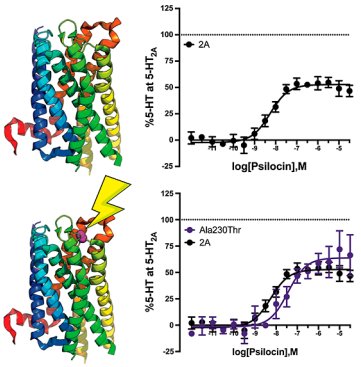
5-HT$_{2A}$ SNPs Alter the Pharmacological Signaling of Potentially Therapeutic Psychedelics

Gavin P. Schmitz, Manish K. Jain, Samuel T. Slocum, and Bryan L. Roth*

ABSTRACT: Serotonin (5-hydroxytryptamine; 5-HT) 2A receptor (5-HT2AR) signaling is essential for the actions of classical psychedelic drugs. In this study, we examined whether sequence variations in the 5-HT2AR gene affect the signaling of four commonly used psychedelic drugs. We examined the *in vitro* pharmacology of seven non-synonymous single-nucleotide polymorphisms (SNPs), which give rise to Ser12Asn, Thr25Asn, Asp48Asn, Ile197Val$^{4.47}$, Ala230Thr, Ala447Val, and His452Tyr variant 5-HT2A serotonin receptors. We found that these non-synonymous SNPs exert statistically significant, although modest, effects on the efficacy and potency of four therapeutically relevant psychedelics. Significantly, the *in vitro* pharmacological effects of the SNP drug actions at 5-HT2AR are drug specific.



KEYWORDS: *5-HT2A, serotonin receptors, hallucinogen, psychedelic, SNP, polymorphism*

1. INTRODUCTION

Psychedelic drugs have been defined as psychoactive compounds that induce changes in cognition, emotion, and perception *via* activation of 5-HT2A serotonin (5-hydroxytryptamine; 5-HT) receptors.[1,2] Recently, there has been renewed interest in psychedelic compounds as potential therapeutics for many neuropsychiatric disorders.[3] For example, psilocybin, the prodrug to the active compound psilocin found in *Psilocybe cubensis* mushrooms, has been granted breakthrough drug status by the Food and Drug Administration due to its potential as a treatment for treatment-resistant depression and anxiety.[4−6] In recent phase II clinical trials, the therapeutic effects of psilocybin are both rapid and apparently enduring.[4,5,7] Similarly, LSD has demonstrated efficacy in treating cluster headaches[8] and alleviating anxiety in terminally ill patients.[9] Finally, anecdotal reports and recent meta-analyses have suggested the potential therapeutic utility for mescaline and 5-methoxy-*N*,*N*-dimethyltryptamine (5-MeO-DMT) in treating anxiety, depression, and other affective disorders.[10−14]

Psychedelics are generally classified by chemical structure (*e.g.*, tryptamines, ergolines, and phenylisopropylamines), and each psychedelic drug has a robust pharmacology, with activities at many serotonin and other biogenic amine receptors.[15−19] 5-HT2A receptor agonism has been shown to be crucial in mediating the psychoactive effects of psychedelics in both animal[20−22] and human studies.[23−26] 5-HT2A receptors are members of the G protein-coupled receptor (GPCR) superfamily and canonically signal through the Gq family of heterotrimeric G proteins activating phospholipase Cβ and many other downstream effector systems.[2,27,28] 5-HT2A receptors also recruit β-arrestin (βArr) proteins *in vitro*,[29] and the 5-HT2A receptor colocalizes with βArr1 and βArr2 in cortical neurons *in vivo*.[30] While many GPCR agonists activate both G protein- and βArr-mediated signaling pathways, certain ligands are known to preferentially activate one signaling pathway *via* a ligand-dependent phenomenon known as functional selectivity or biased signaling.[31] It has been proposed that ligands with biased profiles may be useful for activating desired pathways (*i.e.*, therapeutically efficacious) rather than undesired pathways (*i.e.*, unwanted side effect producing) downstream of a given GPCR.[32] Indeed, LSD is shown to preferentially signal through βArr2 at the 5-HT2A receptor,[18,33] and numerous LSD-elicited responses are either significantly attenuated or completely absent in βArr2-KO mice.[34]

Early clinical studies on psilocybin have shown a wide variability in drug response[35] with a significant portion of respondents showing no statistically significant treatment effect. The reasons for such inter-individual variability in psychedelic drug response are unknown. At the molecular level, random sequence variations in genes (single-nucleotide polymorphisms, SNPs) could explain inter-individual differences in drug response, and such activities are highly relevant as psychedelics

Received: December 10, 2021
Accepted: June 22, 2022
Published: July 27, 2022

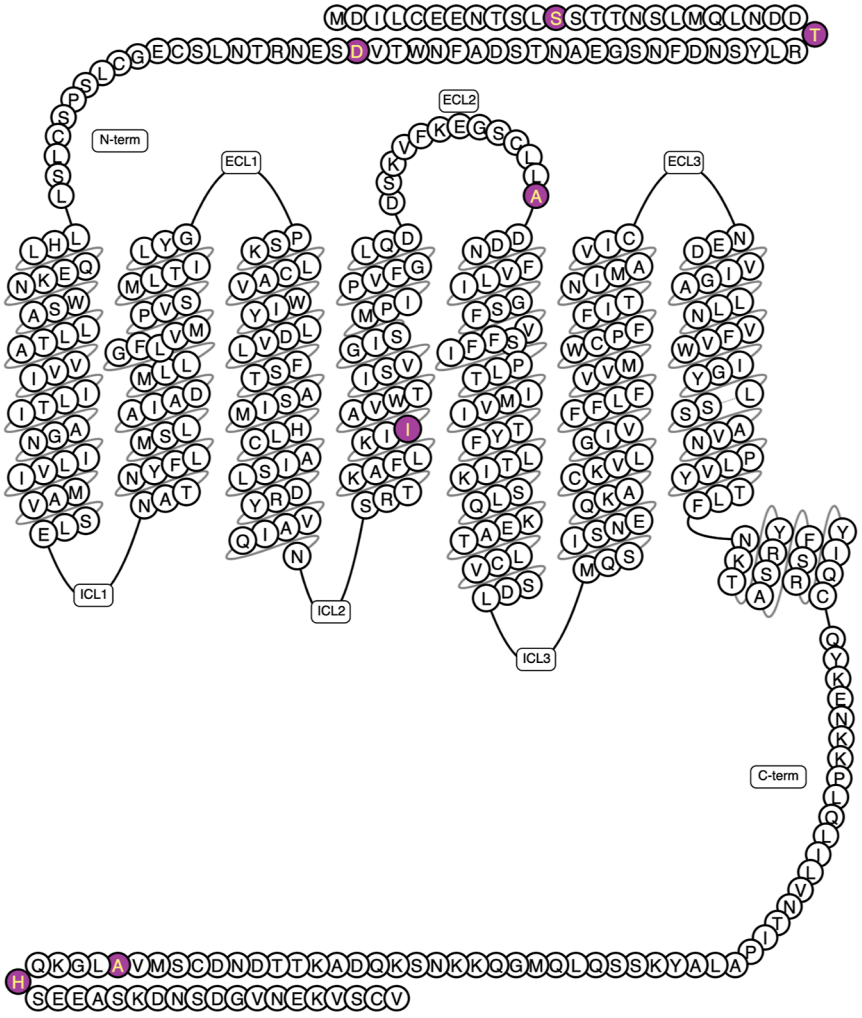


Figure 1. Snake plot representation of the 5-HT$_{2A}$ receptor showing the location of the seven SNPs: Serl2Asn, Thr25Asn, Asp48Asn, lle197Val, Ala230Thr, Ala447Val, and His452Tyr. Snake plot adapted from www.gperdb.org.

become more prevalent in clinical practice. At least seven non-synonymous SNPs are located within the coding region of the human 5-HT2A receptor gene (HTR2A, 13q14-21) (Figure 1). These seven SNPs (Ser12Asn, Thr25Asn, Asp48Asn, Ile197Val[4.47], Ala230Thr, Ala447Val, and His452Tyr) have potential actions on both the receptor structure and function.[36] As shown in Figure 1, the Ser12Asn, Thr25Asn, and Asp48Asn SNPs map to the predicted N-terminal tail of the receptor; the Ile197Val[4.47] SNP resides in the fourth transmembrane helix; the Ala230Thr SNP resides in the putative second extracellular loop (ECL2); and the two remaining non-synonymous SNPs (Ala447Val and His452Tyr) are located within the putative C-terminal tail. These SNPs represent seven of the most common variants observed in human populations with allele frequencies varying from 0.003% (Thr25Asn) to 7.9% (His452Tyr) (Table S1).[37]

Here, we comprehensively examined the potency and signaling bias for four therapeutically promising psychedelics at several 5-HT2A non-synonymous SNPs. We discovered that the effects of individual SNPs were drug selective and that no single SNP had uniform effects on all psychedelics tested. These results imply that future clinical studies into the therapeutic utility of psychedelics might consider particular drug-SNP combinations—paying close attention to those SNPs that cause significant differences in the *in vitro* pharmacologies of the tested agents.

2. RESULTS AND DISCUSSION

2.1. Gaq Protein-Mediated Signaling Activity Potencies for Therapeutically Promising Psychedelics Differ between Wild-Type and Polymorphic 5-HT2A Receptors.

To determine whether naturally occurring, non-synonymous SNPs in the 5-HT2A receptor are likely to affect psychedelic actions *in vivo*, we measured the *in vitro* potencies and efficacies of the wild-type (WT), Ser12Asn, Thr25Asn, Asp48Asn, Ile197Val[4.47], Ala230Thr, Ala447Val, and His452Tyr variant 5-HT2A receptors for 5-HT and four therapeutically promising psychedelics using the TRUPATH Gq bioluminescence resonance energy transfer (BRET) assay. None of the variant 5-HT2A receptors exhibited a difference in the potency of 5-HT from the WT receptor that was statistically significant (Figure S1 and Table S2). As shown in Figure 2 and Table 1, certain polymorphic 5-HT2A receptors exhibited statistically significant, albeit modest, changes in response to specific psychedelics. The Ala230Thr and His452Tyr receptors displayed a 7-fold decrease in potency for psilocin compared to WT (38 nM *vs* 5.4 nM, $P < 0.05$). The Ala447Val 5-HT2A receptor displayed a 3-fold increase in potency for 5-MeO-DMT compared to the WT (3.2 nM *vs* 10.6 nM, $P < 0.05$). Four polymorphic 5-HT2A receptors exhibited statistically significant changes in mescaline potency. Of these, the largest effect was observed for the

