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Research report

Repeated exposure to methylenedioxymethamphetamine (MDMA) alters nucleus accumbens neuronal responses to dopamine and serotonin

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Abstract

The purpose of this experiment was to investigate the effects of repeated exposure to methylenedioxymethamphetamine (MDMA) on responses of neurons in the nucleus accumbens of anesthetized rats to microiontophoretically-applied dopamine and serotonin. In tests conducted 1–4 days or 9–15 days following the last injection of MDMA (20 mg/kg, s.c., twice daily for 4 days), the inhibitory effects of both dopamine and serotonin on glutamate-evoked firing of nucleus accumbens cells were significantly attenuated compared to effects in control rats that were pretreated with saline injections. The inhibitory effect of the D_1 receptor agonist SKF38393 was also significantly attenuated in the MDMA-pretreated rats. In contrast, the amount of inhibition of glutamate-evoked firing produced by application of GABA did not significantly differ between the MDMA-pretreated and the saline-pretreated rats. The neurotoxicity of the MDMA treatment regimen was confirmed by demonstrating that ^{3}H-paroxetine binding was significantly decreased in the medial prefrontal cortex and the nucleus accumbens of the MDMA-pretreated rats. The mechanisms that produce the attenuated inhibitory responses to dopamine and serotonin following repeated injections of MDMA are not known. However, the results of these experiments indicate that repeated MDMA administration induces long-lasting changes in dopaminergic as well as serotonergic neurotransmission in the nucleus accumbens.

Keywords: MDMA; Nucleus accumbens; Dopamine; Serotonin; GABA

1. Introduction

Methylenedioxymethamphetamine (MDMA, 'ecstasy') is an amphetamine derivative that has become a common recreational drug of abuse [7,14,43]. Acutely applied MDMA increases extracellular levels of dopamine (DA) and serotonin (5HT) in the nucleus accumbens [47,53], a forebrain region that has been implicated in the rewarding properties of many abused drugs including cocaine and amphetamine [11,16,24]. In addition to increasing extracellular levels of both DA and 5HT, locally-applied MDMA has an inhibitory effect on the excitability of most neurons within the nucleus accumbens [47]. This MDMA-induced inhibition appears to be mediated by both DA and 5HT since it is mimicked by these monoamines and can be attenuated by depleting DA and 5HT or by applying receptor antagonists [32,47].

The long-term functional consequences of MDMA use are poorly understood, but repeated exposure to MDMA is likely to produce long-term changes in neurotransmission within the nucleus accumbens. MDMA has been demonstrated to be neurotoxic to a subpopulation of fine-diameter 5HT-containing axons in the forebrain of both laboratory rats and monkeys when given repeatedly at moderate doses [25,33,37,49]. Tissue levels of 5HT, densities of 5HT-reuptake sites and densities of fine-diameter, lightly varicose, 5HT-immunoreactive fibers in the forebrain including the nucleus accumbens and dorsal striatum are depleted from several hours to many weeks following multiple injections of MDMA [4,38]. This neurotoxic effect is of concern because the dose of MDMA that has been demonstrated to be neurotoxic in nonhuman primates is near the dose that is typically taken by recreational MDMA users [37]. Reports of psychopathology including depression, anxiety and psychosis that can occur in frequent MDMA users weeks to a month or more after the last ingestion of MDMA [6,28,29] suggest that MDMA is

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capable of producing long-term changes in the human nervous system that may be similar to those produced in laboratory animals.

Whether the excitability of neurons in the nucleus accumbens is altered by MDMA-induced damage to 5HT-containing terminals in the nucleus is not known, but damage to 5HT terminals by the neurotoxin 5,7-dihydroxytryptamine (5,7DHT) alters excitability of postsynaptic neurons in several other brain areas. Development of supersensitivity to the effects of 5HT has been reported in the frontal cortex, amygdala, lateral geniculate nucleus and facial motor nucleus [2,12,27,44] following selective 5HT depletion with the neurotoxin 5,7DHT. Dopamine neurotransmission has also been reported to be altered in the cerebral cortex following selective 5HT depletion with 5,7DHT [2,12]. In view of the importance of the dopaminergic mesoaccumbens pathway in reward [50] and drug abuse [8,24], it is important to determine how repeated exposure to MDMA alters sensitivity of nucleus accumbens cells to DA and to 5HT. The present study used single cell recording combined with microiontophoretic applications of drugs to compare the effects of DA and 5HT on glutamate-evoked firing of nucleus accumbens cells in MDMA-pretreated and control rats.

