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## Support for 5-HT<sub>2C</sub> receptor functional selectivity *in vivo* utilizing structurally diverse, selective 5-HT<sub>2C</sub> receptor ligands and the 2,5-dimethoxy-4-iodoamphetamine elicited head-twitch response model

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### Abstract

There are seemingly conflicting data in the literature regarding the role of serotonin (5-HT) 5-HT<sub>2C</sub> receptors in the mouse head-twitch response (HTR) elicited by the hallucinogenic 5-HT<sub>2A/2B/2C</sub> receptor agonist 2,5-dimethoxy-4-iodoamphetamine (DOI). Namely, both 5-HT<sub>2C</sub> receptor agonists and antagonists, regarding 5-HT<sub>2C</sub> receptor-mediated Gq-phospholipase C (PLC) signaling, reportedly attenuate the HTR response. The present experiments tested the hypothesis that both classes of 5-HT<sub>2C</sub> receptor compounds could attenuate the DOI-elicited-HTR in a single strain of mice, C57Bl/6J. The expected results were considered in accordance with ligand functional selectivity. Commercially-available 5-HT<sub>2C</sub> agonists (CP 809101, Ro 60-0175, WAY 161503, mCPP, and 1-methylpsilocin), novel 4-phenyl-2-N,N-dimethyl-aminotetralin (PAT)-type 5-HT<sub>2C</sub> agonists (with 5-HT<sub>2A/2B</sub> antagonist activity), and antagonists selective for 5-HT<sub>2A</sub> (M100907), 5-HT<sub>2C</sub> (SB-242084), and 5-HT<sub>2B/2C</sub> (SB-206553) receptors attenuated the DOI-elicited-HTR. In contrast, there were differential effects on locomotion across classes of compounds. The 5-HT<sub>2C</sub> agonists and M100907 decreased locomotion, SB-242084 increased locomotion, SB-206553 resulted in dose-dependent biphasic effects on locomotion, and the PATs did not alter locomotion. *In vitro* molecular pharmacology studies showed that 5-HT<sub>2C</sub> agonists potent for attenuating the DOI-elicited-HTR also reduced the efficacy of DOI to activate mouse 5-HT<sub>2C</sub> receptor-mediated PLC signaling in HEK cells. Although there were differences in affinities of a few compounds at mouse compared to human 5-HT<sub>2A</sub> or 5-HT<sub>2C</sub> receptors, all compounds tested retained their selectivity for either receptor, regardless of receptor species. Results indicate that 5-HT<sub>2C</sub> receptor agonists and antagonists attenuate the DOI-elicited-HTR in C57Bl/6J mice, and suggest that structurally diverse 5-HT<sub>2C</sub> ligands result in different 5-HT<sub>2C</sub> receptor signaling outcomes compared to DOI.

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### Conflict of interest

All authors declare that they have no financial conflicts of interest.

## Keywords

Head-twitch response; DOI; Hallucinogen; C57Bl/6J; Mouse serotonin 2C receptor; Mouse serotonin 2A receptor; Functional selectivity

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## 1. Introduction

Serotonin (5-hydroxytryptamine, 5-HT) type 2 receptors (5-HT<sub>2A</sub>, 2B, and 2C) regulate many complex brain functions, including sleep, feeding, emotion, perception, cognition, reward, and memory (Jensen et al., 2010; Landolt and Wehrle, 2009; Millan, 2005; Nichols, 2004). The functions of 5-HT<sub>2</sub> receptors implicate them as potentially useful targets for treating neuropsychiatric disorders, however, drug discovery programs have yielded only one approved 5-HT<sub>2</sub>-selective medicine, the 5-HT<sub>2C</sub>-preferring agonist lorcaserin (BELVIQ<sup>®</sup>), which was approved for obesity recently. A number of factors explain the lack of drugs targeting 5-HT<sub>2</sub> receptors. The amino acid sequences of the transmembrane regions of 5-HT<sub>2</sub> receptors are highly homologous, creating a challenge for designing and developing ligands that activate very selectively one but not the other 5-HT<sub>2</sub> receptor subtypes. This is a serious issue, since most 5-HT<sub>2</sub> receptor agonists that may have clinically useful effects also produce hallucinations, mediated primarily by activation of the 5-HT<sub>2A</sub> receptor subtype (Nichols, 2004). Furthermore, prolonged activation of 5-HT<sub>2B</sub> receptors on heart valve leaflets has been associated with the development of cardiac pulmonary valvulopathy, as seen with fenfluramine (Hutcheson et al., 2011). Finally, selective 5-HT<sub>2</sub> antagonists including 5-HT<sub>2A</sub> antagonists proposed as novel medications to treat schizophrenia, have exhibited only modest antipsychotic effects in clinical trials (Ebdrup et al., 2011), yet are effective for treating L-DOPA-induced psychosis (Abbas and Roth, 2008). Recent clinical studies have shown that 5-HT<sub>2A</sub> antagonists also increase slow wave sleep, and thus may treat certain sleep disorders (Monti, 2010; Teegarden et al., 2008).

Specific (distinguished from “selective”) activation of 5-HT<sub>2C</sub> receptors may produce neuropharmacotherapeutic effects with few side-effects. For example, drugs specific for activating 5-HT<sub>2C</sub> receptors that have zero efficacy for activating 5-HT<sub>2A</sub> and/or 5-HT<sub>2B</sub> receptors may be useful for treating schizophrenia (Rosenzweig-Lipson et al., 2007) and substance abuse (Cunningham et al., 2011) by modulating central dopamine release. Extant 5-HT<sub>2C</sub>-selective agonists including BELVIQ<sup>®</sup>, however, produce 5-HT<sub>2C</sub>-dependent hypolocomotion effects in preclinical animal models and similarly can produce somnolence or fatigue in humans (Arena Pharmaceuticals, 2012; Halberstadt et al., 2009; Siuciak et al., 2007). Importantly, at higher concentrations, nearly all reported 5-HT<sub>2C</sub> selective agonists also activate 5-HT<sub>2A</sub> and/or 5-HT<sub>2B</sub> receptors, including BELVIQ<sup>®</sup> (Thomsen et al., 2008). Thus, the challenge remains to develop compounds that are specific 5-HT<sub>2C</sub> receptor agonists lacking sedative effects.

Most 5-HT<sub>2</sub> receptor agonists (regardless of selectivity) produce a head-twitch response (HTR) in rodents (Canal and Morgan, 2012). Genetic ablation of 5-HT<sub>2A</sub> receptors or treatment with selective 5-HT<sub>2A</sub> antagonists abolishes the HTR in mice elicited by 5-HT<sub>2</sub> agonist hallucinogens (Gonzalez-Maeso et al., 2007, 2003), such as 2,5-dimethoxy-4-iodoamphetamine (DOI), providing strong evidence that activation of 5-HT<sub>2A</sub> receptors is necessary for the HTR in rodents elicited by hallucinogenic 5-HT<sub>2</sub> agonists. Several other neurotransmitter receptors also appear to modulate DOI-elicited-HTRs in mice, including 5-HT<sub>2C</sub> receptors (Canal et al., 2010; Fantegrossi et al., 2010). Interestingly, DOI produces HTRs at doses that also potently and efficaciously activate 5-HT<sub>2C</sub> receptors, yet selective 5-HT<sub>2C</sub> agonists, with lower potency and efficacy for activating 5-HT<sub>2A</sub> receptors, dose-dependently attenuate the DOI-elicited-HTR in rodents (Fantegrossi et al., 2010; Siuciak et

al., 2007). Adding further intrigue, recent reports show that the selective 5-HT<sub>2C</sub> antagonist SB-242084 also attenuates the DOI-elicited-HTR in C57Bl/6J and DBA/2J mice (Canal et al., 2010). This effect was not observed, however, in NIH/Swiss mice, wherein SB-242084 actually enhanced the DOI-elicited-HTR (Fantegrossi et al., 2010).

One aim of the current studies was to resolve the seemingly conflicting observations in the literature by comparing directly the effects of multiple 5-HT<sub>2C</sub> agonists and antagonists on the DOI-elicited-HTR in a single animal model using identical experimental parameters and conditions. It was hypothesized that both classes of compounds could attenuate the DOI-elicited-HTR, and that the results could be in accordance with *in vivo* functional selectivity (Milligan, 1993; Moya et al., 2007; Urban et al., 2007). This hypothesis involves the notion that selective 5-HT<sub>2C</sub> agonists could activate 5-HT<sub>2C</sub> receptors *in vivo* in a different way compared to DOI, similar to reports of other 5-HT<sub>2</sub> agonists acting at 5-HT<sub>2A</sub> receptors (Schmid and Bohn, 2010; Schmid et al., 2008). Therefore, like antagonists, selective 5-HT<sub>2C</sub> agonists could interfere with 5-HT<sub>2</sub> receptor signaling that leads to the DOI-elicited-HTR by acting as functional antagonists of DOI *in vivo*.

Functional selectivity *in vivo* may indeed have therapeutic impact (Mailman, 2007). If 5-HT<sub>2C</sub> receptor ligands behave as functionally-selective agonists *in vivo*, then it may be feasible to develop therapeutic 5-HT<sub>2C</sub> receptor agonists that lack side-effects, such as sedation, by targeting specific 5-HT<sub>2C</sub> signaling patterns. To this end, in addition to effects on the DOI-elicited-HTR, several classes of selective 5-HT<sub>2C</sub> agonists were tested for their effects on locomotion following administration of DOI.

