

Essential role for orbitofrontal 5-HT_{1B} receptors in obsessive-compulsive disorder-like behavior and serotonin reuptake inhibitor response in mice

Supplemental Information

Chemicals

Fluoxetine was purchased from ANAWA (Zurich, Switzerland). Clomipramine, desipramine, 8-hydroxy-2-(dinpropylamino)-tetralin (8-OH-DPAT) and N-[4-Methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2'-methoxy-1,4'-(5-methyl-1,2,4-oxadiazol-3-yl)-1,1'-biphenyl-4-carboxamide hydrochloride (GR127935) were purchased from Sigma-Aldrich (St. Louis, MO). 5-methoxy-3-(1,2,3,6)tetrahydropyridin-4-yl-1H-indole (RU24969) and 3-(1,2,5,6-tetrahydro-4-pyridyl)-5-propoxy pyrrolo[3,2-b]pyridine (CP94253) were purchased from Tocris-Cookson (Ellisville, MO). Radiolabeled chemicals [125I]-iodocyanopindolol (2200 Ci/mmol) and [35S]-GTPγS (1250 Ci/mmol) were purchased from Perkin Elmer (Boston, MA).

Quantitative Autoradiographic Binding Experiments

[125I]-CYP. Slides were preincubated at 4°C in 170 mM Tris-HCl (pH 7.5) for 1 hour. Slides were then incubated for 2 hours at room temperature (RT) in 170 mM Tris-HCl supplemented with 0.01% ascorbic acid, 10 μM pargyline, 70 pM [125I]-CYP, 3 μM isoproterenol, 100 nM 8-OH-DPAT, and 0.3% bovine serum albumin. Slides were then washed twice for 10 min in cold (4°C) Tris buffer. Slides were then briefly dipped in distilled water and air dried. Slides were exposed to Kodak Biomax MR film for 24 – 48 hours.

[35S]-GTPγS. Slides were preincubated at RT for 15 min in 50 mM HEPES buffer (pH 7.5) containing 100 mM NaCl, 1 mM MgCl₂, 0.2 mM EGTA, and 0.3 mM DTT, and for a further 15 min in the same buffer supplemented with 2 mM GDP and 100 nM DPCPX. Then slides were incubated for 60 min at 30°C in the same buffer supplemented with 0.1 nM [35S]-GTPγS and either 0 μM CP94253, 10 μM CP94253, or 10 μM CP94253 + 20 μM GR127935. Slides were

then washed twice for 5 min in cold 50 mM HEPES buffer (pH 7.0), briefly dipped in distilled water and air dried. Slides were exposed to Kodak Biomax MR film for 48 – 72 hours.

Statistical Analysis

Open Field Test. Analyses of variance (ANOVAs) with antidepressant pretreatment (fluoxetine, clomipramine, desipramine) or GR127935 as a between-subjects factor and acute drug treatment (RU24969 or 8-OH-DPAT) as a within-subject factor were applied to two measures: total distance traveled and the spatial scaling exponent “spatial d ”. Spatial d quantifies geometric patterns of locomotion and has been described in detail elsewhere (1). Briefly, spatial d quantifies the degree to which consecutive movements are along a straight line ($d \approx 1$), meandering ($d \approx 1.5$), or include many directional changes ($d \approx 2$). For example, the perseverative paths of locomotion induced by RU24969 are characterized by straight paths along the perimeter of the open field, and are quantified as low spatial d values. For all experiments, significant interactions were resolved using post-hoc ANOVAs for within-subjects factors and/or Newman-Keuls post-hoc tests for between-subjects factors. Significance was set at $p < .05$, and p values were adjusted using the Bonferroni correction when post-hoc ANOVAs were applied. Animals were removed from analysis for total distance traveled if their mean saline value was greater or less than 3 standard deviations (SD) from the group mean. Animals were removed from analysis for spatial d if their mean saline value was greater or less than 2 SD from the group mean.

Prepulse Inhibition (PPI). PPI was calculated as $[100 - (\text{Prepulse} - \text{Pulse trial/averaged Pulse Alone}) \times 100]$. Pulse Alone values were calculated as the mean of startle values from blocks two and three. ANOVAs with pretreatment as a between-subjects factor, and block, prepulse intensity, and drug treatment (RU24969 or 8-OH-DPAT) as within-subject factors were applied to averaged PPI values. A significant main effect of prepulse intensity was found in all experiments, and is not reported.