2. Materials and methods

2.1. Animals and drugs

Male Sprague–Dawley rats (220–260 g on the day of first injection) were obtained from Simonsen Laboratories, Gilroy, CA. The animals were housed separately in a temperature- and humidity-controlled environment with food and water available ad lib. Prior to the neuronal recording or binding experiments, rats were pretreated with either MDMA (MDMA-PreT. group) or saline (Saline-PreT. group) or received no pretreatment (Naive group). The MDMA-PreT. group received morning and evening subcutaneous injections of 20 mg/kg MDMA for 4 successive days (8 injections). The Saline-PreT. group received equivalent injections of saline. All drugs were purchased from Sigma Chemical except for the D_1 dopamine agonist SKF38393 (purchased from Research Biochemicals), (±)3,4-MDMA (gift from NIDA), ^{3}H-paroxetine (purchased from New England Nuclear) and fluoxetine (gift from Eli Lilly Pharm.).

2.2. Neuronal recording

Recording sessions were conducted at an early time period (1–4 days) or late time period (9–15 days) following the last MDMA or saline injection. On the recording day, rats were anesthetized with urethane (1.25 g/kg i.p. with i.v. supplements as needed). The trachea was intubated to assist respiration and the left femoral vein was cannulated for intravenous drug administration. The skull overlying the nucleus accumbens was removed, leaving intact a narrow midline strip of bone overlying the sinus. The head was fixed in a Kopf stereotaxic headholder, the meninges were cut and the electrode was inserted into the brain 10.0–11.0 mm anterior to the interaural line and 1.1–2.0 mm lateral to the midline and advanced 5.7 to 7.2 mm below the surface of the cortex according to the atlas of Paxinos and Watson [35]. All recordings were made from the core region of the nucleus accumbens in the right hemisphere.

Seven barrel glass microdot microelectrodes with tip diameters of 7–10 μm were used for single cell extracellular recording and iontophoretic application of drugs. The center barrel and one side-barrel were filled with 4 M NaCl for recording and automatic current balance. Other barrels contained 0.2 M monosodium-L-glutamate, pH 6.5, to drive the quiescent cells; 2% pontamine sky blue in 0.5 M sodium acetate to mark the position of the electrode tip; and some combination of dopamine HCl (DA), 0.8 M, pH 5; serotonin creatinine sulfate (5HT), 0.04 M, pH 4.5; gamma aminobutyric acid (GABA), 0.1 M, pH 4.5; the selective dopamine D_1 agonist SKF38393 HCl, 0.01 M, pH 5; and acidic saline, 0.01 M, brought to pH 4.5 with hydrochloric acid for pH control. Drugs were made up in double-distilled water and brought to pH 4–5 with NaOH or HCl. Solutions were frozen in aliquots suitable for one experiment and used within 6 h of thawing. A five-channel Medical Systems microiontophoresis system was used to eject and retain the drugs in the microelectrode barrels. Retaining currents were +10 nA for glutamate and −10 nA for all other solutions except the dye which received no retaining current. Glutamate and the dye were ejected as anions, all other drugs were ejected as cations.

Cells in the core region of the nucleus accumbens were driven at stable, relatively low firing rates by cycled 10 s pulses of glutamate delivered every 20 s throughout the recording session. Neuronal activity was monitored with a Tektronix oscilloscope and audio monitor and stored on videocassette tape. Single units were isolated with a Neurolog window discriminator, and spikes from single units were counted with a pulse integrator. Cumulative counts of spikes from individual cells were displayed with a Grass (curvilinear) oscillograph. Glutamate ejection currents which produced a stable baseline response were established empirically for each cell. Following establishment of a glutamate response that remained stable for at least 3 min, effects of 5HT, DA, GABA or SKF38393 were tested. No more than two test drugs were applied to a single cell. Test drugs were applied for 60 s at increasing doses (current intensity) with at least 3 min separating each application.

At the end of a recording session for a cell, the position of the electrode tip was marked by application of pontamine sky blue dye (30 μA negative current for 12 min). Brains were removed at the end of the experiment, fixed in

formalin, sectioned at 50 μm and counterstained with cresyl violet. Locations of dye spots for recording sites were determined histologically.

