Lack of GPR88 enhances medium spiny neuron activity and alters motor- and cue-dependent behaviors

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The striatum is a major component of the basal ganglia circuitry; it receives excitatory cortical and thalamic glutamatergic inputs and modulatory dopaminergic input, which, together with inhibitory inputs from interneurons, are integrated and relayed to other basal ganglia components via GABAergic MSNs. MSNs express either D1 or D2 dopamine receptors (D1R and D2R), constituting the striatonigral (direct) and striatopallidal (indirect) pathways, respectively1. The orphan G protein–coupled receptor GPR88 is robustly expressed in MSNs and is regulated by neuropharmacological drugs, but its contribution to MSN physiology and behavior is unclear. We found that, in the absence of GPR88, MSNs showed increased glutamatergic excitation and reduced GABAergic inhibition, which promoted enhanced firing rates in vivo, resulting in hyperactivity, poor motor coordination and impaired cue-based learning in mice. Targeted viral expression of GPR88 in MSNs rescued the molecular and electrophysiological properties and normalized behavior, suggesting that aberrant MSN activation in the absence of GPR88 underlies behavioral deficits and its dysfunction may contribute to behaviors observed in neuropsychiatric disease.

RESULTS

Gpr88 is highly expressed in striatal MSNs

Mice with the Gpr88 coding region replaced by a cassette encoding a Cre recombinase–EGFP fusion protein (Gpr88–Cre-EGFP; Online Methods and Fig. 1a) were generated by gene targeting. When the Gpr88–Cre-EGFP mice were crossed with Cre-dependent reporter mice3,13, expression of TdTomato marked all of the cells in which Cre had been expressed, and EGFP revealed where Cre was currently being expressed. TdTomato labeling was abundant in the striatum (Fig. 1b); it filled projections to the substantia nigra pars reticulata (SNr) and external globus pallidus (GPe), indicating that both direct and indirect striatal MSNs expressed GPR88 (Fig. 1b). Some TdTomato-expressing cells were visible in other brain regions (Fig. 1b and Supplementary Table 1).

Expression of Gpr88 in the adult brain was more restricted; we readily observed EGFP-positive cells in the striatum and olfactory tubercle (Fig. 1c). Regions of lower Gpr88 expression were identified by immunohistochemistry, which revealed Gpr88 expression in cortex, thalamus and inferior olive (Fig. 1d–g). There were fewer cortical EGFP-positive cells than TdTomato-positive cells, indicating that Gpr88 is expressed during development and silenced later on.

Striatal Gpr88 expression is confined to MSNs

To determine which striatal neurons expressed Gpr88–Cre-EGFP, we bred the Gpr88–Cre-EGFP mice with RiboTag mice14, which allow Cre-dependent expression of hemagglutinin (HA)-tagged ribosomal protein L22. The HA tag enables immunoprecipitation of polysomes and associated mRNAs from cells that express Cre. We compared the

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Received 22 August; accepted 14 September; published online 14 October 2012; doi:10.1038/nn.3239
Figure 1 Characterization of Gpr88-Cre-EGFP mice. (a) Gene targeting strategy to generate a Gpr88-Cre-EGFP mouse line. (b) Composite sagittal image of a Gpr88-Cre-EGFP; TdTomato reporter mouse. Cre-mediated recombination (red) was observed as fluorescence in striatum, olfactory tubercle and cortex. Terminals from striatoginal (filled arrow) and striatopallidal (open arrow) MSNs were visible in the SNr and GPi, respectively. (c) Composite sagittal image of EGFP fluorescence in an adult Gpr88-Cre-EGFP mouse. EGFP-positive cells were observed in the striatum and olfactory tubercle. Scale bar represents 1 mm. (d-g) Immunohistochemistry detected low-level EGFP expression in cortex (ctx), striatum (str), thalamus (thal) and inferior olive (io) of an adult Gpr88-Cre-EGFP mouse; locations are shown in c. Scale bar represents 100 μm. (h) qRT-PCR analysis of transcripts expressed in Gpr88-Cre-EGFP-expressing neurons after immunoprecipitation of HA-tagged polypeptides from Gpr88-Cre-EGFP, RiboTag forebrain slices. MSN-specific mRNAs (Ppp1r1b, Pdyn and Penk) as well as Gpr88 were enriched in the pellet fraction compared with the input. Striatal interneuron-specific marker mRNAs (Sst, Pvalb, Chat and Calb2) were less abundant in the pellet fraction. **P < 0.001, ***P < 0.01, Student’s t test, two-tailed. (f) Immunofluorescence staining for dynorphin (Dyn, red) and EGFP (green). Both Dyn and EGFP double-positive cells (filled arrows) and Dyn-negative, EGFP-positive cells (open arrow) were observed in the striatum of Gpr88-Cre-EGFP mice. Scale bar represents 50 μm. (i) qRT-PCR analysis of Gpr88 mRNA in Gpr88Cre+/+, Gpr88Cre+/− and Gpr88Cre−/− mice (n = 3 each). ***P < 0.001, one-way ANOVA, SNK post-test. Data are shown as mean ± s.e.m.

relative abundance of transcripts in the pellets and the total tissue RNA (input) by quantitative reverse transcription PCR (qRT-PCR) from striatum. Gpr88 mRNA was enriched fourfold in the pellets compared with the input (t1, 841.81 < P < 0.01 versus pellet, Student’s t test, two-tailed; Fig. 1h), confirming immunoprecipitation of polypeptides from Gpr88-expressing cells. There was comparable enrichment of the MSN-specific DARPP32 (Ppp1r1b) met-enkephalin (Penk) and dynorphin (Pdyn) transcripts, indicating that both D1R- and D2R-bearing MSNs express Gpr88 (P < 0.01 versus pellet, Student’s t test, two-tailed; Ppp1r1b, Penk, t4 = 6.18; Pdyn, t4 = 12.02; Fig. 1h). In contrast, expression of the interneuron-specific genes somatostatin (Sst), calretinin (Calb2), parvalbumin (Pvalb) and choline acetyl transferase (Chat) were de-enriched (P < 0.001 versus pellet, Student’s t test, two-tailed; Sst, t4 = 25.58; Calb2, t4 = 23.63; Pvalb, t4 = 83.17; Chat, t4 = 88.86, respectively; Fig. 1h), and all HA-positive cells colocalized with nuclear EGFP (Fig. 1h). Using immunofluorescence, we found dynorphin (marker for direct pathway MSNs) staining in a subset of the EGFP-positive cells (Fig. 1i). These results indicate that striatal Gpr88 expression is restricted to MSNs.

