Increased 5-HT_{2A} receptor signalling efficacy differentiates serotonergic psychedelics from non-psychedelics

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The data that support the findings of this study are available from the corresponding author upon reasonable request.

ABSTRACT

Background and Purpose

Serotonergic psychedelic drugs are under renewed investigation for the potential treatment of several psychiatric disorders. While all serotonergic psychedelics have 5-HT_{2A} receptor activity, the explanation for why some 5-HT_{2A} receptor agonists are not psychedelic is unknown. To address this question, we investigated the 5-HT_{2A} receptor signalling bias and efficacy of a panel of psychedelics and non-psychedelics.

Experimental Approach

G_q-coupled (Ca^{2+} and IP_1) and β-arrestin2 signalling effects of eight chemically diverse psychedelics (psilocin, 5-MeO-DMT, LSD, mescaline, 25B-NBOMe and DOI) and non-psychedelics (lisuride and TBG) were characterised using SH-SY5Y cells expressing recombinant human 5-HT_{2A} receptors. Measurements of signalling efficacy and bias were derived from dose-responses curves for each agonist, compared to 5-HT. Follow-up experiments sought to confirm the generality of findings using rat C6 cells expressing endogenous 5-HT_{2A} receptors.

Key Results

In SH-SY5Y cells, all psychedelics were partial agonists at both 5-HT_{2A} receptor signalling pathways and none showed significant signalling bias. In comparison, in SH-SY5Y cells the non-psychedelics lisuride and TBG were not distinguishable from psychedelics in terms of biased agonist properties, but both exhibited the lowest 5-HT_{2A} receptor signalling efficacy of all drugs tested, a result confirmed in C6 cells.

Conclusion and Implications

In summary, all psychedelics tested were unbiased, partial 5-HT_{2A} receptor agonists. Importantly, the non-psychedelics lisuride and TBG were discriminated from psychedelics, not through biased signalling but rather by relatively low efficacy. Thus, 5-HT_{2A} receptor signalling efficacy and not bias provides a possible explanation for why some 5-HT_{2A} receptor agonists are not psychedelic.

Keywords: serotonin, 5-HT, psychedelic, 5-HT_{2A} receptor, biased agonism, G_q and β-arrestin2 signalling.

INTRODUCTION

Serotonergic psychedelics are in development for the treatment of psychiatric disorders ranging from major depression and anxiety to substance misuse disorder and anorexia[1,2]. Each of psilocybin, 5-methoxy-N,N-dimethyltryptamine (5-MeO-DMT) and lysergic acid diethylamide (LSD) have progressed to clinical trials of treatment-resistant depression and anxiety[3–5]. As a result, there is increasing interest in the molecular mechanisms by which such agents induce their characteristic psychedelic effects.

There is clear evidence that engagement at the 5-HT_{2A} receptor is central to the subjective effects of serotonergic psychedelics. Specifically, clinical PET imaging studies using the 5-HT_{2A} radioligand, [^{11}C]Cimbi-36, report a strong correlation between 5-HT_{2A} receptor occupancy and the intensity of psychedelic effects following psilocybin administration[6,7]. Moreover, the subjective effects of both psilocybin and LSD in human volunteers were attenuated by the 5-HT_{2A} receptor antagonist ketanserin[8–12]. In preclinical studies, 5-HT_{2A} receptor agonist-induced head-twitches in mice are widely considered a surrogate marker of the psychedelic effects of these drugs in humans. Indeed, this head-twitch response is abolished by 5-HT_{2A} receptor knockout or selective antagonists[13,14], and agonist potency in this model correlates with potency to induce psychedelic effects in humans[15].

A recent interesting development is evidence of non-psychedelic 5-HT_{2A} receptor agonists. Thus, there are several reports of 5-HT_{2A} receptor agonists lacking the propensity to evoke head-twitches[16–18]. For example, in mice, administration of the high affinity 5-HT_{2A} receptor ligand tabernanthalog (TBG) did not induce head-twitches but was capable of evoking 5-HT_{2A} receptor-mediated effects in other in vivo models[18]. Similarly, lisuride, another high affinity 5-HT_{2A} receptor agonist, lacked effects on head-twitches in mice[16]. Although it is not yet known whether TBG is non-psychedelic when administered to humans, there are numerous clinical reports showing that lisuride lacks psychedelic effects[19–21]. The explanation for why some 5-HT_{2A} receptor agonists are psychedelic and not others, is unknown.

A common feature of G protein-coupled receptors is their capacity to signal through both G protein-dependent and β-arrestin2-dependent pathways[22–24]. Thus, the 5-HT_{2A} receptor has been shown to signal via $G_{q/11}$ (to activate phospholipase C and increase inositol trisphosphate and intracellular Ca^{2+} [7]) as well as β-arrestin2 and other pathways[25–28]. Divergence in the subjective effects of drugs with 5-HT_{2A} agonist activity could be driven by selective signalling through G_q- or β-arrestin2-mediated pathways (biased agonism)[29]. As an example, biased agonism at μ-opioid receptors was initially thought to explain the preferential sedative versus analgesic effects of certain μ-opioid receptor agonists[30,31]. An alternative explanation for non-psychedelic 5-HT_{2A} receptor agonists is partial agonism; agonists with low of 5-HT_{2A} receptor efficacy may exhibit a more limited repertoire of behavioural effects[32–34]. Partial agonism at the benzodiazepine binding site of the $GABA_A$ receptor was offered to account for the behaviourally selective effects of benzodiazepine agonists[35–37]. Moreover, partial agonism at μ-opioid receptors is the currently favoured alternative explanation for analgesia selective μ-opioid receptor agonists[30,31].

Against this background, the current study investigated the 5-HT_{2A} receptor-mediated G_q or β-arrestin2 signalling properties of a panel psychedelics; the tryptamines psilocin (active metabolite of psilocybin) and 5-MeO-DMT, the ergoline LSD and the phenethylamines mescaline (3,4,5-trimethoxyphenethylamine), 4-bromo-2,5-dimethoxy-N-(2-methoxybenzyl)phenethylamine (25B-NBOMe) and 2,5-dimethoxy-4-iodoamphetamine (DOI). The signalling properties of these agents were compared with two non-psychedelics TBG and lisuride (also of the ergoline chemical class). The drugs were selected to be chemically diverse since receptor stabilization in a particular state might determine signalling bias[16,38–40]. Experiments utilised cell lines expressing human or rat 5-HT_{2A} receptors.

METHODS

Cell culture

SH-SY5Y neuroblastoma cells transfected with the human 5-HT_{2A} receptor[41] were maintained in culture medium; Dulbecco's Modified Eagle Medium (DMEM) containing 2 mM Glutamax, 10 % (v/v) fetal bovine serum (FBS), 100 I.U. μg^{-1} ml^{-1} penicillin/streptomycin, and 480 µg ml^{-1} G418 (to maintain transfection selection pressure). C6 glioma cells which endogenously express the rat 5-HT_{2A} receptor[42,43] (ATCC CCL-107) were maintained in Ham's F12 nutrient mix containing 2 mM Glutamax, 10 % (v/v) FBS and 100 I.U. μg^{-1} ml^{-1} penicillin/streptomycin. Cells were grown at 37°C in a humidified atmosphere of 95 % air and 5 % CO_2.