2.3. Statistics

Dose response curves were generated for each test drug by comparing the average number of spikes that occurred for the cells during the 60 s application of each dose of drug to the average number of spikes that occurred in the 1 min baseline period which immediately preceded drug ejection. The dose-response effects of DA, GABA and 5HT on glutamate-evoked firing were then compared for the MDMA-PreT. group and the Saline-PreT. group using two-way, repeated measures analysis of variance, with Tukey post-hoc comparisons of means as appropriate. Time-response curves were generated for the effects of the 60 nA dose of DA and SKF38393 by comparing the number of spikes that occurred during each 20 s glutamate cycle during and for 2 min after the drug application to the average number of spikes that occurred during the 1 min baseline period which immediately preceded the drug ejection. Time-response curves were compared for MDMA-PreT. and Saline-PreT. groups using two-way, repeated measures analysis of variance with Tukey post-hoc comparisons of means.

2.4. Autoradiography

MDMA-PreT. ($n = 5$) and Saline-PreT. ($n = 4$) rats were killed by decapitation 11–13 days following the last injection. Brains were rapidly removed and frozen in hexane (-20°C). The brains were coronally sectioned through the nucleus accumbens (20 μm) at 16°C using a cryostat. The sections were thaw-mounted onto chrome alum/gelatin-subbed microscope slides and stored at -20°C. Autoradiographic mapping of 5HT uptake sites was performed using a modification of the protocol described by DeSouza and Kuyatt [10]. The slide-mounted sections were brought to room temperature and incubated with ^{3}H-paroxetine, 0.1 nM in 50 mM Tris–HCl containing 120 mM NaCl and 5 mM KCl (pH 7.7) for 120 min at room temperature. Nonspecific binding was evaluated on a parallel set of adjacent sections by incubating them in the same concentration of tritiated ligand along with a 10 μM final concentration of unlabeled fluoxetine. After incubation, tissue sections were washed in the same buffer for two 60 min periods at room temperature, dipped in deionized water and dried rapidly under a stream of cold, dry air. The slide-mounted sections and tritium autoradiographic standards (Amersham) were apposed to tritium-sensitive Hyperfilm (Amersham) for 6 weeks. The film was developed (D-19 developer, Kodak) and fixed (Rapidfix, Kodak) and the autoradiograms were analyzed using a computer-assisted image analyzer, MCID (Imaging Research, St. Catharines, Ontario, Canada). Four readings of optical density were performed in the nucleus accumbens core, in the nucleus accumbens shell and in the medial prefrontal cortex on at least five sets of adjacent sections that had been processed for total binding and nonspecific binding from each rat. Specific binding was obtained by subtracting nonspecific from total binding. Mean optical densities were converted to emitted radioactivity by interpolation on the curve obtained using radiolabeled standards. Binding in each brain region was compared for MDMA-PreT. and Saline PreT. rats using two-tailed *t*-tests.

3. Results

Electrophysiological recordings were made either 1–4 days or 9–15 days after the last MDMA or saline injection. Very few spontaneously active neurons were encountered in the core region of the nucleus accumbens of the urethane-anesthetized rats as has been observed by others [46,52], so all data are reported for cells that were driven by cycled pulses of glutamate. Glutamate-evoked firing of cells in the nucleus accumbens core of the Saline-PreT. and the Naive rats was inhibited by microiontophoretic applications of DA. This inhibitory effect of DA was markedly attenuated in cells from MDMA-PreT. rats. Records illustrating DA effects on glutamate-evoked firing

