

Mice with Decreased Cerebral Dopamine Function following a Neurotoxic Dose of MDMA (3,4-Methylenedioxymethamphetamine, "Ecstasy") Exhibit Increased Ethanol Consumption and Preference

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ABSTRACT

MDMA (3,4-methylenedioxymethamphetamine, "ecstasy") administration to mice produces relatively selective long-term neurotoxic damage to dopaminergic pathways. There is strong evidence indicating that the dopamine system plays a key role in the rewarding effects of ethanol and modulates ethanol intake. Using a two-bottle free-choice paradigm, we examined the voluntary consumption and preference for ethanol in mice deficient in cerebral dopamine concentration and dopamine transporter density by previous repeated MDMA administration. The current study shows that mice pre-exposed to a neurotoxic dose of MDMA exhibited a higher consumption of and preference for ethanol compared with saline-treated animals. The D₁ receptor full agonist SKF81297 [(6-chloro-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrobromide)] attenuated the enhanced ethanol intake, an effect that was reversed by SCH23390 [(R)-(+)-7-chloro-8-hydroxy-

3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride], a D₁ receptor antagonist. MDMA-exposed mice also showed a reduced release of basal dopamine in the nucleus accumbens compared with saline-injected animals and a modest increase in D₁ receptor density in caudate-putamen and nucleus accumbens. Intraperitoneal administration of ethanol elevated extracellular dopamine release in the nucleus accumbens of saline-treated mice, but this effect was almost abolished in MDMA-treated mice. Differences between saline- and MDMA-treated animals did not appear to be secondary to changes in acute ethanol clearance. These results indicate that mice with reduced dopamine activity following a neurotoxic dose of MDMA exhibit increased ethanol consumption and preference and suggest that animals might need to consume more alcohol to reach the threshold for the rewarding effects of ethanol.

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ABBREVIATIONS: MDMA, 3,4-methylenedioxymethamphetamine, ecstasy; WIN 35,428, (–)2β-carbomethoxy-3 β-(4-fluorophenyl)tropane; SKF81297, (6-chloro-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrobromide); SCH23390, ((R)-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride); DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; YM09151-2, *cis-N*-[1-benzyl-2-methyl-pyrrolidin-3-yl]-5-chloro-2-methoxy-4-methylaminobenzamide; ANOVA, analysis of variance; EtOH, ethanol; NAc, nucleus accumbens.

There is strong evidence for an involvement of the dopamine system in some of the central effects of ethanol at cellular and molecular levels and also at the behavioral level. Ethanol increases the firing rate of dopamine neurons of the ventral tegmental area of mice (Brodie, 2002) and dopamine release in the nucleus accumbens after central administration in rats (Tuomainen et al., 2003). Ethanol is self-administered into the ventral tegmental area (Gatto et al., 1994), which supports a role for dopamine in the rewarding properties of ethanol and, therefore, in ethanol consumption. In a study that compared 15 inbred mouse strains, the C57BL/6J strain displayed the highest ethanol consumption and preference (Belknap et al., 1993), and this effect seemed to be associated with low nigrostriatal/mesolimbic dopaminergic activity (George et al., 1995). Dopamine agonists reduce ethanol self-administration in this strain of mice (Ng and George, 1994) and in the high-alcohol-drinking line of rats (Dyr et al., 1993). In line with these findings, several genetic manipulations in mice that produce a functional hypodopaminergic state also cause an increase in ethanol intake (Naassila et al., 2002). However, there are other studies that do not support this hypothesis. For example, ethanol self-administration is reduced in D_1 or D_2 receptor-deficient mice (El-Ghundi et al., 1998; Phillips et al., 1998), and dopamine antagonists either do not modify (Czachowski et al., 2002) or decrease (Czachowski et al., 2001) ethanol intake.

These lines of evidence have led us to hypothesize that exposure to a neurotoxic dose of MDMA might critically influence the regulation of alcohol drinking behavior. To this end, we studied the voluntary consumption and preference for ethanol in mice deficient in dopamine concentration and dopamine transporter by previous repeated MDMA administration. The ability of D_1 dopaminergic agents to modulate ethanol intake and preference in MDMA-lesioned mice was also evaluated. A second purpose was to determine the *in vivo* release of dopamine in the nucleus accumbens induced by an acute dose of ethanol to evaluate the consequences of an MDMA-induced dopaminergic lesion in an area involved in the rewarding properties of ethanol.

Materials and Methods

Animals, Drug Administration, and Experimental Protocol. Adult male C57BL/6J mice (Harlan Iberica, Barcelona, Spain) initially weighing 20 to 25 g were housed in groups of 10 in standard cages (38 cm long \times 22 cm wide \times 15 cm high), in conditions of constant temperature ($21 \pm 2^\circ\text{C}$) and a 12-h light/dark cycle (lights on, 7:00 AM), and given free access to food and water. Mice were randomly assigned to two treatment groups. Group I was injected with saline, whereas group II received MDMA (30 mg/kg *i.p.* three times with a 3-h interval). Seven days later, mice were given access to voluntary consumption of ethanol. The protocol of MDMA administration used in this study induces neurotoxicity in dopamine nerve terminals 7 days later (Escobedo et al., 2005).

(\pm)-MDMA-HCl was obtained from Ultrafine Chemicals Ltd. (Manchester, UK), dissolved in 0.9% w/v NaCl (saline), and injected in a volume of 10 ml/kg. Doses are quoted in terms of the base.

Ethanol-drinking solutions were made up of absolute ethanol aditivo (Panreac, Barcelona, Spain) and diluted with tap water to the different concentrations. For injections, absolute ethanol was diluted with 0.9% saline to a 20% (w/v) solution.

Quinine and sucrose were obtained from Sigma-Aldrich (Madrid, Spain) and were dissolved in tap water. [^3H]WIN 35,428 was purchased from PerkinElmer Life and Analytical Sciences (Boston, MA).

Separate groups of mice were used for each of the behavioral tests and neurochemical studies. All experimental procedures were carried out at a room temperature of $21 \pm 2^\circ\text{C}$ and performed in accordance with the guidelines of the Animal Welfare Committee of the Complutense University (following DC86/609/EU).

Ethanol Intake Test. Voluntary ethanol consumption and preference were examined using a two-bottle free-choice paradigm (Naassila et al., 2002). Fluid was supplied by means of plastic serological pipettes of 25 ml graduated at 0.2-ml intervals [construction of the drinking tubes followed the method by Tordoff and Bachmanov (2005) Monell Mouse Taste Phenotyping Project: Construction of Drinking Tubes, <http://www.monell.org/MMPPTP/>].

Three days after MDMA or saline administration, mice were individually housed in polycarbonate mouse cages (type 2, 22.0 cm long \times 16.5 cm wide \times 14 cm high, floor space 363 cm², EH-II-1264; EHRET GmbH and Co. KG Labor und Pharmatechnik, Emmendingen, Germany), with standard rodent chow available *ad libitum*, and habituated to drinking for 4 days from two pipettes of 25 ml, containing plain water, placed on the stainless steel cage top. Saline (group I) and MDMA-treated (group II) mice were then each divided into two groups (a and b). Groups Ia and IIa were given access to two pipettes containing plain water, whereas for groups Ib and IIb, one pipette contained plain water and the other ethanol in water. The ethanol concentration (v/v) was increased every 7 days, and the mice received solutions containing 3, 6, 10, and finally 20% ethanol over the course of the experiment. All drinking solutions were renewed every 2 days, at which time the position of the bottles was changed to avoid side preferences. Fluid intake was determined daily. Water consumption was calculated throughout the experiment. To obtain a measure of ethanol consumption that corrected for individual differences in mouse size, grams of ethanol consumed per kilogram of body weight per day were calculated for each mouse every day. Average ethanol consumption per day was calculated for each ethanol concentration. The ethanol preference ratio was calculated for each ethanol concentration as volume of ethanol solution consumed per total volume of fluid (water plus ethanol solution) consumed. On days 4 and 7 of each 7-day interval, food intake (grams per kilogram) and body weight were assessed. Cages were cleaned at 7-day intervals in the middle of each ethanol block to avoid interaction with changes in ethanol concentrations.

