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METHYLENEDIOXYMETHAMPHETAMINE DEPRESSES GLUTAMATE-EVOKED NEURONAL FIRING AND INCREASES EXTRACELLULAR LEVELS OF DOPAMINE AND SEROTONIN IN THE NUCLEUS ACCUMBENS *IN VIVO*

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Abstract—The nucleus accumbens has been implicated as an important site for the actions of many drugs that are used recreationally. This study examined the effects of methylenedioxymethamphetamine (MDMA), a euphoric and hallucinogenic drug, on glutamate-evoked neuronal firing and on extracellular levels of dopamine and serotonin in the nucleus accumbens of the rat. Microiontophoretic application of MDMA inhibited glutamate-evoked firing of most of the nucleus accumbens cells that were tested (83 of 86), as did microiontophoretic application of dopamine and serotonin. MDMA-induced inhibition of glutamate-evoked firing was partially blocked by the dopamine antagonist SCH39166 and was attenuated by combined pretreatment with inhibitors of both serotonin and catecholamine synthesis, *p*-chlorophenylalanine and α-methyl-*p*-tyrosine. MDMA applied directly into the nucleus accumbens and adjacent regions of the ventral striatum through a dialysis probe increased extracellular levels of both dopamine and serotonin.

These results indicate that MDMA has inhibitory effects on glutamate-evoked neuronal firing in the nucleus accumbens and suggest that the inhibition is mediated by increased extracellular dopamine and serotonin. Furthermore, these results permit MDMA to be added to the extensive list of abused drugs that have been demonstrated to elevate extracellular levels of dopamine and serotonin in the nucleus accumbens.

Methylenedioxymethamphetamine (MDMA, "ecstasy") is a recreationally-used euphoric and hallucinogenic drug[14] that appears to alter both serotonin (5-HT) and dopamine (DA) neurotransmission. Chronically administered MDMA is selectively neurotoxic to the population of fine-diameter, 5-HT-containing fibers that arise from the dorsal raphe nucleus while sparing larger, beaded 5-HT fibers and catecholamine-containing fibers.[18,34] However, acute administration of MDMA increases extracellular levels of both 5-HT and DA in several forebrain and brainstem regions.[6,8,11,13,15,16,25,37] The consequences of this effect on extracellular levels of 5-HT and DA are not yet understood, but MDMA appears to inhibit neuronal firing in the dorsal raphe nucleus[27] and in the prefrontal cortex[19] by 5-HT-mediated mechanisms.

A property common to many abused drugs including opiates, alcohol, amphetamine and cocaine is the ability to increase extracellular concentrations of DA in the nucleus accumbens,[5,9,38] a forebrain region that is an important component of the brain reward pathway.[22,35] Many of these abused drugs also elevate extracellular 5-HT in the nucleus accumbens.[9,38] MDMA increases extracellular DA measured by *in vivo* voltammetry in the nucleus accumbens,[37] but whether it also increases 5-HT *in vivo* in this nucleus is unknown. Early studies showed that MDMA released both 5-HT and DA from striatal slices and from synaptosomes in whole brain or cortex/striatal homogenates, but the prepotent effect was on 5-HT release.[11,16,25] In contrast to these early *in vitro* findings, a more recent *in vivo* microdialysis study found that extracellular DA was increased at least as much as 5-HT in the caudate nucleus by intraperitoneally administered MDMA.[7]

The nucleus accumbens, like the striatum, receives both serotonergic and dopaminergic innervation.[4,12,28,30] Therefore, MDMA may alter neuronal excitability in the accumbens by increasing extracellular levels of both of these monoamines. The present study used microiontophoresis combined with extracellular recording to determine the effects of MDMA on glutamate-evoked firing of neurons in the nucleus accumbens *in vivo* and to compare MDMA effects with those of 5-HT and DA. In a separate group of

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Abbreviations: AMPT, α-methyl-*p*-tyrosine; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; EDTA, ethylenediaminetetra-acetate; 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, serotonin; MDMA, methylenedioxymethamphetamine; PCPA, *p*-chlorophenylalanine.

animals, *in vivo* microdialysis was used to determine whether local infusion of MDMA into the nucleus accumbens and surrounding ventral striatum alters extracellular levels of DA and/or 5-HT.

EXPERIMENTAL PROCEDURES

Animals

Adult male Sprague–Dawley rats (265–335 g) from Simonsen Breeding Laboratories were used for these experiments. Animals were housed in a temperature- and humidity-controlled environment with food and water available *ad libitum*. Extracellular neuronal recordings were made from the nucleus accumbens in 42 rats and dialysis samples were taken from the nucleus accumbens of 14 other rats.

Materials

(±)-3,4-MDMA was provided by the National Institute on Drug Abuse (Bethesda, MD). R + SCH39166 was a gift from Schering-Plough Corporation and methysergide was a gift from Sandoz Pharmaceuticals. All other drugs used in the experiments were purchased from Sigma Chemical Company (St Louis, MO).

Neuronal recording

Rats were anesthetized with urethane (1.25 g/kg, i.p., with supplements as required). The trachea was intubated to aid respiration and the left femoral vein was cannulated for intravenous drug administration. The dorsal surface of the skull was removed anterior to bregma, leaving a narrow strip of intact skull overlying the superior sagittal sinus. The head was fixed in a Kopf stereotaxic headholder, the meninges were cut and the electrode was inserted into the nucleus accumbens 10.0–11.2 mm anterior to the interaural line, 1.2–2.0 mm lateral to the midline and 6.0–7.0 mm below the surface of the cortex.[21] The exposed brain tissue and skull were covered with 3% agar in saline.

Recording electrodes were seven-barrel glass microdot micropipettes that were made from glass tubing obtained from the Glass Company of America (Millville, NJ). Electrode tips were broken to a diameter of 7–10 μm. The center barrel and one side-barrel were filled with 4 M NaCl for recording and automatic current balance, respectively. Three other barrels contained monosodium-L-glutamate, 0.2 M, pH 6.5 to drive quiescent cells. MDMA, 0.1 M, pH 5 and Pontamine Sky Blue dye (2% in 0.5 M acetic acid) to mark recording sites. The remaining two barrels contained some combination of 5-HT, 0.04 M, pH 4.5, DA hydrochloride, 0.8 M, pH 5, sulpiride hydrochloride, 0.02 M, pH 4.5, SCH39166, 1 mM, pH 5 or 0.01 M acetic acid in physiological saline, pH 4.5, for acid control. Fresh solutions were made up daily in deionized water except for sulpiride and SCH39166, which were dissolved in hydrochloric acid and brought to pH 4.5 with NaOH. Resistances for electrode barrels were 1–4 MΩ for recording and balance barrels and 20–80 MΩ for the drug barrels. Retaining currents were +10 nA for glutamate and −10 nA for all other solutions except for the dye, which received no retaining current. Glutamate and the dye were ejected as anions, all other drugs were ejected as cations.

