GPR88 in D1R-Type and D2R-Type Medium Spiny Neurons Differentially Regulates Affective and Motor Behavior

A. C. Meirsman, Sami Ben Hamida, E. Clarke, A. de Kerchove d'Exaerde, E. Darcq, and B. L. Kieffer

https://doi.org/10.1523/ENEURO.0035-19.2019

1Département de Médecine Translationnelle et Neurogénétique, Institut de Génétique et de Biologie Moléculaire et Cellulaire, Institut National de la Santé et de la Recherche Médicale Unité 964, Centre National de la Recherche Scientifique Unité Mixte de Recherche 7104, Université de Strasbourg, Illkirch, France, 2Neuroscience Paris Seine, Centre National de la Recherche Scientifique Unité Mixte de Recherche 8246/Institut National de la Santé et de la Recherche Médicale Unité 1130/Université Pierre et Marie Curie, Paris F-75005, France, 3Douglas Research Center, Department of Psychiatry, McGill University, Montréal, Canada, 4Laboratory of Neurophysiology, Université Libre de Bruxelles (ULB), ULB Neuroscience Institute, 1070 Brussels, Belgium

Visual Abstract

Significance Statement

GPR88, an orphan G-protein-coupled receptor (GPCR), has been implicated in the regulation of striatum-dependent behaviors. In the striatum, GPR88 is most abundant in both medium spiny neurons (MSNs)-expressing dopamine D1 and D2 receptors. We compared effects of a conditional Gpr88 gene knock-out (KO) in D1 receptor (D1R)-MSNs or D2R-MSNs with effects of the total Gpr88 deletion. Our data suggest that GPR88 in D2R-MSNs shapes defensive and social behavior and contributes in maintaining the inhibition of basal ganglia outputs to control locomotion, stereotypies and motor coordination, while GPR88 in D1R-MSNs promotes novelty habituation and motor learning. Gpr88 therefore plays very distinct roles in modulating D1R-type and D2R-type neurons function and the related behaviors.
The orphan receptor GPR88 is highly expressed in D1 receptor (D1R)- and D2R-medium spiny neurons (MSNs) and has been associated to striatum-dependent functions in rodents. The total deletion of Grpr88 in mice was shown to decrease anxiety-like behaviors, increase stereotypies and locomotion, and impair motor coordination and motor learning. Knowing the opposing role of D1R- and D2R-MSNs, we here investigated the respective roles of GPR88 in the two MSN subtypes for these behaviors. To do so, we compared effects of a conditional Grpr88 gene knock-out (KO) in D1R-MSNs (D1R-Gpr88 mice) or D2R-MSNs (A2AR-Gpr88 mice) with effects of the total Grpr88 KO (CMV-Gpr88 mice). Overall, most phenotypes of CMV-Gpr88 mice were recapitulated in A2AR-Gpr88 mice, including reduced marble burying, increased social interactions, increased locomotor activity and stereotypies in the open field, and reduced motor coordination in the rotarod. Exceptions were the reduced habituation to the open field and reduced motor skill learning, which were observed in CMV-Gpr88 and D1R-Gpr88 mice, but not in A2AR-Gpr88 mice. D1R-Gpr88 mice otherwise showed no other phenotype in this study. Our data together show that GPR88 modulates the function of both D1R- and D2R-MSNs, and that GPR88 activity in these two neuron populations has very different and dissociable impacts on behavior. We suggest that GPR88 in D2R-MSNs shapes defensive and social behavior and contributes in maintaining the inhibition of basal ganglia outputs to control locomotion, stereotypies and motor coordination, while GPR88 in D1R-MSNs promotes novelty habituation and motor learning.