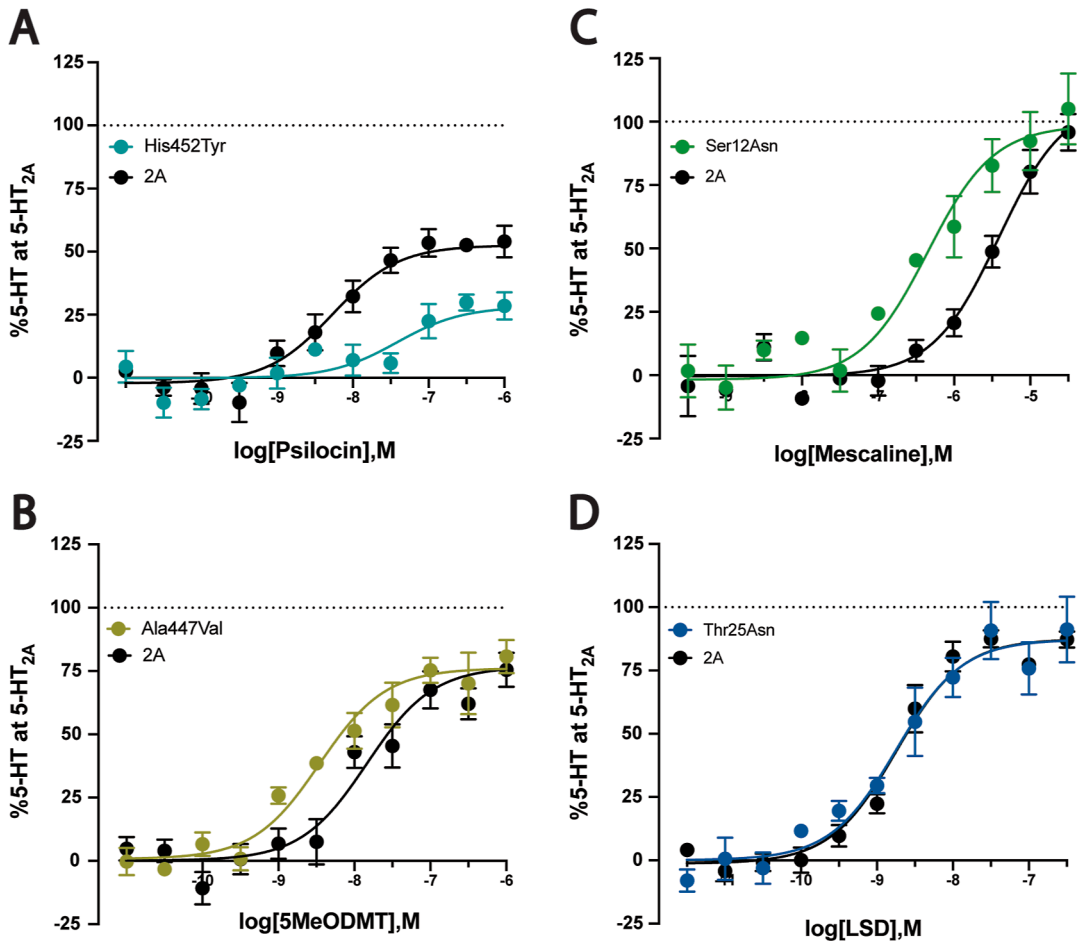


Figure 2. Averaged Gq dissociation concentration−response curves for psychedelic drugs (a) psilocin, (b) 5-MeODMT, (c) mescaline, and (d) LSD at wild-type and polymorphic 5-HT_{2A} receptors using TRUPATH Gq BRET assays. Raw data were normalized, with 0% being the baseline signal and 100% being the maximum 5-HT-stimulated signal at the wild-type receptor, and then fit to a three-parameter logistic equation. For each agonist at each receptor, data from at least three independent experiments (each measured in duplicate) were analyzed simultaneously, with the log EC_{50} shared among data sets. See Tables 1 and S3 for the complete data matrix.

Table 1. Potency (pEC_{50}) Values for Several Potentially Therapeutic Psychedelics at Wild-Type (WT) and Polymorphic 5-HT2A Receptors as Measured Using TRUPATH Gq BRET Assays. *P < 0.05, **P < 0.005, and ***P < 0.0005 by the F-test

	TRUPATH Gq BRET, pEC_{50} ± s.e. (EC_{50}, nM)							
drug	WT	Ser12Asn	Thr25Asn	Asp48Asn	Ile197Val	Ala230Thr	Ala447Val	His452Tyr
psilocin	8.270 ± 0.137 (5.38)	7.98 ± 0.132 (10.47)	8.126 ± 0.088 (7.48)	8.034 ± 0.119 (9.25)	8.223 ± 0.113 (5.99)	**7.421 ± 0.230* (38.0)**	8.422 ± 0.085 (3.78)	**7.419 ± 0.247* (38.2)**
5-MeODMT	7.975 ± 0.135 (10.6)	7.711 ± 0.175 (19.5)	8.246 ± 0.155 (5.67)	7.820 ± 0.117 (15.1)	7.795 ± 0.132 (16.0)	8.386 ± 0.252 (4.11)	**8.489 ± 0.097* (3.24)**	8.442 ± 0.226 (3.61)
mescaline	5.413 ± 0.108 (3860)	**6.350 ± 0.126*** (447)**	**6.060 ± 0.120*** (871)**	**6.149 ± 0.153*** (710)**	5.678 ± 0.145 (2100)	5.775 ± 0.211 (1680)	**6.025 ± 0.170** (943)**	5.577 ± 0.159 (2650)
LSD	8.732 ± 0.074 (1.85)	8.601 ± 0.126 (2.50)	8.773 ± 0.129 (1.69)	8.862 ± 0.111 (1.37)	8.615 ± 0.102 (2.43)	8.716 ± 0.225 (1.92)	8.769 ± 0.076 (1.70)	8.441 ± 0.192 (3.63)

Ser12Asn 5-HT2A receptor, which displayed a 9-fold increase in potency for mescaline compared to the WT (447 nM *vs* 3860 nM, P < 0.0005). None of the SNP-induced alterations in LSD potency were significantly different from the WT value.

The relative amplitude (E_{max} or efficacy) of agonist-induced stimulation for each SNP was analyzed by normalizing to the 5-HT response at the WT 5-HT2A receptor. As shown in Figure S1 and Tables S2 and S3, the His452Tyr 5-HT2A receptor displayed significant reductions from the WT in relative E_{max} for 5-HT and all the psychedelics examined herein. Similarly, the Ile197Val$^{4.47}$ 5-HT2A receptor displayed a significant reduction in relative E_{max} for 5-HT and LSD. The Ser12Asn 5-HT2A receptor was the only SNP to demonstrate a significant increase in relative E_{max} for 5-HT as well as Psilocin, 5-MeO-DMT, and LSD. Several polymorphisms did not significantly alter the relative E_{max} for 5-HT but did cause significant changes in the relative E_{max} of the psychedelics studied. Thr25Asn displayed an increased response to psilocin (73.5% *vs* 54.7%, P < 0.0005) and 5-MeO-DMT (94.4% *vs* 72.3%, P < 0.005). Asp48Asn showed an increased response to psilocin (60.0% *vs* 54.7%, P < 0.05) and 5-MeO-DMT (94.8% *vs* 72.3%, P < 0.0005) but a decreased response to mescaline (81.0% *vs* 109%, P < 0.05). Ala230Thr showed a near 50% reduction in the response to mescaline (58.4% *vs* 109%, P < 0.0005). Finally, Ala447Val showed an

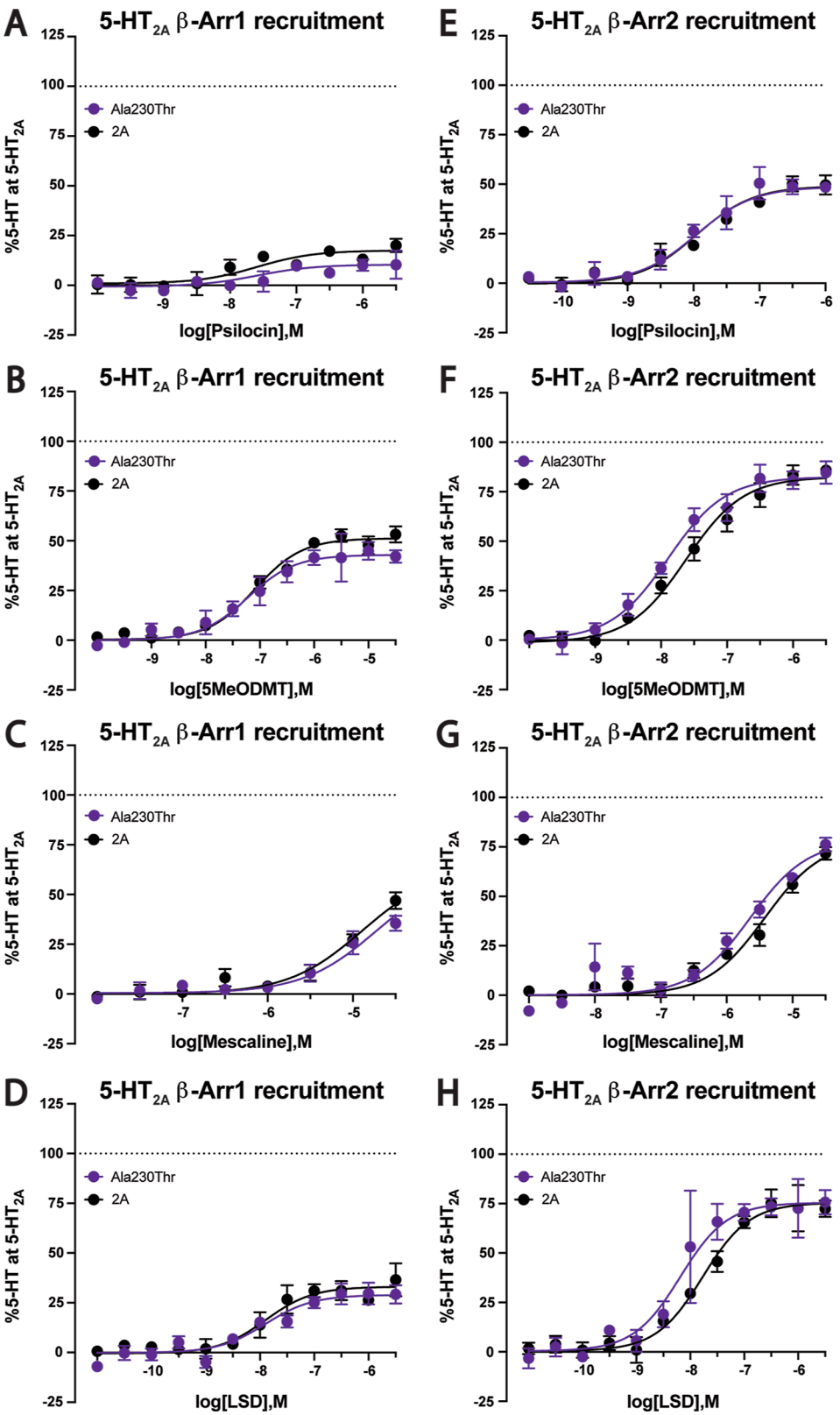


Figure 3. Averaged concentration−response curves for psychedelic drugs (a) psilocin, (b) 5-MeODMT, (c) mescaline, and (d) LSD at wild-type and polymorphic Ala230Thr 5-HT_{2A} receptors using β-arrestin-1 recruitment BRET assay. Averaged concentration−response curves for (e) psilocin, (f) 5-MeODMT, (g) mescaline, and (h) LSD at wild-type and polymorphic Ala230Thr 5-HT_{2A} receptors using β-arrestin-2 recruitment BRET assay. For both assays, raw data were normalized, with 0% being the baseline signal and 100% being the maximum 5-HT-stimulated signal at the wild-type receptor, and then fit to a three-parameter logistic equation. For each agonist at each receptor, data from at least three independent experiments (each measured in duplicate) were analyzed simultaneously, with the log EC_{50} shared among data sets. See Tables 2, 3, S6, and S7 for the complete data matrix.

