Table S1. cryoEM Data collection, model refinement and validation. Related to Figure 1A

Structures	HTR2A-miniGαq
Data Collection	
Magnification	57,050
Voltage (kV)	300
Dose per frame(e-/Å^2)	1.03
Electron exposure (e-/ Å^2)	80
Defocus Range (μm)	-0.8 to -2.6
Pixel size (Å)	0.8521
Symmetry imposed	C1
Number of Micrographs	2,368
Initial particle images (no.)	2,712,613
Final particle images (no.)	166,497
Map resolution (Å)	3.36
FSC threshold	0.143
Refinement Statistics	
Model Resolution (Å)	3.4
FSC threshold	0.5
Map sharpening B factor (Å^2)	-106.4
Model composition	
Total number of atoms	8,576
No. of protein residues	1,122
No. of ligands	1
Model validation	
CC (mask) map vs. model (%)	83
RMSD	
Bond length (Å) / Bond angles (°)	0.004 / 0.647
Ramachandran plot statistics	
Most favored (%)	100
Outliers (%)	0
Rotamer outliers (%)	2.34
C-beta deviations	0.00
Clash score	5.72

Table S2. Data Collection and Refinement Statistics (X-ray crystallography).

Structures	HTR2A-BRIL (LSD)	HTR2A-BRIL (methiothepin)
Data Collection	APS, GMCA/CAT 23ID-B, 1.033 Å, 10-μm NSLS-II, FMX 17-ID-2, 1.033 Å, 10-μm	
Crystals	25	16
Resolution (Å)	34.5 – 3.4 (3.61-3.40)	34.8 -3.4 (3.61-3.40)
Space group	$C222_1$	$C222_1$
Complexes/ASU	3	3
Unit cell dimensions *a, b, c* (Å)	81.64 175.26 280.67	82.38 177.31 280.59
α, β, γ (°)	90 90 90	90 90 90
No. total reflections	171,800 (9,294)	159,514 (23,600)
No. unique reflections	27,254 (3,762)	28,636 (4,578)
Multiplicity	6.3 (2.5)	5.6 (5.2)
Completeness (%)	96.8 (84.9)	99.7 (99.8)
Mean I/σ(I)	5.5 (1.0)	5.8 (1.2)
R_{merge} (%)	20.0 (80.6)	16.4 (119.7)
$CC_{1/2}$ (%)	96.8 (84.9)	99.5 (66.0)
Refinement Statistics		
Resolution used in refinement (Å)	34.5 -3.4 (3.55-3.42)	33.2 -3.4 (3.52-3.40)
No. reflections used in refinement	20,947 (188)	26,099 (1,239)
No. reflections used for R-free	1,072 (9)	1,336 (63)
R-work (%)	26.7 (43.2)	25.9 (28.5)
R-free (%)	30.1 (57.7)	29.8 (30.1)
Number of atoms	8,094	8,095
HTR2A-BRIL	7,874	7,850
Ligands (LSD or methiothepin)	72 (LSD)	72 (methiothepin)
Lipids	148	173
***Overall B*-factors (Å²)**	52.9	45.9
HTR2A-BRIL	53.1	45.8
Ligands (LSD or methiothepin)	41.1(LSD)	30.4 (methiothepin)
Lipids	51.4	56.2
Model Statistics		
RMSD Bond (Å)	0.013	0.012
RMSD Angles (°)	1.45	1.43
Ramachandran Favored (%)[a]	97.37	94.95
Ramachandran Allowed (%)[a]	2.63	5.05
Ramachandran Outliers (%)[a]	0.00	0.00
Rotamer outliers (%)[a]	0.14	0.28
Molprobity score[a]	1.86	2.09

Related to Figure 1B and C.

Highest-resolution shell is shown in parentheses.
[a]As defined in MolProbity.

Table S3. Analysis of the ligand-binding pocket in the HTR2A structures. Related to Figure 2. Solvent accessible (SA) volume and area are analyzed using CASTp 3.0 server. Probe radius of 2.0 is used in this analysis.

	HTR2A/methiothepin	HTR2A/LSD	HTR2A/25CN-NBOH
Area_SA/A^2	427.8	333.7	535.9
Volume_SA/ A^3	188.1	153.1	287.0

Table S4. HTR2A Gq dissociation (BRET 2) and mini-Gq association (BRET 1) assays of wild-type HTR2A. Related Figure 1E and Figure 1F. EC_{50} and Emax estimates represent the average and standard error of the mean (SEM) from three independent experiments performed in triplicate; data for Fig 1E and Fig 1F were from obtained independently. Emax is defined as percent 5-HT maximum response. N.P.=not performed

