COMPREHENSIVE PHARMACOLOGICAL PROFILING OF THE HUMAN 5-HT$_{7A}$ RECEPTOR ISOFORM EXPOSES NOVEL SIGNALING

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INTRODUCTION

The 5-HT (serotonin) receptor type 7 (5-HT$_7$R) is one of the most recently identified members of the 5-HT receptor family. Pharmacological modulation of this G protein coupled receptor (GPCR) is considered a promising approach for the treatment of various neurological and psychiatric disorders including anxiety, depression, schizophrenia and Alzheimer's disease[1].

Human (h) 5-HT$_7$R has been reported to couple primarily to Gαs, which activates adenylate cyclases and leads to the production of cAMP[2]. However, it has now become evident that many GPCRs can couple to more than one signaling pathway, and that different ligands acting at a given receptor can selectively promote the activation of different subsets of these pathways. This so-called "functional selectivity" (or biased signaling) highlights the importance of exhaustively testing multiple ligands on multiple signaling pathways when defining a receptor's signaling repertoire. To date, h5-HT$_7$R's full signaling signature in response to different ligands remains unexplored.

In this study, we applied Domain Therapeutics' bioSens-All® platform of enhanced bystander Bioluminescence Resonance Energy Transfer (ebBRET)-based biosensors to profile the signaling and pharmacology of ten diverse 5-HT$_7$R ligands at the recombinantly expressed h5-HT$_{7a}$R isoform in HEK293 cells. The agonist and antagonist activity of each ligand was characterized on an extensive panel of sixteen pathway-specific biosensors. The results obtained confirmed h5-HT$_{7a}$R's coupling to Gαs and are in alignment with previously published data[3-7]. Moreover, our data exposed novel couplings to brain-enriched Gi/o-family G proteins (i.e., GαoA, GαoB, Gαz), Gα13, Gα14 and (especially) Gα15. Interestingly, comparison of the ligand activity on Gαs and the newly identified Gα15 pathway enabled the broad classification of compounds into five functionally distinct pharmacological clusters. Among the identified clusters, one defined by LP-44 and LSD for example produced a switch in h5-HT$_{7a}$R G protein coupling. Specifically, ligands in this cluster displayed no detectable agonist activity on Gαs but fully antagonized 5-HT-induced Gαs signaling while activating Gα15 and other pathways.

MATERIALS AND METHODS

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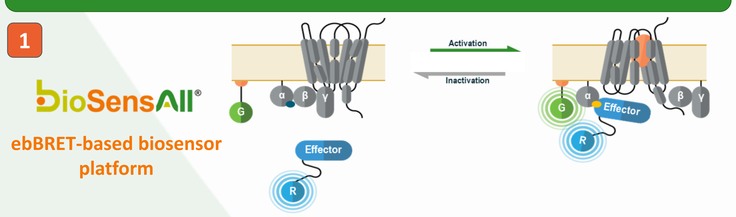
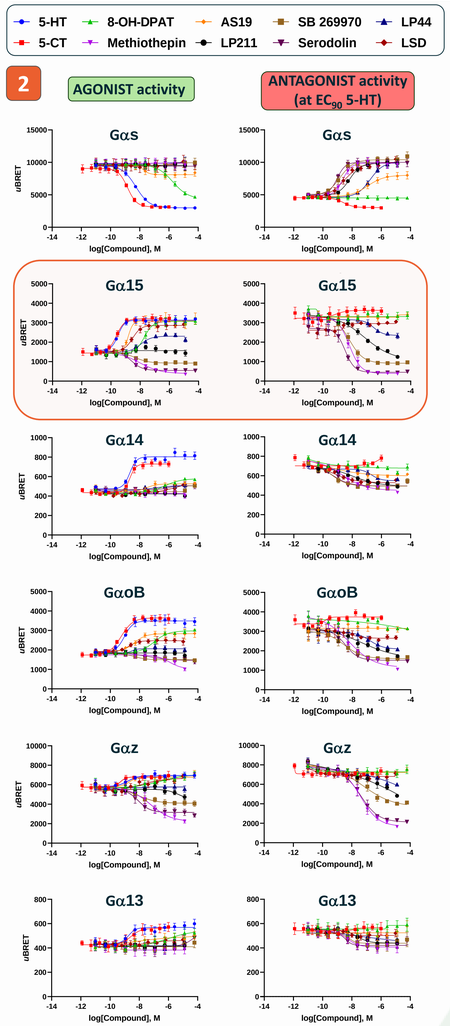