## 2. Materials and methods

### 2.1. Compounds and treatment doses

The chemical structures of all compounds used in the present studies are shown in Table 1. (±)-DOI (DOI), mCPP, SB-242084, SB-206553, and M100907 were purchased from Sigma-Aldrich (MO, USA). WAY 161503, Ro 60-0175, CP 809101, and 1-methylpsilocin were purchased from Tocris (Bristol, UK). Serotonin was purchased from Alfa Aesar (MA, USA). [<sup>3</sup>H]-mesulergine, [<sup>3</sup>H]-ketanserin, and [<sup>3</sup>H]-myo-inositol were purchased from Perkin-Elmer (MA, USA). The (+)-(2*R*, 4*S*)- and (-)-(2*S*, 4*R*)-*trans* enantiomers of 4-phenyl-2-*N,N*-dimethylaminotetralin (PAT) and (-)-(2*S*, 4*R*)-*trans*-4-(4'-methyl)-*N,N*-dimethylaminotetralin (methyl-PAT) were synthesized in our laboratories as racemates that were resolved by chiral stationary-phase HPLC and converted to hydrochloride salts as previously described (Booth et al., 2009; Bucholtz et al., 1999; Vincek and Booth, 2009). All compounds, with the exceptions of SB-242084 and 1-methylpsilocin, were dissolved in sterile MilliQ water which served as the vehicle control. SB-242084 was dissolved in sterile MilliQ water containing Tween-80 (6% final v/v), and 1-methylpsilocin was dissolved in sterile MilliQ water containing acetic acid (5% v/v). Separate MilliQ water vehicles containing these additives were used for control groups for these compounds. All compounds were administered subcutaneously (sc) at a volume of 10 mL/kg. A single dose of DOI (1 mg/kg) was used for all experiments, as it is known to reliably elicit a robust and consistent number of HTRs in C57Bl/6J mice (Canal et al., 2010; Fox et al., 2010). Two doses were used to test the efficacy of 5-HT<sub>2C</sub> selective agonists for attenuating the DOI-elicited-HTR 3 mg/kg and 5.6 mg/kg. Doses for antagonists were as follows: M100907, 0.0025, 0.025, and 0.25 mg/kg, SB-206553 and SB-242084, 0.03, 0.3, and 3 mg/kg. All compounds were weighed on a Mettler-Toledo (OH, USA) XP26 microanalytical balance, and solutions of all compounds were made fresh on the day of testing.

## 2.2. Animal subjects

Male C57Bl/6J mice, purchased at 60 days old from Jackson Laboratories (Bar Harbor, Maine) were used in all experiments. Mice were pair-housed in standard cages with *ad libitum* access to food and water, and were acclimated in the vivarium for at least 4 days prior to testing. Animal procedures were approved by the Institutional Animal Care and Use Committee and are in accordance with the principles in the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

## 2.3. Behavioral studies

Drug or vehicle was injected sc 10 min prior to injection of DOI. Mice were placed in an activity chamber (43 × 43 cm, Med Associates, Inc.) 10-min later, and head-twitches were counted for the next 10 min by an observer blind to experimental treatment. Locomotion (distance traveled, cm) was tracked with an overhead camera and Ethovision XT 7.0 software. A separate group of mice was administered DOI 10 min prior to injection of 0.25 mg/kg M100907 or 3 mg/kg SB-206553 to test whether the HTR becomes independent of 5-HT<sub>2</sub> receptors after their activation, i.e. to test whether M100907 and/or SB-206553 remain effective at attenuating the DOI-elicited-HTR after 5-HT<sub>2</sub> receptor activation.

## 2.4. Mouse 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> receptor constructs

Plasmid DNA containing either mouse htr2A or htr2C-vnv cDNA clones was purchased from Origene (MD, USA). cDNA clones were inserted between the EcoR 1 and Not 1 sites in pCMV6-Kan/Neo vectors. Plasmid DNA was transformed into Subcloning Efficiency DH5a competent cells following the manufacturer's protocol (Invitrogen, CA, USA). Selections of individual colonies of kanamycin-resistant cells were grown in sterile LB broth overnight which served to generate glycerol stocks that were kept stored at -80 °C. Following purification of DNA using Wizard Plus SV minipreps (Promega, USA), samples of htr2A or htr2C DNA were sequenced by the University of Florida DNA sequencing core. Verification of the open-reading frame sequences for the mouse htr2A and mouse htr2C DNA was performed by comparing the results obtained to results published on Pubmed (<http://www.ncbi.nlm.nih.gov/pubmed/>). The sequences obtained were identical to the published sequences, and match the receptor htr2A and htr2C DNA sequences from the C57Bl/6 mouse. During the verification process, it was observed that the mouse htr2C cDNA from Origene coded for the VNV edited isoform of this receptor. cDNA coding for the human 5-HT<sub>2A</sub> or 5-HT<sub>2C</sub>-INI receptors were from previously transformed competent cells, as previously reported (Canal et al., 2011).

## 2.5. Radioreceptor assays

Transfection procedures, membrane receptor preparation, and binding assays were performed as previously described (Canal et al., 2011). Briefly, radioligand competitive displacement and saturation binding assays were performed in 96-well plates, using cell membranes from HEK-293 cells transiently expressing high femtomoles per mg protein mouse or human 5-HT<sub>2A</sub> or 5-HT<sub>2C</sub> receptors. All competition and saturation binding experiments involved the use of triplicates or quadruplicates of samples for each dose of unlabeled (competition) and radiolabeled (saturation) ligands, and each binding experiment was repeated a minimum of three times. Competitive displacement of 1–2 nM [<sup>3</sup>H]-mesulergine (5-HT<sub>2C</sub>) or [<sup>3</sup>H]-ketanserin (5-HT<sub>2A</sub>) was used to obtain approximate  $K_i$  values for unlabeled ligands. Mianserin (30 μM) was used to define non-specific binding. After a 90 min equilibration period at room temperature, incubation mixtures were rapidly passed through GF/B filters using a Mach 2 cell harvester (Tomtec, CT, USA) and subsequently washed with 50 mM Trise -HCl. Filter disks were placed in vials containing scintillation cocktail (ScintiVerse, Fisher Scientific, USA), allowed to equilibrate overnight,

and then were counted for  $^3\text{H}$ -induced scintillation using a Beckman-Coulter LS6500 counter (Indianapolis, IN).

## 2.6. Phosphoinositide hydrolysis assay

Receptor-mediated phosphoinositide hydrolysis by phospholipase C (PLC) was measured as previously described (Canal et al., 2011), with a few changes. Briefly, HEK-293 cells, were grown in DMEM media containing 5% dialyzed fetal bovine serum (growth media) to 80% confluency in 10 cm plates in an incubator at 5%  $\text{CO}_2$ , 37 °C. Cells were then transfected with 10  $\mu\text{g}$  mouse 5-HT2A or mouse 5-HT2C-VNV pcDNA and 33  $\mu\text{L}$  Lipofectamine 2000 reagent (Invitrogen, CA, USA) in 5 mL DMEM containing 5% dialyzed fetal bovine serum and 5 mL Opti-MEM (transfection media). After an overnight incubation, transfection media was removed and replaced with 16 mL inositol-free growth media containing 1.0  $\mu\text{Ci/mL}$  [ $^3\text{H}$ ]-myo-inositol. Cells were apportioned into 48 well CellBind<sup>®</sup> plates (Corning, MA, USA), and [ $^3\text{H}$ ]-myo-inositol incorporation continued for 24 h in an incubator. Cells were then acclimated to serum-free media, treated for 30 min with experimental drugs diluted in media containing a final concentration of 50 mM LiCl per well. Media was discarded, and 50 mM formic acid was added to lyse cells. Plates were stored at  $-80$  °C for at least 24 h. After thawing, solution from each well was added to individual anion-exchange columns. Bound [ $^3\text{H}$ ]-inositol phosphates were eluted with 800 mM ammonium formate, and eluate was added to scintillation fluid.  $^3\text{H}$ -induced scintillations were counted with a Beckman-Coulter LS6500 counter. Experiments were performed with duplicates for 5-HT and triplicates for all other drugs, and each independent experiment was performed a minimum of three times.

## 2.7. Statistical analyses

Competition and saturation binding data were analyzed using nonlinear regression, curve-fitting algorithms in GraphPad Prism, 5.03 for Windows (CA, USA). Data points were limited (seven to nine points per experiment), thus Hill slopes were not calculated (Motulsky and Christopoulos, 2003); data were fit using the “one site fit-Ki” model that constrains the Hill slope to 1.0. Two-site curve-fitting did not result in an improved fit (data not shown). Approximate  $K_i$  values were determined by conversion of the IC50 data using the equation  $K_i = \text{IC}_{50}/(1 + L/K_D)$  where  $L$  is the concentration of radioligand (Cheng and Prusoff, 1973). Unpaired  $t$ -tests were used to compare  $K_i$  values of individual compounds for mouse vs. human 5-HT2A or 5-HT2C receptors. No statistical analyses were performed for results of functional assays characterizing compounds at mouse versus human 5-HT2 receptors, as *a priori*, they were conducted primarily for qualitative purposes. For experiment 6, individual  $t$ -tests were used to compare maximal functional responses between groups.

Separate ANOVAs were performed to analyze effects of experimental compounds on the DOI-elicited-HTR and on locomotion. Dunnett’s post-test compared the number of HTRs elicited by DOI after pretreatment with each experimental compound to the number of HTRs elicited by vehicle plus DOI. Dunnett’s post-test was used for analyzing the effects of each compound on locomotion relative to DOI or vehicle alone. Two-way ANOVA and Student-Newman-Keuls comparisons were used to identify dose-dependent effects and differences in efficacy across test compounds.

## 3. Results

### 3.1. Experiment 1: effect of 5-HT2C agonists on the DOI-elicited-HTR

The effects of selective 5-HT2C agonists on the DOI-elicited-HTR are shown in Fig. 1a and b. In a 10 min period, vehicle plus 1 mg/kg DOI treated mice ( $N= 38$ ) exhibited  $40 \pm 1.0$  (mean  $\pm$  S.E.M.) HTRs. There was a significant main effect of pretreatment with the

selective 5-HT<sub>2C</sub> agonists ( $F_{10,76} = 31.39$ ,  $p < 0.001$ ) and the novel PAT compounds (5-HT<sub>2C</sub> agonists/5-HT<sub>2A</sub> inverse agonist/antagonists) ( $F_{6,57} = 86.03$ ;  $p < 0.001$ ) on the number of HTRs elicited by DOI. Both doses of each 5-HT<sub>2C</sub>-preferring agonist significantly attenuated the DOI-elicited-HTR ( $p$ -values  $< 0.05$ ). Two-way ANOVA ( $F_{7,73} = 2.85$ ;  $p = 0.011$  for the interaction) revealed that there was a significant dose-dependent effect of (+)-PAT and mCPP. WAY 161503, mCPP, Ro 60-0175, and CP 809101, (+)-PAT, and methyl-PAT were equally effective, attenuating the DOI-elicited-HTR by approximately 40%. (–)-PAT and 1-methylpsilocin were significantly more efficacious compared to all other 5-HT<sub>2C</sub> agonists at both doses tested ( $p$ -values  $< 0.05$ ), decreasing the response by 84 and 82%, respectively, at the 5.6 mg/kg dose.