**Key words:** anxiety; locomotion; medium spiny neuron; motor coordination; orphan GPCR; striatum

**Introduction**

Among brain orphan G-protein-coupled receptors (GPCRs), GPR88 shows highest and almost restricted expression in the striatum, a key region in motor control, cognitive functions and motivational processes (Liljeholm and O’Doherty, 2012; Quintana et al., 2012; Ehrlich et al., 2018). Homozygous deleterious mutation of Gpr88 in humans was linked to a familial developmental disorder characterized by a childhood chorea (hyperkinetic movement disorder), learning disabilities and marked speech retardation (Alkufri et al., 2016). Previous reports have shown that mice lacking Gpr88 present hyperlocomotion, increased stereotypies, motor coordination and motor learning deficits (Logue et al., 2009; Quintana et al., 2012; Meirsman et al., 2016b). The total Gpr88 gene deletion in mice also induced failure to habituate to an open field or automated home-cage environment and decreased anxiety-like behaviors (Meirsman et al., 2016b; Maroteaux et al., 2018). Additionally, AAV-mediated re-expression of GPR88 in the dorsal striatum [caudate putamen (CPU)] restored the locomotor hyperactivity and motor learning deficits in knock-out (KO) animals, thus providing a direct link between GPR88 loss in the dorsal striatum and the locomotor phenotype of KO mice (Quintana et al., 2012; Meirsman et al., 2016b).

Within the striatum, GPR88 is expressed in the majority of medium spiny neurons (MSNs) of both the direct (co-expressing dopamine D1 receptors (D1Rs) and substance P, D1R-MSNs) and indirect (co-expressing dopamine D2Rs, adenosine A2A receptor (A2AR) and Enkephalin, D2R-MSNs) pathways (Quintana et al., 2012). Converging evidence support the opposing influence of these two MSNs populations in motor output systems and motivated behavior. For example, optogenetic depolarization of D2R-MSNs decreased locomotor initiation (Kravitz et al., 2010), while ablation or disruption of these neurons increased motor activity (Durieux et al., 2009, 2012; Bateup et al., 2010). In contrast, optical stimulation of D1R-MSNs increased locomotion whereas disruption or ablation of these neurons had the opposite effect (Kravitz et al., 2010; Durieux et al., 2012). Also, cell-specific neuron ablation using an inducible diphtheria toxin receptor (DTR)-mediated cell targeting strategy further suggests a differential role of D2R- and D1R-MSNs in acquisition and expression of motor skill learning (Durieux et al., 2012). Ablation of D2R-MSNs neurons delayed the acquisition of a rotarod task but had no effect in a previously acquired motor skill. Contrarily, ablation of D1R-MSNs neurons impaired motor skill learning regardless of the training extension and also disrupted performance of a previously learned motor sequence (Durieux et al., 2012). Further, in recent years, research on MSNs subtypes function has revealed that these two neuronal populations differentially regulate not only motor behaviors but also responses to rewarding and aversive stimuli: while optogenetic activation of the D1R-
MSNs was shown to increase reinforcement, activation of D2R-MSNs induced transient punishment and depressive-like behavior (Kravitz et al., 2012; Hikida et al., 2013; Francis et al., 2015).

Despite the established overall function of striatal GPR88 in brain functions and deficits (in humans and mice), no study to date has directly compared the specific role of GPR88 in D1R- and D2R-MSNs. A conditional KO mouse line for GPR88 in D2R-MSNs was developed in a previous study, using a A2AR-Cre driver line (A2AR-Gpr88 mice), and mutant mice showed hyperactive behavior, decreased anxiety-like behaviors and increased locomotor response to dopaminergic agonists (Meirsmann et al., 2016a, 2017). In this study, we have generated conditional Gpr88 KO for D1R-MSNs (D1R-Gpr88 mice), and compared behavioral responses of D1R-Gpr88 with those of A2AR-Gpr88 mice and total KO (CMV-Gpr88) mice. Results show that GPR88 in D1R neurons regulates locomotor habituation to novel environments and motor skill learning. In contrast, GPR88 in D2R, but not in D1R neurons, control defensive burying and social approach, and also regulate levels of locomotion, stereotypies and initial motor coordination.

Materials and Methods

Subjects

Mice (male and female) aged 9–15 weeks where bred in house and grouped-house three to five animals per cage. Animals where maintained on a 12/12 h light/dark cycle at controlled temperature (22 ± 1°C). Food and water were available ad libitum throughout all experiments.

