SUPPLEMENTARY MATERIAL

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Article Title:	**In vitro screening of major neurotransmitter systems possibly involved in the mechanism of action of antibodies to S100 protein in released-active form**
Running header:	Released–active form of antibodies to S100 protein
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Supplementary Item & Number	**Title or Caption**
Supplementary Methods	Radioligand assays: ▪ D_{2S}, $D_{4.4}$, D_5, $GABA_A$, $GABA_{B(1b)}$, PCP, AMPA, Kainate, NMDA, 5-HT_{1D}, $sigma_2$ receptors ▪ D_1, D_{2L}, D_3, $GABA_{B1A/B2}$, 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1E}, 5-HT_{2A}, 5-HT_{4e}, 5-HT_6, 5-HT_7 receptors Functional assays: ▪ [^{35}S]GTPγS binding assays on 5-HT_{1E} and 5-HT_{1F} receptors ▪ cAMP HTRF assays on D_1, D_{2L}, 5-HT_{4e}, 5-HT_7 receptors ▪ cAMP HTRF assays on D_{2S}, $D_{4.4}$, D_5 receptors ▪ 5-HT_{2A}, 5-HT_{2B}, 5-$HT2_{Ce}$, 5-HT_3 and 5-HT_6 receptors based on measuring of intracellular concentration of Ca^{2+} ions ▪ Cellular dielectric spectroscopy assay on 5-HT_{1D} receptor
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SUPPLEMENTARY INFORMATION

Supplementary Methods

Radioligand assays: D_{2S}, $D_{4.4}$, D_5, $GABA_A$, $GABA_{B(1b)}$, PCP, AMPA, Kainate, NMDA, 5-HT_{1D}, sigma$_2$ receptors

Homogenates of cell membranes were incubated with the corresponding radioligands in the presence or absence of RAF of Abs to S100 (20 μl or 10% v/v) or placebo (20 μl or 10% v/v), diluted in assay buffer (Supplementary Table 1 and Supplementary Table 2). Non-specific binding was determined in the presence of a saturation concentration of the corresponding non-radioactive ligand. After incubation the samples were quickly filtrated using glass filters, which were preliminary soaked in 0.3% polyethylenemine (PEI) (except for $GABA_{B(1b)}$, kainate, NMDA, AMPA receptors); and then washed several times with cooled buffer. After drying the filters, scintillation cocktail was added and a scintillation counter was used for reading of the radioactivity. The results were expressed as % of inhibition of control specific ligand binding.

Radioligand assays: D_1, D_{2L}, D_3, $GABA_{B1A/B2}$, 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1E}, 5-HT_{2A}, 5-HT_{4e}, 5-HT_6, 5-HT_7 receptors

Extracts of cell membranes were incubated with the appropriate radioligands in the presence or absence of RA dilutions of antibodies to S100 protein or placebo (50 μl or 50% v/v), diluted in assay buffer (Supplementary Table 1 and Supplementary Table 2). Non-specific binding was determined in the presence of a saturation concentration of the corresponding non-specific ligand. After incubation the samples were quickly filtrated (the filters were preliminary soaked for 2 hours at room temperature in washing buffer containing 0.5% PEI; except for: 5-HT_{4e} receptor (0.1% PEI) and $GABA_{B1A/B2}$ receptor (0.05% Brij)).

When the filters were rinsed 5-6 times using 0.5 ml of ice-cold washing buffer, into each well 50 µl of scintillation mixture was added and the samples were incubated 15 minutes on an orbital shaker and then counted with a scintillation counter. The results were expressed as % of inhibition of control specific ligand binding.

