Abuse Liability Profile of Three Substituted Tryptamines

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ABSTRACT

The abuse liability profile of three synthetic hallucinogens, *N,N*-diisopropyltryptamine (DIPT), 5-*N,N*-diethyl-5-methoxytryptamine (5-MeO-DET), and 5-methoxy- α -methyltryptamine (5-MeO-AMT), was tested in rats trained to discriminate hallucinogenic and psychostimulant compounds, including cocaine, methamphetamine, 3,4-methylenedioxymethylamphetamine (MDMA), lysergic acid diethylamide (LSD), (–)-2,5-dimethoxy-4-methylamphetamine (DOM), and dimethyltryptamine (DMT). Because abused hallucinogens act at 5-hydroxytryptamine 1A (5-HT_{1A}) and 5-HT_{2A} receptors, and abused psychostimulants act at monoamine transporters, binding and functional activities of DIPT, 5-MeO-DET, and 5-MeO-AMT at these sites were also tested. DIPT fully substituted in rats trained to discriminate DMT (ED₅₀ = 1.71 mg/kg) and DOM (ED₅₀ = 1.94 mg/kg), but produced only 68% LSD-appropriate responding. 5-MeO-DET fully substituted for DMT (ED₅₀ = 0.41

mg/kg) and produced 59% MDMA-appropriate responding. 5-MeO-AMT did not fully substitute for any of the training drugs, but produced 67% LSD-appropriate responding. None of the compounds produced substitution in rats trained to discriminate cocaine or methamphetamine. All three compounds showed activity at 5-HT_{1A} and 5-HT_{2A} receptors as well as blockade of reuptake by the serotonin transporter. In addition, 5-MeO-AMT produced low levels of serotonin release and low potency blockade of dopamine uptake. DIPT, 5-MeO-DET, and 5-MeO-AMT produced behavioral and receptor effects similar to those of abused hallucinogens, but were not similar to those of psychostimulants. DIPT and 5-MeO-DET may have abuse liability similar to known hallucinogens and may be hazardous because high doses produced activity and lethality.

Introduction

The use of newer “designer” hallucinogens is increasing, although the use of classic hallucinogens has waned (McCambridge et al., 2007). Many compounds are freely available over the internet, and websites that promote their use share descriptions of the subjective effects of these drugs (e.g., www.erowid.org). Not all of these compounds have been specifically scheduled by the Drug Enforcement Agency, but may be if their structures are substantially similar to a

scheduled compound and if they are distributed with intent for human consumption, according to Section 813 of the Controlled Substances Act. Three of these compounds were flagged by the Drug Enforcement Agency for abuse liability evaluation: *N,N*-diisopropyltryptamine (DIPT), 5-methoxy- α -methyltryptamine (5-MeO-AMT), and 5-*N,N*-diethyl-5-methoxytryptamine (5-MeO-DET).

Very little is known about the effects of these three compounds. All are indolealkylamines and structurally similar to DMT (Table 1). The most studied compound, 5-MeO-AMT, bound to 5-HT_{1A} and 5-HT₂ receptors (Glennon et al., 1990; Tomaszewski et al., 1992). It also inhibited the reuptake and increased the release of monoamines in brain synaptosomes at micromolar concentrations (Nagai et al., 2007). Likewise, 5-MeO-DET bound to 5-HT₂ receptors (Lyon et al., 1988) and also inhibited reuptake but did not cause release of monoamines (Nagai et al., 2007). 5-MeO-AMT lowered intraocular

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ABBREVIATIONS: DIPT, *N,N*-diisopropyltryptamine; LSD, lysergic acid diethylamide; 5-MeO-DET, 5-*N,N*-diethyl-5-methoxytryptamine; 5-MeO-AMT, 5-methoxy- α -methyltryptamine; MDMA, 3,4-methylenedioxymethylamphetamine; DOM, (–)-2,5-dimethoxy-4-methylamphetamine; DMT, dimethyltryptamine; DOI, 2,5-dimethoxy-4-iodoamphetamine; 8-OH-DPAT, 8-hydroxy-2-(di-*n*-propylamino)tetralin; HEK, human embryonic kidney; IP-1, inositol-monophosphate; GTP γ S, guanosine 5'-O-[γ -thio]triphosphate; WAY 100635, *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]-ethyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide; 5-HT, 5-hydroxytryptamine (serotonin); AA, arachadonic acid; hDAT, human dopamine transporter; hSERT, human serotonin transporter; hNET, norepinephrine transporter; DMEM, Dulbecco's modified Eagle's medium; DMSO, dimethylsulfoxide; G418, (2*R*,3*S*,4*R*,5*R*,6*S*)-5-amino-6-[(1*R*,2*S*,3*S*,4*R*,6*S*)-4,6-diamino-3-[(2*R*,3*R*,4*R*,5*R*)-3,5-dihydroxy-5-methyl-4-methylaminooxan-2-yl]oxy-2-hydroxycyclohexyl]oxy-2-(1-hydroxyethyl)oxane-3,4-diol; RTI-55, (–)-2 β -carbomethoxy-3 β -(4-iodophenyl)tropane.

TABLE 1

Chemical structures of dimethyltryptamine and three hallucinogenic tryptamines

Compound	
DMT
DIPT
5-MeO-DET
5-MeO-AMT

pressure (May et al., 2003). In rats trained to discriminate DOM (1 mg/kg i.p., 15 min) from saline, 5-MeO-AMT produced little effect at 15 min after administration, but racemate and both (+) and (–) isomers fully substituted when tested 90 min after administration (Glennon et al., 1983). DIPT inhibited transport at serotonin transporter and also vesicular monoamine transporter 2 (Nagai et al., 2007; Cozzi et al., 2009).

The purpose of this study was to identify potential abuse liability of DIPT, 5-MeO-AMT, and 5-MeO-DET. First, locomotor activity was tested to determine the effective dose range and time course of behavioral effects. Second, the ability of these compounds to produce discriminative stimulus effects similar to those of known drugs of abuse was tested. Third, the ability of these compounds to bind to and activate molecular/pharmacological mechanisms used by known drugs of abuse was characterized.

Because very little is known about the behavioral effects of these compounds, it was necessary to test for a range of potential discriminative stimulus effects. The effects of psychedelic compounds can be classified into three categories: hallucinogen, stimulant, and other (Glennon, 1999). Furthermore, the hallucinogens fall into three classes: simple tryptamines (indolealkylamines), ergolines, and phenethylamines (Nichols, 2004). To test the effects of novel hallucinogens across all of these categories, different groups of rats were trained to discriminate each of the following compounds: DMT (indolealkylamine), LSD (ergoline), DOM (phenethylamine hallucinogen), methamphetamine (phenethylamine psychostimulant), MDMA (phenethylamine psychostimulant/hallucinogen), and cocaine (psychostimulant). Each of these compounds represents one of the classes of psychedelic compounds, and each one is also abused.