Startle Reactivity. ANOVAs with pretreatment as a between-subjects factor and RU24969 or 8-OH-DPAT treatment as a within-subject factor were applied to averaged Pulse Alone values from blocks two and three to determine whether any effects of these variables on startle confounded the interpretation of PPI results.

Forced Swim Test. ANOVAs with fluoxetine as a between-subjects factor and block (1 min intervals) as a within-subject factor were applied to three different measures: swimming, climbing, and immobility.

Autoradiography. ANOVAs with antidepressant pretreatment as a between-subjects factor and slice as a within-subjects factor were applied to specific binding (% control) for [125I]-CYP studies or CP94253-induced binding for [35S]-GTP γ S studies. Three slices through areas of interest were analyzed for each mouse. Specific binding was calculated by subtracting nonspecific binding (binding in the presence of 20 μ m GR127935) from total binding.

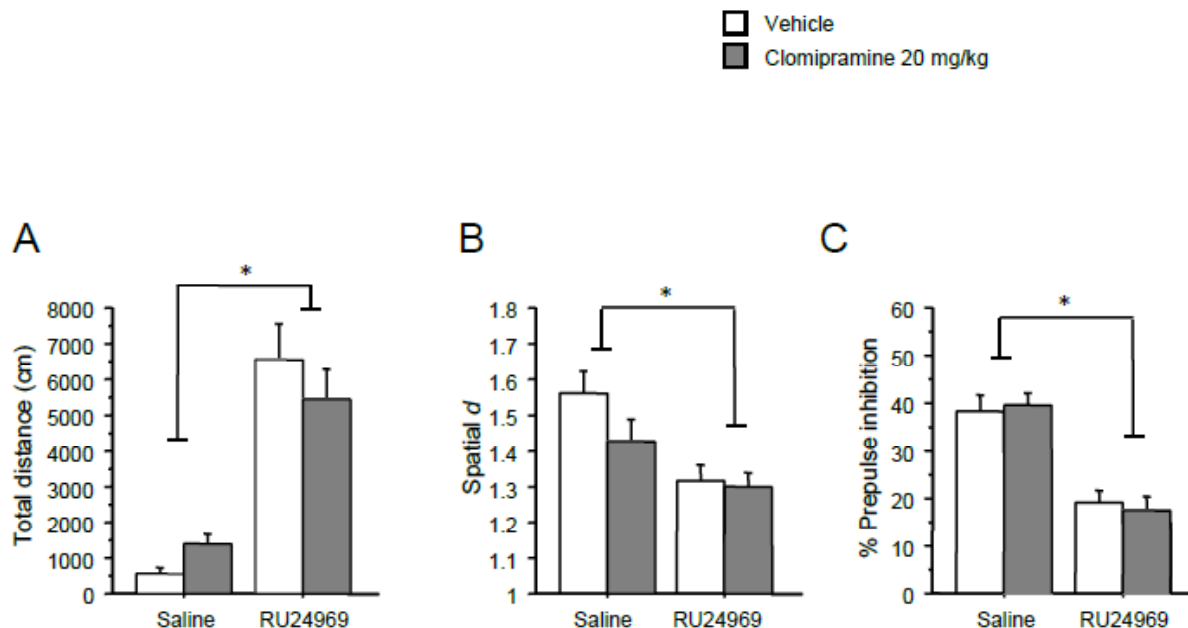


Figure S1. Subchronic clomipramine treatment does not reduce OCD-like behavior. Following 1 week of treatment with 0 mg/kg/d ($n = 13$) or 20 mg/kg/d ($n = 15$) clomipramine, mice received injections of the 5-HT_{1B}R agonist RU24969 (0 or 10 mg/kg) on separate test days. RU24969 increased locomotion [$F(1,26) = 63.63$; $p < 0.001$] (**A**), reduced spatial d [$F(1,26) = 23.70$; $p < 0.001$] (**B**) and decreased prepulse inhibition [$F(1,26) = 20.18$; $p < 0.001$] across groups (**C**). Clomipramine pretreatment did not affect RU24969-induced behavior. Results are presented as means \pm SEM. Asterisk (*) indicates $p < 0.05$ compared to saline. OCD, obsessive-compulsive disorder; SEM, standard error of the mean.