Intravenous injections (1.0–2.0 mg/kg) of the 5-HT antagonist methysergide were administered for some experiments. In another set of experiments, 5-HT, DA, or both were depleted by administering synthesis inhibitors prior to the recording session. α-Methyl-*p*-tyrosine methyl ester (AMPT) was given as two intraperitoneal injections to five rats (300 mg/kg, 4 h and 200 mg/kg, 2 h before recording began) to deplete catecholamines, including DA. The 5-HT synthesis inhibitor *p*-chlorophenylalanine methyl ester (PCPA) was given as a single dose (300 mg/kg, i.p., 68 h prior to recording) to a second group of five rats. A third group of five rats received both PCPA and AMPT to deplete both 5-HT and DA. These dose regimens have been reported to deplete 5-HT and DA in the forebrain by 74–87% and 86–97%, respectively.[33] A fourth group of five control rats received two i.p. injections of saline 4 and 2 h prior to recording.

Nucleus accumbens cells were activated with pulses of glutamate delivered with a Medical Systems microiontophoresis current pump. Spikes were monitored on a Tektronix oscilloscope, led into a Medical Systems analog/digital converter and stored on videocassette tape. Action potentials from single cells were isolated with a Neurolog window discriminator and counted with a pulse integrator. Short glutamate pulses (5–10 s) were automatically delivered every 20 s and cumulative counts of spikes occurring during each glutamate-ejection pulse were displayed using a Grass oscillograph (curvilinear) pen. Glutamate ejection currents and durations 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 microiontophoretically ejected MDMA, 5-HT or DA on the glutamate response were tested. A change of 25% or more from the baseline glutamate response (defined as the mean response to the six glutamate pulses that immediately preceded ejection of the test drug) was considered to be significant. Cells were excluded from the experiment if the action potential amplitude or shape changed during the test drug ejection period.

Dose–response curves for the effects of MDMA on the glutamate responses of nucleus accumbens cells were constructed and compared to the effects of control solution (acidic saline) ejection using two-way repeated measures analysis of variance with post hoc comparisons of means. The effects of monoamine antagonists and synthesis inhibitors on MDMA responses were also tested using two-way repeated measures analysis of variance.

Recording sites were marked by ejecting Pontamine Sky Blue dye (30 μA, negative current) for 10 min. At the conclusion of the recording session, brains were removed, placed in 10% formalin for at least three days and then sectioned (30 μm thick) with a freezing microtome. Sections containing the blue dye spots were mounted on glass slides and counterstained with the pink Nissl stain Safranin O. Section outlines, landmark structures and the dye spots were drawn with the aid of a drawing tube attached to a viewing microscope (Leitz).

Dialysis

Stainless steel guide cannulae (20-gauge; Small Parts, Miami, FL) were implanted unilaterally over the nucleus accumbens of Equithesin-anesthetized rats (0.3 ml/kg, i.p., of a solution of 9.7 g sodium pentobarbital, 42.5 g chloral hydrate, 21.3 g $MgSO_4$ dissolved in 1 l of 11% ethanol, 42% propylene glycol, v/v). Stainless steel obturators (25-gauge; Small Parts) were inserted into the cannulae and the rats were allowed at least seven days recovery prior to insertion of the dialysis probe. The dialysis probe was constructed as described by Robinson and Whishaw.[23] The active region of the dialysis membrane was approximately 2.5 mm long and 0.25 mm in diameter. Probe recovery was determined by placing the probe for 20 min in dialysis buffer (5 mM KCl, 120 mM NaCl, 1.8 mM $CaCl_2$, 1.2 mM $MgCl_2$, 0.15% phosphate-buffered saline, pH 7.4) which contained DA (10^{-7} M), 3,4-dihydroxyphenylacetic acid (DOPAC; 10^{-6} M), 5-HT (10^{-7} M) and 5-hydroxyindoleacetic acid (5-HIAA; 10^{-6} M). The flow rate of the buffer was maintained at 1.9 μl/min with a Harvard Instruments infusion pump. For collecting samples from the brains of unrestrained, conscious rats, the dialysis probe was inserted into a connector that was attached at one end to a liquid swivel located on a balance beam. The other end of the connector

was attached to the exposed portion of the guide cannula and fixed the dialysis probe at a position that extended 3 mm below the guide cannula.[26]

The dialysis probe was inserted into the brain the evening before the experiment. The next day, dialysis buffer was perfused through the probe for at least 2 h prior to sample collection. Four baseline samples of dialysate (20 min each) were then collected over the next 80 min. MDMA was then infused through the dialysis probe at one of four concentrations (0.1, 1.0, 10 or 100 μM). Each concentration was maintained for five 20-min collection periods. Individual rats received only two or three of the different concentrations of MDMA. The dialysis samples were collected into 20 μl of mobile phase (0.1 M citric acid, 0.75 mM Na_2HPO_4, 0.6–1.0 mM heptane sulfonic acid, 0.1 mM EDTA, 13% methanol, v/v, pH 3.8–4.2, with 2.0 pmol dihydroxybenzylamine as the internal standard), and the entire sample was injected into the high performance liquid chromatography system. A reversed-phase column (C-18, 25 cm, Bioanalytical, Indianapolis, IN) was used to separate the biogenic amines and a coulometric electrochemical system (ESA Inc., Beford, MA) utilizing three electrodes (guard electrode = +0.4 V, oxidation working electrode = +0.35 V and reduction working electrode = −0.2 V) was used to detect the amines. Standard curves ranging from 0.01 to 10 pmol were used to quantify DA, 5-HT, DOPAC and 5-HIAA.

Rats were killed with an overdose of pentobarbital and perfused transcardially with saline followed by 10% formalin. Brains were removed and stored in formalin for at least one week. Coronal sections (100 μm thick) of the cannula track were made with a vibratome. Sections were mounted on gelatin-coated slides and stained with Cresyl Violet. The location of the dialysis probe track within the stained sections was determined by a person who was unaware of the neurochemical response of the animal.

RESULTS

Electrophysiology

Glutamate application was required to activate all of the nucleus accumbens cells that were recorded; none were spontaneously active in these urethane-anesthetized rats. Continuous glutamate application usually produced continuously increasing firing frequenty with decreasing spike amplitude and eventual cessation of firing that presumably resulted from depolarization blockade of firing. However, most cells responded to short pulses of glutamate delivered for 5–10 s every 20 s with stable firing patterns during the glutamate application period. Neurons which did not develop stable glutamate-evoked firing were abandoned.