Assay of agonist-evoked cytosolic Ca^{2+}

Cells were plated in 96-well black/clear bottom plates at a density of 40,000 (SH-SY5Y) or 60,000 (C6) cells/well, 48 h (SH-SY5Y) or 24 h (C6) before the day of experiment. The 10 % FBS culture medium was replaced with culture medium containing 10 % dialysed-FBS to avoid potential receptor desensitisation by 5-HT in the FBS.

On the day of experiment, the culture medium was aspirated and cells were washed twice with 200 µl Hanks' Balanced Salt Solution (HBSS) containing calcium (HBSS-Ca^{2+}). Next, 100 µl of assay buffer containing 4 µM Fluo-4-AM (Life Technologies), 0.02 % Pluronic F127 and 2.5 mM probenecid in HBSS-Ca^{2+} was added to each well and the plate was incubated at room temperature (RT) for 1 h to allow dye loading, followed by 37°C for 30 min to allow for intracellular esterase action. The assay buffer was aspirated and cells were washed twice with HBSS-Ca^{2+} before addition of 90 µl/well HBSS-Ca^{2+}. Cells were allowed to equilibrate for 15 min at RT before fluorescence recordings at the same temperature.

Baseline fluorescence was measured on a plate reader (BMG Optima) from the plate bottom at 480/520nm excitation/emission every 5 s for 30 s prior to addition of one of; 10 µl agonist, 10 µl agonist/agonist combination or 10 µl agonist plus 10 µl antagonist (MDL-100,907 15 min before agonist addition), after which fluorescence was recorded for a further 2 min. In each well the final concentration of DMSO was 0.1 % (v/v).

Assay of agonist-evoked inositol monophosphate (IP_1) accumulation

SH-SY5Y cells were plated in 384-well white low-volume plates 24 h before the day of experiment at a density of 20,000 cells/well. As above, the 10 % FBS supplemented culture medium was replaced with 10 % dialysed-FBS. On the day of experiment, the medium was aspirated and 10 µl of buffer containing LiCl and 4 µl of agonist/antagonist was added to each well. The plate was incubated at 37°C for 1 h before addition to each well of 3 µl IP_1-d2 and anti-IP_1-d2 dissolved in lysis buffer (CisBio HTRF IP-One G_q Kit) and then incubated further at RT for 1 h. A calibration curve was run prior to commencing experiments (according to manufacturer's instructions).

Fluorescence was measured on a plate reader (Tecan Infinite F1200) from the top of the plate at 620/340nm and 665/340nm excitation/emission. The final concentration of DMSO in each well was 0.1 % (v/v).

Assay of agonist-evoked β-arrestin2 recruitment

SH-SY5Y cells were plated in 96-well black/clear-bottom plates at a density of 40,000 cells/well 48 h before the day of experiment. The 10 % FBS supplemented culture medium was replaced with 10 % dialysed-FBS. The following day, to each well was added 50 µl of a transfection mix containing 8 µl of β-arrestin2 sensor, 15 µl of human-5-HT_{2A} receptor, 3 µl GPCR kinase 2, 3 µl GPCR kinase 3 (all packaged in Mammalian Baculovirus vectors, Montana Molecular), 0.6 µl sodium butyrate and 21.4 µl media. The transfection mix

was aspirated and cells washed twice with DPBS containing calcium (DPBS-Ca^{2+}) before addition of 100 µl DPBS-Ca^{2+} to each well. The cells were allowed to equilibrate for 30 min at RT.

Baseline fluorescence was measured on a plate reader (BMG Omega) from the bottom of the plate at 485/520 excitation/emission every 15 s for 60 s prior to addition of 50 µl agonist, after which fluorescence was recorded for a further 20 min. The final concentration of DMSO in each well was at 0.1 % (v/v).

Drugs

Psilocin (supplied by Compass Pathways), 5-MeO-DMT (Cambridge Bioscience), DOI (Cambridge Bioscience), mescaline (Cambridge Bioscience), LSD (Chiron), 25B-NBOMe (Chiron), lisuride (Bio-Techne), TBG (supplied by Compass Pathways) and MDL-100,907 (volinanserin; Bio-Techne), were dissolved in DMSO, and 5-HT-HCl (Enzo Life Sciences) was dissolved in deionised water, to obtain 10 mM stock solutions. On the day of experiment, working solutions were obtained by diluting drugs in HBSS-Ca^{2+} (Ca^{2+}/IP_1 assays) or DPBS-Ca^{2+} (β-arrestin2 assays).

Data processing and statistical analysis

Statistical analyses were performed using GraphPad Prism software. For Ca^{2+} assays, baseline fluorescence was averaged and maximum fluorescence reached was expressed as a % of baseline (corrected for vehicle addition). For IP_1 assays, data were converted to emission at 665/620 values which were then interpolated as intracellular IP_1 concentrations using the IP_1 calibration curve. For β-arrestin2 assays, fluorescence was averaged over the first 2.5 min and then measurements after agonist addition over the remaining 20 minutes were normalised to baseline. Steady states were then calculated using the 'Baseline then rise to steady state time course' curve fit on GraphPad Prism and this was used as an endpoint measurement of cumulative response.

Dose-response curves were generated using log[agonist] versus response (three parameter) curve fits (GraphPad Prism), which also provided potency and efficacy values. The dose response for each 5-HT_{2A} receptor agonist was normalised to the response to 10 µM 5-HT and each point was expressed as mean ± SEM value of at least two independent experiments carried out in duplicate.

The relative activity of agonists in different assays were calculated using the method of Kenakin et al[44]. Specifically, $\log(E_{max}/EC_{50})$ values were calculated for each agonist in each pathway using E_{max} and EC_{50} values derived by averaging these parameters across replicates for each assay, and then compared to the reference agonist 5-HT by calculation of $\Delta\log(E_{max}/EC_{50})$ $(\log(E_{max}/EC_{50})_{agonist} - \log(E_{max}/EC_{50})_{5\text{-}HT})$.

RESULTS

Effect of 5-HT_{2A} receptor agonists on cytosolic Ca^{2+} in SH-SY5Y cells

Initial experiments determined the effect of the selected psychedelic and non-psychedelic 5-HT_{2A} receptor agonists on G_q signalling activity via measurement of cytosolic Ca^{2+} increase in SH-SY5Y cells expressing recombinant human 5-HT_{2A} receptors. All drugs tested elicited dose-dependent increases in cytosolic Ca^{2+} (Fig. 1). The psychedelics tested had variable potencies with 25B-NBOMe and LSD being the most potent and mescaline the least potent (Fig. 1, Table 1).

In terms of efficacy, all psychedelics had lower efficacy than 5-HT (Fig. 1), with psilocin and LSD being the least efficacious. Interestingly, the two non-psychedelics, lisuride and TBG, displayed the lowest efficacy of all drugs tested (Fig. 1, Table 1).