Pharmacological experiments with the D_1 receptor full agonist SKF81297 (1 mg/kg *i.p.*) and the D_1 receptor antagonist SCH23390 (100 $\mu\text{g}/\text{kg}$ *i.p.*) were performed to address the central hypothesis that dopamine depletion is responsible for the observed changes in ethanol consumption in MDMA-lesioned mice. In these experiments mice received solutions containing 3% (for 7 days), 6% (for 7 days), and 10% (for 14 days) ethanol. SKF81297 (1 mg/kg *i.p.*) was given daily during the 1st week of 10% ethanol exposure, and both SCH23390 (100 $\mu\text{g}/\text{kg}$ *i.p.*) and SKF81297 were given daily during the 2nd week of 10% ethanol exposure. Both compounds were dissolved in saline and injected in a volume of 10 ml/kg 30 min (SCH23390) and immediately (SKF81297) before the onset of the dark/light cycle. Control mice received saline. The doses of dopamine agents used have been shown previously to be efficacious for studying dopamine mediated behaviors in mice (Liu and Weiss, 2002).

Sucrose and Quinine Consumption Test. Three days after MDMA or saline administration, mice were habituated in their home cage to drinking from two pipettes containing water for 4 days and were then given plain water in one pipette and sucrose or quinine in the other pipette, using a protocol similar to that described for ethanol consumption. Four ascending sucrose concentrations were tested, 0.017, 0.17, 1.7, and 4.25%, as well as two quinine concentrations, 0.03 and 0.1 mM, in these two-bottle preference tests according to previously described protocols (Naassila et al., 2002). The mice had 4 days of access to each solution. The preference for each solution

was calculated by dividing the volume of the taste solution consumed by the total volume of fluid (taste solution plus water) consumed.

Measurement of Locomotor Activity. There is a correlation between sensitization to the locomotor effects and the intake of ethanol (Lessov et al., 2001). To discard that a sensitization to ethanol effects could be responsible for the increased ethanol consumption, locomotor activity was performed 3 weeks after MDMA, the time at which ethanol consumption in MDMA-treated mice was higher than in those injected with saline. The test was performed in an open arena (26 × 21, 10 cm high) with bottom and sides made of methacrylate covered with a brown nonreflecting material. The behavior of the mice was recorded in eight arenas run in parallel using a video camera (Sony model CCD-IRIS with a 1:1.4 lens; Sony, Tokyo, Japan) placed above the arenas and connected to a video monitor (Sony model PVM-145E) and recorder (Panasonic model AG-5700; Panasonic, Barcelona, Spain). Diffuse lighting was used to minimize shadows in the arenas. The mice were placed in the experimental room the day before testing. On the day of the test, saline or MDMA-treated mice received an i.p. injection of 1 g/kg ethanol [20% (w/v) in saline] and immediately placed in the arena. Their behavior was video-tracked for 30 min, after which the mice were returned to their home cages. Testing was conducted between 10:00 AM and 12:00 PM.

Automated analysis of the videotapes was conducted off-line by the EthoVision program (version 1.70; Noldus Information Technologies, Barcelona, Spain). Each 30-min observation period on the videotapes was analyzed by the EthoVision program. This analysis resulted in a track record for each mouse that contained a complete record of the mouse movement pattern in the arena during the observation period. The EthoVision program determines the distance traveled (centimeters) during the total observation period and in 10-min intervals.

Light-Dark Box Test. The light-dark box test was performed 3 weeks after MDMA and saline administration to determine whether MDMA-pretreated mice show an anxiety-like behavior. It is based on the innate aversion of rodents to brightly illuminated places and on the spontaneous exploratory behavior (tendency) in response to a novel environment (Crawley and Goodwin, 1980). Drugs that increase the time spent in the light compartment and decrease the latency to the first transition are considered anxiolytic, whereas drugs that produce the reverse pattern are considered anxiogenic. The light-dark box was located in a dark room and consisted of two compartments, dark (made of black opaque PVC, 20 × 20 × 14 cm) and light (made of methacrylate, 20 × 20 × 14 cm) connected by a tunnel (5 × 7 × 10 cm). A 60-W white light was placed 25 cm above the floor to provide direct illumination to the light compartment. At the beginning of the session, the mouse was individually placed in the dark compartment with its head facing away from the opening, and behavior was recorded by videotape for 5 min. The latency to the first transition, the total time spent in each compartment, and the number of transitions between the two compartments were scored. The mouse was considered to enter a specific compartment when both front and hind paws were inside the chamber.

Plasma Ethanol Concentration. To determine whether MDMA-pretreated mice metabolized ethanol differently to controls, 7 days after MDMA and saline administration, mice were administered an i.p. injection of ethanol [3 g/kg, 20% (w/v) in saline]. Samples of 20 μ l of blood were collected from the tail in heparinized capillary tubes between 30 and 270 min after injection. At the end of collection, samples were centrifuged for 6 min (Microcentrifuge MK5, model 01400-00; Analox, London, UK) and injected into an analyzer (AM1; Analox). The rationale of the method consists of ethanol being oxidized by the enzyme alcohol oxidase in the presence of molecular oxygen; therefore, the rate of oxygen consumption is directly proportional to alcohol concentration. Plasma ethanol levels were calculated as milligrams per deciliter using 300 mg/dl ethanol as standard.

Implantation of a Microdialysis Probe in the Nucleus Accumbens. Six days after MDMA and saline administration, mice were anesthetized with pentobarbitone (Euta-Lender, 40 mg/kg) and secured in a Kopf stereotaxic frame (David Kopf Instrument, Tujunga, CA) coupled to a Kopf mouse adapter with the tooth bar at -3.3 mm below the interaural zero. A guide cannula was implanted just above the nucleus accumbens shell according to the following coordinates: +1.5 mm from bregma, -0.6 mm mediolateral, and 4 mm below the skull (Franklin and Paxinos, 1997). Twenty-four hours later, the dialysis probes (membrane length, 1.0 mm × 240 μ m; CMA/7; CMA Microdialysis AB, Solna, Sweden) were inserted in the guide cannulae such that the membrane protruded its full length from the end of the probe into the nucleus accumbens shell. Microdialysis experiments were conducted in awake animals.

Measurement of Dopamine and Metabolites in the Striatal Dialysate and Tissue. Twenty-four hours after implantation, probes were perfused with artificial cerebrospinal fluid (2.5 mM KCl, 125 mM NaCl, 1.18 mM MgCl₂·6H₂O, 1.26 mM CaCl₂·2H₂O) at a rate of 1 μ l/min and samples collected from the freely moving animals at 30-min intervals in tubes containing 5 μ l of a solution composed of HClO₄ (0.01 M), cysteine (0.2%), and sodium metabisulfite (0.2%). The first 60-min sample after probe insertion was discarded, and the next three 30-min baseline samples were collected. After injection, samples were collected every 30 min for 5 h.

Striatal catechol concentration was always evaluated at the end of each experimental protocol to confirm dopamine neurotoxicity and specifically checked after the 4 weeks of ethanol exposure to determine the long-lasting depletion of dopamine concentration exerted by MDMA and changes induced by ethanol intake. The mice were killed by cervical dislocation and decapitation, and the brains were rapidly removed and the striatum dissected out on ice.

Dopamine and the metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), were measured by high-performance liquid chromatography and electrochemical detection. The mobile phase consisted of KH₂PO₄ (0.05 M), octanesulfonic acid (0.4 mM), EDTA (0.1 mM), and methanol (16%) and was adjusted to pH 3 with phosphoric acid, filtered, and degassed. The flow rate was 1 ml/min. The high-performance liquid chromatography system consisted of a pump (Waters 510) linked to an automatic sample injector (Loop 200 μ l, Waters 717 plus Autosampler), and a stainless steel reversed-phase column (Spherisorb ODS2, 5 μ m, 150 × 4.6 mm; Waters, Milford, MA) with a precolumn and a coulometric detector (Coulchem II; Esa, Chelmsford, MA). The working electrode potential was set at 400 mV with a gain of 1 μ A (for dopamine) and 500 nA (for the remaining compounds). The current produced was monitored by means of integration software (Unipoint; Gilson, Villier Le Bel, France).