MDMA (40 nA, 60 s) depressed glutamate-evoked firing by at least 25% in 83 of 86 nucleus accumbens cells that were tested. Oscilloscope traces of action potentials evoked by glutamate (40 nA, 6 s) in one nucleus accumbens cell prior to (A) and during (B) MDMA application are illustrated in Fig. 1. The line above trace A indicates the period of glutamate application for both traces. The MDMA (40 nA) ejection began 40 s prior to the onset of trace B and continued throughout trace B. MDMA increased the latency and decreased the frequency of glutamate-evoked firing. Note that for this cell, the spike size was beginning to decrease as the frequency increased just prior to the offset of the pre-MDMA glutamate application (Fig. 1A). In the presence of MDMA, an equivalent application of glutamate produced less frequent firing and there was little change in spike amplitude (Fig. 1B). This record is shown to demonstrate that MDMA could inhibit even very rapidly firing glutamate-driven cells. However, for the other cells reported in this study, glutamate ejection current intensities and durations were chosen that did not produce firing so frequent that the spike amplitude decreased.

Responses of four different accumbens cells to repeated applications of MDMA are illustrated in Fig. 2. For these records, the oscillograph pen that recorded cumulative action potentials was reset at the onset of each glutamate application cycle. Therefore, the peak of each histogram indicates the total number of spikes that occurred during the 10 s glutamate pulse. The nearly flat tops of the histograms indicate that the cells fired mainly during the glutamate application pulses and were almost silent during the 10 s intervals between glutamate applications. The inhibition of firing that was produced by MDMA was repeatable within a cell and was dose-dependent, with more inhibition occurring with higher ejection currents (Fig. 2A). Equivalent ejection currents applied to the acidic saline control solution (Na) did not alter glutamate-evoked firing (Fig. 2B).

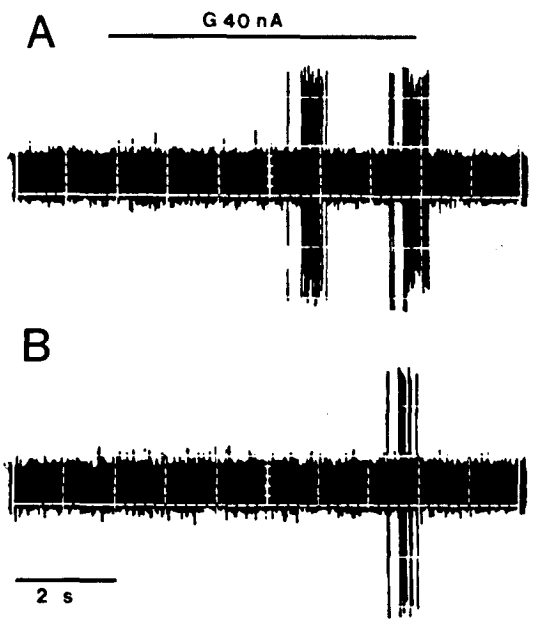

Fig. 1. Microiontophoretically applied MDMA inhibited glutamate-evoked firing of a cell in the nucleus accumbens core. The cell was driven by pulses of glutamate (40 nA, 6 s) that were applied every 20 s. (A) Oscilloscope trace of spikes occurring during a glutamate pulse delivered prior to MDMA application. (B) Oscilloscope trace of spikes occurring during a glutamate pulse delivered during MDMA application. MDMA ejection (40 nA) began 40 s prior to the trace in B and continued throughout the trace. The period of glutamate application for both A and B is indicated by the line above trace A.

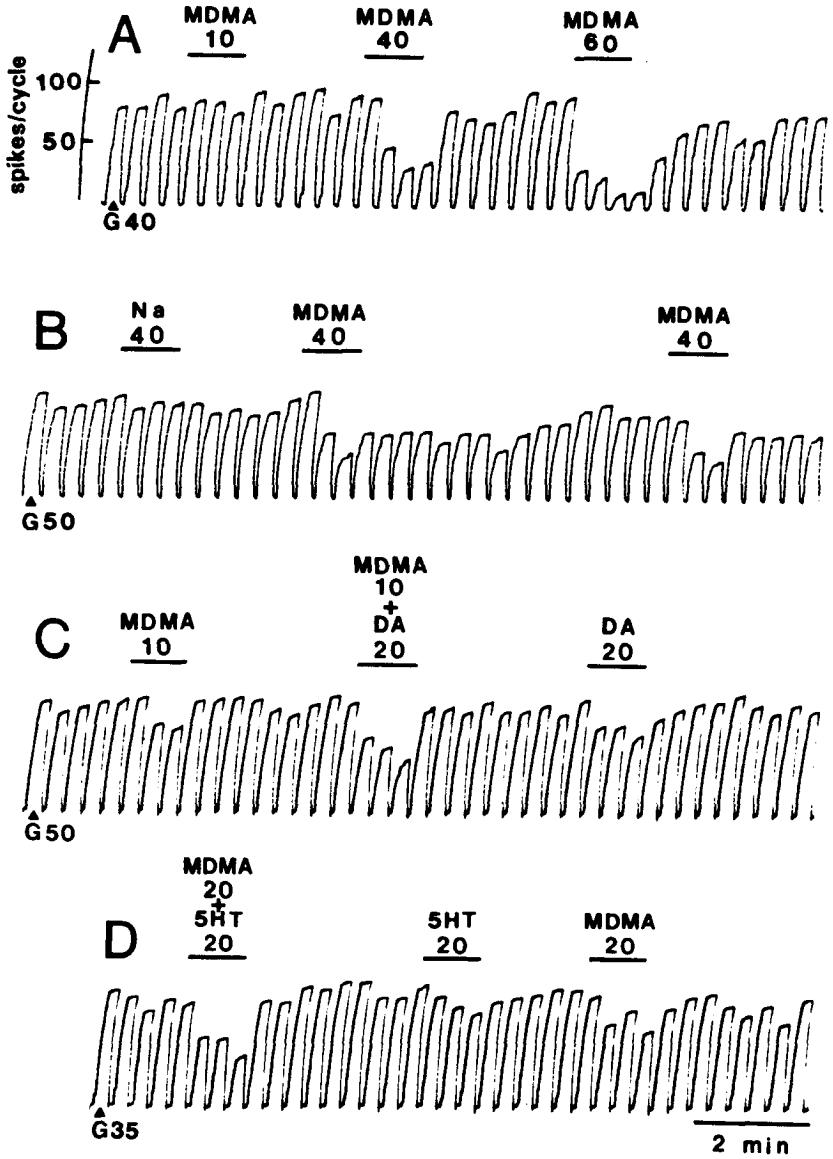

Fig. 2. Effects of MDMA, 5-HT, DA and acidic saline control solution (Na) on glutamate-evoked firing in the nucleus accumbens. Records from four different cells are shown. Cells were driven by 10 s glutamate pulses that were delivered every 20 s. Glutamate (G) ejection current intensities are indicated by the numbers below the records. Onset of each glutamate pulse reset the curvilinear oscillograph pen that recorded cumulative action potentials. The peak of each histogram indicates the total number of spikes that occurred during the glutamate pulse. Lines above the records indicate periods of ejection of MDMA, Na, DA, 5-HT or combined ejection of MDMA and DA or 5-HT. Numbers above the lines indicate ejection current intensities in nA. A–C are records from cells in the accumbens core. D is a record from a cell in the accumbens shell.

