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Supporting Online Material

www.sciencemag.org/cgi/content/full/311/6/77/DC1
 Materials and Methods
 Figs. S1 to S12
 Tables S1 to S10
 References

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Alterations in 5-HT_{1B} Receptor Function by p11 in Depression-Like States

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The pathophysiology of depression remains enigmatic, although abnormalities in serotonin signaling have been implicated. We have found that the serotonin 1B receptor [5-hydroxytryptamine (5-HT_{1B}) receptor] interacts with p11. p11 increases localization of 5-HT_{1B} receptors at the cell surface. p11 is increased in rodent brains by antidepressants or electroconvulsive therapy, but decreased in an animal model of depression and in brain tissue from depressed patients. Overexpression of p11 increases 5-HT_{1B} receptor function in cells and recapitulates certain behaviors seen after antidepressant treatment in mice. p11 knockout mice exhibit a depression-like phenotype and have reduced responsiveness to 5-HT_{1B} receptor agonists and reduced behavioral reactions to an antidepressant.

The serotonin system plays a key modulatory role in a plethora of functions of the central nervous system in physiological and disease states (1, 2). Compounds

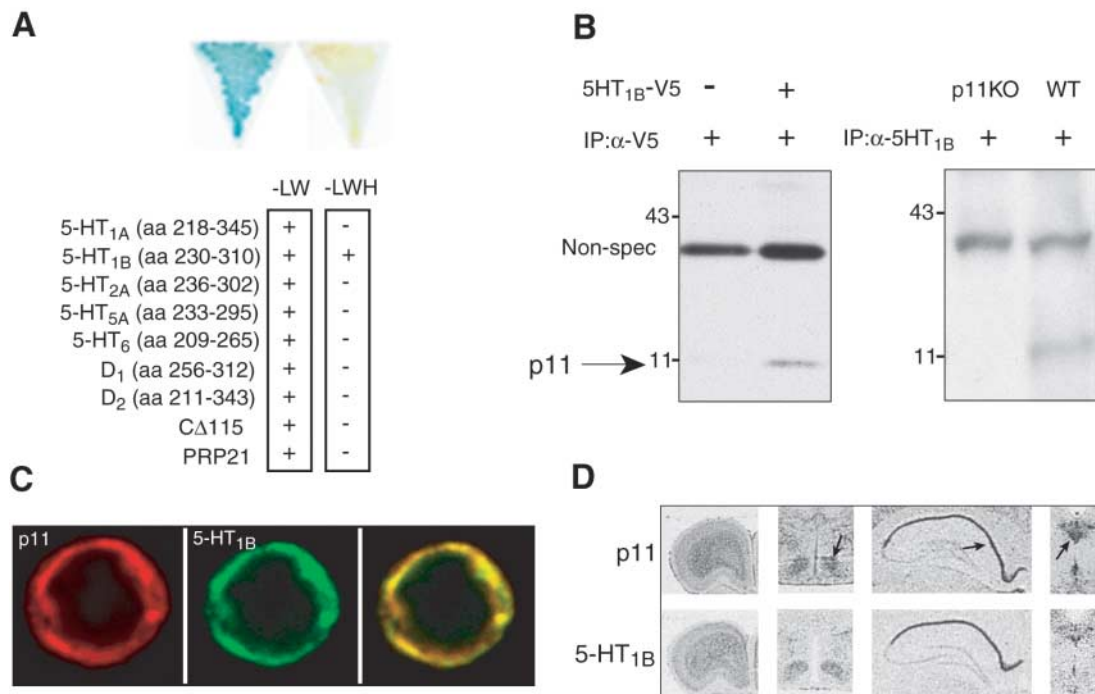
that alter either the reuptake or the metabolism of serotonin are used as medications against many neuropsychiatric disorders (1–4). A better understanding of the role of individ-

ual serotonin receptors in mediating the effects of these medications would improve our comprehension of the etiology of certain neuropsychiatric disease states and enhance our ability to design more effective medications. There are 14 different serotonin receptors (2), some of which have multiple splice variants that enable binding of distinct sets of intracellular proteins (5). 5-HT_{1B} receptors play a crucial role in regulating serotonin neurotransmission, as they serve as both autoreceptors on serotonin-containing neurons originating from the raphe nuclei and heteroreceptors on several neurons that do not

