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Receptor interaction profiles of novel psychoactive tryptamines compared with classic hallucinogens

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Abstract

The present study investigated interactions between the novel psychoactive tryptamines DiPT, 4-OH-DiPT, 4-OH-MET, 5-MeO-AMT, and 5-MeO-MiPT at monoamine receptors and transporters compared with the classic hallucinogens lysergic acid diethylamide (LSD), psilocin, *N,N*-dimethyltryptamine (DMT), and mescaline. We investigated binding affinities at human monoamine receptors and determined functional serotonin (5-hydroxytryptamine [5-HT]) 5-HT_{2A} and 5-HT_{2B} receptor activation. Binding at and the inhibition of human monoamine uptake transporters and transporter-mediated monoamine release were also determined. All of the novel tryptamines interacted with 5-HT_{2A} receptors and were partial or full 5-HT_{2A} agonists. Binding affinity to the 5-HT_{2A} receptor was lower for all of the tryptamines, including psilocin and DMT, compared with LSD and correlated with the reported psychoactive doses in humans. Several tryptamines, including psilocin, DMT, DiPT, 4-OH-DiPT, and 4-OH-MET, interacted with the serotonin transporter and partially the norepinephrine transporter, similar to 3,4-methylenedioxymethamphetamine but in contrast to LSD and mescaline. LSD but not the tryptamines interacted with adrenergic and dopaminergic receptors. In conclusion, the receptor interaction profiles of the tryptamines predict hallucinogenic effects that are similar to classic serotonergic hallucinogens but also MDMA-like psychoactive properties.

Keywords: hallucinogens; LSD; DMT; psilocybin; mescaline; tryptamines

1. Introduction

Classic or serotonergic hallucinogens can be grouped into different chemical groups, including tryptamines (e.g., psilocin and *N,N*-dimethyltryptamine [DMT]), ergolines (lysergic acid diethylamide [LSD]), and phenethylamines (e.g., mescaline). Psychoactive tryptamines are naturally found in toads, plants, and mushrooms. However, many synthetic tryptamine derivatives have been synthesized and are recreationally used as novel psychoactive substances (Araujo et al., 2015; EMCDDA, 2014; Helander et al., 2014; Kamour et al., 2014; Shulgin and Shulgin, 1997; Tittarelli et al., 2015; Winstock et al., 2014). Tryptamines share their core structure with the neurotransmitter serotonin (5-hydroxytryptamine [5-HT]). The psychoactive effects of hallucinogens, including those of tryptamines, are thought to be mediated mainly by the 5-HT_{2A} receptor (Glennon et al., 1984; Nichols, 2004; Titeler et al., 1988; Vollenweider et al., 1998) but may also be modulated by interactions with other targets, including other 5-HT receptors, monoamine transporters, and trace amine-associated receptors (Baumeister et al., 2014; Bunzow et al., 2001; Cozzi et al., 2009; Fantegrossi et al., 2006; McKenna et al., 1990; Nagai et al., 2007; Nichols, 2004; Ray, 2010). Structural alterations of tryptamines have been shown to result in different pharmacological and psychoactive profiles (Araujo et al., 2015; McKenna et al., 1990; Repke et al., 1985; Shulgin and Shulgin, 1997; Tittarelli et al., 2015; Trachsel et al., 2013). For example, compounds that have no substitutions or a 4-hydroxyl group (e.g., DMT or psilocin, respectively; Fig. 1) produce hallucinogenic effects with relative low potency in man (Repke et al., 1985). Psilocin is orally psychoactive above 5-10 mg, and DMT is active at parenteral doses of 20-100 mg (Araujo et al., 2015; Shulgin and Shulgin, 1997; Strassman et al., 1994; Studerus et al., 2011; Tittarelli et al., 2015). In contrast, a 5-methoxy group, such as in 5-MeO-AMT (Fig. 1), resulted in greater compound potency, with subjective effects at 1-5 mg doses, more stimulant-type activation, and less visual perceptual alterations (Repke

et al., 1985). Different *N*-substitutions also influenced *in vivo* potency (Nichols et al., 2015; Repke et al., 1985). The pharmacological profiles of many tryptamines have been studied previously at selected targets (Blough et al., 2014; Gatch et al., 2011; McKenna et al., 1990; Nichols et al., 2015; Repke et al., 1985; Shulgin and Carter, 1980), and new and pharmacologically unknown tryptamine derivatives are constantly emerging on the illicit drug market (Araujo et al., 2015; Corkery et al., 2012; EMCDDA, 2014; Greene, 2013; Helander et al., 2014; Tittarelli et al., 2015). Because small changes in molecular structure can alter the pharmacology of these novel designer drugs, studying the *in vitro* receptor interaction profiles of these novel substances is important. Such data can help predict psychotropic effects and acute clinical toxicity. Therefore, we assessed the receptor interaction profiles of a series of classic and novel tryptamines at human monoamine receptors and assessed 5-HT_{2A} receptor activation. LSD and mescaline were included for comparison. Tryptamines have also been shown to interact with membrane monoamine transporters to inhibit their function or release monoamines through the transporter (Araujo et al., 2015; Cozzi et al., 2009; Nagai et al., 2007), similar to 3,4-methylenedioxymethamphetamine (MDMA) and many other novel psychoactive substances (Rickli et al., 2015a; Simmler et al., 2013; Simmler et al., 2014a; Simmler et al., 2014b). Therefore, inhibition of the norepinephrine (NE), dopamine (DA), and 5-HT transporters (NET, DAT, and SERT, respectively) and the release of NE, DA, and 5-HT were also investigated. MDMA was included as a comparator in these assays.

The present study included recreationally used tryptamines (Araujo et al., 2015; EMCDDA, 2014; Greene, 2013; Schmidt et al., 2011; Tittarelli et al., 2015), including *N,N*-diisopropyltryptamine (DiPT), 4-hydroxy-*N,N*-diisopropyltryptamine (4-OH-DiPT), 4-hydroxy-*N*-methyl-*N*-ethyltryptamine (4-OH-MET), 5-methoxy-α-methyltryptamine (5-MeO-AMT), and 5-methoxy-*N*-methyl-*N*-isopropyltryptamine (5-MeO-MiPT; Fig. 1). DiPT is a ring-unsubstituted tryptamine, similar to DMT. DiPT fully substituted for

DMT in discrimination studies (Gatch et al., 2011) but unlike DMT reportedly induces auditory and not visual alterations in humans (Blough et al., 2014). DiPT is psychoactive at doses of 20-100 mg, with effects that last 4-8 h (Shulgin and Carter, 1980; Tittarelli et al., 2015).

DiPT is an agonist at rat and human 5-HT_{2A} receptors and also blocks the rat and human SERT (Blough et al., 2014; Cozzi et al., 2009; Gatch et al., 2011; Nagai et al., 2007). However, interactions with other receptors have not yet been studied.

4-OH-DiPT has been detected in the urine of substance users (Pichini et al., 2008), and a series of 4-OH-MET intoxications has recently been reported (Helander et al., 2013; Taljemark and Johansson, 2012). The subjective effects of these 4-substituted tryptamines that are used at oral doses of 10-20 mg are reportedly similar to those of psilocybin and last 2-6 h. The receptor interaction profiles of 4-OH-DiPT and 4-OH-MET are unknown (Tittarelli et al., 2015).

5-MeO-AMT and 5-MeO-MiPT (user names: “Alpha” and “Moxy,” respectively) are 5-methoxy-substituted tryptamines that exert psychoactive effects at oral doses of 2-6 mg (Greene, 2013; Shulgin and Shulgin, 1997; Tittarelli et al., 2015). The clinical effects of 5-MeO-AMT reportedly last up to 18 h, and severe toxicity has been associated with this substance (Tittarelli et al., 2015). 5-MeO-AMT is a potent 5-HT_{2A} receptor ligand and agonist with some selectivity for the 5-HT_{2A} receptor over the 5-HT_1 receptor (Gatch et al., 2011; Glennon et al., 1990; Tomaszewski et al., 1992). Data on other receptor interactions are lacking. 5-MeO-AMT has also been shown to act as a substrate releaser at rat monoamine transporters and the human SERT (Gatch et al., 2011). A recent study showed that 5-MeO-MiPT stimulated 5-HT_2 receptors but did not interact with rat monoamine transporters (Blough et al., 2014), in contrast to its close analog 5-methoxy-diisopropyltryptamine (5-MeO-DiPT), which acted at the SERT (Blough et al., 2014; Sogawa et al., 2007). In contrast, a previous study found that both 5-MeO-MiPT and 5-MeO-DiPT were inhibitors of the rat SERT and NET (Nagai et al., 2007). Thus, conflicting data have been reported, and more

comprehensive profiles of 5-MeO-AMT and 5-MeO-MiPT at human transporters and other receptors are needed.