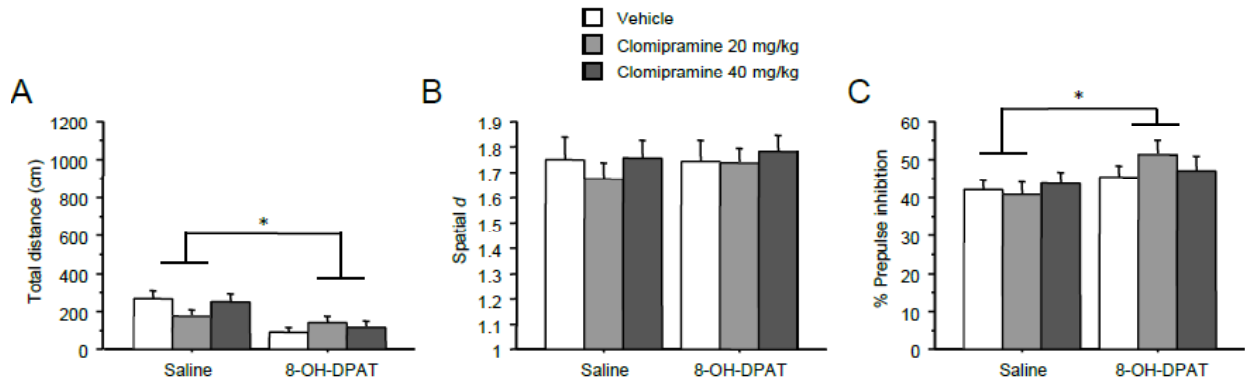


Figure S2. Activation of 5-HT_{1A}Rs does not induce OCD-like behavior. Following 4 weeks of treatment with 0 mg/kg/d ($n = 14$), 20 mg/kg/d ($n = 14$), or 40 mg/kg/d ($n = 14$) clomipramine via the drinking water, mice received injections of the 5-HT_{1A}R agonist 8-OH-DPAT (0 or 1 mg/kg) on separate test days. 8-OH-DPAT reduced locomotion [$F(1,37) = 5.01$; $p < 0.05$] across groups (**A**), had no effect on perseveration (spatial d) (**B**) and increased prepulse inhibition [$F(1,27) = 4.43$; $p < 0.05$] across groups (**C**). Clomipramine pretreatment did not affect 8-OH-DPAT-induced behavior. Results are presented as means \pm SEM. Asterisk (*) indicates $p < 0.05$ compared to saline. See Figure S1 for abbreviations.