### 3.2. Experiment 2: effect of 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> antagonists on the DOI-elicited-HTR

The effects of pretreatment and post-treatment with selective 5-HT<sub>2</sub> antagonists on the DOI-elicited-HTR data are shown in Fig. 1c. Two-way ANOVA examining the effects of the selective 5-HT<sub>2C/2B</sub> inverse agonist/antagonist, SB-206553, and the selective 5-HT<sub>2C</sub> antagonist, SB-242084 revealed a significant interaction ( $F_{2,34} = 18.1$ ;  $p < 0.001$ ). SB-206553 dose-dependently attenuated the DOI-elicited-HTR: 0.03, 0.3, and 3.0 mg/kg significantly decreased the number of HTRs, by 20, 37, and 79%, respectively. SB-242084 significantly decreased the DOI-elicited-HTR at all doses tested. The 0.03 mg/kg dose, however, produced a larger, and the 3.0 mg/kg dose produced a smaller effect relative to SB-206553 ( $p$ -values  $< 0.05$ ). Unlike SB-206553, there was no dose-related difference in the efficacy of SB-242084 to attenuate the HTR; each dose decreased the number of HTRs by approximately 50%. The selective 5-HT<sub>2A</sub> antagonist, M100907, dose-dependently attenuated the DOI-elicited-HTR ( $F_{3,18} = 41.7$ ;  $p < 0.001$ ): 0.0025 mg/kg suppressed the response by 37%, 0.025 mg/kg by 92%, and 0.25 mg/kg by 100%, completely eliminating the HTR. Mice treated only with vehicle exhibit approximately 2–5 HTR's in 10 min (unpublished observations), and thus the 0.25 mg/kg dose of M100907 blocked even the basal HTR in these mice. Finally, SB-206553 (3 mg/kg) and M100907 (0.25 mg/kg) administered 10 min after DOI retained their ability to suppress the HTR ( $p$  values  $< 0.05$ ).

### 3.3. Experiment 3: effect of 5-HT<sub>2C</sub> agonists on locomotion

As shown in Fig. 2a, administration of DOI alone had no statistically significant effect on locomotion relative to vehicle administration (unpaired  $t$ -test,  $p = 0.083$ ). Subsequent analyses compared the effects of 5-HT<sub>2C</sub> agonists (as a 10-min pretreatment to DOI) to either vehicle/vehicle or DOI/vehicle. Both doses of each 5-HT<sub>2C</sub> agonist in combination with DOI resulted in lower levels of activity relative to DOI/vehicle administration ( $F_{10,85} = 21.1$ ;  $p < 0.001$ ). The combinations of DOI and the 5-HT<sub>2C</sub> agonists CP 809101, Ro 60-0175, and WAY 161503 resulted in lower levels of activity relative to vehicle administration ( $F_{10,50} = 8.6$ ;  $p < 0.001$ ). mCPP and 1-methylpsilocin showed dose-dependent effects in that only the 5.6 mg/kg dose altered locomotion levels. In contrast to the 5-HT<sub>2C</sub> agonists described above, the novel 4-phenyl-2-aminotetralin compounds (+)-PAT, (–)-PAT, and methyl-PAT at both the 3.0 and 5.6 mg/kg doses failed to alter locomotion levels relative to either vehicle ( $F_{6,31} = 0.63$ ,  $p = 0.71$ ) or DOI only ( $F_{6,66} = 1.22$ ;  $p = 0.30$ ) administration (Fig. 2b). A two-way ANOVA across all agonists demonstrated that Ro 60-0175 was most effective in suppressing locomotion ( $F_{7,71} = 19.8$ ;  $p < 0.001$ ).

### 3.4. Experiment 4: effect of 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> antagonists on locomotion

Fig. 2c shows the effects of DOI in combination with selective 5-HT<sub>2</sub> antagonists. The highest dose combination of DOI and M100907 (0.25 mg/kg) resulted in decreased activity relative to vehicle administration, whereas the 5-HT<sub>2C</sub> antagonists SB-206553 (0.3 mg/kg) and SB-242084 (3 mg/kg) resulted in increased levels of activity ( $F_{11,59} = 13.4$ ;  $p < 0.001$ ).

Compared to DOI administration ( $F_{11,94} = 14.7$ ;  $p < 0.001$ ), all doses of M100907 decreased locomotion. When DOI was administered 10 min prior to M100907 (0.25 mg/kg, 2nd), the resulting locomotion remained suppressed. Compared to DOI alone, SB-206553 (0.3 mg/kg) and SB-242084 (3 mg/kg) increased activity. A higher dose of SB-206553 (3 mg/kg) in combination with DOI resulted in decreased activity relative to DOI alone. Two-way ANOVA demonstrated that 3.0 mg/kg of SB-206553 resulted in lower activity compared to 0.03 and 0.3 mg/kg SB-206553, and 3.0 mg/kg of SB-242084 ( $F_{2,36} = 15.0$ ;  $p < 0.001$ ).

### 3.5. Experiment 5: ligand affinity and function at mouse and human 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> receptors

To provide a molecular pharmacological context for interpretation of the *in vivo* behavioral results obtained in mice (above), affinity and function of ligands at mouse and human 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> receptors were determined (Table 1 and Table 2). There are no published affinity values of the selective 5-HT<sub>2</sub> antagonists SB-206553, SB-242084, or M100907 at mouse 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> receptors. Thus, it was important to assess whether ligand receptor selectivity remains at the mouse receptors. Similarly, affinity values were assessed for the PAT compounds at mouse receptors, as these values have also not been reported. First, the  $K_d$  values of [<sup>3</sup>H]-ketanserin and [<sup>3</sup>H]-mesulergine at C57Bl/6 mouse 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> receptors were determined using membranes prepared from transiently transfected HEK cells; results were similar to values obtained using the native receptors (Canal et al., 2010; Dougherty and Aloyo, 2011). Mean ( $\pm$ S.E.M.)  $K_d$  values of [<sup>3</sup>H]-ketanserin and [<sup>3</sup>H]-mesulergine were 0.92 (0.18) nM and 2.2 (0.15) nM, respectively. Regarding comparison of affinity of 5-HT<sub>2</sub> ligands at human vs. mouse 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> receptors, no general trend emerged (Table 1). For example, the 5-HT<sub>2</sub> agonists 5-HT and DOI, as well as, the novel 5-HT<sub>2C</sub> agonists (-)-PAT and (+)-PAT, showed no differences in affinity at mouse vs. human 5-HT<sub>2A</sub> receptors ( $p > 0.05$ ), but showed greater affinity at human compared to mouse 5-HT<sub>2C</sub> receptors ( $p < 0.05$ ). In contrast to the other PAT-type 5-HT<sub>2C</sub> agonists, methyl-PAT showed greater affinity at both the human 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> receptors compared to the mouse homologs. The 5-HT<sub>2C</sub> antagonist SB-242084 demonstrated higher affinity at mouse vs. human 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> receptors, but SB-206553 had greater affinity at only mouse relative to human 5-HT<sub>2A</sub> receptors ( $p < 0.05$ ), with no species difference at 5-HT<sub>2C</sub> receptors. The 5-HT<sub>2A</sub> antagonist M100907 showed greater affinity at human compared to mouse 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> receptors ( $p < 0.05$ ). (Notably ligand  $K_i$  values did not significantly differ across different membrane receptor preparations as is illustrated in the low variance associated with the means.)

Table 2 shows the functional activity (EC<sub>50</sub> and percent of maximum 5-HT response (efficacy)) of the 5-HT<sub>2</sub> agonists 5-HT and DOI compared to the novel PATs, at mouse vs. human 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> receptors. Functional data for other 5-HT<sub>2</sub> ligands at both human and rodent receptors has been reported in the literature, so these experiments were not performed. In contrast to affinity differences noted above at mouse vs. human 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> receptors, the potency (EC<sub>50</sub>) and efficacy for most of the 5-HT<sub>2</sub> agonists tested was similar at mouse and human 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> receptors. One notable difference was (+)-PAT. This compound showed agonist activity at both mouse and human 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> receptors (unlike (-)-PAT which activated only 5-HT<sub>2C</sub> receptors), yet showed over 10 $\times$  greater potency for activating human compared to mouse 5-HT<sub>2C</sub> receptors. (Notably the EC<sub>50</sub> values for ligands did not significantly differ across individual experiments, as are illustrated in the low variance associated with the means.)

### 3.6. Experiment 6: functional activity of 5-HT<sub>2C</sub> agonists alone and in combination with DOI

In line with the functional selectivity hypothesis, experiments were performed to determine whether selective 5-HT<sub>2C</sub> agonists alter 5-HT<sub>2C</sub> receptor signaling stimulated by DOI (Fig. 3). HEK cells expressing the mouse 5-HT<sub>2C</sub> receptor were treated with 500 nM (–)-PAT, mCPP, or 1-methylpsilocin simultaneously with increasing concentrations of DOI (0.1 nM – 10 μM), and PLC signaling was assessed. The 500 nM concentration of (–)-PAT, mCPP, and 1-methylpsilocin was chosen based on competitive antagonist studies showing that doses approximately 10× the  $K_i$ 's of antagonists reliably alter agonist signaling yet in a surmountable manner. Individual *t*-tests revealed that each of the three 5-HT<sub>2C</sub> agonists, which were most potent for attenuating the DOI-elicited-HTR, significantly reduced the maximum efficacy of DOI to stimulate mouse 5-HT<sub>2C</sub> receptor-mediated PLC signaling ( $p < 0.05$ ). Notably, each selective 5-HT<sub>2C</sub> agonist stimulated 5-HT<sub>2C</sub> receptor PLC signaling by itself, yet no agonist led to additive or synergistic effects with DOI at the 500 nM dose tested.

## 4. Discussion

Using the common inbred strain of mice, C57Bl/6J, the present experiments show that both 5-HT<sub>2C</sub>-preferring agonists and antagonists, administered at doses commonly used *in vivo* to explore 5-HT<sub>2C</sub> receptor function, significantly attenuate the DOI-elicited-HTR. These data suggest that seemingly contradictory literature reports of both 5-HT<sub>2C</sub> agonists *and* antagonists suppressing the DOI-elicited-HTR are not contradictory, and demand a new explanation.