Table 2. Potency (pEC_{50}) Values for Several Potentially Therapeutic Psychedelics at Wild-Type (WT) and Polymorphic 5-HT2A Receptors as Measured Using βArr1 Recruitment BRET Assays

	Arr1 recruitment BRET, pEC_{50} ± s.e. (EC_{50}, nM)							
drug	WT	Ser12Asn	Thr25Asn	Asp48Asn	Ile197Val	Ala230Thr	Ala447Val	His452Tyr
psilocin	7.853 ± 0.352 (14.04)	6.907 ± 0.351 (123.9)	7.631 ± 0.375 (23.38)	7.681 ± 0.302(20.84)	8.090 ± 0.302(8.13)	7.101 ± 0.390(79.21)	7.385 ± 0.256(41.20)	7.726 ± 0.328(18.81)
5-MeODMT	7.088 ± 0.073(81.68)	7.169 ± 0.190 (67.75)	7.073 ± 0.177 (84.57)	7.154 ± 0.123(70.22)	7.078 ± 0.170(83.48)	7.222 ± 0.144(59.95)	7.459 ± 0.245(34.73)	7.105 ± 0.142(78.44)
mescaline	4.786 ± 0.162 (16,370)	5.421 ± 0.350 (3797)	4.226 ± 0.576 (59,440)	5.296 ± 0.211 (5057)	5.062 ± 0.304 (8669)	5.023 ± 0.167 (9494)	4.926 ± 0.334 (11,870)	5.408 ± 0.243 (3905)
LSD	7.942 ± 0.175 (11.42)	7.742 ± 0.214 (18.10)	7.643 ± 0.186 (22.74)	7.813 ± 0.172 (15.37)	7.780 ± 0.182 (16.61)	7.793 ± 0.164 (16.12)	8.050 ± 0.255 (8.92)	7.583 ± 0.243 (26.12)

Table 3. Potency (pEC_{50}) Values for Several Potentially Therapeutic Psychedelics at Wild-Type (WT) and Polymorphic 5-HT2A Receptors as Measured Using βArr2 Recruitment BRET Assays. *$P < 0.05$ and **$P < 0.005$ by the *F*-test

	Arr2 recruitment BRET, pEC_{50} ± s.e. (EC_{50}, nM)							
drug	WT	Ser12Asn	Thr25Asn	Asp48Asn	Ile197Val	Ala230Thr	Ala447Val	His452Tyr
psilocin	7.812 ± 0.094(15.42)	8.067 ± 0.155 (8.57)	7.802 ± 0.120 (15.77)	7.611 ± 0.129 (24.50)	7.878 ± 0.094 (13.24)	8.083 ± 0.119(8.26)	7.845 ± 0.127(14.30)	7.927 ± 0.097(11.84)
5-MeODMT	7.595 ± 0.064(25.40)	7.629 ± 0.094 (23.51)	7.624 ± 0.074 (23.75)	7.647 ± 0.086 (22.56)	7.707 ± 0.088 (19.62)	**7.916 ± 0.071**(12.13)**	7.482 ± 0.127(32.98)	7.523 ± 0.77(30.00)
mescaline	5.410 ± 0.089(3887)	5.266 ± 0.118 (5421)	5.383 ± 0.105 (4143)	5.343 ± 0.096 (4537)	5.363 ± 0.112 (4333)	**5.675 ± 0.076*(2114)**	5.258 ± 0.154(5527)	5.324 ± 0.076(4748)
LSD	7.781 ± 0.086 (16.57)	7.887 ± 0.090 (12.97)	7.924 ± 0.063 (11.92)	7.868 ± 0.88(13.56)	7.720 ± 0.062 (19.05)	**8.179 ± 0.120*(6.62)**	7.755 ± 0.150(17.59)	7.641 ± 0.091(22.84)

increased response to psilocin (62.5% *vs* 54.7%, $P < 0.005$) but a decreased response to mescaline (74.9% *vs* 109%, $P < 0.005$).

2.2. β-Arrestin Recruitment for Therapeutically Promising Psychedelics Differ between Wild-Type and Polymorphic 5-HT2A Receptors. Having addressed whether several therapeutically promising psychedelics exhibit differential *in vitro* Gaq protein signaling pharmacologies at WT and polymorphic 5-HT2A receptors, we next investigated the effects on βArr recruitment. None of the variant 5-HT2A receptors exhibited a difference in the potency of 5-HT from the WT receptor that was statistically significant (Figures S2, S3) for βArr1 or βArr2 recruitment. As shown in Figure 3 and Table 2, the βArr1 potencies of all psychedelics tested were not significantly altered by any of the SNP 5-HT2A receptors tested. In contrast, the βArr2 potencies of 5-MeO-DMT, mescaline, and LSD tested at the Ala230Thr 5-HT2A receptor were significantly altered. Specifically, the 5-MeO-DMT potency was increased 2-fold compared to WT (12.1 nM *vs* 25.4 nM, $P < 0.005$), the mescaline potency was increased 2-fold compared to WT (2114 nM *vs* 3887 nM, $P < 0.05$), and the LSD potency was increased 3-fold compared to WT (6.62 nM *vs* 16.6 nM, $P < 0.05$). As shown in Figure 3 and Table 3, the Ala230Thr 5-HT2A receptor was the only SNP found to cause significant changes in βArr2 potencies of 5MeODMT, LSD, and mescaline.

The relative amplitudes (E_{max} or efficacy) of agonist-induced stimulation for each SNP were again analyzed by normalizing to the 5-HT response at the WT 5-HT2A receptor. As shown in Figure S2 and Table S4, the Ser12Asn, Thr25Asn, Ile197Val$^{4.47}$, and Ala230Thr receptors exhibited statistically significant reductions in relative E_{max} in βArr1 recruitment for 5-HT. As shown in Figure S3 and Table S5, Ser12Asn, Thr25Asn, and Ile197Val$^{4.47}$ also exhibited statistically significant reductions in relative E_{max} in βArr2 recruitment for 5-HT in addition to Asp48Asn.

None of the polymorphic 5-HT2A receptors demonstrated a significant increase in βArr1 recruitment compared to the WT receptor for any of the psychedelics tested. Additionally, every polymorphic 5-HT2A receptor tested demonstrated a significant reduction in relative E_{max} of βArr1 recruitment for 5-MeO-DMT with Ile197Val$^{4.47}$ displaying the largest reduction (30.1% *vs* 51.0%, $P < 0.0005$). For βArr2 recruitment, the Ala447Val 5-HT2A receptor did not significantly alter the response to 5-HT but displayed significant reductions from the WT in relative E_{max} for all the psychedelics examined herein. Similarly, His452Tyr did not alter the relative E_{max} for 5-HT but did significantly reduce the relative E_{max} for psilocin and LSD.

2.3. Bias Calculations for Wild-Type and Polymorphic 5-HT2A Receptors. Having addressed whether several SNPs exhibit different *in vitro* signaling for several potentially therapeutic psychedelics, we next quantified changes in bias using the approach of Kenakin and colleagues.[38] Using the concentration−response data generated at 5 min for the assays described previously (Figures 2 and 3), we used the operational model to calculate transduction coefficients $\log(E_{max}/EC_{50})$ for each psychedelic at each pathway. Given that we wanted to compare changes in bias across the different receptor polymorphisms, the $\log(E_{max}/EC_{50})$ values of each receptor polymorphism were normalized to that of the WT receptor for each drug $\Delta\log(E_{max}/EC_{50})$. Changes in the relative strengths of each signaling pathway for each polymorphism at a given psychedelic drug were compared to give a $\Delta\Delta\log(E_{max}/EC_{50})$ or LogBias value. The LogBias values were represented graphically in Figure 4 as box and whiskers plots to illustrate the changes in the bias for each polymorphism. In these plots, the $\Delta\Delta\log(E_{max}/EC_{50})$ for the WT receptor is equal to 0. As shown in Figure 4, the overall biases [$\Delta\Delta\log(E_{max}/EC_{50})$ values] for the investigated pathways were significantly altered by multiple SNPs. Psilocin signaling was significantly altered by the Ser12Asn, Ala230Thr, Ala447Val, and His452Tyr SNPs. The Ser12Asn 5-HT2A receptor significantly favored βarr1 recruitment over Gaq signaling but did not affect the relative bias between the βarr2 and Gaq signaling pathways. The His452Tyr 5-HT2A receptor showed the greatest change in signaling, favoring Gaq signaling over βarr1 and βarr2. For 5-MeO-DMT, the His452Tyr 5-

