Behavioral and neurochemical pharmacology of six psychoactive substituted phenethylamines: Mouse locomotion, rat drug discrimination and in vitro receptor and transporter binding and function

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Abstract

Rationale—Psychoactive substituted phenethylamines 2,5-dimethoxy-4-chlorophenethylamine (2C-C); 2,5-dimethoxy-4-methylphenethylamine (2C-D); 2,5-dimethoxy-4-ethylphenethylamine (2C-E); 2,5-dimethoxy-4-iodophenethylamine (2C-I); 2,5-dimethoxy-4-ethylthiophenethylamine (2C-T-2) and 2,5-dimethoxy-4-chloroamphetamine (DOC) are used recreationally and may have deleterious side effects.

Objectives—This study compares behavioral effects and mechanisms of action of these substituted phenethylamines with those of hallucinogens and a stimulant.

Methods—The effects of these compounds on mouse locomotor activity and in rats trained to discriminate dimethyltryptamine, (−)DOM, (+)LSD, (±)MDMA and (S+)methamphetamine were assessed. Binding and functional activity of the phenethylamines at $5\text{-}HT_{1A}$, $5\text{-}HT_{2A}$, $5\text{-}HT_{2C}$ receptors and monoamine transporters were assessed using cells heterologously expressing these proteins.

Results—The phenethylamines depressed mouse locomotor activity, although 2C-D and 2C-E stimulated activity at low doses. The phenethylamines except 2C-T-2 fully substituted for at least one hallucinogenic training compound but none fully substituted for (+)-methamphetamine. At $5\text{-}HT_{1A}$ receptors, only 2C-T-2 and 2C-I were partial-to-full very low potency agonists. In $5\text{-}HT_{2A}$ arachidonic acid release assays, the phenethylamines were partial to full agonists except 2C-I which was an antagonist. All compounds were full agonists at $5\text{-}HT_{2A}$ and $5\text{-}HT_{2C}$ receptor inositol phosphate assays. Only 2C-I had moderate affinity for, and very low potency at, the serotonin transporter.

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Conflict of interest.

All authors declare that they have no conflicts of interest.

Conclusions—The discriminative stimulus effects of 2C-C, 2C-D, 2C-E, 2C-I and DOC were similar to those of several hallucinogens but not methamphetamine. Additionally, the substituted phenethylamines were full agonists at 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors, but for 2C-T-2, this was not sufficient to produce hallucinogenlike discriminative stimulus effects. Additionally, the 5-HT$_{2A}$ inositol phosphate pathway may be important in 2C-I's psychoactive properties.

Keywords

Substituted phenethylamines; Drug discrimination; Serotonin receptor; Locomotor activity; Lysergic acid diethylamide (LSD); (-)-2,5-dimethoxy-4-methylamphetamine; Drug abuse

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Introduction

Synthetic "designer" hallucinogens are psychoactive compounds derived from phenalkylamines such as mescaline and amphetamine, from tryptamines such as *N,N,*-dimethyltryptamine (DMT), or ergolines such as lysergic acid diethylamide (LSD) (Nichols 2004). These psychoactive drugs do not produce any clear withdrawal syndrome (Shulgin and Shulgin 1991), but psychosis in predisposed individuals following LSD ingestion has occurred (reviewed in Cohen 1967;Nichols 2004). The United States Drug Enforcement Agency has categorized some hallucinogenic compounds, including LSD, DMT and substituted phenethylamines 2,5-dimethoxy-4-chlorophenethylamine (2C-C); 2,5-dimethoxy-4-methylphenethylamine (2C-D); 2,5-dimethoxy-4-ethylphenethylamine (2C-E); and 2,5-dimethoxy-4-iodophenethylamine (2C-I); 2,5-dimethoxy-4-ethylthiophenethylamine (2C-T-2) (Fig. 1), as Schedule 1 substances, a category having abuse liability and no recognized therapeutic uses (DEA 2013). 2,5-Dimethoxy-4-chloroamphetamine (DOC) is regulated by the Federal Analog Act. The synthesis and psychoactive properties of the phenethylamines in humans have been described (Shulgin and Shulgin 1991).

The reported effects of these compounds are dose-dependent, with a combination of stimulant and hallucinogenic effects (reviewed in Dean et al. 2013). Generally, stimulation and increased visual, auditory and tactile sensation are seen with low doses, hallucinations with moderate doses, and unpleasant hallucinations, tachycardia, hypertension and excited delirium with higher doses (Dean et al. 2013). While there is a paucity of clinical data, some case reports of adverse side effects include stroke and quadriplegia following ingestion of 2C-I with 3,4-methylenedioxyamphetamine (Drees et al. 2009) and seizures and rhabdomyolysis following ingestion of DOC, MDMA and ethanol (Ovaska et al. 2008).

Although there is agreement that the neuronal serotonergic system is involved in the discriminative stimulus effects of hallucinogenic compounds (Glennon et al. 1984;Winter 2009), debate continues regarding which receptor subtypes are involved and whether the compounds are agonists, partial agonists or antagonists (reviewed in Halberstadt and Geyer 2011;Nichols 2004). Possible biochemical targets of these compounds have been investigated in several systems (Berg et al. 1998;Kurrasch-Orbaugh et al. 2003;Moya et al. 2007). Using antagonists with differential affinity for 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors in rats trained to discriminate LSD and 2,5-dimethoxy-4-methylamphetamine (DOM) from water, Fiorella et al. (1995) found that affinities of antagonists at 5-HT$_{2A}$, but not 5-HT$_{2C}$, receptors correlated with IC$_{50}$ values for blocking LSD and DOM behavioral effects. 5-HT$_{2A}$ receptor antagonists also decreased rhesus monkeys' responding to the phenethylamines DOM and 2,5-dimethoxy-4-(n)-propylthiophenethylamine (2C-T-7) (Li et al. 2010). Thus, the stimulus effects of some substituted ergolines and phenethylamines may be elicited by 5-HT$_{2A}$ receptor agonist activity. 2C-C, 2C-E and 2C-I partially stimulated guanosine 5′-O-[gammathio]triphosphate (GTPγS) binding in cortical membranes, an effect blocked by methiothepin, an antagonist for 5-HT$_{1,6,\&7}$ receptors (Nonaka et al. 2007). In rat

brain synaptosomes, 2C-C, 2C-E and 2C-I inhibited serotonin and norepinephrine uptake at mid-micromolar concentrations but had no effect on dopamine uptake or neurotransmitter release via the transporters (Nagai et al. 2007).

The goal of this study was to assess behavioral effects and mechanisms of action of these substituted phenethylamines. First, drug-induced locomotor changes were characterized in mice to estimate the effective dose range and time course of behavioral effects. Next, the ability of these compounds to produce discriminative stimulus effects similar to those of a range of known drugs of abuse was tested in rats. Because phenethylamines can produce either psychostimulant or hallucinogenic effects, a number of compounds with a range of stimulant and/or hallucinogenic effects were used to screen for psychoactive effects. In addition, the ability of these compounds to bind to and activate pharmacological targets of known abused drugs was examined to confirm and extend the biochemical data available for these substituted phenethylamines. The 4-substituents on the phenyl ring can differentially influence the biochemical activity of phenethylamines (Nichols 1986b). $5-HT_{1A}$, $5-HT_{2A}$ and $5-HT_{2C}$ receptors are primary pharmacological targets for hallucinogens such as DMT and LSD, and the methylated phenethylamine, amphetamine, exerts its initial effects *via* the dopamine, serotonin and norepinephrine transporters, leading to effects at neurotransmitter (dopamine) receptors. For these reasons drug effects on these systems were characterized.

Materials and Methods

Subjects

Male Sprague-Dawley rats were obtained from Harlan-Sprague Dawley (Indianapolis, IN). All rats were housed individually and were maintained on a 12:12 light/dark cycle (lights on at 7:00 AM). Body weights were maintained at 320–350 g by limiting food to 15 g/day which included the food received during operant sessions. Water was freely available. Male Swiss–Webster mice were obtained from Harlan (Indianapolis, IN) 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 were allowed free access to food and water. All housing and procedures were in accordance with the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (National Research Council 2003) and were approved by the University of North Texas Health Science Center Animal Care and Use Committee.