Since the recognition of the phenomenon of G-protein-coupled receptor (GPCR) functional selectivity, it has become clear that describing a ligand as an agonist or antagonist does not have comprehensive explanatory value unless a specific context, e.g. receptor density, cell type, cellular location of the GPCR, *in vitro* versus *in vivo*, or signaling pathway, is described. For example, lisuride is a 5-HT<sub>2C</sub> partial agonist *in vitro* using recombinant CHO cells (Cussac et al., 2008), but an antagonist, lacking no agonist effects on its own, *in vivo* or *ex vivo* using choroid plexus cells that express high densities of 5-HT<sub>2C</sub> receptors (Burriss et al., 1991). Similar observations have been reported recently for ligands acting on the dopamine D<sub>2</sub> receptor (Koener et al., 2012) and the β<sub>2</sub>-adrenergic receptor (Kaya et al., 2012). It is conceivable that these findings are related mechanistically to ligand functional selectivity across receptor contexts: a particular ligand can more favorably stabilize one of many possible GPCR conformations that determine intracellular signaling outcomes (Mary et al., 2012), and the specific conformation(s) that is stabilized may also depend on the receptor's context, e.g. the presence of unique sets of scaffolding proteins (Abbas et al., 2009; Becamel et al., 2004). Thus, every ligand interacting with a GPCR may produce its own unique signaling signature in unique cellular contexts (Flordellis, 2012; Fowler et al., 2012).

The fact that both selective agonists and antagonists of the traditional 5-HT<sub>2C</sub> receptor signaling pathway (5-HT<sub>2C</sub>-Gq-PLC) attenuated the DOI-elicited-HTR in the same strain of mouse, from the same vendor, under identical experimental conditions suggests a few explanations: 1) the 5-HT<sub>2C</sub> agonist ligands tested are functionally selective, orthosterically stabilizing a 5-HT<sub>2C</sub> receptor conformation(s) that is unique from the conformation(s) that DOI stabilizes, and thus prevent (similar to 5-HT<sub>2C</sub> selective antagonists) DOI from binding and activating 5-HT<sub>2C</sub> receptors to positively affect the HTR; 5-HT<sub>2C</sub> receptor knockout mice show a reduction in, but not an elimination of the HTR elicited by DOI (Canal et al., 2010), suggesting they contribute positively to the DOI-elicited-HTR. Similarly, 5-HT<sub>2C</sub> selective agonists, at the doses tested here, suppress but do not eliminate the DOI-elicited-



HTR. Indeed, this functional antagonist explanation can be extended to 5-HT<sub>2A</sub> receptors, as all reported 5-HT<sub>2C</sub> selective agonists, besides (–)-PAT, activate 5-HT<sub>2A</sub> receptors at higher concentrations (discussed below); 2) 5-HT<sub>2C</sub> selective agonists stabilize a 5-HT<sub>2C</sub> receptor conformation(s) that leads to signaling outcomes that antagonize 5-HT<sub>2A</sub> signaling activated by DOI that produces the HTR, i.e., mutually opposing signaling antagonism (cross-talk); or 3) selective 5-HT<sub>2C</sub> ligands are in fact not selective *in vivo*, and other receptors that bind these ligands negatively modulate the DOI-elicited-HTR.

Each of these outcomes, either on their own, or in combination could explain the behavioral data, and the data reported have limitations that preclude specifically defining which outcome accounts for the most variance. Nevertheless, although the third explanation cannot be ruled out without further tests (e.g. whether the behavioral effects of 5-HT<sub>2C</sub> ligands observed are eliminated in 5-HT<sub>2C</sub> receptor knock-out mice), the data support the former explanations, which are in accordance with functional selectivity. First, the 5-HT<sub>2C</sub> agonists most potent for attenuating the DOI HTR *in vivo* also suppressed the efficacy of DOI to stimulate mouse 5-HT<sub>2C</sub> receptor-mediated Gq/PLC signaling *in vitro*; Gq signaling is known to be involved in the production of the DOI-elicited-HTR (Garcia et al., 2007). Notably, there were no additive or synergistic effects of selective 5-HT<sub>2C</sub> agonists with DOI *in vitro*, but suppressive effects consistent with the *in vivo* results. It should be mentioned, however, that exclusive signaling through Gq does not mediate the DOI-elicited-HTR; Gq knock-out mice show only about a 40% reduction in the number of DOI-elicited-HTRs (Garcia et al., 2007). Also, in the present studies, irrespective of the direction of intrinsic efficacy in terms of 5-HT<sub>2C</sub> Gq-PLC signaling, i.e. inverse agonist/antagonist/agonist, all 5-HT<sub>2C</sub>-targeting compounds tested suppressed the HTR elicited by DOI, strongly suggesting that another signaling pathway(s) influences the suppressive effects of 5-HT<sub>2C</sub> selective ligands on the DOI-elicited-HTR. Notably,  $\beta$ -arrestin-2 knock-out mice did not show changes in the DOI-elicited-HTR, suggesting alternative signaling via  $\beta$ -arrestin-2 can be ruled out (Schmid et al., 2008). Although the present experiments do not reveal novel signaling pathways that regulate the DOI-elicited-HTR, they encourage further testing in this area.

Surprisingly, the 5-HT<sub>2C</sub> agonists WAY 161503, Ro 60-0175, mCPP, and CP 809101 showed no difference in potency or efficacy for attenuating the DOI HTR, suggesting that potential pharmacokinetic differences between these compounds (see CLogP values in Table 1) did not affect their behavioral potencies. More attention to pharmacokinetics issues would be in order had one or more compounds within a class had opposite or no effects on the DOI HTR. Also, it is acknowledged that the negative modulation of the DOI elicited HTR by each compound could be due to a metabolite(s) and/or the parent compound, yet the similarities in behavioral effects within classes of compounds imply that the effects are related to 5-HT<sub>2</sub> receptor modulation, and not off-targets. It seems unlikely, with what is presently known about the pharmacology of the structurally diverse compounds utilized, that they and/or their metabolites interact with a common target(s) other than 5-HT<sub>2C</sub> receptors to produce their comparable effects on the HTR (and locomotion, noted below), albeit, 5-HT<sub>2A</sub> and/or 5-HT<sub>2B</sub> receptor activation elicited by selective 5-HT<sub>2C</sub> agonists could play a role in the negative modulation of the HTR observed, as described below. These considerations, nevertheless, illustrate that there are a host of factors intervening between *in vitro* cellular pharmacological assays and behavioral pharmacology phenotypes.

Interestingly, 1-methylpsilocin was more potent for attenuating the DOI-elicited-HTR than all other 5-HT<sub>2C</sub>-preferring agonists that also activate 5-HT<sub>2A</sub> receptors (Sard et al., 2005). Like psilocin, 1-methylpsilocin may also activate 5-HT<sub>1</sub> receptors that are known to negatively modulate the HTR (Darmani et al., 1990), perhaps accounting for the superior potency of 1-methylpsilocin here. Finally, the 4-phenyl-2-aminotetralin compound, (–)-

PAT, that is a 5-HT<sub>2C</sub> agonist and 5-HT<sub>2A/2B</sub> antagonist *in vitro* and lacks appreciable affinity for other GPCRs known to modulate the DOI HTR (Booth et al., 2009), was as potent and efficacious as 1-methylpsilocin in suppressing the DOI HTR, likely due to its unique 5-HT<sub>2</sub> pharmacology (5-HT<sub>2A/2B</sub> antagonism together with 5-HT<sub>2C</sub> agonism).

Regarding the 5-HT<sub>2</sub> antagonists studied, the results are comparable to previous findings from the C57Bl/6J strain of mice (Canal et al., 2010), with the exception that the 5-HT<sub>2C/2B</sub> inverse agonist SB-206553 was more efficacious in suppressing the DOI-elicited-HTR than the 5-HT<sub>2C</sub> antagonist, SB-242084. Also, SB-206553 led to a bi-phasic effect on locomotion after DOI treatment, increasing activity at lower doses, and decreasing activity at higher doses. These data suggest that 5-HT<sub>2B</sub> receptors may be involved in the behaviors tested here, and/or that, at doses above 0.3 mg/kg, SB-206553 may be interacting with off-targets, including alpha-7 nicotinic receptors (Dunlop et al., 2009), that affect behavior.

The present data show clearly that 5-HT<sub>2C</sub> agonists that also activate 5-HT<sub>2A</sub> and/or 5-HT<sub>2B</sub> receptors (e.g. Ro 60-0175, mCPP, CP 809101, WAY 161503, 1-methylpsilocin) are effective at attenuating a 5-HT<sub>2A</sub> receptor-dependent behavior, the DOI-elicited-HTR. Ro 60-0175 is considered a selective 5-HT<sub>2C</sub> agonist and is commonly chosen for studying *in vivo* functions of 5-HT<sub>2C</sub> receptors. This compound, however, also stimulates human 5-HT<sub>2A</sub> and 5-HT<sub>2B</sub> receptor-mediated Ca<sup>++</sup> signaling *in vitro*, and among the 5-HT<sub>2</sub> receptor subtypes is most potent at activating 5-HT<sub>2B</sub> receptors (Porter et al., 1999). Also, in a pilot experiment measuring human 5-HT<sub>2A</sub> receptor-mediated PLC signaling *in vitro*, we observed Ro 60-0175 to be a potent agonist with an EC<sub>50</sub> value and efficacy nearly equivalent to 5-HT (data not shown). Finally, *in vivo*, Ro 60-0175 is known to possess activity at receptors other than 5-HT<sub>2C</sub> (Damjanoska et al., 2003). Thus, suppression of the DOI-elicited-HTR by selective 5-HT<sub>2C</sub> agonists may not be mediated solely by activation of 5-HT<sub>2C</sub> receptors, but may also involve activation of 5-HT<sub>2A</sub> and/or 5-HT<sub>2B</sub> receptors. Indeed, the non-hallucinogenic and non-HTR-eliciting compound lisuride has 5-HT<sub>2A</sub> agonist properties, but attenuates the HTR in mice elicited by the structurally and pharmacologically similar lysergic acid diethylamide (LSD) (Gonzalez-Maeso et al., 2007). Similarly, hallucinogenic 5-HT<sub>2</sub> agonist compounds attenuate the HTR in mice elicited by LSD (Corne and Pickering, 1967). Moreover, although to a lesser extent than DOI, 1-methylpsilocin produces an HTR on its own (Halberstadt et al., 2011), yet, as shown here, 1-methylpsilocin also attenuates the DOI-elicited-HTR. These observations may be accounted for by the functional selectivity hypothesis, i.e., different 5-HT<sub>2</sub> ligands stabilize particular 5-HT<sub>2A</sub>, 5-HT<sub>2B</sub>, and/or 5-HT<sub>2C</sub> receptor conformations leading to different signaling outcomes that have unique influences on behavior. Molecular modeling studies docking DOI and other 5-HT<sub>2</sub> ligands in the 5-HT<sub>2A</sub>, 5-HT<sub>2B</sub>, and/or 5-HT<sub>2C</sub> receptor binding pockets will help resolve whether there are specific amino acid interactions that lead to DOI's HTR-eliciting effect. Alternative explanations for the results obtained include the possibility that different 5-HT<sub>2C</sub> ligands interact uniquely with known homodimerized 5-HT<sub>2C</sub> receptors (Herrick-Davis et al., 2012, 2004) which could impact signaling associated with the HTR. Also, the 5-HT<sub>2C</sub> receptor may heterodimerize with 5-HT<sub>2A</sub> and/or other receptors that might impact the pharmacology of 5-HT<sub>2C</sub> ligands (Gonzalez-Maeso et al., 2008). Finally, DOI may act directly at receptor systems separate from 5-HT (Ray, 2010) that could influence the HTR, complicating the picture.

