding sites was sustained throughout this time period (64–68% of control). The MDMA (L) mice showed a sensitized response to MDMA only on day 5. Both MDMA (L) and MDMA (H) mice were sensitized to the cocaine challenge. *Conclusions:* The induction of sensitization to the locomotor stimulating effects of MDMA and cocaine was independent of MDMA-induced neurotoxicity. However, the long-lasting maintenance of the sensitized response to MDMA may be related to the enduring neurotoxicity caused by MDMA.

Keywords MDMA · Cocaine · Neurotoxicity · Sensitization · Hyperlocomotion

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Introduction

In recent years the amphetamine analog 3,4-methylenedioxymethamphetamine (MDMA; "ecstasy") has become a major drug of abuse, particularly among adolescents in the US and Europe. Like other amphetamines, MDMA causes the release of dopamine (DA) and 5-hydroxytryptamine (5-HT) in the striatum and nucleus accumbens (Yamamoto and Spanos 1988; White et al. 1994). In rats (Commins et al. 1987; Schmidt 1987; Farfel et al. 1992) and non-human primates (Ali et al. 1993; Scheffel et al. 1998) MDMA causes primarily serotonergic neurotoxicity associated with depletion of 5-HT, its metabolite 5-hydroxy-indoleacetic acid (5-HIAA) and a decrease in 5-HT transporter (5-HTT) binding sites in the cortex, hippocampus and striatum. Although mice are less susceptible to serotonergic neurotoxicity, a high dose of MDMA administered to mice resulted in dopaminergic and serotonergic neurotoxicity (Stone et al. 1987; Logan et al. 1988). In human MDMA users, positron emission tomography (PET) studies showed a decrease in global and regional brain 5-HTT binding sites (McCann et al. 1998).

Several studies have investigated the behavioral consequences of exposure to MDMA. However, most of these studies investigated either the outcome of pre-exposure to a non-neurotoxic dose of MDMA or the short-term outcome after exposure to a neurotoxic dose of MDMA. MDMA caused dose-dependent facilitation of acoustic and tactile startle in rats (Schmidt and Kehne 1990). A high dose of MDMA given to adolescent rats did not cause serotonergic neurotoxicity, but animals showed a decrease in social interaction 12–29 days after the drug administration (Fone et al. 2002). A neurotoxic dose of MDMA accompanied by degeneration of 5-HT neurons in rat brain resulted in deficits in open field behavior and the plus-maze paradigm (Mechan et al. 2002), and tolerance to the serotonin syndrome produced by MDMA in rats (Marston et al. 1999). In addition, MDMA-induced neurotoxicity in neonatal rat pups was associated with a decrease in ultrasonic vocalization (Winslow and Insel 1990), and MDMA-induced neurotoxicity in adult rats was accompanied by an increase in the analgesic effect of morphine (Nencini et al. 1988).

We have previously reported that methamphetamine (METH)-induced neurotoxicity in mice was associated with a robust sensitization to the psychomotor stimulating effect of METH (Itzhak et al. 1997, 1998). The long-lasting sensitized response to METH coincided with the sustained depletion of striatal dopaminergic markers over a period of 74 days after the last exposure to METH (Itzhak et al. 2002). Sensitization to the psychomotor stimulating effects of amphetamines and cocaine is considered a relevant animal model of drug addiction and psychostimulant-induced psychosis (Robinson and Becker 1986; Pierce and Kalivas 1997). Based on our previous observation of the outcome of METH-induced neurotoxicity, we postulated that MDMA-induced neurotoxicity would lead to long-lasting behavioral sensitization to psychostimulants. Thus, we investigated a) the degree and time course of MDMA-induced neurotoxicity in mice, and b) the short- and long-term responses of MDMA treated mice to the psychomotor-stimulating effects of MDMA and cocaine.

Materials and methods

Materials

(±)MDMA-HCl was a gift from the National Institute on Drug Abuse (Bethesda, Md., USA). Cocaine-HCl was purchased from Sigma (St Louis, Mo., USA). [^{3}H]Mazindol (24 Ci/mmol) and [^{3}H]citalopram (81 Ci/mol) were purchased from New England Nuclear (Wilmington, Del., USA).

Animals

Male Swiss Webster mice Crl:CFW (SW)BR (8 weeks of age at the beginning of the experiment; 30–32 g; Charles River, Wilmington, Mass., USA) were maintained on a 12-h light/dark lighting schedule at a room temperature of 23°C, and housed in groups of five with free access to food and water. Animals were housed in groups of two following the drug or saline treatments. When an odd number of mice were used, one cage housed three mice. Animal care was in accordance to the Guide for the Care and Use of Laboratory Animals (National Research Council, National Academy Press, 1996) and as approved by the University of Miami Animal Care and Use Committee.

