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A neurotoxic regimen of MDMA suppresses behavioral, thermal and neurochemical responses to subsequent MDMA administration

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Abstract *Rationale:* 3,4-Methylenedioxymethamphetamine (MDMA) produces a long-term depletion of serotonin (5-HT) in the rat brain; this depletion may have some functional consequences. *Objective:* The aim of the present study was to evaluate the acute effects of MDMA on the extracellular concentrations of dopamine and 5-HT, body temperature and the 5-HT behavioral syndrome in rats 7 days following a neurotoxic regimen of MDMA. *Methods:* One week after the rats were treated with a neurotoxic regimen of MDMA (10 mg/kg, IP, every 2 h for a total of four injections), the rats were injected with a subsequent injection of MDMA. In vivo microdialysis combined with HPLC was utilized to measure the extracellular concentration of 5-HT and dopamine in the striatum. The increase in body temperature was determined by rectal temperature measurements, and the 5-HT behavioral syndrome was scored using a rating scale following the administration of MDMA. *Results:* The neurotoxic regimen produced a 45% reduction in brain 5-HT concentrations. The magnitude of the MDMA-induced increase in the extracellular concentration of 5-HT, but not dopamine, in the striatum produced by an acute injection of MDMA (7.5 mg/kg, IP) was reduced in rats treated previously with the neurotoxic regimen of MDMA when compared with that in control animals. In addition, the magnitude of the 5-HT behavioral syndrome, as well as the hyperthermic response, produced by MDMA was markedly diminished in rats that had previously received the neurotoxic regimen of MDMA. *Conclusions:* It is concluded that the long-term depletion of brain 5-HT produced by MDMA is accompanied by impairments in 5-HT function, as evidenced by the deficits in the neurochemical, thermal and behavioral responses to subsequent MDMA administration.

Key words MDMA · Serotonin · Behavior · Hyperthermia · Neurotoxicity

Introduction

The amphetamine analog 3,4-methylenedioxymethamphetamine (MDMA) is a recreational drug of abuse which is considered to be potentially toxic to serotonergic nerve terminals of rodents and non-human primates (Green et al. 1995; Scheffel et al. 1998). The evidence of MDMA-induced 5-HT toxicity is based on biochemical studies in which there is a reduction in the tissue concentration of 5-HT and its major metabolite 5-hydroxyindole acetic acid (Stone et al. 1986; Schmidt 1987); reduction in the activity of the enzyme tryptophan hydroxylase (Schmidt and Taylor 1987; Stone et al. 1987) and reductions in [³H]-paroxetine-labelled 5-HT reuptake sites (Battaglia et al. 1987). Moreover, there is immunocytochemical evidence of 5-HT axon terminal damage (Commins et al. 1987; O'Hearn et al. 1988; Scallet et al. 1988). The long-term effects of MDMA have been shown to persist for at least 32 weeks in rats (Scanzello et al. 1993; Sabol et al. 1995) and may even be permanent in non-human primates (Ricaurte et al. 1992).

In addition to the long-term effects of MDMA on serotonergic neurons, MDMA also exerts effects that are thought to involve the acute release of 5-HT from axon terminals. Indeed, MDMA has been shown to increase the extracellular concentration of 5-HT, as well as dopamine, in several brain regions in vivo (Yamamoto and Spanos 1988; Nash 1990; Schmidt et al. 1992; Gudelsky et al. 1994; Gudelsky and Nash 1996) and enhance the release of 5-HT from brain slices or synaptosomes in vitro (Johnson et al. 1986; Crespi et al. 1997). The acute release of 5-HT elicited by MDMA is thought to underlie the behavioral and thermal responses produced by this amphetamine analog. In rats, the administration of MDMA results in a specific behavioral response known as the 5-HT behavioral syndrome which consists of forepaw treading, head-weaving and low body posture

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(Spanos and Yamamoto 1989). The finding that the behavioral syndrome elicited by *p*-chloroamphetamine and fenfluramine is attenuated by 5-HT antagonists and prior depletion of 5-HT (Trulson and Jacobs 1976; Sloviter et al. 1978a) supports the view that 5-HT, presumably released by these amphetamine analogs, mediates the induction of these behaviors. MDMA also produces a significant increase in body temperature that is thought to be mediated by the activation of 5-HT₂ receptors (Nash et al. 1988; Schmidt et al. 1990).

Although the long-term effect of MDMA on brain 5-HT content has been well documented, the potential functional consequences associated with MDMA-induced depletion of brain 5-HT have not yet been thoroughly evaluated. In the present study, the neurochemical, thermal and behavioral responses to an acute injection of MDMA were evaluated 1 week after the administration of a neurotoxic regimen of MDMA.

Material and methods

Animals

Male rats of the Sprague-Dawley strain (200–275 g, Charles River Labs, Portage, Mich., USA) were used in the studies. The animals were housed three per cage in the vivarium in a temperature and light controlled room (lights on 0600–1800 hours) and allowed food and water ad libitum.