The training compounds used in this study not only come from different structural classes, but they do not have completely overlapping discriminative stimulus effects (Gatch et

al., 2009; Winter, 2009), thus affording a wide range of categories for testing abuse liability of the novel compounds. For example, MDMA is often not considered a hallucinogen in humans, even though it produces visual distortions and produces discriminative stimulus effects similar to both psychostimulants and hallucinogens (Gatch et al., 2009; Winter, 2009). These findings indicate that the discriminative stimulus effects may lie in between, or overlap the two classes, and that the MDMA discrimination may be useful for detecting weak discriminative stimulus effects of either type or for detecting compounds with stimulus properties of both classes.

The third purpose of this study was to identify whether the test compounds act at receptors known to mediate the effects of drugs of abuse. Hallucinogens are typically thought to produce their sensory effects through activation of 5-HT_{2A} receptors, although there is also evidence for a contribution of 5-HT_{1A} receptors and perhaps other monoamine sites as well (Fiorella et al., 1995; Nichols, 2004; Nonaka et al., 2007). In addition, tryptamine compounds, most notably serotonin, act at the monoamine transporters (Adkins et al., 2001). The ability of DIPT, 5-MeO-AMT, and 5-MeO-DET to bind to and/or activate 5-HT_{1A} and 5-HT_{2A} receptors was tested, as well as their ability to bind to dopamine, norepinephrine, and serotonin transporters and block uptake and/or promote release. Arachidonic acid (AA) release after activation of phospholipase A2 and inositol-monophosphate (IP-1) formation after activation of phospholipase C were used as measures of 5-HT_{2A} receptor activation because LSD and other hallucinogens seem to preferentially activate this signal-transduction pathway (see review in Nichols, 2004).

Materials and Methods

Subjects. Male Sprague-Dawley rats were obtained from Harlan (Indianapolis, IN). All rats were housed individually and maintained on a 12:12-h light/dark cycle (lights on at 7:00 AM). Body weights were maintained at 320 to 350 g by limiting food to 20 g/day, which included the food received during operant sessions. Water was readily available. Male Swiss-Webster mice were obtained from Harlan at approximately 8 weeks of age and tested at approximately 10 weeks of age. Mice were group-housed in cages on a 12:12-h light/dark cycle and allowed free access to food and water. All housing and procedures were in accordance with Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (National Research Council, 2003) and approved by the University of North Texas Health Science Center Animal Care and Use Committee.

Discrimination Procedures. Standard operant chambers (Coulbourn Instruments, Allentown, PA) were connected to IBM-PC-compatible computers via LVB interfaces (MED Associates, St. Albans, VT). The computers were programmed in Med-PC for Windows, version IV (Med Associates) for the operation of the chambers and collection of data.

Using a two-lever choice methodology, separate groups comprised of 15 to 32 rats were trained to discriminate one of four compounds from saline: methamphetamine (1 mg/kg), MDMA (1.5 mg/kg), LSD (0.1 mg/kg), and DMT (5 mg/kg) as described previously (Gatch et al., 2009). Rats received an injection of either saline or drug and were subsequently placed in the operant chambers, where food (45 mg of food pellets; Bio-Serve, Frenchtown, NJ) was available as a reinforcer for every 10 responses on a designated injection-appropriate lever. The pretreatment time was 10 min for methamphetamine and MDMA, 15 min for LSD, and 5 min for DMT. Each training session lasted a maximum of 10 min, and the rats could earn up to 20 food pellets. The rats received approximately 60 of these sessions before

they were used in tests for substitution of the experimental compounds. Rats were used in testing once they had achieved 9 of 10 sessions at 85% injection-appropriate responding for both the first reinforcer and total session. The training sessions occurred on separate days in a double alternating fashion (drug-drug-saline-saline-drug, etc.) until the training phase was complete, after which substitution tests of DIPT, 5-MeO-AMT, or 5-MeO-DET were introduced into the training schedule such that at least one saline and one drug session occurred between each test (drug-saline-test-saline-drug-test-drug, etc.). The substitution tests occurred only if the rats had achieved 85% injection-appropriate responding on the two prior training sessions.

Test sessions lasted for a maximum of 20 min. In contrast with training sessions, both levers were active, such that 10 consecutive responses on either lever led to reinforcement. Data were collected until the first reinforcer was obtained or for a maximum of 20 min. Each compound was tested in groups of six rats. Intraperitoneal injections (1 ml/kg) of saline, DIPT (0.5–10 mg/kg), 5-MeO-AMT (0.1–1 mg/kg), or 5-MeO-DET (0.05–2.5 mg/kg) occurred 30 min before the start of the test session. A repeated-measures design was used, such that each rat was tested at all doses of a given drug.

Locomotor Activity. The study was conducted using 40 Digiscan (model RXYZCM; Omnitech Electronics, Columbus, OH) locomotor activity testing chambers (40.5 × 40.5 × 30.5 cm) housed in sets of two, within sound-attenuating chambers. A panel of infrared beams (16 beams) and corresponding photodetectors were located in the horizontal direction along the sides of each activity chamber. A 7.5-W incandescent light above each chamber provided dim illumination, and fans provided an 80-dB ambient noise level within the chamber.

Separate groups of eight mice were injected intraperitoneally with either vehicle (0.9% saline), DIPT (1, 3, 10, 30, or 56 mg/kg), 5-MeO-DET (0.1, 0.3, 1, 3, or 10 mg/kg), or 5-MeO-AMT (0.1, 0.3, 1, 3, or 10 mg/kg), immediately before locomotor activity testing. In all studies, horizontal activity (interruption of photocell beams) was measured for 8 h within 10-min periods, beginning at 8:00 AM (2 h after lights on).