Functional assays: [^{35}S]GTPγS binding assays on 5-HT$_{1E}$ and 5-HT$_{1F}$ receptors

Extracts of recombinant cell membranes of chinese hamster ovary (CHO) were thawed on ice and diluted in assay buffer (Supplementary Table 3 and Supplementary Table 4); they were kept on ice until assays were begun. Membranes were mixed with guanosine diphosphate (GDP) (v/v) diluted in assay buffer to reach the final concentration and incubated for at least 15 minutes on ice. In parallel, GTPγ[^{35}S] (Perkin Elmer, NEG030X) was diluted in assay buffer to reach a final concentration of 0.1 nM and was mixed with granules (PVT-WGA (Amersham, RPNQ0001)) (v/v) directly prior to initiation of reaction.

The following compounds were successively added to the wells of an Optiplate (Perkin Elmer) in order to determine agonistic activity: either 50 µl of RAF of Abs to S100, or 50 µl placebo, or 50 µl of reference ligand (reference agonist diluted in assay buffer in order to reach concentration from 0.01 x the half maximal effective concentration (EC_{50}) to 100 x EC_{50}), 20 µl of membrane mixture with GDP and 20 µl of mixture of GTPγ[^{35}S] with beads.

The following compounds were successively added to the wells of an Optiplate (Perkin Elmer) in order to determine antagonostic activity: either 50 µl of RAF of Abs to S100, or 50 µl placebo, or 50 µl of reference ligand (reference antagonist diluted in assay buffer in order to reach concentration from 0.01 x the half maximal inhibitory concentration (IC_{50}) to 100 x IC50), 10 µl assay buffer, 20 µl of membrane mixture with GDP. After a 15

minute incubation, 10 µl reference agonist in concentration equal to historical EC_{80} and 20 µl of mixture of GTPγ[^{35}S] with beads were added.

The plates were covered with a top seal, shaken on an orbital shaker for 2 minutes, and then incubated for 1 hour at 21°C. Then the plates were centrifuged for 10 minutes at 2000 rpm, incubated at 21°C for 1 hour (except for 5-HT_{1F} no incubation) and counted for 1 minute/well with a PerkinElmer TopCount™ reader.

Functional assays: cAMP HTRF assays on D_1, D_{2L}, 5-HT_{4e}, 5-HT_7 receptors

Recombinant CHO cells were grown in culture media (HAM's F12 with L-Glutamine, 10% FBS for D_1 and D_{2L} receptors; UltraCHO w/ L-Glutamine, 1% dialyzed FBS, 600 ng/ml doxycycline for 5-HT_{4e} receptor; UltraCHO w/ L-Glutamine, 1% dialyzed FBS for 5-HT_7 receptor) without antibiotics and detached with phosphate buffered saline with ethylenediaminetetraacetic acid (PBS-EDTA). The cells were centrifuged and resuspended in assay buffer (Supplementary Table 3 and Supplementary Table 4). Experiments were performed in 96 well plates (Corning 3694).

In order to determine agonistic activity for D_1, 5-HT_{4e}, 5-HT_7 receptors 12 µl cells were mixed with either 12 µl RAF of Abs to S100, or 12 µl of placebo, or 12 µl reference agonist and for D_{2L} receptor 12 µl cells were mixed with either 6 µl RAF of Abs to S100, or 6 µl of placebo, or 6 µl reference agonist and 6 µ forskolin in concentration 10 µM (Calbiochem, lot 344270).

In order to determine antagonistic activity for D_1, 5-HT_{1B}, 5-HT_{4e}, 5-HT_7 receptors 12 µl of cells were mixed with either 6 µl of RAF of Abs to S100, or 6 µl placebo, or 6 µl reference antagonist. After incubation for 10 minute at 21°C, 6 µl reference agonist in the concentration corresponding to EC_{80} was added and for D_{2L} receptor 12 µl cells were mixed with 6 µl RAF of Abs to S100 or 6 µl placebo or 6 µl reference agonist. After a 10 minute

incubation at 21°C, 6 µl mixture containing forskolin at a concentration of 10 µM and reference agonist on the concentration corresponding to EC_{80}.