Numerous studies have demonstrated that compounds interacting with 5-HT<sub>2</sub> receptor subtypes can influence locomotion in mice. Results from the present study are generally consistent with the literature (Fletcher et al., 2009; Gleason et al., 2001; Halberstadt et al., 2009; Heisler and Tecott, 2000). Each of the commercially-available 5-HT<sub>2C</sub> agonists given prior to DOI produced decreases in activity, whereas the antagonists produced dose-dependent increases in activity. Notably, 5-HT<sub>2C</sub> agonist and antagonist effects on

locomotion are abolished in 5-HT<sub>2C</sub> receptor knock-out mice (Fletcher et al., 2009). The 5-HT<sub>2A</sub> antagonist given prior to DOI also suppressed locomotion, as would be expected (Halberstadt et al., 2009). In contrast, the novel 4-phenyl-2-aminotetralin compounds (+)-PAT, (-)-PAT, and methyl-PAT did not alter activity relative to vehicle or DOI only administration. Based on the pharmacological profile of these compounds (5-HT<sub>2C</sub> agonism and 5-HT<sub>2A</sub> antagonism/inverse agonism), this failure to alter locomotion is somewhat surprising, however from a medication development perspective this may represent a promising and desirable side effect profile (e.g. no sedation or hyperlocomotion). The dissociation of behavioral effects (all classes of compounds decreased the DOI-elicited-HTR, while different classes of compounds showed differential effects on locomotion) demonstrates that the suppression of the DOI-elicited-HTR was not a direct consequence of an alteration in locomotor activity.

Few studies have compared directly the potencies of 5-HT<sub>2</sub> ligands for mouse versus human 5-HT<sub>2</sub> receptors (Dougherty and Aloyo, 2011). The current study revealed subtle differences in binding affinities of 4-phenyl-2-aminotetralin ligands and commercially available 5-HT<sub>2</sub> antagonists for mouse versus human 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> receptors, yet the selectivity of ligands for 5-HT<sub>2A</sub> and/or 5-HT<sub>2C</sub> receptors did not change across the receptor species, suggesting that the behavioral pharmacology data cannot be explained by a loss of ligand receptor selectivity in mice. Though ligands show unique potencies for 5-HT<sub>2C</sub> receptors depending on the specific edited 5-HT<sub>2C</sub> isoform analyzed, 5-HT was shown to have equivalent potency for activating the human 5-HT<sub>2C</sub>-VNV and 5-HT<sub>2C</sub>-INI isoforms (Burns et al., 1997), suggesting the difference between mouse (5-HT<sub>2C</sub>-VNV) and human (5-HT<sub>2C</sub>-INI) receptors studied herein did not contribute greatly to the variability in binding potency. The potencies of 4-phenyl-2-aminotetralin ligands for activating mouse 5-HT<sub>2C</sub> receptor-mediated PLC signaling matched closely with their binding affinities, and each ligand was a partial 5-HT<sub>2C</sub> agonist. Interestingly, although (-)-PAT and methyl-PAT were inactive at the mouse 5-HT<sub>2A</sub> receptor, (+)-PAT displayed consistent, yet weak, partial agonist activity at this receptor. Given (+)-PAT's higher potency for activating mouse 5-HT<sub>2A</sub> receptors, relative to mouse 5-HT<sub>2C</sub> receptors, it is notable that (+)-PAT retained efficacy at attenuating the DOI HTR, bolstering previous findings that agonists of 5-HT<sub>2A</sub> receptors unlike DOI may effectively attenuate DOI's HTR-eliciting effects.

Results from this study suggest the possibility that 5-HT<sub>2C</sub> ligands, whether agonists or antagonists of the 5-HT<sub>2</sub>-Gq receptor signaling pathway, block the DOI-elicited-HTR by functionally attenuating or altering 5-HT<sub>2C</sub> receptor signaling activated by DOI and/or by re-directing 5-HT<sub>2C</sub> receptor signaling that subsequently alters 5-HT<sub>2A</sub> receptor signaling initiated by DOI (cross-talk). Selective 5-HT<sub>2C</sub> agonists, therefore, may behave as biased agonists for the receptor, stimulating 5-HT<sub>2C</sub> receptor signaling that is unique from DOI. The DOI-elicited-HTR may thus be an *in vivo* behavioral model to study ligand-specific 5-HT<sub>2C</sub> (and/or 5-HT<sub>2A</sub> or 5-HT<sub>2B</sub>) receptor conformations, and could assist in drug development of 5-HT<sub>2</sub> receptor ligands, like the 4-phenyl-2-aminotetralins, that may have antipsychotic properties, and/or provide other therapeutic outcomes, such as weight reduction, without locomotor or sedative side effects.

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## References

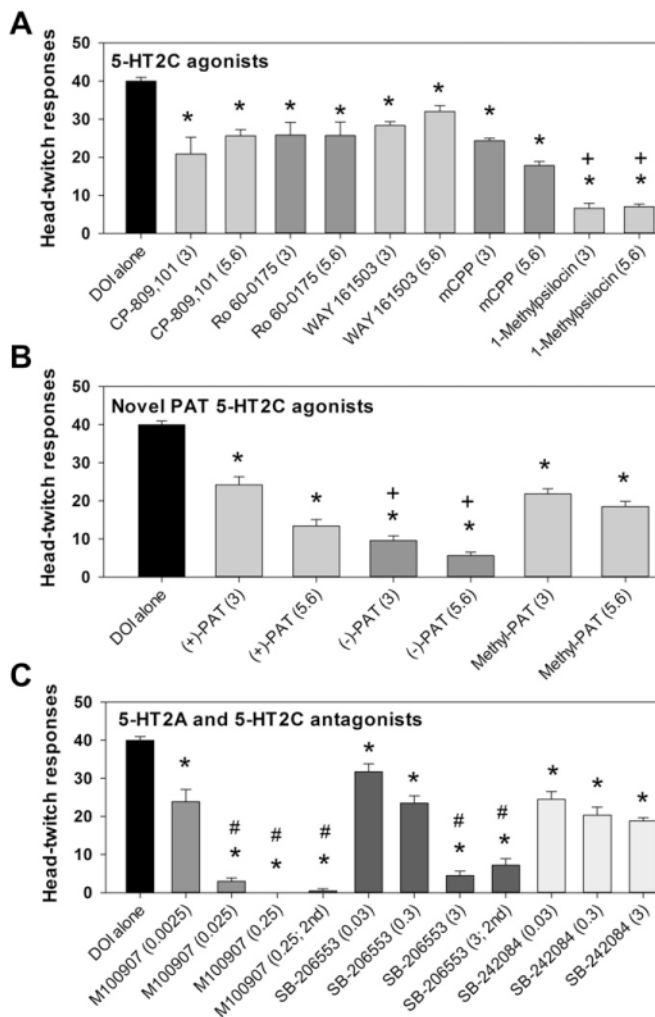
- Abbas A, Roth BL. Pimavanserin tartrate: a 5-HT<sub>2A</sub> inverse agonist with potential for treating various neuropsychiatric disorders. *Expert Opin Pharmacother*. 2008; 9:3251–3259. [PubMed: 19040345]
- Abbas AI, Yadav PN, Yao WD, Arbuckle MI, Grant SG, Caron MG, Roth BL. PSD-95 is essential for hallucinogen and atypical antipsychotic drug actions at serotonin receptors. *J Neurosci*. 2009; 29:7124–7136. [PubMed: 19494135]
- Arena Pharmaceuticals Inc. Arena Pharmaceuticals and Eisai Announce FDA Approval of BELVIQ® (Lorcaserin HCl) for Chronic Weight Management in Adults Who are Overweight with a Comorbidity or Obese. 2012. <http://invest.arenapharm.com/releasedetail.cfm?ReleaseID=687182>
- Becamel C, Gavarini S, Chanrion B, Alonso G, Galeotti N, Dumuis A, Bockaert J, Marin P. The serotonin 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> receptors interact with specific sets of PDZ proteins. *J Biol Chem*. 2004; 279:20257–20266. [PubMed: 14988405]
- Bentley JM, Adams DR, Bebbington D, Benwell KR, Bickerdike MJ, Davidson JE, Dawson CE, Dourish CT, Duncton MA, Gaur S, George AR, Giles PR, Hamlyn RJ, Kennett GA, Knight AR, Malcolm CS, Mansell HL, Misra A, Monck NJ, Pratt RM, Quirk K, Roffey JR, Vickers SP, Cliffe IA. Indoline derivatives as 5-HT(2C) receptor agonists. *Bioorg Med Chem Lett*. 2004; 14:2367–2370. [PubMed: 15081042]
- Booth RG, Fang L, Huang Y, Wilczynski A, Sivendran S. (1R, 3S)-(-)-trans-PAT: a novel full-efficacy serotonin 5-HT<sub>2C</sub> receptor agonist with 5-HT<sub>2A</sub> and 5-HT<sub>2B</sub> receptor inverse agonist/antagonist activity. *Eur J Pharmacol*. 2009; 615:1–9. [PubMed: 19397907]
- Bucholtz EC, Brown RL, Tropsha A, Booth RG, Wyrick SD. Synthesis, evaluation, and comparative molecular field analysis of 1-phenyl-3-amino-1,2,3,4-tetrahydronaphthalenes as ligands for histamine H(1) receptors. *J Med Chem*. 1999; 42:3041–3054. [PubMed: 10447948]
- Burns CM, Chu H, Rueter SM, Hutchinson LK, Canton H, Sanders-Bush E, Emeson RB. Regulation of serotonin-2C receptor G-protein coupling by RNA editing. *Nature*. 1997; 387:303–308. [PubMed: 9153397]
- Burris KD, Breeding M, Sanders-Bush E. (+)Lysergic acid diethylamide, but not its nonhallucinogenic congeners, is a potent serotonin 5HT<sub>1C</sub> receptor agonist. *J Pharmacol Exp Ther*. 1991; 258:891–896. [PubMed: 1679849]
- Canal CE, Cordova-Sintjago TC, Villa NY, Fang LJ, Booth RG. Drug discovery targeting human 5-HT(2C) receptors: residues S3.36 and Y7.43 impact ligand-binding pocket structure via hydrogen bond formation. *Eur J Pharmacol*. 2011; 673:1–12. [PubMed: 22020288]
- Canal CE, Morgan D. Head-twitch response in rodents induced by the hallucinogen 2,5-dimethoxy-4-iodoamphetamine: a comprehensive history, a re-evaluation of mechanisms, and its utility as a model. *Drug Test Anal*. 2012; 4:556–576. [PubMed: 22517680]
- Canal CE, Olaghère da Silva UB, Gresch PJ, Watt EE, Sanders-Bush E, Airey DC. The serotonin 2C receptor potently modulates the head-twitch response in mice induced by a phenethylamine hallucinogen. *Psychopharmacology (Berl)*. 2010; 209:163–174. [PubMed: 20165943]
- Cheng Y, Prusoff WH. Relationship between the inhibition constant (K<sub>1</sub>) and the concentration of inhibitor which causes 50 per cent inhibition (I<sub>50</sub>) of an enzymatic reaction. *Biochem Pharmacol*. 1973; 22:3099–3108. [PubMed: 4202581]
- Corne SJ, Pickering RW. A possible correlation between drug-induced hallucinations in man and a behavioural response in mice. *Psychopharmacologia*. 1967; 11:65–78. [PubMed: 5302272]
- Cunningham KA, Fox RG, Anastasio NC, Bubar MJ, Stutz SJ, Moeller FG, Gilbertson SR, Rosenzweig-Lipson S. Selective serotonin 5-HT(2C) receptor activation suppresses the reinforcing efficacy of cocaine and sucrose but differentially affects the incentive-saliency value of cocaine- vs. sucrose-associated cues. *Neuropharmacology*. 2011; 61:513–523. [PubMed: 21575646]
- Cussac D, Boutet-Robinet E, Ailhaud MC, Newman-Tancredi A, Martel JC, Danty N, Raully-Lestienne I. Agonist-directed trafficking of signalling at serotonin 5-HT<sub>2A</sub>, 5-HT<sub>2B</sub> and 5-HT<sub>2C</sub>-VSV receptors mediated Gq/11 activation and calcium mobilisation in CHO cells. *Eur J Pharmacol*. 2008; 594:32–38. [PubMed: 18703043]
- Cussac D, Newman-Tancredi A, Quentric Y, Carpentier N, Poissonnet G, Parmentier JG, Goldstein S, Millan MJ. Characterization of phospholipase C activity at h5-HT<sub>2C</sub> compared with h5-HT<sub>2B</sub>