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Fig. 1. Identification of an interaction between 5-HT_{1B} receptors and p11. **(A)** Results from a yeast two-hybrid screen showing an interaction of p11 with the 5-HT_{1B} receptor (left; blue color), but not with an unrelated bait (CΔ115; right; no color), or with pRP21, 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2A}, 5-HT_{5A}, 5-HT₆, D₁, or D₂ receptors. **(B)** Coimmunoprecipitation confirming that p11 interacts with (left panel) V5 epitope-tagged 5-HT_{1B} receptors in HeLa cells and with (right panel) native 5-HT_{1B} receptors in brain tissue from wild-type, but not p11 KO, mice. The immunoprecipitates were analyzed by Western blotting using a p11-specific antibody. The nonspecific band corresponds to the light chains of the antibodies against V5 or 5-HT_{1B} receptors (α-V5 and α-5HT_{1B}). **(C)** Immunofluorescence staining of p11 (left, red fluorescence), V5 epitope-tagged 5-HT_{1B} receptors (middle, green fluorescence) and their colocalization (right, yellow fluorescence) at the cell surface in HeLa cells. **(D)** In situ hybridization made on



coronal sections from a rat brain showing that the distribution of p11 mRNA is similar to that of 5-HT_{1B} receptor mRNA in (left to right) frontal cortex, ventromedial hypothalamus (arrow), hippocampus (arrow), and raphe nuclei (arrow).

contain serotonin (6, 7). Pharmacologic and genetic studies have suggested a role for 5-HT_{1B} receptors in the pathophysiology of

obsessive compulsive disorder, drug addiction, depression, anxiety, aggression, and sleep (1, 7, 8).

To better understand the function of 5-HT_{1B} receptors, we used the third intracellular loop of this receptor as bait in a yeast two-hybrid

Fig. 2. Regulation of p11 expression by antidepressant treatments and in depression-like states. In situ hybridization illustrating an up-regulation of p11 mRNA in the forebrain following (A) repeated treatment with imipramine [10 mg/kg per day, intraperitoneally (i.p.) for 14 days] in mice ($n = 8$ per group) and (B) electroconvulsive therapy (ECT) for 10 days in rats ($n = 5$ per group). Conversely, p11 mRNA was down-regulated in (C) the forebrain in helpless H/Rouen versus nonhelpless NH/Rouen mice ($n = 10$ per group) and (D) in patients who suffered from unipolar major depression ($n = 15$ per group). Data from the anterior (A; B; C, left; D) and posterior (C, right) cingulate cortices were normalized to the corresponding controls and represent means \pm SEM. * $P < 0.05$, *** $P < 0.001$ versus control by Student's t test.