Ligand	BRET 2 HTR2A/Gq		BRET 1 HTR2A/mini-Gq	
	EC_{50}, nM (pEC_{50} ± SEM)	Emax % 5-HT	EC_{50}, nM (pEC_{50} ± SEM)	Emax % 5-HT
25CN-NBOH (Fig 1E)	0.87 (9.06 ± 0.08)	93.1 ± 2.2	1.71 (8.77 ± 0.10)	89.2 ± 2.8
Ligands (Hallucinogens; Fig 1F)			EC_{50}, nM (pEC_{50} ± SEM)	Emax % 5-HT
25CN-NBOH	N.P.	N.P.	1.87 (8.73 ± 0.09)	89.4 ± 2.5
LSD	N.P.	N.P.	1.57 (8.80 ± 0.15)	54.8 ± 2.4
DOI	N.P.	N.P.	6.22 (8.21 ± 0.10)	83.9 ± 2.7
Psilocin	N.P.	N.P.	25.60 (7.59 ± 0.24)	33.8 ± 3.1

Table S5. Gq dissociation (BRET 2) and arrestin association (BRET1) parameter estimates for various ligands at wild-type and mutant HTR2A receptors. Related to Figure 3D, Figure 5C and Figure 7. Data were acquired by BRET 2 assay conducted in parallel. EC_{50} and Emax estimates represent the average and standard error of the mean (SEM) from three independent experiments performed in triplicate. Emax is defined as percent WT maximum response. N.D; no detected activity. (**** p<0.0001, *** p<0.001, ** p<0.01 and * p<0.05 vs WT)

HTR2A Constructs	5-HT		25CN-NBOH		LSD	
	EC_{50}, nM (pEC_{50} ± SEM)	Emax % WT	EC_{50}, nM (pEC_{50} ± SEM)	Emax % WT	EC_{50}, nM (pEC_{50} ± SEM)	Emax % WT
Fig 3D; BRET 2 (HTR2A /Gq)						
WT	4.65 (8.33 ± 0.07)	100.3 ± 2.2	0.59 (9.23 ± 0.05)	99.4 ± 1.8	0.96 (9.02 ± 0.08)	99.7 ± 2.9
D155A	N.D	N.D	N.D	N.D	92.53 (7.03 ± 0.80)****	39.6 ± 19.0****
S159A	732.00 (6.14 ± 0.16)****	97.9 ± 8.8	95.17 (7.02 ± 0.39)****	74.9 ± 17.6	4.30 (8.37 ± 0.20)	119.4 ± 9.1
L229A	172.30 (6.77 ± 0.13)****	82.0 ± 4.7*	4.64 (8.33 ± 0.14)**	67.6 ± 3.7**	46.79 (7.33 ± 0.33)****	64.6 ± 10.9**
G238A	7.45 (8.13 ± 0.18)	64.7 ± 3.8****	0.50 (9.31 ± 0.15)	63.6 ± 3.1***	0.68 (9.17 ± 0.17)	61.7 ± 3.7**
S239A	126.70 (6.90 ± 0.12)****	89.2 ± 5.0	9.88 (8.01 ± 0.14)****	89.4 ± 5.5	3.03 (8.52 ± 0.17)	98.5 ± 6.1
S242A	7.74 (8.11 ± 0.14)	84.3 ± 4.1	1.50 (8.82 ± 0.09)	107.2 ± 3.5	3.75 (8.43 ± 0.14)	113.2 ± 6.2
W336A	N.D	N.D	N.D	N.D	N.D	N.D
F339L	82.81 (7.08 ± 0.35)****	44.4 ± 6.7****	152.90 (6.82 ± 0.22)****	51.4 ± 7.4****	7.90 (8.10 ± 0.18)	47.4 ± 3.7****
G369A	14.01 (7.85 ± 0.34)	33.2 ± 4.2****	N.D	N.D	10.68 (7.97 ± 0.25)*	44.6 ± 4.9****
S372A	8.02 (8.10 ± 0.21)	77.6 ± 5.7**	0.74 (9.13 ± 0.28)	87.0 ± 8.5	1.10 (8.96 ± 0.20)	89.2 ± 0.2
Fig 7; BRET 2 (HTR2A /Gq)						
WT	1.52 (8.82 ± 0.10)	99.8 ± 3.0	0.38 (9.42 ± 0.11)	100.1 ± 3.5	0.66 (9.18 ± 0.08)	101.4 ± 3.0
I181A	163.1 (6.79 ± 0.10)	61.7 ± 2.7	N.D	N.D	N.D	N.D
I181E	404.4 (6.39 ± 0.23)	48.9 ± 5.5	N.D	N.D	N.D	N.D
Fig 7; BRET 1 (HTR2A /βArr2)						
WT	43.18 (7.37 ± 0.12)	99.3 ± 4.7	5.48 (8.26 ± 0.14)	100.3 ± 0.1	15.37 (7.81 ± 0.15)	98.9 ± 5.6
I181A	15.1 (7.82 ± 0.14)	172.2 ± 9.2	4.27 (8.37 ± 0.19)	136.3 ± 8.3	4.21 (8.38 ± 0.17)	125.5 ± 6.9