Drug treatment

Rats were housed two per cage at 22–24°C and given either the vehicle or a neurotoxic regimen of MDMA which consisted of 4 IP injections of MDMA (10 mg/kg) at 2-h intervals (Scanzello et al. 1993). Neurochemical, behavioral and thermal responses to a subsequent injection of MDMA were assessed in separate groups of rats 1 week after the neurotoxic regimen of MDMA during the period of 0900–1600 hours.

The racemic mixture of MDMA hydrochloride was provided by the National Institute on Drug Abuse. MDMA was dissolved in 0.15 M NaCl and administered IP in a volume of 1 ml/kg. Doses are expressed in terms of the salt.

In vivo microdialysis procedures

One group of rats treated with the neurotoxic regimen of MDMA or the vehicle was implanted with a stainless steel guide cannula under ketamine/xylazine (70/7 mg/kg, IM) induced anesthesia 48–72 h prior to the insertion of the dialysis probe. On the day before the dialysis experiment, a concentric style dialysis probe was inserted through the guide cannula. The coordinates for the striatum were A, 1.2 mm; L, 3.1 mm; V, –7 mm from bregma according to the stereotaxic atlas of Paxinos and Watson (1986). The microdialysis probes were constructed as described previously (Yamamoto and Pehek 1990). The dialysis surface of the membrane (Spectra Por, 6000 MW cut-off, 210 µm outside diameter) for the striatum was 4.5 mm in length. The probe placement was verified at the end of the experiment by visual examination of brain sections. The in vitro recovery of the probes was 10–15% for dopamine, and no correction was made for recovery.

On the day of the dialysis experiment, the probe was connected to an infusion pump set to deliver Dulbecco's phosphate buffered saline containing 1.2 mM CaCl₂ and 10 mM glucose at a rate of 1.8 µl/min. After a 2-h equilibration period, dialysis samples were

collected every 30 min. At least three baseline samples were obtained prior to drug treatment.

Biochemical measurements

The extracellular concentrations of dopamine and 5-HT and the tissue concentrations of 5-HT were quantified with high performance liquid chromatography (HPLC) with electrochemical detection using methods similar to those described elsewhere (Gudelsky et al. 1994). Briefly, samples were injected onto a C18 column connected to a LC-4B amperometric detector (Bioanalytical Systems, West Lafayette, Ind., USA). The mobile phase consisted of 35 mM citric acid, 54 mM sodium acetate, 50 mg/l disodium ethylenediamine tetraacetate, 50 mg/l octane sulfonic acid sodium salt, 3% methanol, 3% acetonitrile, pH 4.2, and was pumped at a flow rate of 0.4 ml/min. Peak heights following injection of 50 µl samples were recorded with an integrator, and the quantity of dopamine and 5-HT were calculated on the basis of known standards. The retention times for dopamine and 5-HT were approximately 6 and 18 min, respectively.

For the analysis of 5-HT in tissue samples, rats were killed by decapitation and the striatum was isolated and stored at –80°C until analyzed for 5-HT content. The tissue samples were homogenized in 0.2 N perchloric acid and centrifuged at 10 000 g for 10 min. An aliquot of the supernatant was analyzed by HPLC as described above.

5-HT behavioral syndrome

In separate groups of rats treated with the neurotoxic regimen of MDMA or vehicle, the 5-HT behavioral syndrome was assessed according to the methods of Spanos and Yamamoto (1989). The following components of the 5-HT syndrome were evaluated: forepaw treading, headweaving and low body posture. On the day of the experiment, the rats were placed individually in clear Plexiglas cages and allowed to acclimate for 1 h. Separate groups of rats were injected IP with 7.5 or 15 mg/kg MDMA and the 5-HT behavioral syndrome was observed and rated for a 1-min period every 10 min for a duration of 90 min after MDMA administration. The scale used for rating was: 0=absent; 1=occasional (the behavior is present for <30 s during the 1-min observation period); 2=frequent (the behavior is present for >30 s during the 1-min observation period); and 3=constant (the behavior is present for the entire 1-min observation period). The scores for each behavior were summed for the nine observation periods.

Body temperature measurements

Separate groups of rats treated with a neurotoxic regimen of MDMA or the vehicle were transferred to a temperature-controlled room (26°C) and allowed to acclimate for 1 h prior to the measurement of body temperature. Rectal temperatures were recorded using a telethermometer and a thermistor probe. Measurements were taken every 20 min for a 1-h period prior to the administration of MDMA (7.5, 10 or 20 mg/kg, IP) and for a 2-h period following the injection of the drug. Separate groups of rats were used at each dose of MDMA.