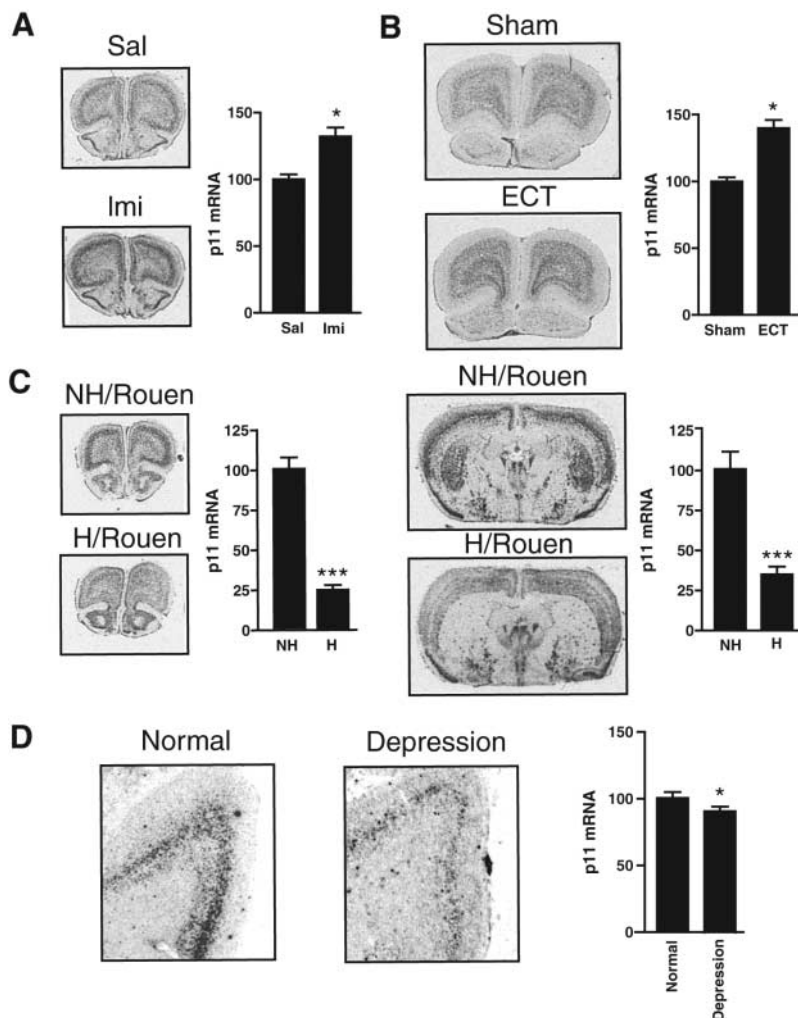
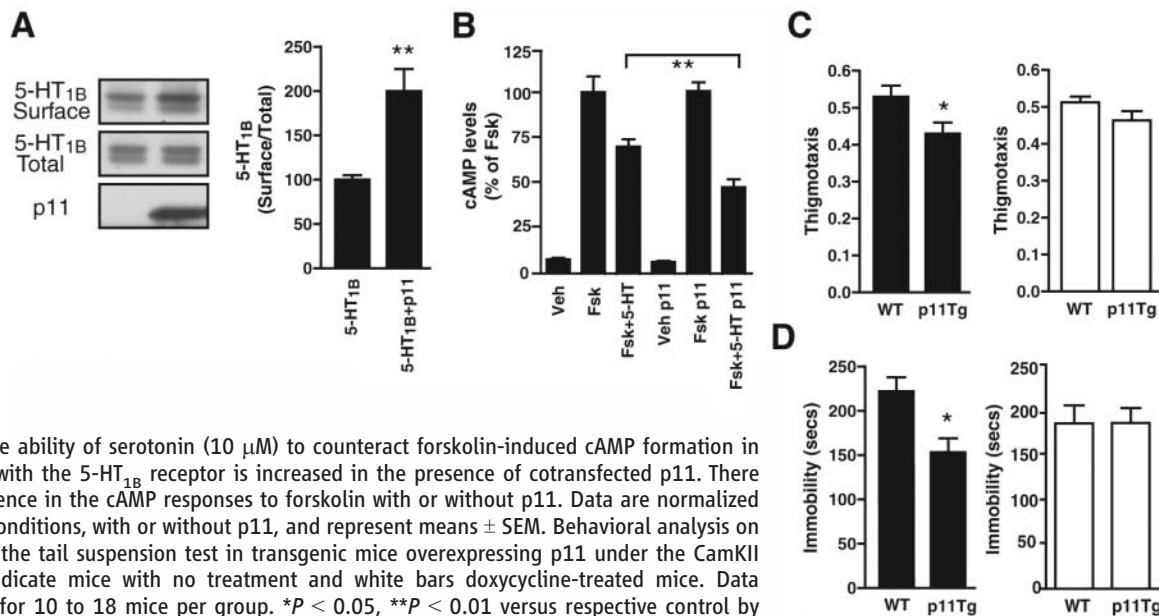