Locomotor Activity

The study was conducted using 40 Digiscan (model RXYZCM, Omnitech Electronics, Columbus, OH) locomotor activity testing chambers ($40.5 \times 40.5 \times 30.5$ cm) housed in sets of two, within sound-attenuating chambers as previously described (Gatch et al. 2011). Separate groups of 8 mice were injected intraperitoneally with either vehicle (0.9% saline), 2C-C (1, 3, 10, 30 and 100 mg/kg), 2C-D (1, 3, 10 and 30 mg/kg), 2C-E (0.03, 0.1, 0.3, 1, 3, 10 and 30 mg/kg), 2C-I (0.3, 1, 3, 10 and 30 mg/kg), 2C-T-2 (0.1, 0.3, 1, 3 and 10 mg/kg), or DOC (0.1, 0.3, 1, 3 and 10 mg/kg), immediately prior to locomotor activity testing. In all studies, horizontal activity (interruption of photocell beams) was measured for 8 h within 10-min periods, beginning at 0800 h (2 h after lights on).

Discrimination Procedures

Standard behavior-testing chambers (Coulbourn Instruments, Allentown, PA) were connected to IBM-PC compatible computers via LVB interfaces (Med Associates, East Fairfield, VT). The computers were programmed in MED-PC IV (Med Associates, East Fairfield, VT) 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 five compounds from saline: METH (1 mg/kg), MDMA (1.5 mg/kg), LSD (0.1 mg/kg), DOM (0.5 mg/kg)and DMT (5 mg/kg) as previously described (Gatch et al. 2009;Gatch et al. 2011). Rats were injected i.p. with either saline or drug and then placed in the operant chambers, where food (45 mg food pellets; Bio-Serve, Frenchtown, NJ) was available under a fixed-ratio 10 schedule of reinforcement. Each training session lasted a maximum of 10 min, and rats could earn up to 20 food pellets. Pretreatment times were 5 min for DMT, 10 min for METH, 15 min for LSD and MDMA, and 30 min for DOM.

The substitution 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 20 reinforcers were obtained, or for a maximum of 20 min. 2C-C, 2C-D, 2C-E, 2C-I, 2C-T-2, or DOC were tested for substitution in subsets of 6 rats from each training drug group. Doses of these compounds were presented incrementally in separate sessions, using a repeated measures design (i.e., each of 6 rats was tested at all doses). All compounds were tested 15 min after i.p. injection, based on the earliest time a locomotor effect was observed. DOC and 2C-D were also tested 60 and 70 min, respectively, after injection in separate groups of rats to investigate whether stimulus control was different during the stimulant phase.

5-HT receptors, 5-HT$_{1A}$ receptor

Human embryonic kidney (HEK) cells expressing the human 5-HT$_{1A}$ receptor (HEK-5-HT$_{1A}$) were used; the cell culture, membrane preparation, [^{3}H]8-hydroxy-N,N-dipropyl-2-aminotetralin ([^{3}H]8-OH-DPAT) binding assay and [^{35}S]GTPγS binding assay were conducted as described previously (Gatch et al. 2011;Newman-Tancredi et al. 1998). *5-HT$_{2A}$ and 5-HT$_{2C}$ receptors* HEK cells expressing the human 5-HT$_{2A}$ receptor (HEK-5-HT$_{2A}$ cells) or the human 5-HT$_{2C}$ receptor (HEK-5-HT$_{2C}$ cells) were used. [^{125}I]2,5-dimethoxy-4-iodoamphetamine ([^{125}I]DOI) binding, accumulation of inositol monophosphate using the IP-1 Elisa kit (Cisbio, Bedford, MA), and drug-induced facilitation of release of [^{3}H]AA from HEK-5-HT$_{2A}$ cells were conducted as previously described (Eshleman et al. 2013;Gatch et al. 2011;Knight et al. 2004;Kurrasch-Orbaugh et al. 2003)

Human dopamine (hDAT), serotonin (hSERT) and norepinephrine (hNET) transporters, Binding, Uptake, and Release

[^{125}I]RTI-55 binding, [^{3}H]neurotransmitter uptake, and neurotransmitter release assays were conducted as previously described (Eshleman et al. 1999;Eshleman et al. 2013;Gatch et al. 2011). HEK cells expressing recombinant hDAT, hSERT, or hNET were used.

Dopamine D1, D2 and D3 receptors, [^{3}H]SCH-23390 and [^{3}H]YM-09151-2 binding

Mouse fibroblast cells expressing the human dopamine D1 receptor, and chinese hamster ovary (CHO) cells expressing the human dopamine D2 or D3 receptor were obtained from Stanford Research Institute (SRI, Menlo Park, CA). The dopamine receptor assays were conducted as described previously (Toll et al. 1998).

Drugs

For behavioral assays, all drugs were dissolved in 0.9% saline. Hydrochloride salts of 2C-C, 2C-D, 2C-E, 2C-I, 2C-T-2, DOC, (−)-cocaine, (S+)-METH, (±) and (+)-3,4-methylenedioxymethamphetamine (MDMA), and (−)DOM, N,N-DMT fumarate and (+)-LSD(+)tartrate were provided by the National Institute on Drug Abuse Drug Supply

Program. [^{125}I]DOI, [^{3}H]8-OH-DPAT, [^{125}I]RTI-55, [^{3}H]dopamine, [^{3}H]serotonin, [^{3}H]norepinephrine, [^{3}H]AA and [^{35}S]GTPγS were purchased from Perkin Elmer (Boston, MA). Most other chemicals were purchased from Sigma (St. Louis, MO).

Data Analysis

Locomotor activity data were expressed as the mean number of photocell counts in the horizontal plane during each 10-min testing period. A 30-min period, beginning when maximal stimulation of locomotor activity first appeared as a function of dose, was used for analysis of dose-response data and calculation of ED50 values. from the ascending linear portion of the dose response curve. 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. Drug discrimination data were expressed as the mean percentage of training-drug-appropriate responses occurring in each substitution test. Response rates were expressed as the number of responses made, divided by total session time. Percent training-drug-appropriate responding was not calculated if a rat failed to complete at least 10 responses on one of the levers, and doses for which fewer than three rats met this criterion were not considered in the discrimination data analysis. Full substitution was defined as 80% drug-appropriate responding and not statistically different from the training drug, and partial substitution as 40% and <80% drug-appropriate responding and not statistically different from the training drug. The ED_{50} values were calculated by fitting straight lines to the dose-response data for each compound by means of TableCurve 2D (Jandel Scientific, San Rafael, CA). Response rate data were analyzed by one-way repeated measures ANOVA. Effects of individual doses were compared to the appropriate control value using single degree-of-freedom F tests. Criterion for significance was set at p<0.05.

For binding and functional assays, IC_{50} or EC_{50} values were calculated with GraphPad Prism. Binding IC_{50} values were converted to K_i values using the Cheng-Prusoff correction (Cheng and Prusoff 1973), Kd values used are listed in (Eshleman et al. 2013). Fractional release was defined as the amount of radioactivity in a fraction divided by the total radioactivity remaining in the sample. For serotonin receptor functional assays, drug effect each day was normalized to maximal serotonin effect. One-way ANOVAs were conducted using the logarithms of $K_i, IC_{50}, or EC_{50}$ values followed by Tukey's posthoc analysis. For release assays, area under the curve (AUC) was calculated using GraphPad Prism. Criterion for significance was set at p<0.05.

Results

Locomotor Activity

Figure 2 shows time course data for the test compounds. Because of the large amount of data, only doses which produced peak depressant or stimulant effects are shown, along with vehicle data for comparison. Treatment with 2C-C resulted in time- and dose-dependent depression of locomotor activity following 30 and 100 mg/kg [$F_{(5,41)}$=32.94, p<.001]; these effects occurred within 10 min following injection and lasted 30 to 120 min. Convulsions were observed in 2/8 mice and tremors in 6/8 mice at 30 min following 100 mg/kg 2C-C. Lethality occurred in 1/8 mice within 120 min following 100 mg/kg. Treatment with 2C-I resulted in time- and dose-dependent depression of locomotor activity following 3–30 mg/kg [$F_{(5,42)}$= 20.90, p<0.001]; depressant effects occurred within 10 min following injection and lasted 30–60 min (Fig 1). Treatment with 2C-T-2 resulted in time- and dose-dependent depression of locomotor activity following 3 and 10 mg/kg [$F_{(5,42)}$=19.88, p<0.001]; depressant effects occurred within 20 min following injection and lasted 30–50 min. Time-

and dose-dependent depression of locomotor activity following 3 and 10 mg/kg DOC occurred within 10 min following injection [$F_{(5,42)}$=44.58, p<.001] and lasted 30–80 min.