[³H]8-OH-DPAT Binding. Human embryonic kidney cells expressing the human 5-HT_{1A} receptor (HEK-5-HT_{1A}) were developed and selected as described for other cell lines (Eshleman et al., 1999). The cDNA for the 5-HT_{1A} receptor was purchased from the Missouri S&T cDNA Resource Center (Rolla, MO). The cells were grown to confluence in Dulbecco's modified Eagle's medium (DMEM) containing 10% FetalClone (HyClone Laboratories, Logan, Utah), 0.05% penicillin-streptomycin, and 300 µg/ml of (2*R*,3*S*,4*R*,5*R*,6*S*)-5-amino-6-[(1*R*,2*S*,3*S*,4*R*,6*S*)-4,6-diamino-3-[(2*R*,3*R*,4*R*,5*R*)-3,5-dihydroxy-5-methyl-4-methylaminooxan-2-yl]oxy-2-hydroxycyclohexyl]oxy-2-(1-hydroxyethyl)oxane-3,4-diol (G418). The cells were scraped from 150-mm plates into phosphate-buffered saline and centrifuged at 270g for 10 min. The cell pellet was homogenized in 50 mM Tris-HCl, pH 7.7, with a polytron, and centrifuged at 27,000g. The homogenization and centrifugation were repeated to wash any remaining serotonin from the growth media. The final pellet was resuspended at 0.5 mg of protein/ml in assay buffer (25 mM Tris-HCl, pH 7.4, containing 100 µM ascorbic acid and 10 µM pargyline). The reaction mixture contained test compound, cell homogenate (0.05 mg of protein/well), and [³H]8-OH-DPAT (0.5 nM final concentration, 170 Ci/mmol; PerkinElmer Life and Analytical Sciences, Waltham, MA) in a final volume of 1 ml. Assays were conducted in duplicate, and nonspecific binding was determined with 1.0 µM dihydroergotamine. The plates were incubated at room temperature for 60 min and then filtered through polyethylenimine-soaked (0.05%) "A" filtermats on a Tomtec 96-well cell harvester (Tomtec, Hamden, CT). The filters were washed with cold 50 mM Tris buffer, pH 7.7 for 6 s, dried, and spotted with scintillation cocktail, and radioactivity was determined by liquid scintillation counting.

[³⁵S]GTPγS Binding. Activation of 5-HT_{1A} receptors was tested by facilitation of [³⁵S]GTPγS binding to G proteins (Newman-Tancredi et al., 1998). GTPγS assay buffer (20 mM HEPES, pH 7.4, 10 mM MgCl₂, 100 mM NaCl, and 0.2 mM dithiothreitol) was used throughout the assay. HEK-5-HT_{1A} cells were scraped from tissue

culture plates into assay buffer and centrifuged at 515g for 15 min. The supernatant was removed, and the pellet was homogenized in 10 ml of buffer/plate of cells. The homogenate was centrifuged at 40,000g for 15 min, and the resulting pellet was washed two times by homogenization in 10 ml of buffer and centrifugation to remove serotonin that was present in the growth medium. The final pellet from four plates was resuspended in 10 ml of assay buffer.

Cell membranes (40–75 µg of protein) were preincubated (10 min, room temperature) with test compound in duplicate in assay buffer. The reaction was initiated by the addition of GDP (3 µM) and [³⁵S]GTPγS (0.1 nM, 1350 Ci/mmol; PerkinElmer Life and Analytical Sciences; ~150,000 cpm) in a final volume of 1 ml. The reaction was incubated for 60 min at room temperature on a rotating platform. Nonspecific binding was defined with 1 µM *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]-ethyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide (WAY-100635). A dose-response curve with the full agonist serotonin was conducted in each experiment to identify full and partial agonist compounds. Experiments were terminated by rapid filtration over Filtermat A with ice-cold saline using a Tomtec cell harvester and radioactivity was determined by liquid scintillation counting.

[¹²⁵I]DOI Binding. Binding to 5-HT_{2A} receptors was tested in human embryonic kidney cells expressing the human 5-HT_{2A} receptor (HEK-h5HT_{2A}) adapting methods described previously (Knight et al., 2004). The cDNA for the h5HT_{2A} receptor was purchased from Missouri S&T cDNA Resource Center. The cells were grown until confluent on 15-cm plates. Medium was removed, and cells were washed with phosphate-buffered saline, scraped into 2 ml of phosphate-buffered saline, and frozen at –20°C until needed. Cell suspension was thawed, 10 ml of assay buffer (50 mM Tris, pH 7.4 at 37°C, with 0.1% ascorbic acid and 5 mM CaCl₂) was added per plate of cells and polytroned at setting 6 for 5 s. The homogenate was centrifuged at 30,000g for 20 min. To minimize the residual serotonin concentration, the pellet was resuspended in buffer, polytroned, and centrifuged as above. The final pellet was resuspended in 2 ml of buffer/plate of cells.

The binding assay included test compound, serotonin or buffer, cell homogenate, [¹²⁵I]DOI (~0.1 nM), and buffer in a final volume of 250 µl. Specific binding was defined as the difference between total binding and binding in the presence of 10 µM serotonin. The reaction was incubated for 1 h at 37°C and terminated by filtration through Wallac A filtermats presoaked in 0.05% polyethylenimine using a Tomtec cell harvester, and radioactivity was determined by liquid scintillation counting.

[³H]Arachidonic Acid Release. Activation of 5-HT_{2A} receptors was tested by facilitation of release of AA as adapted from methods described previously (Kurrasch-Orbaugh et al., 2003). HEK-5-HT_{2A} cells were plated onto poly-L-lysine (1%) coated 24-well plates and grown until 90 to 95% confluent. Wells were rinsed with 0.5 ml of unsupplemented DMEM. Medium was removed, [³H]AA (0.5 µCi/well) in 1 ml of DMEM was added, and cells were incubated for 4 h at 37°C. Medium was removed, and cells were rinsed three times for 5 min each with supplemented DMEM (0.02% ascorbic acid and 2% fatty acid-free bovine serum albumin) at 34°C without or with release inhibitors. After the final wash, medium was removed, fresh supplemented DMEM without or with inhibitors was added, and release was initiated by addition of a receptor agonist or test compound. Plates were incubated at 34°C for 60 min. To terminate the assay, plates were placed on ice and medium was transferred from each well to a microfuge tube. To pelletize any cells that may have become detached during the assay, the medium was centrifuged at 13,000 rpm for 1 min. Aliquots of media (400 µl) were transferred to scintillation vials and counted on a Beckman L4801 liquid scintillation counter (Beckman Coulter, Brea, CA). Ketanserin (30 µM) was used to define nonspecific release.

Inositol-1-Phosphate Accumulation. Activation of 5-HT_{2A} receptors was tested by measuring the accumulation of inositol monophosphate using the IP-1 Elisa kit (Cisbio, Bedford, MA). Cells were plated at a density of 400,000 cells per well in 24-well plates in

DMEM supplemented with 10% charcoal-stripped FetalClone. The next day, medium was removed and cells were rinsed and then preincubated with DMEM for 1 h. After removal of medium, stimulation buffer without or with antagonists was added. After 10-min incubation, agonists were added, and the plates were incubated for 60 min. Cells were lysed for 30 min, and 50- μ l aliquots of the lysates were added to the IP-1 plate. The assay was conducted according to kit instructions. Stimulated IP-1 formation was normalized to the maximal effect of serotonin, which was determined in each assay.

hDAT, hSERT, or hNET Binding, Uptake, and Release. The methods for characterizing the effects of the monoamine transporters have been described previously in detail (Eshleman et al., 1999). Human embryonic kidney cells expressing the recombinant human dopamine transporter (HEK-hDAT), serotonin transporter (HEK-hSERT), or norepinephrine transporter (HEK-hNET) were used. Cells were grown to 80% confluence on 150-mm-diameter tissue culture dishes.