The Ca^{2+} responses of SH-SY5Y cells to both psychedelic and non-psychedelic drugs (10 µM) were abolished by pre-treatment with MDL-100,907 (1 µM), confirming the role of 5-HT_{2A} receptor activation in this G_q signalling activity (Suppl. Fig. 1A).

Effect of 5-HT_{2A} receptor agonists on IP_1 accumulation in SH-SY5Y cells

Next, the effect of the psychedelics and non-psychedelics on G_q signalling activity was determined upstream of the Ca^{2+} response by measuring of accumulation of intracellular IP_1 in the SH-SY5Y cells. As with cytosolic Ca^{2+}, all drugs tested elicited dose-dependent increases in IP_1 (Fig. 2). The rank order of potency for IP_1 accumulation varied across the psychedelics but was similar to the Ca^{2+} response, with 25B-NBOMe and LSD being the most potent psychedelics and mescaline the least potent (Fig. 2, Table 1).

In the IP_1 assay, most drugs were less efficacious than 5-HT, except 5-MeO-DMT and DOI. Interestingly, as noted for the Ca^{2+} assay, the non-psychedelics lisuride and TBG displayed the lowest efficacy of all drugs tested (Fig. 2, Table 1).

Effect of 5-HT_{2A} receptor agonists on β-arrestin2 recruitment in SH-SY5Y cells

Next, experiments measured the effects of psychedelic and non-psychedelic drugs on 5-HT_{2A} receptor signalling via β-arrestin2 in the SH-SY5Y cells. As with the assays of G_q signalling, all drugs tested elicited dose-dependent increases in β-arrestin2 recruitment (Fig. 3). Moreover, 25B-NBOMe and LSD were amongst the most potent psychedelics tested, and mescaline amongst the least potent (Fig. 3, Table 1).

As observed in the Ca^{2+} assay, a feature of the β-arrestin2 assay was that drugs were less efficacious than 5-HT (Fig. 3, Table 1), in this case with psilocin being the least efficacious psychedelic. Moreover, of the drugs tested the non-psychedelics lisuride and TBG were ranked lowest in terms of efficacy.

The β-arrestin2 response to both psychedelics and non-psychedelics (10 µM) was abolished by pre-treatment with MDL-100,907 (1 µM), confirming that the β-arrestin2 signalling was 5-HT_{2A} receptor mediated (Suppl. Fig. 1B).

Biased agonist properties of psychedelic and non-psychedelic agents

With agonist activity data generated from the IP_1 and β-arrestin2 assays, ligand bias in 5-HT_{2A} receptor-mediated signalling was next assessed. For each assay, to cancel out system-specific differences such as downstream signalling amplification, agonist potencies and maximal efficacies were converted to $\Delta\log(E_{max}/EC_{50})$ values with 5-HT being the reference agonist. A scatter plot of $\Delta\log(E_{max}/EC_{50})$ values then allowed comparison of the activity of each agonist in these two pathways[44] (Fig. 4). In this plot, agonists

falling close to the line of unity (i.e. possessing similar $\Delta\log(E_{max}/EC_{50})$ values in each assay) have low bias, whereas agonists that deviate from the line of unity (i.e. possessing different $\Delta\log(E_{max}/EC_{50})$ values in each assay) display bias.

There was a generally linear relationship between the IP_1 versus β-arrestin2 signalling activity of the different agonists tested (Fig. 4). Of the psychedelic drugs, all were unbiased with the exception of LSD which showed a modest bias towards IP_1 signalling versus β-arrestin2 signalling (Fig. 4). In comparison, one of the two non-psychedelics tested, lisuride, showed a bias towards IP_1 versus β-arrestin2 signalling but TBG showed no signalling bias (Fig. 4).

Thus, comparison of agonist activity at IP_1 versus β-arrestin2 signalling failed to distinguish between psychedelic and non-psychedelic 5-HT_{2A} receptor agonists. Overall, there was no clear pattern between signalling bias and chemical structure although it is noteworthy that the two ergolines LSD and lisuride showed bias towards IP_1 signalling, albeit to varying degrees.

As with the plot of IP_1 versus β-arrestin2 signalling, a scatter plot of $\Delta\log(E_{max}/EC_{50})$ values to compare agonist activity in the Ca^{2+} and β-arrestin2 signalling pathways did not discriminate between the psychedelic and non-psychedelic agonists (Suppl. Fig. 2A).

Finally, a scatter plot of $\Delta\log(E_{max}/EC_{50})$ values obtained from the Ca^{2+} and IP_1 assays allowed comparison of agonist activity of what should be the same G_q signalling pathway. This revealed a generally linear relationship between the activity of the different agonists in the two pathways, although it was notable that LSD and lisuride showed increased activity in the IP_1 assay compared to in the Ca^{2+} assay (Suppl. Fig. 2B).

Overall, the different scatter plots revealed, importantly, that signalling bias pattern did not distinguish between psychedelic and non-psychedelic agents.

Effect of 5-HT_{2A} receptor agonists on cytosolic Ca^{2+} in C6 cells

Results obtained from the SH-SY5Y cells suggested that psychedelics could be distinguished from non-psychedelics on the basis of the latter having a very low efficacy at 5-HT_{2A} receptors and not differences in biased signalling activity. To test this observation further, the effect of drugs on G_q activity was examined in a different cell model, specifically C6 glioma cells endogenously expressing the rat 5-HT_{2A} receptor.

These experiments revealed that, as observed in SH-SY5Y cells, all psychedelics caused a dose-related increase in cytosolic Ca^{2+} in C6 cells (Fig. 5) with lower efficacy than 5-HT. Furthermore, the Ca^{2+} response to the psychedelics (10 µM) was abolished by pre-treatment with MDL-100,907 (1 µM), confirming 5-HT_{2A} receptor involvement (Suppl. Fig. 3B). The relative potency of the psychedelics in the C6 cells was similar to that observed in SH-SY5Y cells, with 25B-NBOMe and LSD being the most potent and mescaline being the least potent (Fig. 5; Table 2).

A $\Delta\log(E_{max}/EC_{50})$ scatter plot of Ca^{2+} responses emphasised the similarity in agonist activity in the C6 and SH-SY5Y cells (Suppl. Fig. 3A). However, it is noteworthy that mescaline was more active in C6 cells, and psilocin was more active in SH-SY5Y cells, potentially highlighting differences in the activity of these psychedelics at the rat versus human 5-HT_{2A} receptors.

Importantly, and also in keeping with results from the SH-SY5Y cells, the non-psychedelic lisuride had relatively low efficacy in the C6 cells and TBG had no measurable efficacy at the concentrations tested (Fig. 5; Table 2). Given their low efficacy both lisuride and TBG were run in combination with 5-HT, and Schild

plots were constructed to confirm partial agonist properties. As expected of a partial agonist, the presence of both lisuride and TBG increased the response of low 5-HT concentrations and reduced the response of higher 5-HT concentrations (Fig. 6). Schild plots for the psychedelic agonists also confirmed the partial agonist properties of these drugs (Suppl. Table 1).

Overall, the data from the C6 cells confirmed that the non-psychedelic drugs lisuride and TBG were very low efficacy 5-HT_{2A} receptor agonists, as observed in the SH-SY5Y cells.