Treatment with 2C-D resulted in both stimulation and depression of locomotor activity. Depressant effects of 10 and 30 mg/kg 2C-D [$F_{(4,35)}$=11.62, p<.001] occurred within 10 min following injection and lasted 20–40 min. Stimulant effects of 3 mg/kg 2C-D [$F_{(4,35)}$=1.46, p=.236] occurred within 50 min following injection and lasted 180 min. Lethality occurred in 8/8 mice within 30 min following 100 mg/kg 2C-D. 2C-E stimulated locomotor activity at low doses and depressed locomotor activity at higher doses. Depressant effects of 10 and 30 mg/kg 2C-E [$F_{(5,42)}$=23.56, p<.001] occurred within 10 min following injection and lasted 50–70 min. Stimulant effects of 0.3 and 1 mg/kg 2C-E [$F_{(7,56)}$=2.71, p=.017] occurred within 50 min after injection and lasted 70–100 min.

Drug Discrimination

Table 1 shows summary data for the drug discrimination studies. 2C-C fully substituted for the discriminative stimulus effects of DOM (ED$_{50}$=0.95± 0.09 mg/kg) and MDMA (ED$_{50}$=1.48±0.15 mg/kg). 2C-C (5 mg/kg) produced a maximum of 75% drug-appropriate responding in both DMT- and LSD-trained rats, whereas 2C-C (10 mg/kg) produced minimal drug-appropriate responding in METH-trained rats. 2C-C decreased response rates following 2.5 mg/kg in MDMA-trained rats [$F_{(4,20)}$=6.18, p=.002], 10 mg/kg in DMT-trained rats [$F_{(5,25)}$=6.41, p=.001] and LSD-trained rats [$F_{(6,30)}$=5.51, p=.001], and 25 mg/kg in METH-trained rats [$F_{(4,8)}$=5.98, p=.016]. Adverse effects were observed following 25 mg/kg 2C-C, including reddening of the extremities (3/3 rats) and salivation (1/3 rats), and this dose was not tested in DMT- or LSD-trained rats.

2C-D was tested at two time points, 15 and 70 min, which corresponded with peak depressant and peak stimulant locomotor activity effects, respectively. At 15 min after administration, 2C-D fully substituted for the discriminative stimulus effects of DMT (ED$_{50}$=3.14±0.15 mg/kg), DOM (ED$_{50}$=0.77±0.10 mg/kg) and LSD (ED$_{50}$=0.71±0.12 mg/kg). 2C-D (2.5 mg/kg) produced 61% drug-appropriate responding in MDMA-trained rats, and 10 mg/kg produced 15% METH-appropriate responding (Table 1). Response rate was decreased following 1 and 2.5 mg/kg in DOM-trained rats [$F_{(4,20)}$=6.23, p=.002], 5 mg/kg in MDMA-trained rats [$F_{(5,25)}$=9.04, p<.001], 10 mg/kg in DMT-trained rats [$F_{(4,20)}$=5.13, p=.005], and 5 and 10 mg/kg in METH-trained rats [$F_{(4,20)}$=6.86, p=.001]. In METH-trained rats, 2/6 rats exhibited reddening of the extremities following 10 mg/kg 2C-D, and 4/6 rats failed to complete the first fixed ratio. At 70 min after administration, 2C-D fully substituted for the discriminative stimulus effects of DMT (ED$_{50}$=2.99±0.13 mg/kg) and LSD (ED$_{50}$=3.04±0.13 mg/kg). 2C-D (10 mg/kg) produced partial substitution of drug-appropriate responding in DOM-trained rats and MDMA-trained rats, and 25 mg/kg 2C-D partially substituted in (+)-METH-trained rats. Response rate was decreased following 10 mg/kg in DOM-trained rats [$F_{(4,20)}$=4.34, p=.011], 25 mg/kg in MDMA-trained rats [$F_{(5,25)}$=4.18, p=.007], and 25 and 50 mg/kg in (+)-METH-trained rats [$F_{(6,12)}$=5.49, p=.006]. Two of three rats receiving 50 mg/kg 2C-D exhibited salivation and failed to complete the first fixed ratio.

2C-E fully substituted for the discriminative stimulus effects of DMT (ED$_{50}$= 0.95±0.20 mg/kg), DOM (ED$_{50}$= 0.84±0.08 mg/kg), LSD (ED$_{50}$= 0.62±0.10 mg/kg), and MDMA (ED$_{50}$= 2.48±0.10 mg/kg), but produced minimal METH-appropriate responding. Response rate was decreased following 2.5, 5, and 10 mg/kg in MDMA-trained rats [$F_{(9,45)}$=10.25, p<.001] and 5 and 25 mg/kg in METH-trained rats [$F_{(10,50)}$=4.01, p<.001]. Loss of muscle tone was observed at 10 mg/kg in MDMA-trained rats, and 25 mg/kg 2C-E completely suppressed responding.

2C-I fully substituted for the discriminative stimulus effects of DMT ($ED_{50}=0.68\pm0.11$ mg/kg) and LSD ($ED_{50}= 1.66$ mg/kg±0.12). In MDMA-trained rats, 2.5–10 mg/kg 2C-I produced a maximal 65% drug-appropriate responding, and 1 mg/kg 2C-I produced only 38% drug-appropriate responding in METH-trained rats (Table 1). Response rate was decreased with doses of 2.5–10 mg/kg 2C-I in MDMA-trained rats [$F_{(4,20)}=3.66$, $p=.022$] and with 1 and 5 mg/kg in METH-trained [$F_{(4,20)}=5.08$, $p=.005$].

2C-T-2 produced 73% drug-appropriate responding following 2.5 mg/kg in DMT-trained rats. A 10 mg/kg dose of 2C-T-2 elicited hind limb paralysis, salivation and loss of muscle tone and was not tested further. 2C-T-2 failed to substitute for LSD, MDMA, or (+)-METH. 2C-T-2 substantially decreased response rates following 2.5 and 5 mg/kg in rats trained to DMT [$F_{(5,25)}=6.66$, $p<.001$], LSD [$F_{(4,20)}=4.60$, $p=.008$], MDMA [$F_{(3,15)}=3.46$, $p=.043$], and (+)-METH [$F_{(3,15)}=14.75$, $p<.001$]. In each case, 5/6 rats failed to complete the first fixed ratio at the highest dose tested. Decreased muscle tone was observed in 3/6 rats following 5 mg/kg 2C-T-2 in LSD-trained rats.

DOC was tested at two time points, 15 and 60 min, which corresponded with the peak depressant and peak stimulant locomotor activity effects, respectively. At 15 min following administration, DOC fully substituted for the discriminative stimulus effects of DOM ($ED_{50}=0.13\pm0.16$ mg/kg) and LSD ($ED_{50}=0.39\pm0.33$ mg/kg). DOC produced 65% DMT-appropriate responding following 1 mg/kg, and less than 50% drug-appropriate responding in MDMA-trained and METH-trained rats. Response rate was decreased following 2.5 mg/kg DOC in rats trained to LSD [$F_{(7,35)}=3.86$, $p=.003$], MDMA [$F_{(5,25)}=3.81$, $p=.011$], and METH [$F_{(5,25)}=3.86$, $p=.010$]. With 2.5 mg/kg DOC, substantial rate suppression was observed, such that 4/6 or 5/6 rats tested in each case failed to respond and decreased muscle tone was observed in 12/24 rats. At 60 min, DOC fully substituted for the discriminative stimulus effects of DMT ($ED_{50}=0.61\pm0.19$ mg/kg), DOM ($ED_{50}=0.26\pm0.19$ mg/kg), and LSD ($ED_{50}= 0.23\pm0.10$ mg/kg). In MDMA-trained rats, DOC produced 60% drug-appropriate responding following 1 mg/kg, and no drug-appropriate responding at any dose in METH-trained rats. Doses of 1 mg/kg and higher decreased response rates in rats trained to DMT [$F_{(5,25)}=3.66$, $p=.013$], LSD [$F_{(5,25)}=2.96$, $p=.031$], MDMA [$F_{(6,30)}=3.96$, $p=.005$], and METH [$F_{(6,30)}=4.07$, $p=.004$]. Substantial rate suppression and failure to complete the first fixed ratio was observed following 2.5 mg/kg DOC in MDMA-trained rats (4/6 rats) and 5 mg/kg in METH-trained rats (5/6 rats).