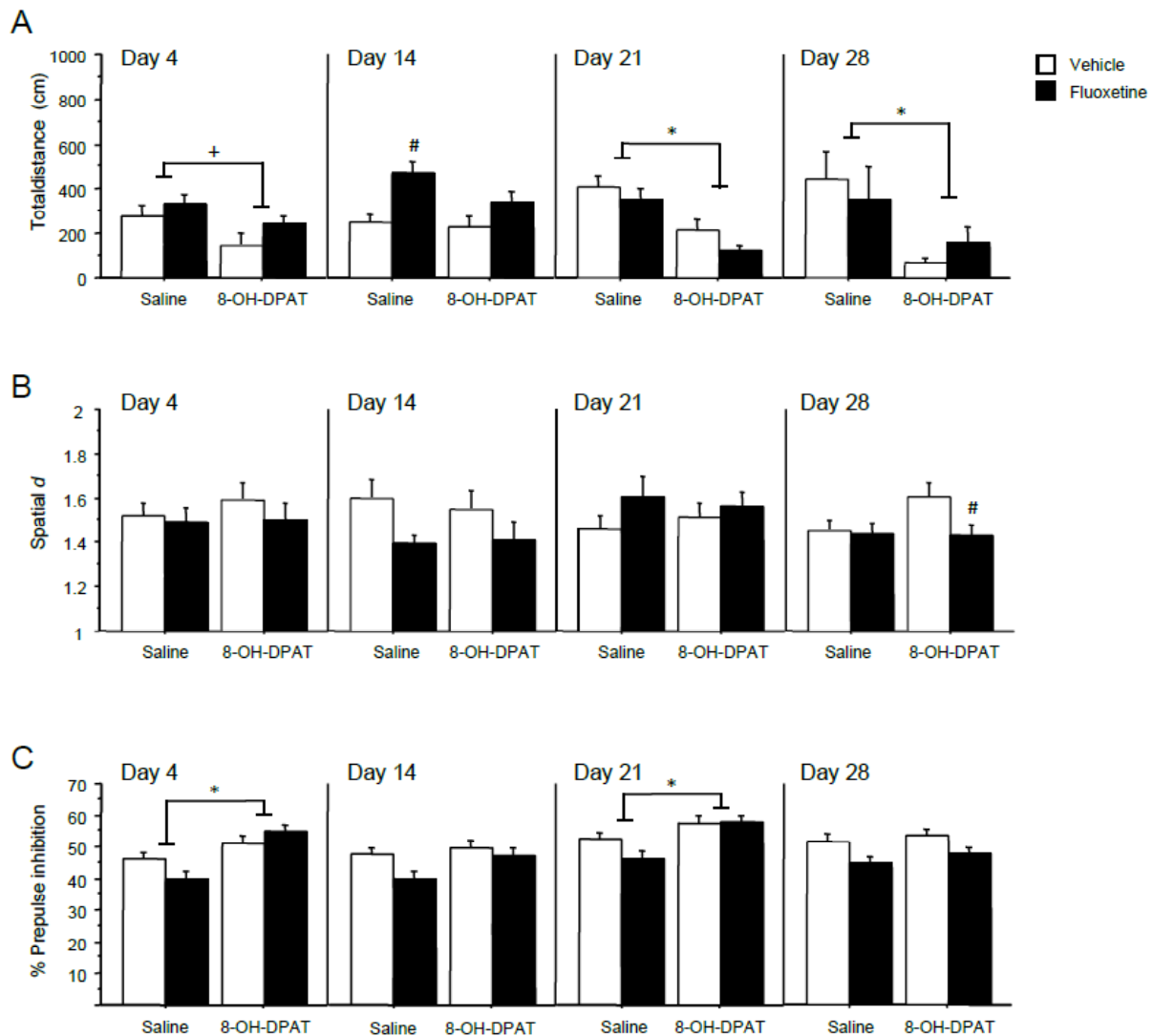


Figure S3. Separate groups of mice received fluoxetine (0 or 10 mg/kg/d) via the drinking water for 4, 14, 21, or 28 days ($n = 15/\text{group}$), received injections with the 5-HT_{1A}R agonist 8-OH-DPAT (0 or 1 mg/kg), and were tested for total distance traveled (**A**) and perseveration (**B**) in the open field, and prepulse inhibition (**C**). A strong trend was found for 8-OH-DPAT to reduce locomotion on day 4 [$F(1,22) = 3.82$; $p = 0.06$]. On day 14, a main effect of pretreatment [$F(1,24) = 4.42$; $p < 0.05$] and Newman-Keuls post-hoc tests revealed that fluoxetine increased locomotion following saline treatment. 8-OH-DPAT reduced locomotion on days 21 [$F(1,23) = 11.44$; $p < 0.01$] and 28 [$F(1,22) = 15.45$; $p < 0.001$]. 8-OH-DPAT had no effect on spatial *d* on days 4, 14 and 21. On day 28, a significant drug \times pretreatment interaction [$F(1,24) = 4.59$; $p < 0.05$] and post hoc analyses revealed that fluoxetine-pretreated mice exhibited reduced spatial *d* compared to controls following 8-OH-DPAT. 8-OH-DPAT increased prepulse inhibition on days 4 [$F(1,23) = 9.67$; $p < 0.01$] and 21 [$F(1,24) = 6.72$; $p < 0.05$]. Results are presented as means \pm

SEM. Asterisk (*) indicates $p < 0.05$ compared to saline. Pound sign (#) indicates $p < 0.05$ compared to chronic vehicle treatment. Plus sign (+) indicates a strong trend ($p = 0.06$). See Figure S1 for abbreviations.