Schedules of MDMA administration: neurotoxicology

Four groups of mice received one of the following treatments (intraperitoneal injections): a) saline (n=24), b) MDMA (40 mg/kg×2; 8 h apart; total 80 mg/kg; n=15), c) MDMA (30 mg/kg×2; 8 h apart; total 60 mg/kg; n=15), and d) MDMA (15 mg/kg×2; 8 h apart, for 2 days; total 60 mg/kg; n=15). The number of MDMA injections was matched and substituted with saline injections in the other groups tested. Routinely, mice were housed at an ambient temperature of 23°C in groups of two per cage (or in the event of an odd number of mice, one cage housed three mice) following the administration of the first MDMA injection, and until day 5 after the last MDMA injection was given. For the initial neurochemical studies, mice were killed on day 5, and the striatum, frontal cortex and hippocampus were freshly dissected and frozen at −80°C. Tissues were used for the determination of the levels of DA, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), DA transporter (DAT) binding sites (striatum), and 5-HT, 5-HIAA and 5-HTT binding sites (frontal cortex, hippocampus and striatum). To determine if MDMA-induced neurotoxicity is long lasting, mice from the behavioral experiments were killed on day 83.

Determination of dopaminergic and serotonergic markers

Routinely, tissue from one hemisphere was used for monoamine assays and the tissue from the other hemisphere was used for [^{3}H]mazindol binding to the DAT and [^{3}H]citalopram binding to the 5-HTT. High performance liquid chromatography (HPLC) combined with electrochemical detection (Itzhak et al. 1998) was used to determine the concentrations of DA, DOPAC, HVA, 5-HT and 5-HIAA. The density of striatal DAT binding sites was determined by saturation binding assays using [^{3}H]mazindol (1.0–20.0 nmol/l) as described previously (Itzhak et al. 1998). Binding of [^{3}H]citalopram (0.5–5.0 nmol/l) was carried out in the absence and presence of 5-HT (10 mmol/l) in the P2 fraction of the frontal cortex that was resuspended in Tris-HCl buffer (50 mmol/l; pH 7.4) containing NaCl (120 mmol/l) and KCl (5 mmol/l). The reaction mixture was incubated for 60 min at room temperature and was stopped by a rapid vacuum filtration (Brandel model M-12) through Whatman GF/B filters. The filters were washed twice with 4 ml ice-cold buffer, and radioactivity was determined by scintillation counting. The binding parameters, e.g. the maximal number of binding sites (B_{max}) and the dissociation constant (K_d), were determined by the LIGAND program.

Schedules of MDMA administration: behavior

Experiment 1: effect of pretreatment with a neurotoxic dose of MDMA on the locomotor response to a low dose of MDMA and cocaine

Two groups of mice were injected with either saline (twice a day; 8 h apart; n=10) or MDMA (30 mg/kg×2; 8 h apart; n=12; 8/12 survived). On day 5 after the last injection was given, both groups were challenged with MDMA (10 mg/kg) and locomotor activity was recorded for 60 min. To determine if the previous exposures to MDMA produced cross-sensitization to cocaine, mice were challenged with cocaine (20 mg/kg) on day 12. An additional group (n=6) of naive animals served as a control for the acute effect of cocaine, and locomotor activity was recorded for 30 min. Subsequently, mice received second, third and fourth MDMA (10 mg/kg) challenges on days 35, 50 and 80, respectively, after the pre-drug

treatment. On day 83, after the completion of the behavioral experiments, the following groups were killed to determine the levels of dopaminergic and serotonergic markers: a) mice that were pretreated with the neurotoxic dose of MDMA and b) a control group (n=10) that received only saline injections throughout the same time period (see Table 2).

Experiment 2: comparison between the effects of pretreatment with a low and a high dose of MDMA on the subsequent response to challenge injections of MDMA and cocaine

Three groups of mice were injected with saline (n=10), a low dose of MDMA [10 mg/kg; designated MDMA (L); n=8] or a high dose of MDMA [15 mg/kg×2; 8 h apart for 2 days; designated MDMA (H); n=10; 8/10 survived]. On day 5 after the last injection was given, all three groups were challenged with MDMA (10 mg/kg) and locomotor activity was recorded for 60 min. On day 12, the MDMA (L), MDMA (H) and naive animals (n=5) that served as controls were challenged with cocaine (20 mg/kg). Locomotor activity was recorded for 30 min. Subsequently, the last three groups received a second, third and fourth injection of MDMA (10 mg/kg) on days 35, 50 and 80, respectively. On day 83 after the completion of the behavioral experiments, the following groups were killed to determine the levels of dopaminergic and serotonergic markers: a) mice that were pretreated with the neurotoxic dose of MDMA [MDMA(H)] and b) mice that were pretreated with the low dose of MDMA [MDMA(L)] (see Table 2).