[³H]WIN 35,428 Binding in Tissue Homogenates. [³H]WIN 35,428 binding was measured by modification of the method described in detail by Segal et al. (2003). Four weeks after ethanol exposure, animals were killed, and the brain was rapidly removed and dissected on ice within 2 min. Striata from individual animals were sonicated in ice-cold sodium phosphate buffer (20 mM; pH 7.4) containing sucrose (0.32 M). The homogenate was centrifuged at 30,000g for 15 min at 4°C. The supernatant was discarded, and the wash procedure was repeated twice more. The pellet was finally resuspended in 60 volumes of homogenization buffer. The assay solution (500 μ l) contained [³H]WIN 35,428 (5 nM), desipramine (300 nM), and 100 μ l of tissue preparation (approximately 80 μ g of protein). Nonspecific binding was carried out in the presence of cocaine (30 μ M). The reaction mixture was incubated for 90 min at 4°C. The assay was terminated by rapid filtration, and radioactivity was counted by scintillation spectrometry.

Quantitative Autoradiography of D₁ and D₂ Dopamine Receptor. One and 4 weeks after MDMA injection, brains were rapidly removed and frozen by slow immersion in isopentane cooled on dry ice. Frozen tissues were kept at -80°C until the day of the assays.

Twenty-micrometer brain coronal sections were taken serially through the caudate-putamen (from 1.0 mm anterior to bregma) and the shell and core of nucleus accumbens (from 1.78 mm anterior to bregma) according to the mouse brain atlas of Franklin and Paxinos (1997) using a Microm cryostat at -20°C . The brain sections were mounted onto gelatin-coated slides and stored at -80° until the day of the assay. Triplicate tissue sections of each brain level were incubated with either 1 nM [^3H]SCH23390 (D_1 receptors) or 1 nM [^3H]YM09151-2 (D_2 receptors) (PerkinElmer Life and Analytical Sciences) in 50 mM Tris-HCl buffer, pH 7.4, containing 120 mM NaCl, 5 mM KCl, 1.5 mM CaCl_2 , and 4 mM MgCl_2 at room temperature for 60 min. Nonspecific binding was determined in the presence of 20 μM SCH23390 (D_1) or 45 μM butaclamol. After the incubation, sections were dipped quickly into 50 mM Tris-HCl incubation buffer ($0-4^{\circ}\text{C}$), dipped quickly again into H_2O_2 water, and blown dry under cold air. After both D_1 and D_2 binding assays, sections were placed in X-ray cassettes with standard ^3H microscales (GE Healthcare, Little Chalfont, Buckinghamshire, UK) and apposed to [^3H]Hyperfilm film (GE Healthcare). After 8 weeks of exposure at 4 to 8°C , films were developed in D-19 (Eastman Kodak, Rochester, NY), fixed, and dried. All data presented were analyzed from resultant autoradiographic images. Three slides per level (three slices/slide; two measurements/slice) in each animal were analyzed with a personal computer using the public domain NIH Image software.

Statistics. Data from water and food consumption, weight gain, locomotor activity, and the effect of the D_1 agonist and antagonist on ethanol consumption and preference were analyzed using one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test where significant differences occurred. The same method was used to compare data from dopamine transporters, dopamine levels, and D_1 and D_2 receptors. Statistical analyses of ethanol consumption, preference measurements, ethanol plasma levels, and dialysis were performed using the statistical computer package BMDP/386 Dynamic (BMDP Statistical Solutions, Cork, UK). Data were analyzed by ANOVA with repeated measures (program 2V), or, where missing values occurred, an unbalanced repeated measure model (program 5V) was used. Both used treatment as the between subjects factor and time as the repeated measure. ANOVA was performed on both pretreatment and post-treatment data. Student's *t* test for unpaired observations was used to compare basal dialysate dopamine levels in nucleus accumbens between saline- and MDMA-injected mice. Differences were considered significant at $P < 0.05$.

Results

Alcohol Consumption Test. Saline (control)- or MDMA-injected mice showed no side preference or difference in the volumes of fluid consumed from the two pipettes when they were offered continuous ad libitum access to two pipettes containing tap water for 4 weeks (saline-treated mice, $n = 9$, left pipette, 2.2 ± 0.14 ml/day; right pipette, 2.1 ± 0.14 ml/day; and MDMA-treated mice, $n = 9$, left pipette, 2.2 ± 0.09 ml/day; right pipette, 2.2 ± 0.12 ml/day). In addition, the total volume of water consumed daily by MDMA-exposed mice (4.4 ± 0.15 ml/day, $n = 9$) was similar to that measured in saline mice (4.3 ± 0.13 ml/day, $n = 9$). These side preference tests ensure that alcohol preference in control and MDMA-exposed mice are not influenced by side preference. In addition, they allow us to state that MDMA exposure does not produce an increase in fluid intake.

Figure 1 shows the intake and preference for ethanol of control and MDMA-exposed mice in the two-bottle free-choice procedure with increasing concentrations of ethanol. MDMA-pretreated mice showed increased consumption of ethanol solutions (Fig. 1a) and greater preference

ratios (Fig. 1b) compared with controls. Both saline- and MDMA-treated mice showed increasing preference with increasing ethanol concentration up to 10%, with the 20% solution presenting a lower preference that is consistent with an aversive component of taste at higher ethanol concentrations. Total (milliliters) and relative (milliliters per kilogram; Fig. 1c) fluid consumption was similar in the two groups, indicating that the increased ethanol consumption by MDMA-treated mice was not caused by an overall increase in total amount of fluid consumed. No significant loss or gain in body weight was observed during ethanol exposure. At the end of ethanol exposure (4 weeks), the gain in body weight (grams) for the different groups was as follows: saline treated-mice exposed to water, 5.5 ± 0.58 ($n = 7$) or ethanol, 5.9 ± 0.89 ($n = 9$); and MDMA-treated mice exposed to water, 6.9 ± 1.6 ($n = 9$) or ethanol, 5.2 ± 1.02 ($n = 10$). There was no difference in food consumption (grams per kilogram per day) among groups: saline treated-mice exposed to water, 129 ± 2.1 ($n = 9$) or ethanol, 126 ± 1.4 ($n = 10$); and MDMA-treated mice exposed to water, 134 ± 2.3 ($n = 9$) or ethanol, 123 ± 3.1 ($n = 9$).

Administration of SKF81297 (1 mg/kg) to MDMA-pretreated mice on each of the 7 days of 10% ethanol exposure significantly reduced ethanol consumption (Fig. 1d) and tended to reduce preference (Fig. 1e), although the effect was not significant. SKF81297 did not modify the relative liquid intake (Fig. 1f). SCH23390 (100 $\mu\text{g}/\text{kg}$) reversed the changes induced by the D_1 agonist SKF81297 (Fig. 1, d and e). SKF81297 and SCH23390 did not modify ethanol consumption (for SKF81297-treated versus nontreated mice, 6.2 ± 0.6 versus 6.4 ± 0.8 g/kg/day; for SCH23390-treated versus nontreated mice, 7.1 ± 0.7 versus 6.8 ± 0.7 g/kg/day; $n = 6-8$) or preference (for SKF81297-treated versus nontreated mice, 0.62 ± 0.06 versus 0.59 ± 0.07 g/kg/day; for SCH23390-treated versus nontreated mice, 0.67 ± 0.06 versus 0.61 ± 0.06 g/kg/day; $n = 6-8$) in nonlesioned mice.