Psilocin (the active metabolite of psilocybin that is contained in magic mushrooms) and DMT (contained in ayahuasca) are classic tryptamines that continue to be used recreationally (Winstock et al., 2014). Psilocin and DMT are 5-HT_{2A} receptor agonists and SERT inhibitors (Blough et al., 2014; Cozzi et al., 2009). DMT also releases 5-HT (Blough et al., 2014). Less is known about interactions with other receptors. Clinically, psilocybin has been extensively studied in laboratory studies in healthy subjects and substance-assisted psychotherapy in patients (Carhart-Harris et al., 2013; Griffiths et al., 2006; Grob et al., 2011; Hasler et al., 2004). Similarly, the acute psychotropic effects of DMT have been described in controlled studies in humans (Dos Santos et al., 2011; Gouzoulis-Mayfrank et al., 2005; Strassman and Qualls, 1994; Strassman et al., 1994; Winstock et al., 2014). Furthermore, the prototypical hallucinogen LSD has received renewed attention in psychiatric research (Dolder et al., 2015; Geyer, 2015; Kupferschmidt, 2014; Schmid et al., 2015) and as an adjunct to psychotherapy to treat anxiety (Gasser et al., 2014). Thus, the classic hallucinogens psilocin, DMT, and LSD were also included in the present study to provide more up-to-date basic *in vitro* pharmacological data on these clinically important substances, including profiles at human receptors, and allow direct comparisons between these classic substances with novel tryptamines and other novel psychoactive substances within the same assays (Liechti, 2015; Rickli et al., 2015a; Rickli et al., 2015b; Rickli et al., 2015c; Simmler et al., 2013; Simmler et al., 2016; Simmler et al., 2014a; Simmler et al., 2014b).

2. Experimental procedures

2.1. Drugs

Psilocin, LSD, DMT, mescaline, and MDMA were obtained from Lipomed (Arlesheim, Switzerland). DiPT, 4-OH DiPT, 4-OH MET, 5-MeO AMT, and 5-MeO

MiPT were obtained from Cayman Chemicals (Adipogen, Switzerland). The compounds were used as racemates. The purity of the compounds was at least 98%. The radiolabeled chemicals [^{3}H]NE and [^{3}H]DA were purchased from Perkin-Elmer (Schwerzenbach, Switzerland), and [^{3}H]5-HT was obtained from Anawa (Zürich, Switzerland). The data on LSD and mescaline were previously published but included herein for comparison because the data were obtained at the same time and by the same researcher using the same cell batches and assays as for the other compounds that are newly presented herein.

2.2. Radioligand receptor and transporter binding assays

The radioligand binding assays were performed as described previously (Simmler et al., 2013). Briefly, membrane preparations of human embryonic kidney (HEK) 293 cells (Invitrogen, Zug, Switzerland) that overexpress the respective transporters (Tatsumi et al., 1997) or receptors (human genes, with the exception of rat and mouse genes for $TAAR_1$; (Revel et al., 2011) were incubated with the radiolabeled selective ligands at concentrations equal to K_d, and ligand displacement by the compounds was measured. Specific binding of the radioligand to the target receptor was defined as the difference between the total binding and nonspecific binding that was determined in the presence of selected competitors in excess. The following radioligands and competitors, respectively, were used: *N*-methyl-[^{3}H]-nisoxetine and indatraline (NET), [^{3}H]citalopram and indatraline (SERT), [^{3}H]WIN35,428 and indatraline (DAT), [^{3}H]8-hydroxy-2-(di-*n*-propylamine)tetralin and indatraline (5-HT_{1A} receptor), [^{3}H]ketanserin and spiperone (5-HT_{2A} receptor), [^{3}H]mesulgerine and mianserin (5-HT_{2C} receptor), [^{3}H]prazosin and risperidone (adrenergic α_1 receptor), [^{3}H]rauwolscine and phentolamine (adrenergic α_2 receptor), [^{3}H]SCH 23390 and butaclamol (D_1 receptor), [^{3}H]spiperone and spiperone (D_2 and D_3 receptors), [^{3}H]pyrilamine and clozapine (histaminergic H_1 receptor), and [^{3}H]RO5166017 and RO5166017 ($TAAR_1$). IC_{50} values were determined by

calculating nonlinear regression curves for a one-site model using three to five independent 10-point concentration-response curves for each compound. K_i (affinity) values, which correspond to the dissociation constants, were determined using the Cheng-Prusoff equation.

2.3. Activity at the serotonin 5-HT_{2A} receptor

Human 5-HT_{2A} receptor-expressing mouse embryonic fibroblasts (NIH-3T3 cells) were incubated in HEPES-Hank's Balanced Salt Solution (HBSS) buffer (70,000 cells/100 µl) for 1 h at 37ºC in 96-well poly-D-lysine-coated plates. To each well 100 µl of dye solution (fluorescence imaging plate reader [FLIPR] calcium 5 assay kit; Molecular Devices, Sunnyvale, CA, USA) was added, and the plates were incubated for 1 h at 37°C. The plates were then placed in a FLIPR, and 25 µl of the test substances diluted in HEPES-HBSS buffer that contained 250 mM probenicid was added online. The increase in fluorescence was then measured. EC_{50} values were derived from the concentration-response curves using nonlinear regression. Efficacy (maximal activity) is expressed relative to the activity of 5-HT, which was used as a control set to 100%.

2.4. Activity at the serotonin 5-HT_{2B} receptor

Human 5-HT_{2B} receptor-expressing HEK293 cells were incubated in growth medium (Dulbecco's Modified Eagle Medium [DMEM] high glucose [Invitrogen, Zug, Switzerland], 10 ml/L PenStrep [Gibco, Life Technologies, Zug, Switzerland], 10% fetal calf serum [non-dialysed, heat-inactivated], and 250 mg/l geneticin) at a density of 50,000 cells/well at 37ºC in 96-well poly-D-lysine-coated plates overnight. The next day, the growth medium was removed by snap inversion, and 100 µl of the calcium indicator Fluo-4 solution (Molecular Probes, Eugene, OR, USA) was added to each well. The plates were incubated for 45 min at 31°C. The Fluo-4 solution was removed by snap inversion, and 100 µl of Fluo-4 solution was added a second time. The cells

were then incubated for another 45 min at 31°C. Immediately before testing, the cells were washed with HBSS (Gibco) and 20 mM HEPES (assay buffer; Gibco) using an EMBLA cell washer, and 100 µl assay buffer was added. The plates were placed in a FLIPR, and 25 µl of the test substances diluted in assay buffer was added online. The increase in fluorescence was then measured. EC_{50} values were derived from the concentration-response curves using nonlinear regression. Efficacy (maximal activity) is expressed relative to the activity of 5-HT, which was used as a control set to 100%.

2.5. Monoamine uptake transporter inhibition

We assessed inhibition of the human NET, DAT, and SERT in transfected HEK293 cells that stably expressed the monoamine transporters as previously specified (Hysek et al., 2012). Briefly, cells were suspended in uptake buffer, treated with different concentrations of the test substances, and incubated for 10 min at room temperature. The corresponding radiolabeled monoamine, [^{3}H]NE, [^{3}H]DA, or [^{3}H]5-HT was then added at a final concentration of 5 nM at room temperature. After 10 min, the cells were centrifuged through silicone oil to separate them from the uptake buffer. The tubes were then frozen in liquid nitrogen immediately after centrifugation. The cell pellets were then lysed, and scintillation fluid was added. Radioactivity was measured with a beta counter, and nonspecific uptake in the presence of specific transporter inhibitors (10 µM nisoxetine for NET cells, 10 µM mazindol for DAT cells, and 10 µM fluoxetine for SERT cells) was determined for each experiment. This nonspecific uptake was subtracted from the total counts to yield specific uptake (100%). The data were fitted with nonlinear regression to variable-slope sigmoidal dose-response curves, and IC_{50} values were calculated using Prism software (GraphPad, San Diego, CA, USA). MDMA was included for comparison.