Threshold responsiveness to MDMA varied among cells, with the lowest dose of MDMA (10 nA, 60 s) producing detectable inhibition in only about 50% of the cells (compare Fig. 2A and C). MDMA-induced inhibition of glutamate-evoked firing developed slowly, peaking near the end of the ejection period. Recovery to baseline firing rates after the offset of the MDMA ejection current was slower for higher doses (60 nA for 60 s) than for lower doses (40 nA for 60 s) of MDMA (Fig. 2A). At the 60 nA ejection current, MDMA also decreased spike amplitude of some cells, requiring the exclusion of those data from the analysis. Consequently, 40 nA was the maximum current intensity used for most of the experimental analysis.

MDMA inhibited glutamate-evoked firing of the same nucleus accumbens cells that were inhibited by DA or 5-HT (Fig. 2C, D). Combined applications of MDMA and DA or 5-HT had additive inhibitory effects on cell firing (Fig. 2C, D). Inhibition of glutamate-evoked firing by MDMA occurred for cells in both the nucleus accumbens core (Fig. 2A–C) and in

the shell (Fig. 2D). However, of the three accumbens cells that failed to be inhibited by MDMA in this study, two were located in the shell region.

MDMA-induced inhibition of glutamate-evoked firing was quantified by constructing time–response curves for responses to MDMA (40 nA, 60 s, $N = 86$), MDMA (20 nA, 60 s, $N = 51$), MDMA (10 nA, 60 s, $N = 24$) and acidic saline (Na; 40 nA, 60 s, $N = 14$). The cells that received the two lower doses of MDMA and the control ejection were subpopulations of the 86 cells that received the 40 nA ejection of MDMA. Total responses during each pulse of glutamate that occurred during and after MDMA application were compared to the average of the total responses that occurred for the six glutamate pulses that immediately preceded the MDMA application (baseline). Means and standard errors for each of these time–response curves are shown in Fig. 3. The control solution did not depress glutamate-evoked firing, but MDMA produced a dose-dependent inhibition. A two-way repeated measures analysis of variance indicated that the dose main effect, the time main effect and the interaction were all significant ($F_{3,171} = 25.57$, $P < 0.001$; $F_{9,1539} = 127.67$, $P < 0.001$; and $F_{27,1539} = 8.78$, $P < 0.001$, respectively). *Post hoc* comparisons of means indicated that all three MDMA ejection doses significantly inhibiting firing compared to the control solution during the 60 s ejection period ($P < 0.05$). The 40 nA dose of MDMA continued to significantly inhibit firing compared to the control ejection for more than a minute post-ejection offset, whereas firing returned to baseline levels by 20 s post-ejection offset for the 10 nA dose of MDMA and by 40 s post-ejection offset for the 20 nA dose (Fig. 3).

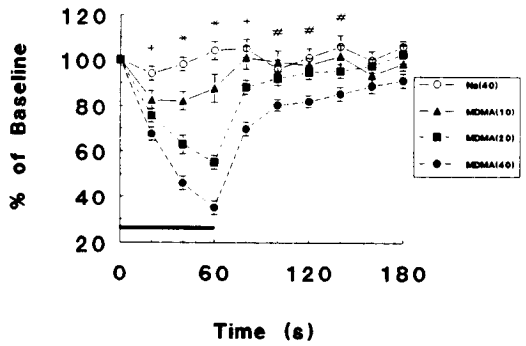

Fig. 3. Time course of effects of three doses of MDMA and the control solution on glutamate-evoked firing of nucleus accumbens cells. Means ± S.E.M. are plotted for 86 cells for the 40 nA dose of MDMA, 51 cells for the 20 nA dose, 24 cells for the 10 nA dose and 14 cells for the 40 nA dose of the control solution (Na). Glutamate-evoked responses occurring during and after MDMA were compared to baseline glutamate responses. Baseline for each cell was defined as the mean number of responses that occurred during the six glutamate cycles that immediately preceded MDMA application for that cell. The thick bar below the curves indicates the period of ejection current application. *Significant differences between all three MDMA doses and control, $P < 0.05$; +significant differences between the two highest doses of MDMA and control, $P < 0.05$, #significant difference between the 40 nA dose of MDMA and control, $P < 0.05$.

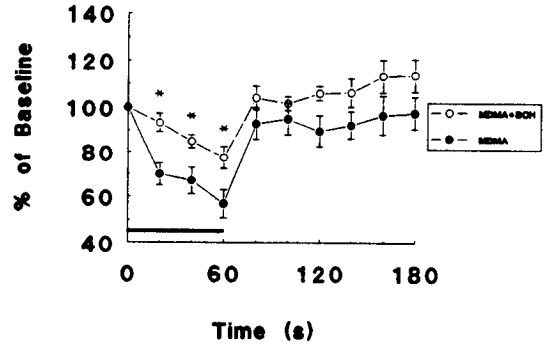

Fig. 4. Microiontophoretic application of the dopamine antagonist SCH39166 (10 nA, 3 min) partially antagonized the inhibitory effect of MDMA (20 nA, 60 sec) on glutamate-evoked firing of nucleus accumbens cells. The partial antagonism of MDMA-induced inhibition was statistically significant. Means ± S.E.M. are plotted for seven cells in the nucleus accumbens core. The thick bar below the curves indicates the period of MDMA (20 nA) application. *Significant differences between MDMA alone and MDMA + SCH39166, $P < 0.05$.

Attempts to antagonize MDMA-induced inhibition of glutamate-evoked firing were made using two antagonists, the selective D_1 antagonist SCH39166[3] and the selective D_2 antagonist sulpiride. Iontophoretic application of SCH39166 (10 nA, 3–5 min) partially antagonized the inhibition of glutamate-evoked firing that was produced by MDMA (20 nA, 60 s) for all seven nucleus accumbens cells that were tested. Mean effects of MDMA before and during SCH39166 (10 nA, 3 min) application are plotted for all seven cells in Fig. 4. SCH39166 significantly attenuated MDMA-induced inhibition of firing but did not completely block it. Higher ejection currents of SCH39166 (20–40 nA) produced greater inhibition alone than did the lower dose, but still failed to completely antagonize MDMA-induced inhibition of firing. Iontophoretic application of sulpiride (10–40 nA, 3–9 min) did not alter the inhibitory effects of MDMA on any of the eight cells for which it was tested (data not shown).