DISCUSSION

It is unknown why some 5-HT_{2A} receptor agonists are psychedelic and others are not, with both biased agonism and partial agonism being plausible explanations (see Introduction). The present study characterised the 5-HT_{2A} receptor signalling properties of a chemically diverse panel of psychedelics (psilocin, 5-MeO-DMT, LSD, mescaline, 25B-NBOMe, DOI) and non-psychedelics (lisuride, TBG), with a focus on G_q (cytosolic Ca^{2+}, IP_1 accumulation) versus β-arrestin2 signalling. Key findings were: (i) the psychedelics were 5-HT_{2A} receptor agonists in models of both G_q and β-arrestin2 signalling, with these drugs typically being unbiased and having with lower efficacy than 5-HT, (ii) the non-psychedelics, lisuride and TBG were indistinguishable from psychedelics in terms of their biased agonist properties but both exhibited the lowest 5-HT_{2A} receptor signalling efficacy of all drugs tested, and (iii) whilst there was no clear correlation between chemical structure and signalling bias or efficacy, it is noteworthy that the two ergolines, lisuride and LSD, showed evidence of G_q signalling bias.

5-HT_{2A} receptor signalling bias did not discriminate between psychedelic and non-psychedelic drugs

A potential explanation for why some 5-HT_{2A} receptor agonists are psychedelic and not others is biased agonism - that is, preference for one 5-HT_{2A} receptor signalling pathway versus another. Here, all the psychedelics and non-psychedelics tested activated both 5-HT_{2A} receptor-mediated G_q and β-arrestin2 signalling. More importantly, signalling bias did not discriminate between psychedelics and non-psychedelics. Thus, none of the psychedelics showed significant G_q versus β-arrestin2 signalling bias (although modest bias was observed for LSD, see below) and of the non-psychedelics, lisuride showed evidence of bias towards G_q signalling whereas TBG did not.

Our principal model of G_q and β-arrestin2 signalling bias used the human 5-HT_{2A} receptor and measurement of IP_1 accumulation and β-arrestin2 recruitment combined with a scatter plot of $\Delta\log(E_{max}/EC_{50})$ values for each agonist and in each signalling pathway[44]. A strength of this model is that the use of a reference agonist

(here 5-HT) in each assay accounts for assays with different receptor reserves and cell backgrounds as well as any differences in signal amplification. The current study also generated a scatter plot of $\Delta\log(E_{max}/EC_{50})$ values for Ca^{2+} versus β-arrestin2 signalling and this also did not discriminate between the psychedelic and non-psychedelic drugs. However, it should be noted that the latter plot is limited to the extent that Ca^{2+} was measured under non-equilibrium conditions whereas β-arrestin2 measurements were performed at equilibrium (see below).

There are currently few studies of 5-HT_{2A} receptor signalling bias of psychedelic versus non-psychedelic drugs. Our data showing that psilocin and other psychedelics lack bias is in accord with a very recent study also reporting that psilocin, LSD, DOI and 5-MeO-DMT have similar activity at human 5-HT_{2A} receptor-mediated G_q and β-arrestin2 signalling[34] although signalling bias was not quantified using $\Delta\log(E_{max}/EC_{50})$ values. Interestingly, an earlier study of human 5-HT_{2A} receptor signalling via IP_1 and arachidonic acid pathways (downstream of the PLA_2 pathway) by Berg *et al* reported that DOI, LSD and lisuride showed signalling bias towards the AA pathway[26]. It is possible that psychedelic and non-psychedelics could be discriminated by 5-HT_{2A} receptor signalling via pathways other than G_q versus β-arrestin2. However, the finding by Berg *et al* that lisuride showed similar bias to LSD and DOI with regards to IP_1 versus AA pathways argues against this[26].

Non-psychedelic drugs had low efficacy 5-HT_{2A} receptor signalling compared to psychedelic drugs

A general feature of the drugs tested here is that they had lower efficacy compared to 5-HT itself in both G_q and β-arrestin2 signalling and were thereby partial 5-HT_{2A} receptor agonists. This finding was robust and consistent across two cell systems (human neuroblastoma SH-SY5Y, rat C6 glioma). Interestingly, the non-psychedelics lisuride and TBG consistently displayed the lowest efficacies of all agonists tested in both cell systems. This finding is in accordance with a recent study by Cao *et al*[16] which reported that compared to psychedelic drugs, three putative non-psychedelic 5-HT_{2A} receptor agonists (lisuride, IHCH-7079, and IHCH-7086) each exhibited low efficacy in both G_q and β-arrestin2 signalling pathways mediated by human 5-HT_{2A}

receptors. Similarly, other recent papers have reported that the substituted phenethylamine Ariadne[32] and ergoline 2-Br-LSD[33], which are both putative non-psychedelics, show low efficacy in human 5-HT_{2A} receptor coupled G_q and β-arrestin2 signalling pathways compared to psychedelic drugs of similar chemical structure (DOM and LSD, respectively). These findings taken together with the current data suggest that a defining feature of non-psychedelics that differentiates them from psychedelics, is their very low efficacy at 5-HT_{2A} receptors.

Recent evidence suggests that a certain level of efficacy is required for a 5-HT_{2A} receptor agonist to elicit a head-twitch response[34], a commonly accepted surrogate marker of the psychedelic effect. Specifically, in a study of the 5-HT_{2A} receptor signalling efficacy of 14 phenethylamines, those drugs with low signalling efficacy lacked ability to evoke a head-twitch response. Low efficacy 5-HT_{2A} receptor agonists are apparently also capable of evoking effects claimed to be similar to known antidepressants in preclinical models. For example, low efficacy 5-HT_{2A} receptor agonists such as lisuride, TBG and IHCH-7086 were reported to have such effects in behavioural models and also exhibit increases molecular and cellular markers of plasticity[16,18,33]. It is currently unknown whether this capacity of low efficacy 5-HT_{2A} receptor agonists is of functional relevance in humans.

Partial agonism rather than biased agonism has also been proposed to explain why certain μ-opioid receptor agonists elicit weak respiratory depressant effects (eg. oliceridine) compared to others (eg. morphine and fentanyl)[45–48]. Thus, drugs with weak respiratory depressant effects such as oliceridine had low μ-opioid receptor mediated G_i signalling efficacy without significant G_i and β-arrestin2 signalling bias[45]. Similarly, it has been proposed that partial agonism explains the actions of behaviourally-selective benzodiazepines (notwithstanding the alternative explanation of $GABA_A$ subunit selectivity). In this case, low efficacy drugs such as bretazenil show reduced sedative effects while maintaining anxiolytic effects[35–37].

5-HT$_{2A}$ receptor signalling bias of ergolines

Analysis of G_q (IP_1) versus β-arrestin2 signalling bias revealed some evidence that the ergolines LSD and lisuride had signalling bias properties. Specifically, LSD showed a modest bias towards G_q (IP_1) over β-arrestin2 signalling while lisuride showed a stronger bias in this regard. However, comparison of the data from the IP_1 and Ca^{2+} assays also revealed that both ergolines exhibited bias towards IP_1, which should be measuring the same G_q signalling pathway as the Ca^{2+} readout. These data suggests that in some cases, measurement of signalling bias may be influenced by assay format.