Statistical analysis

Tissue concentrations of 5-HT and dopamine, and the data from temperature studies were evaluated using a one-way analysis of variance or Student's *t*-test, respectively (Sigmastat, Jandel Scientific). The behavioral syndrome elicited by a low dose (7.5 mg/kg) of MDMA was analyzed by a non-parametric procedure (Kruskal-Wallis test). The scores for each component of the behavioral syndrome in response to a high dose (15 mg/kg) of MDMA were analyzed with a one-way analysis of variance. Differences between

treatment groups were determined with the use of the Student-Newman-Keuls test. Data from dialysis experiments were analyzed using a two-way repeated measures analysis of variance followed by a post-hoc analysis with Student-Newman-Keuls test for multiple pairwise comparisons. Treatment differences for all the data were considered statistically significant at $P < 0.05$.

Results

The administration of MDMA (10 mg/kg, IP, every 2 h for a total of four injections) produced a significant decrease in the tissue concentration of 5-HT in the striatum 1 week after drug administration [$F(1,13)=35$, $P < 0.0001$] (Table 1). However, this dosage regimen of MDMA did not alter the tissue concentration of dopamine in the striatum.

The acute injection of MDMA produced a significant increase in the extracellular concentration of dopamine and 5-HT in the striatum. The increase in the extracellular concentration of 5-HT produced by MDMA

(7.5 mg/kg, IP) in rats that had received the neurotoxic regimen of MDMA (10 mg/kg, IP, every 2 h for a total of four injections) 1 week earlier was significantly less than that in rats that had received the vehicle [main drug effect, $F(1,16)=4.83$, $P < 0.05$] (Fig. 1A). However, the increase in the extracellular concentration of dopamine evoked by an acute injection of MDMA was not significantly different in rats treated with the neurotoxic regimen of MDMA or the vehicle [main drug effect, $F(1,16)=0.01$, $P=0.92$] (Fig. 1B). The neurotoxic regimen of MDMA did not alter the basal extracellular concentration of dopamine or 5-HT in the striatum.

The 5-HT behavioral syndrome produced by an acute injection of MDMA (7.5 mg/kg, IP) predominantly consisted of low body posture, and this response was significantly attenuated in animals that had received the neurotoxic regimen of MDMA 1 week earlier [$H(1)=8.22$, $P < 0.005$] (Fig. 2). The acute IP administration of MDMA at a dose of 15 mg/kg produced all the behaviors of the syndrome, and the score for each component of the syndrome in rats treated earlier with the neurotoxic dose regimen of MDMA was significantly less than the scores for

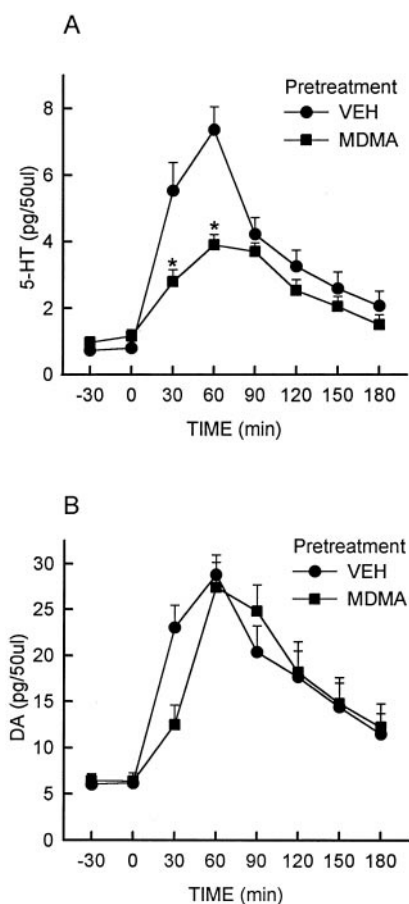


Fig. 1A, B Effect of a neurotoxic regimen of MDMA on the MDMA-induced increase in the extracellular concentration of 5-HT (A) and dopamine (B) in the striatum. Seven days after treatment of rats with vehicle (VEH) or the neurotoxic regimen of MDMA, all animals were injected at time 0 with MDMA (7.5 mg/kg, IP). The values represent the mean \pm SE of six to eight rats. *Indicates values that differ significantly (drug \times time interaction $P < 0.05$) from vehicle-pretreated animals. ● VEH, ■ MDMA pretreatment

Table 1 Dopamine and 5-HT concentrations in the striatum 7 days following a neurotoxic regimen of MDMA. The rats were injected with vehicle (VEH) or MDMA (10 mg/kg, IP; every 2 h for a total of four injections). All animals were killed 7 days after drug administration. The values represent the mean \pm SE of six to eight rats

	DA (ng/mg tissue)	5-HT
VEH	10.0 \pm 1.5	0.33 \pm 0.02
MDMA	8.6 \pm 1.5	0.18 \pm 0.02*