Fig. 3. Biochemical and behavioral effects of overexpression of p11 in cells and in transgenic mice. (A) Biotinylation experiment from COS-7 cells showing that p11 increases the amount of 5-HT_{1B} receptors at the cell surface. Data were normalized to the amount in cells transfected only with 5-HT_{1B} receptors and represent means \pm SEM for three experiments, each in triplicate. (B) The ability of serotonin (10 μ M) to counteract forskolin-induced cAMP formation in COS-7 cells transfected with the 5-HT_{1B} receptor is increased in the presence of cotransfected p11. There was no significant difference in the cAMP responses to forskolin with or without p11. Data are normalized to forskolin-stimulated conditions, with or without p11, and represent means \pm SEM. Behavioral analysis on (C) thigmotaxis and (D) the tail suspension test in transgenic mice overexpressing p11 under the CamKII promoter. Black bars indicate mice with no treatment and white bars doxycycline-treated mice. Data represent means \pm SEM for 10 to 18 mice per group. * $P < 0.05$, ** $P < 0.01$ versus respective control by Student's t test.



screen (9). Twenty-six out of 29 double-positive prey clones encoded the gene for p11 (also called S100A10, 42C, calpactin I light chain, and annexin II light chain), a member of the S100 EF-hand protein family (10). p11 interacted with 5-HT_{1B} receptors, but not with 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2A}, 5-HT_{5A}, 5-HT₆, dopamine D₁ or D₂ receptors, two irrelevant baits (CA115 and pRP21), or the empty plasmid, which showed the specificity of this interaction (Fig. 1A). p11 was found to coimmunoprecipitate with 5-HT_{1B} receptors in HeLa cells and brain tissue (Fig. 1B). Immunofluorescence studies showed a prominent colocalization between p11 and 5-HT_{1B} receptors at the cell surface (Fig. 1C). The distribution of p11 mRNA in the brain resembled that of 5-HT_{1B} receptor mRNA (Fig. 1D).

Long-term administration of the tricyclic antidepressant imipramine (Fig. 2A), but not of haloperidol, risperidone, or diazepam (+6, +3, and +1% versus saline), increased the amount of p11 mRNA (+30 ± 5% versus saline) in cortex. This effect of imipramine was restricted to the forebrain, e.g., it did not occur in the raphe nuclei (-3% versus saline). In subsequent experiments, long-term administration of another antidepressant, tranylcypromine (+29 ± 4% versus saline; $P < 0.05$ Student's *t* test), and repeated electroconvulsive therapy (Fig. 2B) also increased the amounts of p11 mRNA in cortex. Moreover, imipramine and electroconvulsive therapy increased p11 protein in cortex (fig. S1). The amounts of p11 were compared in helpless H/Rouen mice, a genetic animal model of depression, and nonhelpless NH/Rouen mice (11). The amounts of p11 mRNA (Fig. 2C) and protein (fig. S1) were markedly reduced in H/Rouen mice. We also found down-regulated p11 mRNA (Fig. 2D) and protein (fig. S1) in the anterior cingulate cortex in patients who had suffered from unipolar major depression disorder.

p11 regulates the translocation of annexin II and ion channels (NaV1.8, TASK-1, TRP5/6) to the cell surface (12–15). Likewise, COS-7 cells cotransfected with 5-HT_{1B} receptors and p11 exhibited more 5-HT_{1B} receptors at the cell surface than cells transfected only with 5-HT_{1B} receptors (Fig. 3A). In contrast, the ratio of surface-to-total dopamine D₁ receptors was similar in the absence or presence of p11 (100 ± 7.4 versus 103 ± 13.0). We determined whether cotransfection with p11 would enhance the signaling efficacy of 5-HT_{1B} receptors, which are negatively coupled to adenylyl cyclase. Serotonin counteracted forskolin-induced adenosine 3',5'-monophosphate (cAMP) formation more efficiently in COS-7 cells expressing both 5-HT_{1B} receptors and p11 than in cells expressing only 5-HT_{1B} receptors (Fig. 3B).