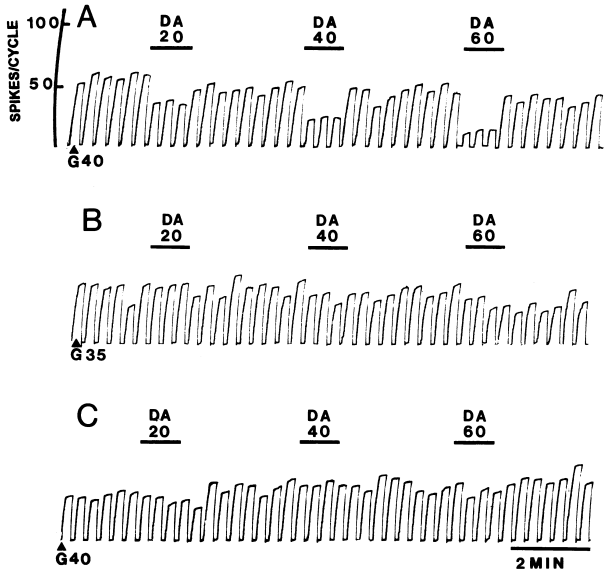

Fig. 1. Effects of DA application on glutamate-evoked firing of three cells in the nucleus accumbens core. (A) Saline-PreT. rat tested 14 days after the last injection of saline. (B) MDMA-PreT. rat tested 2 days after the last injection of MDMA. (C) MDMA-PreT. rat tested 14 days after the last injection of MDMA. Lines above the records indicate periods of DA ejection and numbers indicate current intensity (nA). The cells were driven by cycled pulses of glutamate (G) 10 s on, 10 s off throughout the records. Onset of each G application reset the curvilinear oscillograph pen that recorded cumulative action potentials. The peak of each histogram indicates the number of spikes that occurred during each cycle. The calibration lines for spikes (top record) and time (below bottom record) apply to all three records.

of single accumbens neurons are shown in Fig. 1. The top record (Fig. 1A) is from a cell in a Saline-PreT. rat that was tested 14 days after the last saline injection. DA produced a dose-dependent inhibition of glutamate-evoked firing that recovered to baseline after ejection offset. The peak of each histogram in the record indicates the number of spikes that were evoked during each glutamate application cycle and the flat tops of the histograms represent the fact that the cell was silent between glutamate applications. In contrast to the cell from the control animal (Fig. 1A), DA had only a slight inhibitory effect on the cells from MDMA-PreT. rats tested two days (Fig. 1B) or 14 days (Fig. 1C) after the last MDMA injection.

The attenuation of the inhibitory effect of 40 nA and 60 nA doses of DA that was observed in the MDMA-PreT. rats was statistically significant ($p < 0.01$) for animals that were tested soon after the last MDMA injection (1–4 days) and for animals that were tested more than a week after the last MDMA injection (9–15 days) compared to saline-injected and naive control animals (Fig. 2). DA was tested

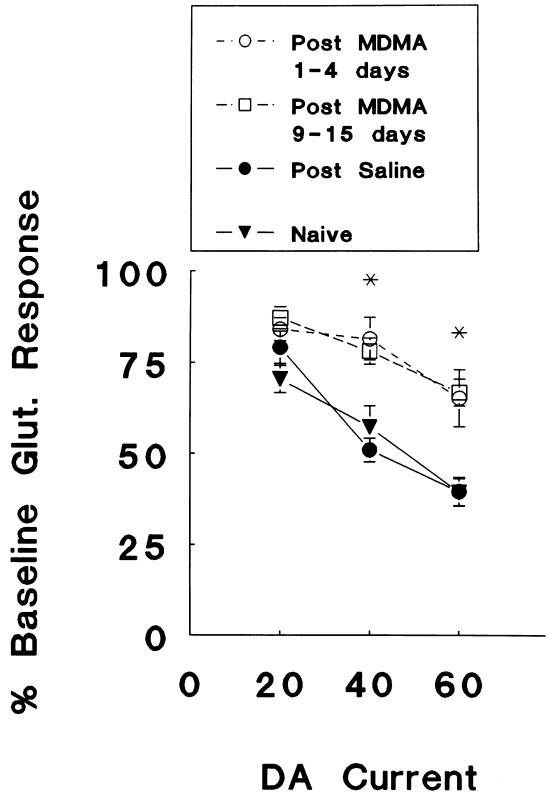

Fig. 2. Dose (current)-response curves for the effects of DA (20, 40 and 60 nA, 60 s) on glutamate-evoked firing of nucleus accumbens cells from MDMA-PreT. rats tested at early and late time periods after the last injection of MDMA compared to dose-response curves for cells from rats in the two control groups (Saline-PreT. rats and naive rats). Means and standard errors of the mean are plotted for 22 cells from 5 MDMA-PreT. rats at the early test period (1–4 days post-MDMA), 36 cells from 7 MDMA-PreT. rats at the late test period (9–15 days post-MDMA), 31 cells from 6 Saline PreT. rats, and 34 cells from 6 Naive rats. * Significant differences between the two MDMA-PreT. groups compared to the two control groups, $p < 0.01$.