Figure 1: Assay principle underlying our ebBRET-based biosensors[8]. G protein-specific *Renilla* luciferase (RlucII)-tagged (R) effector proteins are recruited to activated (GTP-bound) untagged G alpha subunits following activation of untagged receptor. This event brings RlucII in close proximity to the plasma membrane-anchored *Renilla* green fluorescent protein (G), leading to an increase in ebBRET. Exceptionally for the Gαs biosensor, RlucII is directly fused to the Gαs protein. GPCR-mediated Gαs activation leads to its dissociation from the plasma membrane, resulting in a reduction in ebBRET.

RESULTS



		AGONIST activity									
		5-HT	5-CT	8-OH-DPAT	Methiothepin	AS19	LP211	SB 269970	LP44	Serodolin	LSD
Gs	% Agonism (vs. 5-HT)	100.00	97.84	79.28	ND	21.79	ND	ND	ND	ND	ND
	Std. Error	1.57	3.25	11.75	ND	3.47	ND	ND	ND	ND	ND
	AVERAGE EC_{50} (nM)	5.36	1.47	1828.00	ND	17.54	ND	ND	ND	ND	ND
	Std. Error	0.67	0.27	1058.00	ND	13.83	ND	ND	ND	ND	ND
G15	% Agonism (vs. 5-HT)	100.00	104.60	96.01	-76.31	97.70	ND	-41.68	46.92	-66.07	82.52
	Std. Error	3.26	3.85	4.70	5.21	5.21	ND	2.21	3.83	2.59	4.76
	AVERAGE EC_{50} (nM)	0.30	0.23	19.04	9.63	1.51	ND	5.13	10.86	2.52	3.00
	Std. Error	0.07	0.05	4.90	5.07	0.48	ND	2.57	4.62	0.80	0.96
G14	% Agonism (vs. 5-HT)	100.00	77.53	31.53	ND	15.54	ND	ND	ND	ND	ND
	Std. Error	3.73	3.53	4.82	ND	3.89	ND	ND	ND	ND	ND
	AVERAGE EC_{50} (nM)	2.16	2.15	559.20	ND	28.37	ND	ND	ND	ND	ND
	Std. Error	0.53	0.38	293.30	ND	26.51	ND	ND	ND	ND	ND
GoB	% Agonism (vs. 5-HT)	100.00	109.80	68.38	-45.22	59.24	ND	-23.93	10.83	-27.20	36.70
	Std. Error	4.18	4.58	3.88	8.25	4.16	ND	1.92	2.60	6.62	3.14
	AVERAGE EC_{50} (nM)	0.93	0.56	97.34	1067.00	4.37	ND	4.82	3.17	29.36	1.96
	Std. Error	0.28	0.14	31.21	820.80	2.34	ND	3.47	4.55	50.74	1.14
Gz	% Agonism (vs. 5-HT)	100.00	99.90*	102.19*	-314.20	103.78*	ND	-149.60	ND	-232.50	77.63
	Std. Error	10.99	5.18*	3.02*	40.04	3.16*	ND	15.30	ND	16.89	32.77
	AVERAGE EC_{50} (nM)	1.66	0.49	152.20	59.06	2.09	ND	7.53	ND	8.55	13.62
	Std. Error	1.43	0.32	226.40	41.98	1.89	ND	7.13	ND	4.94	24.05
G13	% Agonism (vs. 5-HT)	100.00	94.83	81.93	-17.55	51.41	ND	ND	ND	ND	20.80
	Std. Error	9.70	16.07	60.22	16.82	49.76	ND	ND	ND	ND	16.01
	AVERAGE EC_{50} (nM)	1.84	2.73	849.10	1.12	0.47	ND	ND	ND	ND	104.20
	Std. Error	1.17	2.22	2647.00	1272.00	7.05	ND	ND	ND	ND	306.70