receptors: influence of novel ligands upon membrane-bound levels of [3H]phosphatidylinositols. *Naunyn Schmiedebergs Arch Pharmacol.* 2002; 365:242–252. [PubMed: 11882920]

- Damjanoska KJ, Muma NA, Zhang Y, D'Souza DN, Garcia F, Carrasco GA, Kindel GH, Haskins KA, Shankaran M, Petersen BR, Van De Kar LD. Neuroendocrine evidence that (S)-2-(chloro-5-fluoro-indol-1-yl)-1-methylethylamine fumarate (Ro 60-0175) is not a selective 5-hydroxytryptamine(2C) receptor agonist. *J Pharmacol Exp Ther.* 2003; 304:1209–1216. [PubMed: 12604698]
- Darmani NA, Martin BR, Pandey U, Glennon RA. Do functional relationships exist between 5-HT1A and 5-HT2 receptors? *Pharmacol Biochem Behav.* 1990; 36:901–906. [PubMed: 2145593]
- Dougherty JP, Aloyo VJ. Pharmacological and behavioral characterization of the 5-HT2A receptor in C57BL/6N mice. *Psychopharmacology (Berl).* 2011; 215:581–593. [PubMed: 21340474]
- Dunlop J, Lock T, Jow B, Sitzia F, Grauer S, Jow F, Kramer A, Bowlby MR, Randall A, Kowal D, Gilbert A, Comery TA, Larocque J, Soloveva V, Brown J, Roncarati R. Old and new pharmacology: positive allosteric modulation of the alpha7 nicotinic acetylcholine receptor by the 5-hydroxytryptamine(2B/C) receptor antagonist SB-206553 (3,5-dihydro-5-methyl-N-3-pyridinylbenzo[1,2-b:4,5-b']di pyrrole-1(2H)-carboxamide). *J Pharmacol Exp Ther.* 2009; 328:766–776. [PubMed: 19050173]
- Ebdrup BH, Rasmussen H, Arnt J, Glenthøj B. Serotonin 2A receptor antagonists for treatment of schizophrenia. *Expert Opin Investig Drugs.* 2011; 20:1211–1223.
- Fantegrossi WE, Simoneau J, Cohen MS, Zimmerman SM, Henson CM, Rice KC, Woods JH. Interaction of 5-HT2A and 5-HT2C receptors in R(-)-2,5-dimethoxy-4-iodoamphetamine-elicited head twitch behavior in mice. *J Pharmacol Exp Ther.* 2010; 335:728–734. [PubMed: 20858706]
- Fletcher PJ, Tampakeras M, Sinyard J, Slassi A, Isaac M, Higgins GA. Characterizing the effects of 5-HT(2C) receptor ligands on motor activity and feeding behaviour in 5-HT(2C) receptor knockout mice. *Neuropharmacology.* 2009; 57:259–267. [PubMed: 19501602]
- Flordellis CS. The plasticity of the 7TMR signaling machinery and the search for pharmacological selectivity. *Curr Pharm Des.* 2012; 18:145–160. [PubMed: 22229579]
- Fowler JC, Bhattacharya S, Urban JD, Vaidehi N, Mailman RB. Receptor conformations involved in dopamine D2L receptor functional selectivity induced by selected transmembrane 5 serine mutations. *Mol Pharmacol.* 2012; 81:820–831. [PubMed: 22416052]
- Fox M, French H, LaPorte J, Blackler A, Murphy D. The serotonin 5-HT2A receptor agonist TCB-2: a behavioral and neurophysiological analysis. *Psychopharmacology.* 2010; 212:13–23. [PubMed: 19823806]
- Garcia EE, Smith RL, Sanders-Bush E. Role of G(q) protein in behavioral effects of the hallucinogenic drug 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane. *Neuropharmacology.* 2007; 52:1671–1677. [PubMed: 17493641]
- Gleason SD, Lucaites VL, Shannon HE, Nelson DL, Leander JD. m-CPP hypolocomotion is selectively antagonized by compounds with high affinity for 5-HT(2C) receptors but not 5-HT(2A) or 5-HT(2B) receptors. *Behav Pharmacol.* 2001; 12:613–620. [PubMed: 11856898]
- Gonzalez-Maeso J, Ang RL, Yuen T, Chan P, Weisstaub NV, Lopez-Gimenez JF, Zhou M, Okawa Y, Callado LF, Milligan G, Gingrich JA, Filizola M, Meana JJ, Sealfon SC. Identification of a serotonin/glutamate receptor complex implicated in psychosis. *Nature.* 2008; 452:93–97. [PubMed: 18297054]
- Gonzalez-Maeso J, Weisstaub NV, Zhou M, Chan P, Ivic L, Ang R, Lira A, Bradley-Moore M, Ge Y, Zhou Q, Sealfon SC, Gingrich JA. Hallucinogens recruit specific cortical 5-HT(2A) receptor-mediated signaling pathways to affect behavior. *Neuron.* 2007; 53:439–452. [PubMed: 17270739]
- Gonzalez-Maeso J, Yuen T, Ebersole BJ, Wurmbach E, Lira A, Zhou M, Weisstaub N, Hen R, Gingrich JA, Sealfon SC. Transcriptome fingerprints distinguish hallucinogenic and nonhallucinogenic 5-hydroxytryptamine 2A receptor agonist effects in mouse somatosensory cortex. *J Neurosci.* 2003; 23:8836–8843. [PubMed: 14523084]
- Halberstadt AL, Koedood L, Powell SB, Geyer MA. Differential contributions of serotonin receptors to the behavioral effects of indoleamine hallucinogens in mice. *J Psychopharmacol.* 2011; 25:1548–1561. [PubMed: 21148021]