The 5-HT antagonist methysergide (1–2 mg/kg, i.v.) was tested in four rats and failed to alter inhibition of accumbens cell firing produced by MDMA (20 or 40 nA, 60 s). Depletion of 5-HT by pretreatment with PCPA (13 cells in five rats) also failed to antagonize MDMA effects on glutamate-evoked firing (Fig. 5). Depletion of DA (and other catecholamines) by pretreatment with AMPT (18 cells in five rats) tended to attenuate MDMA-induced inhibition, but the effect was not statistically significant. However, depletion of both 5-HT and catecholamines by pretreatment with both PCPA and AMPT (16 cells in five rats) significantly attenuated the inhibitory effects of MDMA on glutamate-evoked firing of nucleus accumbens cells compared to effects

on 14 cells from five saline-injected control rats (Fig. 5).

Dialysis

MDMA infused into the nucleus accumbens and adjacent regions of the ventral striatum at 10 and 100 μM concentrations significantly increased extracellular levels of both 5-HT and DA (Figs 6, 7; Table 1). However, the time course and the magnitude of the response differed markedly for the two monoamines. Basal 20 min samples of dialysate contained 70 $\pm$ 8 fmol of DA (uncorrected for probe recovery), which was well above the detection limit of 10 fmol/sample (defined as 2 $\times$ background). The increase in extracellular DA produced by the 10 μM dose of MDMA was somewhat delayed, just beginning to appear in the sample collected 20–40 min after the beginning of the infusion and abruptly increasing to a peak that was maintained for the remaining three samples, which were collected 40–100 min after the beginning of the infusion. Increasing the MDMA concentration to 100 μM produced another delayed increase in extracellular DA which also began in the sample collected 20–40 min after the beginning of the infusion of the higher dose and then also abruptly increased to a peak that was maintained for the remaining three samples (Fig. 6).

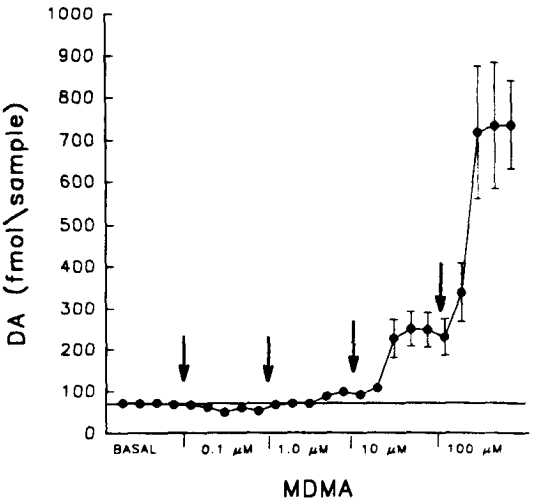

Fig. 6. MDMA infused through the dialysis probe increased DA in dialysate samples. Means $\pm$ S.E.M. of DA concentrations in 20 min dialysate samples are plotted. DA was measured in four consecutive basal samples that were taken from all 14 rats prior to MDMA infusion. DA was then measured in five consecutive 20-min samples that were taken during 0.1 μM MDMA infusion for four of the rats, during 1.0 μM MDMA infusion for 11 of the rats, during 10 μM MDMA infusion for all 14 rats, and during 100 μM MDMA infusion for nine of the rats. Arrows indicate periods when MDMA concentrations in the probe were changed. Probe recovery was determined to be 9.0 $\pm$ 1.5% in six experiments.

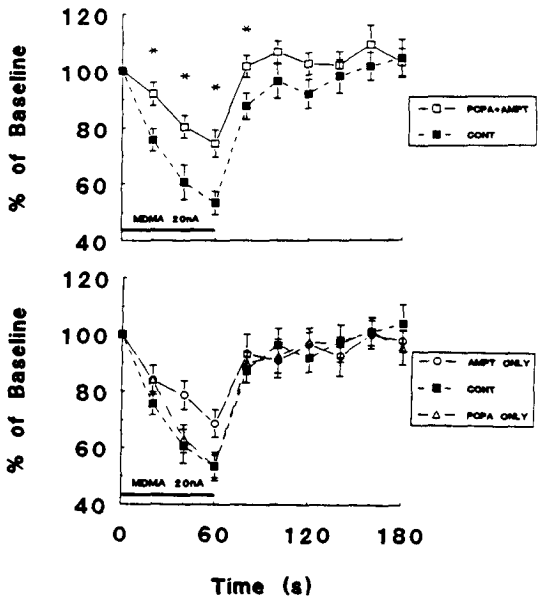

Fig. 5. Depletion of DA and 5-HT by pretreatment with combined PCPA and AMPT partially prevented the inhibitory effect of MDMA (20 nA, 60 s) on nucleus accumbens cells compared to effects in saline-injected control (CONT) rats. PCPA alone failed to alter the MDMA effect. AMPT alone tended to antagonize the MDMA-induced inhibition but the antagonism was not statistically significant. The control curve (saline) is the same in the top and bottom records. It has been compared separately for PCPA + AMPT (top record) and PCPA or AMPT only (bottom record) for visual clarity. Periods of MDMA application are indicated by the thick bars below the curves. *Significant difference between cells in control (CONT) and PCPA + AMPT pretreated rats, $P < 0.05$.

5-HT could not be measured in basal samples from five of the 14 rats. Basal samples from the remaining nine rats contained 25 $\pm$ 4 fmol of 5-HT (uncorrected for probe recovery), which was near the detection limit of 10 fmol/sample. There was a tendency (not significant) for the 1 μM dose of MDMA to increase extracellular 5-HT for the six rats which had detectable 5-HT in basal samples (Fig. 7). Increasing the MDMA concentration to 10 μM produced in immediate near peak increase in extracellular 5-HT, in contrast to the more delayed DA increase. Little further increase in extracellular 5-HT was produced by increasing the MDMA concentration to 100 μM.

For statistical comparisons of MDMA effects, monoamine levels in the last two samples for each dose of MDMA were averaged and compared to corresponding basal levels using two-tailed paired Student's t-tests followed by Bonferonni adjustments for multiple comparisons. Both the 10 and the 100 μM doses of MDMA produced statistically significant increases in extracellular DA and 5-HT (Table 1). The 10 μM dose of MDMA increased extracellular DA levels about three-fold compared to basal levels and the 100 μM dose increased extracellular DA about 10-fold. Both the 10 and the 100 μM doses of MDMA increased extracellular

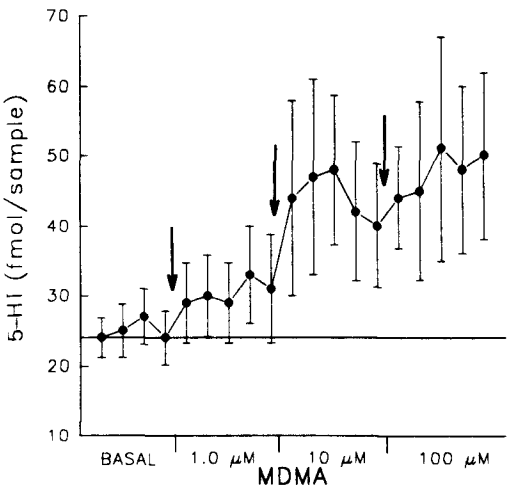

Fig. 7. MDMA infused through the dialysis probe also increased 5-HT in the dialysis samples. Means ± S.E.M. of 5-HT concentrations in 20 min dialysate samples are plotted. 5-HT was measured in four consecutive basal samples from nine rats prior to MDMA infusion. (Basal samples from five of the 14 rats did not contain detectable levels of 5-HT.) 5-HT was then measured in five consecutive 20 min samples that were taken during 1.0 μM MDMA infusion for six of the rats, during 10 μM MDMA infusion for all nine rats, and during 100 μM MDMA infusion for six of the rats. Arrows indicate periods when MDMA concentrations in the probe were changed. Probe recovery was determined to be 11.2 ± 2.0% in four experiments.