Experiment 3: influence of pretreatment with a single versus multiple injections of cocaine on the locomotor response to MDMA challenge

The aim of the third experiment was to investigate if a single or multiple cocaine injections caused cross-sensitization to MDMA. Mice received a) saline (n= 8; 5 days), b) a single injection of cocaine (20 mg/kg; 4 days saline and cocaine on day 5; n=9), or c) five daily cocaine (20 mg/kg) injections (n=8) in their home cage. Three days after the last injection was given, mice were challenged with MDMA (10 mg/kg), and locomotor activity was recorded for 60 min.

Measurement of locomotor activity

The locomotor activity cages were standard transparent rectangular rodent cages (42×24×20 cm high), and locomotion was monitored by an activity meter (Opto-Varimex-Mini Model B; Columbus Instruments, Columbus, Ohio, USA). The activity meter consisted of an array of 15 infrared emitter/detector pairs, spaced at 2.65 cm intervals, measuring activity along a single axis of motion. Each emitter and detector was mounted alongside the length of the cage (42 cm long). Both the total counts and the ambulatory counts were recorded and transferred by a counter interface to a computer. The Opto-Varmix-Mini Model B can separate counts (beam interruptions) associated with ambulatory activity from total activity. This is accomplished by memorizing the location of the last broken beam, and blocking additional count information from being scored on an additional ambulatory front panel counter until a different beam is broken. Subtraction of ambulatory counts from total counts provides an index of vertical activity. Based on our previous observations (Itzhak 1997) and the current results, it appears that the fraction of non-ambulatory counts (25–30% of total counts) increased or decreased in parallel to corresponding changes in ambulatory counts. Because of this relationship, only ambulatory counts are reported. Counts were registered every 10 min for 30 or 60 min. Results are represented as cumulative horizontal counts registered for 30 min following cocaine administration and for 60 min following MDMA administration. Routinely, each mouse was placed in the test cage for a 30-min habituation period to the new environment before the recording of drug-induced locomotor activity.

Statistical analysis

Differences between control and MDMA treated mice in the level of dopaminergic and serotonergic markers were analyzed by one-way ANOVA followed by post-hoc Newman-Keuls test. The effect of a challenge injection of MDMA on locomotion on days 5, 35, 50 and 80 was analyzed by a two-way ANOVA, drug pre-exposure effect×time, with time as the repeated measure. Post-hoc Scheffe's test was used to determine differences between the various groups. The effect of cocaine on locomotion on day 12 was analyzed by one-way ANOVA followed by post-hoc Newman-Keuls' test. A P value <0.05 was considered to be statistically significant.

Results

MDMA-induced neurotoxicity

Different doses and schedules of MDMA were investigated to determine the degree of neurotoxicity induced by the drug. The administration of MDMA (40 mg/kg×2; 8 h apart) to Swiss Webster mice resulted in 54% mortality within a 24 h period following the second injection (Table 1). The remaining animals (7/15) were killed on day 5 after MDMA administration. The level of DA in the striatum was only 21% of control (Table 1); the levels of DOPAC and HVA were also reduced by 70–75% of control values (data not shown). The concentration of 5-HT in the striatum, frontal cortex and hippocampus was reduced by 39–67% from control levels

Table 1 Effect of MDMA on Swiss Webster mouse survival and the levels of DA, DAT, 5-HT and 5-HTT in brain. Animals were killed 4 days after drug administration. *N/T* not tested. Numbers in parentheses represent percentages of control

MDMA	Survival	Striatum			Frontal cortex		Hippocampus
		DA[a]	DAT[b]	5-HT[a]	5-HT	5-HTT[b]	5-HAT
0 mg/kg	24/24 (100)	584±9	1147±33	56±5	25±2	355±28	23±2
40 mg/kg×2 (1 day)	7/15 (46)	125±6 (21)*	N/T	34±3 (61)*	11±2 (44)*	N/T	8±1 (33)*
30 mg/kg×2 (1 day)	9/15 (60)	266±11 (45)*	585±23 (51)*	43±4 (77)*	23±2 (92)	223±18 (63)*	19±3 (82)
15 mg/kg×2×2 (2 days)	13/15 (86)	351±10 (60)*	630±31 (55)*	42±3 (75)*	24±3 (96)	234±10 (66)*	25±2 (108)