2.6. Transporter-mediated monoamine release

We investigated the effects of a single high dose (100 µM) of the test compounds on transporter-mediated NE, DA, and 5-HT efflux in HEK293 cells that stably overexpressed the respective human monoamine transporter as previously described (Simmler et al., 2013). Briefly, adherent cells were incubated with the respective radiolabeled monoamine (10 nM [^{3}H]NE and 10 µM unlabeled NE, 10 nM [^{3}H]DA and 1 µM unlabeled DA, and 10 nM [^{3}H]5-HT) for 20 min at 37°C. The cells were then washed twice with KHB buffer, and 1 ml of buffer that contained the test compound was added (100 µM final concentration). [^{3}H]5-HT and [^{3}H]DA release was stopped after 15 min, and [^{3}H]NE release was stopped after 45 min by washing with ice-cold buffer. Release was quantified by measuring the radioactivity that remained in the cells. Nonspecific "pseudo-efflux," which arises from nonspecific substrate release and subsequent reuptake inhibition (Scholze et al., 2000), was assessed for each experiment using the transporter inhibitors nisoxetine (NET cells), citalopram (SERT cells), and mazindol (DAT cells) at 10 µM as negative controls. Analysis of variance (ANOVA) followed by Dunnett's test was used to compare compound-induced release with the negative controls. Substances that exhibited significant higher outflow than the controls were considered monoamine releasers.

2.7. Cytotoxicity

To confirm cell integrity during the pharmacological assays, cytotoxicity was assessed using the ToxiLight bioassay (Lonza, Basel, Switzerland) according to the manufacturer's instructions. The assay quantitatively measures the release of adenylate kinase from damaged cells, providing a highly sensitive method of measuring cytolysis (Crouch et al., 1993). Cells that were grown in 96-well plates were exposed to the compounds at a high concentration of 100 µM. All of the test conditions contained 0.1% (v:v) dimethylsulfoxide, which is nontoxic at this concentration and was also used as a negative control. Triton X-100 (0.1%, Sigma-Aldrich, Buchs, Switzerland) lyses cells and was used as a positive control. After 4 h

incubation at 37°C, 10 µl of the supernatant per well was removed and combined with 50 µl of ToxiLight reagent, and luminescence was recorded using a Tecan Infinite 200 Pro plate reader (Tecan, Männedorf, Switzerland).

3. Results

3.1. Interactions with serotonin receptors

Table 1 presents binding to serotonin 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2C} receptors and activation potency and efficacy at 5-HT_{2A} and 5-HT_{2B} receptors. All of the tested hallucinogens bound to or activated 5-HT receptors mostly at submicromolar concentrations. LSD was the compound that most potently bound to the 5-HT_{2A} receptor, which is considered the primary target of hallucinogenic compounds. Binding affinity to the 5-HT_{2A} receptor was lower for all of the tryptamines, including psilocin and DMT, compared with LSD. Mescaline was the least potent. Among all of the substances tested in the present study, the K_i values for 5-HT_{2A} receptor binding were not associated with the EC_{50} values for 5-HT_{2A} receptor activation (Spearman rank correlation, R_s = 0.4, p > 0.1, n = 10). Similarly, 5-HT_{2A} receptor binding (K_i values) was not correlated with receptor activation when only the tryptamines were analyzed (R_s = 0.4, p > 0.1, n = 7). For the tryptamines, 5-HT_{2A} receptor binding affinity (R_s = 0.9, p < 0.05, n = 7) but not 5-HT_{2A} receptor activation potency (R_s = 0.6, n = 7, p > 0.1) was significantly correlated with the estimated mean doses that produced hallucinogenic subjective effects (Table 1; (Tittarelli et al., 2015).

5-HT_{2A} receptor activation potency was highest for 5-MeO-AMT, followed by all of the tryptamines, for which 5-HT_{2A} receptor activation potency was higher than the binding affinity. In contrast, for LSD, psilocin, and mescaline, 5-HT_{2A} receptor activation potency was low compared with their high binding affinity. As a result, all of the tryptamines, including DMT but with the exception of psilocin, were more potent agonists at the 5-HT_{2A} receptor than LSD in the assay that was used in the present study. LSD and psilocin were 5-HT_{2A} receptor partial agonists, with 28% and 16%

activation efficacy, respectively, whereas all of the other compounds presented higher 5-HT_{2A} receptor activation efficacies (up to > 80% for DiPT and 5-MeO-MiPT). Generally, the hallucinogens bound more potently to the 5-HT_{2A} receptor than to the 5-HT_{2C} receptor. However, 5-HT_{2A} over 5-HT_{2C} receptor binding selectivity was low (binding ratios < 10) for all of the compounds. LSD and most of the other drugs were relatively equally potent at 5-HT_{2A} and 5-HT_{1A} receptors, and LSD was the only compound to exhibit 5-HT_{1A} receptor affinity at 1-digit nanomolar concentrations. Only 5-MeO-AMT and 4-OH-DiPT activated the 5-HT_{2B} receptor at submicromolar concentrations.

3.2. Binding to monoamine receptors and transporters

Table 2 shows the binding affinities to monoamine transporters and receptors. Binding to transporters was generally weak, with the exception of DiPT and 4-OH-MET, which exhibited submicromolar binding affinity to the SERT. LSD was the only compound that bound to α_1-adrenergic receptors with submicromolar affinity. DMT also exhibited moderate binding to α_1-adrenergic receptors (K_i = 1.3 μM). LSD bound to α_2-adrenergic receptors at submicromolar concentrations. Only LSD showed affinity to dopaminergic $D_{1\text{-}3}$ receptors and exhibited submicromolar affinity to $TAAR_{1rat}$.

3.3. Monoamine uptake transporter inhibition

Monoamine uptake inhibition for at least one transporter was found for all of the substances, with the exception of mescaline and LSD (Table 3). Psilocin, DMT, DiPT, and 4-OH-DiPT inhibited the SERT, with IC_{50} values in the low micromolar range, similar to MDMA. Additionally, psilocin, DMT, DiPT, and 4-OH-MET inhibited NET but with lower potency than MDMA. All of the substances were no or very weak DAT inhibitors, in contrast to MDMA (Table 3).

3.4. Transporter-mediated monoamine release by tryptamines

Monoamine release was assessed only for tryptamines because LSD and mescaline did not interact with the monoamine transporters in the transporter inhibition assay and were shown not to release monoamines in previous studies (Rickli et al., 2015c). DMT released 5-HT, and 5-MeO-AMT released 5-HT and DA, whereas none of the other substances acted as substrate releasers (Fig. 2).

3.5. Cytotoxicity

None of the substances produced cytotoxicity after 4 h incubation in the adenylate-kinase release assay, indicating no cell damage.

4. Discussion

All of the tryptamines that were tested in the present study bound to and activated the 5-HT_{2A} receptor, extending previous *in vitro* studies on this group of hallucinogens (Blough et al., 2014; Gatch et al., 2011; McKenna et al., 1990; Nichols et al., 2015; Repke et al., 1985; Shulgin and Carter, 1980). None of the tryptamines was very selective for the 5-HT_{2A} receptor over other 5-HT_{1A} receptor.

The present study confirms and extends previous characterizations of tryptamines. DiPT was a full 5-HT_{2A} receptor agonist (101% efficacy) in the present study, consistent with high efficacy in an inositol-1-phosphate formation assay (82%; Gatch et al., 2011) and a calcium mobilization assay (110%; Blough et al., 2014). DiPT inhibited the human SERT (IC_{50} = 0.9 µM) as shown in studies that utilized rat brain synaptosomes (Blough et al., 2014) and human cells (Gatch et al., 2011) at a potency similar to MDMA (IC_{50} = 1.4 µM). DiPT also weakly inhibited the NET (IC_{50} = 9.9 µM) as previously shown for the human transporter (Blough et al., 2014) but not rat transporter (Gatch et al., 2011). DiPT activity at the DAT was low (IC_{50} > 10 µM) and even weaker than previously reported (Blough et al., 2014; Gatch et al., 2011). Confirming a previous study that used rat synaptosomes (Blough et al., 2014), DiPT

did not release monoamines via the human transporter in the present study. Our new data on 4-OH-DiPT and 4-OH-MET showed that these 4-ring-substituted compounds were 5-HT_{2A} receptor partial agonists, SERT inhibitors, and weak NET inhibitors, exhibiting a similar profile to psilocin and consistent with their reportedly common clinical effects (Tittarelli et al., 2015). 5-MeO-AMT was the most potent 5-HT_{2A} receptor ligand and agonist among the tryptamines that were evaluated in the present study and previous studies (Gatch et al., 2011). 5-MeO-AMT was a very weak monoamine transporter inhibitor, especially considering its much higher potency at the 5-HT_{2A} receptor. 5-MeO-AMT also induced the transporter-mediated release of DA and 5-HT as previously shown for the rat DAT and SERT (Nagai et al., 2007) and human SERT (Gatch et al., 2011). 5-MeO-AMT did not directly interact with adrenergic receptors. Together with the low-potency interaction with catecholamine transporters, our data indicate that the reported cardiostimulant effects of 5-MeO-AMT (Repke et al., 1985; Tittarelli et al., 2015) likely depend on the relatively potent 5-HT_{2A} receptor interactions rather than direct effects on the adrenergic systems, similar to hallucinogenic benzodifurans (Rickli et al., 2015b). 5-MeO-MiPT was a near-full (83%) agonist at the 5-HT_{2A} receptor, consistent with previous data (101%; Blough et al., 2014). 5-MeO-MiPT had no relevant action at the human monoamine transporters, consistent with recent data (Blough et al., 2014). Psilocin and DMT were agonists at the 5-HT_{2A} receptor with low efficacy. Additionally, psilocin was a SERT inhibitor, and DMT was a dual SERT-NET inhibitor and 5-HT releaser as reported previously (Blough et al., 2014; Cozzi et al., 2009).