Sucrose and Quinine Voluntary Consumption Tests. To determine whether differences in ethanol consumption and preferences might reflect changes in taste preferences or caloric needs caused by MDMA exposure, drinking studies with sucrose and quinine were performed. Figure 2a shows significant differences in sucrose preference across concentrations in control mice, the greater preference appearing across the two highest sucrose concentrations (1.7 and 4.25%). This pattern was not altered by MDMA exposure. As expected, preference for the highest concentration of quinine (0.1 mM) was significantly lower than that observed for 0.03 mM, the effect being similar in MDMA-injected mice (Fig. 2b).

Ethanol-Induced Locomotor Activity. Both MDMA- and saline-pretreated mice showed a reduction of locomotor activity in the second and third 10-min periods after saline injection and placement of the animals in the chamber area compared with the first 10-min period. This response reflects a drop in the initial exploratory behavior of the novelty of being placed in the arena (Fig. 3). Administration of ethanol increased the mean activity at the end of the second (40%) and third (80%) 10-min periods, respectively, the effect being similar in MDMA- and saline-pretreated mice (Fig. 3).

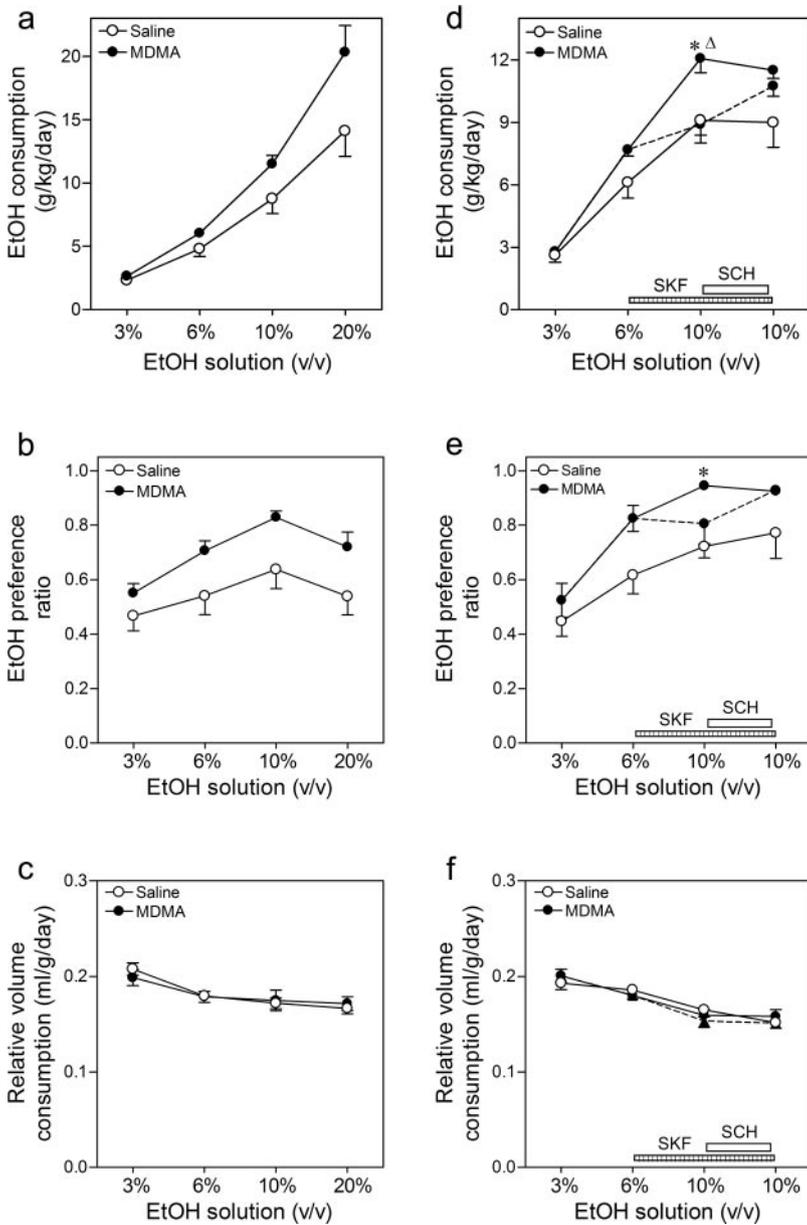


Fig. 1. Consumption (a) and preference (b) for ethanol (EtOH) and relative intake of fluid (c) in mice exposed to MDMA (30 mg/kg, three times every 3 h i.p.) or saline 7 days before starting the two-bottle free-choice paradigm. Mice had 7 days of access to each EtOH solution (3, 6, 10, and 20% EtOH). a, consumption (grams per kilogram per day) of each EtOH solution (average of 7 days). There was significant effect of pretreatment [$F(1,19) = 3.48, P < 0.05$] and of EtOH concentration [$F(3,57) = 76.16, P < 0.001$] but no significant interaction effect [$F(3,57) = 2.73, N.S.$]. b, preference ratio (volume of EtOH consumed/total volume of fluid consumed). There were significant effects of pretreatment [$F(1,19) = 6.45, P < 0.01$] and of EtOH concentration [$F(3,57) = 15.40, P < 0.001$] but no significant interaction effect [$F(3,57) = 0.85, N.S.$]. c, relative fluid intake (milliliters per gram per day) at each EtOH concentration (average of 7 days). Graphs on the right show consumption (d) and preference (e) for EtOH and relative intake of fluid (c) in mice pretreated with MDMA and saline as described above but exposed to 10% EtOH solution during 14 days either receiving saline or dopamine receptor ligands (dotted line) in the following order: the first 7 days receiving SKF81297 (1 mg/kg i.p. every day, lined bar) and the latter 7 days receiving both SCH23390 (100 μ g/kg i.p. every day) and SKF81297 (open bar). Results shown as mean \pm S.E.M., $n = 10$ to 11. Different from saline-pretreated mice: *, $P < 0.05$. Different from the MDMA-pretreated group injected with SKF81297: Δ , $P < 0.05$.

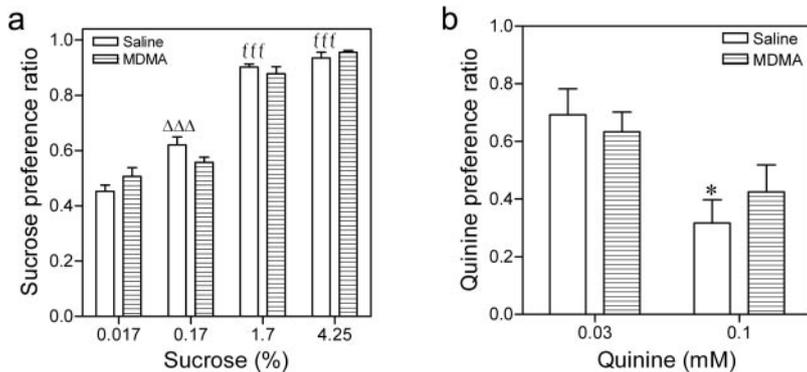


Fig. 2. Preference ratios for sucrose (a) and quinine (b) in mice exposed to MDMA or saline 7 days before starting the two-bottle free-choice paradigm. Mice had 4 days of access to each sucrose or quinine solution with an interval of 4 days in the highest sucrose concentration and the lowest quinine concentration. Results shown as mean \pm S.E.M., $n = 7$ to 10. Different from 0.017% sucrose consumption in saline-pretreated mice: $\Delta\Delta\Delta$, $P < 0.001$. Different from 0.17% sucrose consumption in saline-pretreated mice: fff , $P < 0.001$. Different from 0.03 mM quinine consumption in saline-pretreated mice: *, $P < 0.05$.