Generation of mutant mice

Gpr88-floxed mice, total Gpr88 KO (CMV-Gpr88) and A2AR-Gpr88 mice were produced as previously described (Meirsmann et al., 2016a, 2016b). To generate CMV-Gpr88, Gpr88-floxed mice (C57BL/6 background) were crossed with CMV-Cre mice (50%-C57BL/6J; 50%-129/sv) expressing Cre recombinase under the cytomegalovirus promoter. To generate a conditional KO of Gpr88 in D2R-MSNs (A2AR-Gpr88) or D1R-MSNs (D1R-Gpr88) Adora2a-Cre (Durieux et al., 2009) and Drd1a-Cre (gensat.org; congenic on C57BL/6J) mice were, respectively, crossed with Gpr88-floxed mice (Meirsmann et al., 2016b). First generation animals expressing the Cre under the control of A2AR or D1R promoter (Gpr88\textsuperscript{A2AR-Cre\textsuperscript{+}} and Gpr88\textsuperscript{D1R-Cre\textsuperscript{+}}) were crossed a second time to eliminate the wild-type Gpr88 gene. We therefore generated 3 mouse lines with different mixed genetic background.

For all experiments, and considering the different genetic background, A2AR-Gpr88 and D1R-Gpr88 mice were compared to their Gpr88-floxed littermates (A2AR-R-CTL and D1R-CTL, respectively) and CMV-Gpr88 mice were compared to their wild-type controls (CMV-CTL). Baseline responses may therefore slightly differ when comparing the three mouse colonies.

Tissue preparation and fluorescent in situ hybridization

RNAscope was used as previously described (Meirsmann et al., 2016a). Mice (n = 3 D1R-CTL; n = 3 D1R-Gpr88) were killed by cervical dislocation and fresh brains were extracted and embedded in optimal cutting temperature (OCT) medium (Thermo Scientific) frozen and kept at −80°C. Frozen brains were coronally sliced into 20-μm serial sections by using cryostat (CM3050 Leica), placed in superfrost slides (Thermo Scientific) and kept at −80°C until processing. In situ hybridizations were performed using the RNAscope Multiplex Fluorescent Assay. GPR88 and D1R probes were alternatively coupled to FITC or TRITC while D2R probes were coupled with Cy5.

Relative expression of D1R and D2R mRNA in GPR88-positive cells

Image acquisition was performed using the slide scanner Olympus VS120 (Olympus Corporation). Regions of interest (ROIs) were selected using Olyvira software (Olympus) and saved as PNG files. Three brain regions where analyzed: rostral CPu (from 1.42 to 0.98 mm from bregma), caudal CPu (from 0.98 to −0.58 mm from bregma), and nucleus accumbens (Nacc; from 1.42 to 1.10 mm from bregma).

For the CPu (rostral and caudal), at least four ROIs were selected: two for the dorso-lateral striatum (DLS) and two for dorso-median striatum (DMS). Counting was balanced between right and left hemispheres. To evaluate expression of D1R and D2R mRNA in GPR88-expressing cells, counting was performed manually using the FIJI (ImageJ) cell counter. First, cells expressing GPR88 mRNA were marked and counted (±55 cells/ROI in D1R-CTL mice; ±21 cells/ROI in D1R-Gpr88 mice). For each GPR88-positive cell, co-expression of D1R or D2R was verified and counted separately. Relative co-expression (GPR88/ D1R or GPR88/D2R) is represented as a percentage of total GPR88-positive cells counted ([number of GPR88-expressing cells co-expressing D1R or D2R × 100]/total number of GPR88-expressing cells). Statistical analysis where realized with percentages of each ROI calculated using excel. Given the lack of difference in GPR88 expression between right and left medial CPUs, relative percentage of each was pooled for graphical representation and statistical analysis.