Because antidepressant treatments elevated p11, we investigated whether increased amounts of p11 would affect behaviors relevant to antidepressant drug action. Transgenic mice with doxycycline-regulatable overexpression of p11 under the calcium/calmodulin-dependent protein kinase II (CamKII) promoter were generated. In the absence of doxycycline, transgenic mice had elevated p11 in neurons that do not contain serotonin in the forebrain, but not in serotonin neurons in the raphe nuclei (fig. S2). These mice had increased functional 5-HT_{1B} receptors in substantia nigra (fig. S3) and exhibited reduced thigmotaxis (an index of anxiety-related distress) (Fig. 3C) and increased horizontal activity (fig. S4) in the open-field test. They also showed a decreased immobility in the tail suspension test (an index of depression-like state) (Fig. 3D). Thus, mice overexpressing p11 acted as if they were treated with antidepressants, although a confounding factor was that they appeared to be generally hyperactive. Transgenic mice treated with doxycycline had normalized p11 expression (fig. S4) and no significant alterations of thigmotaxis (Fig. 3C), immobility (Fig. 3D), or horizontal activities (fig. S4).

We also generated p11 knockout (KO) mice (fig. S5). Autoradiographic ligand-binding experiments showed that there were fewer binding sites for the 5-HT_{1B} receptor antagonist radioligands [¹²⁵I]iodocyanopindolol (Fig. 4A; fig. S6) and [³H]GR125743 (fig. S6) in globus pallidus in p11 KO than in wild-type mice. Similarly, [¹²⁵I]iodocyanopindolol binding was lower in substantia nigra pars reticulata in p11 KO than in wild-type mice (77.3 ± 5.8 versus 98.8 ± 6.2 fmol/mg protein; $P < 0.05$ Student's *t* test). There was no difference in the affinity of serotonin to displace bound [¹²⁵I]iodocyanopindolol between wild-type and p11 KO mice [median effective concentration (EC₅₀) values: 57 versus 52 nM]. No differences in the amounts of 5-HT_{1A}, D₁, or D₂ receptors were detected between the wild-type and p11 KO mice (fig. S6). [¹²⁵I]iodocyanopindolol binding was also reduced in H/Rouen mice versus NH/Rouen mice (fig. S7).

The reduced number of 5-HT_{1B} receptors at the cell membrane in p11 KO mice was reflected in a reduced ability of the 5-HT_{1B} receptor agonist anpirtoline (16) to increase [³⁵S]guanosine 5'-O-(3'-thiotriphosphate (GTP-γ-S) binding in globus pallidus in these mice (Fig. 4B). In contrast, there was no difference in [³⁵S]GTP-γ-S binding by 8-OH-DPAT [(+/-)-8-hydroxy-2-(di-*n*-propylamino) tetralin], a 5-HT_{1A} receptor agonist, in wild-type and p11 KO mice (6.0 ± 2.1 versus 5.0 ± 2.0 optical density units). The decreased number of functional 5-HT_{1B} receptors at the cell surface of p11 KO mice was also reflected in a loss of ability of

serotonin and of anpirtoline to down-regulate phospho-Thr²⁰²/Tyr²⁰⁴-ERK1/2 (extracellular signal-regulated kinase) levels in primary cortical cultures from p11 KO mice (Fig. 4C) and of anpirtoline to decrease phospho-Ser⁹-synapsin I, a site phosphorylated by cAMP-dependent protein kinase, in striatal slices from p11 KO mice (Fig. 4D).

Serotonin, via 5-HT_{1B} receptors, reduces glutamate release at terminals of neurons originating from the cerebral cortex and inhibits synaptic transmission at corticostriatal synapses (17). We monitored the amplitude of field excitatory postsynaptic potentials (fEPSPs) evoked by brief electrical stimulation of glutamatergic fibers and recorded extracellularly in the nucleus accumbens. fEPSPs were mediated by AMPA receptors activated by endogenous glutamate released by electrical stimulation of the slice in both wild-type and p11 KO mice [fEPSP/population spike (PS) reduction 77 and 81%, respectively, compared with baseline, 15 min after the AMPA receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX)]. When applied in the perfusion solution, serotonin depressed the amplitude of the fEPSP/PS in slices from wild-type, but not from p11 KO, mice (Fig. 4E).