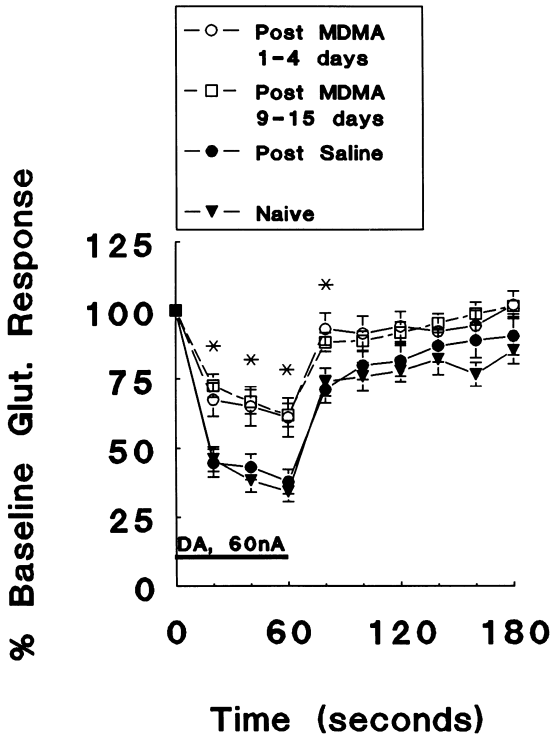

Fig. 3. Time-response curves for effects of DA (60 nA, 60 s) on glutamate-evoked firing of nucleus accumbens core cells in MDMA-PreT. and control rats. The heavy line below the records indicates the time of DA ejection. * Times at which the responses of the two MDMA-PreT. groups were significantly different from the responses of the two control groups, $p < 0.01$.

on 22 cells from 5 MDMA-PreT. rats at the early test period, 36 cells from 7 MDMA-PreT. rats at the late test period, 31 cells from 6 Saline PreT. rats, and 34 cells from 6 Naive rats. The highest dose of DA that was tested (60 nA, 60 s) reduced glutamate-evoked firing of nucleus accumbens cells from all of the rats in all of the groups by at least 25%. However, comparison of the time course of the inhibitory effect of the highest dose of DA indicated that DA was significantly ($p < 0.01$) less inhibitory for the MDMA-PreT. rats than for the Saline-PreT. or naive rats both during the DA ejection period and during the first glutamate cycle following offset of DA ejection (Fig. 3). Thus, the inhibition was both greater in magnitude and longer in duration in the Saline PreT. rats compared to the MDMA-PreT. rats.

The selective D_1 receptor agonist SKF38393 was applied to 20 cells from 5 MDMA-PreT. rats that were tested 9–15 days after the last injection of MDMA and effects on glutamate-evoked responses were compared to those of 19 cells from 5 Saline-PreT. rats. Similarly to DA, the inhibition of glutamate-evoked firing by SKF38393 (60 nA, 60 s) was significantly greater in magnitude and longer in duration in the control animals that in the MDMA-pretreated animals (Fig. 4).

Inhibition of glutamate-evoked firing by 5HT was also significantly attenuated in MDMA-PreT. rats (tested 9–15

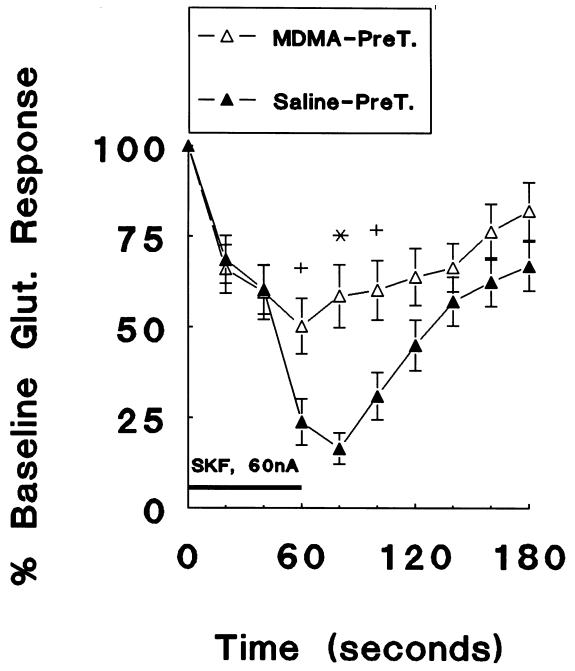

Fig. 4. Time-response curves for effects of the D_1 agonist SKF38393 (60 nA, 60 s) on glutamate-evoked firing of nucleus accumbens core cells in MDMA-PreT. and Saline-PreT. rats. The heavy line below the records indicates the time of SKF38393 ejection. Means and standard errors of the mean are plotted for 20 cells from 5 MDMA-PreT. rats that were tested 9–15 days after the last injection and 19 cells from 5 Saline-PreT. rats. $^{+*}$ Times at which the responses from the two groups were significantly different: $^{+}p < 0.05$; $^{*}p < 0.01$.