*Indicates values that differ significantly ($P < 0.0001$) from VEH-treated animals

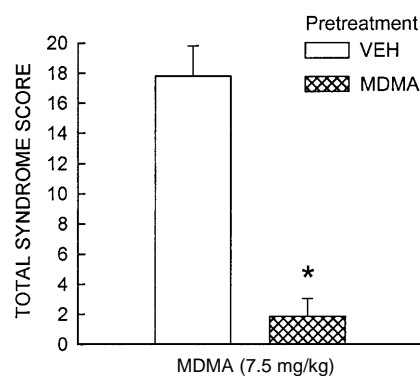


Fig. 2 Effect of a neurotoxic regimen of MDMA on the 5-HT behavioral syndrome elicited by subsequent MDMA treatment. Seven days after treatment of rats with vehicle (VEH, clear bars) or the neurotoxic regimen of MDMA (hatched bars), the animals received an injection of MDMA (7.5 mg/kg, IP). Each component of the syndrome was rated for a 1-min period at 10-min intervals for a total duration of 90 min. The scores for all three components were added and a total score was obtained by summation of the values for the nine observation periods. The values represent the mean \pm SE of the total score for six to eight rats. *Indicates values that differ significantly ($P < 0.005$) from vehicle-pretreated animals

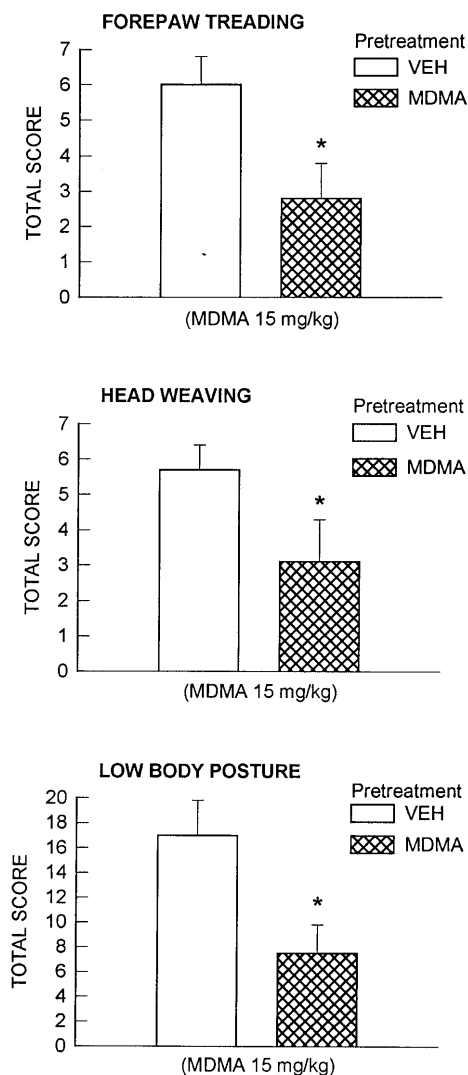


Fig. 3 Effect of a neurotoxic regimen of MDMA on the 5-HT behavioral syndrome elicited by subsequent MDMA treatment. Seven days after treatment of rats with vehicle (VEH, clear bars) or the neurotoxic regimen of MDMA (hatched bars), the animals received an injection of MDMA (15 mg/kg, IP) and each component of the syndrome was rated for a 1-min period at 10 min intervals for a duration of 90 min. The total score was obtained by summation of the values for the nine observation periods. The values represent the mean±SE of the total score for six to eight rats. *Indicates values that differ significantly ($P<0.05$) from vehicle-pretreated animals

the control rats [forepaw treading, $F(1,11)=5.77$, $P<0.05$; head weaving, $F(1,11)=7.23$, $P<0.05$; low body posture, $F(1,11)=6.84$, $P<0.05$] (Fig. 3).

The IP administration of MDMA (7.5–20 mg/kg) produced a dose-dependent hyperthermic response of approximately 1.2–2.2°C. The administration of 7.5 mg/kg MDMA produced a hyperthermic response that was significantly less [$t(12)=3.24$, $P<0.001$] in rats treated with a neurotoxic regimen of MDMA, when compared to that in the control rats (Fig. 4); the hyperthermic response to 10 mg/kg MDMA was also significantly diminished [$t(27)=2.87$, $P<0.001$] in rats treated with the neurotoxic