[a] The values of DA and 5-HT are in ng/100 mg tissue. Changes in the concentration of DA metabolites DOPAC and HVA, and in 5-HT metabolite 5-HIAA, were proportional to the changes in DA and 5-HT levels, respectively
[b] The values of DAT and 5-HTT are in fmol/mg protein
*P<0.05 drug treated versus saline (Newman-Keuls test)

(Table 1). The administration of MDMA (30 mg/kg×2; 8 h apart) resulted in 40% mortality (6/15; Table 1). The rest of the animals were killed on day 5. The concentrations of DA and DAT binding sites in the striatum were 45 and 51% of control, respectively. The level of 5-HT in the striatum was 77% of control. No significant change in 5-HT level was detected in the frontal cortex and hippocampus (Table 1). However, a significant decrease in the number of 5-HTT binding sites in the frontal cortex was observed (63% of control; Table 1). The administration of MDMA (15 mg/kg×2; 8 h apart, for 2 days) resulted in only 13.3% mortality (2/15; Table 1). The extent of depletion of DA and 5-HT, and their corresponding transporter binding sites was similar to the depletion observed after the administration of the previous schedule MDMA (30 mg/kg×2; 8 h apart in 1 day; Table 1).

Effect of pretreatment with a neurotoxic dose of MDMA on the locomotor response to a low dose of MDMA and cocaine (experiment 1)

In preliminary experiments we measured the locomotor activity generated in Swiss Webster mice after the administration of 0, 5, 10 and 20 mg/kg MDMA. Saline and 5 mg/kg MDMA resulted in 568±79 and 798±92 ambulatory counts per 60 min, respectively. The doses of 10 and 20 mg/kg resulted in 4261±845 and 13,881±1759 ambulatory counts per 60 min, respectively (data not shown). Since the lowest dose of 5 mg/kg MDMA had no significant effect on locomotor activity, while the highest dose tested (20 mg/kg) resulted in intense locomotion that was accompanied by stereotypic movements, the dose of 10 mg/kg was chosen for all subsequent experiments.

In the first experiment, the short- and long-term outcome of the administration of MDMA (30 mg/kg×2) was investigated. The administration of a challenge injection of MDMA (10 mg/kg) to mice pretreated with MDMA [30 mg/kg×2; MDMA(H)] resulted in a sensitized response to the locomotor stimulating effect of MDMA on days 5, 35, 50 and 80 following the initial drug pre-exposure compared to control group that received saline instead of MDMA (30 mg/kg×2) (Fig. 1). A two-way ANOVA for the effects of drug pre-exposure×time (with time as the repeated measure) revealed a significant drug pre-exposure effect [$F(1,64)=71.577$; $P<0.001$], a non-significant time effect [$F(3,64)=0.611$; $P=0.609$], and non-significant interaction [$F(3,64)=0.682$; $P=0.569$]. Post-hoc Scheffe's test revealed significant differences ($P<0.05$) between the responses of the saline/MDMA group and the MDMA (H)/MDMA group on days 5, 35, 50 and 80 (Fig. 1). The results suggest that the MDMA(H) group remained sensitized to the locomotor stimulating effect of MDMA from days 5 through 80, while the repeated injections of MDMA (10 mg/kg) to the control group did not induce sensitization.

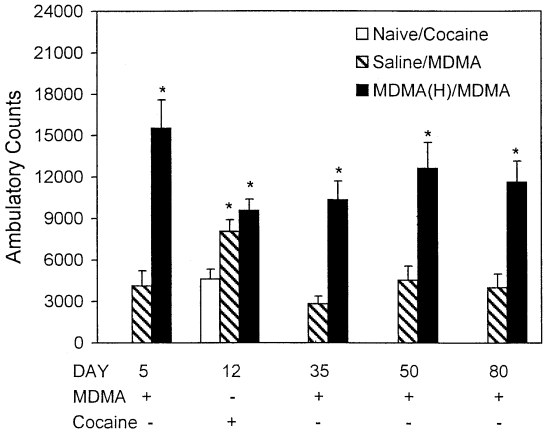

Fig. 1 Comparison between the responses of mice pre-exposed to saline or a high dose of MDMA to challenge injections of MDMA and cocaine. The *y*-axis represents mean±SEM ambulatory counts registered for 60 min after MDMA, and 30 min after cocaine were administered. The *x*-axis represents the days after the pretreatment with saline or MDMA [30 mg/kg×2; MDMA(H)] and the drugs given on each test day. On day 5, saline and MDMA(H) pretreated mice received MDMA (10 mg/kg) challenge, and on day 12 they received cocaine (20 mg/kg) challenge; control naive mice also received cocaine challenge. On days 35, 50 and 80 the saline/MDMA and MDMA(H)/MDMA mice received MDMA (10 mg/kg) challenge. Results show that the MDMA(H) mice were sensitized to the locomotor stimulating effect of MDMA from days 5 through 80 compared to the control group (*$P<0.05$). Mice pre-exposed to either a single MDMA (10 mg/kg; day 5; saline/MDMA group) or multiple MDMA treatments [MDMA(H) group] both showed a sensitized response to cocaine challenge on day 12 compared with the response of naive mice that received cocaine (*$P<0.05$)