To assay neurotransmitter release, cells were scraped into Krebs HEPES buffer (pH 7.4; 122 mM NaCl, 2.5 mM CaCl_2 , 1.2 mM MgSO_4 , 10 μ M pargyline, 100 μ M tropolone, 0.2% glucose, and 0.02% ascorbic acid, buffered with 25 mM HEPES), centrifuged at 500 rpm for 7 min, and resuspended in 5 to 8 ml of Krebs HEPES. Uptake was conducted for 15 min at 30°C (hDAT and hNET) or 30 min at 25°C (hSERT) with 120 nM [^3H]dopamine (9.9 Ci/mmol), 120 nM [^3H]norepinephrine (6.5 Ci/mmol), or 20 nM [^3H]serotonin (29 Ci/mmol). Cells were then centrifuged and resuspended in 7 ml of Krebs HEPES, and 280 μ l of cells were added to the superfusion device. Release was conducted at 30°C (hDAT) or 25°C (hNET and hSERT). Buffer was perfused for 12 to 15 min, with the last 6 min (three fractions) collected. Drug was added, and 24 min (12 fractions) of effluent was collected. SDS (1%) was then perfused, and four fractions, 2.5 min each, were collected. Positive controls were methamphetamine (hDAT and hNET) and *p*-chloroamphetamine, (hSERT). Radioactivity in the samples was determined using conventional liquid scintillation spectrometry. Fractional release was the amount of radioactivity in a fraction divided by the total radioactivity remaining in the sample.

Drugs. (–)-Cocaine hydrochloride, (+)-methamphetamine hydrochloride, ($\pm$)-3,4-methylenedioxymethamphetamine hydrochloride, (+)-lysergic acid diethylamide (+)-tartrate, (–)-2,5-dimethoxy-4-methylamphetamine hydrochloride, *N,N*-dimethyltryptamine fumarate, 5-methoxy-*N,N*-diethyltryptamine hydrochloride, 5-methoxy- α -methyltryptamine, and *N,N*-diisopropyltryptamine hydrochloride were provided by the National Institute on Drug Abuse Drug Supply Program (Rockville, MD). All drugs were dissolved in 0.9% saline and were administered intraperitoneally in a volume of 1 ml/kg. Dose increments were based on 1/3 logs. [^3H]8-OH-DPAT, [^{125}I]DOI, [^{125}I](–)-2 β -carbomethoxy-3 β -(4-iodophenyl)tropane (RTI-55), [^3H]dopamine, [^3H]serotonin, and [^3H]arachidonic acid were obtained from PerkinElmer Life and Analytical Sciences, and [^3H]norepinephrine was obtained from GE Healthcare (Chalfont St. Giles, Buckinghamshire, UK). Other reagents were obtained from Sigma-Aldrich (St. Louis, MO).

For binding and functional assays, the test compounds were weighed and dissolved in dimethylsulfoxide (DMSO) to make a 10 mM stock solution. An initial dilution to 50 μ M was made in assay buffer for binding or to 0.1 or 1 mM in buffer for uptake. Subsequent dilutions were made with assay buffer supplemented with DMSO, maintaining a final concentration of 0.1% DMSO. Because of the limitation of the concentration of DMSO, the highest concentration of test compounds tested was 10 μ M. Pipetting was conducted using a Biomek 2000 robotic workstation (Beckman Coulter).

Data Analysis. Drug discrimination data are expressed as the mean percentage of drug-appropriate responses occurring in each test period. Rates of responding were expressed as a function of the number of responses made divided by the total session time. Graphs for percentage of drug-appropriate responding and response rate were plotted as a function of dose of test compound (log scale). Percentage of drug-appropriate responding was shown only if at

least three rats completed the first fixed ratio. Full substitution was defined as $\geq 80\%$ drug-appropriate responding and was not statistically different from the training drug.

The potencies of DIPT, 5-MeO-AMT, and 5-MeO-DET were calculated by fitting straight lines to the dose-response data for each compound by means of TableCurve 2D (SPSS Inc., Chicago, IL). Straight lines were fitted to the linear portion of dose-effect curves, including not more than one dose producing $<20\%$ of the maximal effect and not more than one dose producing $>80\%$ of the maximal effect. Other doses were excluded from the analyses. Rates of responding were expressed as a function of the number of responses made divided by the total session time. Response rate data were analyzed by one-way repeated-measures analysis of variance. Effects of individual doses were compared with the vehicle control value using *a priori* contrasts. The criterion for significance was set *a priori* at $p < 0.05$.

Locomotor activity data were expressed as the mean number of photocell counts in the horizontal plane (ambulation counts) during each 10-min period of testing. A 30-min period, beginning when maximal suppression of locomotor activity first appeared as a function of dose, was used for analysis of dose-response data. The ID_{50} (dose producing half-maximal depressant activity, where maximal depression = 0 counts/30 min) was calculated based on the linear portion of the $\log_{10}$ dose-response curve. A two-way repeated-measures analysis of variance was conducted on horizontal activity counts/10-min interval. A one-way analysis of variance was conducted on horizontal activity counts for the 30-min period of maximal effect, and planned comparisons were conducted for each dose against saline control using single degree-of-freedom *F* tests.

For data from binding and functional assays, EC_{50} values were calculated with Prism (GraphPad Software Inc., San Diego, CA), and maximal effect for a given drug each day was normalized to the maximal effect of serotonin (GTP γ S binding and IP-1 formation) or LSD ([^3H]AA release). IC_{50} values were calculated with Prism and converted to K_i values using the Cheng-Prusoff correction (Cheng and Prusoff, 1973). For release assays, area under the curve was calculated using Prism, and EC_{50} values were determined using nonlinear regression. One-way analysis of variances were conducted using the log values of the K_i , IC_{50} , or EC_{50} values followed by Tukey's post hoc analysis.

Results

Drug Discrimination

DIPT. As shown in Fig. 1, DIPT fully substituted for the discriminative stimulus effects produced by 5 mg/kg DMT (ED_{50} of 2.20 ± 0.77 mg/kg) and 0.5 mg/kg DOM (ED_{50} of 1.81 ± 0.47 mg/kg), and the maximal effects of DIPT were not different from those of the training dose of DMT ($p = 0.961$) or DOM ($p = 0.363$). DIPT did not alter response rate in either the DMT-trained rats ($F_{4,20} = 0.59$, $p = 0.671$) or DOM-trained rats ($F_{4,16} = 0.88$, $p = 0.495$). DIPT did not fully substitute for LSD, producing a maximum of 68% LSD-appropriate responding. Response rate in the LSD-trained rats was decreased after 10 mg/kg DIPT ($F_{3,15} = 5.37$, $p = 0.010$). Higher doses were not tested because of convulsions at 25 mg/kg (2/2 rats).