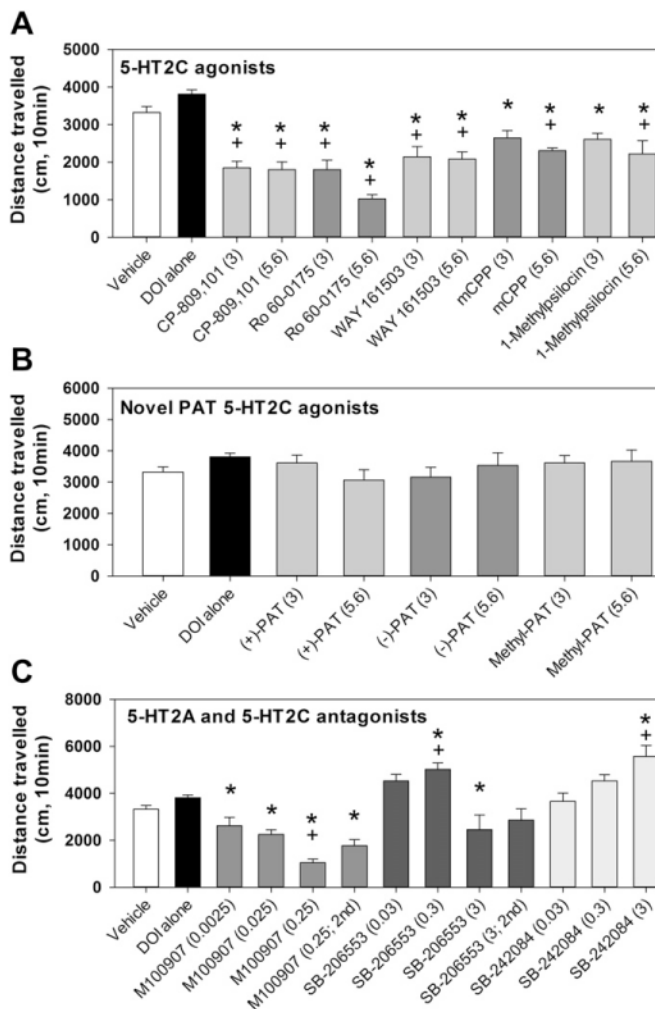
- Halberstadt AL, van der Heijden I, Ruderman MA, Risbrough VB, Gingrich JA, Geyer MA, Powell SB. 5-HT(2A) and 5-HT(2C) receptors exert opposing effects on locomotor activity in mice. *Neuropsychopharmacology*. 2009; 34:1958–1967. [PubMed: 19322172]
- Heisler LK, Tecott LH. A paradoxical locomotor response in serotonin 5-HT(2C) receptor mutant mice. *J Neurosci*. 2000; 20:RC71. [PubMed: 10751458]
- Herrick-Davis K, Grinde E, Lindsley T, Cowan A, Mazurkiewicz JE. Oligomer size of the serotonin 5-hydroxytryptamine 2C (5-HT2C) receptor revealed by fluorescence correlation spectroscopy with photon counting histogram analysis: evidence for homodimers without monomers or tetramers. *J Biol Chem*. 2012; 287:23604–23614. [PubMed: 22593582]
- Herrick-Davis K, Grinde E, Mazurkiewicz JE. Biochemical and biophysical characterization of serotonin 5-HT2C receptor homodimers on the plasma membrane of living cells. *Biochemistry*. 2004; 43:13963–13971. [PubMed: 15518545]
- Hutcheson JD, Setola V, Roth BL, Merryman WD. Serotonin receptors and heart valve disease it was meant 2B. *Pharmacol Ther*. 2011; 132:146–157. [PubMed: 21440001]
- Jensen NH, Cremers TI, Sotty F. Therapeutic potential of 5-HT2C receptor ligands. *ScientificWorldJournal*. 2010; 10:1870–1885. [PubMed: 20852829]
- Kaya AI, Onaran HO, Ozcan G, Ambrosio C, Costa T, Balli S, Ugur O. Cell contact-dependent functional selectivity of beta2-adrenergic receptor ligands in stimulating cAMP accumulation and extracellular signal-regulated kinase phosphorylation. *J Biol Chem*. 2012; 287:6362–6374. [PubMed: 22241475]
- Knight AR, Misra A, Quirk K, Benwell K, Revell D, Kennett G, Bickerdike M. Pharmacological characterisation of the agonist radioligand binding site of 5-HT(2A), 5-HT(2B) and 5-HT(2C) receptors. *Naunyn Schmiedeberg Arch Pharmacol*. 2004; 370:114–123. [PubMed: 15322733]
- Koener B, Focant MC, Bosier B, Maloteaux JM, Hermans E. Increasing the density of the D2L receptor and manipulating the receptor environment are required to evidence the partial agonist properties of aripiprazole. *Prog Neuropsychopharmacol Biol Psychiatry*. 2012; 36:60–70. [PubMed: 21871520]
- Landolt HP, Wehrle R. Antagonism of serotonergic 5-HT2A/2C receptors: mutual improvement of sleep, cognition and mood? *Eur J Neurosci*. 2009; 29, 1795–1809. [PubMed: 20092553]
- Mailman RB. GPCR functional selectivity has therapeutic impact. *Trends Pharmacol Sci*. 2007; 28:390–396. [PubMed: 17629962]
- Mary S, Damian M, Louet M, Floquet N, Fehrentz JA, Marie J, Martinez J, Baneres JL. Ligands and signaling proteins govern the conformational landscape explored by a G protein-coupled receptor. *Proc Natl Acad Sci USA*. 2012; 109:8304–8309. [PubMed: 22573814]
- Millan MJ. Serotonin 5-HT2C receptors as a target for the treatment of depressive and anxious states: focus on novel therapeutic strategies. *Therapie*. 2005; 60:441–460. [PubMed: 16433010]
- Milligan G. Mechanisms of multifunctional signalling by G protein-linked receptors. *Trends Pharmacol Sci*. 1993; 14:239–244. [PubMed: 8396793]
- Monti JM. Serotonin 5-HT(2A) receptor antagonists in the treatment of insomnia: present status and future prospects. *Drugs Today (Barc)*. 2010; 46:183–193. [PubMed: 20467592]
- Motulsky, HJ.; Christopoulos, A. *A Practical Guide to Curve-fitting*. GraphPad Software, Inc.; San Diego, CA: 2003. *Fitting Models to Biological Data Using Linear and Non-linear Regression*.
- Moya PR, Berg KA, Gutierrez-Hernandez MA, Saez-Briones P, Reyes-Parada M, Cassels BK, Clarke WP. Functional selectivity of hallucinogenic phenethylamine and phenylisopropylamine derivatives at human 5-hydroxytryptamine (5-HT)2A and 5-HT2C receptors. *J Pharmacol Exp Ther*. 2007; 321:1054–1061. [PubMed: 17337633]
- Nichols DE. Hallucinogens. *Pharmacol Ther*. 2004; 101:131–181. [PubMed: 14761703]
- Porter RH, Benwell KR, Lamb H, Malcolm CS, Allen NH, Revell DF, Adams DR, Sheardown MJ. Functional characterization of agonists at recombinant human 5-HT2A, 5-HT2B and 5-HT2C receptors in CHO-K1 cells. *Br J Pharmacol*. 1999; 128:13–20. [PubMed: 10498829]
- Ray TS. Psychedelics and the human receptorome. *PLoS One*. 2010; 5:e9019. [PubMed: 20126400]

- Rosenzweig-Lipson S, Zhang J, Mazandarani H, Harrison BL, Sabb A, Sabalski J, Stack G, Welmaker G, Barrett JE, Dunlop J. Antiobesity-like effects of the 5-HT<sub>2C</sub> receptor agonist WAY-161503. *Brain Res.* 2006;1073–1074.
- Rosenzweig-Lipson S, Dunlop J, Marquis KL. 5-HT<sub>2C</sub> receptor agonists as an innovative approach for psychiatric disorders. *Drug News Perspect.* 2007; 20:565–571. [PubMed: 18176661]
- Sard H, Kumaran G, Morency C, Roth BL, Toth BA, He P, Shuster L. SAR of psilocybin analogs: discovery of a selective 5-HT<sub>2C</sub> agonist. *Bioorg Med Chem Lett.* 2005; 15:4555–4559. [PubMed: 16061378]
- Schmid CL, Bohn LM. Serotonin, but not N-methyltryptamines, activates the serotonin 2A receptor via a ss-arrestin2/Src/Akt signaling complex in vivo. *J Neurosci.* 2010; 30:13513–13524. [PubMed: 20926677]
- Schmid CL, Raehal KM, Bohn LM. Agonist-directed signaling of the serotonin 2A receptor depends on beta-arrestin-2 interactions in vivo. *Proc Natl Acad Sci USA.* 2008; 105:1079–1084. [PubMed: 18195357]
- Siuciak JA, Chapin DS, McCarthy SA, Guanowsky V, Brown J, Chiang P, Marala R, Patterson T, Seymour PA, Swick A, Iredale PA. CP-809,101, a selective 5-HT<sub>2C</sub> agonist, shows activity in animal models of antipsychotic activity. *Neuropharmacology.* 2007; 52:279–290. [PubMed: 16949622]
- Teegarden BR, Al Shamma H, Xiong Y. 5-HT(2A) inverse-agonists for the treatment of insomnia. *Curr Top Med Chem.* 2008; 8:969–976. [PubMed: 18673166]
- Thomsen WJ, Grottick AJ, Menzaghi F, Reyes-Saldana H, Espitia S, Yuskin D, Whelan K, Martin M, Morgan M, Chen W, Al-Shamma H, Smith B, Chalmers D, Behan D. Lorcaserin, a novel selective human 5-hydroxytryptamine<sub>2C</sub> agonist: in vitro and in vivo pharmacological characterization. *J Pharmacol Exp Ther.* 2008; 325:577–587. [PubMed: 18252809]
- Urban JD, Clarke WP, von Zastrow M, Nichols DE, Kobilka B, Weinstein H, Javitch JA, Roth BL, Christopoulos A, Sexton PM, Miller KJ, Spedding M, Mailman RB. Functional selectivity and classical concepts of quantitative pharmacology. *J Pharmacol Exp Ther.* 2007; 320:1–13. [PubMed: 16803859]
- Vincek AS, Booth RG. New Approach to 4-Phenyl-beta-aminotetralin from 4-(3-Halophenyl)tetralen-2-ol Phenylacetate. *Tetrahedron Lett.* 2009; 50:5107–5109. [PubMed: 20161011]
- Welmaker GS, Nelson JA, Sabalski JE, Sabb AL, Potoski JR, Graziano D, Kagan M, Coupet J, Dunlop J, Mazandarani H, Rosenzweig-Lipson S, Sukoff S, Zhang Y. Synthesis and 5-hydroxytryptamine (5-HT) activity of 2,3,4,4a-tetrahydro-1H-pyrazino[1,2-a]quinoxalin-5-(6H)ones and 2,3,4,4a,5,6-hexahydro-1H-pyrazino[1,2-a]quinoxalines. *Bioorg Med Chem Lett.* 2000; 10:1991–1994. [PubMed: 10987434]