To determine if pre-exposure to MDMA results in cross-sensitization to the locomotor stimulating effect of cocaine, mice were challenged with cocaine (20 mg/kg) on day 12 after the initial pre-exposure to the high dose of MDMA (Fig. 1). One-way ANOVA analysis for the effect of cocaine on naive mice, mice pre-exposed to a single MDMA injection on day 5, and mice pre-exposed to the neurotoxic dose of MDMA revealed a significant drug pre-exposure effect [$F(2,21)=11.837$; $P=0.0004$]. Post-hoc Newman-Keuls test showed significant differences ($P<0.05$) between the naive/cocaine versus saline/MDMA mice, and naive/cocaine versus MDMA(H)/MDMA mice (Fig. 1; day 12). These results suggest that the pre-exposure to either a low or a high dose of MDMA induced cross-sensitization to cocaine.

Investigation of the levels of dopaminergic and serotonergic markers 82 days after the administration of the neurotoxic dose of MDMA (30 mg/kg×2) revealed that the concentration of striatal DA and the density of DAT binding sites were below control levels (71 and 64%, respectively, Table 2). The density of 5-HTT binding sites in the frontal cortex was 71% of control (Table 2).

Table 2 Long-term effect of MDMA on the levels of DA, DAT, 5-HT and 5-HTT in mouse brain. Animals were killed 82 days after pretreatment with high and low dose of MDMA. Numbers in parentheses represent percentages of control

MDMA[a]	Striatum			Frontal cortex	
	DA[b]	DAT[c]	5-HT[b]	5-HT	5-HTT[c]
Control					
(0 mg/kg; *n*=10)	601±13	1328±58	63±6	28±3	298±31
High dose					
30 mg/kg×2 (1 day) (*n*=8)	426±16 (71)*	850±43 (64)*	66±4 (106)	25±2 (89)	211±13 (71)*
15 mg/kg×2×2 (2 days) (*n*=8)	449±21 (75)*	863±26 (65)*	61±4 (97)	27±3 (96)	202±14 (68)*
Low dose					
10 mg/kg×1 (1 day) (*n*=8)	618±25 (103)	1159±63 (87)	68±6 (108)	24±3 (86)	311±27 (104)

[a] Doses of MDMA correspond to the pretreatment regimen
[b] The values of DA and 5-HT are in ng/100 mg tissue. Changes in the concentration of DA metabolites DOPAC and HVA were proportional to the changes in DA levels
[c] The values of DAT and 5-HTT are in fmol/mg protein
*P<0.05 drug treated versus saline (Newman-Keuls' test)

Comparison between the effects of pretreatment with a low and a high dose of MDMA on the subsequent response to challenge injections of MDMA and cocaine (experiment 2)

The goals of the second experiment were: a) to compare the effect of a different high dose regimen of MDMA (15 mg/kg×2; 8 h apart, for 2 days) with the results from the first experiment, b) to compare directly between the results of pre-exposure to high- and low-dose (10 mg/kg) MDMA and c) to determine whether a single exposure to cocaine yields cross-sensitization to MDMA challenge. The effect of a challenge MDMA injection (10 mg/kg) on mice pre-exposed to a) saline, b) a low dose of MDMA [10 mg/kg; MDMA(L)], and c) a high dose of MDMA [15 mg/kg×2, for 2 days; MDMA(H)] is illustrated in Fig. 2. A two-way ANOVA (drug pre-exposure×time) was performed to analyze the effect of the challenge injection of MDMA on days 5, 35, 50 and 80. There was a significant drug pre-exposure effect [$F(1,76)=24.112$; $P<0.0001$], a significant time effect [$F(3,76)=6.943$; $P=0.0003$] and non-significant interaction [$F(3,76)=1.577$; $P=0.201$]. Post hoc Scheffe's test revealed the following: on day 5, MDMA (10 mg/kg) caused significantly higher ($P<0.05$) locomotor activity in the MDMA(L) and MDMA(H) groups compared to the control group (Fig. 2). On days 35, 50 and 80, MDMA (10 mg/kg) caused significantly higher ($P<0.05$) locomotion in the group that was pre-exposed to the neurotoxic dose of MDMA [MDMA(H)] compared to the other groups tested [MDMA(L) and naive/cocaine] (Fig. 2). These findings indicate the following: a) A single exposure to a low dose of MDMA was sufficient to induce sensitization to locomotor stimulating effect of MDMA. b) While the sensitized response to MDMA was maintained through day 80 in the MDMA(H) group, the sensitized response dissipated in the MDMA(L) group by day 35. c) A single exposure to cocaine (on day 12; Fig. 2) did not induce subsequent cross-sensitization to an MDMA challenge.