The plates were then incubated for 30 minutes at 21°C. During the incubation, the anti-cAMP (cyclic adenosine monophosphate) cryptate antibody (K) and the cAMP-D2 (D2) were prepared according to the manufacturer specifications (HTRF kit from Cis-Bio International (cat # 62AM2PEB)). 12 µl of cAMP-D2 solution followed by 12 µl of K solution were added to each well. The plate was then covered by a top-seal and incubated for at least 1 hour at 21°C. The plates were then read on the Rubystar plate reader (BMG Labtech) and the data were analyzed by non-linear regression using a single site model.

Functional assays: cAMP HTRF assays on D_{2S}, $D_{4.4}$, D_5 receptors

Recombinant CHO-K1 cells (D4.4), Human Embryonic Kidney 293 cells (HEK-293) cells (D_{2S}) or tumor cell lines derived from rat pituitary (GH4 cells) (D5) were suspended in Hank's buffered salt solution (HBSS buffer) (Invitrogen) supplemented with 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) 20 mM (pH 7.4) and 500 µM IBMX, then distributed in microplates at a density of 10^4 cells/well (Supplementary Table 3 and Supplementary Table 4).

In the agonist mode, cells were incubated for 10 minutes ($D_{4.4}$ and D_{2S}) or 30 minutes (D_5) at room temperature in the presence of one of the followings: 2 µl HBSS with Hepes 20 mM (for basal control), 2 µl of reference agonist dopamine at 8 concentrations (for EC_{50} determination) and at 300nM ($D_{4.4}$), 100 nM (D_{2S}) or 1µM (D_5) (for stimulated control), 2 µl of RAF of Abs to S100 or 2 µl placebo.

In the antagonist mode, cells were incubated for 5 minutes at room temperature in the presence of one of the following: 2 µl of the reference antagonist at 10 µM ($D_{4.4}$), 1 µM (D_{2S}) (for basal control), 2 µl of HBSS with Hepes 20 mM (for basal control for D_5 and stimulated

control), 2µl of the reference antagonist clozapine (for $D_{4.4}$), butaclamol (for D_{2S}) or SCH 23390 (for D_5) (for IC_{50} determination), 2µl of RAF of Abs to S100 or placebo. Thereafter, cells were incubated for 10 minutes ($D_{4.4}$ and D_{2S}) or 30 minutes (D_5) at room temperature in the absence (for basal control) or presence of the reference agonist dopamine at 100 nM ($D4_{.4}$), 30 nM (D_{2S}) or 50 nM (D_5).

After incubation, for both effects, the adenylyl cyclase activator NKH 477 was added at final concentration of 0.7 µM ($D_{4.4}$) or 1 µM (D_{2S}) for an additional incubation of 10 minutes ($D_{4.4}$) or 20 minutes (D_{2S}) at 37°C.

Following the incubation, the cells were lysed and the fluorescence acceptor (D2-labeled cAMP) and fluorescence donor (anti-cAMP antibody labeled with europium cryptate) were added.

After 60 minutes at room temperature, the fluorescence transfer was measured at Lambdaex=337 nm and Lambdaem=620 and 665 nm using a microplate reader (Rubystar, BMG).

The cAMP concentration was determined by dividing the signal measured at 665 nm by that measured at 620 nm (ratio).

Functional assays: 5-HT_{2A}, 5-HT_{2B}, 5-$HT2_{Ce}$, 5-HT_3 and 5-HT_6 receptors based on measuring of intracellular concentration of Ca^{2+} ions