Here, both IP_1 and β-arrestin2 measurements were made under conditions in which agonists had likely reached equilibrium with the receptor, and measures were taken as an accumulation of response. Therefore, it is reasonable to conclude that comparison of these two assays provides a more accurate measure of signalling bias. On the other hand, the Ca^{2+} readout was obtained immediately after agonist addition when the drug and receptor were likely under non-equilibrium conditions. When comparisons of agonist activity are made under equilibrium and non-equilibrium conditions, kinetic differences in agonist on- and off-rates as well as receptor residency times can influence measures of agonist efficacy and potency[49,50], and thereby potentially lead to inaccurate measures of signalling bias.

Our observation that lisuride and LSD had increased 5-HT$_{2A}$ receptor signalling activity in the equilibrium IP_1 assay versus the non-equilibrium Ca^{2+} assay agrees with previous findings with other ergolines at the 5-HT$_{2B}$ receptor[51,52], which has high structural homology to the 5-HT$_{2A}$ receptor. Interestingly, it is reported that LSD has a unique 5-HT$_{2B}$ receptor binding mode in which a molecular 'lid' hinders drug on- and off-rates and prolongs residency times[38,40]. Presumably this also applies to lisuride. Thus, the Ca^{2+} assay may underestimate the activity of LSD and lisuride due to these drugs not having reached equilibrium with the receptor leading to an apparent IP_1 versus Ca^{2+} bias. On the other hand, the finding that LSD and lisuride have a G_q signalling bias using the IP_1 and β-arrestin2 assays may be a more accurate finding because both assay conditions were at equilibrium.

Thus, our findings with LSD and lisuride support the contention that the kinetics and equilibrium state of signalling assays is an important consideration when measuring signalling bias parameters.

Role of 5-HT_{2A} receptor signalling pathways in mediating behavioural effects

Some critical level of 5-HT_{2A} receptor signalling efficacy is required to elicit psychedelic effects in that, as noted above, low efficacy 5-HT_{2A} receptor agonists lack an ability to elicit head-twitches in mice. In the current study there was no clear pattern of 5-HT_{2A} receptor signalling bias from the psychedelic and non-psychedelic drugs tested to inform on the likely pathways mediating the psychedelic effects. Moreover, the literature is currently unclear on this point. Some evidence suggests a role for G_q signalling in the head-twitch response. For example, inhibitors of inositol monophosphatase, a key enzyme in the G_q signalling pathway, reduced head-twitches induced by DOI and psilocin[53]. Also, G_q but not β-arrestin2 signalling efficacy was correlated with propensity to evoke head twitches[34]. However, data generated using β-arrestin2 knockout mice are inconsistent. Whilst one study reported that LSD-induced head-twitches were attenuated in this mouse[54], other studies found that DOI-induced head-twitches were not[55,56].

There are similarly conflicting findings regarding how 5-HT_{2A} receptor signalling pathways may mediate different behavioural and neuroplastic effects. Thus, inositol monophosphatase inhibitors were reported to reduce DOI-evoked expression of markers of neural plasticity [53], suggesting a role for G_q signalling. Accordingly, a phospholipase C inhibitor prevented the increase in plasticity genes in cultured mouse cortical neurons exposed to LSD and lisuride[13]. On the other hand, another study reported that several β-arrestin2-biased 5-HT_{2A} receptor agonists attenuated acute restraint stress-induced freezing behaviour in tail suspension and forced swim tests in mice[16]. A further complication is recent evidence that –some behavioural and neuroplastic effects of psychedelic drugs are not mediated by 5-HT_{2A} receptors alone but that the neurotrophic factor receptor TrkB may also play a role[57].

A final point is that whilst the 5-HT_{2A} receptor signalling pathways underlying the behavioural effects are uncertain, other factors add further uncertainty. In particular, most (if not all) psychedelic and non-psychedelic drugs are not selective 5-HT_{2A} receptor agonists and exhibit affinity for other 5-HT receptors and receptors for other neurotransmitters. For example, in addition to having agonist activity at the 5-HT_{2A} receptor psilocin also exhibits agonist activity at 5-$HT_{2B/C}$ and 5-HT_{1A} receptors, and there is evidence that 5-HT_{2C} receptor activity opposes 5-HT_{2A}-mediated effects[58,59]. Thus, the polypharmacology of 5-HT_{2A} agonists likely plays on the behavioural effects of these drugs.

Conclusion

The present data suggests that the psychedelic drugs tested are not biased towards either 5-HT_{2A} receptor-mediated G_q or β-arrestin2 signalling pathways. The non-psychedelic 5-HT_{2A} receptor agonists lisuride and TBG also did not have a consistent bias. Rather a feature of the latter drugs was their low 5-HT_{2A} receptor efficacy in both G_q and β-arrestin2 signalling pathways. This finding combined with other recent studies reporting low efficacy of other non-psychedelic 5-HT_{2A} receptor agonists, suggests that low efficacy rather than signalling bias plays a key role in their lack of psychedelic effect.

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Conflict of Interest Statement

GG, FW and SH are all employees of Compass Pathways plc.

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Figure Legends

Figure 1. Effect of psychedelic and non-psychedelic drugs on cytosolic Ca^{2+} in SH-SY5Y cells expressing the human 5-HT_{2A} receptor. Each point is the mean ± SEM value of triplicates in two independent experiments. Responses are relative to 10 μM 5-HT.

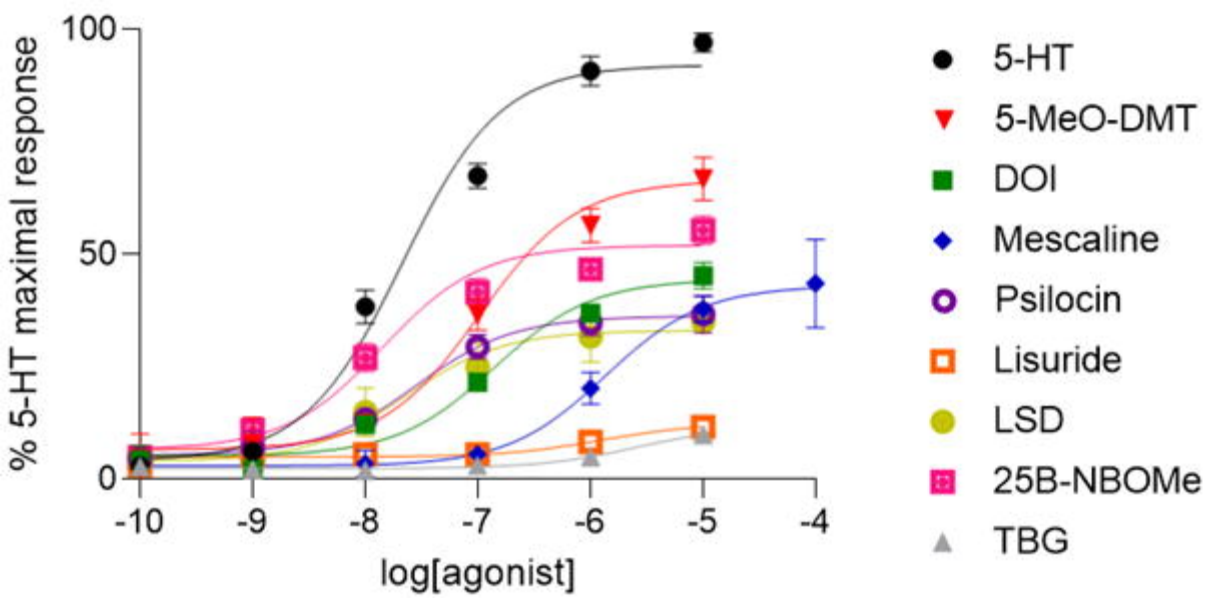
Figure 2. Effect of psychedelic and non-psychedelic drugs on IP_1 accumulation in SH-SY5Y cells expressing the human 5-HT_{2A} receptor. Each point is the mean ± SEM value of triplicates in two independent experiments. Responses are relative to 10 μM 5-HT.