To evaluate the role of Gpr88 in MSN physiology, we generated homozygous Gpr88-Cre-EGFP (Gpr88Cre+/Cre) mice. Gpr88 expression, assessed by qRT-PCR, was absent in the striatum of Gpr88Cre+/Cre mice compared with Gpr88+/+ mice; Gpr88Cre−/− mice had half as much Gpr88 mRNA as Gpr88+/+ mice (F2,9 = 248.3, P < 0.001, genotype, one-way ANOVA, Student-Newman-Keuls (SNK) post-test; Fig. 1j). Gpr88 deficiency had no apparent effect on striatal cell populations (Supplementary Fig. 1).

Figure 2 Hyperlocomotion and motor deficits in Gpr88Cre−/− mice. (a) Locomotion (48 h) of Gpr88+/+ (n = 9) and Gpr88Cre−/− (n = 12) mice. Animals were placed in a novel chamber and their activity was acquired in 15-min bins. Inset, novelty-induced locomotion (1 h) in Gpr88+/+ and Gpr88Cre−/− mice. ***P < 0.001, Student’s t test versus Gpr88+/+, two-tailed. (b) Diurnal and nocturnal locomotion in Gpr88+/+ and Gpr88Cre−/− mice. *P < 0.05, genotype, rmANOVA, Bonferroni post-test. (c) Rotarod performance by Gpr88+/+ (n = 15) and Gpr88Cre−/− (n = 14) mice. Animals were placed in an accelerating rotating rod (4–40 rpm) for 3 min and the latency to fall was recorded. ***P < 0.001, genotype, rmANOVA, Bonferroni post-test. Data are shown as mean ± s.e.m.
Altered behavior of Gpr88<sup>Cre/Cre</sup> mice

To elucidate the role of Gpr88 in basal locomotor activity, we placed mice in activity chambers for 48 h. Activity during the first few hours, which reflects the response to novelty, was higher in Gpr88<sup>Cre/Cre</sup> mice than in Gpr88<sup>+/+</sup> mice (t<sub>18</sub> = 3.988, P < 0.001, Student’s t test, two-tailed; Fig. 2a). All of the mice increased their activity during the nocturnal cycle, and this response was greater in Gpr88<sup>Cre/Cre</sup> mice than in Gpr88<sup>+/+</sup> mice (F<sub>2,18</sub> = 4.90, P < 0.05, genotype, repeated-measures ANOVA [rmANOVA], Bonferroni post-test; Fig. 2a,b); daytime activities were comparable.

Motor coordination and balance were assessed using a rotarod; we placed mice on top of an accelerating rod and scored their latency to fall. Gpr88<sup>+/+</sup> mice improved their performance with each experimental session (F<sub>3,14</sub> = 6.566, P < 0.001, time, one-way ANOVA; Fig. 2c), whereas Gpr88<sup>Cre/Cre</sup> mice fell more quickly and showed no improvement with training (F<sub>2,12</sub> = 32.70, P < 0.001, genotype, rmANOVA, SNK post-test; Fig. 2c). The latency to fall from an inverted wire mesh was shorter for Gpr88<sup>Cre/Cre</sup> (n = 13) mice than for Gpr88<sup>+/+</sup> (n = 15) mice (Gpr88<sup>+/+</sup>, 133.70 ± 12.65; Gpr88<sup>Cre/Cre</sup>, 62.83 ± 12.52; t<sub>26</sub> = 3.957, P < 0.001, mean ± s.e.m., Student’s t test, two-tailed), confirming that there were impairments in motor coordination or strength of Gpr88<sup>Cre/Cre</sup> mice.

Mastery of the Morris water maze requires intact striatal function. Gpr88<sup>Cre/Cre</sup> mice had longer latencies to find the platform compared to Gpr88<sup>+/+</sup> during the first four training sessions (F<sub>2,19</sub> = 6.06, P < 0.05, genotype, rmANOVA; Fig. 3a); however, with more training the latencies between genotypes were equivalent (sessions 6–9; Fig. 3a). Probe trials showed that both Gpr88<sup>+/+</sup> and Gpr88<sup>Cre/Cre</sup> mice preferred the quadrant where the platform had been hidden (F<sub>2,17</sub> = 15.88 (probe 1) and F<sub>2,17</sub> = 58.65 (probe 2), P < 0.001, quadrant, rmANOVA, Bonferroni post-test; Fig. 3b,c). Gpr88<sup>Cre/Cre</sup> and Gpr88<sup>+/+</sup> mice had the same swim speed (Supplementary Fig. 2a,b). Hence, Gpr88<sup>Cre/Cre</sup> had mild impairment in initial performance of the task but visuospatial memory and learning were intact.

Associative learning depends on intact striatal function<sup>17</sup>. We tested the ability of the mice to learn the position of an escape platform in a water-based, U maze procedure<sup>18</sup> (Fig. 3d). Gpr88<sup>+/+</sup> and Gpr88<sup>Cre/Cre</sup> mice were trained daily for 3 d to learn a turn-based strategy to find a platform placed at the end of one maze arm. The percentage of correct choices was the same between genotypes (Fig. 3d), although Gpr88<sup>Cre/Cre</sup> mice had higher latencies to reach the decision point and platform...
(Supplementary Fig. 2cd). The mice were then forced to shift from the turn-based to a cue-based strategy, in which the platform was associated to the color of the arms, for seven more daily sessions. After the shift, the number of correct choices decreased and then improved with further training; Gpr88+/+ mice reached >90% correct choices after 4 d, but Gpr88Cre/Cre mice were slower to learn the new escape strategy (F1,19 = 4.39, P < 0.05, genotype, rmANOVA, Bonferroni post-test; Fig. 3d) and had higher latencies to reach the platform (Supplementary Fig. 2d). We tested a second cohort of mice exclusively in the cue-based, U-water maze for 5 d. Gpr88Cre/Cre mice were slower to learn the task than the Gpr88+/+ mice (F1,12 = 6.70, P < 0.05, genotype, rmANOVA, Bonferroni post-test; Fig. 3e), indicating that the Gpr88Cre/Cre mice had impairments in cue-based learning.