CHO-K1 cells co-expressing the recombinant receptor of interest along with mitochondrial aequorin and the promiscuous Gα16 protein (an exception was for the assay on 5-HT_3 receptor: HEK-293 cells expressing mitochondrial aequorin and no Gα16 was used) were grown 18 hours in a humidified 37°C/5% CO_2 incubator, prior to the test in media without antibiotics (UltraCHO w/ L-Glutamine, 1% dialyzed FB for 5-HT_{2A}, 5-HT_{2B}, 5-$HT_{2Cedited}$ receptors; Eagle's minimal essential medium (EMEM) w/o Earle's balanced salt

solution (EBSS) & Non-essential amino acid (NEAA), w/o $CaCl_2$, 10% fetal bovine serum (FBS) for 5-HT_3 receptor) (Supplementary Table 3 and Supplementary Table 4). Cells were detached by gentle flushing with PBS-EDTA (5 mM EDTA), recovered by centrifugation and resuspended in assay buffer. All the cells were incubated at 21°C for at least 4h with Coelenterazine h (Molecular Probes).

In order to determine agonistic activity, 50 µl of the cell suspension was added to either 50 µl of RAF of Abs to S100, placebo, or reference agonist plated in a 96-well plate (NUNC 265301).

In order to determine antagonistic activity, 50 µl of the cell suspension was added to either 50 µl of RAF of Abs to S100, placebo, or reference agonist plated in a 96-well plate (NUNC 265301). After a 15 minute incubation 100 µl (or 50 µl for 5-HT_3 receptor) of the reference agonist at a concentration corresponding to its EC_{80} was injected.

The resulting emission of light was recorded using Hamamatsu Functional Drug Screening System 6000 (FDSS 6000).

To standardize the emission of recorded light (determination of the "100% signal") across plates and across different experiments, some of the wells contained 100 µM digitonin or a saturating concentration of adenosine triphosphate (ATP) (20 µM). Plates also contained the reference agonist at a concentration equivalent to the EC_{100} and the EC_{80}.

For 5-HT_3 assay, the calcium concentration was adapted in the assay buffer or in the compound to have a 3 mM $CaCl_2$ final concentration in agonist mode and a 4mM $CaCl_2$ final concentration in antagonist mode.

Functional assays: cellular dielectric spectroscopy assay on 5-HT_{1D} receptor

CHO-S cells were seeded onto 96-well plate coated with fibronectin at $8x10^4$ cells/well in HBSS buffer with HEPES 20 mM (Invitrogen) and 0.1% BSA and allowed to

equilibrate for 60 minutes at 28°C before the start of the experiment. Plates were placed onto the system and measurements were made at a temperature of 28°C (Supplementary Table 3 and Supplementary Table 4).

In order to determine agonistic activity, solutions were added simultaneously to all 96 wells using an integrated fluidics system: either 15 µl RAF of Abs to S100, or 15 µl placebo, or 15 µl HBSS with HEPES 20 mM (for basal control) or 15 µl reference agonist 5-HT (for EC_{50} determination) and at 100 nM (for stimulated control).

In order to determine antagonistic activity, solutions were added simultaneously to all 96 wells using an integrated fluidics system: either 15 µl RAF of Abs to S100, or 15 µl placebo, 15 µl HBSS with HEPES 20 mM (for basal and stimulated controls) or 15 µl reference antagonist methiothepin (for IC_{50} determination) were incubated for 15 minutes before the addition of HBSS with Hepes 20 mM (basal control) or the reference agonist 5-HT at 3 nM. Impedance measurements were monitored for about 10 minutes.

Supplementary Table 1. Experimental conditions: radioligand assays (part 1)