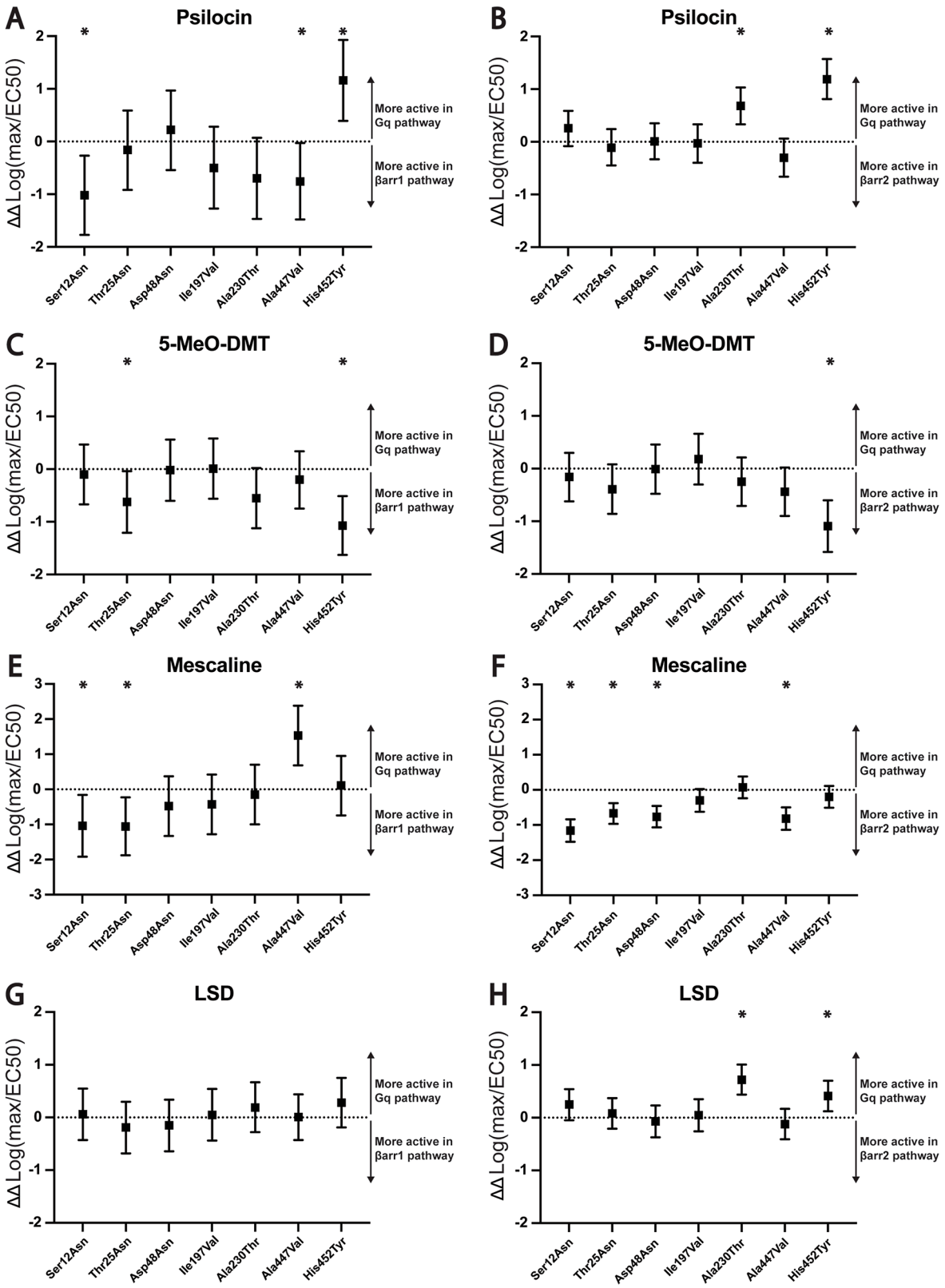


Figure 4. Bias plots showing $\Delta\Delta\log(E_{max}/EC_{50})$ values as a measure of ligand bias between signaling pathways. Bias of the wild-type receptor to each ligand is calculated to be zero. The error bars represent 95% confidence interval; when the interval includes zero, the polymorphisms are not signification biased in respect to the WT receptor. (A,B) Psilocin, (C,D) 5-MeO-DMT, (C,F) mescaline, and (G,H) LSD. (A,C,E,G) Gq vs βarr1 and (B,D,F,H) Gq vs βarr2.

HT2A receptor also demonstrated the largest change conversely increasing βarr1 and βarr2 recruitment relative to Gaq signaling. Mescaline signaling was significantly altered by the Ser12Asn, Thr25Asn, Asp48Asn, and Ala447Val 5-HT2A receptors with the largest changes observed for the Ala447Val 5-HT2A receptor. Only the Ala230Thr and His452Tyr 5-HT2A

receptors demonstrated altered LSD signaling profiles, favoring Gaq over βarr2 in both cases.

2.4. Psychedelic Binding and Signaling Kinetics. Changes in receptor agonism and bias over time have been documented.[39] The βarr2 signaling of LSD for instance has proven to increase in potency and efficacy over time;[18] however, the signaling kinetics of psilocin, 5-MeO-DMT, and mescaline have not been well elucidated. To determine whether such changes take place, we measured the *in vitro* Gaq and βArr2 protein signaling pharmacologies at 5 min, 30 min, 1 h, 2 h, and 3 h time points for the WT 5-HT2A receptor. Importantly, all agonists maintained a robust signal up to the 3 h time point in both the TRUPATH Gq BRET and βArr2 recruitment BRET assays. As shown in Figure 5 and Tables 4 and 5, Psilocin exhibited a significant increase in Gaq signaling over the 3 h time course (27.2 nM at 5 min *vs* 3.68 nM at 3 h, $P < 0.05$) and a larger decrease in βArr2 recruitment over the same time course (71.1 nM at 5 min *vs* 730 nM at 3 h, $P < 0.05$), suggesting an increased biased toward Gaq signaling over time. 5-MeO-DMT exhibited a similar increase in Gaq signaling over the 3 h time course (33.0 nM at 5 min *vs* 6.97 nM at 3 h, $P < 0.0001$) with no significant change in βArr2 recruitment (78.6 nM at 5 min *vs* 132 nM at 3 h), again suggesting an increased biased toward Gaq signaling over time. Mescaline also exhibited a similar increase in Gaq signaling over the 3 h time course (2380 nM at 5 min *vs* 425 nM at 3 h, $P < 0.0001$) and no apparent change in βArr2 recruitment (due to poor curve fit, a pEC_{50} was unable to be attained at the 5 min time point in these kinetic experiments).

Consistent with previous studies, we measured the *in vitro* Gaq and βArr2 protein signaling pharmacology of LSD at 5 min, 30 min, 1 h, 2 h, and 5 h time points for the WT 5-HT2A receptor. As shown in Figure 5 and Tables 6 and 7, of the compounds tested, LSD displayed the largest changes in potency of Gaq signaling and βArr2 recruitment over time. Gaq signaling over the 5 h time course displayed an approximately 550-fold increase in potency (5.91 nM at 5 min *vs* 0.0105 nM at 5 h, $P < 0.0001$), and βArr2 recruitment displayed a 200-fold increase in potency (16.0 nM at 5 min *vs* 0.0780 nM at 5 h, $P < 0.0001$). These large changes in the potencies of the concentration response curves for LSD reflected its long residence time. Of note, the kinetic profile of 5-HT (Figure S4) shows modest increases in the potency and efficacy of Gaq signaling and modest reductions in the potency and efficacy of βArr2 recruitment.

Given that LSD displayed the largest changes in signaling kinetics, we measured the *in vitro* signaling profiles of the WT and variant 5-HT2A receptors at LSD over a 5 h time course. Consistent with our earlier study, we observed no changes in Gaq signaling at either the initial 5 min time point or any later time points. For βArr2 recruitment, we again saw that the Ala230Thr 5-HT2A receptor was the only SNP found to cause significant changes at the 5 min time point compared to WT (2.40 nM *vs* 16.0 nM, $P < 0.05$). None of the SNPs differed in βArr2 potencies at the 30 min or 1 h time points; however, the His452Tyr SNP caused significant changes in βArr2 potency for LSD at the 2 h (0.387 nM *vs* 0.109 nM, $P < 0.05$) and 5 h time points (0.185 nM *vs* 0.0780 nM, $P < 0.05$) compared to WT.

2.5. Effects of 5-HT2A Receptor SNPs on Receptor Expression. To determine whether the 5-HT2A receptor SNPs affected the receptor density, we measured the maximum binding (B_{max}) for each by radioligand saturation binding assay as shown in Figure 6 and Table 8. Of note, in non-transfected cells, no significant binding to ^{3}H ketanserin was observed, suggesting that non-transfected cells do not endogenously express any receptors that bind to the radioligand (Figure S5).

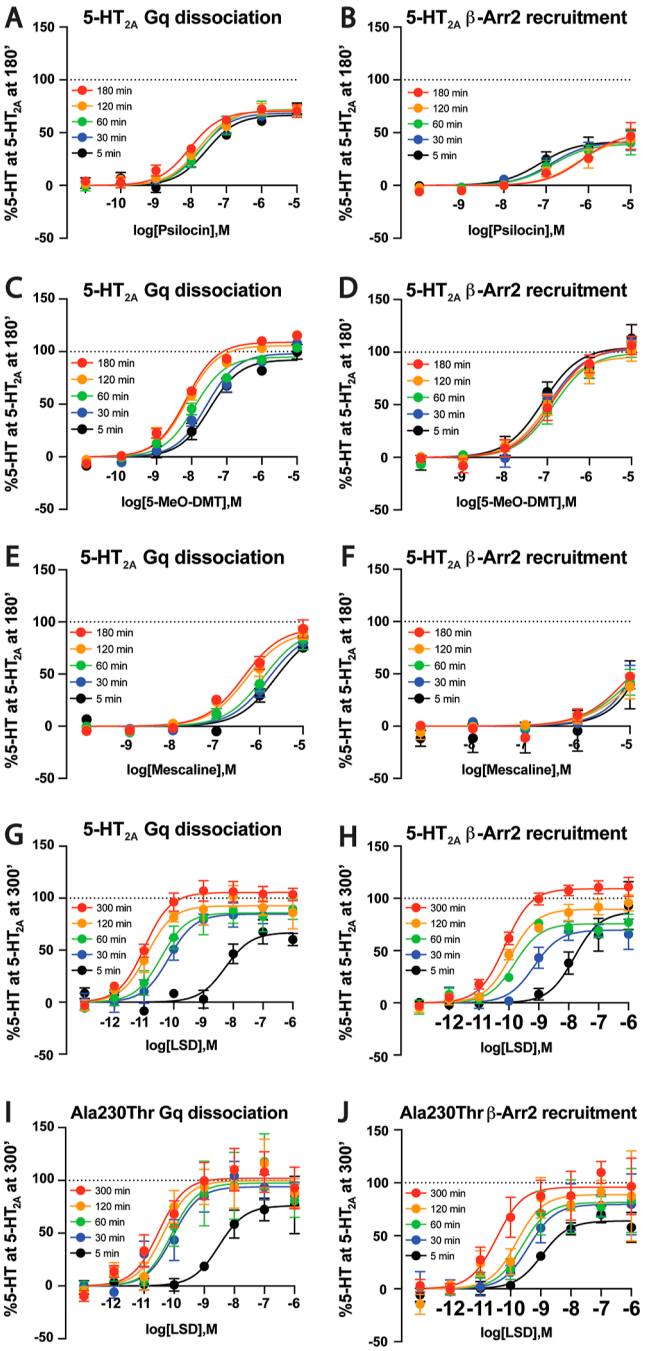


Figure 5. Kinetic measurements of Gq dissociation for (a) psilocin, (c) 5-MeODMT, (e) mescaline, and (g) LSD at wild-type 5-HT_{2A} receptor using TRUPATH Gq BRET assays. Kinetic measurements of β-arrestin-2 recruitment for (b) psilocin, (d) 5-MeODMT, (f) mescaline, and (h) LSD at wild-type 5-HT_{2A} receptor using β-arrestin-2 recruitment BRET assay. Kinetic measurements of Gq dissociation (i) and (β-arrestin-2 recruitment) (j) for LSD at the polymorphic Ala230Thr 5-HT_{2A} receptor. Raw data were normalized, with 0% being the baseline signal and 100% being the maximum 5-HT-stimulated signal at the wild-type receptor, and then fit to a three-parameter logistic equation. For each agonist at each receptor, data from at least three independent experiments (each measured in duplicate) were analyzed simultaneously, with the log EC_{50} shared among data sets. See Tables 4, 5, 6, and 7 for the complete data matrix.