We also tested mice in a two-way active avoidance procedure. Performance in this task depends on striatal integrity, and is affected by neuropsychiatric drugs and striatal lesions17,19. Mice learn that a sound cue predicts a foot shock and learn to avoid the shock by moving to the other side of the box. Mice were trained with 100 trials per day for 5 d and the percentage of avoidance responses was measured. Acquisition of this task was impaired in Gpr88Cre/Cre mice relative to Gpr88+/+ mice (F1,22 = 11.07, P < 0.05; genotype, rmANOVA, Bonferroni post-test; Fig. 3f). Both the latencies to escape after the foot shock or the sound cue during the first ten trials (when neither group had mastered the task) were not different (Supplementary Fig. 2f), suggesting that the impaired acquisition of the task by Gpr88Cre/Cre mice is not attributable to decreased pain sensitivity or motor deficits. The results of these tests indicate that Gpr88Cre/Cre mice have deficits in the acquisition and integration of visual or auditory cues, leading to impaired performance on these behavioral tasks14,22.

**Enhanced firing rate of MSNs of Gpr88Cre/Cre mice in vivo**

To determine whether absence of GPR88 affected MSN activity, we recorded freely moving Gpr88+/+ and Gpr88Cre/Cre mice with chronically implanted electrodes in the dorsal (predominantly dorsomedial) striatum, a region controlling initiation and acquisition of goal-directed behaviors3 and cognitive flexibility18 (Supplementary Fig. 2a). Mice were habituated to the recording environment for 1 week, at which point basal locomotion and rearing activity were comparable (Supplementary Fig. 3c). Putative MSNs (Gpr88+/+, n = 70 cells; Gpr88Cre/Cre, n = 52 cells; six mice per group), identified as units having >300-µs valley width as described20, had similar waveform properties between the two groups (valley width: Gpr88+/+, 484 ± 10 µs; Gpr88Cre/Cre, 480 ± 10 µs; mean ± s.e.m., P > 0.05, Student’s t test, two-tailed; peak amplitude: Gpr88+/+, 14.193 ± 536 arbitrary units (a.u.); Gpr88Cre/Cre, 14.050 ± 798; mean ± s.e.m., P > 0.05, Student’s t test, two-tailed; Fig. 4a and Supplementary Fig. 3d). MSNs from Gpr88Cre/Cre mice had a higher average firing rate than those from Gpr88+/+ mice (f11 = 2.096, P < 0.05 versus Gpr88+/+, Student’s t test, two-tailed; Fig. 4b).

MSNs transition between a relatively depolarized “up-state,” where most action potentials occur, and a less active, hyperpolarized
Altered neurotransmitter signaling in Gpr88<sup>Cre/Cre</sup> MSNs

To determine how the absence of Gpr88 increases striatal excitability, we obtained whole-cell, patch-clamp recordings from dorsal MSNs in striatal slices. Current-clamp recordings revealed inward rectification following depolarizing current pulses and equivalent resting membrane potentials in cells from Gpr88<sup>+/+</sup> and Gpr88<sup>Cre/Cre</sup> mice (Gpr88<sup>+/+</sup>, −71.56 ± 0.25 mV; Gpr88<sup>Cre/Cre</sup>, −71.38 ± 0.23 mV; mean ± s.e.m., n = 14 cells each, P > 0.05, Student’s t-test, two-tailed), but input resistance was increased in Gpr88<sup>Cre/Cre</sup> cells (Gpr88<sup>+/+</sup>, 22.90 ± 2.28 MΩ; Gpr88<sup>Cre/Cre</sup>, 34.78 ± 4.9 MΩ; mean ± s.e.m., n = 16–18 cells, f<sub>1</sub> = 0.02, P < 0.05, Student’s t-test, two-tailed; Fig. 4a). Enhanced input resistance in MSNs suggests alterations in synaptic drive, as it is not a result of inwardly rectifying K<sup>+</sup> currents<sup>22</sup> (Supplementary Fig. 4a–b).

Baseline tonic GABA currents in MSNs from Gpr88<sup>Cre/Cre</sup> mice were reduced. The GABA<sub>α</sub> receptor antagonist bicuculline (10 µM) provoked a smaller change in the holding current (t<sub>16</sub> = 3.39, P < 0.01) and root mean square noise in Gpr88<sup>Cre/Cre</sup> cells than in Gpr88<sup>+/+</sup> mice (t<sub>16</sub> = 2.26, P < 0.05, Student’s t-test, two-tailed; Fig. 5b,c). Furthermore, GABA<sub>α</sub> receptor-mediated currents in Gpr88<sup>Cre/Cre</sup> cells were less responsive to bath-applied GABA than in Gpr88<sup>+/+</sup> mice (t<sub>16</sub> = 2.12, P < 0.05, Student’s t-test, two-tailed; Fig. 5d). In response to local intrastral stimulation (in the presence of glutamate and dopamine receptor antagonists), the peak evoked inhibitory postsynaptic currents (IPSCs) were suppressed in MSNs from Gpr88<sup>Cre/Cre</sup> mice (F<sub>9,225</sub> = 6.04, P < 0.05, genotype, two-way ANOVA, Bonferroni post-test; Fig. 5e,f) and the mean stimulation current required to evoke an IPSC in MSNs was higher in cells from Gpr88<sup>Cre/Cre</sup> mice (F<sub>10</sub> = 4.40, P < 0.05, two-way ANOVA, Bonferroni post-test; Fig. 5g,h) and the stimulation threshold required to evoke an EPSC was lower in MSNs from Gpr88<sup>Cre/Cre</sup> (t<sub>34</sub> = 2.82, P < 0.01, Student’s t-test, two-tailed; Fig. 5h).