Light-Dark Box Test in MDMA-Lesioned Mice. There were no significant differences between saline- and MDMA-pretreated mice for any of the parameters measured. The latency until the first entry into the light compartment was

for saline-pretreated mice, 20 ± 2.9 s ($n = 16$), and for MDMA-pretreated mice, 21 ± 2.7 s ($n = 19$). The total time spent in the dark box was for saline-pretreated mice, 181 ± 10 s ($n = 16$), and for MDMA-pretreated mice, 183 ± 15 s

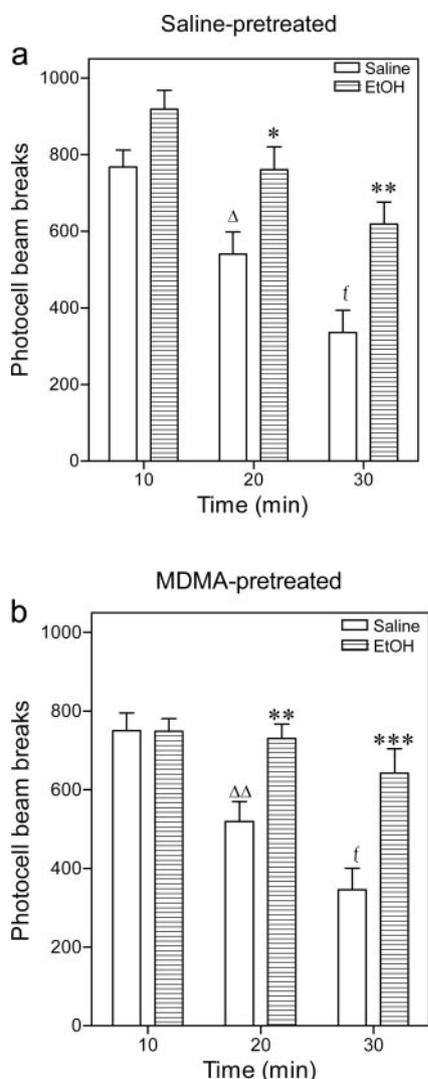


Fig. 3. Effect of EtOH (1 g/kg i.p.) on locomotor activity of mice exposed to MDMA or saline (a) or MDMA (b) 3 weeks before. Mice were placed in the locomotor activity chamber immediately after EtOH injection, and the total number of photocell beams breaks was counted for 30 min at 10-min intervals. MDMA- and saline-pretreated mice showed a progressive reduction in locomotor activity throughout the 30-min test period. EtOH administration increased locomotion 20 and 30 min after injection in both saline- and MDMA-pretreated mice. Each value is the mean \pm S.E.M., $n = 13$ to 16. Different from the corresponding saline group at the same interval: *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$. Different from the saline group at 10-min interval: Δ , $P < 0.05$; and $\Delta\Delta$, $P < 0.01$. Different from the saline group at 20-min interval: f , $P < 0.05$.

($n = 23$). The total time spent in the light box was for saline-pretreated mice, 119 ± 10 s ($n = 16$), and for MDMA-pretreated mice, 117 ± 10 s ($n = 23$). The total number of transitions was for saline-pretreated mice, 17 ± 1.3 ($n = 16$), and for MDMA-pretreated mice, 16 ± 1.2 ($n = 23$).

Ethanol-Induced Dopamine Release in Nucleus Accumbens. Basal extracellular levels of dopamine in the nucleus accumbens of MDMA-pretreated mice were lower than those observed in the saline group (Fig. 4). To compare the two curves independently of the difference in their basal values, post-treatment values were expressed as a percentage of basal values (Fig. 4, inset). Ethanol administration significantly increased dopamine levels in the dialysate of saline-pretreated mice, whereas it had no effect on MDMA-

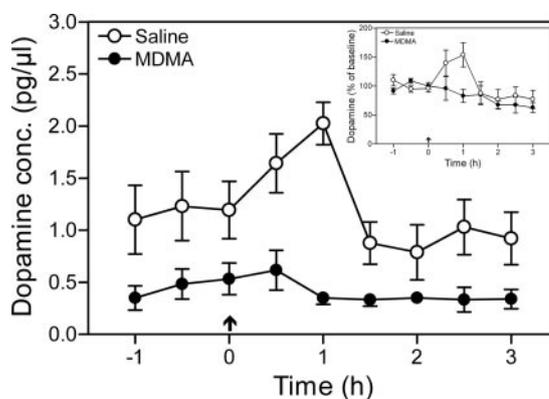


Fig. 4. Effect of EtOH (3 g/kg) on the extracellular levels of dopamine in the nucleus accumbens of mice that received MDMA or saline 7 days before. Basal values for extracellular dopamine, expressed as a mean of three measurements before EtOH administration, were higher ($P < 0.001$) in saline-treated mice (1.18 ± 0.04 pg/ μ l, $n = 3$) than in the MDMA group (0.45 ± 0.05 pg/ μ l, $n = 3$). To compare the two curves independently of the difference in their basal values, post-treatment values were expressed as percentage values of dopamine in dialysate with respect to basal samples (inset). EtOH produced an increase in dopamine levels in the dialysate of saline- but not MDMA-treated mice [$F(1,10) = 3.57$, $P < 0.05$]. Each value is the mean \pm S.E.M. of five to seven animals.

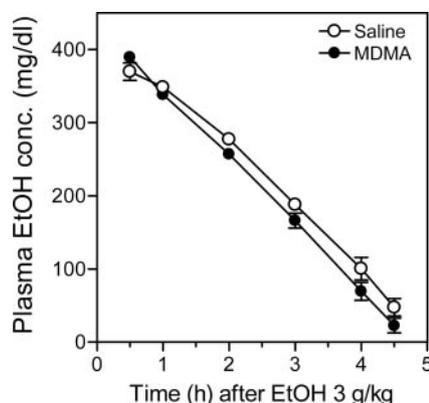


Fig. 5. Plasma EtOH concentration after EtOH injection (3 g/kg) in mice exposed to MDMA or saline 7 days before. Samples were collected from the tail up to 4.5 h after EtOH administration. Results shown as mean \pm S.E.M., $n = 5$.

pretreated mice (Fig. 4, inset). These differences in ethanol-induced dopamine release do not appear to be secondary to differences in acute ethanol clearance because plasma ethanol concentration for 4.5 h after 3 g/kg ethanol administration did not differ between saline- and MDMA-treated animals (Fig. 5).

D₁ and D₂ Receptor Density in the Nucleus Accumbens and Caudate-Putamen of MDMA-Lesioned Mice. Binding of [³H]SCH23390 to dopamine D₁ receptors significantly increased in the caudate-putamen and in the core of nucleus accumbens 7 days following MDMA compared with that observed in saline-injected mice (Fig. 6). There was also a trend toward increased D₁ density in the shell of nucleus accumbens, but this was not statistically significant (Fig. 6). Values had returned to baseline level 4 weeks after the treatment. No change was observed in [³H]YM09151-2 binding in either of the brain areas examined 1 or 4 weeks after MDMA injection (Fig. 6).