Table 4. Potency (pEC_{50}) Values for Kinetic Measurements of Gq Dissociation for Psilocin, 5-MeODMT, and Mescaline at Wild-Type (WT) 5-HT2A Receptors as Measured Using TRUPATH Gq BRET Assays

	TRUPATH Gq BRET, pEC_{50} ± s.e. (EC_{50}, nM)		
drug	psilocin	5-MeODMT	mescaline
5 min	7.565 ± 0.144 (27.20)	7.482 ± 0.120 (32.97)	5.624 ± 0.128 (2378)
30 min	7.726 ± 0.109 (18.81)	7.574 ± 0.101 (26.67)	5.792 ± 0.117 (1615)
60 min	7.672 ± 0.130 (21.30)	7.919 ± 0.075 (12.04)	5.917 ± 0.124 (1212)
120 min	7.777 ± 0.098 (9.160)	8.134 ± 0.079 (7.351)	6.309 ± 0.113 (491.3)
180 min	8.038 ± 0.130 (3.683)	8.157 ± 0.068 (6.965)	6.371 ± 0.111 (425.2)

Table 5. Potency (pEC_{50}) Values for Kinetic Measurements of βArr2 Recruitment for Psilocin, 5-MeODMT, and Mescaline at Wild-Type (WT) 5-HT2A Receptors as Measured Using βArr2 Recruitment BRET Assays

	βarr2 recruitment BRET, pEC_{50} ± s.e. (EC_{50}, nM)		
drug	psilocin	5-MeODMT	mescaline
5 min	7.148 ± 0.235 (71.11)	7.104 ± 0.143 (78.62)	NA
30 min	6.882 ± 0.221 (131.3)	6.952 ± 0.112 (111.8)	2.463 ± 161.507
60 min	6.850 ± 0.237 (141.2)	6.848 ± 0.109 (142.0)	5.074 ± 0.539 (8437)
120 min	6.240 ± 0.245 (575.0)	7.007 ± 0.124 (98.46)	5.058 ± 0.732 (8752)
180 min	6.137 ± 0.232 (729.5)	6.879 ± 0.130 (132.0)	5.035 ± 0.523 (9217)

None of the SNPs altered the receptor density more than 3-fold compared to the WT. The largest alteration of receptor density was seen in the Ser12Asn 5-HT2A receptor which was, on average, increased less than 3-fold relative to the WT receptor. As we previously demonstrated, our BRET-based assays are relatively insensitive to differences in receptor density.[40] We note that even though the Ser12Asn and Ala230Thr polymorphisms were both expressed at levels higher than WT, no uniform differences in agonist potencies or efficacies were observed. Thus, the differences observed between the *in vitro* pharmacology of the seven variant 5-HT2A receptors tested probably do not arise due to differential expression.

3. DISCUSSION

The major finding of this study is that certain 5-HT2A receptor non-synonymous SNPs can alter the *in vitro* pharmacology of the tested psychedelics. As we have demonstrated in the current study, even polymorphisms far removed from the orthosteric binding site of the receptor can have significant effects on the *in vitro* pharmacology of a given drug. Previous clinical studies have already demonstrated statistically significant associations between 5-HT2A receptor SNPs and atypical antipsychotic drug response.[41,42] Given that several psychedelic drugs are in clinical trials, including the ones in this study, this information can aid in the design and ultimate interpretation of clinical studies.

Our prior study[36] examined some of these polymorphisms on the agonist and antagonist actions of several drugs, although functional selectivity was not addressed. Additionally, of the compounds tested in the current study, only 5-MeO-DMT was examined. Based on the current results, our data imply that individuals carrying the Ala230Thr variant might show altered responses to all of the psychedelics studied given that Gaq and/or βarr2 signaling was altered. Additionally, we would predict that individuals carrying the His452Tyr variants might show altered responses to psilocin and psilocybin, as large decreases in psilocin Gaq potency were found to significantly alter the signaling bias. Additionally, for the His452Tyr polymorphism, we saw significant reductions in efficacy for the reference compound 5-HT and all drugs included in this study. These results are consistent with prior work showing a reduced ability of the His452Tyr variant to activate phospholipases C and D, thus hindering the Gq signaling pathway.[43,44] For mescaline, the most dramatic change was seen at the Ser12Asn polymorphism with an approximately 10-fold increase in potency.

Of note, examination of functional potency and efficacy at a single 5 min time point may mask important signaling phenomena. βarr2 signaling of LSD, for instance, has been shown to increase in potency and efficacy over time.[18] Therefore, we collected signaling data for several time points after initial receptor activation by drug. These data revealed significant differences among drug chemotypes as well as between different 5-HT2A SNPs. Of the drugs tested, psilocin, 5-MeO-DMT, and mescaline exhibited moderate shifts in potency and efficacy over the time course. However, LSD exhibited dramatic kinetic effects both in WT and in mutant 5HT2A, and additionally, two SNPs (Ala230Thr and His452Tyr) had significantly altered pharmacology during βarr2 signaling. Interestingly, Ala230Thr appears to increase the potency of LSD at the earliest time point but does not alter the maximal βarr2 potency and efficacy

Table 6. Potency (pEC_{50}) Values for Kinetic Measurements of Gq Dissociation for LSD at Wild-Type (WT) and Polymorphic 5-HT2A Receptors as Measured Using TRUPATH Gq BRET Assays

	TRUPATH Gq BRET, pEC_{50} ± s.e. (EC_{50}, nM)							
LSD	WT	Ser12Asn	Thr25Asn	Asp48Asn	Ile197Val	Ala230Thr	Ala447Val	His452Tyr
5 min	8.228 ± 0.233 (5.91)	8.661 ± 0.257 (2.18)	8.658 ± 0.378 (2.20)	8.418 ± 0.317 (3.82)	8.844 ± 0.333 (1.43)	8.498 ± 0.315 (3.18)	8.565 ± 0.281 (2.72)	8.529 ± 0.358 (2.96)
30 min	10.177 ± 0.189 (0.0665)	10.240 ± 0.357 (0.0576)	10.271 ± 0.324 (0.0536)	9.780 ± 0.460 (0.166)	10.026 ± 0.208 (0.0942)	9.998 ± 0.258 (0.101)	10.338 ± 0.262 (0.0459)	10.005 ± 0.233 (0.0989)
60 min	10.456 ± 0.257 (0.0350)	10.664 ± 0.201 (0.0217)	10.520 ± 0.378 (0.0302)	10.070 ± 0.335 (0.0851)	9.988 ± 0.228 (0.103)	10.079 ± 0.390 (0.0834)	10.378 ± 0.252 (0.0418)	10.231 ± 0.205 (0.0588)
120 min	10.880 ± 0.201 (0.0132)	10.461 ± 0.164 (0.0346)	10.935 ± 0.325 (0.116)	10.633 ± 0.212 (0.0233)	10.842 ± 0.452 (0.0144)	10.350 ± 0.306 (0.0447)	10.566 ± 0.162 (0.0272)	10.427 ± 0.284 (0.0374)
300 min	10.979 ± 0.130 (0.0105)	11.236 ± 0.308 (0.00581)	11.163 ± 0.188 (0.00687)	10.653 ± 0.249 (0.0222)	10.873 ± 0.243 (0.0134)	10.463 ± 0.301 (0.0344)	10.586 ± 0.135 (0.0259)	10.629 ± 0.172 (0.0235)

Table 7. Potency (pEC_{50}) Values for Kinetic Measurements of βArr2 Recruitment for LSD at Wild-Type (WT) and Polymorphic 5-HT2A Receptors as Measured Using βArr2 Recruitment BRET Assays. *$P < 0.05$ by the *F*-test

	βarr2 recruitment BRET, pEC_{50} ± s.e. (EC_{50}, nM)							
LSD	WT	Ser12Asn	Thr25Asn	Asp48Asn	Ile197Val	Ala230Thr	Ala447Val	His452Tyr
5 min	7.797 ± 0.245 (16.0)	7.811 ± 0.359 (15.5)	7.895 ± 0.478 (12.7)	7.822 ± 0.207 (15.1)	8.043 ± 0.190 (9.05)	**8.620 ± 0.205*** **(2.40)**	7.606 ± 0.298 (24.8)	7.613 ± 0.247 (24.4)
30 min	9.151 ± 0.226 (0.707)	9.446 ± 0.254 (0.358)	9.035 ± 0.330 (0.922)	9.009 ± 0.253 (0.978)	9.435 ± 0.335 (0.367)	9.385 ± 0.368 (0.412)	9.649 ± 0.138 (0.224)	9.355 ± 0.217 (0.442)
60 min	9.798 ± 0.153 (0.159)	9.686 ± 0.209 (0.206)	9.581 ± 0.349 (0.263)	9.678 ± 0.290 (0.210)	9.635 ± 0.269 (0.232)	9.561 ± 0.380 (0.275)	10.111 ± 0.269 (0.0774)	9.492 ± 0.240 (0.322)
120 min	9.961 ± 0.129 (0.109)	9.643 ± 0.311 (0.227)	9.869 ± 0.232 (0.135)	9.492 ± 0.351 (0.322)	9.806 ± 0.328 (0.156)	9.676 ± 0.357 (0.211)	10.022 ± 0.321 (0.0950)	**9.412 ± 0.204*** **(0.387)**
300 min	10.108 ± 0.096 (0.0780)	10.136 ± 0.130 (0.0731)	10.239 ± 0.296 (0.0577)	10.000 ± 0.240 (0.100)	10.121 ± 0.185 (0.0757)	10.428 ± 0.313 (0.0373)	10.345 ± 0.239 (0.0452)	**9.732 ± 0.150*** **(0.185)**

achieved by LSD at the WT receptor. His452Tyr, in contrast, does not alter the early time point kinetics but rather limits the potency and efficacy of LSD at later time points when compared to other mutants and WT. These results suggest that these residues play important roles in 5HT2A dynamics during LSD binding, such as alteration of receptor-active state conformations or βarr2 engagement.

In the current study, we observed differences in measured maximum binding (B_{max}) for each SNP *versus* WT as measured by radioligand saturation binding assay. As we note, all of the SNPs demonstrated a higher B_{max} than the WT receptor; however, these differences did not predict uniform changes in the potencies or efficacies of 5-HT or the psychedelics tested. Additionally, the BRET-based assays used in the study are relatively insensitive to differences in receptor density;[40] therefore, the observed changes between the *in vitro* pharmacology of the seven variant 5-HT2A receptors tested probably do not arise due to differential expression. Nevertheless, future studies should examine whether these changes in receptor density are replicated in 5-HT2A expressing neurons and examine whether translation is affected by these polymorphisms as such changes could affect the 5-HT2A expressing neuronal activity and alter the circuit activity and connectivity. Additionally, given the prevalence of animal models, future studies should examine the differences in the pharmacologies among 5-HT2A receptors of different species, especially as more preclinical data regarding the effects of psychedelics is generated.

In summary, our findings indicate that 5-HT2A receptor SNPs can alter the *in vitro* pharmacology of some therapeutically promising psychedelics. Prior studies have already demonstrated that the His452Tyr, Thr25Asn, and Ile197Val$^{4.47}$ 5-HT2A receptor polymorphisms can exhibit pronounced differences from the WT receptor in the pharmacologies of atypical antipsychotics.[36] Our results extend these findings and suggest that these polymorphisms also alter the signaling properties of psychedelics. Additionally, we find that the polymorphisms Ser12Asn, Ala230Thr, and Ala447Val 5-HT2A receptors also exhibit pronounced differences. Our results suggest that patients and populations with certain polymorphisms may be differentially amenable to psychedelic-assisted treatments. Taken together, these results may have relevance for the design and interpretation of future clinical trials.

4. MATERIALS AND METHODS

4.1. Cell Culture. HEK293T cells were obtained from ATCC (Manassas, VA). The cells were maintained, passaged, and transfected in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS), 100 units/mL penicillin, and 100μg/mL streptomycin (Gibco-Thermo Fisher, Waltham, MA) in a humidified atmosphere at 37 °C and 5% CO_2. After transfection, the cells were plated in DMEM containing 1% dialyzed FBS, 100 units/mL penicillin, and 100μg/mL streptomycin for BRET assays.