D1R- and D2R-containing MSNs differ in their electrophysiological properties and their contributions to animal behavior<sup>23–26</sup>. We took advantage of the segregation of the direct and indirect pathway projections to label D1R- and D2R-expressing MSNs<sup>1</sup> by injecting retrogradely transported, fluorescent latex beads into the SNr or the GPe of Gpr88<sup>Cre/Cre</sup> mice (Supplementary Fig. 4c). We found no differences in response to cortical excitatory input or the tonic GABA currents in whole-cell, patch-clamp recordings in D1R- or D2R-expressing MSNs from striatal slices (Supplementary Fig. 4d–f), suggesting that both populations are affected by GPR88 deficiency. We evaluated the D1R- and D2R-mediated responses in <i>vivo</i> by analyzing the locomotor response to amphetamine<sup>14</sup>. Gpr88<sup>Cre/Cre</sup> mice had greater locomotion response after exposure to repeated doses of amphetamine (2.5 mg per kg of body weight) than Gpr88<sup>+/+</sup> mice (F<sub>1,8</sub> = 9.80, P < 0.05, genotype, rmANOVA; Supplementary Fig. 4g). To tease apart the contribution of each MSN subpopulation, we administered varying concentrations of a D1R (SKF-81297) or a D2R (quinpirole) agonist. SKF-82197 increased locomotion more in Gpr88<sup>+/+</sup> mice than in Gpr88<sup>Cre/Cre</sup> mice (F<sub>1,10</sub> = 9.18, P < 0.05, genotype, rmANOVA; Supplementary Fig. 4h), whereas quinpirole reduced locomotion in Gpr88<sup>+/+</sup> mice, but increased locomotion in Gpr88<sup>Cre/Cre</sup> mice (F<sub>1,9</sub> = 49.91, P < 0.05, genotype, rmANOVA; Supplementary Fig. 4i).
Striatal mRNA and neurotransmitter content in Gpr88<sup>Cre/Cre</sup> mice

To determine whether the increased activity of MSNs in Gpr88<sup>Cre/Cre</sup> mice involves alterations in neurotransmitter levels, we measured amino acids and their neurotransmitter-related metabolites in Gpr88<sup>−/−</sup> and Gpr88<sup>Cre/Cre</sup> striatal punches by high-performance liquid chromatography. We found no differences in the levels of dopamine, norepinephrine or serotonin. (Supplementary Table 2).

Microarray analysis of mRNA abundance in striatal punches from Gpr88<sup>−/−</sup> and Gpr88<sup>Cre/Cre</sup> mice coupled with a gene ontology analysis revealed that most of the over-represented mRNAs encoded proteins that negatively regulate cell communication (Supplementary Table 3a), confirming an inhibitory role for GPR88. Of this list, 50 mRNAs were downregulated and 39 were upregulated in Gpr88<sup>Cre/Cre</sup> mice compared with Gpr88<sup>−/−</sup> mice by >1.35-fold (Supplementary Table 3b,c), including genes associated with striatal disorders, such as Ads (ref. 27), Fosl21 (ref. 28) and Rgs4 (ref. 29) (Supplementary Table 3b,c). We used qRT-PCR to measure mRNA levels from genes encoding neurotransmitter receptors, namely dopaminergic (Drd1, Drd2), glutamatergic (Grin1, Grin2a, Grin2b, Grm1, Grm2, Grm3, Grm5) and GABAergic (Gabab1, Gabab2, Gabbr1, Gabbr3, Gad1) neuropeptides (Pdyn, Penk), and molecules involved in intracellular signaling (Ppp1r1b, Rgs4) in whole striata from Gpr88<sup>−/−</sup> and Gpr88<sup>Cre/Cre</sup> mice (Fig. 6a). Among these mRNAs, only Rgs4 mRNA was significantly reduced in Gpr88<sup>Cre/Cre</sup> mice (t<sub>s</sub> = 5.417, P < 0.01 versus Gpr88<sup>−/−</sup>, Student’s t test, two-tailed; Fig. 6a). RGS4 is a GTPase-activating protein that is involved in the regulation of G<sub>qi</sub> and G<sub>q/11</sub> GPCRs<sup>50</sup> and has been linked to schizophrenia and motor deficits<sup>29,31–33</sup>.

Changes in GluR1 and GABA<sub>A</sub> receptors in Gpr88<sup>Cre/Cre</sup> mice

Alterations in Rgs4 mRNA are suggestive of abnormal G<sub>qi</sub>/G<sub>q/11</sub> GPCR signaling and potential changes in receptor phosphorylation. We examined the phosphorylation at serine 897 (S897) of NR1, the essential subunit of NMDA glutamate receptor, and at serines 831 and 845 (S831 and S845) of GluR1, a subunit of the AMPA glutamate receptor, by Western blot analysis. Protein kinase A– and protein kinase C–dependent S831 and S845 phosphorylation of GluR1 (pGluR1) was increased in Gpr88<sup>Cre/Cre</sup> mice, which facilitates AMPA receptor signaling<sup>4,54</sup> (S845, t<sub>14</sub> = 9.72, P < 0.001; S831, t<sub>14</sub> = 6.45, P < 0.01; Student’s t test, two-tailed), but pNR1 phosphorylation was unchanged. The total amounts of expressed GluR1, NR1 and the synaptic protein PSD-95 were the same in Gpr88<sup>−/−</sup> and Gpr88<sup>Cre/Cre</sup> mice (Fig. 6b,c).