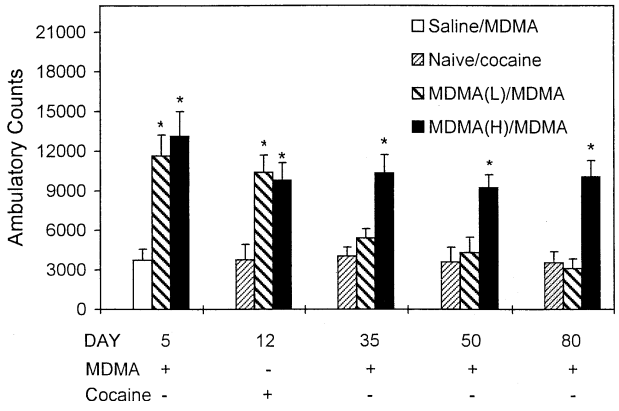

Fig. 2 Comparison between the responses of mice pre-exposed to saline, a low or a high dose of MDMA to challenge injections of MDMA and cocaine. The *y*-axis represents mean±SEM ambulatory counts registered for 60 min after MDMA, and 30 min after cocaine were administered. The *x*-axis represents the days after the pretreatment with saline, a low dose of MDMA [10 mg/kg; MDMA(L)] or a high dose of MDMA [15 mg/kg×2 for 2 days; MDMA(H)]. On day 5, the three groups were challenged with MDMA (10 mg/kg). Both the MDMA(L) and MDMA(H) groups showed a sensitized response to the locomotor stimulating effect of MDMA compared to the control group (*$P<0.05$ saline/MDMA versus MDMA(L)/MDMA and saline/MDMA versus MDMA(H)/MDMA). On day 12, MDMA(L), MDMA(H) and naive mice received cocaine (20 mg/kg) challenge. Both the MDMA(L) and MDMA(H) groups showed a sensitized response to cocaine compared with the control group (*$P<0.05$ naive/cocaine versus MDMA(L)/MDMA and naive/cocaine versus MDMA(H)/MDMA). On days 35, 50 and 80 the naive/cocaine, MDMA(L)/MDMA and MDMA(H)/MDMA groups were challenged with MDMA (10 mg/kg). In the last three tests, the MDMA(H)/MDMA group showed a sensitized response to MDMA compared to the response of the naive/cocaine group (*$P<0.05$) as well as the response of the MDMA(L)/MDMA group (*$P<0.05$)

As described in the first experiment, the effect of a challenge injection of cocaine on mice pre-exposed to MDMA was investigated on day 12. One-way ANOVA for the effect of cocaine on a) naive/control mice, b) MDMA(L) and c) MDMA(H) mice yielded the following

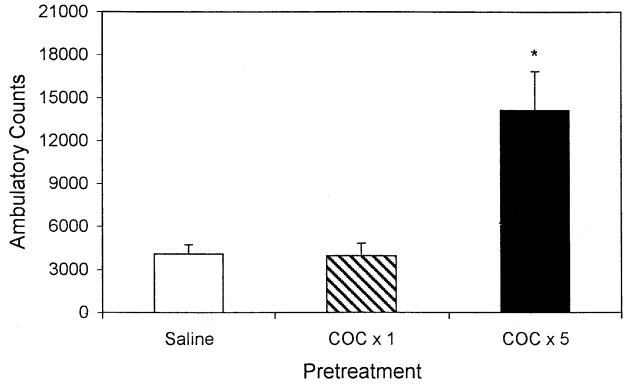

Fig. 3. Effect of a challenge injection of MDMA on mice pretreated with cocaine. The *y*-axis represents mean±SEM ambulatory counts registered for 60 min and the *x*-axis shows the saline and cocaine pretreatments. No significant difference was observed between the level of locomotor activity induced by MDMA (10 mg/kg) in mice pretreated with saline or a single injection of cocaine (20 mg/kg; COC×1). However, MDMA-induced locomotion in mice pretreated with cocaine (20 mg/kg, for 5 days; COC×5) was significantly higher than that recorded in the saline group (*P<0.05) and the COC×1 group (*P<0.05)

[F(2,20)=9.641; P=0.003]. Post hoc Newman-Keuls' test indicated significant differences (P<0.05) between naive/cocaine versus MDMA(L)/MDMA groups and naive/cocaine versus MDMA(H)/MDMA groups. No significant differences were detected between the MDMA(L)/MDMA and MDMA(H)/MDMA groups in their response to cocaine challenge (Fig. 2). These findings are similar to the results of the first experiment, except that in the second experiment the mice that received the non-neurotoxic dose of MDMA were pre-exposed to two injections of MDMA (10 mg/kg).