Receptor Subtype	Origin	Protein (µg/well)	Radioligand	Nonspecific Ligand	Incubation Conditions
D_1 *(h)*	human recombinant (CHO-K1 cells)	4	[^{3}H]-SCH23390 (1 nM)	SCH23390 (100 nM)	60 min / 25°C
D_{2L} *(h)*	human recombinant (CHO-K1 cells)	0.5	[^{3}H]-Spiperone (0.3 nM)	Risperidone (10 µM)	60 min / 25°C
D_{2S} *(h)*	human recombinant (HEK-293 cells)	30	[^{3}H]7-OH-DPAT (1 nM)	(+)butaclamol (10 µM)	60 min / 22°C
D_3 *(h)*	human recombinant (CHO-K1 cells)	1	R-(+)-7-OH-[3H]DPAT (0.23 nM)	R-(+)-7-OH-DPAT (10 µM)	30 min / 25°C
$D_{4.4}$ *(h)*	human recombinant (CHO cells)	100	[^{3}H]spiperone (0.3 nM)	(+)butaclamol (10 µM)	60 min / 22°C
D_5 *(h)*	human recombinant (GH4 cells)	150	[^{3}H]dopamine (7.5 nM)	SCH 23390 (10 µM)	60 min / 22°C
GABA A	rat cerebral cortex	500	[^{3}H]muscimol (5 nM)	Muscimol (10 µM)	10 min / 4°C
GABA B (1b) *(h)*	human recombinant (HEK-293 cells)	1,5	[^{3}H]CGP 54626 (2.5 nM)	GABA (10 mM)	60 min / 22°C
GABA $B_{1A/B2}$ *(h)*	human recombinant (CHO-K1 cells)	2	[^{3}H]-CGP54626 (15 nM)	CGP54626 (10 µM)	30 min / 25°C
PCP	rat cerebral cortex	100	[^{3}H]TCP (5 nM)	MK 801 (10 µM)	60 min / 22°C
AMPA	rat cerebral cortex	600	[^{3}H]AMPA (8 nM)	L-glutamate (1 mM)	60 min / 4°C
Kainate	rat cerebral cortex	600	[^{3}H]kainic acid (5 nM)	L-glutamate (1 mM)	60 min / 4°C

Receptor Subtype	Origin	Protein (µg/well)	Radioligand	Nonspecific Ligand	Incubation Conditions
NMDA	rat cerebral cortex	1	[^{3}H]CGP 39653 (5 nM)	L-glutamate (100 µM)	60 min / 4°C
5-HT$_{1A}$ *(h)*	human recombinant (CHO-K1 cells)	2,5	[^{3}H]-8-OH-DPAT (0.39 nM)	5-HT (1 µM)	60 min / 25°C
5-HT$_{1B}$ *(h)*	human recombinant (CHO cells)	10	[^{3}H]-5-CT (0.6 nM)	5-HT (10 µM)	60 min / 25°C
5-HT$_{1D}$	rat recombinant (CHO cells)	20	[^{3}H]-5-CT (1 nM)	serotonin (10 µM)	60 min / 22°C
5-HT$_{1E}$ *(h)*	human recombinant (CHO cells)	5	[^{3}H]-LSD (14 nM)	5-HT (100 µM)	60 min / 37°C
5-HT$_{2A}$ *(h)*	human recombinant (CHO-K1 cells)	5	[^{3}H]-Ketanserin (1.48 nM)	Ketanserin (1 µM)	60 min / 25°C
5-HT$_{4e}$ *(h)*	human recombinant (CHO-K1 cells)	10	[^{3}H]-GR113808 (0.3 nM)	5-HT (100 µM)	60 min / 37°C
5-HT$_{6}$ *(h)*	human recombinant (CHO-K1 cells)	2,5	[^{3}H]-LSD (1.16 nM)	Mianserin (100 µM)	60 min / 25°C
5-HT$_{7}$ *(h)*	human recombinant (CHO-K1 cells)	5	[^{3}H]-LSD (1 nM)	5-CT (100 µM)	30 min / 37°C
Sigma$_2$	rat cerebral cortex	500	[^{3}H]DTG (+ 300 nM (+)pentazocine) (5 nM)	Haloperidol (10 µM)	120 min / 22°C

Supplementary Table 2. Experimental conditions: radioligand assays (part 2)