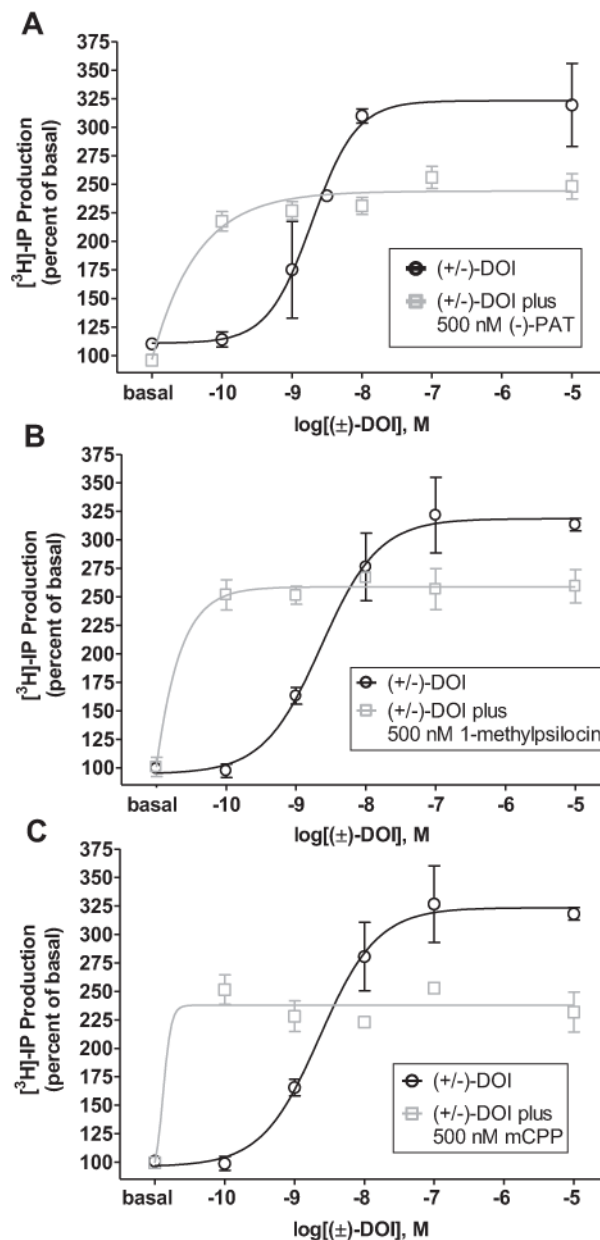


**Fig. 1.** The effects of commercially available 5-HT2C agonists (A) 4-phenyl-2-aminotetralin 5-HT2C agonists (B) and selective 5-HT2 antagonists (C) on the HTR elicited by DOI (1 mg/kg). The dose (mg/kg) is indicated in parentheses. “2nd” refers to test sessions in which the antagonist was administered after DOI. \*Indicates a statistically significant difference from DOI alone. +Indicates a statistically significant difference from the other 5-HT2C agonists. #Indicates a statistically significant difference from lower doses.





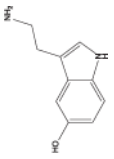
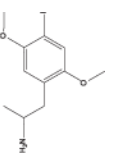
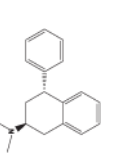
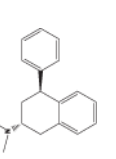
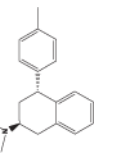
**Fig. 2.** The effects of commercially available 5-HT2C agonists (A), 4-phenyl-2-aminotetralin 5-HT2C agonists (B), and selective 5-HT2 antagonists (C) on locomotion following administration of saline, DOI (1 mg/kg), or DOI in combination with the various compounds. The dose (mg/kg) is indicated in parentheses. “2nd” refers to test sessions in which the antagonist was administered after DOI. \*Indicates a statistically significant difference from DOI alone. +Indicates a statistically significant difference from vehicle alone.

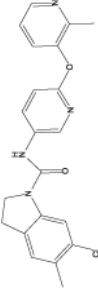
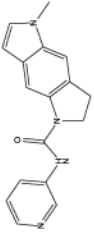
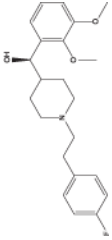
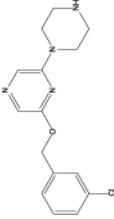
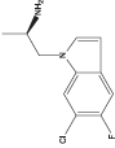
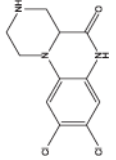
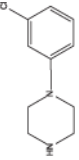
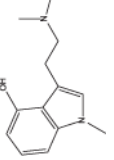


**Fig. 3.** Representative mouse 5-HT<sub>2C</sub>-VNV-mediated PLC functional data showing the effects of co-administration of 500 nM (-)-PAT (A), 1-methylpsilocin (B), or mCPP (C) with DOI on the ability of DOI to stimulate 5-HT<sub>2C</sub> receptor-mediated inositol phosphate (IP) production in HEK cells. Note that each of the selective 5-HT<sub>2C</sub> agonists significantly suppressed the maximal IP response to DOI.

Table 1

Approximate  $K_i$  values of 5-HT<sub>2</sub> ligands at mouse vs. human 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> receptors.  $K_i$  values for novel 4-phenyl-2-aminotetrahydroquinoline ligands ((-)-PAT, (+)-PAT, methyl-PAT) and selective 5-HT<sub>2</sub> antagonists (SB-242084, SB-206553, M100907) were obtained experimentally via competition with [<sup>3</sup>H]-ketanserin (5-HT<sub>2A</sub>) or [<sup>3</sup>H]-mesulergine (5-HT<sub>2C</sub>). Data are expressed as means ( $\pm$ SEM's).  $K_i$  values of commercially available selective 5-HT<sub>2C</sub> agonists are from the literature; data from receptors labeled with ligands other than [<sup>3</sup>H]-ketanserin (5-HT<sub>2A</sub>) or [<sup>3</sup>H]-mesulergine (5-HT<sub>2C</sub>) are reported when these data were not available: CP 809101 (<sup>3</sup>Siuciak et al., 2007, [<sup>3</sup>H]-5-HT), Ro 60-0175 (<sup>3</sup>Cussac et al., 2002; <sup>3</sup>Bentley et al., 2004 and <sup>3</sup>Knigh et al., 2004, [<sup>125</sup>I]-DOI), WAY 161503 (<sup>3</sup>Welmaker et al., 2000; <sup>3</sup>Rosenzweig-Lipson et al., 2006, [<sup>125</sup>I]-DOI labeled), mCPP (<sup>3</sup>S. <sup>3</sup>Data from the NIMH Psychoactive Drug Screening Program), and 1-methylpilociclin (<sup>3</sup>Sard et al., 2005). CLogP values were obtained from ChemBioDraw software version 12. \*5-HT does not readily cross the blood-brain barrier, and was not tested *in vivo*. m5-HT<sub>2A</sub> = C57Bl/6 mouse 5-HT<sub>2A</sub> receptor; m5-HT<sub>2C</sub> = C57Bl/6 mouse 5-HT<sub>2C</sub>-VNV receptor; h5-HT<sub>2A</sub> = human 5-HT<sub>2A</sub> receptor; h5-HT<sub>2C</sub> = human 5-HT<sub>2C</sub>-INI receptor. NR = not reported.

Ligand	Structure	CLogP	$K_i$ , nM (SEM)		
			m5-HT <sub>2A</sub>	h5-HT <sub>2A</sub>	m5-HT <sub>2C</sub> h5-HT <sub>2C</sub>
5-HT		0.76*	138 (27.7)	128 (19.7)	50.4 (10.2) 15.4 (2.3)
(±)-DOI		2.67	4.0 (1.2)	3.2 (0.8)	46.7 (4.4) 19.1 (1.7)
(-)-PAT		4.12	116 (18.7)	102 (12.2)	53 (2.7) 29 (3.3)
(+)-PAT		4.12	332 (46.8)	463 (47.3)	1734 (156.6) 492 (73.0)
Methyl-PAT		4.62	326 (29.9)	179 (17.9)	868 (86.8) 128 (6.12)

Ligand	Structure	CLogP				$K_p$ , nM (SEM)			
		m5-HT2A	h5-HT2A	m5-HT2C	h5-HT2C	m5-HT2A	h5-HT2A	m5-HT2C	h5-HT2C
SB-242084		5.05	146 (45.0)	0.1 (0.03)	1.5 (0.2)	96 (20)	676 (69.3)	3.2 (0.5)	3.1 (0.6)
SB-206553		3.18	342 (44.4)	74.2 (12.2)	36.6 (4.4)	0.45 (0.05)	0.2 (0)	6 <sup>a</sup>	1.6 <sup>a</sup>
MI00907		3.29	NR	NR	NR	NR	NR	NR	NR
CP 809101		2.90	NR	NR	NR	NR	NR	NR	NR
Ro 60-0175		3.07	NR	NR	NR	NR	NR	NR	NR
WAY161503		2.12	NR	NR	NR	NR	NR	NR	NR
mCPP		2.47	NR	NR	NR	NR	NR	NR	NR
1-Methylpsilocin		2.14	NR	NR	NR	NR	NR	NR	NR

**Table 2**

Approximate potency (EC50) and efficacy values of novel 4-phenyl-2-aminotetralin ligands relative to 5-HT and (±)-DOI for activating mouse and human 5-HT2A and 5-HT2C receptor-mediated PLC signaling. EC50 data are expressed in nM (±SEM). Efficacy data represent percent of maximum 5-HT response (±SEM). m5-HT2A = C57Bl/6 mouse 5-HT2A receptor; m5-HT2C = C57Bl/6 mouse 5-HT2C-VNV receptor; h5-HT2A = human 5-HT2A receptor; h5-HT2C = human 5-HT2C-INI receptor; N/A = not applicable. \*Pilot test only; #results from Booth et al., 2009 using CHO-K1 cells.

Ligand	m5-HT2A		h5-HT2A		m5-HT2C		h5-HT2C	
	EC50	Efficacy	EC50	Efficacy	EC50	Efficacy	EC50	Efficacy
5-HT	71 (2.6)	100	118 (7.50)	100	16 (2.5)	100	4.2 (1.3)	100
(±)-DOI	2.6 (0.1)	98 (4.6)	1.8 (0.7)	97 (3.5)	3.4 (0.9)	77 (2.6)	5.5*	106*
(-)-PAT	Inactive	N/A	Inactive	N/A	39 (1.7)	72 (2.9)	20#	100#
(+)-PAT	352 (18.5)	31 (1.2)	233(20.7)	18 (0.5)	2115 (87.50)	69 (1.2)	171 (37.0)	88 (9.9)
Methyl-PAT	Inactive	N/A	Inactive	N/A	808 (24.3)	67 (2.6)	1120 (145.5)	96 (3.0)