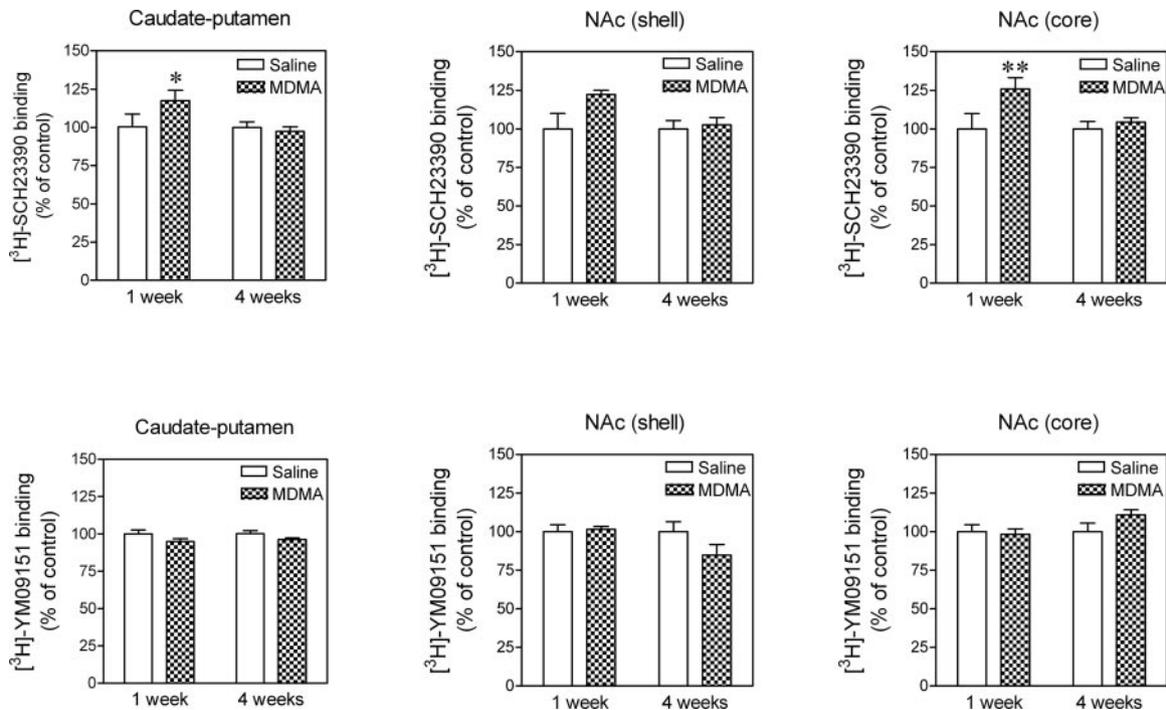


Fig. 6. Quantitative autoradiographic measurements of [^3H]SCH23390 binding to D_1 receptors and [^3H]YM09151-2 binding to D_2 receptors in caudate-putamen and in the shell and core of nucleus accumbens (NAc) of mice exposed to MDMA or saline 1 or 4 weeks before. Results shown as mean \pm S.E.M., $n = 5$ to 8. Different from the corresponding saline-pretreated mice: *, $P < 0.05$; and **, $P < 0.01$. Absolute values (femtomoles per milligram of tissue) for D_1 and D_2 receptor density were as follows: caudate-putamen, 172 ± 9 ($n = 14$) and 176 ± 3 (15), respectively; shell of NAc, 146 ± 8 ($n = 14$) and 137 ± 5 ($n = 13$), respectively; and core of NAc, 152 ± 8 ($n = 14$) and 127 ± 5 ($n = 15$), respectively.

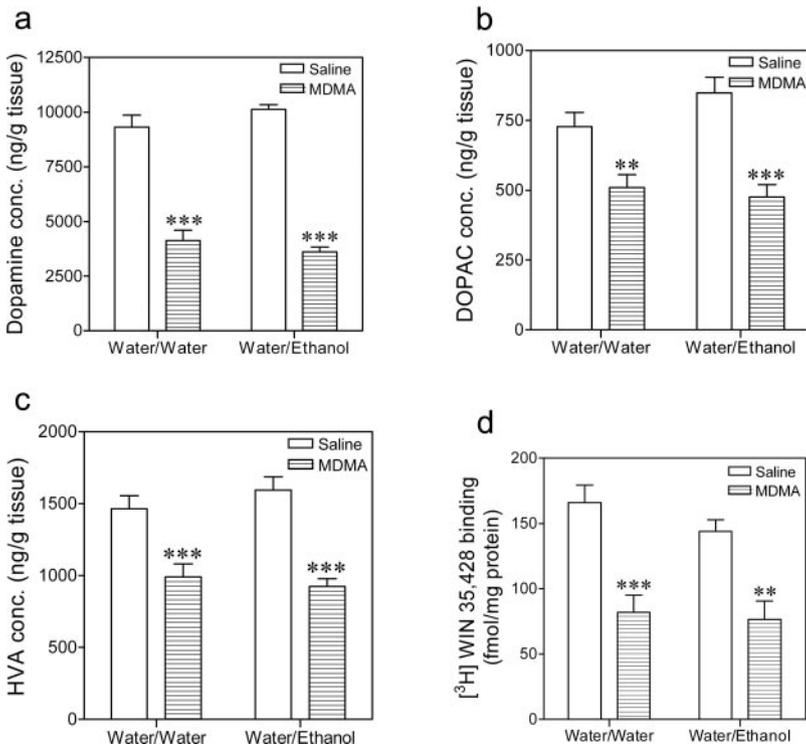


Fig. 7. Concentration of dopamine (a), DOPAC (b), and HVA (c) and density of dopamine transporter (d) in the striatum of mice pretreated with MDMA or saline 5 weeks before. Seven days after drug injection, both groups of mice given access to two pipettes containing plain water or different solutions of EtOH in water [3, 6, 10, and finally 20% (v/v)] for 4 weeks. The EtOH concentration was increased every 7 days. Results shown as mean \pm S.E.M., $n = 7$ to 11. Different from the corresponding saline-pretreated mice: **, $P < 0.01$; and ***, $P < 0.001$.

Dopamine Concentration and Dopamine Transporter Density in MDMA-Lesioned Mice. The last day of ethanol exposure, mice were killed and striatum dissected out to evaluate the effect induced by MDMA on dopamine terminals and whether this effect was altered by exposure to different concentrations of ethanol. Five weeks after

MDMA (30 mg/kg, three times at three hourly intervals) administration, there was a reduction of 56% in striatal dopamine content measured by HPLC and a decrease of 50% in the density of dopamine transporters in the striatum quantified by [^3H]WIN 35,428 radioligand binding. DOPAC and HVA levels were reduced approximately 30%.

These changes were similar in mice exposed and not exposed to ethanol (Fig. 7).

Discussion

Using a two-bottle choice paradigm, this study shows that mice exposed to a neurotoxic dose of MDMA and consequently showing a long-term loss of striatal dopamine concentration and dopamine transporters exhibit higher ethanol consumption than saline-treated mice. An increased ethanol preference was also observed in MDMA-treated mice, and because the ethanol preference ratios were high (>0.5), this effect was indicative of a high preference for ethanol. These differences between the two groups of mice do not reflect a more global change in taste preferences or caloric needs since MDMA-lesioned mice showed a consumption of solutions containing either sucrose or quinine similar to that observed in saline-treated animals. Preference ratios for ethanol, sucrose, and quinine are in accordance with those described previously (Naassila et al., 2004; Lewis et al., 2005). In the current study, MDMA- and saline-injected animals did not differ in terms of volume of fluid consumed, indicating that the increased ethanol consumption by MDMA-injected mice was not caused by an overall increase in the total amount of fluid consumed. Food consumption was also similar in both groups, indicating that the altered ethanol intake does not result from changes in appetite.

Previous studies (Escobedo et al., 2005) have reported that 7 days after repeated administration of MDMA, time at which ethanol exposure was started, there is a decrease in striatal dopamine concentration and in the density of dopamine uptake sites (Mann et al., 1997). The MDMA-induced loss in the dopaminergic markers of mice presumably reflects a neurotoxic degeneration of dopamine nerve terminals similar to that seen following methamphetamine administration to both rats and mice (Baldwin et al., 1993). The current study extends these findings by showing that the reduction of striatal dopamine concentration and dopamine transporters induced by MDMA persists for at least 5 weeks after injection. Nevertheless, it is possible that some degree of recovery occurs since the loss of dopamine content observed 7 days after MDMA (90%, Escobedo et al., 2005) is more pronounced than that detected 5 weeks after MDMA (56%, this study). The decrease in dopamine function caused by MDMA is consistent with the up-regulation of D₁ receptors observed in the caudate-putamen and nucleus accumbens (core) observed 1 week after injection. D₁ receptors are restricted to a postsynaptic localization, and decreased dopamine content usually leads to increased D₁ receptor density under different experimental paradigms (Betarbet and Greenamyre, 2004). On the other hand, D₂ receptors function both as auto- and postsynaptic receptors, and a hypothetic up-regulation at the postsynaptic level caused by MDMA might be compensated for by the loss of presynaptic D₂ density as a result of dopamine terminal loss. Despite the reduction of striatal dopamine concentration and transporters induced by MDMA persisting for at least 5 weeks after injection (this study), there was no change in D₁ receptor density 4 weeks after MDMA, suggesting that early changes in dopamine receptor function may reflect reversible compensatory adaptations in response to the loss of dopamine concentration.