4.2. Mutagenesis. The human 5-HT2A cDNA cloned into the *Not*I site pIRES-neo (Clonetech) was obtained through the resources of the National Institute of Mental Health Psychoactive Drug Screening Program (http:kidb. pdsp.unc.edu). The following mutagenesis primers were obtained from Eton Bioscience:

Ser12Asn forward: 5′ TCACTGAACAGCACAACTAACTCTCT-CATG 3′

Ser12Asn reverse: 5′ TGTGCTGTTCAGTGAGG-TATTCTCCTC 3′

Thr25Asn forward: 5′ GATGATAACCGACTTTACTCCAAC-GACTTCAAC 3′

Thr25Asn reverse: 5′ AAGTCGGTTATCATCATTGAGCTG-CATGAG 3′

Asp48Asn forward: 5′-ACAGTAAACAGTGAAAACCFFAC-CAATCTGTCC

Asp48Asn reverse: 5′-TTCACTGTTTACTGTCCAATT-GAAGGCGTC

Ile197Val$^{4.47}$ forward: 5′ AAAATCGTCGCGGTCTGGAC-CATTTCA 3′

Ile197Val$^{4.47}$ reverse: 5′ GACCGCGACGATTTTCA-GAAAGGCTTT 3′

Ala230Thr forward: 5′ CTTCTGACTGATGACAATTTCG-TACTTATAGGAAGC 3′

Ala230Thr reverse: 5′ GTCATCAGTCAGAAGG-CAACTGCCCTC 3′

Ala447Val forward: 5′ ATGGTGGTCCTGGGCAAACAGCA-CAGTGAAGAAGCC 3′

Ala447Val reverse: 5′ GCCCAGGACCACCATACTG-CAGTCGTTGTC 3′

His452Tyr forward: 5′ AAACAGTACAGTGAAGAAGCCTC-CAAAGAC 3′

His452Tyr reverse: 5′ TTCACTGTACTGTTTGCCCAGGGC-CAC 3′

SNPs were introduced into the cloned human 5-HT2A receptor template using the QuikChange site directed, PCR-based mutagenesis kit exactly as described by the manufacturer (Stratagene, La Jolla, CA, USA). Mutagenized clones were isolated and subjected to Sanger DNA sequencing to verify the entire coding sequence for the presence of the desired mutation and the absence of any PCR-induced sequence errors.

4.3. TRUPATH Gq BRET Assays. Cells were plated either in six-well dishes at a density of 700,000−800,000 cells/well or 10 cm dishes at 7−8 million cells/dish. The cells were transfected 2−4 h later using a 1:1:1:1 DNA ratio of 5-HT2A receptor/Gαq-RLuc8/Gβ3/Gγ9-GFP2 (100 ng/construct for six-well dishes and 1000 ng/construct for 10 cm dishes). Transit 2020 (Mirus Biosciences, Madison, WI) was used to complex the DNA at a ratio of 3 μL Transit/μg DNA in OptiMEM (Gibco-Thermo Fisher, Waltham, MA) at a concentration of 10 ng DNA/μL OptiMEM. The next day, the cells were harvested from the plate and plated in poly-D-lysine-coated white, clear-bottom 96-well assay plates (Greiner Bio-One, Monroe, NC) at a density of 30,000−50,000 cells/well.

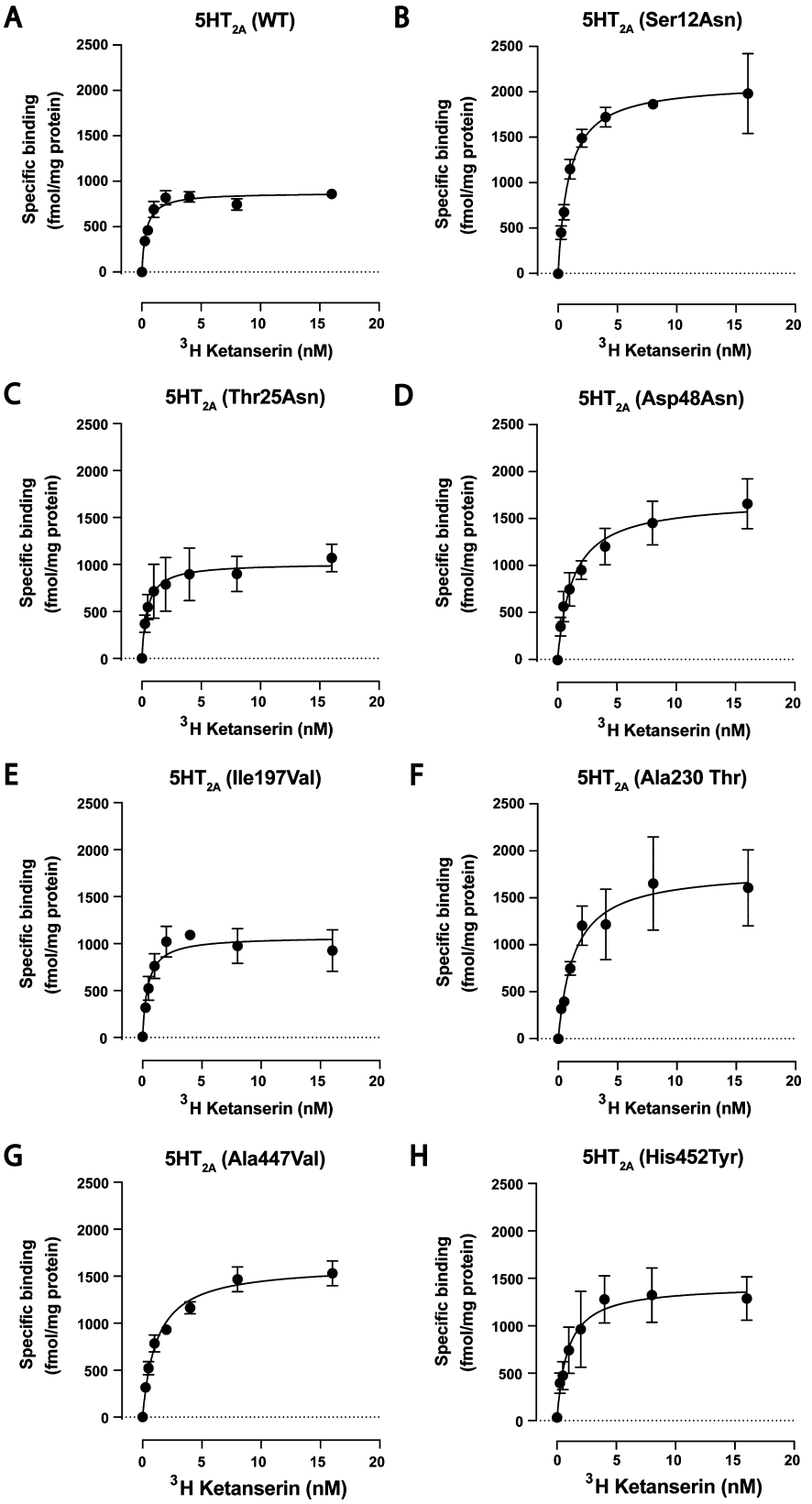


Figure 6. Saturation radioligand binding analysis of WT and polymorphic $5HT_{2A}$ receptors as a measure of receptor expression. (A) WT 5HT2A, (B) Ser12Asn, (C) Thr25Asn, (D) Asp48Asn, (E) Ile197Val, (F) Ala230Thr, (G) Ala447Val, and (H) His452Tyr.

One day after plating in 96-well assay plates, white backings (PerkinElmer, Waltham, MA) were applied to the plate bottoms, and the growth medium was carefully aspirated and replaced immediately with 50 μL of assay buffer (1× HBSS + 20 mM HEPES, pH 7.4) containing 10μL of freshly prepared 50 μM coelenterazine 400a (Nanolight Technologies, Pinetop, AZ). After a five minute equilibration period, the cells were treated with 50 μL of drug (2×) for an additional 5 min. For kinetic experiments, the plates were

Table 8. B_{max} Values at Wild-Type (WT) and Polymorphic 5-HT2A Receptors as Measured Using Radioligand Saturation Binding Using [3H]-Ketanserin

	WT	Ser12Asn	Thr25Asn	Asp48Asn	Ile197Val	Ala230Thr	Ala447Val	His452Tyr
B_{max} (fmol/mg prot)	874.4 ± 38.4	2100 ± 113	1013 ± 120	1698 ± 148	1073 ± 91.1	1802 ± 215	1618 ± 72.4	1429 ± 185
K_d	0.357 ± 0.078	0.903 ± 0.186	0.448 ± 0.246	1.293 ± 0.396	0.443 ± 0.171	1.358 ± 0.562	1.194 ± 0.191	0.872 ± 0.391

incubated at 37 °C for at least 20 min prior to receiving drug stimulation. Afterward, 50 μL of drug (2×) was added per well and incubated for designated time points. Before reading, 10 μL of coelenterazine 400a (Nanolight Technologies, Pinetop, AZ) was added per well and incubated for an additional 5 min to allow for substrate diffusion, and the plates were immediately read. The plates were read in a Pherastar FSX microplate reader (BMG Labtech Inc., Cary, NC) with 395 nm (RLuc8-coelenterazine 400a) and 510 nm (GFP2) emission filters at 1 s/well integration times. BRET2 ratios were computed as the ratio of the GFP2 emission to RLuc8 emission. Results are from at least three independent experiments, each performed in duplicate. Data were normalized to 5-HT stimulation and analyzed using nonlinear regression "log(agonist) *vs* response" in Prism 9 (Graphpad Software Inc., San Diego, CA).

4.4. BRET Arrestin Assay. To measure 5-HT2AR-mediated β-arrestin-1 recruitment, HEK293T cells were co-transfected in a 1:1:5 ratio with human 5-HT2AR containing C-terminal *Renilla* luciferase (*R*Luc8), GRK2, and Venus-tagged N-terminal β-arrestin-1. To measure 5-HT2AR-mediated β-arrestin-2 recruitment, HEK293T cells were co-transfected in a 1:1:5 ratio with human 5-HT2AR containing C-terminal *Renilla* luciferase (*R*Luc8), GRK2, and Venus-tagged N-terminal β-arrestin-2. For both experiments, after at least 24 h, transfected cells were plated in poly-lysine-coated 96-well white clear-bottom cell culture plates in plating medium (DMEM + 1% dialyzed FBS) at a density of 40,000−50,000 cells in 200 μL per well and incubated overnight. The next day, the medium was decanted, and the cells were washed with 50 μL of drug buffer (1× HBSS, 20 mM HEPES, 0.1% bovine serum albumin (BSA), and 0.01% ascorbic acid, pH 7.4), and then 50 μL of drug buffer with the *R*Luc substrate coelenterazine h (Promega, 5 μM final concentration) was added per well. After 5 min, 50 μL of drug (2×) was added per well and incubated for 5 min before being read. For kinetic experiments, the plates were incubated at 37 °C for at least 20 min prior to receiving drug stimulation. Afterward, 50 μL of drug (2×) was added per well and incubated for designated time points. Before reading, 10 μL of the *R*Luc substrate, coelenterazine h (Promega, 5 μM final concentration), was added per well and incubated for an additional 5 min to allow for substrate diffusion, and the plates were immediately read. The plates were read for both luminescence at 485 nm and fluorescent eYFP emission at 530 nm for 1 s per well using a Pherastar FSX microplate reader (BMG Labtech Inc., Cary, NC). The ratio of eYFP/*R*Luc was calculated per well, and the net BRET ratio was calculated by subtracting the eYFP/*R*Luc per well from the eYFP/*R*Luc ratio in wells without Venus-β-arrestin present. The results are from at least three independent experiments, each performed in duplicate. The net BRET ratio was plotted as a function of drug concentration using Prism 9 (Graphpad Software Inc., San Diego, CA). Data were normalized to 5-HT stimulation and analyzed using nonlinear regression "log(agonist) *vs* response" in Prism 9.