In Vitro Pharmacology, Interaction with Serotonin Receptors

In HEK-h5-HT$_{1A}$ cells, the phenethylamines were tested for their affinities for the [^{3}H]8-OH-DPAT binding site and their effect on 5-HT$_{1A}$ function (Table 2). The agonist [^{3}H]8-OH-DPAT binds to the 5-HT$_{1A}$ high affinity state and agonist activation of the receptor results in increased binding of [^{35}S]GTPγS to the Gαi/o subunit of G proteins and reflects receptor function. All six compounds had lower affinities (high nanomolar to low micromolar) for the [^{3}H]8-OH-DPAT binding site than serotonin and LSD (ps<0.001, one way ANOVA followed by Tukey's multiple comparison test). 2C-C, 2C-I, and 2C-T-2 had higher affinities than DOM, DOC, MDMA and METH (ps<0.05); 2C-E had higher affinity than MDMA and METH (ps<0.05); and DOC had lower affinity than DMT ($p<0.001$). In the [^{35}S]GTPγS functional assay, serotonin and LSD had very high potency, higher than all other compounds (ps<0.001). 2C-I and 2C-T-2 had similar low micromolar potencies and 2C-T-2 was more potent than MDMA and METH (ps<0.001). The four other phenethylamines had minimal efficacy (<25%). 2C-I and 2C-T-2 had similar efficacies to serotonin and LSD and 2C-I had higher efficacy than DOM, MDMA and METH ($p<0.05$).

In HEK-h5-HT$_{2A}$ cells, the phenethylamines were tested for their affinity for the [^{125}I]DOI binding site and effects on 5-HT$_{2A}$ signaling pathways. Agonist binding to 5-HT$_{2A}$

receptors can activate both phospholipase A2 increasing AA release from plasma membranes and phospholipase C increasing the inositol phosphate cascade (IP-1 assay). Binding affinities of the drugs were significantly different ($p < 0.0001$, one way ANOVA, Table 2). LSD had higher affinity than all other tested compounds ($ps < 0.001$). The phenethylamines had high affinities, and their Ki values did not differ from each other or from serotonin or DOM ($p > 0.05$), and were higher than DMT, MDMA and METH ($ps < 0.001$).

In the 5-HT$_{2A}$ [^{3}H]AA release assay, 2C-C, 2C-D, 2C-E, 2C-T-2, DOC, LSD and 5-HT were agonists with similar efficacies ($p = 0.26$, one way ANOVA, Table 2) while potencies differed significantly ($p < 0.0001$, one way ANOVA). LSD and serotonin had potencies in the low nanomolar range, with LSD having higher potency ($p < 0.01$). 2C-T-2, 2C-E, and DOC were very potent with EC$_{50}$ values that did not differ from serotonin and LSD. 2C-T-2, 2C-C, 2C-E, DOC, LSD and serotonin were more potent than 2C-D and DMT ($ps < 0.05$ to 0.001). In contrast, 2C-I minimally stimulated release (Fig 3A) and no EC$_{50}$ was determined. This finding was unexpected, since 2C-I substituted for the discriminative effects of LSD and DMT, which stimulated [^{3}H]AA release (Table 2 and Fig 3A). 2C-I fully inhibited serotonin-stimulated [^{3}H]AA release with low potency, similar to ketanserin ($p > 0.05$, two-tailed t-test, Fig 3B). To confirm that the same compound was used in behavioral and biochemical assays, an aliquot of 2C-I from the behavioral assays was tested and confirmed 2C-I antagonism of serotonin-mediated [^{3}H]AA release.

In the 5-HT$_{2A}$ IP-1 functional assay, all compounds tested were agonists (except METH with no measurable efficacy), with significantly different potencies ($p < 0.001$, one way ANOVA, Table 2, Fig 4A). LSD had higher potency ($ps < 0.001$) while MDMA had lower potency ($ps < 0.001$) than all other compounds. 2C-I and 2-C-T-2 were the most potent phenethylamines. 2C-I had higher potency than serotonin, 2C-D, and 2C-E ($ps < 0.05$ to 0.001). 2C-T-2 had higher potency than serotonin, DOM, 2C-D and 2C-E ($ps < 0.05$ to 0.001). 2C-C, 2C-D, 2C-E, DOC, DOM and serotonin had similar potencies. Efficacies differed significantly ($p < 0.0001$, one way ANOVA). 2C-E had higher efficacy than LSD, 2C-I and 2C-T-2 ($ps < 0.05$). 2C-C, 2C-D, 2C-I, 2C-T-2, DOC, serotonin, LSD and DOM had similar efficacies. DMT and MDMA had similar efficacy that was lower than all other compounds ($ps < 0.01$).

In HEK-h5-HT$_{2C}$ cells, the phenethylamines were tested for their affinities for the [^{125}I]DOI binding site and effects on 5-HT$_{2C}$-mediated IP-1 turnover. In the [^{125}I]DOI binding assay, there were significant differences in affinities ($p < 0.0001$, one way ANOVA). 2C-C, 2C-D, 2C-E, 2C-I, 2C-T-2, DOC, serotonin and LSD had similar, low nanomolar, affinities (Table 2). DOM had lower affinity than serotonin and LSD ($p < 0.01$) but was similar to the phenethylamines. DMT had similar affinity to DOM. MDMA and METH had similar affinities which were lower than all other compounds ($p < 0.001$).

All compounds were 5-HT$_{2C}$ agonists, activating the phospholipase C-inositol phosphate cascade, with significant differences in potencies ($p < 0.001$, one way ANOVA, Table 2, Fig 4B). The highest potency drugs were LSD, serotonin, 2C-I, 2C-T-2 and DOM. 2C-I was more potent than 2C-D, DMT, MDMA and METH ($ps < 0.01$). 2C-T-2 was more potent than DMT, MDMA and METH ($ps < 0.001$). 2C-C, 2C-D, 2C-E and DOC had similar low-to-mid nanomolar potencies and were less potent than serotonin ($ps < 0.05$) and LSD (except DOC). DMT, MDMA, and METH had very low potencies. Efficacies did not differ ($p = 0.28$, one way ANOVA).

In Vitro Pharmacology, Interaction with hDAT, hSERT and hNET and dopamine receptors

In the transporter assays, the phenethylamines had no measurable or very low affinity for hDAT and hNET in the binding assays, and very low potency (at least micromolar) in the [^{3}H]dopamine and [^{3}H]norepinephrine uptake assays (Table 3). Only 2C-I had measurable affinity (high nanomolar) for hSERT and very low potency in the [^{3}H]serotonin uptake assay. In release assays, the compounds had no releasing efficacy, while METH elicited robust release in all cell lines. The phenethylamines also had no measurable affinity for the dopamine D1, D2 and D3 receptors (data not shown). Thus, the substituted phenethylamines have minimal interaction with hDAT and hNET and dopamine receptors and very low potency at hSERT.

Discussion

Discriminative stimulus effects of six substituted phenethylamines were tested in separate groups of rats trained to discriminate DMT, DOM, LSD, MDMA, or METH from saline. Previously, we reported that these training compounds produced non-identical patterns of cross substitution: LSD fully substituted for all training compounds except METH; MDMA fully substituted for all compounds except partial substitution for LSD; DOM substituted fully for DMT and LSD, partially for MDMA but not for METH; DMT substituted fully for DOM, partially for LSD and MDMA but not for METH; while METH only substituted for MDMA (Gatch et al. 2009).

In the current study, five of the six phenethylamines produced full substitution to at least one of the three prototypic hallucinogens, LSD (ergoline), DMT (tryptamine) and DOM (phenalkylamine). In LSD-trained rats, 2C-D, 2C-E, 2C-I and DOC fully substituted (80% DAR), whereas 2C-C produced 75% LSD-appropriate responding. The pattern of substitution for DMT-trained rats was similar, with the addition of partial substitution by 2C-T-2. The four compounds tested in DOM-trained rats (2C-C, 2C-D, 2C-E, and DOC) all fully substituted, suggesting that these phenethylamines have very similar discriminative stimuli to DOM, consistent with their structural similarities. In contrast, 2C-T-2 partially substituted only for DMT, indicating that it does not share discriminative stimulus effects with most serotonergic hallucinogens or with psychostimulants.