Interestingly, ethanol consumption did not alter the de-

creased dopamine, DOPAC, and HVA levels found in the striatum of MDMA-lesioned mice, nor did it modify catechol content in the saline group. Yoshimoto and Komura (1989) also showed that voluntary ethanol consumption using a two-bottle free-choice paradigm (10% ethanol) did not alter dopamine levels during time of exposure (4 weeks) in the alcohol-preferring C57BL/6J strain, but in those with less preference for alcohol, DBA/2Cr, there were significant increases in dopamine levels. Genetic alterations in dopamine receptor expression and function in the nigrostriatal and mesolimbic pathways of C57BL/6J compared with DBA mice might account for these differences (Ng et al., 1994).

In addition to a reduction in dopamine markers, MDMA-pretreated mice also showed functional changes in the mesolimbic system reflected by a reduced release of basal dopamine in the nucleus accumbens compared with saline-injected animals. This finding might be a consequence of a reduced dopamine activity caused by the loss of dopamine nerve terminals induced by the drug. As expected, peripheral ethanol administration elevates extracellular dopamine release in the nucleus accumbens of saline-treated mice (Kiianmaa et al., 1995; this study) but this response is almost abolished in MDMA-treated mice, this being further evidence of an impaired dopamine function (this study). The fact that these animals consumed more ethanol might appear to be at odds with the hypothesis that the mesolimbic dopamine system mediates the reinforcing actions of drugs of abuse (Gatto et al., 1994). However, chronic exposure to ethanol sensitizes dopaminergic neurons in the mesolimbic pathway to subsequent ethanol exposure (Brodie, 2002). This sensitization may be responsible for the neuroadaptive changes following chronic ethanol consumption that result in significant changes in the function of dopamine transporters (Carroll et al., 2006). These alterations could reflect an increase in dopamine release. In fact, animals pre-exposed to ethanol respond with a higher number of ethanol self-infusions than those pre-exposed to water (Rodd et al., 2005). Although it is not clear how these neuroadaptive changes produce the differences observed between lesioned and nonlesioned mice, studies on reinforcement using operant procedures show that severe 6-hydroxydopamine-induced lesions in the nucleus accumbens do not affect responding to ethanol (Gonzales et al., 2004). Therefore, it appears that a hypodopaminergic state does not inhibit reinforcing behaviors.

Administration of the full D₁ agonist, SKF81297, is sufficient to reduce ethanol consumption in MDMA-lesioned mice, suggesting that the impairment is caused by the reduced D₁ receptor stimulation subsequent to a deficit in dopamine neurotransmission. The reversal of the D₁ receptor agonist effect by SCH23390, a D₁ receptor antagonist, confirms actions at the D₁ receptor rather than nonspecific actions. Data reported in this study are in agreement with drinking data obtained in pharmacological studies using dopaminergic agonists. Pretreatment with dopamine D₁ or D₂ agonists cause marked reductions in voluntary ethanol intake in comparison with control mice and rats (Dyr et al., 1993; Ng and George, 1994). Therefore, this would confirm that mice with a hypodopaminergic activity might need to consume more alcohol to reach the threshold for the rewarding effects of ethanol.

It has been suggested that a correlation exists between sensitization to the locomotor effects and the intake of drugs

of abuse, including ethanol (Lessov et al., 2001). Both behavioral sensitization to the locomotor stimulant effects of ethanol and an increase in voluntary ethanol consumption develop following repeated ethanol administration (Kampov-Polevoy et al., 2000; Correa et al., 2003), but sensitization also appears after pre-exposure to drugs of abuse other than ethanol; for example, following repeated cocaine or morphine administration (Lessov and Phillips, 2003). Based on the evidence, the current study also evaluated the relationship between ethanol self-administration and the locomotor stimulant effects of ethanol in MDMA-lesioned mice. Ethanol induced an increase in locomotion in MDMA-pretreated mice, the effect being similar to that observed in saline-pretreated animals. These findings indicate that the lesioning protocol used in this study (acute administration of repeated MDMA) does not induce sensitization to the locomotor stimulant effects of ethanol. It remains to be determined whether chronic MDMA administration interferes with the stimulant effect of ethanol on locomotion.

The differences in ethanol consumption could also be related to the basal level of anxiety between saline- and MDMA-injected mice. Based on animal and human studies, it has been hypothesized that the anxiolytic property of ethanol or, in the case of humans, the belief that alcohol would relieve anxiety, could play a role in the drug-seeking behavior (Thomas et al., 2003) and that anxiety problems precede alcohol abuse (Cox et al., 1990). A recent study has shown that methamphetamine (which also induces long-term damage to dopamine neurons) caused persistent anxiety-related behavioral symptoms lasting for at least 5 days (Hayase et al., 2005). Nevertheless, the current study clearly shows that pretreatment with MDMA does not alter the behavior of the animals in the dark-light box 3 weeks after MDMA, which is the time at which ethanol consumption is greater than that observed in saline-treated mice. Therefore, it is reasonable to propose that the enhancement of ethanol consumption observed in MDMA-treated mice is not related to increased anxiety. Although other studies have shown that MDMA causes an anxiogenic-like activity as indicated by the elevated plus maze (Navarro and Maldonado, 2002) and the light-dark box (Maldonado and Navarro, 2000), it is interesting to point out that these tests were performed shortly after MDMA administration and that there is no data other than that reported in the current study on the long-term effects of MDMA on anxiety. Differences in alcohol consumption and ethanol-induced dopamine release between saline- and MDMA-treated mice are not likely related to changes in ethanol metabolism because the blood ethanol concentration in MDMA-pretreated mice was not different from that observed in saline-pretreated mice during the 4.5 h after the injection of a single dose of ethanol.

In summary, this study indicates for the first time that the long-lasting dopamine neurotoxicity induced by MDMA predisposed the mice to high voluntary consumption of ethanol and suggests that higher levels of ethanol are required in these animals to achieve similar rewarding effects as those attained in nonlesioned mice. This study does not preclude the possibility of the cannabinoid or opioid systems participating in the drinking behavior of MDMA-lesioned mice.