Western blot analysis revealed that the β1 subunit of the GABA<sub>A</sub> receptor was unaltered, but expression of the β3 protein was...
Cells from VR-KO mice that were infused with the RGS4 inhibitor CCG-63802 showed targeting of the Gpr88-TdTomato protein to the membrane (Supplementary Fig. 6a, b) while reducing the cortical stimulation threshold required to evoke an EPSC was increased (t(15) = 5.21, P < 0.001, Student’s t test; Fig. 8c), with values comparable to those of MSNs from Gpr88Cre/Cre mice (Fig. 5h). Dialysis of the RGS4 inhibitor CCG-63802 (100 µM) via the recording electrode into MSNs from VR-KO mice decreased tonic GABA currents (t(9) = 2.03, P < 0.05, Student’s t test versus VR-KO; Fig. 8a) and increased mean peak excitatory response amplitudes were reduced (F(9,207) = 4.61, P < 0.01, genotype, two-way ANOVA; Fig. 8b) and the cortical stimulation threshold required to evoke an EPSC was increased (t(15) = 5.21, P < 0.001, Student’s t test; Fig. 8c), thereby mimicking the loss of GPR88 and implicating RGS4 in the altered properties of MSNs from Gpr88Cre/Cre mice.

**DISCUSSION**

Our results link the activity of GPR88 to normal striatal MSN function. We confirmed that Gpr88 is highly expressed in the striatum, with its expression being limited to D1R- and D2R-containing MSNs. Inactivation of the Gpr88 locus leads to increased glutamate receptor phosphorylation and altered GABA A receptor composition, which together enhance excitability in both D1R- and D2R-expressing MSNs in vitro and elevate MSN firing in vivo. The enhanced excitability of MSNs in Gpr88Cre/Cre mice results in behavioral hyperactivity, deficits in motor coordination, impaired acquisition, and integration of visual or auditory cues, leading to poor performance in cue-based tasks. We restored electrophysiological responses and prevented hyperactivity and learning deficits in Gpr88Cre/Cre mice by re-expressing a human GPR88-TdTomato fusion protein in MSNs. Although we cannot rule out the possibility that GPR88 has additional roles in other brain regions, the behavioral restoration observed after viral rescue indicates the importance of GPR88 function in the striatum.

GABA-induced responses in slices and tonic GABA conductance were reduced in MSNs of Gpr88Cre/Cre mice. These findings are likely a result of decreased GABA A receptor subunit β3 levels37. The tonic GABA input in MSNs is mediated by extrasynaptic (β3 and β3 expressing) GABA A receptors38,39. Lack of phospho-specific antibodies has impeded the determination of phosphorylation status of β3 subunit-containing GABA A receptors, but they are regulated by protein kinases A and C35. Gpr88Cre/Cre mice had less striatal RGS4, which promotes signaling by other Gαi- and Gq-coupled GPCRs in MSNs35, resulting in altered kinase activity and abnormal MSN excitability. Changes in kinase activity could mediate the increase in

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**Figure 8** Intracellular RGS4 activity in MSNs is required for viral-mediated electrophysiological rescue. (a) Mean tonic GABA current following bicuculline in MSNs from sham-KO (n = 12 cells, 3 mice) and VR-KO (n = 7 cells, 3 mice) mice and in VR-KO mice 15 min after patch pipette dialysis of the RGS4 inhibitor CCG-63802 (n = 4 cells). *P < 0.05, Student’s t test versus VR-KO. Gray line denotes Gpr88Cre/Cre mice (t(9) = 6.016, P < 0.01, Student’s t test, two-tailed; Fig. 6b,c and Supplementary Fig. 5), despite normal mRNA levels, suggesting post-translational regulation35. The β3 subunit is required for normal tonic GABA A receptor currents in MSNs36, suggesting that alterations in receptor abundance and/or phosphorylation may account for some of the electrophysiological observations.

**Striatal Gpr88 re-expression rescues mutant phenotype**

To confirm the contribution of striatal GPR88 expression to the phenotype of Gpr88Cre/Cre mice, we produced an adeno-associated virus (AAV) encoding a human GPR88-TdTomato fusion protein (Supplementary Fig. 6a). Transduction of primary neuronal cultures allowed visualization of the fusion protein in dendritic spines (Fig. 6b,c). Expressing GPR88 in cultured MSNs in a set of primers designed to hybridize to both mouse (endogenous) and human (viral rescue) Gpr88. Similar levels of Gpr88 expression were observed in VR-KO and sham-WT mice (F(2,11) = 4.295, P < 0.001, one-way ANOVA, SNK post-test; Fig. 7g). Furthermore, both striatal pGluR1 S845 (F(3,14) = 5.58, P < 0.05, one-way ANOVA, SNK post-test; Fig. 7i) and GABA A receptor β3 (F(3,14) = 5.02, P < 0.05, one-way ANOVA, SNK post-test; Fig. 7j) abundance were normalized in VR-KO mice, suggesting a direct link between GPR88 deficiency and the molecular alterations observed in Gpr88Cre/Cre mice.

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To confirm the contribution of striatal GPR88 expression to the phenotype of Gpr88Cre/Cre mice, we produced an adeno-associated virus (AAV) encoding a human GPR88-TdTomato fusion protein (Supplementary Fig. 6a). Transduction of primary neuronal cultures allowed targeting of the Gpr88-TdTomato protein to the membrane (Supplementary Fig. 6b,c). Colocalization with postsynaptic protein PSD-95 and proximity to presynaptic boutons (Supplementary Fig. 6b,c), suggesting that it was targeted to dendritic spines, consistent with previous observations using an antibody to GPR88 (ref. 8).

To rescue the behavioral phenotype, we injected a Cre-dependent version of the virus (Fig. 7a) at multiple coordinates in Gpr88Cre/Cre mice to achieve maximum striatal coverage (viraally rescued Gpr88Cre/Cre mice, VR-KO mice). As a control, an AAV vector expressing only TdTomato (Fig. 7b) was injected into Gpr88 Cre/Cre mice (sham-WT) and Gpr88Cre/Cre mice (sham-KO). By measuring immunofluorescence of TdTomato, we found that ~45% of total striatal area was transduced in VR-KO mice (Fig. 7d), confirming that the protein was targeted to MSNs (Fig. 7d).