4.5. Receptor Expression Measurements. HEK293T cells were plated in 10 cm dishes and transfected with 1000 ng of the wild-type 5-HT2A R, Ser12Asn, Thr25Asn, Asp48Asn, Ile197Val[4,47], Ala230Thr, Ala447Val, and His452Tyr variants of the 5-HT2A receptor. After 36 h of transfection, the cell medium was replaced with a medium containing 1% dialyzed FBS. After 48 h, the medium was aspirated, and the cells were washed twice with cold phosphate-buffered saline (PBS). The cells were scraped in 10 mL ice-cold PBS and centrifuged at 3000 rpm at 4 °C for 15 min, and the supernatant was discarded. The cell pellet was resuspended in ice-cold lysis buffer (50 mM Tris−HCl, pH 7.4) and incubated on ice for 20 min. After incubation, the cells were triturated gently for hypotonic lysis. The suspension was centrifuged at 21,000*g* for 20 min at 4 °C to obtain a crude membrane pellet. The supernatant was discarded, and the membrane pellets were stored at −80 °C for further use.

Saturation binding assays were performed to determine the equilibrium dissociation constant (K_d) and B_{max}. The membrane pellets were dissolved in lysis buffer, and the amount of protein was estimated using Bradford assays. Binding assays were performed in standard binding buffer (50 mM Tris, 10 mM MgCl2, 0.1 mM EDTA, 0.1% BSA, and 0.01% ascorbic acid, pH 7.4) using [^{3}H] ketanserin (PerkinElmer, Waltham, MA; specific activity 22.8 Ci/mmol) as the radioligand.

Saturation binding assays were carried out in 96-well plates in a final volume of 125 μL per well. In brief, 25 μL of radioligand was added to a 96-well plate, followed by addition of 25 μL of binding buffer (for total binding) or 25 μL of the reference compound (clozapine) at a final concentration of 10 μM to measure non-specific binding. After, 75 μL of fresh membrane protein (15 μg/well) was added, and plates were incubated in the dark at room temperature for 90 min. The reaction was stopped by vacuum filtration onto cold 0.3% polyethyleneimine (PEI)-soaked 96-well filter mats using a 96-well Packard FilterMate harvester, followed by three washes with cold wash buffer (50 mM Tris, pH 7.4). The filters were dried, and scintillation cocktail (MeltiLex, PerkinElmer) was melted onto the dried filters and allowed to cool to room temperature. Then, the filters were placed into cassettes and radioactivity was measured in a MicroBeta counter (Molecular Devices). Data were analyzed using "One Site-Total and non-specific binding" equation using Prism 9. Specific binding was calculated after subtracting the non-specific binding.

4.6. Data Analysis. All concentration−response curves were fit to a three-parameter logistic equation in Prism (Graphpad Software, San Diego, CA). TRUPATH Gq BRET concentration−response curves were analyzed by normalizing to the reference agonist (5-HT response at the wild-type receptor) for each experiment. Efficacy (E_{max}) calculations were performed according to Kenakin and colleagues:[38] stimulus−response amplitudes (net BRET2) were normalized to the maximal responding agonist (maximal system response). EC_{50} and E_{max} values were estimated from the simultaneous fitting of all biological replicates. Transduction coefficients were calculated as $\log(E_{max}/EC_{50})$ as described in Kenakin and colleagues,[38] and propagation of error was conducted at all steps. EC_{50}, E_{max}, and transduction coefficient values were analyzed first by ANOVAs (*F*-test of curve fit, one-way ANOVA, or two-way ANOVA as described in the text). *Post hoc* pairwise comparisons used Tukey-adjusted *p* values to control for multiple comparisons. The significance threshold was set at $\alpha = 0.05$.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acschemneuro.1c00815.

Gq dissociation concentration−response curves for 5-HT at polymorphic 5-HT2A receptors; β-arrestin-1 recruitment BRET assay concentration−response curves for 5-HT at polymorphic 5-HT2A receptors; β-arrestin-2 recruitment BRET assay dissociation concentration−response curves for 5-HT at polymorphic 5-HT2A receptors; kinetic measurements of Gq dissociation and β-arrestin-2 recruitment for 5-HT at WT 5-HT2A receptor; saturation radioligand binding analysis of non-transfected HEK293 cells as a measure of receptor density; allele frequencies (%) for each of the SNPs included in the study; potency (pEC_{50}) and efficacy

(span) values for 5-HT at wild-type (WT) and polymorphic 5-HT2A receptors as measured using TRUPATH Gq BRET assays; efficacy (span) values for several potentially therapeutic psychedelics at wild-type (WT) and polymorphic 5-HT2A receptors as measured using TRUPATH Gq BRET assays; potency (pEC_{50}) and efficacy (span) values for 5-HT at wild-type (WT) and polymorphic 5-HT2A receptors as measured using βArr1 recruitment BRET assays; potency (pEC_{50}) and efficacy (span) values for 5-HT at wild-type (WT) and polymorphic 5-HT2A receptors as measured using βArr2 recruitment BRET assays; efficacy (span) values for several potentially therapeutic psychedelics at wild-type (WT) and polymorphic 5-HT2A receptors as measured using βArr1 recruitment BRET assays; and efficacy (span) values for several potentially therapeutic psychedelics at wild-type (WT) and polymorphic 5-HT2A receptors as measured using βArr2 recruitment BRET assays (PDF)

AUTHOR INFORMATION

Corresponding Author

Bryan L. Roth − *Department of Pharmacology, University of North Carolina at Chapel Hill School of Medicine, Chapel Hill, North Carolina 27599-7365, United States;* orcid.org/0000-0002-0561-6520; Email: bryan_roth@med.unc.edu

Authors

Gavin P. Schmitz − *Department of Pharmacology, University of North Carolina at Chapel Hill School of Medicine, Chapel Hill, North Carolina 27599-7365, United States*

Manish K. Jain − *Department of Pharmacology, University of North Carolina at Chapel Hill School of Medicine, Chapel Hill, North Carolina 27599-7365, United States;* orcid.org/0000-0001-8173-3326

Samuel T. Slocum − *Department of Pharmacology, University of North Carolina at Chapel Hill School of Medicine, Chapel Hill, North Carolina 27599-7365, United States*

Complete contact information is available at: https://pubs.acs.org/10.1021/acschemneuro.1c00815

Author Contributions

G.P.S. and B.L.R. designed the study. G.P.S. generated the mutagenized clones. G.P.S., M.K.J., and S.T.S. performed the BRET experiments. M.K.J. performed the saturation binding experiments. G.P.S. analyzed the data and wrote the manuscript. G.P.S. prepared all the figures and tables. All authors reviewed the manuscript and approved the final draft.

Funding

This work was supported by the National Institutes of Health (RO1MH112205 and R37DA045657) and funding from the Defense Advanced Research Projects Agency. https://www.darpa.mil/.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors gratefully thank Terry Kenakin for his help with statistical analysis as well as Dewran Kocak and Jeffery Diberto for their training.

REFERENCES

(1) Nichols, D. E. Psychedelics. *Pharmacol. Rev.* **2016**, *68*, 264−355.

(2) Slocum, S. T.; Diberto, J. F.; Roth, B. L. Molecular insights into psychedelic drug action. *J. Neurochem.* **2021**, *2021*, 162 DOI: 10.1111/jnc.15540.

(3) Nutt, D.; Erritzoe, D.; Carhart-Harris, R. Psychedelic Psychiatry's Brave New World. *Cell* **2020**, *181*, 24−28.

(4) Ross, S.; Bossis, A.; Guss, J.; Agin-Liebes, G.; Malone, T.; Cohen, B.; Mennenga, S. E.; Belser, A.; Kalliontzi, K.; Babb, J.; Su, Z.; Corby, P.; Schmidt, B. L. Rapid and sustained symptom reduction following psilocybin treatment for anxiety and depression in patients with life-threatening cancer: a randomized controlled trial. *J. Psychopharmacol.* **2016**, *30*, 1165−1180.

(5) Carhart-Harris, R. L.; Bolstridge, M.; Day, C. M. J.; Rucker, J.; Watts, R.; Erritzoe, D. E.; Kaelen, M.; Giribaldi, B.; Bloomfield, M.; Pilling, S.; Rickard, J. A.; Forbes, B.; Feilding, A.; Taylor, D.; Curran, H. V.; Nutt, D. J. Psilocybin with psychological support for treatment-resistant depression: six-month follow-up. *Psychopharmacology* **2018**, *235*, 399−408.

(6) Carhart-Harris, R.; Giribaldi, B.; Watts, R.; Baker-Jones, M.; Murphy-Beiner, A.; Murphy, R.; Martell, J.; Blemings, A.; Erritzoe, D.; Nutt, D. J. Trial of Psilocybin versus Escitalopram for Depression. *N. Engl. J. Med.* **2021**, *384*, 1402−1411.

(7) Griffiths, R. R.; Johnson, M. W.; Carducci, M. A.; Umbricht, A.; Richards, W. A.; Richards, B. D.; Cosimano, M. P.; Klinedinst, M. A. Psilocybin produces substantial and sustained decreases in depression and anxiety in patients with life-threatening cancer: A randomized double-blind trial. *J. Psychopharmacol.* **2016**, *30*, 1181−1197.

(8) Sewell, R. A.; Halpern, J. H.; Pope, H. G., Jr. Response of cluster headache to psilocybin and LSD. *Neurology* **2006**, *66*, 1920−1922.

(9) Gasser, P.; Kirchner, K.; Passie, T. LSD-assisted psychotherapy for anxiety associated with a life-threatening disease: A qualitative study of acute and sustained subjective effects. *J. Psychopharmacol.* **2015**, *29*, 57−68.

(10) Davis, A. K.; Barsuglia, J. P.; Lancelotta, R.; Grant, R. M.; Renn, E. The epidemiology of 5-methoxy-N, N-dimethyltryptamine (5-MeO-DMT) use: Benefits, consequences, patterns of use, subjective effects, and reasons for consumption. *J. Psychopharmacol.* **2018**, *32*, 779−792.

(11) Davis, A. K.; So, S.; Lancelotta, R.; Barsuglia, J. P.; Griffiths, R. R. 5-methoxy-N,N-dimethyltryptamine (5-MeO-DMT) used in a naturalistic group setting is associated with unintended improvements in depression and anxiety. *Am. J. Drug Alcohol Abuse* **2019**, *45*, 161−169.

(12) Johnson, M. W.; Hendricks, P. S.; Barrett, F. S.; Griffiths, R. R. Classic psychedelics: An integrative review of epidemiology, therapeutics, mystical experience, and brain network function. *Pharmacol. Ther.* **2019**, *197*, 83−102.

(13) Agin-Liebes, G.; Haas, T. F.; Lancelotta, R.; Uthaug, M. V.; Ramaekers, J. G.; Davis, A. K. Naturalistic Use of Mescaline Is Associated with Self-Reported Psychiatric Improvements and Enduring Positive Life Changes. *ACS Pharmacol. Transl. Sci.* **2021**, *4*, 543−552.