Clear differences were found between the pharmacological profiles of the tryptamines and classic and well-studied hallucinogen LSD. First, LSD more potently bound to 5-HT_{2A} receptors compared with all of the tryptamines. Second, LSD was absolutely and in most cases also relatively more potent than all of the tryptamines at the 5-HT_1 receptor, which may moderate the *in vivo* effects of hallucinogens (Halberstadt et al., 2011; Krebs-Thomson et al., 2006; Sipes and Geyer, 1994;

Strassman, 1996). Third, all of the novel tryptamines were full agonists at the 5-HT_{2A} receptor or presented > 50% activation efficacy, whereas LSD, DMT, and psilocin were partial agonists with < 40% activation efficacy. Fourth, LSD bound to adrenergic and dopaminergic receptors at submicromolar concentrations, which was not the case for any of the other substances. Dopamine D_2 receptors may therefore contribute to the effects of LSD (Giacomelli et al., 1998; Marona-Lewicka et al., 2005) but are not or less involved in the action of tryptamines. Finally, all of the tryptamines inhibited the SERT in the case of DiPT at submicromolar concentrations, similar to MDMA. Additionally, NET inhibition was observed for DMT and DiPT, and transporter-mediated 5-HT release was observed for DMT and 5-MeO-AMT. In contrast, LSD and mescaline did not interact with the monoamine transporters. Consistent with our findings, DMT was previously shown to release 5-HT from 5-HT-preloaded rat synaptosomes (Blough et al., 2014), and DiPT and psilocin but not 5-MeO-MiPT inhibited the rat SERT (Blough et al., 2014). DMT, DiPT, and 5-MeO-AMT have also been previously shown to inhibit the human SERT (Cozzi et al., 2009; Gatch et al., 2011), and 5-MeO-AMT released 5-HT through the human SERT, similar to our findings (Gatch et al., 2011). Altogether, the results indicate that activity at the SERT may contribute to the pharmacology of several tryptamines. This is most likely relevant for DiPT and 4-OH-DiPT where the SERT inhibition potency is in the range of the binding potency at the 5-HT_{2A} receptor. It remains to be determined how interactions with SERT contribute to the effects of these tryptamines *in vivo*.

The pharmacological profiles of psilocin and LSD are particularly interesting because both substances currently receive high interest as research tools and potential therapeutic substances in psychiatry (Geyer, 2015; Halberstadt, 2015; Kupferschmidt, 2014). Clinically, the acute effects of psilocybin last shorter than those of LSD but are qualitatively very similar (Passie et al., 2008). The present study showed that LSD was a more potent 5-HT_{2A} receptor ligand compared with psilocin, consistent with its higher clinical potency (Schmid et al., 2015; Studerus et

al., 2011). At 5-HT receptors, both substances were 5-HT_{2A} receptor partial agonists with low efficacy and low selectivity for the 5-HT_{2A} receptor over the 5-HT_{1A} or 5-HT_{2C} receptor. LSD was also 10- to 100-fold more potent at α_1- and α_2-adrenergic and dopaminergic D_{1-3} receptors than psilocin in the present study. However, because psilocin (psilocybin) is used at approximately 100-fold higher doses than LSDthe profile was overall quite similar, with the exception that psilocin inhibited the SERT. Whether this is clinically relevant needs to be determined, and modern clinical studies that directly compare the effects of psilocybin and LSD are currently lacking.

The affinity of hallucinogens at the 5-HT_{2A} receptor but not at the 5-HT_{1A} receptor has been shown to correlate with psychoactive potency in humans (Sadzot et al., 1989; Titeler et al., 1988). The tryptamines that were tested in the present study all exhibited significantly lower affinity to the 5-HT_{2A} receptor compared with LSD. Tryptamines can be expected to be psychoactive at higher doses than LSD. Indeed, LSD is psychoactive at oral doses of 0.05-0.1 mg (Passie et al., 2008), whereas the tryptamine with the highest 5-HT_{2A} receptor binding affinity in the present study (5-MeO-AMT) is psychoactive at 2-5 mg (Shulgin and Shulgin, 1997; Tittarelli et al., 2015). The tryptamine with the lowest 5-HT_{2A} receptor affinity, DiPT, is psychoactive at 20-100 mg (Shulgin and Carter, 1980; Tittarelli et al., 2015). For the seven tryptamines that were evaluated in the present study, we found a significant correlation between 5-HT_{2A} receptor binding affinity and the estimated average doses at which the tryptamines are psychoactive in humans (Tittarelli et al., 2015) as previously shown for other hallucinogens (Sadzot et al., 1989; Titeler et al., 1988). In contrast, 5-HT_{2A} receptor activation potency did not correlate with the human doses. Notably, 5-HT_{2A} receptor activation potency did not reflect binding potency for the series of substances that were tested. Consistent within other hallucinogens, no clear correlation was found between binding affinity to the 5-HT_{2A} receptor and its functional activation potency (Nichols et al., 2015). Importantly, 5-HT_{2A} receptor activation is measured using various *in vitro* assays that reflect the activation of

different second messenger systems (Moya et al., 2007; Nichols, 2004), and these measures may apparently not reflect the mechanisms that mediate the subjective effects of these hallucinogens.

Many psychoactive compounds bind to $TAAR_1$ (Bunzow et al., 2001; Simmler et al., 2013; Simmler et al., 2016), a potential target for the treatment of addiction (Cotter et al., 2015; Jing and Li, 2015; Pei et al., 2015). Psilocin, DMT, 4-OH-MET, and 5-MeO-AMT had low-micromolar affinity to $TAAR_{1rat}$. LSD and the hallucinogenic phenethylamines and benzofurans were shown to be more potent $TAAR_1$ ligands (Rickli et al., 2015b; Rickli et al., 2015c). $TAAR_1$ agonism was reported to reduce monoamine system stimulation and the stimulant properties of MDMA (Di Cara et al., 2011). Thus, greater $TAAR_1$ interactions may be linked to lower stimulant-type properties (Simmler et al., 2013; Simmler et al., 2014a; Simmler et al., 2014b). The relevance of $TAAR_1$ binding to the subjective and reinforcing properties of tryptamines requires further study.

As expected, we observed several structure-activity relationships. Among the tryptamines, 5-MeO-AMT exhibited the highest 5-HT_{2A} receptor affinity and activation potency. The α-methylation and 5-methoxylation of tryptamines have both been shown to increase their potency. α-Methyl-5-HT and fluoro-α-methyltryptamines were shown to be more potent than their non-α-methylated analogs in inducing hallucinogen-typical head-twitch responses in mice (Nakagawasai et al., 2004; Tadano et al., 1995). Similarly, the α-methylation of phenethylamines increased 5-HT_{2A} receptor stimulation efficacy and head-twitch responses (Moya et al., 2007). The 5-methoxylation of tryptamines has been shown to increase 5-HT_{2A} receptor affinity and receptor activation potency for 5-MeO-DiPT *vs*. DiPT and for 5-MeO-MiPT *vs*. MiPT (Blough et al., 2014; Rogawski and Aghajanian, 1981). In the present study, 4-hydroxylation also increased 5-HT_{2A} receptor binding for psilocin *vs*. DMT and for 4-OH-DiPT *vs*. DiPT, consistent with previous studies (McKenna et al., 1990;

Repke et al., 1985; Rogawski and Aghajanian, 1981). *N*-substitutions are also known to alter the receptor interaction profiles of psychoactive tryptamines and phenethylamines (Braden et al., 2006; McKenna et al., 1990; Nichols et al., 2015; Repke et al., 1985; Rickli et al., 2015c). In our series, *N,N*-isopropylation, such as in DiPT, reduced binding affinity at 5-HT_2 receptors compared with *N,N*-methylation, such as in DMT. In contrast, asymmetrical *N*-methyl-*N*-isopropyl substitutions (MiPTs) resulted in greater activity compared with symmetrical *N*-alkyl substitution (Repke et al., 1985). Importantly, *N*-benzyl-substitutions with an ortho-substituent on the benzyl group resulted in highly potent phenethylamines (NBOMes; Braden et al., 2006; Rickli et al., 2015c), whereas *N*-benzyl-substitution with a meta-substituent on the benzyl group produced potent tryptamines (Nichols et al., 2015).