We evaluated the performance of mice in several motor and learning tests 2 weeks after AAV injection. Sham-KO and naive-KO mice had similar responses, and so we pooled those data. The locomotor activity of the VR-KO mice was the same as that of the sham-WT mice and less than that of the sham-KO mice (P < 0.01, Kruskal-Wallis test, Dunn post-test; Fig. 7e). More demanding behaviors, such as rotarod performance (F(2,33) = 12.04, P < 0.05, one-way ANOVA, SNK post-hoc test; Fig. 7f) and two-way active avoidance (F(2,20) = 4.70, P < 0.05, genotype, rmANOVA, Bonferroni post-test; Fig. 7g), were also restored to normal levels in the VR-KO mice (Fig. 7f,g). Expression of Gpr88 mRNA in striatum of VR-KO mice was confirmed by qRT-PCR with a set of primers designed to hybridize to both mouse (endogenous) and human (viral rescue) Gpr88. Similar levels of Gpr88 expression were observed in VR-KO and sham-WT mice (F(2,11) = 4.295, P < 0.001, one-way ANOVA, SNK post-test; Fig. 7h). Furthermore, both striatal pGluR1 S845 (F(3,14) = 5.58, P < 0.05, one-way ANOVA, SNK post-test; Fig. 7i) and GABA A receptor β3 (F(3,14) = 5.02, P < 0.05, one-way ANOVA, SNK post-test; Fig. 7j) abundance were normalized in VR-KO mice, suggesting a direct link between GPR88 deficiency and the molecular alterations observed in Gpr88Cre/Cre mice.

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**DISCUSSION**

Our results link the activity of GPR88 to normal striatal MSN function. We confirmed that Gpr88 is highly expressed in the striatum, with its expression being limited to D1R- and D2R-containing MSNs. Inactivation of the Gpr88 locus leads to increased glutamate receptor phosphorylation and altered GABA A receptor composition, which together enhance excitability in both D1R- and D2R-expressing MSNs in vitro and elevate MSN firing in vivo. The enhanced excitability of MSNs in Gpr88Cre/Cre mice results in behavioral hyperactivity, deficits in motor coordination, impaired acquisition, and integration of visual or auditory cues, leading to poor performance in cue-based tasks. We restored electrophysiological responses and prevented hyperactivity and learning deficits in Gpr88Cre/Cre mice by re-expressing a human GPR88-TdTomato fusion protein in MSNs. Although we cannot rule out the possibility that GPR88 has additional roles in other brain regions, the behavioral restoration observed after viral rescue indicates the importance of GPR88 function in the striatum.

GABA-induced responses in slices and tonic GABA conductance were reduced in MSNs of Gpr88Cre/Cre mice. These findings are likely a result of decreased GABA A receptor subunit β3 levels37. The tonic GABA input in MSNs is mediated by extrasynaptic (β3 and β3 expressing) GABA A receptors38,39. Lack of phospho-specific antibodies has impeded the determination of phosphorylation status of β3 subunit–containing GABA A receptors, but they are regulated by protein kinases A and C35. Gpr88Cre/Cre mice had less striatal RGS4, which promotes signaling by other Gαi- and Gq-coupled GPCRs in MSNs35, resulting in altered kinase activity and abnormal MSN excitability. Changes in kinase activity could mediate the increase in...
observed GluR1 phosphorylation that underlies the increased AMPA receptor–mediated responses in Gpr88<sup>Cre</sup>/Cre mice. Accordingly, RGS4 inhibition was sufficient to abolish the restoration of the electrophysiological properties of MSNs after virally mediated GPR88 re-expression. We propose that the absence of GPR88 modifies transcription and intracellular signaling, leading to changes in RGS4 activity and increased MSN excitability.

The behavioral changes observed in Gpr88<sup>Cre</sup>/Cre mice resemble neurological disease processes associated with the striatum<sup>3,5–7</sup>. The Gpr88<sup>Cre</sup>/Cre mice showed turn-based learning (egocentric) in the U maze, but were slower to learn cue-based (allocentric) tasks, such as the Morris water maze. U maze and two-way active avoidance. Burst-firing in dopamine neurons is thought to facilitate LTP and thereby contribute to cue-based learning through modulation of the signal-to-noise ratio in MSNs<sup>40,41</sup>. Thus, the increased firing rate of MSNs in the Gpr88<sup>Cre</sup>/Cre mice may dampen the dynamic range of MSNs in response to burst firing by dopamine neurons, and this could impair cue-based learning. We confirmed D2R agonist hypersensitivity<sup>12</sup> and observed reduced D1R agonist effects in Gpr88<sup>Cre</sup>/Cre mice, suggesting altered dopaminergic signaling in both MSN populations of Gpr88<sup>Cre</sup>/Cre mice<sup>39</sup>. Although alterations in dopaminergic signaling have received the most attention in neurological disorders<sup>3,12</sup>, our results illustrate that alterations in MSNs may also be involved.

Our findings suggest that tonic activation of MSNs in Gpr88<sup>Cre</sup>/Cre mice impairs integration and filtering of information in the striatum. Accordingly, Gpr88<sup>Cre</sup>/Cre mice have impaired pre-pulse inhibition (data not shown), as shown previously<sup>2,12</sup>, an indicator of defective sensorimotor gating that is observed at striatal lesions and found in many neuropsychiatric diseases<sup>33–45</sup>. Furthermore, administration of antidepressants<sup>11,12</sup>, lithium<sup>46</sup> and valproate<sup>47</sup> alter Gpr88 expression, which may mediate some of the drug effects. It remains unclear how GPR88 is activated, as identification of its ligand has been elusive. Perhaps GPR88 functions without ligand activation; it may be regulated by altering the abundance of the protein rather than by a typical ligand-activation mechanism. Alternatively, MSN-specific chaperones and/or partners may be necessary for proper targeting and/or signaling of GPR88, which would preclude common high-throughput, heterologous-expression approaches. Regardless of these uncertainties, our data suggest that GPR88 is important for the regulation of MSN excitability and that alteration in its function may contribute to the behavioral deficits caused by neuropsychiatric diseases.

METHODS

and any associated references are available in the online version of the paper.

Accession codes. ArrayExpress: E-MTAB-1282.

Note: Supplementary information is available in the online version of the paper.