On day 83 after the completion of the behavioral experiments, the following groups were killed to determine the levels of dopaminergic and serotonergic markers: a) mice that were pretreated with the neurotoxic dose of MDMA [MDMA(H)] and b) mice that were pretreated with the low dose of MDMA [MDMA(L)] (Table 2). The levels of DA and DAT binding sites in the striatum of MDMA (H) mice were still below control values (75 and 65%, respectively, Table 2). Also, the number of 5-HTT binding sites in the frontal cortex was reduced by 32% of control. However, the levels of the dopaminergic and serotonergic markers in the MDMA (L) group were not significantly different from control values (Table 2). These results suggest that the pre-exposure to the high dose of MDMA, but not the low dose, caused a long-lasting deficit in the number of DAT and 5-HTT binding sites.

Influence of pretreatment with a single versus multiple injections of cocaine on the locomotor response to MDMA challenge (experiment 3)

We investigated whether a single or multiple (five) injections of cocaine (20 mg/kg) and a short interval (3 days) between the MDMA challenge and the last cocaine injection would induce cross-sensitization to MDMA. One-way ANOVA for the effect of MDMA on mice pretreated with a) saline, b) cocaine (20 mg/kg×1) or c) cocaine (20 mg/kg×5) showed a significant difference [F(2,22)=11.98; P<0.001]. Post-hoc Newman-Keuls test indicated a significant difference (P<0.05) between saline/control versus cocaine (20 mg/kg×5) as well as cocaine (20 mg/kg×1) versus cocaine (20 mg/kg×5). No significant difference between saline/control versus cocaine (20 mg/kg×1) was observed (Fig. 3). The results indicate that a single injection of cocaine was insufficient to cause cross-sensitization to MDMA despite the short interval between the two injections. Only the multiple injections of cocaine induced cross-sensitization to the locomotor stimulating effect of MDMA.

Discussion

The major finding of the present study is the persistent sensitization to the psychomotor stimulating effect of MDMA, which lasted more than 2.5 months following the exposure of mice to a neurotoxic dose of MDMA. It appears that the sensitized response coincided with the preserved depletion of the DAT and the 5-HTT in the striatum and frontal cortex, respectively.

Several studies have documented the serotonergic neurotoxicity that develops after the administration of high doses of MDMA to rats (Commins et al. 1987; Schmidt 1987; Farfel et al. 1992) and non-human primates (Ali et al. 1993; Scheffel et al. 1998). In mice, however, the degree of dopaminergic and serotonergic neurotoxicity depends on the dose and the frequency of MDMA administration; mice are more susceptible to dopaminergic than serotonergic neurotoxicity (Stone et al. 1987; Logan et al. 1988). Results of the present study are in general agreement with the previous reports. The degree of neurotoxicity we observed was proportional to the dose of the drug, and dopaminergic neurotoxicity was more prominent than serotonergic neurotoxicity (Table 1). Although several studies have addressed the behavioral effects of MDMA in rats (see Introduction), the long-term consequences of exposure to a neurotoxic dose of MDMA are less clear. In the present study we investigated the long-term effects of exposure to neurotoxic and non-neurotoxic doses of MDMA.

Results of the first behavioral experiment (Fig. 1) indicate that pre-exposure to a neurotoxic dose of MDMA resulted in long-lasting sensitization to the psychomotor stimulating effect of MDMA. A relationship between MDMA-induced neurotoxicity and the long-lasting sensitized response is suggested by the finding that the levels of DAT and 5-HTT binding sites remained below control values only in the group that was pretreated with the high dose of MDMA (Table 2) and had expressed sensitization to MDMA challenges from days 5 through 80 (Fig. 1). The group that was challenged with 10 mg/kg MDMA on days 5, 35, 50 and 80 did not develop sensitization to

MDMA (Fig. 1). This is likely due to the large intervals between the repeated injections of MDMA in this experiment, since the daily administration of a low dose of MDMA to rats resulted in sensitization to subsequent MDMA challenge (Kalivas et al. 1998).