The presents study has limitations. The *in vitro* mechanism of action helps to predict clinical effects. However, additional factors such as absorption, protein binding, brain penetration, and metabolism also play a role and need to be addressed in future studies. For example, LSD may exhibit better brain penetration than tryptamines (Passie et al., 2008).

In conclusion, all of the tested tryptamines were partial or full 5-HT_{2A} receptor agonists and mostly interacted with the 5-HT transporter, similar to MDMA. In contrast to LSD, the tryptamines exhibited no or only low-potency interactions with adrenergic and dopaminergic receptors. The *in vitro* pharmacological data indicate that these tryptamines exert both hallucinogenic and MDMA-like effects in humans.

Conflict of interest

M.C. Hoener is an employee of Hoffmann-La Roche. The authors do not have any conflicts of interest to declare for this work.

Contributors

AR, MCH and MEL designed the study. MEL and MCH obtained funding. AR, OM, and MCH conducted experiments. AR, MCH, and ML analyzed the data and wrote the manuscript. All the authors reviewed and approved the manuscript.

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References

Araujo, A.M., Carvalho, F., de Lourdes Bastos, M., Guedes de Pinho, P., Carvalho, M., 2015. The hallucinogenic world of tryptamines: an updated review. Arch Toxicol 89, 1151-1173.

Baumeister, D., Barnes, G., Giaroli, G., Tracy, D., 2014. Classical hallucinogens as antidepressants? A review of pharmacodynamics and putative clinical roles. Ther Adv Psychopharmacol 4, 156-169.

Blough, B.E., Landavazo, A., Decker, A.M., Partilla, J.S., Baumann, M.H., Rothman, R.B., 2014. Interaction of psychoactive tryptamines with biogenic amine transporters and serotonin receptor subtypes. Psychopharmacology (Berl) 231, 4135-4144.

Braden, M.R., Parrish, J.C., Naylor, J.C., Nichols, D.E., 2006. Molecular interaction of serotonin 5-HT_{2A} receptor residues Phe339(6.51) and Phe340(6.52) with superpotent N-benzyl phenethylamine agonists. Mol Pharmacol 70, 1956-1964.

Bunzow, J.R., Sonders, M.S., Arttamangkul, S., Harrison, L.M., Zhang, G., Quigley, D.I., Darland, T., Suchland, K.L., Pasumamula, S., Kennedy, J.L., Olson, S.B., Magenis, R.E., Amara, S.G., Grandy, D.K., 2001. Amphetamine, 3,4-methylenedioxymethamphetamine, lysergic acid diethylamide, and metabolites of the catecholamine neurotransmitters are agonists of a rat trace amine receptor. Mol Pharmacol 60, 1181-1188.

Carhart-Harris, R.L., Leech, R., Erritzoe, D., Williams, T.M., Stone, J.M., Evans, J., Sharp, D.J., Feilding, A., Wise, R.G., Nutt, D.J., 2013. Functional connectivity measures after psilocybin inform a novel hypothesis of early psychosis. Schizophr Bull 39, 1343-1351.

Corkery, J.M., Durkin, E., Elliott, S., Schifano, F., Ghodse, A.H., 2012. The recreational tryptamine 5-MeO-DALT (N,N-diallyl-5-methoxytryptamine): a brief review. Prog Neuropsychopharmacol Biol Psychiatry 39, 259-262.

Cotter, R., Pei, Y., Mus, L., Harmeier, A., Gainetdinov, R.R., Hoener, M.C., Canales, J.J., 2015. The trace amine-associated receptor 1 modulates methamphetamine's neurochemical and behavioral effects. Front Neurosci 9, 39.

Cozzi, N.V., Gopalakrishnan, A., Anderson, L.L., Feih, J.T., Shulgin, A.T., Daley, P.F., Ruoho, A.E., 2009. Dimethyltryptamine and other hallucinogenic tryptamines exhibit substrate behavior at the serotonin uptake transporter and the vesicle monoamine transporter. J Neural Transm 116, 1591-1599.

Crouch, S.P., Kozlowski, R., Slater, K.J., Fletcher, J., 1993. The use of ATP bioluminescence as a measure of cell proliferation and cytotoxicity. J Immunol Methods 160, 81-88.

Di Cara, B., Maggio, R., Aloisi, G., Rivet, J.M., Lundius, E.G., Yoshitake, T., Svenningsson, P., Brocco, M., Gobert, A., De Groote, L., Cistarelli, L., Veiga, S., De Montrion, C., Rodriguez, M., Galizzi, J.P., Lockhart, B.P., Coge, F., Boutin, J.A., Vayer, P., Verdouw, P.M., Groenink, L., Millan, M.J., 2011. Genetic deletion

of trace amine 1 receptors reveals their role in auto-inhibiting the actions of ecstasy (MDMA). J Neurosci 31, 16928-16940.

Dolder, P.C., Schmid, Y., Haschke, M., Rentsch, K.M., Liechti, M.E., 2015. Pharmacokinetics and concentration-effect relationship of oral LSD in humans. Int J Neuropsychopharmacol doi: 10.1093/ijnp/pyv072.

Dos Santos, R.G., Valle, M., Bouso, J.C., Nomdedeu, J.F., Rodriguez-Espinosa, J., McIlhenny, E.H., Barker, S.A., Barbanoj, M.J., Riba, J., 2011. Autonomic, neuroendocrine, and immunological effects of ayahuasca: a comparative study with d-amphetamine. J Clin Psychopharmacol 31, 717-726.

EMCDDA, 2014. European Drug Report 2014. European Monitoring Center for Drugs and Drug Addiction (EMCDDA) www.emcdda.europa.eu; accessed January 198, 2016

Fantegrossi, W.E., Harrington, A.W., Kiessel, C.L., Eckler, J.R., Rabin, R.A., Winter, J.C., Coop, A., Rice, K.C., Woods, J.H., 2006. Hallucinogen-like actions of 5-methoxy-N,N-diisopropyltryptamine in mice and rats. Pharmacol Biochem Behav 83, 122-129.

Gasser, P., Holstein, D., Michel, Y., Doblin, R., Yazar-Klosinski, B., Passie, T., Brenneisen, R., 2014. Safety and efficacy of lysergic acid diethylamide-assisted psychotherapy for anxiety associated with life-threatening diseases. J Nerv Ment Dis 202, 513-520.

Gatch, M.B., Forster, M.J., Janowsky, A., Eshleman, A.J., 2011. Abuse liability profile of three substituted tryptamines. J Pharmacol Exp Ther 338, 280-289.

Geyer, M.A., 2015. Lysergic acid diethylamide and psilocybin revisited. Biol Psychiatry 78, 516-518.

Giacomelli, S., Palmery, M., Romanelli, L., Cheng, C.Y., Silvestrini, B., 1998. Lysergic acid diethylamide (LSD) is a partial agonist of D_2 dopaminergic receptors and it potentiates dopamine-mediated prolactin secretion in lactotrophs in vitro. Life Sci 63, 215-222.

Glennon, R.A., Chaurasia, C., Titeler, M., 1990. Binding of indolylalkylamines at 5-HT_2 serotonin receptors: examination of a hydrophobic binding region. J Med Chem 33, 2777-2784.

Glennon, R.A., Titeler, M., McKenney, J.D., 1984. Evidence for 5-HT_2 involvement in the mechanism of action of hallucinogenic agents. Life Sci 35, 2505-2511.