Receptor Subtype	Assay buffer	Filtres	Washing cooled buffer	Scintillation cocktail	Scintillation counter
D_1 *(h)*	50 mM Tris (pH 7.4), 5 mM MgCl2, 10µg/ ml saponin	Filtermate Harvester (Perkin Elmer)	50 mM Tris (pH 7.4), 5 mM MgCl2	Microscint 20, Packard	Topcount, Packard
D_{2L} *(h)*	25 mM Hepes (pH 7.4), 5 mM MgCl2, 1 mM CaCl2,10 µg/ml 0.5% BSA	Filtermate Harvester (Perkin Elmer)	25 mM Hepes (pH 7.4), 5 mM MgCl2, 1 mM CaCl2	Microscint 20, Packard	Topcount, Packard
D_{2S} *(h)*	50 mM Tris-HCl (pH 7.4), 5mM KCl, 5mM MgCl2, 1 mM EDTA and 0.1% BSA	GF/B, Packard	50 mM Tris-HCl	Microscint 0, Packard	Topcount, Packard
D_3 *(h)*	50 mM Tris (pH 7.4), 5 mM MgCl2, 10µg/ ml saponin	Filtermate Harvester (Perkin Elmer)	50 mM Tris (pH 7.4), 5 mM MgCl2	Microscint 20, Packard	Topcount, Packard
$D_{4.4}$ *(h)*	50 mM Tris-HCl (pH 7.4), 120 mM NaCl, 5 mM KCl, 5 mM MgCl2 and 1 mM EDTA	GF/B, Packard	50 mM Tris-HCl	Microscint 0, Packard	Topcount, Packard
D_5 *(h)*	20 mM Tris-HCl (pH 7.4), 5 mM MgCl2, 0.8 mM DTT, 0.3 mM ascorbic acid and 0.1 mM pyrocatechol	GF/B, Packard	50 mM Tris-HCl	Microscint 0, Packard	Topcount, Packard

Receptor Subtype	Assay buffer	Filtres	Washing cooled buffer	Scintillation cocktail	Scintillation counter
GABA A	25 mM Hepes/Tris (pH 7.0)	Filtermat A, Wallac	50 mM Tris-HCl	Meltilex B/HS, Wallac	Betaplate 1204, Wallac
GABA B (1b) *(h)*	50 mM Tris-HCl (pH 7.4) and 2.5 mM CaCl2	GF/B, Packard	50 mM Tris-HCl and 150 mM NaCl	Microscint 0, Packard	Topcount, Packard
GABA $B_{1A/B2}$ *(h)*	50 mM Tris (pH 7.4), 5 mM MgCl2	Filtermate Harvester (Perkin Elmer)	50 mM Tris (pH 7.4)	Microscint 20, Packard	Topcount, Packard
PCP	5 mM Tris (pH 7.7)	GF/B, Packard	50 mM Tris-HCl and 150 mM NaCl	Microscint 0, Packard	Topcount, Packard
AMPA	50 mM Tris-HCl (pH 7.1), 2.5 mM CaCl2 and 100 mM KSCN	Filtermat B, Wallac	50 mM Tris-HCl (pH 7.1), 2.5 mM CaCl2 and 100 mM KSCN	Meltilex B/HS, Wallac	Betaplate 1204, Wallac
Kainate	50 mM Tris-HCl (pH 7.4) and 2.5 mM CaCl2	Filtermat A, Wallac	50 mM Tris-HCl	Meltilex B/HS, Wallac	Betaplate 1204, Wallac
NMDA	5 mM Tris-HCl (pH 7.7) and 10 mM EDTA-Tris	GF/B, Whatman	5 mM Tris-HCl (pH 7.7) and 10 mM EDTA-Tris	Formula 989, Packard	LS series, Beckman
5-HT_{1A} *(h)*	50 mM Tris (pH 7.4), 0.1% ascorbic acid, 5 mM CaCl2, 10 µg/ ml saponin	Filtermate Harvester (Perkin Elmer)	50 mM Tris (pH 7.4)	Microscint 20, Packard	Topcount, Packard