ACKNOWLEDGMENTS

the authors thank G. Froelick for assistance with histology, J. Parker for help making the targeting construct, L. Zweifel and M. Carter for helpful discussions, and D. Durman for editing. This work was supported by grants NS005236, NS060803, HD02274 (N.S.B.), MH06396 (G.S.M. and P.S.A.), DA007278 (G.P.S.) and GM032875 (G.S.M.) from the US National Institutes of Health. A.Q. and E.S. were recipients of Spanish Ministry of Science and Innovation postdoctoral mobility program fellowships.

AUTHOR CONTRIBUTIONS

A.Q. and R.D.P. designed the study. R.D.P. generated the Gpr88<sup>Cre</sup>/Cre mice. A.Q. performed the histological experiments. A.Q. and E.S. performed the Ribotag and biochemical experiments. E.S. performed the microarray experiment and A.Q. analyzed the data. W.W., G.P.S. and M.J.W. performed the in vitro electrophysiological studies and analyzed the data. A.Q. and A.D.G. performed the in vivo electrophysiological experiments and A.D.G. analyzed the data. A.Q. and B.A.B. performed the behavioral experiments and analyzed the data. A.Q. made viral vectors and performed stereotaxic surgery. A.L.T. performed the mouse primary culture experiments. P.S.A. and G.S.M. supervised the biochemical experiments. N.S.B. supervised the electrophysiological experiments. R.D.P. supervised the study. A.Q., N.S.B. and R.D.P. wrote the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

Published online at http://www.nature.com/doi/10.1038/nn.3239. Reprints and permissions information is available online at http://www.nature.com/reprints/index.html.

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ONLINE METHODS

Animals. All animal experiments were approved by the Animal Care and Use Committee at the University of Washington. Group-housed, male and female C57Bl/6j (2–4 months old) mice were maintained with rodent diet (5053, Picolab) and water available ad libitum with a 12-h light-dark cycle at 22 °C. Generation of RiboTag® and Rosa26-loxP-stop-loxP-TdTomato (line A114) mice have been described.

Generation of Gpr88-Cre mice. The two-exon Gpr88 gene was subcloned from a C57Bl/6j BAC clone. Most of the open reading frame of Gpr88 from just upstream of the initiation codon was replaced by a Cre-EGFP cassette with a Myc-tag and nuclear localization signal at the N terminus. Both arms of the targeting construct were inserted into a targeting vector with FRT-flanked Sve-Neo, Pkgk-DT 1 and HSV-TK genes and electroporated into C57Bl/6 embryonic stem cells; clones were screened by Southern blot (NcoI digestion) using a unique probe S 5′ of one arm. After germ-line transmission, the Sve-Neo gene was removed by breeding with FLPer mouse and backcrossed to C57Bl/6 mice. Heterozygous (Gpr88Cre+C) mice were bred to obtain mice homozygous for Cre (Gpr88Cre/Cre); heterozygous mice (Gpr88+Cre) and wild-type mice (Gpr88−/−) at the expected Mendelian ratios. No differences were observed in body weight or lifespan between genotypes. Genotyping by PCR was performed with two different strategies (Supplementary Table 4).

Behavioral assays. Spontaneous locomotion was assessed in chambers (Columbus Instruments) with ad libitum access to food and water for 48 h. Distance traveled was calculated by Optomax software.

Rotarod was performed by placing the mice on an accelerating rod (4–40 rpm over the course of a 3-min trial, three trials per day, 20-min intertrial interval (ITI), for 3 d)49. Latency to fall or fail to stay on top of the rod was recorded.

Hanging wire grip test was performed by placing mice on an inverted 15.5-cm-wide wire grid 42 cm above a padded surface with a maximum trial time of 3 min (ref. 50) (three trials, 15-min ITI). The average latency to fall was recorded.

The water maze test was carried out in a metal U maze with arms (50 cm, one black and one white) bent back toward the stem (45 cm)55. An escape platform, not visible from the end of stem, was present at the end of one of the two arms. Maze arms were alternated in a non-repetitive, pseudo-random sequence daily (ten trials per day, 3–5 min ITI)55. The percentage of correct trials and latencies to reach the platform (escape latencies) and the end of the stem (decision point) were recorded. Mice remained in the maze until a correct turn was made to ensure an equal number of reinforced responses. The strategy-shifting procedure consisted of two phases. For phase 1, the mice had to learn a turn-based strategy for 3 d with the escape platform always on the same side of the ramp. For phase 2 (days 4–7), the platform was associated to the arm color (cue-based strategy). For cue-based experiments, naive mice were only trained in phase 2 (5 d).

For the Morris water maze, mice were trained to locate a hidden platform over a period of 8 d (days 1–4 and 6–9) with four 90-s trials per day in a circular pool (84 cm diameter)55. On each trial, mice were released into the pool from a different location. Sessions were video recorded and analyzed with EthoVision software (Noldus). Latency to reach the hidden platform and swim speed was recorded. On day 5 and 10, mice performed a no-platform 90-s spatial memory probe trial; the percentage of time spent in each quadrant was recorded.

On day 125 mM Scm methanesulfonate, 5 mM KCl, 4 mM NaCl, 1 mM MgCl2, 5 mM MgATP, 5 mM EGTA, 10 mM HEPES, 10 mM NaHCO3, 0.5 mM CaCl2, 35 mM glucose, 100 µM dopamine, 300 µM MPP+, 10 µM TTX and 100 µM MPEP were added to the medium in which the cells were maintained. GABAergic neurons were isolated by blocking ionotropic and metabotropic glutamate and dopamine receptors, respectively. EPSCs and IPSCs in striatal MSNs were evoked by tungsten bipolar electrical stimulation of the dorsal cortex or striatum, respectively26.