5-HT_{1B} receptors act as autoreceptors and inhibit serotonin release (2, 7). Because p11 is expressed in the raphe nuclei, the amounts of serotonin and its major metabolite 5-hydroxyindoleacetic acid (5-HIAA) were measured in projection areas, namely, cortex, striatum, and hippocampus in wild-type and p11 KO mice. In accordance with a negative regulation of 5-HT turnover and/or metabolism by 5-HT_{1B} receptors, and a potentiating role of p11 on 5-HT_{1B} receptor function, p11 KO mice have increased levels of serotonin turnover and/or metabolism (Fig. 4F).

To evaluate behavioral effects of p11 deletion, we compared thigmotaxis in wild-type and p11 KO mice under basal conditions and in response to anpirtoline in drug-naïve mice and in mice that had been treated long-term with imipramine. In animals treated with imipramine, anpirtoline caused a significant reduction in thigmotaxis in wild-type mice, but not in p11 KO mice (Fig. 4G). In addition, there was less thigmotaxis in saline-injected wild-type than p11 KO mice (Fig. 4G). Drug-naïve wild-type and p11 KO mice exhibited similar thigmotaxis either in the absence or presence of anpirtoline. There was an increased immobility in the tail suspension test in p11 KO mice compared with wild-type mice, both under baseline conditions and after acute treatment with either anpirtoline or imipramine (Fig. 4H). These behavioral results indicate that p11 KO mice exhibit a depression-like phenotype and that p11 mediates behavioral responses to imipramine via 5-HT_{1B} recep-