Receptor Subtype	Assay buffer	Filtres	Washing cooled buffer	Scintillation cocktail	Scintillation counter
5-HT$_{1B}$ *(h)*	50 mM Tris (pH 7.4), 0.1% ascorbic acid, 12.5 mM MgCl2, 1 mM EDTA	Filtermate Harvester (Perkin Elmer)	50 mM Tris (pH 7.4)	Microscint 20, Packard	Topcount, Packard
5-HT$_{1D}$	50 mM Tris-HCl (pH 7.7), 4 mM $CaCl_2$, 0.1% ascorbic acid and 10 µM pargyline	GF/B, Packard	50 mM Tris-HCl	Microscint 0, Packard	Topcount, Packard
5-HT$_{1E}$ *(h)*	50 mM Tris (pH 7.4), 0.1% ascorbic acid, 4 mM CaCl2	Filtermate Harvester (Perkin Elmer)	50 mM Tris (pH 7.4)	Microscint 20, Packard	Topcount, Packard
5-HT$_{2A}$ *(h)*	50 mM Tris (pH 7.4), 0.1% ascorbic acid, 5 mM CaCl2	Filtermate Harvester (Perkin Elmer)	50 mM Tris (pH 7.4)	Microscint 20, Packard	Topcount, Packard
5-HT$_{4e}$ *(h)*	50 mM Hepes (pH 7.4)	Filtermate Harvester (Perkin Elmer)	50 mM Hepes (pH 7.4)	Microscint 20, Packard	Topcount, Packard
5-HT$_{6}$ *(h)*	50 mM Tris (pH 7.4), 0.1% ascorbic acid, 4 mM CaCl2, 10µg/ ml saponin	Filtermate Harvester (Perkin Elmer)	50 mM Tris (pH 7.4)	Microscint 20, Packard	Topcount, Packard
5-HT$_{7}$ *(h)*	50 mM Tris (pH 7.4), 0.1% ascorbic acid, 4 mM CaCl2, 10µg/ ml saponin	Filtermate Harvester (Perkin Elmer)	50 mM Tris (pH 7.4)	Microscint 20, Packard	Topcount, Packard
Sigma$_2$	50 mM Tris-HCl (pH 8.0) and 0.3 µM	Filtermat A, Wallac	50 mM Tris-HCl and 150 mM NaCl	Meltilex B/HS, Wallac	Betaplate 1204, Wallac

Receptor Subtype	Assay buffer	Filtres	Washing cooled buffer	Scintillation cocktail	Scintillation counter
	penthazocine)				

Supplementary Table 3. Experimental conditions: functional assays (part 1).

Receptor Subtype	Origin	Stimulus (antagonist mode)	Incubation Conditions	Assay buffer
D_1 *(h)*	human recombinant (CHO-K1 cells)	SKF81297 (0.12 nM)	30 min / 21°C	KRH, 1 mM IBMX
D_{2L} *(h)*	human recombinant (CHO-K1 cells)	Quinpirole (12.4 nM)	30 min / 21°C	KRH, 1 mM IBMX
D_{2S} *(h)*	human recombinant (HEK-293 cells)	dopamine (30 nM)	20 min / 37°C	HBSS buffer, HEPES 20 mM (pH 7.4) and 500 µM IBMX
$D_{4.4}$ *(h)*	human recombinant (CHO cells)	dopamine (100 nM)	10 min / 37°C	HBSS buffer, HEPES 20 mM (pH 7.4) and 500 µM IBMX
D_5 *(h)*	human recombinant (GH4 cells)	dopamine (50 nM)	30 min / 22°C	HBSS buffer, HEPES 20 mM (pH 7.4) and 500 µM IBMX
5-HT_{1D}	rat recombinant (CHO-S cells)	serotonin (3 nM)	28°C	HBSS buffer, 20 mM HEPES (pH 7.4), 0.1% BSA
5-HT_{1E} *(h)*	human recombinant (CHO cells)	5-CT (31.6 nM)	60 min / 21°C	20 mM HEPES (pH 7.4), 100 mM NaCl, 10 µg/ml saponin, 3 mM MgCl2
5-HT_{1F} *(h)*	human recombinant (CHO cells)	5-HT (316 nM)	60 min / 21°C	20 mM HEPES (pH 7.4), 100 mM NaCl, 10 µg/ml saponin, 1 mM MgCl2, 0.1% BSA