[S35]-GTP\textsubscript{Y}S binding assay

[S35]-GTP\textsubscript{Y}S assays were performed on membrane preparations as described in previous report (Pradhan et al., 2009). To perform [S35]-GTP\textsubscript{Y}S assays on whole striatum mice were killed by cervical dislocation and both striatum were rapidly manually removed, frozen in dry ice, and stored at −80°C until use. Two (CMV-Gpr88 and CMV-CTL) and three (D1R-Gpr88 and D1R-CTL) membrane preparations were used. Each membrane preparation was generated using striatum from three animals (males and females). Results are expressed by mean measures from the three-membrane preparation. All assays were performed on membrane preparations. Membranes were prepared by homogenizing the tissue in ice-cold 0.25 M sucrose solution 10 vol (ml/g wet weight of tissue). Samples were then centrifuged at 2500 × g for 10 min. Supernatants were collected and diluted 10 times in buffer containing 50 mM TrisHCl (pH 7.4), 3 mM MgCl2, 100 mM NaCl, and 0.2 mM EGTA, following which they
were centrifuged at 23,000 \times g for 30 min. The pellets were homogenized in 800-\mu l ice-cold sucrose solution (0.32 M) and kept at -80°C. For each [35S]GTP\gammaS binding assay 2 \mu g of protein per well was used. Samples were incubated with and without ligands, for 1 h at 25°C in assay buffer containing 30 mM GDP and 0.1 nM [35S]GTP\gammaS. Bound radioactivity was quantified using a liquid scintillation counter. \( B_{\text{max}} \) and \( K_d \) values were calculated. Non-specific binding was defined as binding in the presence of 10 \mu M GTP\gammaS and binding in the absence of agonist was defined as the basal binding.

Gene expression analysis

Mice were killed by cervical dislocation. Brains structures (Nacc n = 8 D1R-CTL; n = 7 D1R-Gpr88 and CPu n = 9 D1R-CTL; n = 9 D1R-Gpr88, hippocampus: n = 9 D1R-CTL; n = 7 D1R-Gpr88 and amygdala: n = 6 D1R-CTL; n = 7 D1R-Gpr88) from D1R-Gpr88 and controls were quickly dissected out, frozen on dry ice and stored at -80°C until used. RNA was isolated using TRIzol reagent (Invitrogen) following the manufacturer's instructions. cDNA was synthesized using the first-strand Superscript II kit (Invitrogen, Life Technologies). Quantitative real-time PCR (qRT-PCR) was performed in triplicates on a LightCycler 480 RT- PCR (Roche) and SyberGreen masterMix (Roche). Thermal cycling parameters were 1 min at 95°C followed by 40 amplification cycles of 15 s at 95°C, 15 s at 60°C, and 30 s at 72°C. Relative expression ratios were normalized to the level of actin and the respective mice were exclude from analysis. Method of contrasts was used to compare day 1 and day 6 (days 1 and 5), first and last rotarod session analysis. Statistics

For in situ hybridization cell counting and GPR88 agonist-induced binding assay data were analyzed using two-way ANOVA followed by Sidak’s and Tukey’s multiple comparisons, respectively. Repeated measures (RM) two-way ANOVA was used to analyze global open field and rotarod results with genotypes as the between-subject factor and time as the RM. One-way ANOVA was used for open field habituation analysis (days 1 and 5), first and last rotarod session analysis. Method of contrasts was used to compare day 1 and day 6 performance on the rotarod. Stereotypes, marble burying and social interaction contacts were analyzed using t test (unpaired with Welch’s correction). All statistical analyses were realized using GraphPad Prism 7 (GraphPad Software, Inc) and the accepted level of significance was \( p < 0.05 \). All the statistical methods are summarized in Table 1.

Results

D1R-Gpr88 mice show Gpr88 mRNA deletion in D1R-expressing neurons

To conditionally delete Gpr88 exon 2 in cells expressing D1R, mice carrying two LoxP sites flanking the second
Table 1. Detailed statistical analysis