Within the dose range of 2.5 to 10 mg/kg, DIPT failed to substitute for the discriminative stimulus effects produced by 10 mg/kg cocaine, 1 mg/kg (+)-methamphetamine, or 1.5 mg/kg MDMA (Fig. 1). DIPT produced a maximum of 38% cocaine-appropriate responding and less than 20% drug-appropriate responding in the methamphetamine- and MDMA-trained rats. A nearly complete suppression of response rates was observed after 10 mg/kg DIPT in cocaine-trained rats

Fig. 1. Discriminative stimulus effects of DIPT, 5-MeO-DET, and 5-MeO-AMT in separate groups of rats trained to discriminate DMT, LSD, DOM, MDMA, cocaine, or methamphetamine from saline. Top of each pair, drug-appropriate responding of the test compounds in DMT-, LSD-, DOM-, MDMA-, cocaine-, and methamphetamine-trained rats. Bottom of each pair, rate of responding in responses per second. Each point represents the average of six rats except where indicated.

($F_{3,15} = 12.88$, $p < 0.001$) and methamphetamine-trained rats ($F_{3,15} = 15.01$, $p < 0.001$). Response rate was less affected in MDMA-trained rats, being decreased to 55% of vehicle control after 10 mg/kg DIPT ($F_{3,12} = 1.48$, $p = 0.269$).

5-MeO-DET. As shown in Fig. 1, 5-MeO-DET fully substituted for the discriminative stimulus effects produced by 5 mg/kg DMT ($ED_{50} = 0.42 \pm 0.12$ mg/kg), and the maximal effect was not different from that of the training dose ($p = 1.00$). Response rate was decreased after 0.5 and 1 mg/kg ($F_{5,25} = 17.05$, $p < 0.001$). In contrast, 5-MeO-DET did not fully substitute for the discriminative stimulus effects produced by 0.5 mg/kg DOM and 1.5 mg/kg MDMA, producing maximum DOM-appropriate responding of 39% and MDMA-appropriate responding of 59%. Response rate was dose-dependently decreased by 5-MeO-DET in both DOM-trained rats ($F_{6,24} = 2.26$, $p = 0.072$) and MDMA-trained rats ($F_{6,30} = 7.17$, $p < 0.001$).

Within the dose range of 0.05 to 2.5 mg/kg, 5-MeO-DET failed to substitute for the discriminative stimulus effects of cocaine (10 mg/kg), (+)-methamphetamine (1 mg/kg), and LSD (0.1 mg/kg). Response rate was decreased after 1 and 2.5 mg/kg 5-MeO-DET in both cocaine-trained rats ($F_{6,30} = 3.94$, $p = 0.005$) and methamphetamine-trained rats ($F_{6,24} = 12.61$, $p < 0.001$) and after 1 mg/kg in LSD-trained rats ($F_{5,25} = 8.14$, $p < 0.001$). Adverse effects occurred after 5 mg/kg 5-MeO-DET, including tremors (2 of 3 rats) and death (1 of 3 rats), an outcome that precluded further testing.

5-MeO-AMT. 5-MeO-AMT did not fully substitute for any of the training drugs. 5-MeO-AMT (0.5 mg/kg) produced a maximum of 67% LSD-appropriate responding in the LSD-trained rats, but higher doses resulted in suppression of responding, leading to uncertainty as to whether or not substitution could be detected in this dose range. In DMT-trained rats, 5-MeO-AMT produced nonlinear effects such that 0.5 mg/kg 5-MeO-AMT resulted in 43% DMT-appropriate responding, but 1 mg/kg yielded only 9% DMT-appropriate responding. 5-MeO-AMT (0.1–1 mg/kg) failed to substitute for the discriminative stimulus effects produced by DOM, MDMA, (+)-methamphetamine, and cocaine. 5-MeO-AMT decreased response rate after 0.5 and/or 1 mg/kg in all training groups ($p < 0.001$), with near total suppression of responding after 1 mg/kg.

Locomotor Activity

DIPT. Treatment with DIPT resulted in time-dependent depression of locomotor activity after 30 mg/kg (Fig. 2). A two-way analysis of variance conducted on horizontal activity counts/10 min failed to indicate significant effects of treatment ($F_{5,40} = 1.75$, $p = 0.145$), although significant effects were observed for the 10-min periods ($F_{47,1880} = 23.42$, $p < 0.001$) and the interaction of periods and treatment ($F_{235,1880} = 1.62$, $p < 0.001$). Depressant effects of 30 mg/kg occurred within 10 min after injection and lasted 80 min. A one-way analysis of variance conducted on horizontal activity counts for the 10- to 40-min time period (maximal depressant effect) indicated a significant effect of treatment ($F_{5,40} = 8.28$, $p < 0.001$), and planned comparisons (a priori contrast) against the vehicle group showed a significant depressant effect for 30 mg/kg ($p < 0.05$ denoted on Fig. 2 by *). The mean average horizontal activity counts/10 min for this 30-min period were fit to a linear function of $\log_{10}$ dose of the descending portion of the dose-effect

curve (3–30 mg/kg dose range). The ID_{50} was estimated to be 26.3 mg/kg.

5-MeO-DET. Treatment with 5-MeO-DET resulted in time- and dose-dependent stimulation of locomotor activity in doses from 0.3 to 10 mg/kg (Fig. 2). Stimulant effects of 0.3 and 1 mg/kg occurred within 30 min after injection and lasted 80 to 90 min. A two-way analysis of variance conducted on horizontal activity counts/10 min indicated significant effects of treatment ($F_{5,42} = 5.55$, $p < 0.001$), 10-min periods ($F_{47,1974} = 39.23$, $p < 0.001$), and the interaction of periods and treatment ($F_{235,1974} = 1.96$, $p < 0.001$). A one-way analysis of variance conducted on horizontal activity counts for the 20- to 50-min time period (maximal stimulant effect) indicated a significant effect of treatment ($F_{5,42} = 5.64$, $p = 0.001$), and planned comparisons (a priori contrast) against the vehicle group showed a significant stimulant effect for 0.3, 1 and 3 mg/kg ($p < 0.05$ denoted on Fig. 2 by *). The ED_{50} for the stimulant effect was estimated to be 0.24 mg/kg.