5-HT nearly two-fold compared to basal levels (Table 1).

Histology

Locations of dialysis probes and neuronal recording sites within the nucleus accumbens are shown in Fig. 8. Most recording sites were within the accumbens core, with only seven cells located in the shell (indicated by small arrows). There was overlap between the regions that were sampled by electrophysiology and those that were sampled by dialysis in the core of the nucleus accumbens. The dialysis probe locations included ventromedial caudate nucleus and olfactory tubercle regions, in addition to the nucleus accumbens.

DISCUSSION

Cycled pulses of glutamate elicited stable firing patterns in most of the nucleus accumbens cells that were tested. The pattern of firing usually consisted of bursts of action potentials separated by short pauses, as has been reported previously for nucleus accumbens cells driven by iontophoretically-applied excitatory amino acids.[36] Microiontophoretically applied MDMA depressed glutamate-evoked firing of nucleus accumbens cells, as did 5-HT and DA. The inhibitory effects of 5-HT on glutamate-evoked firing were consistent with previous demonstrations of inhibitory effects of microiontophoretically applied 5-HT on glutamate-evoked or cholecystokinin-evoked firing of nucleus accumbens cells.[31,32] However, this inhibitory effect of 5-HT on glutamate-evoked firing of accumbens cells was probably indirect, since 5-HT produces a tetrodotoxin-insensitive depolarization of nucleus accumbens cells in brain slices.[17] DA has also been reported previously to inhibit glutamate-evoked firing of nucleus accumbens cells.[1,33]

The mechanism by which MDMA inhibited glutamate-evoked firing in the present experiment is unknown. However, since MDMA was found to increase extracellular levels of both DA and 5-HT in the nucleus accumbens, its inhibitory effect may be mediated by either or both of these monoamines. The partial antagonism of MDMA-induced inhibition by the D_1 antagonist SCH39166[3] suggests that at least part of the inhibition was mediated by DA. In nucleus accumbens slices, hyperpolarizing effects of DA appear to be mediated by D_1 receptors, whereas sulpiride antagonized only depolarizing effects of DA.[29] These observations are consistent with the apparent partial antagonism of MDMA-induced inhibition produced by SCH39166 but not by sulpiride in the present study. Evidence for a role for 5-HT in MDMA-induced inhibition of glutamate-evoked neuronal firing in the nucleus accumbens was also obtained in the present study. Although neither methysergide nor pretreatment with PCPA alone antagonized the inhibitory effects of MDMA, pretreatment with combined PCPA and AMPT produced greater antagonism than pretreatment with AMPT alone. However, further studies with other

Table 1. Effects of MDMA on monoamine levels (fmol) in dialysate samples from the nucleus accumbens and adjacent ventral striatum

Monoamine	Basal	MDMA 1.0 μM	MDMA 10.0 μM	MDMA 100 μM
DA	70 ± 8.0	90 ± 7.9	251 ± 39.6*	725 ± 121.1**
(*N*)	(14)	(11)	(14)	(9)
5-HT	25 ± 4.0	32 ± 6.7	42 ± 9.5*	49 ± 9.7*
(*N*)	(9)	(11)	(9)	(6)

Means ± S.E.M. are presented for the last two samples that were collected from the rats for each dose of MDMA and for the pre-MDMA basal sampling period. Numbers of rats (*N*) for each dose are indicated in parentheses. Differences between means for samples during MDMA infusion and for corresponding basal samples are significant at $*P < 0.05$ and $**P < 0.01$.

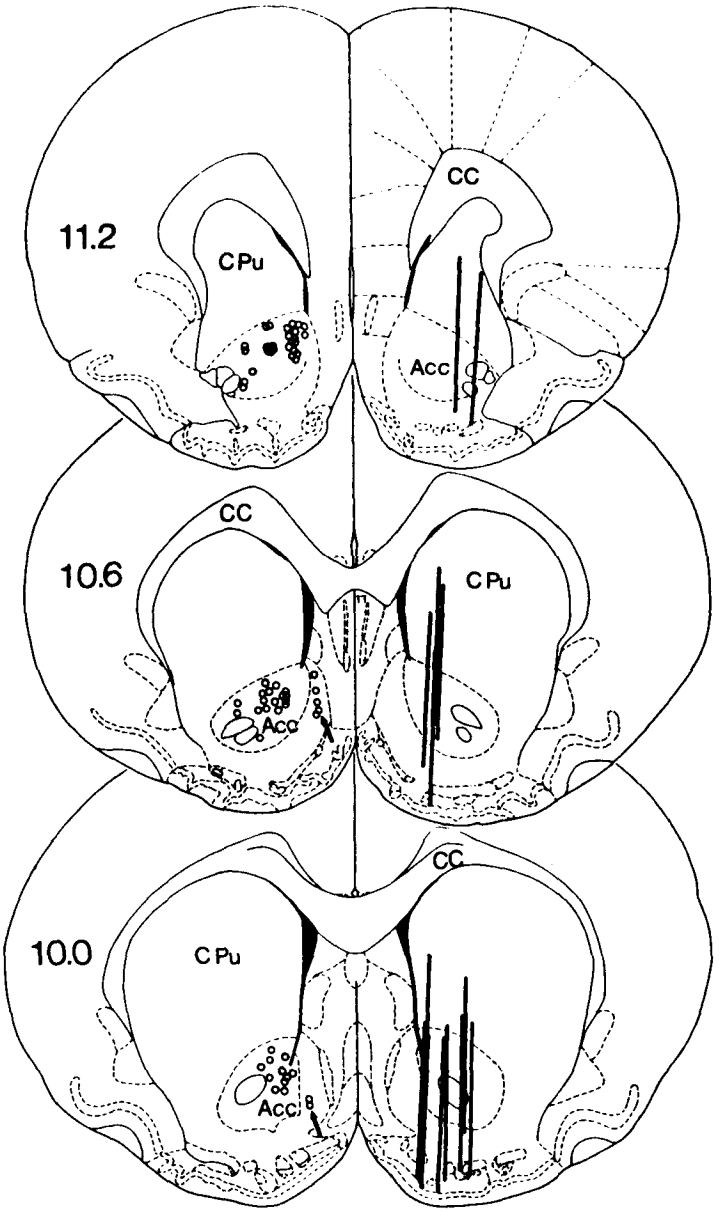

Fig. 8. Locations of recording sites (indicated by small circles on the left) and dialysis probes (indicated by bars on the right) within the nucleus accumbens and adjacent ventral striatum. Coronal sections were modified from Paxinos and Watson.[21] Numbers on the left indicate the locations of the sections (in mm) anterior to the interaural line. Small arrows in the middle and bottom sections point toward recording sites within the nucleus accumbens shell. Most recording sites were within the core region. Acc, nucleus accumbens; CC, corpus callosum; CPu, caudate–putamen.