Figure 3. Effect of psychedelic and non-psychedelic drugs on β-arrestin2 recruitment in SH-SY5Y cells expressing the human 5-HT_{2A} receptor. Each point is the mean ± SEM value of triplicates in two independent experiments. Responses are relative to 10 μM 5-HT.

Figure 4. Scatter plot comparing the activity ($\Delta\log(E_{max}/EC_{50})$ values) of psychedelic and non-psychedelic drugs on 5-HT_{2A} receptor-mediated IP_1 and β-arrestin2 signalling pathways in SH-SY5Y cells. In this plot, the more positive the x or y value, the greater activity in a particular pathway. The further a drug deviates from the line of unity, the more biased the agonist. Each point represents a mean ± SEM value.

Figure 5. Effect of psychedelic and non-psychedelic drugs on cytosolic Ca^{2+} in C6 cells expressing the rat 5-HT_{2A} receptor. Each point is the mean ± SEM value of triplicates in two independent experiments. Responses are relative to 10 μM 5-HT.

Figure 6. Effect of the non-psychedelic drugs lisuride (A) and TBG (B) on cytosolic Ca^{2+} in C6 cells expressing the rat 5-HT_{2A} receptor in the presence of 5-HT (10 μM). Each point is the mean ± SEM value of triplicates.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Psilocin
Lisuride
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25B-NBOMe
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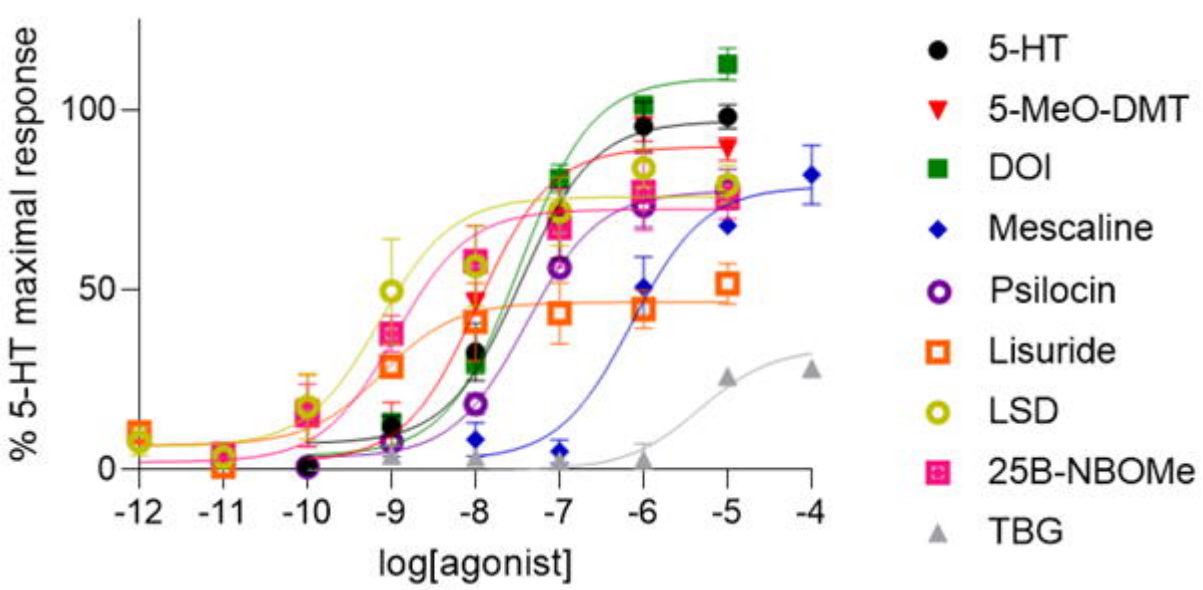
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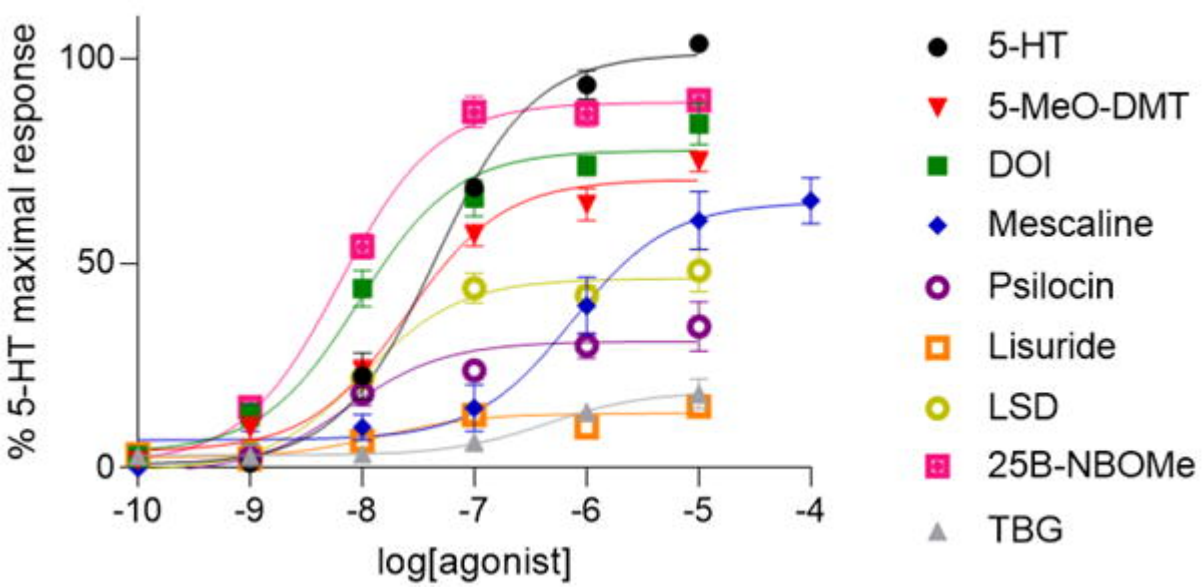
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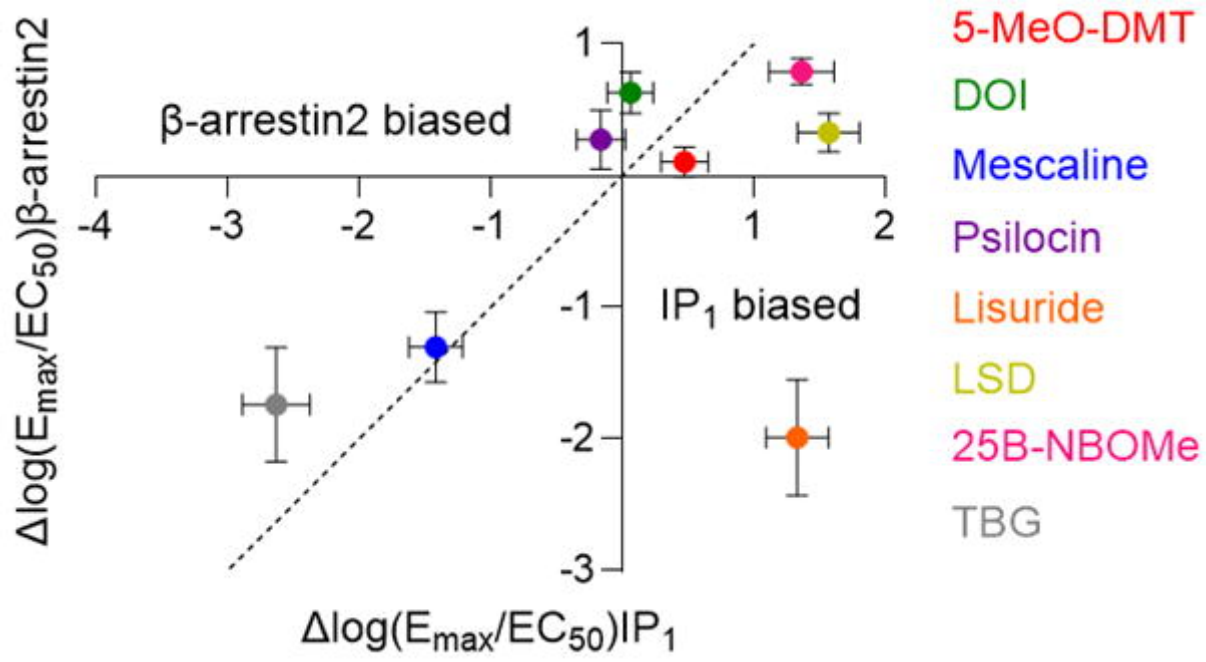
β-arrestin2 biased
IP1 biased
Δlog(Emax/EC50)β-arrestin2
Δlog(Emax/EC50)IP1
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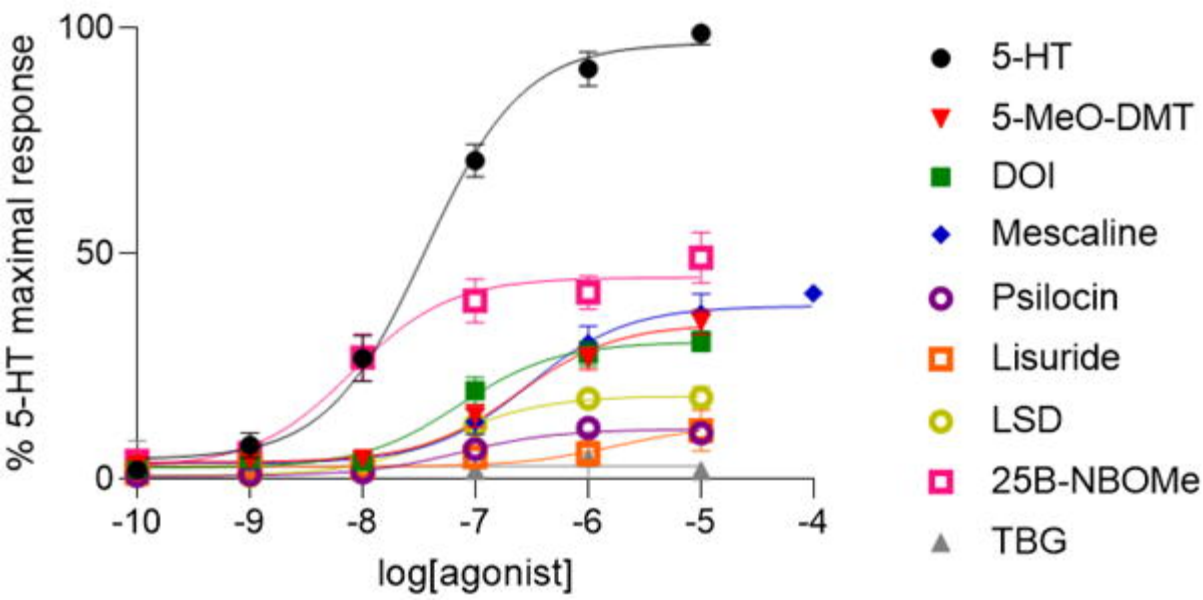
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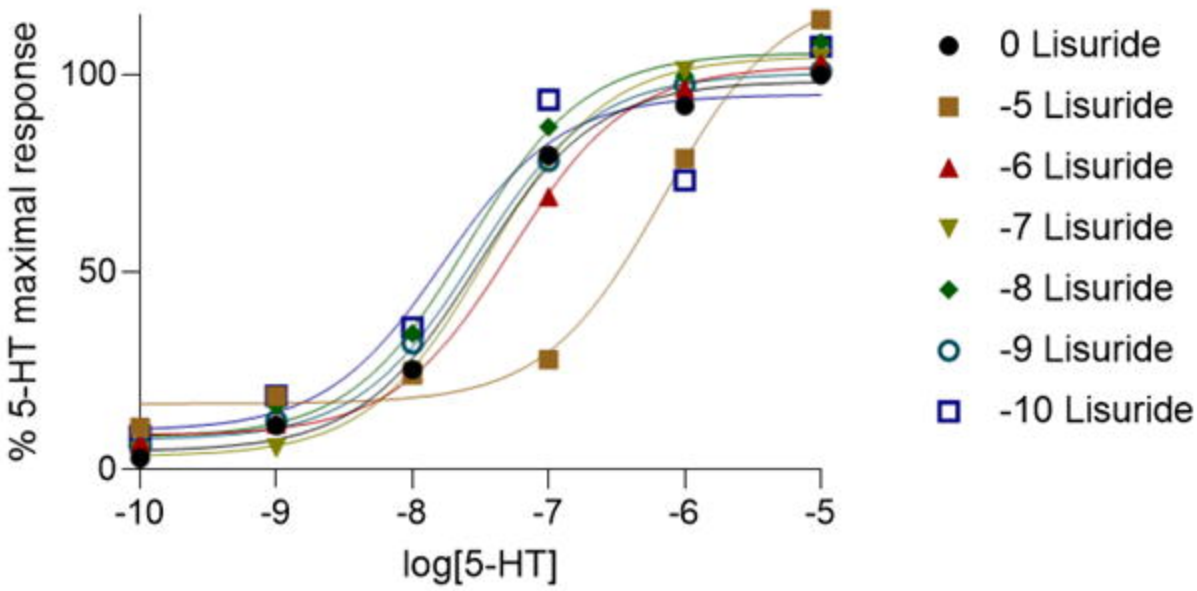