Gouzoulis-Mayfrank, E., Heekeren, K., Neukirch, A., Stoll, M., Stock, C., Obradovic, M., Kovar, K.A., 2005. Psychological effects of (S)-ketamine and N,N-dimethyltryptamine (DMT): a double-blind, cross-over study in healthy volunteers. Pharmacopsychiatry 38, 301-311.

Greene, S., 2013. Tryptamines, in: Dargan, P.I., Wood, D.M. (Eds.), Novel Psychoactive Substances: Classification, Pharmacology and Toxicology. Amsterdam; Elsevier, pp. 363-381.

Griffiths, R.R., Richards, W.A., McCann, U., Jesse, R., 2006. Psilocybin can occasion mystical-type experiences having substantial and sustained personal meaning and spiritual significance. Psychopharmacology (Berl) 187, 268-283; discussion 284-292.

Grob, C.S., Danforth, A.L., Chopra, G.S., Hagerty, M., McKay, C.R., Halberstadt, A.L., Greer, G.R., 2011. Pilot study of psilocybin treatment for anxiety in patients with advanced-stage cancer. Arch Gen Psychiatry 68, 71-78.

Halberstadt, A.L., 2015. Recent advances in the neuropsychopharmacology of serotonergic hallucinogens. Behav Brain Res 277, 99-120.

Halberstadt, A.L., Koedood, L., Powell, S.B., Geyer, M.A., 2011. Differential contributions of serotonin receptors to the behavioral effects of indoleamine hallucinogens in mice. J Psychopharmacol 25, 1548-1561.

Hasler, F., Grimberg, U., Benz, M.A., Huber, T., Vollenweider, F.X., 2004. Acute psychological and physiological effects of psilocybin in healthy humans: a double-blind, placebo-controlled dose-effect study. Psychopharmacology 172, 145-156.

Helander, A., Backberg, M., Hulten, P., Al-Saffar, Y., Beck, O., 2014. Detection of new psychoactive substance use among emergency room patients: results from the Swedish STRIDA project. Forensic Sci Int 243C, 23-29.

Helander, A., Beck, O., Hagerkvist, R., Hulten, P., 2013. Identification of novel psychoactive drug use in Sweden based on laboratory analysis: initial experiences from the STRIDA project. Scand J Clin Lab Invest 73, 400-406.

Hysek, C.M., Simmler, L.D., Nicola, V.G., Vischer, N., Donzelli, M., Krahenbühl, S., Grouzmann, E., Huwyler, J., Hoener, M.C., Liechti, M.E., 2012. Duloxetine inhibits effects of MDMA ("ecstasy") in vitro and in humans in a randomized placebo-controlled laboratory study. PLoS One 7, e36476.

Jing, L., Li, J.X., 2015. Trace amine-associated receptor 1: a promising target for the treatment of psychostimulant addiction. Eur J Pharmacol 761, 345-352.

Kamour, A., James, D., Spears, R., Cooper, G., Lupton, D.J., Eddleston, M., Thompson, J.P., Vale, A.J., Thanacoody, H.K., Hill, S.L., Thomas, S.H., 2014. Patterns of presentation and clinical toxicity after reported use of alpha methyltryptamine in the United Kingdom: a report from the UK National Poisons Information Service. Clin Toxicol (Phila) 52, 192-197.

Krebs-Thomson, K., Ruiz, E.M., Masten, V., Buell, M., Geyer, M.A., 2006. The roles of 5-HT_{1A} and 5-HT_2 receptors in the effects of 5-MeO-DMT on locomotor activity and prepulse inhibition in rats. Psychopharmacology (Berl) 189, 319-329.

Kupferschmidt, K., 2014. High hopes. Science 345, 18-23.

Liechti, M., 2015. Novel psychoactive substances (designer drugs): overview and pharmacology of modulators of monoamine signaling. Swiss Med Wkly 145, w14043.

Marona-Lewicka, D., Thisted, R.A., Nichols, D.E., 2005. Distinct temporal phases in the behavioral pharmacology of LSD: dopamine D_2 receptor-mediated effects in the rat and implications for psychosis. Psychopharmacology (Berl) 180, 427-435.

McKenna, D.J., Repke, D.B., Lo, L., Peroutka, S.J., 1990. Differential interactions of indolealkylamines with 5-hydroxytryptamine receptor subtypes. Neuropharmacology 29, 193-198.

Moya, P.R., Berg, K.A., Gutierrez-Hernandez, M.A., Saez-Briones, P., Reyes-Parada, M., Cassels, B.K., Clarke, W.P., 2007. Functional selectivity of hallucinogenic phenethylamine and phenylisopropylamine derivatives at human 5-hydroxytryptamine $(5\text{-HT})_{2A}$ and 5-HT_{2C} receptors. J Pharmacol Exp Ther 321, 1054-1061.

Nagai, F., Nonaka, R., Satoh Hisashi Kamimura, K., 2007. The effects of non-medically used psychoactive drugs on monoamine neurotransmission in rat brain. Eur J Pharmacol 559, 132-137.

Nakagawasai, O., Arai, Y., Satoh, S.E., Satoh, N., Neda, M., Hozumi, M., Oka, R., Hiraga, H., Tadano, T., 2004. Monoamine oxidase and head-twitch response in mice: mechanisms of alpha-methylated substrate derivatives. Neurotoxicology 25, 223-232.

Nichols, D.E., 2004. Hallucinogens. Pharmacol Ther 101, 131-181.

Nichols, D.E., Sassano, M.F., Halberstadt, A.L., Klein, L.M., Brandt, S.D., Elliott, S.P., Fiedler, W.J., 2015. N-Benzyl-5-methoxytryptamines as potent serotonin 5-HT receptor family agonists and comparison with a series of phenethylamine analogues. ACS Chem Neurosci 6, 1165-1175.

Passie, T., Halpern, J.H., Stichtenoth, D.O., Emrich, H.M., Hintzen, A., 2008. The pharmacology of lysergic acid diethylamide: a review. CNS Neurosci Ther 14, 295-314.

Pei, Y., Mortas, P., Hoener, M.C., Canales, J.J., 2015. Selective activation of the trace amine-associated receptor 1 decreases cocaine's reinforcing efficacy and prevents cocaine-induced changes in brain reward thresholds. Prog Neuropsychopharmacol Biol Psychiatry 63, 70-75.

Pichini, S., Pujadas, M., Marchei, E., Pellegrini, M., Fiz, J., Pacifici, R., Zuccaro, P., Farre, M., de la Torre, R., 2008. Liquid chromatography-atmospheric pressure ionization electrospray mass spectrometry determination of "hallucinogenic designer drugs" in urine of consumers. J Pharm Biomed Anal 47, 335-342.

Ray, T.S., 2010. Psychedelics and the human receptorome. PLoS One 5, e9019.

Repke, D.B., Grotjahn, D.B., Shulgin, A.T., 1985. Psychotomimetic N-methyl-N-isopropyltryptamines: effects of variation of aromatic oxygen substituents. J Med Chem 28, 892-896.

Revel, F.G., Moreau, J.L., Gainetdinov, R.R., Bradaia, A., Sotnikova, T.D., Mory, R., Durkin, S., Zbinden, K.G., Norcross, R., Meyer, C.A., Metzler, V., Chaboz, S., Ozmen, L., Trube, G., Pouzet, B., Bettler, B., Caron, M.G., Wettstein, J.G., Hoener, M.C., 2011. $TAAR_1$ activation modulates monoaminergic neurotransmission, preventing hyperdopaminergic and hypoglutamatergic activity. Proc Natl Acad Sci U S A 108, 8485-8490.

Rickli, A., Hoener, M.C., Liechti, M.E., 2015a. Monoamine transporter and receptor interaction profiles of novel psychoactive substances: para-halogenated amphetamines and pyrovalerone cathinones. Eur Neuropsychopharmacol 25, 365-376.

Rickli, A., Kopf, S., Hoener, M.C., Liechti, M.E., 2015b. Pharmacological profile of novel psychoactive benzofurans. Br J Pharmacol 172, 3412-3425.

Rickli, A., Luethi, D., Reinisch, J., Buchy, D., Hoener, M.C., Liechti, M.E., 2015c. Receptor interaction profiles of novel N-2-methoxybenzyl (NBOMe) derivatives of 2,5-dimethoxy-substituted phenethylamines (2C drugs). Neuropharmacology 99, 546-553.

Rogawski, M.A., Aghajanian, G.K., 1981. Serotonin autoreceptors on dorsal raphe neurons: structure-activity relationships of tryptamine analogs. J Neurosci 1, 1148-1154.