| Assay                  | Mouse line                  | Number | Figure | Genotype effect | ANOVA           | t test         |
|------------------------|-----------------------------|--------|--------|-----------------|-----------------|----------------|
| RT-qPCR                | D1R-Gpr88                   | N = 9  | 1A (CPu)|                 |                 |                |
|                        | N = 9 D1R-CTL; N = 9 D1R-Gpr88 |        |        |                 |                 |                |
|                        | N = 8 D1R-CTL; N = 7 D1R-Gpr88 | 1A (Nacc) |        |                 |                 |                |
|                        | N = 9 D1R-CTL; N = 7 D1R-Gpr88 | 1A (Hippo) |        |                 |                 |                |
|                        | N = 6 D1R-CTL; N = 7 D1R-Gpr88 | 1A (Amy) |        |                 |                 |                |
| [35S]-GTPγS binding   | CMV-Gpr88; D1R-Gpr88        | N = 3  | 1B    |                 |                 |                |
|                        | CMV-CTL, CMV-Gpr88          |        |        |                 |                 |                |
| In situ hybridization/ | D1R-Gpr88                   | N = 3  | 2B (CPu) |                 |                 |                |
| cell counting          | D1R-CTL                      |        |        |                 |                 |                |
| Marble burying         | CMV-Gpr88                   | N = 9  | 3A, left |                 |                 |                |
|                        | D1R-Gpr88                   | N = 14 | 3B, left |                 |                 |                |
|                        | A2AR-Gpr88                  | N = 10 | 3C, left |                 |                 |                |
| Nose contact in social | CMV-Gpr88                   | N = 8  | 3D, right |                 |                 |                |
| interaction            | D1R-Gpr88                   | N = 14 | 3E, right |                 |                 |                |
| Open field (all sessions) | CMV-Gpr88                  | N = 21 | 4A, left |                 |                 |                |
|                        | D1R-Gpr88                   | N = 13 | 4B, left |                 |                 |                |
|                        | A2AR-Gpr88                  | N = 17 | 4C, left |                 |                 |                |
| Open field (sessions 1 and 5) | CMV-Gpr88                  | N = 21 | 4A, right |                 |                 |                |
|                        | D1R-Gpr88                   | N = 13 | 4B, right |                 |                 |                |
|                        | A2AR-Gpr88                  | N = 17 | 4C, right |                 |                 |                |
| Stereotypies           | CMV-Gpr88                   | N = 21 | 5A     |                 |                 |                |
|                        | D1R-Gpr88                   | N = 13 | 5B     |                 |                 |                |
|                        | A2AR-Gpr88                  | N = 17 | 5C     |                 |                 |                |
| Rotorod (all sessions) | CMV-Gpr88                   | N = 21 | 6A, left |                 |                 |                |
|                        | CMV-CTL, CMV-Gpr88          |        |        |                 |                 |                |
|                        | D1R-Gpr88                   | N = 13 | 6B, left |                 |                 |                |
|                        | A2AR-Gpr88                  | N = 17 | 6C, left |                 |                 |                |
| Rotorod (sessions 1 and 6) | CMV-Gpr88                  | N = 21 | 6A, right |                 |                 |                |
|                        | CMV-CTL, CMV-Gpr88          |        |        |                 |                 |                |
|                        | D1R-Gpr88                   | N = 13 | 6B, right |                 |                 |                |
|                        | A2AR-Gpr88                  | N = 17 | 6C, right |                 |                 |                |

exon of the Gpr88 gene (Meirsm et al., 2016b) were crossed with mice expressing the Cre recombinase under the control of the Drd1a gene promoter (Gensat). We first tested whether GPR88 transcript and protein are reduced in the striatum. We quantified Gpr88 mRNA levels by RT-qPCR for CPu and Nacc from D1R-Gpr88 and their control littermates. As shown in Figure 1A, Gpr88 expression was significantly decreased in striatal regions of conditional KO compared to controls (CPu: t(16) = 3.01, p = 0.008; Nacc: t(13) = 4.19, p = 0.001; Table 1). Testing Gpr88 mRNA levels in the hippocampus and amygdala showed a milder but significant reduction in hippocampus.
Figure 1. GPR88 agonist-induced activation and mRNA levels in D1R-Gpr88 mice. We measured levels of Gpr88 mRNA in D1R-CTL and D1R-Gpr88 mice (A) and show a significant reduction of GPR88 expression in the CPu, Nacc, hippocampus (Hipp), and amygdala (Amy). We also performed GPR88-mediated [35S]-GTPγS assay (B) and show that protein activation was totally and partially abolished in the striatum of CMV-Gpr88 and D1R-Gpr88 mice, respectively. Two (CMV-Gpr88 and control mice) and three (D1R-Gpr88 and control mice) membrane preparations were used per genotype. Data are presented as mean ± SEM. A, CPu: n = 9 D1R-CTL; n = 9 D1R-Gpr88; Nacc: n = 8 D1R-CTL; n = 7 D1R-Gpr88; Hipp: n = 9 D1R-CTL; n = 7 D1R-Gpr88; Amy: n = 6 D1R-CTL; n = 7 D1R-Gpr88; two black stars p < 0.01; three black stars p < 0.001 (Welch’s t test). B, n = 3 D1R-CTL; n = 3 D1R-Gpr88; n = 2 CMV-Gpr88 and n = 2 CMV-CTL. Three text stars p < 0.001 Tukey’s multiple comparisons of D1R-CTL or CMV-CTL versus D1R-Gpr88 and CMV-Gpr88 versus D1R-Gpr88.