I181E	14 (7.85 ± 0.14)	162.8 ± 8.5	3.69 (8.43 ± 0.15)	204.6 ± 10.1	8.4 (8.08 ± 0.12)	137.1 ± 6.0
Fig 5C; BRET2 (HTR2A /Gq)						
WT	5-HT		TRYPTAMINE		LSD	
	EC_{50}, nM (pEC50 ± SEM)	Emax % WT	EC_{50}, nM (pEC50 ± SEM)	Emax % WT	EC_{50}, nM (pEC50 ± SEM)	Emax % WT
WT	9.83 (8.00 ± 0.16)	100.0 ± 5.5	71.32 (7.15 ± 0.13)	100.0 ± 4.6	1.15 (8.94 ± 0.09)	100.0 ± 2.6
N343A	335.1 (6.48 ± 0.1611)	100.5 ± 5.0	173 (6.76 ± 0.12)	90.5 ± 4.5	0.56 (9.25 ± 0.16)	85.2 ± 3.8

Table S6. Binding affinities for 5-HT, 25CN-NBOH, LSD, and methiothepin at binding pocket mutants. Related to Figure 3D. Competitive binding was performed at 0.42 nM of [^{3}H]-LSD. All values indicate mean ± SEM from at least three independent experiments. ND=no detectible binding

HTR2A	Bmax ± SEM (pmol/mg protein)	Kd, nM ± SEM	[3H]-LSD, 0.42 nM pKi ± SEM			
			5-HT	25CN-NBOH	LSD	METHIOTHEPIN
WT	763.8 ± 27.9	1.09 ± 0.13	7.49 ± 0.07	9.08 ± 0.03	8.63 ± 0.07	9.70 ± 0.05
D155A	N.D	N.D	N.D	N.D	N.D	N.D
S159A	886.1 ± 122.1	2.33 ± 0.82	5.49 ± 0.33	6.92 ± 0.11	8.84 ± 0.05	9.68 ± 0.03
L229A	671.9 ± 299.5	6.94 ± 6.17	7.04 ± 0.14	8.54 ± 0.08	9.12 ± 0.05	10.05 ± 0.04
G238A	251.5 ± 20.6	0.42 ± 0.12	7.43 ± 0.07	8.39 ± 0.06	8.25 ± 0.07	9.42 ± 0.03
S239A	999.9 ± 74.2	3.36 ± 0.66	6.20 ± 0.10	8.06 ± 0.06	8.10 ± 0.05	9.24 ± 0.06
S242A	1,098.0 ± 70.5	2.04 ± 0.40	7.47 ± 0.15	8.64 ± 0.09	8.17 ± 0.09	9.49 ± 0.06
W336L	N.D	N.D	N.D	N.D	N.D	N.D
F339L	N.D	N.D	N.D.	N.D.	N.D.	N.D.
G369A	221.8 ± 43.7	1.91 ± 1.01	7.74 ± 0.14	8.89 ± 0.09	8.56 ± 0.11	8.50 ± 0.11

Table S7. Gq dissociation assay (BRET 2) with wild-type and mutants (Gq interface) of HTR2A and Gq. Related to Figure 6. Data were acquired by BRET 2 assay conducted in parallel. EC_{50} and Emax estimates represent the average and standard error of the mean (SEM) from three independent experiments performed in triplicate. Emax is defined as percent WT maximum response. N.D; no detected activity. (****p<0.0001 and *p<0.05 vs WT)

HTR2A Constructs	25CN-NBOH	
	EC_{50}, nM (pEC_{50} ± SEM)	Emax, % WT
WT	0.41 (9.39 ± 0.05)	99.6 ± 1.7
N107A	1.53 (8.81 ± 0.26)*	60.8 ± 5.9****
D172A	0.30 (9.53 ± 0.20)	45.6 ± 2.9****
I181A	N.D	N.D
I181E	N.D	N.D
R185A	0.76 (9.12 ± 0.15)	75.2 ± 4.0****
N317A	0.58 (9.23 ± 0.13)	111.3 ± 4.9
L325A	198.30 (6.70 ± 0.22)****	55.8 ± 8.2****
N384A	17.34 (7.76 ± 0.22)****	66.1 ± 6.6****
K385A	0.22 (9.67 ± 0.13)	86.1 ± 3.6
Gq Constructs	25CN-NBOH	
	EC_{50}, nM (pEC_{50} ± SEM)	Emax, % WT
WT	0.49 (9.31 ± 0.06)	100.1 ± 2.0
Q350A	N.D	N.D
E355A	0.87 (9.06 ± 0.13)	115.4 ± 5.4*
Y356A	0.38 (9.42 ± 0.17)	108.2 ± 6.0
Y356F	0.54 (9.27 ± 0.12)	107.8 ± 4.3
N357A	6.16 (8.21 ± 0.08)****	75.6 ± 2.6****
L358A	N.D	N.D