For the MDMA-trained rats, 2C-C and 2C-E fully substituted, 2C-D and 2C-I and DOC produced 60 to 65% MDMA-appropriate responding, but DOC produced only 44%. These findings are similar to those of earlier studies in which MDMA, an entactogen that induces feelings of empathy and emotional closeness to others (Nichols 1986a), did not consistently cross-substitute for the classic serotonergic hallucinogens (Baker et al. 1995;Gatch et al. 2009;Oberlender and Nichols 1988;Schechter 1998).

In agreement with very low potencies of 2C-C, 2C-E, and 2C-I for inhibition of uptake and minimal stimulation of release in rat brain synaptosomes (Nagai et al. 2007), the phenethylamines minimally substituted for METH, consistent with their low to negligible affinity and potency at the transporters. This suggests that the compounds have minimal psychostimulant properties and agrees with the general depression of locomotor activity by other hallucinogens (Krebs and Geyer 1994;Krebs-Thomson et al. 1998), although lower doses of 2C-D, 2C-E, and DOC had a delayed stimulant activity.

Many studies have demonstrated the involvement of G-protein-coupled 5-HT$_2$ receptors in the actions of hallucinogenic drugs including LSD, DOM, DOI, and 2,5-dimethoxy-4-bromophenethylamine (2C-B) (Egan et al. 2000;Fantegrossi et al. 2008;Glennon et al. 1984;Kurrasch-Orbaugh et al. 2003;Titeler et al. 1988). More specifically, 5-HT$_{2A/2C}$ receptors, which have high sequence homology and similarity in second messenger

signaling, are important targets of hallucinogenic compounds (reviewed in Fantegrossi et al. 2008;Halberstadt and Geyer 2011;Nichols 2004;Winter 2009). Both 5-HT$_{2A/2C}$ receptors activate phospholipase A$_2$ via G$\alpha_{12/13}$, liberating AA from membrane phospholipids (reviewed in Raymond et al. 2001) and activate phospholipase C via G$_{q/11}$, generating the second messengers inositol triphosphate and diacylglycerol with subsequent release of intracellular stores of calcium and protein kinase C activation (Backstrom et al. 1999). Among the training compounds, LSD and DOM were full agonists at the 5-HT$_{2A/2C}$ receptors although in rat brain membranes and NIH3T3-5HT$_{2A}$ cells, these compounds are partial agonists (Kurrasch-Orbaugh et al. 2003;Sanders-Bush et al. 1988). In contrast, at the 5-HT$_{2A}$ receptor, DMT and MDMA were very low potency partial agonists, and METH had no efficacy, whereas at the 5-HT$_{2C}$ receptor, DMT and MDMA were low to very low potency full agonists and METH was a very low potency partial agonist. Activation of 5-HT$_{1A}$ receptors may not play a role in the discriminative stimulus (Nichols 2004); while LSD was a very potent full 5-HT$_{1A}$ agonist, all other psychoactive exogenous compounds tested had potencies in the micromolar range, and only 2C-I and 2C-T-2 had partial to full efficacy among the phenethylamines. These results partly agree with the partial stimulation of GTPγS binding in brain preparations by 2C-C, 2C-E and 2C-I (Nonaka et al. 2007)

In CHO cells expressing the 5-HT$_{2A}$ or 5-HT$_{2C}$ receptors, 2C-D, 2C-I and 2C-B were partial, low potency agonists for the phospholipase C or A2 pathways. (Moya et al. 2007). Herein, 2C-C and DOC had similar affinities for 5-HT$_{2A/2C}$ receptors, and each was a full agonist with similar potencies at both receptors, similar to LSD and DOM. 2C-D had higher affinity for, and was a full agonist with slightly higher potency at, the 5-HT$_{2C}$ compared to the 5-HT$_{2A}$ receptor. 2C-E had similar affinity for the 5-HT$_{2A/2C}$ receptors and was a partial agonist in the 5-HT$_{2A}$ AA assay, but a full agonist in stimulating the inositol phosphate pathway by both 5-HT$_{2A/2C}$ receptors. Although 2C-T-2 substituted partially only for DMT, it was a full high potency agonist at the 5-HT$_{2A/2C}$ receptors, similar to LSD. A structurally similar compound, 2,5-dimethoxy-4-propylthiophenethylamine (2C-T-7), partially substitutes for LSD and serves as a discriminative stimulus, an effect blocked by a 5-HT$_{2A}$ antagonist (Fantegrossi et al. 2005). 2C-T-2 has been sold on the internet and in Europe (de Boer and Bosman 2004) and anecdotal evidence (Erowid.com) suggests that it is psychoactive.

In contrast, 2C-I had differential effect on 5-HT$_{2A}$-mediated pathways. 2C-I had high affinity for both 5-HT$_{2A/2C}$ receptors, consistent with Ki and K_d values obtained using stable HEK-5HT$_{2A}$ cells (Parrish et al. 2005) and [^{125}I]2C-I in rat frontal cortex, which did not discriminate between 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors (Johnson et al. 1990). 2C-I fully antagonized serotonin's activation of the phospholipase A2/[^{3}H]AA release pathway. However, 2C-I was a full agonist, with efficacy similar to LSD, in 5-HT$_{2A/2C}$ receptor-mediated IP-1 formation. Because 2C-I fully substituted for DMT and LSD and stimulated phospholipase C, but not phospholipase A2, pathway, the former pathway may be more significant in 2C-I drug discrimination. Similarly, Parrish et al. (2005) observed 2C-I to be a partial agonist with high potency at the phospholipase C pathway. Consistent with the possible importance of the phospholipase C pathway, a conformationally restricted 2C-B analog ((R)-4-bromo-3,6-dimethoxybenzocyclobuten-1-yl)methyl-amine) was equipotent to LSD in rats trained to discriminate LSD from saline and was 65-fold more potent at stimulating inositol phosphate turnover than AA release, although the authors suggested that preferential stimulation of this pathway may indicate lower psychoactivity (McLean et al. 2006). Using a phospholipase C inhibitor, Schindler et al. (2013) found that phospholipase C activation is necessary for DOI-elicited head bobs in mice while LSD-elicited head bobs are independent of this pathway, suggesting distinct pathways for these structurally dissimilar hallucinogens. A caveat to the possible relative contribution of 5-HT$_{2A}$ pathways to hallucinogenic activity is the abundant evidence that the cell types used to express serotonin

receptors, as well as the second messenger systems characterized, have impact on whether a drug is an agonist, partial agonist, or antagonist (Acuna-Castillo et al. 2002;Kurrasch-Orbaugh et al. 2003;Moya et al. 2007;Rabin et al. 2002;Villalobos et al. 2004).

To summarize, the relationship between drug substitution, mechanism of action, and potential for abuse for these drugs is not uniform. 2C-C, 2C-D, 2C-E and DOC fully substituted for the discriminative stimuli of the hallucinogenic phenethylamine DOM and fully to partially substituted for LSD, and have agonist profiles similar to DOM and LSD at 5-HT$_{2A/2C}$ receptors. In contrast, 2C-I substituted fully for hallucinogenic compounds, but its pharmacology is more complex, as it fully stimulated the 5-HT$_{2A/2C}$ receptors phospholipase C pathways but inhibited the 5-HT$_{2A}$ receptor phospholipase A2 pathway, as discussed above. Finally, 2C-T-2 was a full, high potency agonist at 5-HT$_{2A/2C}$ receptors but produced appreciable drug-appropriate responding for only one training drug (DMT). Overall, there was not a good correlation between potencies and efficacies in drug discrimination assays and binding/functional assays, suggesting that complex mechanisms or additional receptor systems may modulate behavior.

These behavioral and biochemical findings, together with ongoing reports of use on the internet (Erowid.com), suggest that the compounds have high abuse liability. In addition, the adverse effects we observed in rat and mouse behavioral assays included tremor, muscle spasms, hind limb paralysis and lethality and indicate toxicity at higher doses. Recent reports of seizures and serotonin syndrome following 2C-I ingestion (Bosak et al. 2013), fatal toxic leukoencephalopathy and acute kidney failure following 2C-E ingestions (Sacks et al. 2012;Van Vrancken et al. 2013), and seizures and rhabdomyloysis following DOC and MDMA co-ingestion (Ovaska et al. 2008) suggest consumption of these phenethylamines carries significant health risk.