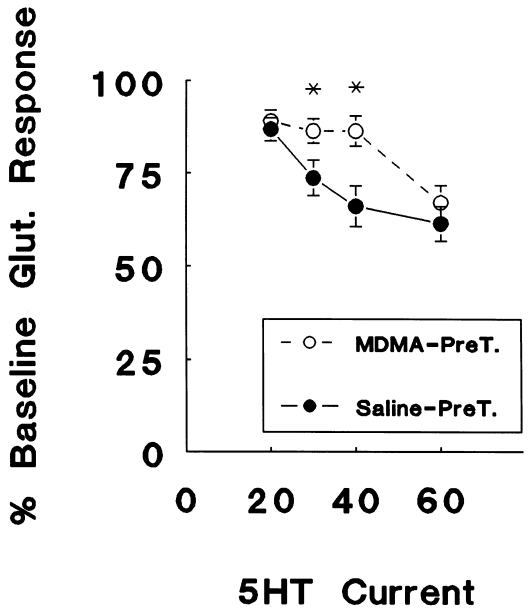

Fig. 5. Dose (current)-response curves for the effects of 5HT (20, 30, 40 and 60 nA, 60 s) on glutamate-evoked firing of nucleus accumbens core cells in rats pretreated with MDMA or saline. Means and standard errors of the mean are plotted for 34 cells from 10 MDMA-PreT. rats tested 9–15 days after the last injection and 20 cells from 6 Saline-PreT. rats. * Significant differences between groups: $p < 0.01$.

days after the last MDMA injection) compared to Saline-PreT. rats (Fig. 5). Effects of 5HT were tested on 34 cells from 10 MDMA-PreT. rats and 20 cells from 6 Saline-PreT. rats. However, although 5HT was significantly less inhibitory at the 30 nA and 40 nA ejection currents in MDMA-pretreated rats than in saline-pretreated rats, this difference was not evident for the 60 nA ejection current (Fig. 5). At the highest dose of 5HT tested (60 nA, 60 s), inhibition was equivalent in the two groups, and significantly greater than an equivalent ejection current applied to the acidic–saline control solution. The acidic saline control solution applied at 60 nA for 60 s slightly reduced glutamate-evoked firing of the cells to $90 \pm 3\%$ of the baseline response (data not shown).

In contrast to DA, SKF38393 and 5HT, the inhibitory effects of GABA on glutamate-evoked firing did not significantly differ between MDMA-PreT. rats and Saline-PreT. rats (Fig. 6). GABA was tested on 19 cells from 4 MDMA-PreT. rats that were examined 9–15 days after the last MDMA injection and 17 cells from 4 Saline-PreT. rats.

Glutamate ejection currents were chosen to produce equivalent baseline response rates in the cells that were tested in the study. Statistical comparisons indicated that the baseline glutamate-evoked firing rates were, indeed equivalent for the cells tested from MDMA-pretreated and control rats (Saline-PreT. plus naive rats). Baseline firing rates were 52.5 ± 1.3 spikes/10 s glutamate application cycle for the cells from the MDMA rats and 51.6 ± 1.8 spikes/10 s glutamate application cycle for the cells from

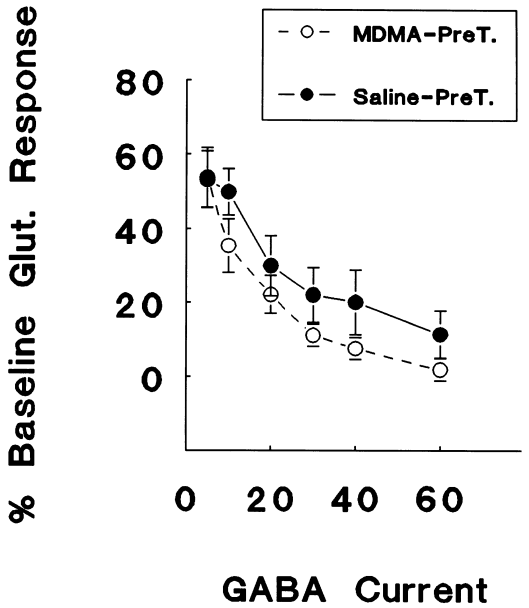

Fig. 6. Dose (current)-response curves for the effects of GABA (5, 10, 20, 30, 40 and 60 nA, 60 s) on glutamate-evoked firing of nucleus accumbens core cells in rats in MDMA-PreT. (19 cells from 4 rats) and Saline-PreT. (17 cells from 4 rats) groups. The animals were tested 9–15 days after the last MDMA or saline injection.