The second behavioral experiment was done after the pretreatment with a regimen of MDMA (15 mg/kg×2 for 2 days) that produced a lower mortality rate than the previous one (30 mg/kg×2; Table 1). Results from the second experiment confirmed and extended the results of the first. An additional finding was that a single injection of a low dose of MDMA (10 mg/kg) was sufficient to produce a sensitized response to MDMA challenge (10 mg/kg) if the challenge was given shortly after the first injection (5 days; Fig. 2). This finding suggests that the induction of sensitization to the psychomotor stimulating effect of MDMA is independent of MDMA-induced neurotoxicity. However, while the sensitized response to MDMA in the MDMA (L) mice dissipated by day 35, the sensitized response to MDMA in the MDMA (H) mice was maintained through day 80 (Fig. 2). Thus, results from the first and second experiments indicate that the enduring sensitized response to MDMA was maintained only in the mice that received the neurotoxic dose of the drug. This response coincided with reduced levels of DAT and 5-HTT binding sites in the striatum and frontal cortex.

Results from the cocaine cross-sensitization studies indicate that a single (first experiment; Fig. 1) or two injections (second experiment; Fig. 2) of a low dose of MDMA (10 mg/kg) were sufficient to cause cross-sensitization to cocaine. These findings suggest that the cross-sensitization to cocaine is independent of MDMA-induced neurotoxicity. However, a single cocaine challenge given to naive mice (day 12) did not produce further cross-sensitization to challenge MDMA (day 35) (Fig. 2). We postulated that this might be due to a) the large interval between the cocaine and MDMA injections (23 days) and b) the fact that it was only a single exposure to cocaine. Yet, results of the third experiment (Fig. 3) indicated that even a short interval (3 days) between a single cocaine injection and MDMA challenge did not produce cross-sensitization to MDMA. Only the repeated injection of cocaine for 5 days induced cross-sensitization to MDMA (Fig. 3). Together, these results suggest that a) the induction of sensitization following administration of MDMA or cocaine is independent of neurotoxicity, and b) the neural adaptation that leads to a sensitized response after MDMA administration is more rapid than the one caused by cocaine.

Previous studies have shown that rats treated repeatedly with a low (5 mg/kg) or a high (20 mg/kg) dose of MDMA were sensitized to MDMA challenge given 11 days after the last exposure to the drug. Thus, it has been suggested that MDMA-induced serotonergic neurotoxicity is not necessary for the expression of sensitization (Kalivas et al. 1998). However, since the long-term consequences of the low and the high dose of MDMA were not investigated, it is unclear if the similar behavioral outcome of the two regimens of MDMA would have persisted at a later time. Kalivas et al. (1998) also reported that a cocaine challenge caused cross-sensitization only in rats pretreated with the high dose of MDMA. This finding, however, may suggest that in rats the neurotoxicity was necessary for the expression of the cross-sensitization to cocaine. The results of the present study with mice, in which a different schedule was utilized, suggest that the cross-sensitization to cocaine challenge is not dependent on MDMA-induced neurotoxicity. Yet, if the cross-sensitization to cocaine is dependent on the time that elapsed between the last MDMA injection and the cocaine challenge, different schedules would be expected to show different results.

The relevance of MDMA-induced neurotoxicity for the long-lasting sensitization to MDMA is supported by our previous studies of the influence of METH-induced neurotoxicity on sensitization to METH. First, we have shown that METH-induced depletion of striatal dopaminergic markers was associated with a robust sensitization to challenge METH given 3 days after the neurotoxic dose (Itzhak et al. 1997, 1998). Second, we found that METH-induced depletion of dopaminergic markers in Swiss Webster mice was long-lasting (95 days) and was accompanied by a sensitized response to challenge METH (day 74) (Itzhak et al. 2002). METH did not affect serotonergic markers in the striatum and frontal cortex. Since MDMA induced depletion of both the DAT and the 5-HTT, it is unclear which deficit is associated with the long-lasting sensitized response to MDMA. In view of the results of METH studies, it is likely that the reduced number of striatal DAT sites contributed to the long-lasting sensitization. This premise is supported by the finding that the dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which caused a marked depletion of the DAT in both the striatum and the nucleus accumbens of mice, potentiated the expression of sensitization to cocaine (Itzhak et al. 1999). The mechanism underlying the sensitized response in relation to dopaminergic neurotoxicity is unclear. Regardless, the decrease in the number of DAT binding sites leads to a diminished reuptake of DA. Under these conditions, it is likely that the effect of psychostimulants that cause DA release will be exacerbated (e.g. sensitized response).

In summary, the data presented suggest that while a transient sensitization to the psychomotor stimulating effect of MDMA is not dependent on MDMA-induced neurotoxicity, the long-lasting sensitization is correlated with the enduring neurotoxic effect of the drug. These findings may be relevant to the psychopathology and the neural adaptation that develop with chronic MDMA abuse.

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