Acknowledgments

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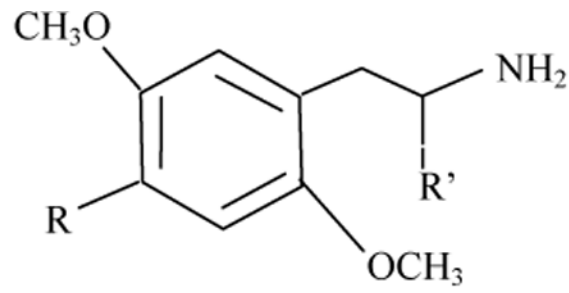
Fig. 1.
Structures of 2C-C, 2C-D, 2C-E, 2C-I, 2C-T-2 and DOC
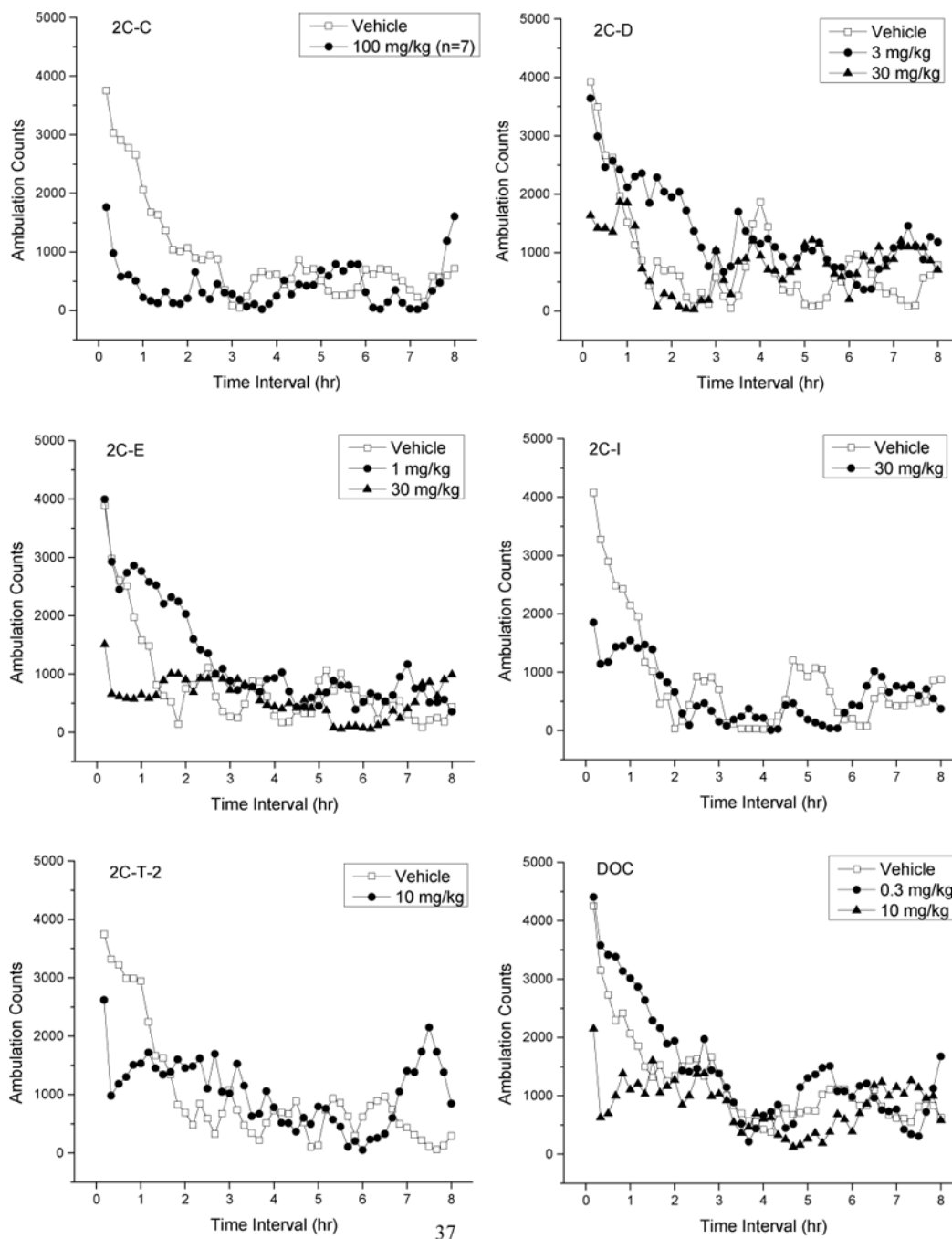
Fig. 2.
Average horizontal activity counts/10 min (ambulation counts) as a function of time (0–8 hr) and dose of test compound. Data for the vehicle and the dose which produced peak depressant effects are shown in each panel. 2C-D and DOC also produced stimulant effects, and data for the dose which produced peak stimulant effects are also shown. N=8 for each treatment.
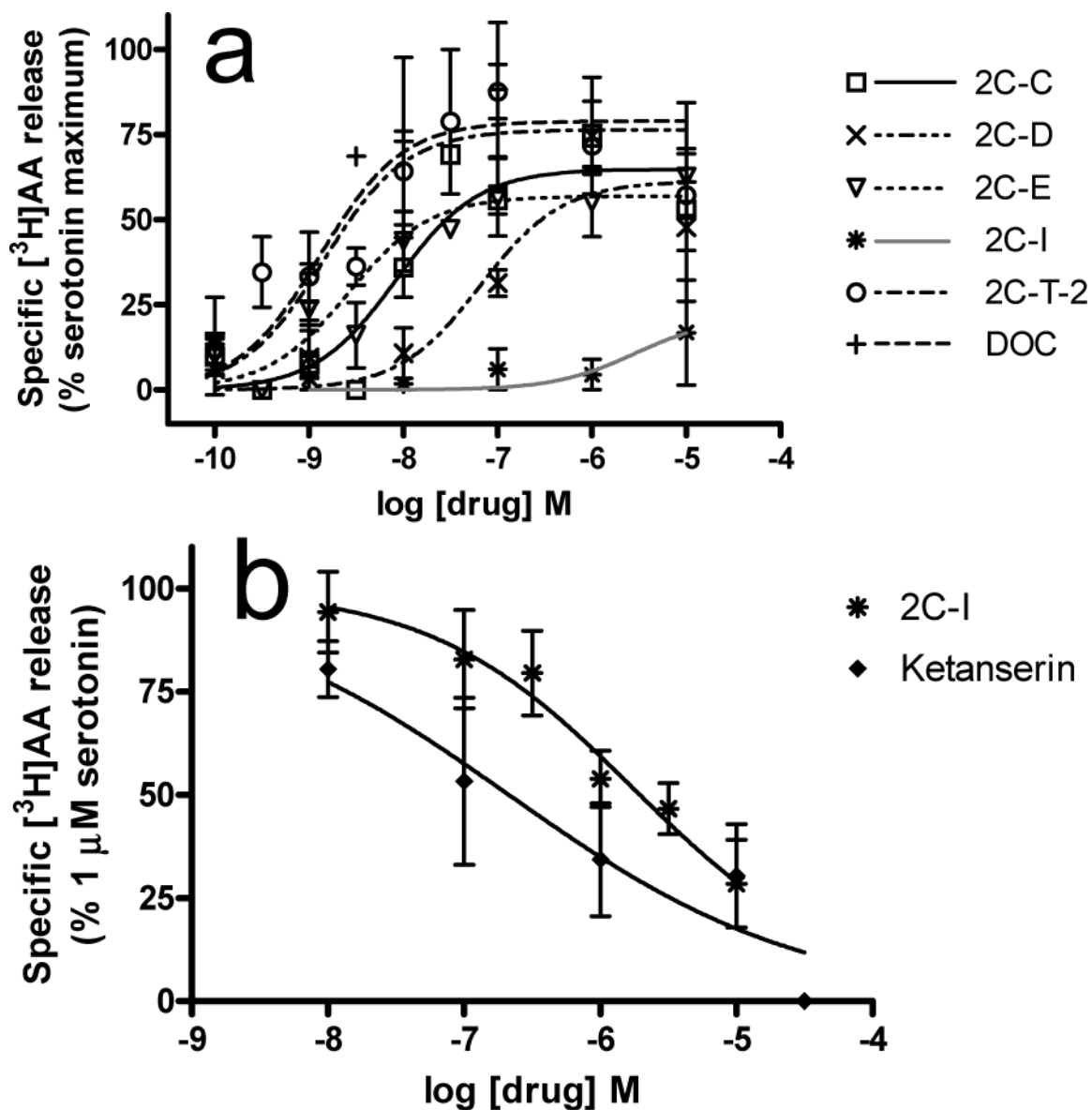
Fig. 3.
[^{3}H]AA release from HEK-5-HT$_{2A}$ cells. Experiments were conducted as described in methods. Data presented are means ± sem. (A) Agonist assay. Basal activity is subtracted, and data are normalized to the maximal stimulation by serotonin on each experimental day. n=3–5 except n=2 for 2C-I. (B) Antagonist assay. Nonspecific release, measured in the presence of 30 μM ketanserin, is subtracted from all data and data are normalized to the maximal release stimulated by serotonin. n=3–4.
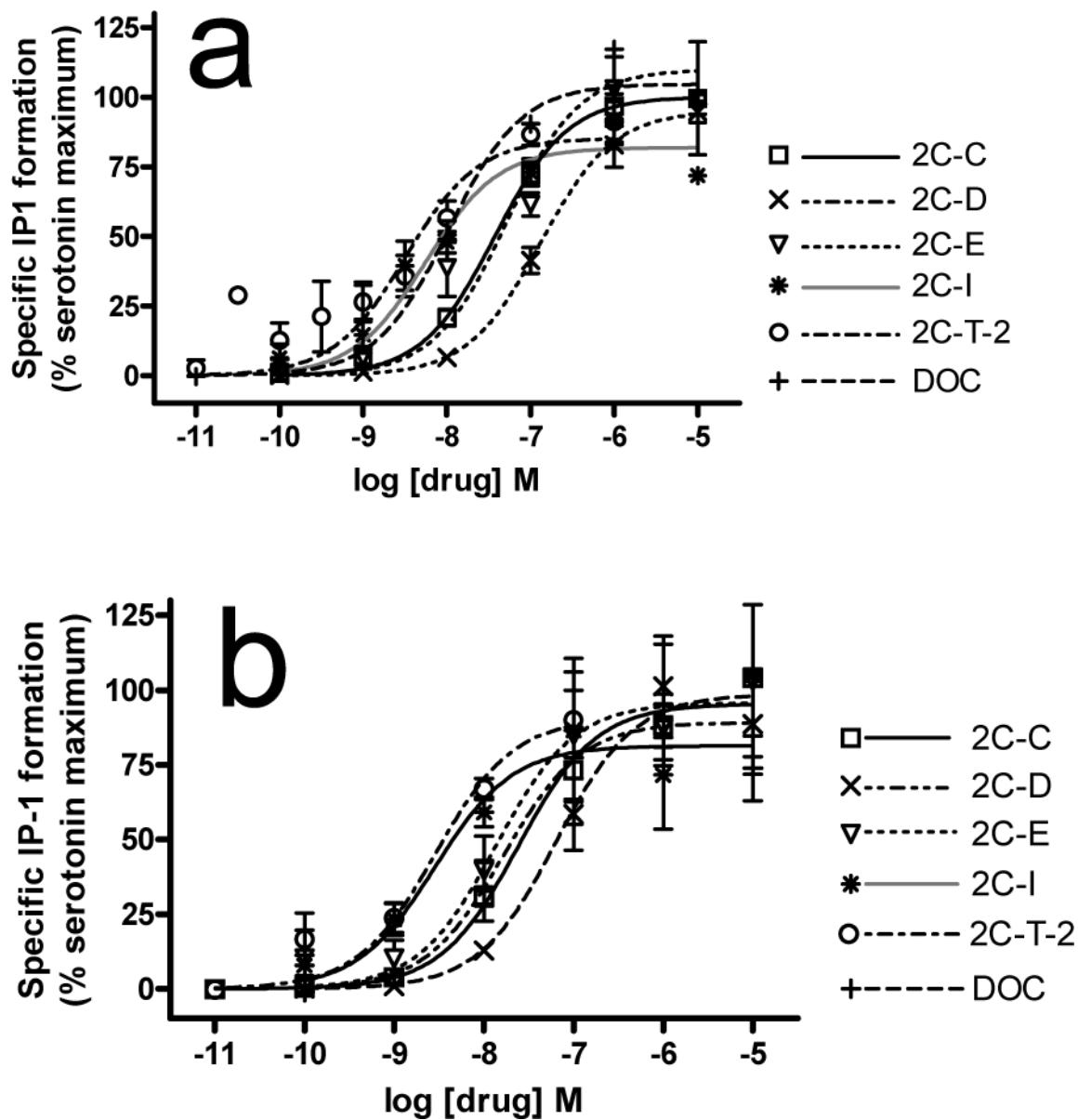
Fig. 4.
Stimulation of IP-1 formation in HEK-5-HT$_{2A}$ and HEK-5-HT$_{2C}$ cells. Experiments were conducted as described in methods. A. HEK-5-HT$_{2A}$ cells. All compounds are full or partial agonists. The average maximal stimulation by serotonin was 565 ± 46 nM IP1. n=3–8. B. HEK-5-HT$_{2C}$ cells. All compounds are full agonists. The average maximal stimulation by serotonin was 1390 ± 180 nM. n=4–7.