5-HT antagonists are required to verify whether part of the MDMA-induced inhibition is mediated by increased extracellular levels of 5-HT because combined PCPA and AMPT pretreatment tends to deplete DA more than pretreatment with AMPT alone.[33]

Several differences in neurotransmitter content and anatomical connections suggest that the core and shell regions of the nucleus accumbens may mediate different functions.[4] Although the number of cells sampled from the shell region was very small in the present study, the shell contained two of the three cells that were not inhibited by MDMA application. Whether this observation reflects a true regional difference in MDMA effects in the nucleus accumbens remains to be determined by recording from a larger sample of cells in the shell. However, the predominant effect of MDMA applied locally in both the shell and the core was to inhibit glutamate-evoked neuronal firing. A recent abstract reported that intravenous injections of MDMA inhibited spontaneous firing of nucleus accumbens cells.[2] The results of the

present study suggest that systemically administered MDMA may inhibit accumbens neuronal excitability, at least in part, by local action within the accumbens.

MDMA infusion directly into the nucleus accumbens via a dialysis probe caused a detectable increase in extracellular DA levels that was somewhat delayed compared to the increase in extracellular 5-HT. However, the partial antagonism of MDMA-induced inhibition of accumbens neuronal excitability that was produced by the DA antagonist SCH39166 suggests that locally applied MDMA may rapidly increase DA by small amounts in the accumbens, with large increases developing more slowly. The lowest concentration of MDMA that was effective for increasing extracellular 5-HT in the present study was at least as effective for increasing extracellular DA, and the highest dose of MDMA increased DA several fold more than 5-HT. These findings are similar to those reported by Gough *et al.*[7] for changes in extracellular DA and 5-HT measured in dialysate samples from the caudate nucleus of freely moving rats following intraperitoneal injections of MDMA. In contrast, MDMA has been reported to be a more potent releaser of 5-HT than DA from striatal slices and from brain synaptosomes.[25] These discrepancies are most likely due to the altered conditions of the *in vitro* slice and synaptosome preparations, and illustrate the importance of comparing *in vitro* findings with measures made *in vivo*.

The increase in extracellular DA that was observed following local infusion of MDMA into the nucleus accumbens and adjacent ventral striatum in the present study extends previous observations that DA levels increased in the nucleus accumbens and the caudate nucleus following systemic application of MDMA.[15,37] Locally infused MDMA also increased extracellular 5-HT in the nucleus accumbens in the present study, as has been reported previously for amphetamine[9] and ethanol.[38] These observations strengthen the hypothesis that both 5-HT and DA may play a role in the rewarding effects of abused drugs.[9]

Local application of 5-HT itself has been reported to increase extracellular DA in the nucleus accumbens.[10,20] The mechanisms by which MDMA increased extracellular DA and 5-HT in the nucleus accumbens were not examined in the present study. However, both 5-HT and MDMA have been reported to increase DA release, at least in part, by acting on a DA transport mechanism,[10,15] whereas MDMA was found to induce 5-HT release by interacting with 5-HT transporters.[24] The delay in extracellular DA increase compared to 5-HT following the onset of MDMA infusion that was seen in the present study suggests the possibility that at least part of the DA release may have resulted from increased extracellular 5-HT.

CONCLUSIONS

Local application of MDMA inhibited glutamate-evoked firing of nucleus accumbens cells. The partial antagonism of this inhibition by the D_1 DA antagonist SCH39166[3] and the catecholamine synthesis inhibitor AMPT suggests that the MDMA effect was mediated at least partially by DA. The enhanced antagonism of the MDMA effect that was produced by AMPT combined with the 5-HT synthesis inhibitor PCPA suggests that part of the MDMA effect may also be mediated by 5-HT. Extracellular levels of both DA and 5-HT were elevated in the nucleus accumbens and adjacent ventral striatum following local application of MDMA, leading further support to the hypothesis that both monoamines mediate MDMA-induced inhibition in the nucleus accumbens, and that both may play a role in the rewarding effects of this recreationally used drug.

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REFERENCES

1. Akaike A., Sasa M. and Takaori S. (1983) Effects of haloperidol and sulpiride on dopamine-induced inhibition of nucleus accumbens neurons. *Life Sci.* **32,** 2649–2653.
2. Ashby C. R. Jr., Minabe Y., Toor A., Emori K. and Wang R. Y. (1993) The effect of (+)-, (−)- and (±)-methylenedioxymethamphetamine on the activity of A10 dopamine neurons in the ventral tegmental area and A10 target neurons in the nucleus accumbens: an electrophysiological study. *Soc. Neurosci. Abstr.* **19,** 828.
3. Chipkin R. E., Iorie L. C., Coffin V. L., McQuade R. D., Berger J. G. and Barnett A. (1988) Pharmacological profile of SCH39166: a dopamine D_1 selective benzonaphthazepine with potential antipsychotic activity. *J. Pharmac. exp. Ther.* **247,** 1093–1102.
4. Deutsch A. Y. and Cameron D. S. (1992) Pharmacological characterization of dopamine systems in the nucleus accumbens core and shell. *Neuroscience* **46,** 49–56.
5. Di Chiara G. and Imperato A. (1988) Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proc. natn. Acad. Sci. U.S.A.* **85,** 5274–5278.
6. Fitzgerald J. L. and Reid J. J. (1990) Effects of methylenedioxymethamphetamine on the release of monoamines from rat brain slices. *Eur. J. Pharmac.* **191,** 217–220.
7. Gough B., Ali S. F., Slikker W. and Holson R. R. (1991) Acute effects of 3,4-methylenedioxymethamphetamine (MDMA) on monoamines in rat caudate. *Pharmac. Biochem. Behav.* **39,** 619–623.