Receptor Subtype	Origin	Stimulus (antagonist mode)	Incubation Conditions	Assay buffer
5-HT$_{2A}$ *(h)*	human recombinant (CHO-K1 cells)	Alpha-Methyl-5-HT (1.75 nM)	15 min / 21°C	DMEM/HAM's F12 with HEPES, without phenol red, 0.1% BSA protease free
5-HT$_{2B}$ *(h)*	human recombinant (CHO-K1 cells)	Alpha-Methyl-5-HT (2.75 nM)	15 min / 21°C	DMEM/HAM's F12 with HEPES, without phenol red, 0.1% BSA protease free
5-HT$_{2Cedited}$ *(h)*	human recombinant (CHO-S cells)	serotonin (3 nM)	22°C	HBSS buffer, HEPES 20 mM (pH 7.4)
5-HT$_{3}$ *(h)*	human recombinant (HEK-293 cells)	5-HT (1227 nM)	15 min / 21°C	DMEM/HAM's F12 with HEPES, without phenol red, 0.1% BSA protease free
5-HT$_{4e}$ *(h)*	human recombinant (CHO-K1 cells)	5-HT (3.30 nM)	30 min / 21°C	KRH, 1 mM IBMX
5-HT$_{6}$ *(h)*	human recombinant (CHO-K1 cells)	5-HT (7.52 nM)	15 min / ???	DMEM/HAM's F12 with HEPES, without phenol red, 0.1% BSA protease free
5-HT$_{7}$ *(h)*	human recombinant (CHO-K1 cells)	5-CT (4 nM)	30 min / 21°C	KRH, 1 mM IBMX

Supplementary Table 4. Experimental conditions: functional assays (part 2).

Receptor Subtype	Reference agonist	Reference antagonist	Reaction Product	Method of Detection
D_1 *(h)*	SKF81297	SCH23390	cAMP	HTRF
D_{2L} *(h)*	Quinpirole	Haloperidol	cAMP	HTRF
D_{2S} *(h)*	dopamine	butaclamol	cAMP	HTRF
$D_{4.4}$ *(h)*	dopamine	clozapine	cAMP	HTRF
D_5 *(h)*	dopamine	SCH 23390	cAMP	HTRF
5-HT_{1D}	serotonin	methiothepin	impedance	cellular dielectric spectroscopy
5-HT_{1E} *(h)*	5-CT	No validated reference antagonist	Activated G-protein (bound to GTPyS)	GTPyS assay
5-HT_{1F} *(h)*	5-HT	No validated reference antagonist	Activated G-protein (bound to GTPyS)	GTPyS assay
5-HT_{2A} *(h)*	5-HT, Alpha-Me	Ketanserin	intracellular [Ca2+]	Luminescence measurement
5-HT_{2B} *(h)*	5-HT, Alpha-Me	SB 204541	intracellular [Ca2+]	Luminescence measurement
5-$HT_{2Cedited}$ *(h)*	serotonin	SB 206553	intracellular [Ca2+]	Fluorimetry
5-HT_3 *(h)*	5-HT	MDL72222	intracellular [Ca2+]	Luminescence measurement

Receptor Subtype	Reference agonist	Reference antagonist	Reaction Product	Method of Detection
5-HT$_{4e}$ *(h)*	5-HT	GR113808	cAMP	HTRF
5-HT$_6$ *(h)*	a-methyl-5-HT	Mianserin	intracellular [Ca2+]	Luminescence measurement
5-HT$_7$ *(h)*	5-CT	Risperidone	cAMP	HTRF