5-MeO-AMT. Treatment with 5-MeO-AMT resulted in time-dependent depression of locomotor activity after 3 and 10 mg/kg. A two-way analysis of variance conducted on horizontal activity counts/10 min indicated significant effects of treatment ($F_{6,49} = 2.68$, $p = 0.025$), 10-min periods ($F_{47,2303} = 33.34$, $p < 0.001$), and the interaction of periods and treatment ($F_{282,2303} = 2.17$, $p < 0.001$). Depressant effects of 3 and 10 mg/kg occurred within 10 min after injection and lasted 30 to 50 min. A one-way analysis of variance conducted on horizontal activity counts for the 0- to 30-min time period (maximal depressant effect) indicated a significant effect of treatment ($F_{6,49} = 19.18$, $p < 0.001$), and planned comparisons (a priori contrast) against the vehicle group showed a significant depressant effect for 3 and 10 mg/kg ($p < 0.05$ denoted on Fig. 2 by *). The ID_{50} for the depressant effect was estimated to be 2.3 mg/kg.

5-HT_{1A} Receptors

DIPT, 5-MeO-DET, and 5-MeO-AMT all bound to 5-HT_{1A} receptors in HEK cells at nanomolar concentrations (Table 2). Analysis using one-way analysis of variance and Tukey's post hoc test revealed that the rank order of affinity (K_i value) was 5-MeO-DET > 5-MeO-AMT ($p < 0.001$) > DIPT ($p < 0.001$). All three test compounds were less potent than the endogenous ligand serotonin ($p < 0.001$), and the standard compound dihydroergotamine ($p < 0.001$). The Hill coefficients were different from one for 5-MeO-DET and 5-MeO-AMT, which may suggest complex binding interactions. In the 5-HT_{1A} functional assay, DIPT, 5-MeO-DET, and 5-MeO-AMT all enhanced binding of [³⁵S] GTPγS (Table 2). The rank order of potency (EC_{50}) was 5-MeO-DET = 5-MeO-AMT ($p > 0.05$) > DIPT ($p < 0.05$). All three compounds were less potent than the endogenous ligand serotonin ($p < 0.001$) and the standard compound dihydroergotamine ($p < 0.001$). 5-MeO-DET and 5-MeO-AMT had equal efficacy compared with serotonin; however, DIPT produced only 58% of the maximal effect of serotonin.

5-HT_{2A} Receptors

DIPT, 5-MeO-DET, and 5-MeO-AMT all bound to 5-HT_{2A} receptors in HEK cells at nanomolar concentrations (Table 2). The rank order of affinity (K_i value) was 5-MeO-AMT > 5-MeO-DET ($p < 0.01$) > DIPT ($p < 0.05$). 5-MeO-DET and DIPT had lower affinity than the endogenous ligand sero-

Fig. 2. Effect of DIPT, 5-MeO-DET, and 5-MeO-AMT on horizontal activity counts/10 min as a function of dose (top to bottom) and time interval during an 8-h session. Each panel shows one dose group compared with vehicle control. *, $p < 0.05$ compared with vehicle for depressant or stimulant effect (10–40 min).

tonin ($p < 0.001$), and all three test compounds had lower affinity than the standard compound LSD ($p < 0.001$). The Hill coefficients were different from one. All three test compounds were agonists in the functional assay for 5-HT_{2A} receptors, release of arachidonic acid (Table 2). The rank order of potency was 5-MeO-AMT > 5-MeO-DET ($p < 0.001$) > DIPT ($p < 0.05$). 5-MeO-AMT had similar potency to serotonin and LSD ($p > 0.05$), and 5-MeO-DET and DIPT were less potent than serotonin ($p < 0.05$) and LSD ($p < 0.001$). However, the efficacy of the three test compounds at the release of arachidonic acid was the same as for the standard, LSD. Consistent with these results, all three compounds were agonists in the 5-HT_{2A} IP-1 functional assay (Table 2). The rank order of potency was 5-MeO-AMT > 5-MeO-DET ($p < 0.01$) = DIPT ($p > 0.05$). 5-MeO-AMT was more potent than serotonin ($p < 0.05$) and less potent than LSD ($p < 0.05$), whereas DIPT and DET were less potent than LSD ($p < 0.001$) but had similar potency to serotonin ($p > 0.05$). The efficacies of the three test compounds were similar to those of serotonin and LSD ($p > 0.05$).

Biogenic Amine Transporters

5-MeO-AMT, 5-MeO-DET, and DIPT produced little or no interaction with dopamine and norepinephrine transport-

ers at concentrations less than 10 μ M (Table 3). None of the three test compounds had measureable affinity for the dopamine and norepinephrine transporters. However, 5-MeO-AMT blocked dopamine uptake with an IC₅₀ in the low micromolar range (2.69 μ M). None of the three test compounds elicited release of dopamine or norepinephrine.

In contrast, DIPT, 5-MeO-DET, and 5-MeO-AMT all produced measurable effects at the serotonin transporter at nanomolar or low micromolar concentrations (Table 3). The rank order of binding affinity was DIPT > 5-MeO-DET ($p < 0.001$) > 5-MeO-AMT ($p < 0.01$). The affinity of DIPT was comparable with that of cocaine ($p > 0.05$), whereas 5-MeO-DET and 5-MeO-AMT had 12- to 30-fold lower affinity ($p < 0.001$). Again, the Hill coefficients were different from one. All three compounds blocked serotonin uptake, with the rank order of potency being DIPT > 5-MeO-AMT ($p < 0.001$) $\geq$ 5-MeO-DET ($p > 0.05$). Compared with the standard compound cocaine, DIPT had similar potency ($p > 0.05$) and 5-MeO-DET and 5-MeO-AMT had lower potency ($p < 0.001$). Only 5-MeO-AMT elicited serotonin release. 5-MeO-AMT was approximately equipotent to *p*-chloroamphetamine at serotonin release (unpaired two-tailed *t* test; $p > 0.05$), but was less efficacious, producing only 37% of the maximal effect of *p*-chloroamphetamine (Table 3).

TABLE 2

Pharmacology of DIPT, 5-MeO-DET, and 5-MeO-AMT at 5-HT_{1A} and 5-HT_{2A} receptors: effects on binding and functionFor test compounds, $n = 3-8$ for each assay. Data are expressed as mean $\pm$ S.E.M. The standard compounds for purposes of comparison for 5-HT_{1A} binding and functional assays were serotonin and dihydroergotamine. The standard compounds for 5-HT_{2A} binding and functional assays were serotonin and LSD.