Table 1
Effects of repeated administration of MDMA on ^{3}H-paroxetine labeled 5HT uptake sites (fmol/mg tissue equivalent) measured by autoradiography[a]

Brain region	Saline-PreT.	MDMA-PreT.	Depletion
Prefrontal cortex	61.99 ± 16.24	14.98 ± 5.55[b]	76%
Accumbens core	79.15 ± 20.29	21.48 ± 10.47[b]	73%
Accumbens shell	50.61 ± 3.75	26.78 ± 2.07[b]	47%

[a]These data represent the means ± S.E.M. for measurements from at least five adjacent sections per brain region for each rat (Saline-Pret.: $n = 4$; MDMA-Pret.: $n = 5$).
[b]Significant difference between the Saline-PreT. control rats and the MDMA-PreT. animals, $p < 0.05$, *t*-test, two-tailed.

the control rats. The glutamate ejection current required to achieve this equivalent baseline firing was also not different for the two groups (39 ± 1.1 nA for the MDMA-pretreated rats, 41.4 ± 1.8 nA for the control rats).

The neurotoxicity of the MDMA treatment regimen that was used was confirmed by measuring specific binding of ^{3}H-paroxetine to 5HT uptake sites in the medial prefrontal cortex, the nucleus accumbens core and the nucleus accumbens shell. ^{3}H-paroxetine binding was significantly decreased in the MDMA-PreT. rats compared to the Saline-PreT. rats for all three brain regions that were measured (Table 1).

4. Discussion

The inhibitory effects of DA, the D_1 receptor agonist SKF38393, 5HT and GABA on glutamate-evoked firing of neurons in the core region of the nucleus accumbens of the control rats are consistent with previous reports of inhibition of glutamate-evoked firing by these substances in anesthetized-rats [15,45–48]. Pretreatment with repeated injections of MDMA significantly attenuated the inhibitory effects of DA and SKF38393 on glutamate-evoked firing of the nucleus accumbens cells. These changes were observed both soon after (1–4 days) and more than a week after (9–15 days) the last MDMA injection. The injection regimen of MDMA that was used was neurotoxic to 5HT-containing terminals in the nucleus accumbens core as demonstrated by the 73% decrease in the density of 5HT-uptake sites (^{3}H-paroxetine binding sites) that are located on 5HT-containing axon terminals. The degree of MDMA-induced depletion of 5HT-uptake sites in the nucleus accumbens and prefrontal cortex was very similar to that reported previously by Battaglia et al. [4].

The observation that MDMA-pretreatment decreased the inhibitory effect of exogenously applied 5HT in the nucleus accumbens is surprising and the mechanism for this change is not known. Although no previous studies have examined the effects of 5HT terminal damage on neuronal excitability in the nucleus accumbens to our knowledge, damage produced by the neurotoxin 5,7DHT significantly increased the inhibitory effect of 5HT on frontal cortex pyramidal cells [2,12] and amygdala cells [44] and significantly increased the excitatory effect of 5HT on facial motoneurons [27]. However, hippocampal pyramidal cell responses to 5HT were unaltered after 5,7DHT injections, indicating that there are regional differences in the consequences of 5HT terminal damage [9]. It is unlikely that the difference in direction of the excitability changes induced by neurotoxic doses of MDMA compared to 5,7DHT can be attributed to differences in basal extracellular levels of 5HT in the animals because dialysis studies have revealed that basal levels of extracellular 5HT remain unchanged compared to control animals after either neurotoxic doses of 5,7DHT [22] or MDMA [13]. Compensatory mechanisms in spared 5HT terminals appear to allow basal efflux of 5HT but not stimulus-induced efflux of 5HT to remain normal in the 5,7DHT or MDMA-pretreated animals [22,40,42]. Since MDMA, unlike 5,7DHT, is selectively toxic to a subpopulation of fine-diameter, unbeaded 5HT-containing terminals [25,33], it is possible that this difference in spared terminals contributes to the differential effect of the two neurotoxins on subsequent neuronal sensitivity to 5HT. It would be interesting to determine whether neurotoxic doses of MDMA might also decrease the inhibitory effect of 5HT in the frontal cortex where both subtypes of 5HT-containing terminals exist and where 5,7DHT is known to increase the inhibitory effect of 5HT.