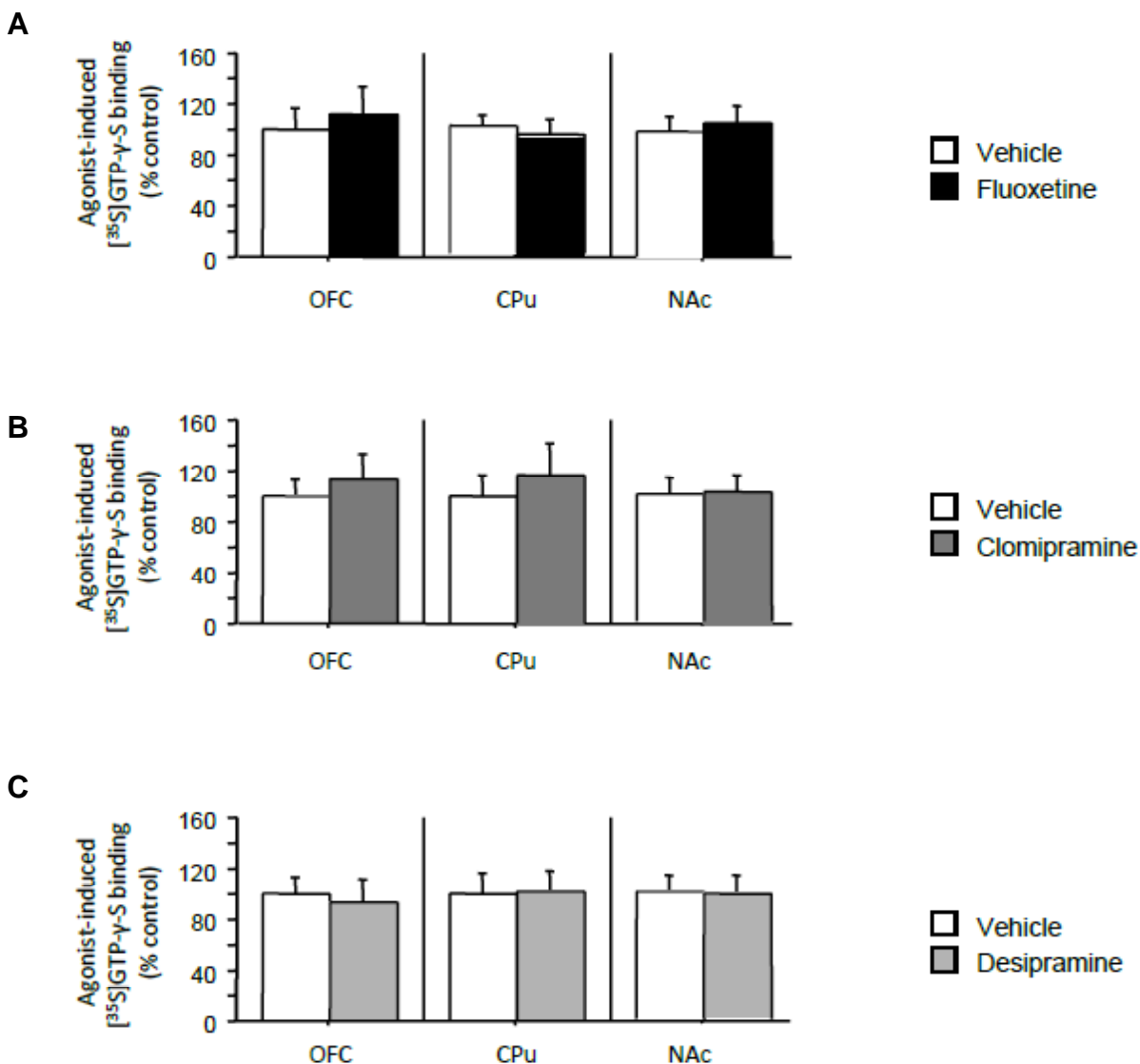
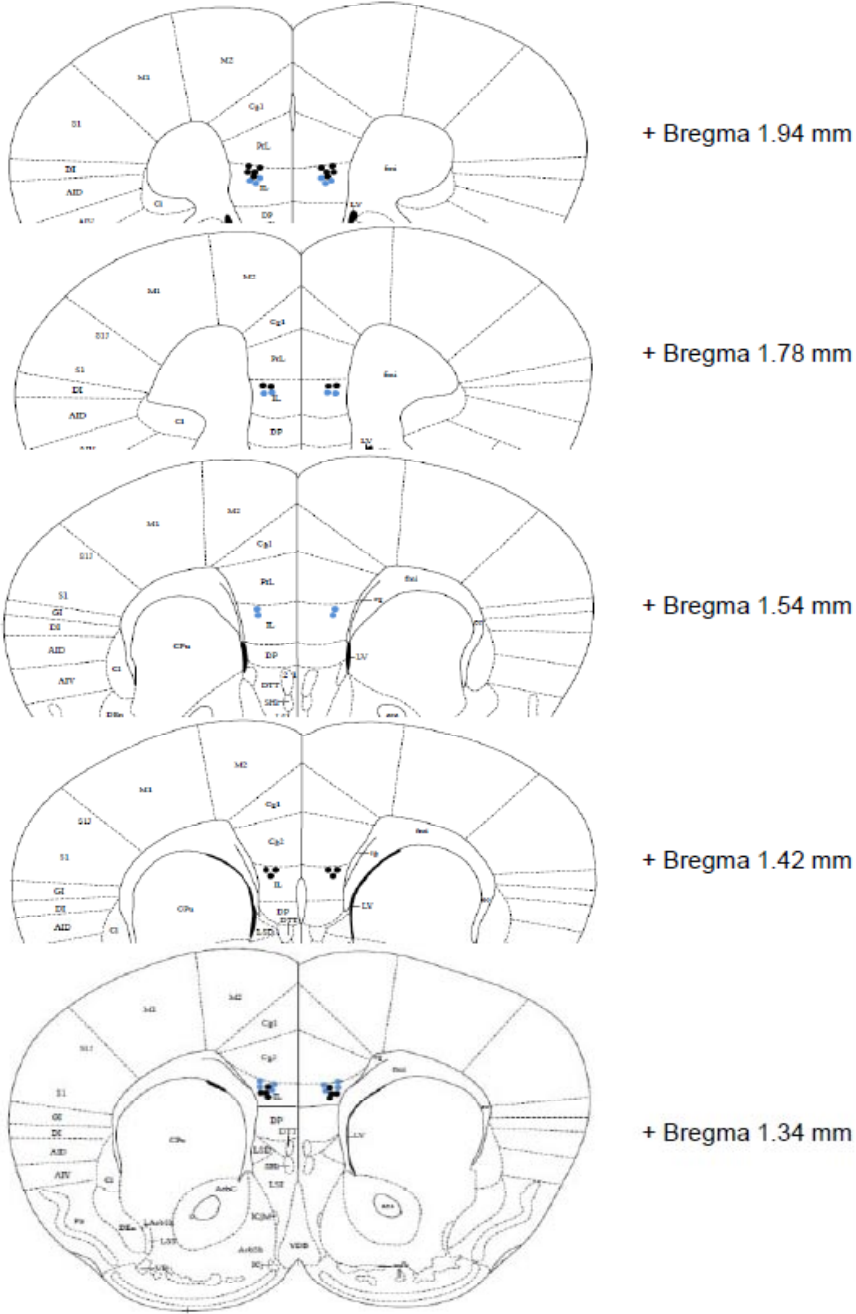