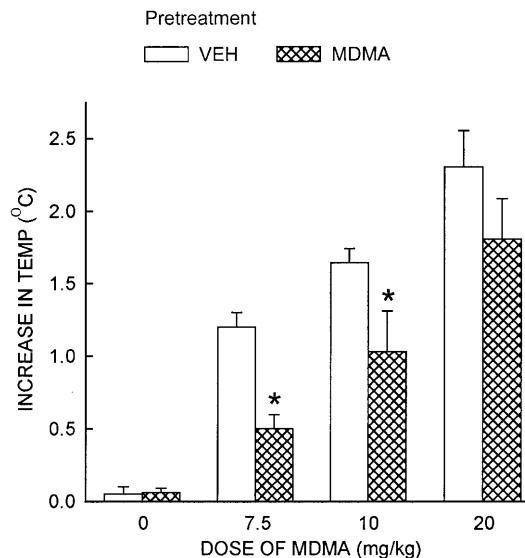


Fig. 4 Effect of a neurotoxic regimen of MDMA on subsequent MDMA-induced hyperthermia. Seven days after treatment of rats with vehicle (VEH, clear bars) or the neurotoxic regimen of MDMA (hatched bars), body temperatures were recorded prior to and following the acute injection of MDMA (7.5, 10 or 20 mg/kg, IP). The values (mean±SE for six to eight rats) represent the increase in body temperature, as calculated from the peak body temperature following MDMA treatment minus the body temperature at time 0. *Indicates values that differ significantly ($P<0.001$) from vehicle-pretreated animals

regimen of MDMA. However, the increase in body temperature produced by larger dose of MDMA (20 mg/kg, IP) was not significantly different in the two groups of animals [$t(11)=1.32$, $P=0.2$].

Discussion

The administration of a single dose of MDMA produced an increase in the extracellular concentration of dopamine and 5-HT in the striatum, consistent with previous studies (Yamamoto and Spanos 1988; Nash 1990; Schmidt et al. 1992; Gudelsky et al. 1994; Gudelsky and Nash 1996). However, the MDMA-induced increase in the extracellular concentration of 5-HT in the striatum was suppressed in rats that had received a neurotoxic regimen of MDMA. The suppression of MDMA-induced 5-HT release in rats in which brain 5-HT has been depleted is consistent with the findings of Series et al. (1994), who reported that treatment of rats with a neurotoxic regimen of amphetamine analogs, such as *p*-chloroamphetamine, MDMA and *d*-fenfluramine, attenuated the release of 5-HT in the cortex in response to a subsequent injection of *d*-fenfluramine. In addition Gartside et al. (1996) have reported that treatment of rats with high doses of MDMA results in a marked reduction in 5-HT release in the cortex following electrical stimulation of the dorsal raphe nucleus. These results are consistent with the view that the suppression of the MDMA-induced increase in the extracellular concentration of 5-HT

may be due to the loss of 5-HT nerve terminals following the repeated dose regimen of MDMA. The resulting defect in 5-HT neurotransmission may only be evident under conditions of stimulated 5-HT release, inasmuch as the basal extracellular concentration of 5-HT in the striatum, as well as in other brain regions (Gartside et al. 1996), is not altered by neurotoxic regimens of MDMA. In accordance with the view that MDMA produces selective neurotoxicity to 5-HT axon terminals, the neurotoxic regimen employed in the present study did not alter the tissue concentration of dopamine in the striatum or the basal or MDMA-induced increase in the extracellular concentration of dopamine in the striatum.

The acute administration of MDMA produced a characteristic 5-HT behavioral syndrome response, similar to that observed by others (Slikker et al. 1989; Spanos and Yamamoto 1989). However, the neurotoxic regimen of MDMA resulted in a marked attenuation of the 5-HT syndrome elicited by a subsequent injection of MDMA. Series et al. (1995) and Baumann et al. (1998) have reported an attenuation of behavioral responses to an acute injection of *d*-fenfluramine, following treatment with neurotoxic amphetamines such as *p*-chloroamphetamine, MDMA and fenfluramine. Moreover, Frederick et al. (1995, 1998) have reported decreased behavioral responses to MDMA in monkeys following a short-course, high-dose exposure to MDMA. Slikker et al. (1989) have also reported a decrease in the behavioral syndrome during the repeated, daily, oral administration of MDMA, although this attenuation may be attributed to the rapid phase of MDMA-induced 5-HT depletion that occurs within 24 h of drug treatment (Schmidt 1987). The mechanism involved in the reduced behavior during daily administration of MDMA (Slikker et al. 1989) may be different from that observed with the dosage regimen of the present study, which may be a consequence of the long-term degeneration of 5-HT axon terminals. Inasmuch as the behavioral response (e.g., head weaving, fore-paw treading, flat body posture) evoked by MDMA and other amphetamine analogs is considered to be mediated by 5-HT receptors following the enhanced release of 5-HT from pre-synaptic neurons (Jacobs 1976; Sloviter et al. 1978b), it is proposed that the reduction in the MDMA-induced 5-HT behavioral syndrome in rats in which brain 5-HT had been depleted is the result of a reduction in the MDMA-induced release of 5-HT, and reflects the functional consequences of the long-term depletion of brain 5-HT.