Assay	Drug					
	DIPT	5-MeO-DET	5-MeO-AMT	Serotonin	Dihydroergotamine	LSD
5-HT _{1A} binding and functional assay						
[³ H]8-OH-DPAT binding K_i , nM	2270 $\pm$ 600	23.8 $\pm$ 7.9	194 $\pm$ 71	3.42 $\pm$ 0.41	1.01 $\pm$ 0.35	
Hill coefficient	-1.01 $\pm$ 0.15	-0.84 $\pm$ 0.07	-0.83 $\pm$ 0.04	-0.89 $\pm$ 0.10	-0.58 $\pm$ 0.04	
[³⁵ S]GTP γ S binding EC ₅₀ , nM	4570 $\pm$ 640	380 $\pm$ 110	680 $\pm$ 250	16.1 $\pm$ 5.7	0.82 $\pm$ 0.13	
5-HT maximal effect, %	58.1 $\pm$ 9.8	103.0 $\pm$ 6.2	100.6 $\pm$ 7.9	100	91 $\pm$ 24	
5-HT _{2A} binding and functional assays						
[¹²⁵ I]DOI binding K_i , nM	910 $\pm$ 120	251 $\pm$ 92	32.9 $\pm$ 5.5	20.4 $\pm$ 4.3		0.92 $\pm$ 0.16
Hill coefficient	-0.72 $\pm$ 0.05	-0.76 $\pm$ 0.08	-0.68 $\pm$ 0.07	-0.70 $\pm$ 0.06		-1.07 $\pm$ 0.13
[³ H]AA release EC ₅₀ , nM	450 $\pm$ 220	89 $\pm$ 34	3.35 $\pm$ 0.96	10.3 $\pm$ 1.0		1.01 $\pm$ 0.31
LSD maximal effect, %	88 $\pm$ 10	106 $\pm$ 16	108 $\pm$ 14			100
IP-1 formation EC ₅₀ , nM	420 $\pm$ 140	280 $\pm$ 120	8.4 $\pm$ 4.4	49 $\pm$ 13		0.277 $\pm$ 0.055
Serotonin maximal effect, %	81.4 $\pm$ 3.9	102 $\pm$ 11	84.2 $\pm$ 8.7	99.7 $\pm$ 1.4		83.1 $\pm$ 5.6

TABLE 3

Radioligand binding and functional activity of DIPT, 5-MeO-DET, and 5-MeO-AMT at monoamine transporters

For test compounds, $n = 3-6$ for binding and uptake assays, $n = 2-4$ for release assays. Data are mean $\pm$ S.E.M. The standard compounds were, for purposes of comparison, cocaine for the binding and uptake assays, methamphetamine for [³H]dopamine, and *p*-chloroamphetamine for [³H]serotonin release.

Assay	Drug					
	DIPT	5-MeO-DET	5-MeO-AMT	<i>p</i> -Chloroamphetamine	Methamphetamine	Cocaine
HEK-hDAT cells						
[¹²⁵ I]RTI-55 binding K_i , nM	>10 μ M	>10 μ M	>10 μ M			520 $\pm$ 69
Hill coefficient						-0.84 $\pm$ 0.06
[³ H]Dopamine uptake IC ₅₀ , nM	>7300*	>10 μ M	2690 $\pm$ 940			270 $\pm$ 83
[³ H]Dopamine release EC ₅₀ , nM	N.D.	N.D.	N.D.		970 $\pm$ 250	
Methamphetamine maximal effect, %	Minimal	Minimal	Minimal		100	
HEK-hSERT cells						
[¹²⁵ I]RTI-55 binding K_i , nM	265 $\pm$ 21	3520 $\pm$ 190	8270 $\pm$ 350			277 $\pm$ 33
Hill coefficient	-0.82 $\pm$ 0.04	-1.29 $\pm$ 0.03	-1.96 $\pm$ 0.20			-0.88 $\pm$ 0.07
[³ H]Serotonin uptake IC ₅₀ , nM	215 $\pm$ 72	2990 $\pm$ 680	1980 $\pm$ 530			320 $\pm$ 78
[³ H]Serotonin release EC ₅₀ , nM	N.D.	N.D.	810 $\pm$ 380	1350 $\pm$ 420	27,800 $\pm$ 7700	
<i>p</i> -Chloroamphetamine maximal effect, %	Minimal	Minimal	37 $\pm$ 10	100	Minimal	
HEK-hNET cells						
[¹²⁵ I]RTI-55 binding K_i , nM	>10 μ M	>10 μ M	>10 μ M			1890 $\pm$ 380
Hill coefficient						-0.74 $\pm$ 0.04
[³ H]Norepinephrine uptake IC ₅₀ , nM	>8100*	>10 μ M	>10 μ M			302 $\pm$ 41
[³ H]Norepinephrine release EC ₅₀ , nM	N.D.	N.D.	N.D.		67 $\pm$ 18	
Methamphetamine maximal effect, %	Minimal	Minimal	Minimal		100	

N.D., not determined.

* If some experiments yielded IC₅₀ or K_i values less than 10 μ M and other experiments yielded IC₅₀ or K_i values more than 10 μ M, the latter experiments were assigned a value of 10 μ M, and averages were calculated. The actual value is greater than that average, and no standard error is reported.

Discussion

The discriminative stimulus effects of DIPT, 5-MeO-DET, and 5-MeO-AMT were tested in separate groups of rats trained to discriminate LSD, DOM, DMT, MDMA, methamphetamine, or cocaine from saline. In addition, the effects of DIPT, 5-MeO-DET, and 5-MeO-AMT on locomotor activity were tested. Finally, the ability of DIPT, 5-MeO-DET, and 5-MeO-AMT to bind and activate 5-HT_{1A} and 5-HT_{2A} receptors or serotonin, dopamine, and norepinephrine transporters was tested. The receptor mechanisms and behavioral effects of DIPT, 5-MeO-DET, and, to a lesser extent, 5-MeO-AMT were similar to those of abused hallucinogens such as LSD, DOM, and DMT, but not to those of the psychostimulants, such as methamphetamine and cocaine.

DIPT showed the most overlap with the discriminative stimuli produced by hallucinogens: full substitution for DOM and DMT and relatively high levels of LSD-appropriate re-

sponding (68%). DIPT produced little or no drug-appropriate responding in the rats trained to discriminate methamphetamine, MDMA, and cocaine. The highest dose of DIPT tested (25 mg/kg) produced convulsions in both of the two rats tested. In terms of its mechanism of action, DIPT was most potent at binding to the serotonin transporter and blocking serotonin uptake, but also bound to and activated 5-HT_{2A} receptors with full efficacy at nanomolar concentrations in both functional assays. DIPT was less efficacious at activating 5-HT_{1A} receptors, but 5-HT_{1A} activity is not thought to be necessary for hallucinogenic effects (Nichols, 2004). These findings are similar to earlier reports that DIPT blocked serotonin uptake (Nagai et al., 2007; Cozzi et al., 2009). One study also reported that DIPT blocked uptake of dopamine and norepinephrine, but at micromolar concentrations, which were higher than those tested in the present study (Nagai et al., 2007). Because DIPT shared discriminative

stimulus effects and mechanism of action with the currently abused hallucinogens DOM, DMT, and LSD, DIPT may also have substantial potential for abuse, which agrees with the large number of reports of human use on the internet (e.g., at www.erowid.org). Furthermore, the incidence of convulsions indicates that DIPT has dangerous effects at high doses.