Figure S4. Chronic antidepressant treatments had no effect on 5-HT1BR G-protein coupling. Autoradiography using [³⁵S]-GTPγS was performed on coronal brain slices from mice treated for 4 weeks with vehicle ($n = 13$), 10 mg/kg/d fluoxetine ($n = 13$) (**A**), 20 mg/kg/d clomipramine ($n = 12$) (**B**), or 20 mg/kg/d desipramine ($n = 12$) (**C**). Agonist-induced [³⁵S]-GTPγS binding was assessed in the presence of 5-HT1BR agonist CP94253 (10 μm). Basal binding (in the presence of 0 μm CP94253) was subtracted from agonist-induced binding and data are expressed as percent of vehicle-treated agonist-induced [³⁵S]-GTPγS binding. Nonspecific [³⁵S]-GTPγS binding was determined in the presence of CP94253 and the 5-HT1B/1DR antagonist GR127935 (20 μm). Results are presented as means ± SEM. OFC, orbitofrontal cortex; CPu, caudate-putamen; NAc, nucleus accumbens.

A



B

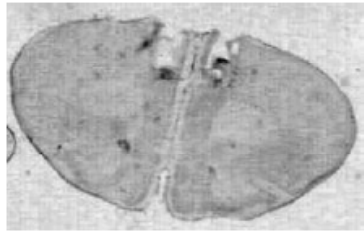


Figure S5. Schematic representation of successive coronal sections (rostral to caudal) of the mouse brain **(A)** and representative pictomicrograph **(B)** showing the histological verification of cannula placements in the infralimbic cortex. Black dots indicate cannula placement for animals infused with antagonist, and blue dots indicate cannula placement for animals infused with agonist. Schematics reproduced and reprinted with permission from Franklin and Paxinos (2).

Supplemental References

1. Paulus MP, Callaway CW, Geyer MA (1993): Quantitative assessment of the microstructure of rat behavior: II. Distinctive effects of dopamine releasers and uptake inhibitors. *Psychopharmacology (Berl)*. 113:187-98.
2. Franklin KBJ, Paxinos G (2007): *The Mouse Brain in Stereotaxic Coordinates, 3rd ed.* San Diego, CA: Academic Press.