Slice electrophysiology. Coronal brain slices (300 µm) were prepared using 4–8-week-old male Gpr88+/− (n = 21) and Gpr88Cre/Cre mice (n = 22). For current-clamp experiments, patch pipettes (4–6 MΩ) contained 112 mM potassium glutamate, 17.5 mM KCl, 4 mM NaCl, 1 mM MgCl2, 0.5 mM CaCl2, 5 mM MgATP, 5 mM EGTA, 10 mM HEPES, 10 mM NaHCO3, and 5 mM K3-ATP (pH 7.2–7.3, 270–280 mOsm). For voltage-clamp experiments, pipettes contained 125 mM cesium methanesulfonate, 5 mM KCl, 4 mM NaCl, 1 mM MgCl2, 5 mM MgATP, 5 mM EGTA, 8 mM HEPES, 1 mM Tris-GTP, 10 mM di-sodium phosphocreatine, 0.1 mM leupeptin and QX-314. For tonic GABA currents, pipettes contained 137 mM CsCl, 3 mM NaCl, 4 mM NaCl, 1 mM MgCl2, 10 mM HEPES, 10 mM EGTA, 12 mM phosphocreatine, 5 mM Na2ATP and 0.2 mM NaGTP. The mean holding current (recorded in the presence of 10 µM NBBQ, 50 µM (-)-2-amino-5-phosphonovaleric acid (AP5), 40 µM CPCPCOET and 10 µM MPEP) was determined at the peak value of a Gaussian fit of an all-points histogram obtained from two 30-s windows. Ihold was calculated by averaging 60 individual 50-ms epochs containing 250 amplitude measurements. Inwardly rectifying K+ currents were measured using voltage steps from −30 to −140 mV with the K+-channel blocker Cs+ (1 mM)53,54. Pipettes contained 135 mM potassium methylsulfate, 5 mM KCl, 0.5 mM CaCl2, 5 mM HEPES, 5 mM EGTA, 12 mM phosphocreatine, 2 mM Na2ATP, and 0.3 mM NaGTP. Voltage-dependent Na+ and Ca2+ conductances were blocked using 3 µM TTX and 100 µM Cd2+, respectively. Maximal GABA A receptor–mediated currents during GABA application were recorded with 100 µM AP5, 10 µM CNXZ, 1 µM strychnine, 0.1 µM eticlopride and 10 µM CGP-52432 to block ionotropic glutamate, glycine, dopamine and GABA A receptors, respectively. EPSCs and IPSCs in striatal MSNs were evoked by tungsten bipolar electrical stimulation of the dorsal cortex or striatum, respectively26.

EPSCs were isolated by blocking GABA A receptors with bicuculline. IPSCs were isolated by blocking ionotropic and metabotropic glutamate and dopamine receptors with 10 µM NBBQ, 50 µM AP5, 40 µM CPCPCOET, 10 µM MPEP, 100 µM SCH23390 and 10 µM sulpiride. Cells showing variable latencies or durations or those with series resistance greater than 20 MΩ or changing by >20% were rejected. Analysis was performed using Clampfit (Molecular Devices).
Freely moving in vivo electrophysiology. Electrophysiological recordings in striatum of freely moving animals (n = 6 per group) were obtained as described48. MSNs were identified as cells with valley widths greater than 300 µs (ref. 20). Four-tetrode microdrives (Neuralynx) were implanted in the dorsal striatum of anesthetized mice (stereotaxic coordinates: anteroposterior (AP) = +0.5 mm, mediolateral (ML) = +1.75 mm, dorsoventral (DV) = −3.5 mm). After 2 weeks of recovery, mice were habituated for a week to the recording environment and subsequently connected to a digital Lynx (10S) acquisition system through an HS-16 head-stage preamplifier (Neuralynx). Signals were amplified, filtered (600–6,000 Hz) and data acquired by using Cheetah software (Neuralynx). After a 10-min habituation baseline, striatal neuron firing properties were recorded for 10 min. Tetrodes were lowered by 50-µm increments each day. Tetrode placement was assessed postmortem by hematoxylin staining of striatal sections. Units were isolated by cluster analysis using Offline Sorter software (Plexon) optimizing clusters for J3 statistics with a minimum clustering probability of P < 0.001. Inter-spike intervals (ISI) of clustered waveforms were subsequently analyzed by using MATLAB (MathWorks).

The distributions of log(ISI) were plotted and the median and mean values were calculated. Two main distributions were observed: neurons with large differences between log(ISI) median and mean values, representing bouts of activity alternated with silent periods, and neurons with a more continuous firing and closer median and mean values. To classify all recorded units, we used the ratio of the ISI median/mean values. A ratio of 0.3 was considered to be the cutoff between the two types of MSN firing patterns.

AAV vector generation and delivery. An AAV-Gpr88-TdTomato vector was generated by fusing human GPR88 to the TdTomato coding region and inserting it downstream of the CBA promoter; it was made Cre dependent by inserting a polyadenylation signal flanked by loxp sequences between the promoter and GPR88. As a control, an AAV with TdTomato under control of the Fos promoter was generated. Encapsidation in AAV1 coat serotype was performed as described55.

For cell culture studies, 1 µl of the virus was used per well. For in vivo viral delivery, 4 µl of the virus (0.5 µl per injection site) were stereotactically injected bilaterally in the dorsal striatum at the coordinates ML = ±1.50, AP = +1.2, DV = −3.25/3.75 and ML = ±2.25, AP = 0, DV = −3.25/3.75 from Bregma. Animals recovered for 2 weeks before behavioral testing or electrophysiological recordings.

Intracerebral retrobead delivery. Red fluorescent latex beads (Retrobeads, Lumafluor; 300 µl) were injected unilaterally in the SNr at the coordinates ML = +1.50, AP = −3.08, DV = −4.75 and ML = +1.50, AP = −3.45, DV = −4.50. Green fluorescent beads (300 µl) were injected unilaterally in the GPe at the coordinates ML = −2.00, AP = −0.58, DV = −4.00. Animals recovered for 2 weeks before obtaining slices for recording.

Statistical analyses. Data are shown as the means ± s.e.m. GraphPad Prism v5.0 software was used for statistical analyses. For normally distributed parameters, either Student’s t test (two-group comparisons) or ANOVA tests (multiple comparisons) were used. Otherwise, non-parametrical tests were used. For in vivo recordings and microarray analysis, built-in packages were used.

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