Table 1

Maximum percent drug-appropriate responding (DAR) of each test compound in each of the training groups and effects on rate of responding at that dose.

1st rfr

Test Compound	Training Drug	Dose (mg/kg)	N Test	%DAR	Rate (resp/s)
2C-C	DMT	5	4/6	75±25[†,1]	0.235±0.098
2C-C	DOM	2.5	6/6	82±16[†]	0.366±0.070
2C-C	LSD	5	4/6	75±25[†,2]	0.200±0.079
2C-C	MDMA	2.5	5/6	89±11[†]	0.244±0.086
2C-C	Meth	10	3/6	33±33[2]	0.156±0.096
2C-D 15 min	DMT	10	3/6	100±0[†]	0.154±0.070
2C-D	DOM	2.5	6/6	100±0[†]	0.383±0.038
2C-D	LSD	2.5	6/6	83±17[†]	0.385±0.060
2C-D	MDMA	2.5	6/6	61±20[†,2]	0.402±0.073
2C-D	Meth	5	4/6	15±15[2]	0.190±0.086
2C-D 70 min	DMT	10	6/6	83±17[†]	0.332±0.067
2C-D	DOM	10	6/6	76±17[†]	0.338±0.187
2C-D	LSD	5	6/6	83±17[†]	0.379±0.054
2C-D	MDMA	10	6/6	50±22[2]	0.547±0.127
2C-D	Meth	25	5/6	44±22[2]	0.072±0.032
2C-E	DMT	5	6/6	80±16[†]	0.466±0.166
2C-E	DOM	2.5	6/6	97±2[†]	0.576±0.087
2C-E	LSD	2.5	6/6	97±3[†]	0.413±0.061
2C-E	MDMA	10	3/6	100±0[†]	0.053±0.036
2C-E	Meth	5	5/6	23±16[1]	0.228±0.024
2C-I	DMT	2.5	6/6	93±4[†]	0.480±0.092
2C-I	DOM	NT			

Test Compound	Training Drug	Dose (mg/kg)	N Test	%DAR	Rate (resp/s)
2C-I	LSD	5	5/6	80±20[†]	0.233±0.075
2C-I	MDMA	2.5	4/6	65±21[†] [1]	0.453±0.169
2C-I	Meth	1	4/6	38±17[1]	0.447±0.058
2C-T-2	DMT	2.5	4/6	73±24[†] [1]	0.170±0.106
2C-T-2	DOM	NT			
2C-T-2	LSD	2.5	3/6	33±33[2]	0.208±0.117
2C-T-2	MDMA	1	6/6	12±7[2]	0.448±0.130
2C-T-2	Meth	0.5	6/6	7±5[2]	0.748±0.109
DOC 15 min	DMT	1	5/6	65±20[2]	0.354±0.125
DOC	DOM	0.5	6/6	83±17[†]	0.708±0.072
DOC	LSD	1	6/6	81±16[†]	0.402±0.049
DOC	MDMA	0.5	6/6	44±19[1]	0.449±0.104
DOC	Meth	0.5	6/6	24±16[1]	0.876±0.330
DOC 60 min	DMT	1	6/6	83±17[†]	0.777±0.095
DOC	DOM	1	6/6	87±13[†]	0.493±0.058
DOC	LSD	1	5/6	95±3[†]	0.410±0.139
DOC	MDMA	1	5/6	60±24[†] [2]	0.356±0.141
DOC	Meth	2.5	4/6	0±0[2]	0.276±0.098

N Test = number of rats which completed the first fixed ratio/total number tested.

resp/s = responses per second.