8. Hekmatpanah C. R. and Peroutka S. J. (1990) 5-Hydroxytryptamine uptake blockers attenuate the 5-hydroxytryptamine-releasing effect of 3,4-methylenedioxymethamphetamine and related agents. *Eur. J. Pharmac.* **177,** 95–98.
9. Hernandez L., Lee F. and Hoebel B. G. (1987) Simultaneous microdialysis and amphetamine infusion in the nucleus accumbens and striatum of freely moving rats: increase in extracellular dopamine and serotonin. *Brain Res. Bull.* **19,** 623–628.
10. Jacocks H. M. and Cox B. M. (1992) Serotonin-stimulated release of [^{3}H]dopamine via reversal of the dopamine transporter in rat straitum and nucleus accumbens: a comparison with release elicited by potassium, *N*-methyl-D-aspartic acid, glutamic acid and D-amphetamine. *J. Pharmac. exp. Ther.* **262,** 356–364.
11. Johnson M. P., Hoffman A. J. and Nichols D. E. (1986) Effects of the enantiomers of MDA, MDMA and related analogues on [^{3}H]serotonin and [^{3}H]dopamine release from superfused rat brain slices. *Eur. J. Pharmac.* **132,** 269–276.
12. Lavoie B. and Parent A. (1990) Immunohistochemical study of the serotoninergic innervation of the basal ganglia in the squirrel monkey. *J. comp. Neurol.* **299,** 1–16.
13. McKenna D. J., Guan X.-M. and Shulgin A. T. (1991) 3,4-Methylenedioxyamphetamine (MDA) analogues exhibit differential effects on synaptosomal release of ^{3}H-dopamine and ^{3}H-5-hydroxytryptamine. *Pharmac. Biochem. Behav.* **38,** 505–512.
14. McKenna D. J. and Peroutka S. J. (1990) Neurochemistry and neurotoxicity of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy"). *J. Neurochem.* **54,** 14–22.
15. Nash J. F. and Brodkin J. (1991) Microdialysis studies on 3,4-methylenedioxymethamphetamine-induced dopamine release: effect of dopamine uptake inhibitors. *J. Pharmac. exp. Ther.* **259,** 820–825.
16. Nichols D. E., Lloyd D. H., Hoffman A. J., Nichols M. B. and Yim G. K. W. (1982) Effects of certain hallucinogenic amphetamine analogues on the release of [^{3}H]serotonin from rat brain synaptosomes. *J. med. Chem.* **25,** 530–535.
17. North R. A. and Uchimura N. (1989) 5-Hydroxytryptamine acts as 5-HT$_2$ receptors to decrease potassium conductance in rat nucleus accumbens neurones. *J. Physiol., Lond.* **417,** 1–12.
18. O'Hearn E., Battaglia G., DeSouza E. B., Kuhar M. J. and Molliver M. E. (1988) Methylenedioxyamphetamine (MDA) and methylenedioxymethamphetamine (MDMA) cause selective ablation of serotonergic axon terminals in forebrain: immunocytochemical evidence for neurotoxicity. *J. Neurosci.* **8,** 2788–2803.
19. Pan H. S. and Wang R. Y. (1991) The action of MDMA on medial prefrontal cortical neurons is mediated through the serotonergic system. *Brain Res.* **543,** 56–60.
20. Parsons L. H. and Justice J. B. Jr (1993) Perfusate serotonin increases extracellular dopamine in the nucleus accumbens as measured by *in vivo* microdialysis. *Brain Res.* **606,** 195–199.
21. Paxinos G. and Watson C. (1986) *The Rat Brain in Stereotaxic Coordinates.* Academic Press, New York.
22. Phillips A. G. (1984) Brain reward circuitry: a case for separate systems. *Brain Res. Bull.* **12,** 195–201.
23. Robinson T. E. and Whishaw I. Q. (1988) Normalization of extracellular dopamine in striatum following recovery from a partial unilateral 6-OHDA lesion of the substantia nigra: a microdialysis study in freely moving rats. *Brain Res.* **450,** 209–224.
24. Rudnick G. and Wall S. C. (1992) The molecular mechanism of "ecstasy" [3,4-methylenedioxymethamphetamine (MDMA)]: serotonin transporters are targets for MDMA-induced serotonin release. *Proc. natn. Acad. Sci. U.S.A.* **89,** 1817–1821.
25. Schmidt C. J., Levin J. A. and Lovenberg W. (1987) *In vitro* and *in vivo* neurochemical effects of methylenedioxymethamphetamine on striatal monoaminergic systems in the rat brain. *Biochem. Pharmac.* **36,** 747–755.
26. Sorg B. A. and Kalivas P. W. (1991) Effects of cocaine and footshock stress on extracellular dopamine levels in the ventral striatum. *Brain Res.* **559,** 29–36.
27. Sprouse J. S., Bradberry C. W., Roth R. H. and Aghajanian G. K. (1989) MDMA (3,4-methylenedioxymethamphetamine) inhibits the firing of dorsal raphe neurons in brain slices via release of serotonin. *Eur. J. Pharmac.* **167,** 375–383.
28. Steinbusch H. W. M. (1981) Distribution of serotonin-immunoreactivity in the central nervous system of the rat: cell bodies and terminals. *Neuroscience* **6,** 557–618.
29. Uchimura N., Higashi H. and Nishi S. (1986) Hyperpolarizing and depolarizing actions of dopamine via D-1 and D-2 receptors on nucleus accumbens neurons. *Brain Res.* **375,** 368–372.
30. Ungerstedt U. (1971) Stereotaxic mapping of the monoamine pathways in the rat brain. *Acta physiol. scand.* **82,** Suppl. 367, 1–48.
31. Wang R. Y. and Hu X.-T. (1986) Does cholecystokinin potentiate dopamine action in the nucleus accumbens? *Brain Res.* **380,** 363–367.
32. White F. J. (1986) Comparative effects of LSD and lisuride: clues to specific hallucinogenic drug actions. *Pharmac. Biochem. Behav.* **24,** 365–379.
33. White F. J., Xiu-Ti H. and Henry D. J. (1993) Electrophysiological effects of cocaine in the rat nucleus accumbens: microiontophoretic studies. *J. Pharmac. exp. Ther.* **266,** 1075–1084.
34. Wilson M. A., Ricaurte G. A. and Molliver M. E. (1989) Distinct morphologic classes of serotonergic axons in primates exhibit differential vulnerability to the psychotropic drug 3,4-methylenedioxymethamphetamine. *Neuroscience* **28,** 121–137.
35. Wise R. A. and Bozarth M. A. (1984) Brain reward circuitry: four circuit elements "wired" in apparent series. *Brain Res. Bull.* **12,** 203–208.
36. Woodruff G. N., McCarthy P. S. and Walker R. J. (1976) Studies on the pharmacology of neurones in the nucleus accumbens of the rat. *Brain Res.* **115,** 233–242.
37. Yamamoto B. K. and Spanos L. J. (1988) The acute effects of methylenedioxymethamphetamine on dopamine release in the awake-behaving rat. *Eur. J. Pharmac.* **148,** 195–203.
38. Yoshimoto K., McBride W. J., Lumeng L. and Li T.-K. (1991) Alcohol stimulates the release of dopamine and serotonin in the nucleus accumbens. *Alcohol* **9,** 17–22.

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