		ANTAGONIST activity							
		5-CT	8-OH-DPAT	Methiothepin	AS19	LP211	SB 269970	LP44	Serodolin	LSD
Gs	% Antagonism (vs. EC_{90} 5-HT)	ND	ND	107.10	67.76	107.10	118.40	111.50	110.00	108.70
	Std. Error	ND	ND	4.46	6.80	3.91	5.26	6.81	4.27	4.91
	AVERAGE IC_{50} (nM)	ND	ND	2.33	114.70	6.68	1.48	174.30	0.96	3.04
	Std. Error	ND	ND	0.62	66.41	1.54	0.55	44.36	0.27	1.05
G15	% Antagonism (vs. EC_{90} 5-HT)	ND	ND	167.70	ND	128.40	137.70	55.85	164.40	ND
	Std. Error	ND	ND	5.03	ND	14.41	5.11	8.04	4.02	ND
	AVERAGE IC_{50} (nM)	ND	ND	6.33	ND	66.65	6.25	291.80	3.24	ND
	Std. Error	ND	ND	1.22	ND	39.55	1.84	193.20	0.71	ND
G14	% Antagonism (vs. EC_{90} 5-HT)	ND	ND	109.10	54.66	92.54	92.60	72.28	92.13	80.25
	Std. Error	ND	ND	5.14	6.76	9.17	3.52	6.97	3.46	4.22
	AVERAGE IC_{50} (nM)	ND	ND	2.17	ND	11.13	0.41	209.90	0.41	0.36
	Std. Error	ND	ND	0.99	ND	10.57	0.23	132.50	0.26	0.37
GoB	% Antagonism (vs. EC_{90} 5-HT)	ND	ND	155.70	ND	120.90	121.70	84.66	127.70	43.26
	Std. Error	ND	ND	11.79	ND	69.07	9.05	23.82	6.75	9.87
	AVERAGE IC_{50} (nM)	ND	ND	5.88	ND	43.40	2.81	161.10	3.46	6.11
	Std. Error	ND	ND	2.79	ND	141.00	2.48	260.20	1.60	12.80
Gz	% Antagonism (vs. EC_{90} 5-HT)	ND	ND	334.80	ND	156.26*	221.20	89.86*	310.20	ND
	Std. Error	ND	ND	21.03	ND	7.99*	26.93	6.75*	14.69	ND
	AVERAGE IC_{50} (nM)	ND	ND	41.05	ND	ND	61.86	ND	40.27	ND
	Std. Error	ND	ND	14.15	ND	ND	45.13	ND	13.22	ND
G13	% Antagonism (vs. EC_{90} 5-HT)	ND	ND	121.90	ND	93.89	95.02	123.00	110.80	39.75
	Std. Error	ND	ND	18.17	ND	21.81	15.41	451.00	10.04	9.16
	AVERAGE IC_{50} (nM)	ND	ND	5.16	ND	21.24	6.74	0.56	3.68	4.33
	Std. Error	ND	ND	5.22	ND	27.76	8.04	22.09	2.54	9.75

Figure 2. Multiparametric profiling of tool compound pharmacology at the h5-HT$_{7a}$R reveals G protein coupling diversity and novel signaling via Gα15. HEK293 cells were co-transfected with pathway-specific biosensor-coding plasmids and a plasmid encoding for h5-HT$_{7a}$R. Ligands were assessed for both agonist and antagonist (at an EC_{90} of 5-HT) activity. Curves were fitted using the log(agonist) vs. response - Variable slope (four parameters) non-linear regression model (GraphPad 10). Data are presented as mean values ± SEM, n=3-4. *In the absence of a clear quantifiable (EC_{50} value) concentration response, % activity was calculated using the highest ligand concentration on the ligand's dose response curve. ND: no response or not determinable. h5-HT$_{7a}$ exhibits a wide G protein signaling repertoire, coupling to at least one member from each of the four G protein families.