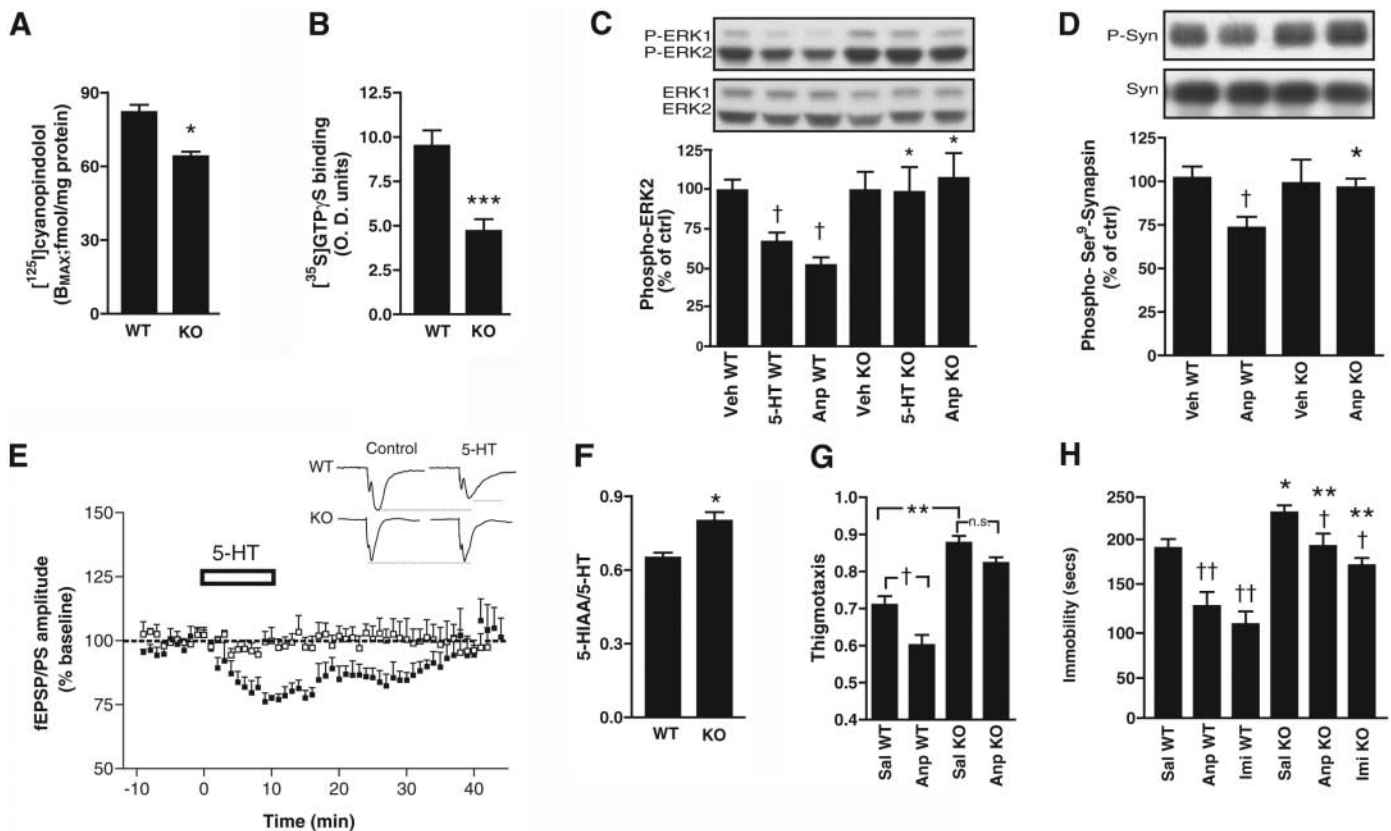


Fig. 4. Comparison of biochemical, electrophysiological, and behavioral responses in wild-type versus p11 KO mice. **(A)** Maximum specific binding (B_{max}) values for [125 I]iodocyanopindolol binding to 5-HT $_{1B}$ receptors in globus pallidus in wild-type and p11 KO mice. **(B)** Anpirtoline (50 μ M)-stimulated [35 S]GTP- γ -S binding in globus pallidus in wild-type and p11 KO mice. For (A and B), data represent means \pm SEM for five to eight mice per group. **(C)** Regulation of phospho-Thr 202 /Tyr 204 -ERK1/2 by serotonin (10 μ M) and anpirtoline (10 μ M) in primary cortical cultures of wild-type and p11 KO mice. **(D)** Regulation of phospho-Ser 9 -synapsin I by anpirtoline (50 μ M) in striatal slices from wild-type and p11 KO mice. For (C and D), data represent means \pm SEM for three experiments, each in triplicate. **(E)** Ability of serotonin (10 μ M) to inhibit corticoaccumbal glutamatergic synaptic transmission seen in wild-type mice (filled symbols) is significantly attenuated in p11 KO mice (open symbols). Data represent means \pm

SEM for five slices per group. **(F)** Serotonin turnover (5-HIAA/5-HT ratio) in striatal tissue is increased in p11 KO mice as compared with wild-type mice. Data represent means \pm SEM for eight mice per group. **(G)** Thigmotaxis in the open field in wild-type and p11 KO mice treated long-term with imipramine (10 mg/kg per day, i.p., for 4 weeks) and challenged with saline or anpirtoline (5 mg/kg, i.p.). Data represent means \pm SEM for 12 to 16 mice per group. **(H)** Behavioral analysis in the tail suspension test in wild-type and p11 KO mice treated acutely with saline, anpirtoline (5 mg/kg, i.p.), or imipramine (10 mg/kg, i.p.). Data represent means \pm SEM for 14 to 16 mice per group. (A, B, F) $*P < 0.05$, $***P < 0.001$ versus respective control by Student's t test. (C, D, G, H) $*P < 0.05$, $**P < 0.01$ versus respective wild-type group; n.s., nonsignificant, $\dagger P < 0.05$, $\dagger\dagger P < 0.01$ versus respective control group by two-way ANOVA followed by Newman-Keul's test.

tors. In further support of a depression-like phenotype of p11 KO mice, we found that p11 KO mice consumed less of a palatable 2% sucrose solution than their wild-type littermates (1.74 ± 0.07 versus 2.17 ± 0.11 ml/g body weight per day; $P < 0.05$ Student's t test), which indicated a decreased responsiveness to sweet reward. Water intake was similar in the p11 KO mice and their wild-type littermates (1.51 ± 0.05 versus 1.42 ± 0.05 ml/g body weight per day), which ruled out a role of altered fluid balance in this behavior.

Taken together, the results of the present study indicate that the dynamic modulation of 5-HT $_{1B}$ receptor function by p11 may be involved in molecular adaptations occurring in neuronal networks that are dysfunctional in depression-like states.

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 Materials and Methods
 Figs. S1 to S7
 References

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