The nucleus accumbens contains several different 5HT receptor subtypes [3,18,21,30,36] and it is possible that an MDMA-induced change in the relative proportions of these receptor subtypes may contribute to the MDMA-induced decrease in the inhibitory effect of 5HT that was observed in the present study. Pretreatment with neurotoxic doses of MDMA has been reported to produce a transient decrease in $5HT_2$ receptors in several brain regions [39], a long-term increase in $5HT_{2C}$ receptor mRNA expression in the hippocampus [54] and an increase in $5HT_{1A}$ receptor density and receptor mRNA expression in the frontal cortex [1]. Whether receptor densities are altered in the nucleus accumbens at early or late times after neurotoxic doses of MDMA is not known, but studies to investigate this question are underway in our laboratory. A nonspecific increase in the excitability state of the nucleus accumbens cells following repeated MDMA injections could also make the cells less sensitive to the inhibitory effects of exogenously-applied 5HT. This possibility is, however, unlikely because neither ejection currents of GABA required to inhibit glutamate-evoked firing of the cells nor ejection currents of glutamate required to drive the cells at equivalent baseline firing rates differed between MDMA-pretreated and control animals.

The blunted inhibitory effects of DA and SKF38393 on glutamate-evoked firing of nucleus accumbens cells in MDMA-pretreated rats resemble the decreases in the in-

hibitory effects of DA that have been reported in the frontal cortex following neurotoxic doses of 5,7DHT [2,12,44]. The mechanism for this change in sensitivity to DA following damage to 5HT-containing terminals is not known and further studies are necessary to elucidate the mechanism. It is possible that DA receptor densities, DA transporter activity, and/or post-DA receptor signal transduction mechanisms are altered following repeated exposure to MDMA. However, the change in sensitivity to DA cannot be attributed to MDMA-induced damage to DA-containing terminals in the accumbens because in a similarly treated group of rats in our laboratory, tissue levels of DA in the nucleus accumbens 2 weeks after the last MDMA injection were slightly but significantly increased rather than decreased [19]. Furthermore, basal extracellular levels of DA measured by dialysis in awake, behaving rats were unchanged in MDMA-pretreated compared to control animals [19]. Several other laboratories also have reported that the dose regimen of MDMA that was used in the present study is selectively neurotoxic to 5HT-containing terminals in the rat and spares catecholamine-containing terminals [4,5,33].

It is noteworthy that the blunted inhibitory effects of DA and the D_1 receptor agonist on glutamate-evoked firing in the MDMA-pretreated animals are exactly opposite to the increased inhibitory effect of DA and D_1 receptor agonists on glutamate-evoked firing that has been observed in the nucleus accumbens of rats that were repeatedly exposed to cocaine [15,48], low doses of amphetamine [51] or low doses of methamphetamine [17]. All three of the latter drugs, like MDMA, increase extracellular DA and 5HT levels in the nucleus accumbens and dorsal striatum when administered acutely [11,16,31,47], but unlike MDMA, repeated administration of these drugs at low doses is not neurotoxic to 5HT-containing terminals in the brain [23,31]. Thus, it is tempting to speculate that the 5HT depletion that is present in the MDMA-pretreated animals may prevent sensitization to the inhibitory effect of DA that follows repeated administration of cocaine or amphetamine.

The decreased sensitivity of nucleus accumbens cells to the inhibitory effects of DA and 5HT that was demonstrated at both early (1–4 day) and late (9–15 day) test periods after the last injection of MDMA is the first observation, to our knowledge, of persistent changes in neuronal excitability following repeated exposure to MDMA. It is possible that repeated MDMA exposure also produces persistent changes in neuronal excitability in other limbic brain regions that contain dual innervation by fine-diameter 5HT-containing axons and by DA-containing axons. Acute local or systemic applications of MDMA not only decrease glutamate-evoked firing of cells in the core region of the nucleus accumbens [32,47], but also inhibit firing of serotonin neurons in the dorsal raphe nucleus [41], dopamine cells in the ventral tegmental area and substantia nigra [20,26] and pyramidal cells in the prefrontal cortex [34]. All of these acute actions appear to be mediated by MDMA-induced increases in extracellular levels of 5HT and/or DA [20,26,32,41,47]. Therefore, it is possible that repeated exposure to MDMA may cause a widespread blunting of neuronal responses to DA and 5HT inputs in many other brain regions that receive dual innervation by these two monoamines.

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