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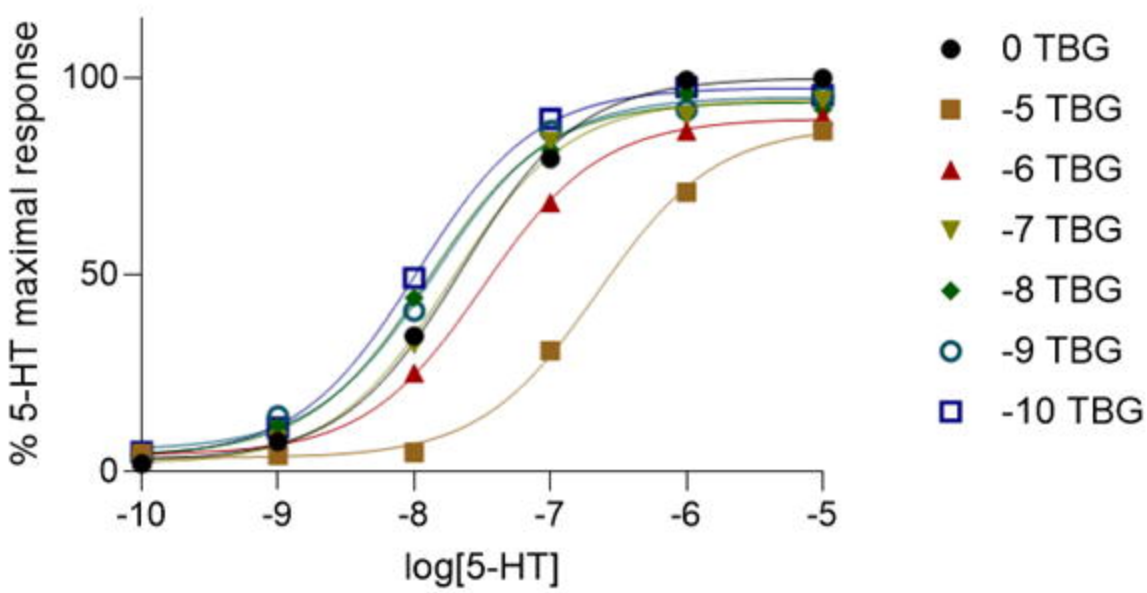

Table 1. Signalling parameters of psychedelic and non-psychedelic drugs at the human 5-HT_{2A} receptor. Data were derived from dose-response curves for Ca^{2+}, IP_1 and β-arrestin2 readouts in SH-SY5Y cells expressing the human 5-HT_{2A} receptor. E_{max}, pEC_{50} and $\Delta\log(E_{max}/EC_{50})$ values are the mean ± SEM of triplicates in two independent experiments. E_{max} and $\Delta\log(E_{max}/EC_{50})$ values are relative to 10 µM 5-HT. Non-psychedelic drugs are denoted by asterisk.

Drug	Ca^{2+}		IP_1		β-arrestin2		Ca^{2+} $\Delta\log(E_{max}/EC_{50})$	IP_1 $\Delta\log(E_{max}/EC_{50})$	β-arrestin2 $\Delta\log(E_{max}/EC_{50})$
	E_{max}	pEC_{50}	E_{max}	pEC_{50}	E_{max}	pEC_{50}			
5-HT	1.00 ± 0.031	7.67 ± 0.10	1.00 ± 0.049	7.46 ± 0.17	1.00 ± 0.018	7.35 ± 0.063	0.00	0.00	0.00
5-MeO-DMT	0.73 ± 0.033	6.99 ± 0.18	0.93 ± 0.027	7.97 ± 0.10	0.70 ± 0.020	7.62 ± 0.095	-0.82 ± 0.21	0.48 ± 0.18	0.10 ± 0.11
DOI	0.49 ± 0.019	6.85 ± 0.13	1.12 ± 0.025	7.48 ± 0.08	0.73 ± 0.027	8.12 ± 0.14	-1.12 ± 0.17	0.06 ± 0.17	0.63 ± 0.16
Mescaline	0.47 ± 0.031	5.87 ± 0.18	0.81 ± 0.050	6.14 ± 0.18	0.64 ± 0.059	6.25 ± 0.26	-2.12 ± 0.21	-1.41 ± 0.20	-1.30 ± 0.27
Psilocin	0.40 ± 0.019	7.55 ± 0.19	0.80 ± 0.030	7.40 ± 0.13	0.28 ± 0.019	8.19 ± 0.21	-0.52 ± 0.22	-0.16 ± 0.19	0.27 ± 0.22
Lisuride*	0.14 ± 0.014	5.91 ± 0.33	0.48 ± 0.031	9.11 ± 0.26	0.21 ± 0.044	6.06 ± 0.43	-2.62 ± 0.35	1.33 ± 0.24	-1.99 ± 0.44
LSD	0.36 ± 0.027	7.64 ± 0.29	0.78 ± 0.039	9.14 ± 0.26	0.44 ± 0.019	8.04 ± 0.13	-0.46 ± 0.31	1.57 ± 0.24	0.33 ± 0.15
25B-NBOMe	0.57 ± 0.021	7.84 ± 0.15	0.75 ± 0.040	8.95 ± 0.28	0.88 ± 0.017	8.20 ± 0.079	-0.06 ± 0.18	1.37 ± 0.25	0.79 ± 0.10
TBG*	0.13 ± 0.033	5.59 ± 0.52	0.35 ± 0.064	5.29 ± 0.29	0.19 ± 0.026	6.35 ± 0.42	-2.96 ± 0.54	-2.63 ± 0.26	-1.74 ± 0.43

Table 2. Potency and efficacy of psychedelic and non-psychedelic drugs at the rat 5-HT_{2A} receptor. Data were derived from dose-response curves for Ca^{2+} readout in C6 cells expressing the rat 5-HT_{2A} receptor. E_{max} and pEC_{50} values are the mean ± SEM of triplicates in two independent experiments. E_{max} values are relative to 10 µM 5-HT. Non-psychedelic drugs are denoted by asterisk. N.D. – not detectable.

Drug	Ca^{2+}	
	E_{max} ± SEM	pEC_{50} ± SEM
5-HT	1.00 ± 0.024	7.44 ± 0.084
5-MeO-DMT	0.36 ± 0.021	6.62 ± 0.16
DOI	0.32 ± 0.017	7.14 ± 0.16
Mescaline	0.40 ± 0.025	6.53 ± 0.19
Psilocin	0.12 ± 0.0079	7.15 ± 0.20
Lisuride*	0.13 ± 0.043	5.74 ± 0.74
LSD	0.19 ± 0.0092	7.25 ± 0.14
25B-NBOMe	0.46 ± 0.027	8.10 ± 0.23
TBG*	N.D.	N.D.