An increase in body temperature was also produced by an acute injection of MDMA, as has been reported in earlier studies (Nash et al. 1988; Dafters et al. 1995; Malberg et al. 1996). In the present study, the hyperthermic responses produced by low doses of MDMA (7.5 and 10 mg/kg, IP) in rats that had previously received the neurotoxic regimen were significantly less than those in control rats; a larger dose of MDMA (20 mg/kg) produced similar hyperthermic responses in both groups of animals. The attenuated hyperthermic response to low doses of MDMA may be the consequence

of a suppression in stimulated 5-HT release in rats treated with the neurotoxic regimen of MDMA. It is conceivable that the administration of a large dose of MDMA evokes 5-HT release that activates sufficient 5-HT₂ receptors to elicit a maximal hyperthermic response. Thus, MDMA pretreatment appears to produce a shift to the right in the dose-response curve of hyperthermia induced by MDMA. Recently, Dafters and Lynch (1998) have also found evidence of long term changes in thermoregulation following the repeated administration of MDMA.

An alternative explanation for the diminished functional responses to MDMA is the possibility of altered 5-HT receptor sensitivity. There is evidence to suggest that the repeated administration of MDMA results in up-regulation of 5-HT_{1A} receptor density and m-RNA expression in certain brain regions, such as the frontal cortex and hypothalamus (Aguirre et al. 1997, 1998). In addition, Obradovic et al. (1998) have reported increased 5-HT_{2C} receptor density in the nucleus accumbens following repeated injections of MDMA. Although it seems unlikely that an up-regulation of 5-HT receptors could contribute to the diminished responses to MDMA, it is necessary to consider the role of multiple 5-HT receptor subtypes in some functions, e.g., thermoregulation. Whereas the activation of 5-HT₂ receptors may result in an hyperthermic response (Nash et al. 1988; Schmidt et al. 1990), activation of 5-HT_{1A} receptors may produce hypothermic responses (Gudelsky et al. 1986). Moreover, functional interactions between 5-HT₁ and 5-HT₂ receptors have been shown to play a role in thermoregulatory mechanisms in the rat (Salmi and Ahlenius 1998). In view of the above findings, the diminished hyperthermic response to MDMA may be reflective of an up-regulation of 5-HT_{1A} sites that negates functionally the 5-HT₂ receptor mediated hyperthermic response.

The diminished functional responses to an acute injection of MDMA following the repeated administration of the drug also may be due to pharmacokinetic alterations, viz., alterations in the metabolism or distribution of MDMA. Gygi et al. (1996) have reported that the brain concentration of methamphetamine following an acute injection was reduced in rats that had received 12 doses of methamphetamine during a 3-day period. Although it is not possible to preclude pharmacokinetic differences in the present study, it is noteworthy that the multiple dose regimen of MDMA employed in the present study resulted only in a suppression of serotonergic responses; the increase in the extracellular concentration of dopamine elicited by MDMA was unaffected by prior treatment. In addition, the dosage regimen in the present study involved only 1 day of treatment in contrast to the 3 days of treatment employed by Gygi et al. (1996).

In summary, the extent to which an acute injection of MDMA increased the extracellular concentration of 5-HT, increased body temperature and evoked the 5-HT behavioral syndrome was diminished in rats that had received a neurotoxic regimen of MDMA. These findings suggest that MDMA-induced depletion of brain 5-HT

content is accompanied by functional deficits in 5-HT neurotransmission.