(14) Galvão-Coelho, N. L.; Marx, W.; Gonzalez, M.; Sinclair, J.; De Manincor, M.; Perkins, D.; Sarris, J. Classic serotonergic psychedelics for mood and depressive symptoms: a meta-analysis of mood disorder patients and healthy participants. *Psychopharmacology* **2021**, *238*, 341−354.

(15) Wacker, D.; Wang, C.; Katritch, V.; Han, G. W.; Huang, X.-P.; Vardy, E.; McCorvy, J. D.; Jiang, Y.; Chu, M.; Siu, F. Y.; Liu, W.; Xu, H. E.; Cherezov, V.; Roth, B. L.; Stevens, R. C. Structural Features for Functional Selectivity at Serotonin Receptors. *Science* **2013**, *340*, 615−619.

(16) Wang, C.; Jiang, Y.; Ma, J.; Wu, H.; Wacker, D.; Katritch, V.; Han, G. W.; Liu, W.; Huang, X.-P.; Vardy, E.; McCorvy, J. D.; Gao, X.; Zhou, X. E.; Melcher, K.; Zhang, C.; Bai, F.; Yang, H.; Yang, L.; Jiang, H.; Roth, B. L.; Cherezov, V.; Stevens, R. C.; Xu, H. E. Structural Basis for Molecular Recognition at Serotonin Receptors. *Science* **2013**, *340*, 610−614.

(17) Rickli, A.; Moning, O. D.; Hoener, M. C.; Liechti, M. E. Receptor interaction profiles of novel psychoactive tryptamines compared with

classic hallucinogens. *Eur. Neuropsychopharmacol.* **2016**, *26*, 1327−1337.
(18) Wacker, D.; Wang, S.; McCorvy, J. D.; Betz, R. M.; Venkatakrishnan, A. J.; Levit, A.; Lansu, K.; Schools, Z. L.; Che, T.; Nichols, D. E.; Shoichet, B. K.; Dror, R. O.; Roth, B. L. Crystal Structure of an LSD-Bound Human Serotonin Receptor. *Cell* **2017**, *168*, 377−389.
(19) Peng, Y.; McCorvy, J. D.; Harpsøe, K.; Lansu, K.; Yuan, S.; Popov, P.; Qu, L.; Pu, M.; Che, T.; Nikolajsen, L. F.; Huang, X.-P.; Wu, Y.; Shen, L.; Bjørn-Yoshimoto, W. E.; Ding, K.; Wacker, D.; Han, G. W.; Cheng, J.; Katritch, V.; Jensen, A. A.; Hanson, M. A.; Zhao, S.; Gloriam, D. E.; Roth, B. L.; Stevens, R. C.; Liu, Z.-J. 5-HT2C Receptor Structures Reveal the Structural Basis of GPCR Polypharmacology. *Cell* **2018**, *172*, 719−730.
(20) Glennon, R. A.; Titeler, M.; McKenney, J. D. Evidence for 5-HT2 involvement in the mechanism of action of hallucinogenic agents. *Life Sci.* **1984**, *35*, 2505−2511.
(21) González-Maeso, J.; Weisstaub, N. V.; Zhou, M.; Chan, P.; Ivic, L.; Ang, R.; Lira, A.; Bradley-Moore, M.; Ge, Y.; Zhou, Q.; Sealfon, S. C.; Gingrich, J. A. Hallucinogens recruit specific cortical 5-HT(2A) receptor-mediated signaling pathways to affect behavior. *Neuron* **2007**, *53*, 439−452.
(22) Keiser, M. J.; Setola, V.; Irwin, J. J.; Laggner, C.; Abbas, A. I.; Hufeisen, S. J.; Jensen, N. H.; Kuijer, M. B.; Matos, R. C.; Tran, T. B.; Whaley, R.; Glennon, R. A.; Hert, J.; Thomas, K. L. H.; Edwards, D. D.; Shoichet, B. K.; Roth, B. L. Predicting new molecular targets for known drugs. *Nature* **2009**, *462*, 175−181.
(23) Vollenweider, F. X.; Vollenweider-Scherpenhuyzen, M. F. I.; Bäbler, A.; Vogel, H.; Hell, D. Psilocybin induces schizophrenia-like psychosis in humans via a serotonin-2 agonist action. *Neuroreport* **1998**, *9*, 3897−3902.
(24) Kometer, M.; Schmidt, A.; Jancke, L.; Vollenweider, F. X. Activation of Serotonin 2A Receptors Underlies the Psilocybin-Induced Effects on Oscillations, N170 Visual-Evoked Potentials, and Visual Hallucinations. *J. Neurosci.* **2013**, *33*, 10544−10551.
(25) Preller, K. H.; Burt, J. B.; Ji, J. L.; Schleifer, C. H.; Adkinson, B. D.; Stämpfli, P.; Seifritz, E.; Repovs, G.; Krystal, J. H.; Murray, J. D.; Vollenweider, F. X.; Anticevic, A. Changes in global and thalamic brain connectivity in LSD-induced altered states of consciousness are attributable to the 5-HT2A receptor. *eLife* **2018**, *7*, No. e35082.
(26) Holze, F.; Vizeli, P.; Ley, L.; Müller, F.; Dolder, P.; Stocker, M.; Duthaler, U.; Varghese, N.; Eckert, A.; Borgwardt, S.; Liechti, M. E. Acute dose-dependent effects of lysergic acid diethylamide in a double-blind placebo-controlled study in healthy subjects. *Neuropsychopharmacology* **2021**, *46*, 537−544.
(27) Roth, B.; Nakaki, T.; Chuang, D.; Costa, E. Aortic recognition sites for serotonin (5HT) are coupled to phospholipase C and modulate phosphatidylinositol turnover. *Neuropharmacology* **1984**, *23*, 1223−1225.
(28) Roth, B.; Willins, D. L.; Kristiansen, K.; Kroeze, W. K. 5-Hydroxytryptamine2-Family Receptors (5-Hydroxytryptamine2A, 5-Hydroxytryptamine2B, 5-Hydroxytryptamine2C) Where Structure Meets Function. *Pharmacol. Ther.* **1998**, *79*, 231−257.
(29) Gray, J. A.; Bhatnagar, A.; Gurevich, V. V.; Roth, B. L. The Interaction of a Constitutively Active Arrestin with the Arrestin-Insensitive 5-HT2AReceptor Induces Agonist-Independent Internalization. *Mol. Pharmacol.* **2003**, *63*, 961−972.
(30) Gelber, E. I.; Kroeze, W. K.; Willins, D. L.; Gray, J. A.; Sinar, C. A.; Hyde, E. G.; Gurevich, V.; Benovic, J.; Roth, B. L. Structure and function of the third intracellular loop of the 5-hydroxytryptamine2A receptor: the third intracellular loop is alpha-helical and binds purified arrestins. *J. Neurochem.* **1999**, *72*, 2206−2214.
(31) Urban, J. D.; Clarke, W. P.; von Zastrow, M.; Nichols, D. E.; Kobilka, B.; Weinstein, H.; Javitch, J. A.; Roth, B. L.; Christopoulos, A.; Sexton, P. M.; Miller, K. J.; Spedding, M.; Mailman, R. B. Functional selectivity and classical concepts of quantitative pharmacology. *J. Pharmacol. Exp. Ther.* **2007**, *320*, 1−13.
(32) Wacker, D.; Stevens, R. C.; Roth, B. L. How Ligands Illuminate GPCR Molecular Pharmacology. *Cell* **2017**, *170*, 414−427.
(33) Kim, K.; Che, T.; Panova, O.; DiBerto, J. F.; Lyu, J.; Krumm, B. E.; Wacker, D.; Robertson, M. J.; Seven, A. B.; Nichols, D. E.; Shoichet, B. K.; Skiniotis, G.; Roth, B. L. Structure of a Hallucinogen-Activated Gq-Coupled 5-HT2A Serotonin Receptor. *Cell* **2020**, *182*, 1574−1588.
(34) Rodriguiz, R. M.; Nadkarni, V.; Means, C. R.; Pogorelov, V. M.; Chiu, Y.-T.; Roth, B. L.; Wetsel, W. C. LSD-stimulated behaviors in mice require β-arrestin 2 but not β-arrestin 1. *Sci. Rep.* **2021**, *11*, 17690.
(35) Aday, J. S.; Mitzkovitz, C. M.; Bloesch, E. K.; Davoli, C. C.; Davis, A. K. Long-term effects of psychedelic drugs: A systematic review. *Neurosci. Biobehav. Rev.* **2020**, *113*, 179−189.
(36) Davies, M. A.; Setola, V.; Strachan, R. T.; Sheffler, D. J.; Salay, E.; Hufeisen, S. J.; Roth, B. L. Pharmacologic analysis of non-synonymous coding h5-HT2A SNPs reveals alterations in atypical antipsychotic and agonist efficacies. *Pharmacogenomics J.* **2006**, *6*, 42−51.
(37) Pándy-Szekeres, G.; Munk, C.; Tsonkov, T. M.; Mordalski, S.; Harpsøe, K.; Hauser, A. S.; Bojarski, A. J.; Gloriam, D. E. GPCRdb in 2018: adding GPCR structure models and ligands. *Nucleic Acids Res.* **2018**, *46*, D440−D446.
(38) Kenakin, T.; Watson, C.; Muniz-Medina, V.; Christopoulos, A.; Novick, S. A simple method for quantifying functional selectivity and agonist bias. *ACS Chem. Neurosci.* **2012**, *3*, 193−203.
(39) Klein Herenbrink, C.; Sykes, D. A.; Donthamsetti, P.; Canals, M.; Coudrat, T.; Shonberg, J.; Scammells, P. J.; Capuano, B.; Sexton, P. M.; Charlton, S. J.; Javitch, J. A.; Christopoulos, A.; Lane, J. R. The role of kinetic context in apparent biased agonism at GPCRs. *Nat. Commun.* **2016**, *7*, 10842.
(40) Olsen, R. H. J.; DiBerto, J. F.; English, J. G.; Glaudin, A. M.; Krumm, B. E.; Slocum, S. T.; Che, T.; Gavin, A. C.; McCorvy, J. D.; Roth, B. L.; Strachan, R. T. TRUPATH, an open-source biosensor platform for interrogating the GPCR transducerome. *Nat. Chem. Biol.* **2020**, *16*, 841−849.
(41) Roth, B. L.; Hanizavareh, S. M.; Blum, A. E. Serotonin receptors represent highly favorable molecular targets for cognitive enhancement in schizophrenia and other disorders. *Psychopharmacology* **2004**, *174*, 17.
(42) Malhotra, A. K.; Murphy, G. M.; Kennedy, J. L. Pharmacogenetics of Psychotropic Drug Response. *Am. J. Psychiatry* **2004**, *161*, 780−796.
(43) Hazelwood, L. A.; Sanders-Bush, E. His452Tyr polymorphism in the human 5-HT2A receptor destabilizes the signaling conformation. *Mol. Pharmacol.* **2004**, *66*, 1293−1300.
(44) Harvey, L.; Reid, R. E.; Ma, C.; Knight, P. J.; Pfeifer, T. A.; Grigliatti, T. A. Human genetic variations in the 5HT2A receptor. *Pharmacogenetics* **2003**, *13*, 107−118.