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References

- Baldwin HA, Colado MI, Murray TK, De Souza RJ, and Green AR (1993) Striatal dopamine release in vivo following neurotoxic doses of methamphetamine and effect of the neuroprotective drugs, chlormethiazole and dizocilpine. *Br J Pharmacol* **108**:590–596.
- Belknap JK, Crabbe JC, and Young ER (1993) Voluntary consumption of ethanol in 15 inbred mouse strains. *Psychopharmacology* **112**:503–510.
- Betarbet R and Greenamyre JT (2004) Regulation of dopamine receptor and neuropeptide expression in the basal ganglia of monkeys treated with MPTP. *Exp Neurol* **189**:393–403.
- Brodie MS (2002) Increased ethanol excitation of dopaminergic neurons of the ventral tegmental area after chronic ethanol treatment. *Alcohol Clin Exp Res* **26**:1024–1030.
- Carroll MR, Rodd ZA, Murphy JM, and Simon JR (2006) Chronic ethanol consumption increases dopamine uptake in the nucleus accumbens of high alcohol drinking rats. *Alcohol* **40**:103–109.
- Colado MI, Camarero J, Mehan AO, Sanchez V, Esteban B, Elliott JM, and Green AR (2001) A study of the mechanisms involved in the neurotoxic action of 3,4-methylenedioxymethamphetamine (MDMA, “ecstasy”) on dopamine neurons in mouse brain. *Br J Pharmacol* **134**:1711–1723.
- Correa M, Arizzi MN, Betz A, Mingote S, and Salamone JD (2003) Locomotor stimulant effects of intraventricular injections of low doses of ethanol in rats: acute and repeated administration. *Psychopharmacology* **170**:368–375.
- Cox BJ, Norton GR, Swinson RP, and Endler NS (1990) Substance abuse and panic-related anxiety: a critical review. *Behav Res Ther* **28**:385–393.
- Crawley J and Goodwin FK (1980) Preliminary report of a simple animal behavior model for the anxiolytic effects of benzodiazepines. *Pharmacol Biochem Behav* **13**:167–170.
- Czachowski CL, Chappell AM, and Samson HH (2001) Effects of raclopride in the nucleus accumbens on ethanol seeking and consumption. *Alcohol Clin Exp Res* **25**:1431–1440.
- Czachowski CL, Santini LA, Legg BH, and Samson HH (2002) Separate measures of ethanol seeking and drinking in the rat: effects of remoxipride. *Alcohol* **28**:39–46.
- Dyr W, McBride WJ, Lumeng L, Li TK, and Murphy JM (1993) Effects of D1 and D2 dopamine receptor agents on ethanol consumption in the high-alcohol-drinking (HAD) line of rats. *Alcohol* **10**:207–212.
- El-Ghundi M, George SR, Drago J, Fletcher PJ, Fan T, Nguyen T, Liu C, Sibley DR, Westphal H, and O'Dowd BF (1998) Disruption of dopamine D1 receptor gene expression attenuates alcohol-seeking behavior. *Eur J Pharmacol* **353**:149–158.
- Escobedo I, O'Shea E, Orio L, Sanchez V, Segura M, de la Torre R, Farre M, Green AR, and Colado MI (2005) A comparative study on the acute and long-term effects of MDMA and 3,4-dihydroxymethamphetamine (HHMA) on brain monoamine levels after i.p. or striatal administration in mice. *Br J Pharmacol* **144**:231–241.
- Franklin KBJ and Paxinos G (1997) *The Mouse Brain in Stereotaxic Coordinates*, Academic Press Inc., San Diego, CA.
- Gatto GJ, McBride WJ, Murphy JM, Lumeng L, and Li TK (1994) Ethanol self-infusion into the ventral tegmental area by alcohol-preferring rats. *Alcohol* **11**:557–564.
- George SR, Fan T, Ng GY, Jung SY, O'Dowd BF, and Naranjo CA (1995) Low endogenous dopamine function in brain predisposes to high alcohol preference and consumption: reversal by increasing synaptic dopamine. *J Pharmacol Exp Ther* **273**:373–379.
- Gonzales RA, Job MO, and Doyon WM (2004) The role of mesolimbic dopamine in the development and maintenance of ethanol reinforcement. *Pharmacol Ther* **103**:121–146.
- Green AR, Mehan AO, Elliott JM, O'Shea E, and Colado MI (2003) The pharmacology and clinical pharmacology of 3,4-methylenedioxymethamphetamine (MDMA, “ecstasy”). *Pharmacol Rev* **55**:463–508.
- Hayase T, Yamamoto Y, and Yamamoto K (2005) Persistent anxiogenic effects of a single or repeated doses of cocaine and methamphetamine: interactions with endogenous cannabinoid receptor ligands. *Behav Pharmacol* **16**:395–404.
- Kampov-Polevoy AB, Matthews DB, Gause L, Morrow AL, and Overstreet DH (2000) P rats develop physical dependence on alcohol via voluntary drinking: changes in seizure thresholds, anxiety, and patterns of alcohol drinking. *Alcohol Clin Exp Res* **24**:278–284.
- Kiianmaa K, Nurmi M, Nykanen I, and Sinclair JD (1995) Effect of ethanol on extracellular dopamine in the nucleus accumbens of alcohol-preferring AA and alcohol-avoiding ANA rats. *Pharmacol Biochem Behav* **52**:29–34.
- Lessov CN, Palmer AA, Quick EA, and Phillips TJ (2001) Voluntary ethanol drinking in C57BL/6J and DBA/2J mice before and after sensitization to the locomotor stimulant effects of ethanol. *Psychopharmacology* **155**:91–99.
- Lessov CN and Phillips TJ (2003) Cross-sensitization between the locomotor stimulant effects of ethanol and those of morphine and cocaine in mice. *Alcohol Clin Exp Res* **27**:616–627.
- Lewis SR, Ahmed S, Dym C, Khaimova E, Kest B, and Bodnar RJ (2005) Inbred mouse strain survey of sucrose intake. *Physiol Behav* **85**:546–556.
- Liu X and Weiss F (2002) Reversal of ethanol-seeking behavior by D1 and D2 antagonists in an animal model of relapse: differences in antagonist potency in previously ethanol-dependent versus nondependent rats. *J Pharmacol Exp Ther* **300**:882–889.
- Maldonado E and Navarro JF (2000) Effects of 3,4-methylenedioxy-methamphetamine (MDMA) on anxiety in mice tested in the light-dark box. *Prog Neuropsychopharmacol Biol Psychiatry* **24**:463–472.
- Mann H, Ladenheim B, Hirata H, Moran TH, and Cadet JL (1997) Differential toxic effects of methamphetamine (METH) and methylenedioxymethamphetamine (MDMA) in multidrug-resistant (mdr1a) knockout mice. *Brain Res* **769**:340–346.
- Naassila M, Ledent C, and Daoust M (2002) Low ethanol sensitivity and increased ethanol consumption in mice lacking adenosine A2A receptors. *J Neurosci* **22**:10487–10493.
- Naassila M, Pierrefiche O, Ledent C, and Daoust M (2004) Decreased alcohol self-

- administration and increased alcohol sensitivity and withdrawal in CB1 receptor knockout mice. *Neuropharmacology* **46**:243–253.
- Navarro JF and Maldonado E (2002) Acute and subchronic effects of MDMA (“ecstasy”) on anxiety in male mice tested in the elevated plus-maze. *Prog Neuropsychopharmacol Biol Psychiatry* **26**:1151–1154.
- Ng GY and George SR (1994) Dopamine receptor agonist reduces ethanol self-administration in the ethanol-preferring C57BL/6J inbred mouse. *Eur J Pharmacol* **269**:365–374.
- Ng GY, O’Dowd BF, and George SR (1994) Genotypic differences in brain dopamine receptor function in the DBA/2J and C57BL/6J inbred mouse strains. *Eur J Pharmacol* **269**:349–364.
- Phillips TJ, Brown KJ, Burkhardt-Kasch S, Wenger CD, Kelly MA, Rubinstein M, Grandy DK, and Low MJ (1998) Alcohol preference and sensitivity are markedly reduced in mice lacking dopamine D2 receptors. *Nat Neurosci* **1**:610–615.
- Rodd ZA, Bell RL, McQueen VK, Davids MR, Hsu CC, Murphy JM, Li TK, Lumeng L, and McBride WJ (2005) Chronic ethanol drinking by alcohol-preferring rats increases the sensitivity of the posterior ventral tegmental area to the reinforcing effects of ethanol. *Alcohol Clin Exp Res* **29**:358–366.
- Segal DS, Kuczenski R, O’Neil ML, Melega WP, and Cho AK (2003) Escalating dose methamphetamine pretreatment alters the behavioral and neurochemical profiles associated with exposure to a high-dose methamphetamine binge. *Neuropsychopharmacology* **28**:1730–1740.
- Thomas SE, Randall CL, and Carrigan MH (2003) Drinking to cope in socially anxious individuals: a controlled study. *Alcohol Clin Exp Res* **27**:1937–1943.
- Tuomainen P, Patsenka A, Hyytia P, Grinevich V, and Kiianmaa K (2003) Extracellular levels of dopamine in the nucleus accumbens in AA and ANA rats after reverse microdialysis of ethanol into the nucleus accumbens or ventral tegmental area. *Alcohol* **29**:117–124.
- Yoshimoto K and Komura S (1989) Genetic differences in the effects of voluntary ethanol consumption on brain monoamine levels in inbred strains of mice, C57BL/6J, C3H/He and DBA/2Cr. *Alcohol Alcohol* **24**:225–229.

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