Sadzot, B., Baraban, J.M., Glennon, R.A., Lyon, R.A., Leonhardt, S., Jan, C.R., Titeler, M., 1989. Hallucinogenic drug interactions at human brain 5-HT2 receptors: implications for treating LSD-induced hallucinogenesis. Psychopharmacology (Berl) 98, 495-499.

Schmid, Y., Enzler, F., Gasser, P., Grouzmann, E., Preller, K.H., Vollenweider, F.X., Brenneisen, R., Muller, F., Borgwardt, S., Liechti, M.E., 2015. Acute effects of lysergic acid diethylamide in healthy subjects. Biol Psychiatry 78, 544-553.

Schmidt, M.M., Sharma, A., Schifano, F., Feinmann, C., 2011. "Legal highs" on the net: evaluation of UK-based websites, products and product information. Forensic Sci Int 206, 92-97.

Scholze, P., Zwach, J., Kattinger, A., Pifl, C., Singer, E.A., Sitte, H.H., 2000. Transporter-mediated release: a superfusion study on human embryonic kidney cells stably expressing the human serotonin transporter. J Pharmacol Exp Ther 293, 870-878.

Shulgin, A.T., Carter, M.F., 1980. N,N-Diisopropyltryptamine (DIPT) and 5-methoxy-N,N-diisopropyltryptamine (5-MeO-DIPT): two orally active tryptamine analogs with CNS activity. Commun Psychopharmacol 4, 363-369.

Shulgin, A.T., Shulgin, A., 1997. TiHKAL the continuation. Berkeley: Transform Press.

Simmler, L., Buser, T., Donzelli, M., Schramm, Y., Dieu, L.H., Huwyler, J., Chaboz, S., Hoener, M., Liechti, M.E., 2013. Pharmacological characterization of designer cathinones in vitro. Br J Pharmacol 168, 458-470.

Simmler, L.D., Buchy, D., Chaboz, S., Hoener, M.C., Liechti, M.E., 2016. In vitro characterization of psychoactive substances at rat, mouse, and human trace amine-associated receptor 1. J Pharmacol Exp Ther (10.1124/jpet.115.229765).

Simmler, L.D., Rickli, A., Hoener, M.C., Liechti, M.E., 2014a. Monoamine transporter and receptor interaction profiles of a new series of designer cathinones. Neuropharmacology 79, 152-160.

Simmler, L.D., Rickli, A., Schramm, Y., Hoener, M.C., Liechti, M.E., 2014b. Pharmacological profiles of aminoindanes, piperazines, and pipradrol derivatives. Biochem Pharmacol 88, 237-244.

Sipes, T.A., Geyer, M.A., 1994. Multiple serotonin receptor subtypes modulate prepulse inhibition of the startle response in rats. Neuropharmacology 33, 441-448.

Sogawa, C., Sogawa, N., Tagawa, J., Fujino, A., Ohyama, K., Asanuma, M., Funada, M., Kitayama, S., 2007. 5-Methoxy-N,N-diisopropyltryptamine (Foxy), a selective and high affinity inhibitor of serotonin transporter. Toxicol Lett 170, 75-82.

Strassman, R.J., 1996. Human psychopharmacology of N,N-dimethyltryptamine. Behav Brain Res 73, 121-124.

Strassman, R.J., Qualls, C.R., 1994. Dose-response study of N,N-dimethyltryptamine in humans: I. Neuroendocrine, autonomic, and cardiovascular effects. Arch Gen Psychiatry 51, 85-97.

Strassman, R.J., Qualls, C.R., Uhlenhuth, E.H., Kellner, R., 1994. Dose-response study of N,N-dimethyltryptamine in humans: II. Subjective effects and preliminary results of a new rating scale. Arch Gen Psychiatry 51, 98-108.

Studerus, E., Kometer, M., Hasler, F., Vollenweider, F.X., 2011. Acute, subacute and long-term subjective effects of psilocybin in healthy humans: a pooled analysis of experimental studies. J Psychopharmacol 25, 1434-1452.

Tadano, T., Neda, M., Hozumi, M., Yonezawa, A., Arai, Y., Fujita, T., Kinemuchi, H., Kisara, K., 1995. alpha-Methylated tryptamine derivatives induce a 5-HT receptor-mediated head-twitch response in mice. Neuropharmacology 34, 229-234.

Taljemark, J., Johansson, B.A., 2012. Drug-induced acute psychosis in an adolescent first-time user of 4-HO-MET. Eur Child Adolesc Psychiatry 21, 527-528.

Tatsumi, M., Groshan, K., Blakely, R.D., Richelson, E., 1997. Pharmacological profile of antidepressants and related compounds at human monoamine transporters. Eur J Pharmacol 340, 249-258.

Titeler, M., Lyon, R.A., Glennon, R.A., 1988. Radioligand binding evidence implicates the brain 5-HT_2 receptor as a site of action for LSD and phenylisopropylamine hallucinogens. Psychopharmacology (Berl) 94, 213-216.

Tittarelli, R., Mannocchi, G., Pantano, F., Romolo, F.S., 2015. Recreational use, analysis and toxicity of tryptamines. Curr Neuropharmacol 13, 26-46.

Tomaszewski, Z., Johnson, M.P., Huang, X., Nichols, D.E., 1992. Benzofuran bioisosteres of hallucinogenic tryptamines. J Med Chem 35, 2061-2064.

Trachsel, D., Lehmann, D., Enzensperger, C., 2013. Phenethylemine: Von der Struktur zur Funktion. Solothurn: Nachtschatten Verlag.

Vollenweider, F.X., Vollenweider-Scherpenhuyzen, M.F., Babler, A., Vogel, H., Hell, D., 1998. Psilocybin induces schizophrenia-like psychosis in humans via a serotonin-2 agonist action. Neuroreport 9, 3897-3902.

Winstock, A.R., Kaar, S., Borschmann, R., 2014. Dimethyltryptamine (DMT): prevalence, user characteristics and abuse liability in a large global sample. J Psychopharmacol 28, 49-54.

Figure Legends

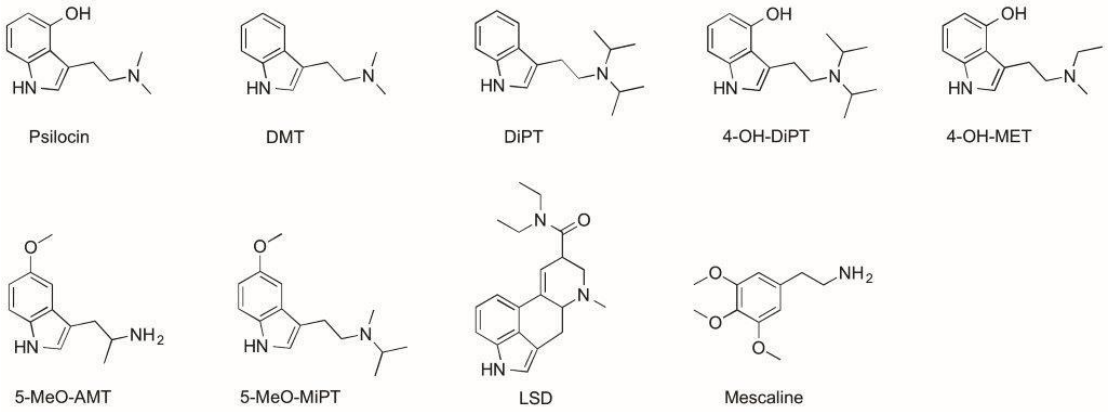

Figure 1. Chemical structures of tryptamines, LSD, and mescaline.