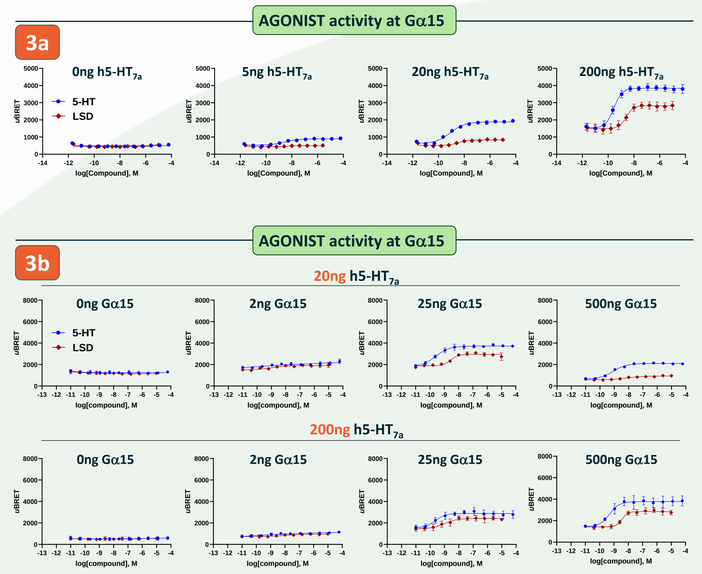


Figure 3. h5-HT$_{7a}$R coupling to Gα15 is specific and receptor-dependent. HEK293 cells were co-transfected with the Gα15 biosensor and increasing amounts of either h5-HT$_{7a}$R coding plasmid (**a**) or Gα15 protein-coding plasmid (**b**). The agonist activity of 5-HT and LSD was assessed and resulting curves were fitted using the log(agonist) vs. response - Variable slope (four parameters) non-linear regression model (GraphPad 10). Data are presented as mean values ± SEM, n=2-3. Coupling to Gα15 is lost in cells not transfected with h5-HT$_{7a}$R (**a**) or Gα15 protein (**b**). Coupling efficacy was also h5-HT$_{7a}$R dose-dependent and Gα15 protein dose-dependent .

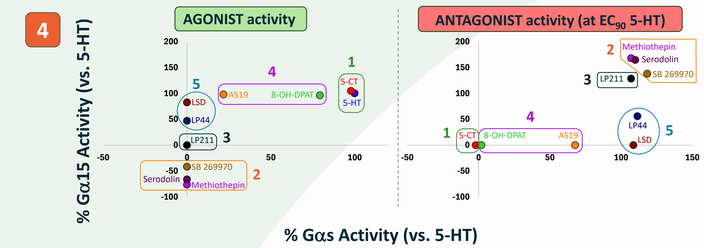


Figure 4. Analysis of compound activity on Gαs and Gα15 enables the classification of compounds into five pharmacological clusters: 1) 5-HT like compounds; **2)** Inverse agonists; **3)** Neutral antagonist; **4)** Compounds with full agonist activity on Gα15 and partial agonist activity on Gαs and other pathways; **5)** Compounds with no detectable agonist activity on Gαs but with agonist activity on Gα15 and other pathways, resulting in antagonism of 5-HT-induced Gαs signaling with agonism (partial) on other pathways.

CONCLUSIONS

> h5-HT$_{7a}$R can signal through various G protein pathways in addition to the Gαs pathway, potentially influencing a wide range of cellular processes and physiological functions.
> Novel coupling through Gα15 was identified; tool compound activity on this pathway allows for their classification into five functionally distinct pharmacological clusters.
> The data and methods reported herein enable deeper pharmacological characterization and understanding of 5-HT$_{7a}$R function and may help inform its therapeutic exploitation.

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