[1] Higher dose(s) produced less drug-appropriate responding.

[2] A higher dose suppressed response rate.

[†] Indicates significantly different from vehicle control/not different from drug control

Table 2

Pharmacology of 2C-C, 2C-D, 2C-E, 2C-I, 2C-T-2 and DOC at 5-HT$_{1A}$, 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors: Effects on binding and function.

Drug	5-HT$_{1A}$		5-HT$_{2A}$			5-HT$_{2C}$	
	[³H]8-OH-DPAT binding Ki (nM) ± sem	Function [³⁵S]GTPγS Binding EC$_{50}$ (nM) EC$_{50}$ (nM) ± sem % maximal effect[a]	[¹²⁵I]DOI binding Ki (nM) ± sem	Function [³H]AA release EC$_{50}$ (nM) ± sem % maximal effect[a]	Function IP-1 formation EC$_{50}$ (nM) ± sem % maximal effect[a]	[¹²⁵I]DOI binding Ki (nM) ± sem	Function IP-formation EC$_{50}$ (nM) ± sem % maximal effect[a]
2C-C	740 ± 170	>10 μM* <25%	5.47 ± 0.68	13.9 ± 6.1 64.2 ± 2.2%	40.1 ± 4.7 102.1 ± 5.5%	5.4 ± 1.2	24.2 ± 5.6 93.5 ± 6.5%
2C-D	1630 ± 150	>10 μM* <25%	23.9 ± 3.9	153 ± 28 81.1 ± 9.7%	145 ± 39 93.0 ± 6.7%	12.7 ± 1.3	71.1 ± 8.0 100 ± 14%
2C-E	1190 ± 250	>10 μM* <20%	4.50 ± 0.71	7.1 ± 3.3 65 ± 11%	84 ± 16 125 ± 13%	5.4 ± 1.1	18.0 ± 5.9 98 ± 16%
2C-I	970 ± 430	4900 ± 2000 102.1 ± 4.7%	9.3 ± 3.2	ND 32.5 ± 1.5%	7.9 ± 2.2 82.4 ± 6.4%	10.2 ± 1.7	2.8 ± 1.0 79 ± 16
2C-T-2	1740 ± 660	3000 ± 1300 76 ± 10%	12.5 ± 4.4	2.30 ± 0.69 106.6 ± 6.3%	7.9 ± 2.0 87.4 ± 6.5%	14.2 ± 3.6	3.8 ± 1.1 93.0 ± 1.8%
DOC	>9200	>10 μM* <10%	4.00 ± 0.45	2.91 ± 0.66 81 ± 16%	10.5 ± 2.6 102.4 ± 4.2%	3.57 ± 0.56	14.6 ± 3.0 97 ± 19%
Serotonin	4.3 ± 1.0	15.7 ± 5.4 105.3 ± 2.2%	25.8 ± 4.6	7.8 ± 2.0 100%	43 ± 10 99.7 ± 0.9%	3.74 ± 0.71	1.94 ± 0.49 93.9 ± 2.0%
LSD	2.50 ± 0.95	6.4 ± 1.8 109.6 ± 4.5%	0.47 ± 0.12	1.01 ± 0.31 87.8 ± 3.4%	0.264 ± 0.052 80.3 ± 4.9%	3.22 ± 0.57	1.14 ± 0.36 74.6 ± 9.1%
DOM	11900 ± 5600	13900 ± 1500 53.8 ± 9.7%	8.1 ± 2.2		31.2 ± 9.6 106 ± 11%	25.9 ± 4.2	8.4 ± 3.0 94 ± 14%
DMT	400 ± 190	1490 ± 570 100.4 ± 5.2%	201 ± 48	260 ± 120 105.1 ± 3.0%	269 ± 61 39.0 ± 4.6%	111 ± 34	114 ± 35 99 ± 11%
MDMA	11900 ± 1200	36000 ± 16000 64.3 ± 9.4%	20900 ± 4600		5.5 ± 2.3 mM 40.2 ± 7.1%	9600 ± 2400	9100 ± 1500 92.2 ± 8.0%
METH	9700 ± 1800	23600 ± 8100 68 ± 11%	41900 ± 9600		>1 mM[a] <3%	10100 ± 3200	74000 ± 16000 52 ± 10%

For test compounds, n=3–11 for each assay. Data are expressed as mean ± S.E.M. Hill slopes for 5-HT$_{1A}$ [^{3}H]8-OH-DPAT binding ranged from –0.73 to –1.04, for 5-HT$_{2A}$ [^{125}I]DOI binding ranged from –0.53 to –1.10 and for 5-HT$_{2C}$ [^{125}I]DOI binding ranged from –0.65 to –1.32.

ND- the EC$_{50}$ value was not determined. 2C-I was an antagonist at the 5-HT$_{2A}$ [^{3}H]AA release functional assay, with an IC$_{50}$ of 2060 ± 860 nM and 84 ± 11% inhibition, compared to the maximal inhibition by ketanserin, of 100 nM serotonin stimulation.

*These compounds also had minimal effect as antagonists at the 5-HT$_{1A}$ receptor, with maximal inhibition <30% of the maximal inhibition by WAY 100,635.

^{a}Maximal effect of each drug was normalized to the maximal effect of serotonin.

Table 3

Pharmacology of 2C-C, 2C-D, 2C-E, 2C-I, 2C-T-2 and DOC at DAT, SERT and NET: Effects on binding and function.

Drug	HEK-hDAT cells			HEK-hSERT cells			HEK-hNET cells		
	[125I]RTI-55 binding Ki, nM	[3H]Dopamine uptake IC$_{50}$, nM	[3H]Dopamine release EC$_{50}$, nM % METH maximal effect	[125I]RTI-55 binding Ki, nM	[3H]Serotonin uptake IC$_{50}$, nM	[3H]Serotonin release EC$_{50}$, nM % PCA maximal effect	[125I]RTI-55 binding Ki, nM	[3H]NE uptake IC$_{50}$, nM	[3H]NE release EC$_{50}$, nM % METH maximal effect
2C-C	>10 μM	>10 μM	ND Minimal	>10 μM	>9600#	ND Minimal	>7600#	>9500	ND Minimal
2C-D	>10 μM	>10 μM	ND Minimal	>10 μM	>8400#	ND Minimal	>8500#	>9200	ND Minimal
2C-E	>10 μM	>10 μM	ND Minimal	>10 μM	>7500#	ND Minimal	>7600#	>9800	ND Minimal
2C-I	>10 μM	>9600#	ND Minimal	950 ± 440	5600 ± 2100	ND Minimal	>10 μM	>8200#	ND Minimal
2C-T-2	>10 μM	>10 μM	ND Minimal	>9400#	>8600#	ND Minimal	>10 μM	>7200#	ND Minimal
DOC	>10 μM	>10 μM	ND Minimal	>10 μM	>8600#	ND Minimal	>7700#	>8400#	ND Minimal
Cocaine	462 ± 97	374 ± 36	ND Minimal	321 ± 26	383 ± 49	ND Minimal	1280 ± 150	332 ± 29	ND Minimal
METH	4750 ± 530	56 ± 15	970 ± 250[a] 100%	188000 ± 14000	4100 ± 1300	27800 ± 7700[a] Minimal	1480 ± 130	23.0 ± 5.5	67 ± 18[a] 100%
LSD	>10 μM	>10 μM	ND Minimal	>10 μM	>10 μM	ND Minimal	5600 ± 260	>10 μM	ND Minimal

Abbreviations: NE, norepinephrine. PCA, p-chloroamphetamine. Hill slopes for [125I]RTI-55 binding ranged from −0.97 to −1.05 for hDAT, −0.70 to −1.15 for hSERT and −0.99 to −2.13 for hNET.

For test compounds, n=2 (when >10 μM) to 7. Data are expressed as mean ± S.E.M. The standard compounds were, for purposes of comparison, cocaine for the binding and uptake assays, methamphetamine for [3H]dopamine release and PCA for [3H]serotonin release. The EC50 value for PCA for stimulating [3H]serotonin release was 1350 ± 420 nM.

\# If some experiments yielded IC$_{50}$ or K$_i$ values less than 10 μM and other experiments yielded IC$_{50}$ or K$_i$ values greater than 10 μM, the latter experiments were assigned a value of 10 μM and averages calculated (n 3). The actual value is greater than that average and no standard error is reported.

ND- The EC$_{50}$ value could not be determined.

[a] Values from Gatch et al., 2011.