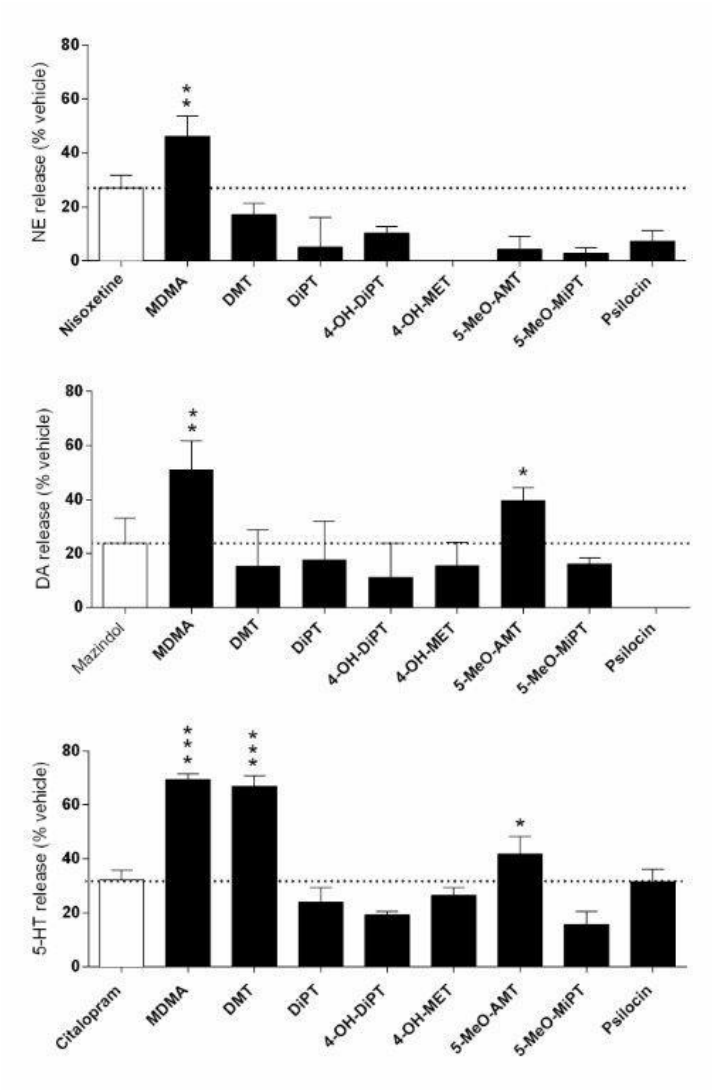

Figure 2. Monoamine release induced by tryptamines. Monoamine release was induced by a high concentration of the test substances (100 µM) after preloading HEK293 cells that stably expressed NET, DAT, or SERT. Most of the tryptamines did not release monoamines. Only DMT released 5-HT, and 5-MeO-AMT released 5-HT and DA. MDMA was added as a positive control, which releases all monoamines. Transporter blockers were included as negative controls (open bars) that produce non-transporter-mediated “pseudo-efflux” (horizontal dashed line). Compounds that produced significantly more monoamine efflux (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$) compared with the respective non-releasing uptake inhibitors (nisoxetine, mazindol, or citalopram) were considered monoamine releasers. The data are expressed as the mean ± SEM of three to four independent experiments.

Table 1. Serotonin receptor interactions

	5-HT_{1A}	5-HT_{2A}			5-HT_{2B}		5-HT_{2C}	
	receptor binding K_i ± SD [µM]	receptor binding K_i ± SD [µM]	activation potency EC_{50} ± SD [µM]	activation efficacy % maximum ± SD	activation potency EC_{50} ± SD [µM]	activation efficacy % maximum ± SD	receptor binding K_i ± SD [µM]	psychoactive dose[b] [mg]
Tryptamines								
Psilocin	0.123 ± 0.02	0.049 ± 0.01	0.721 ± 0.55	16 ± 8	> 20		0.094 ± 0.009	12
DMT	0.075 ± 0.02	0.237 ± 0.04	0.076 ± 0.03	40 ± 11	3.4 ± 3.2	19 ± 6	0.424 ± 0.15	40
DiPT	0.538 ± 0.05	1.2 ± 0.3	0.240 ± 0.08	101 ± 2	1.0 ± 0.2	103 ± 15	6.5 ± 1.6	75
4-OH-DiPT	5.7 ± 0.9	0.728 ± 0.07	0.093 ± 0.03	74 ± 14	0.460 ± 0.36	55 ± 10	2.8 ± 1.2	18
4-OH-MET	0.228 ± 0.06	0.057 ± 0.01	0.037 ± 0.01	72 ± 11	> 20		0.141 ± 0.03	15
5-MeO-AMT	0.046 ± 0.005	0.034 ± 0.01	0.002 ± 0.0002	60 ± 5	0.004 ± 0.001	104 ± 19	0.090 ± 0.03	3
5-MeO-MiPT	0.058 ± 0.01	0.163 ± 0.03	0.023 ± 0.004	83 ± 7	1.5 ± 0.9	12 ± 7	1.3 ± 0.3	5
Ergoline								
LSD[a]	0.003 ± 0.0005	0.004 ± 0.001	0.261 ± 0.15	28 ± 10	12 ± 0.4	71 ± 31	0.015 ± 0.003	0.1
Phenethylamine								
Mescaline[a]	4.6 ± 0.4	6.3 ± 1.8	10 ± 1.8	56 ± 15	> 20		17 ± 2.0	300

Values are K_i given as microM (mean± SD); [a]Values have previously been published in Rickli et al. 2015c; [b]Estimated average from Tittarelli et al. 2015; Passie et al. 2008, Nichols, 2004.

Table 2. Monoamine transporter and receptor binding affinities.

	NET	DAT	SERT	α_{1A}	α_{2A}	D_1	D_2	D_3	H_1	$TAAR_{1rat}$[b]	$TAAR_{1mouse}$[b]
Tryptamines											
Psilocin	13 ± 1.7	> 30	6.0 ± 0.3	6.7 ± 1.1	2.1 ± 0.01	> 14	3.7 ± 0.6	8.9 ± 0.8	1.6 ± 0.2	1.4 ± 0.2	17 ± 1.7
DMT	6.5 ± 1.3	22 ± 3.9	6.0 ± 0.6	1.3 ± 0.2	2.1 ± 0.4	6.0 ± 0.9	3.0 ± 0.4	6.3 ± 2.1	0.22 ± 0.03	2.2 ± 0.2	3.3 ± 0.4
DiPT	8.9 ± 0.9	4.1 ± 0.1	0.18 ± 0.02	> 12	3.6 ± 0.1	> 25	> 25	> 25	0.92 ± 0.36	> 15	> 15
4-OH-DiPT	11 ± 2.8	> 26	1.8 ± 0.4	> 12	15 ± 1.6	> 25	> 25	> 25	9.8 ± 0.9	> 15	> 15
4-OH-MET	13 ± 4.9	> 26	0.20 ± 0.06	9.7 ± 1.7	2.4 ± 0.2	> 25	4.0 ± 0.2	6.7 ± 2.0	0.82 ± 0.09	3.1 ± 0.2	12 ± 3.4
5-MeO-AMT	> 22	> 26	12 ± 1.1	> 12	11 ± 0.3	> 25	> 25	> 25	> 25	1.1 ± 0.2	4.8 ± 0.9
5-MeO-MiPT	> 22	> 26	3.3 ± 0.3	> 12	5.3 ± 0.3	> 25	> 25	> 25	3.9 ± 0.5	> 15	> 15
Ergoline											
LSD[a]	> 30	> 30	> 30	0.67 ± 0.18	0.012 ± 0.002	0.31 ± 0.09	0.025 ± 0.0004	0.10 ± 0.01	1.1 ± 0.2	0.45 ± 0.05	10 ± 2.9
Phenethylamines											
Mescaline[a]	> 30	> 30	> 30	> 15	1.4 ± 0.2	> 14	> 10	> 17	> 25	3.3 ± 0.5	11 ± 3.6

Values are K_i given as microM (mean ± SD). [a]Values have previously been published in Rickli et al. 2015c. [b]Values have previously been published in Simmler et al. 2016.

Table 3. Monoamine transporter inhibition

	NET	DAT	SERT
	IC_{50} [µM] (95% CI)	IC_{50} [µM] (95% CI)	IC_{50} [µM] (95% CI)
Tryptamines			
Psilocin	14 (10-19)	>100	3.9 (3.1-4.8)
DMT	3.9 (2.8-5.3)	52 (37-72)	3.1 (2.4-4.0)
DiPT	9.9 (7.4-13)	35 (28-45)	0.9 (0.7-1.2)
4-OH-DiPT	46 (33-62)	>100	2.4 (1.7-3.4)
4-OH-MET	11 (8-14)	>100	9.0 (6.7-12)
5-MeO-AMT	78 (67-90)	43 (35-53)	17 (11-24)
5-MeO-MiPT	84 (65-109)	>100	22 (17-28)
Ergoline			
LSD[a]	>100	>100	>100
Phenethylamines			
Mescaline[a]	>100	>100	>100
MDMA	0.20 (0.11-0.36)	14 (8.4-23)	1.4 (1.0-2.0)

Values are means of three to four independent experiments and 95% confidence intervals (CI).

[a]Values have previously been published in Rickli *et al.* 2015c