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References

- Aguirre N, Frenchilla D, Garcia-Osta A, Lasheras B, Del Rio J (1997) Differential regulation by methylenedioxyamphetamine of 5-hydroxytryptamine_{1A} receptor density and m-RNA expression in rat hippocampus, frontal cortex and brain stem: the role of corticosteroids. *J Neurochem* 68:1099–1105
- Aguirre N, Ballaz S, Lasheras B, Del Rio J (1998) MDMA (“Ecstasy”) enhances 5-HT_{1A} receptor density and 8-OH-DPAT-induced hypothermia: blockade by drugs preventing 5-hydroxytryptamine depletion. *Eur J Pharmacol* 346:181–188
- Battaglia G, Yeh SY, O’Hearn E, Molliver ME, Kuhar MJ, DeSouza EB (1987) 3,4-Methylenedioxy methamphetamine and 3,4-methylenedioxy amphetamine destroy serotonin terminals in rat brain: quantification of neurodegeneration by measurement of [³H] paroxetine-labelled serotonin uptake sites. *J Pharmacol Exp Ther* 242:911–16
- Baumann MH, Ayestas MA, Rothman RB (1998) Functional consequences of central serotonin depletion produced by repeated fenfluramine administration in rats. *J Neurosci* 18:9069–9077
- Commings DL, Bosmer G, Virus RM, Woolverton WL, Schuster CR, Seiden LS (1987) Biochemical and histological evidence that methylenedioxyamphetamine (MDMA) is toxic to neurons in the rat brain. *J Pharmacol Exp Ther* 240:338–345
- Crespi D, Mennini T, Gobbi M (1997) Carrier-dependent and Ca²⁺-dependent 5-HT and dopamine release induced by (+)-amphetamine, 3,4-methylenedioxy-methamphetamine, *p*-chloroamphetamine and (+)-fenfluramine. *Br J Pharmacol* 121:1735–1743
- Dafters RI (1995) Hyperthermia following MDMA administration in rats: effects of ambient temperature, water consumption, and chronic dosing. *Physiol Behav* 58:877–882
- Dafters RI, Lynch E (1998) Persistent loss of thermoregulation in the rat induced by 3,4-methylenedioxyamphetamine (MDMA or “Ecstasy”) but not by fenfluramine. *Psychopharmacology* 138:207–212
- Frederick DL, Ali SF, Slikker W Jr, Gillam MP, Allen RR, Paule MG (1995) Behavioral and neurochemical effects of chronic methylenedioxyamphetamine (MDMA) treatment in rhesus monkeys. *Neurotoxicol Teratol* 17:531–543
- Frederick DL, Ali SF, Gillam MP, Gossett J, Slikker W Jr, Paule MG (1998) Acute effects of dexfenfluramine (d-FEN) and methylenedioxyamphetamine (MDMA) before and after short-course, high-dose treatment. *Ann NY Acad Sci* 844:183–190
- Gartside SE, McQuade R, Sharp T (1996) Effects of repeated administration of 3,4-methylenedioxy methamphetamine on 5-hydroxytryptamine neuronal activity and release in the rat brain in vivo. *J Pharmacol Exp Ther* 279:277–283
- Green AR, Cross AJ, Goodwin GM (1995) Review of the pharmacology and clinical pharmacology of 3,4-methylenedioxy-methamphetamine (MDMA or Ecstasy). *Psychopharmacology* 119:247–260
- Gudelsky GA, Nash JF (1996) Carrier-mediated release of serotonin by 3,4-methylenedioxy methamphetamine: implications for serotonin-dopamine interactions. *J Neurochem* 66:243–249
- Gudelsky GA, Koenig JJ, Meltzer HY (1986) Thermoregulatory responses to serotonin receptor stimulation in the rat: evidence for opposing roles of 5-HT_{2A} and 5-HT_{1A} receptors. *Neuropharmacology* 25:1307–1313
- Gudelsky GA, Yamamoto BK, Nash JF (1994) Potentiation of 3,4-methylenedioxy-methamphetamine-induced dopamine release and serotonin neurotoxicity by 5-HT₂ agonists. *Eur J Pharmacol* 264:325–330
- Gygi MP, Gygi SP, Johnson M, Wilkins DG, Gibb, JW, Hanson GR (1996) Mechanisms for tolerance to methamphetamine effects. *Neuropharmacology* 35:751–757
- Jacobs BL (1976) An animal behavior model for studying central serotonergic synapses. *Life Sci* 19:777–786
- Johnson MP, Hoffman AJ, Nichols DE (1986) Effects of enantiomers of MDA, MDMA and related analogues on [³H] serotonin and [³H] dopamine release from superfused rat brain slices. *Eur J Pharmacol* 132:269–276
- Malberg JE, Sabol KE, Seiden LS (1996) Co-administration of MDMA with drugs that protect against MDMA neurotoxicity produces different effects on body temperature in the rat. *J Pharmacol Exp Ther* 278:258–267
- Nash JF (1990) Ketanserin pretreatment blocks MDMA-induced dopamine release in the striatum as measured by in vivo microdialysis. *Life Sci* 47:2401–2408
- Nash JF, Meltzer HY, Gudelsky GA (1988) Elevation of serum prolactin and corticosterone concentrations after administration of 3,4-methylenedioxy-methamphetamine. *J Pharmacol Exp Ther* 245:873–879
- Obrovic T, Churchill L, White SR (1998) 5-HT_{2C} receptor density is increased in the nucleus accumbens following repeated injections of methylenedioxyamphetamine (MDMA). *Abstr Soc Neurosci* 24:1369
- O’Hearn E, Battaglia G, De Souza EB, Kuhar MJ, Molliver ME (1988) Methylenedioxyamphetamine (MDA) and methylenedioxyamphetamine (MDMA) ablation cause selective ablation of serotonergic axon terminals in forebrain: immunocytochemical evidence for neurotoxicity. *J Neurosci* 8:2788–2803
- Paxinos G, Watson C (1986) The rat brain in stereotaxic coordinates. Academic Press, Orlando
- Ricaurte GA, Martello AL, Katz JL, Martello MB (1992) Lasting effects of (±) 3,4-methylenedioxyamphetamine (MDMA) on central serotonergic neurons in non-human primates: neurochemical observations. *J Pharmacol Exp Ther* 261:616–622
- Sabol KE, Lew R, Richards JB, Vosmer GL, Seiden LS (1996) Methylenedioxy methamphetamine-induced serotonin deficits are followed by partial recovery over a 52-week period. Part 1: synaptosomal uptake and tissue concentrations. *J Pharmacol Exp Ther* 276:846–854
- Salmi P, Ahlenius S (1998) Evidence for functional interactions between 5-HT_{1A} and 5-HT_{2A} receptors in rat thermoregulatory mechanisms. *Pharmacol Toxicol* 82:122–127
- Scallet AC, Lipe GW, Ali SF, Holson RR, Frith CH, Slikker W Jr (1988) Neuropathological evaluation by combined immunohistochemistry and degeneration-specific methods: application to methylenedioxyamphetamine. *Neurotoxicology* 9:529–538
- Scanzello CR, Hatzidimitriou G, Martello AL, Katz JL, Ricaurte GA (1993) Serotonergic recovery after (±) 3,4-(methylenedioxy)methamphetamine injury: observation on rats. *J Pharmacol Exp Ther* 264:1484–1491
- Scheffel U, Szabo Z, Mathews WB, Finley PA, Dannals RF, Ravert HT, Szabo K, Yuan J, Ricaurte GA (1998) In vivo detection of short- and long-term MDMA neurotoxicity – a positron emission tomography study in the living baboon brain. *Synapse* 29:183–192
- Schmidt CJ (1987) Neurotoxicity of the psychedelic amphetamine, methylenedioxy methamphetamine. *J Pharmacol Exp Ther* 240:1–7
- Schmidt CJ, Taylor VL (1987) Depression of rat brain tryptophan hydroxylase activity following the acute administration of methylenedioxy methamphetamine. *Biochem Pharmacol* 36:4095–5102
- Schmidt CJ, Black CK, Abbate GM, Taylor VL (1990) Methylenedioxyamphetamine-induced hyperthermia and neurotoxicity are independently mediated by 5-HT₂ receptors. *Brain Res* 529:85–90
- Schmidt CJ, Fadayel GM, Sullivan CK, Taylor VL (1992) 5-HT₂ receptors exert a state-dependent regulation of dopaminergic function: studies with MDL 100,907 and the amphetamine an-