5-MeO-DET fully substituted for the discriminative stimulus effects of DMT, a component of the shamanistic entheogen ayahuasca, which has attracted large increases in use (Halpern, 2004; McKenna, 2004; Gable, 2007). 5-MeO-DET also produced relatively high levels of MDMA-appropriate responding (59%), but produced little drug-appropriate responding in rats trained to discriminate LSD, DOM, cocaine, or methamphetamine. 5-MeO-DET had highest affinity for the 5-HT_{1A} receptors, having 10- and 150-fold higher affinity at 5-HT_{1A} than at 5-HT_{2A} receptors and the serotonin transporter, respectively. 5-MeO-DET was fully efficacious at 5-HT_{1A} and 5-HT_{2A} receptors. These findings are in agreement with the observation that 5-MeO-DET binds to 5-HT₂ receptors (Lyon et al., 1988) and suggest further that 5-MeO-DET binds to the 5-HT_{2A} subtype. Taken together, these findings suggest that 5-MeO-DET may have some potential for abuse. There is not much evidence of current use of 5-MeO-DET unlike DIPT (e.g., www.erowid.org), which may be related to its smaller degree of substitution for well known hallucinogens such as DOM and LSD. The highest dose of 5-MeO-DET tested (5 mg/kg) produced tremors and lethality, which indicates that 5-MeO-DET may be dangerous in humans at high doses.

5-MeO-AMT produced a maximum of 67% LSD-appropriate responding. In DMT-trained rats, 5-MeO-AMT produced an inverted u-shaped function, such that the 0.5 mg/kg dose elicited DMT-appropriate responding (43%), but a higher dose produced markedly lower levels of DMT-appropriate responding (9%) as well as substantial suppression of responding. The rats that were most sensitive to the stimulus effects of 5-MeO-AMT and chose the DMT-appropriate lever were also the most sensitive to rate suppression. This suggests that there may be a subpopulation of rats in which low doses of 5-MeO-AMT produce discriminative stimulus effects similar to DMT and another subpopulation in which they do not. In the DOM-, MDMA-, methamphetamine-, and cocaine-trained rats, 5-MeO-AMT produced only small amounts of drug-appropriate responding, but suppression of the response rate prevented testing higher doses. It is a limitation of the drug discrimination assay that the discriminability of a drug cannot be assessed if the subjects do not press the levers. Because 5-MeO-AMT suppressed responding, we cannot tell whether those or higher doses would produce greater amounts of substitution. It should be noted that an earlier report using rats trained to discriminate DOM from saline also reported little or no substitution by 5-MeO-AMT at 15 min, but saw full substitution for DOM at 90 min (Glennon et al., 1983). Longer pretreatment times were not tested in the present study because the locomotor activity data showed the largest decrease in activity at 10 to 20 min and no effects at 90 min. These findings suggest that the time course of locomotor activity may not always predict the time course of the discriminative stimulus effects of hallucinogens, nor do locomotor effects predict discrimination performance, because DIPT decreased locomotor activity, whereas 5-MeO-DET increased locomotor activity, but both substituted for at least

one compound. Nevertheless, the locomotor effects were good predictors of the potency of the compounds in the discrimination tests and for suppression of response rate.

5-MeO-AMT had the highest affinity and potency of the three compounds for 5-HT_{2A} receptors and had affinity for the 5-HT_{1A} receptors between 5-MeO-DET and DIPT. These findings are in agreement with earlier reports that 5-MeO-AMT binds to 5-HT_{1A} and 5-HT_{2A} receptors (Glennon et al., 1990; Tomaszewski et al., 1992). 5-MeO-AMT was actually more efficacious at the activation of 5-HT_{1A} receptors than was DIPT in the present study even though DIPT had stronger stimulus effects than 5-MeO-AMT. This finding suggests that 5-HT_{1A} receptors may not be a major contributor to the discriminative stimulus effects of hallucinogens or at least those of DIPT. There is a substantial number of reports of recreational use of 5-MeO-AMT, which is known by the street name Alpha-O (e.g., www.erowid.org). Large doses have been reported to lead to long "trips" and reports of unpleasant subjective effects, vomiting, seizures, and possibly lethality. The rats in the present study may have been more sensitive to these adverse effects than to the hallucinogen-like stimulus effects, leading to the rate suppression and lack of substitution.

Psychedelic compounds have various chemical structures, so it is important to test the effects of novel compounds across a range of categories. Although activation of 5-HT_{2A} receptors is thought to be a primary mechanism of action for hallucinogens, the precise mechanism is not known (Nichols, 2004; Winter 2009). It was also possible that these compounds have other mechanisms of action that could contribute to abuse liability, so a variety of molecular mechanisms were tested. Not surprisingly, these three tryptamines produced behavioral effects most similar to those of DMT, which is structurally similar. Overall, each of the compounds produced a different profile of substitution for the discriminative stimulus effects of several psychoactive compounds and a different profile of molecular mechanism. Testing a wide range of discriminations and molecular mechanisms was important; for example, if only the discriminative stimulus effects of LSD had been tested, it would probably have been concluded that these compounds had limited abuse liability.

It should be noted that different species were used in the different assays. Mice were used in the locomotor activity studies, rats were used in the discrimination studies, and human cell lines were used in the binding and functional assays. The mouse data accurately predict the behaviorally active dose range in the rat and are fairly good at predicting time course. Human cell lines were chosen because they are more directly relevant to human brain mechanisms and behavior. In addition, inference about abuse liability was made based on stimulus similarity to known drugs of abuse and mechanisms of action similar to those of known drugs of abuse. Other classic methods of abuse liability testing include self-administration and testing for development of dependence and withdrawal. Unfortunately, these assays are of limited use with hallucinogens because these compounds are typically not self-administered in nonhuman animals and do not produce dependence (Deneau et al., 1969; Nichols, 2004; Fantegrossi et al., 2008).

In conclusion, DIPT and 5-MeO-DET shared stimulus effects with various abused hallucinogens, so they may have similar abuse liability. In addition, both compounds showed

serious adverse effects such as tremors/convulsions or lethality. In contrast, 5-MeO-AMT showed only low levels of drug-appropriate responding for the various abused hallucinogens at the doses and time tested. However, the large rate of suppressant effects may have prevented detection of hallucinogen-like discriminative effects. These studies provide evidence for increased control of DIPT and 5-MeO-DET, but further testing of 5-MeO-AMT will be necessary to confirm its abuse liability.

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Authorship Contributions

Participated in research design: Gatch, Forster, Janowsky, and Eshleman.

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Wrote or contributed to the writing of the manuscript: Gatch, Forster, Janowsky, and Eshleman.

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