- alogue, 3,4-methylenedioxymethamphetamine. *Eur J Pharmacol* 223:65–74
- Series HG, Cowen PJ, Sharp T (1994) *p*-Chloroamphetamine (PCA), 3,4-methylenedioxy methamphetamine (MDMA) and *d*-fenfluramine pretreatment attenuates *d*-fenfluramine-evoked release of 5-HT in vivo. *Psychopharmacology* 116:508–514
- Series HG, Masurier M, Gartside SE, Franklin M, Sharp T (1995) Behavioral and neuroendocrine responses to *d*-fenfluramine in rats treated with neurotoxic amphetamines. *J Psychopharmacol* 9:214–222
- Slikker W Jr, Holson RR, Ali SF, Kolta MG, Paule MG, Scallet AC, McMillan DE, Bailey JR, Hong JS, Scalzo FM (1989) Behavioral and neurochemical effects of orally administered MDMA in the rodent and nonhuman primate. *Neurotoxicology* 10:529–542
- Sloviter RS, Drust EG, Connor JD (1978a) Specificity of a rat behavioral model for serotonin receptor activation. *J Pharmacol Exp Ther* 206:339–347
- Sloviter RS, Drust EG, Connor JD (1978b) Evidence that serotonin mediates some behavioral effects of amphetamine. *J Pharmacol Exp Ther* 206:348–352
- Spanos LJ, Yamamoto BK (1989) Acute and subchronic effects of methylenedioxy methamphetamine [(±)MDMA] on locomotion and serotonin syndrome behavior in the rat. *Pharmacol Biochem Behav* 32:835–840
- Stone DM, Stahl D, Hanson GR, Gibb JW (1986) The effects of 3,4-methylenedioxy methamphetamine (MDMA) on monoaminergic systems in rat brain. *Eur J Pharmacol* 128: 41–49
- Stone DM, Merchant KM, Hanson GR, Gibb JW (1987) Immediate and long-term effects of 3,4-methylenedioxymethamphetamine on serotonin pathways in brain of rat. *Neuropharmacology* 26:1677–1683
- Trulson ME, Jacobs BL (1976) Behavioral evidence for the rapid release of CNS serotonin by PCA and fenfluramine. *Eur J Pharmacol* 36:149–154
- Yamamoto B, Pehek E (1990) A neurochemical heterogeneity of the rat striatum as measured by in vivo electrochemistry and microdialysis. *Brain Res* 506:236–242
- Yamamoto BK, Spanos LJ (1988) The acute effects of methylenedioxymethamphetamine on dopamine release in the awake-behaving rat. *Eur J Pharmacol* 148:195–203