but not amygdala (Hipp: t14 = 2.7, p = 0.017; Amy t11 = 0.53, p = 0.6), indicating that GPR88 KO may also have occurred in some extrastriatal regions containing D1R-type neurons (see Discussion). There was no significant difference in D1R expression levels across genotypes (data not shown). To establish whether reduced mRNA level translates into lower protein level, we tested GPR88 signaling in the striatum. To this aim, we performed GPR88 agonist-induced [35S]-GTPγS binding assays (Fig. 1B) with membranes prepared from whole striatum (CPu and Nacc) of D1R-Gpr88 mice and their controls, as well as with total KO CMV-Gpr88 mice (negative control) and their wild-type control mice (positive control). Two-way RM ANOVA revealed a significant genotype effect (F1,66 = 185.2, p < 0.0001) and interaction effect (F30,66 = 23.19, p < 0.0001). Post hoc analysis (Tukey’s multiple comparisons) revealed significant differences (p < 0.0001) between D1R-Gpr88 mice (118.4 ± 1.17%) and their control littermates (209.5 ± 2.47%) as well as between D1R-Gpr88 mice and CMV-Gpr88 mice (95.61 ± 1.72%; p < 0.0001). This result confirms that the Drd1a-Cre-driven conditional Gpr88 gene deletion produced a significant reduction of GPR88 expression. Importantly, deletion of GPR88 does not affect the function of D1R. Indeed, locomotor response to D1R agonist SKF 81297 is comparable in D1R-Gpr88 mice and their corresponding controls (data not shown).

We then tested whether the genetic deletion was specific to D1R MSNs, using in situ hybridization. As depicted in Figure 2A, we first demonstrate that, in control mice cells expressing Gpr88 mRNA colocalize with both Drd1a (left panel), and Drd2 mRNA-expressing cells (right panel). In D1R-Gpr88 mice, however, cells expressing Gpr88 do not colocalize with Drd1a-expressing cells (left panel), but still colocalize with Drd2-expressing cells (right panel). Quantitative analysis (Fig. 2B) in the CPu and Nacc confirmed that, in control animals, Gpr88 mRNA is found in both Drd1a-positive (CPu: 43.96 ± 1.54% and Nacc: 45.13 ± 3.57%) and Drd2-positive (CPu: 62.11 ± 1.95% and Nacc: 59.03 ± 4.47%) cells whereas in D1R-Gpr88 mice the great majority of cells expressing Gpr88 are found in Drd2-expressing cells (CPU: 92.83 ± 1.45% and Nacc: 91.88 ± 2.48%) with significantly reduced number of cells co-expressing Drd1a mRNA (CPU: 11.16 ± 1.43% and Nacc: 15.50 ± 2.18%; CPu: genotype: F1,134 = 0.42; p = 0.52; cell type: F1,134 = 957.2; p < 0.0001; interaction: F1,134 = 387.8, p < 0.0001; Nacc: genotype: F1,136 = 0.26, p = 0.6134; cell type: F1,136 = 204.3, p < 0.0001; interaction: F1,136 = 97.83, p < 0.0001; n = 3 genotype), indicating that the Gpr88 deletion had occurred mostly in D1R-type MSNs.

A2aR-Gpr88 but not D1R-Gpr88 mice show altered defensive burying and social approach

The deletion of Gpr88 specifically in D2R-neurons is sufficient to decrease anxiety-like behaviors and increase social approach (Meirsman et al., 2016a). Here, we investigated whether deletion of Gpr88 in D1R-neurons also modifies anxiety-related and/or social behaviors. As depicted in Figure 3A–C, using the defensive burying paradigm we first confirmed that CMV-Gpr88 (t13 = 2.03, p = 0.059; n = 9–10) buried less marbles than their control littermates. D1R-Gpr88 mice showed equal numbers of buried marbles compared to D1R-CTL mice (t22 = 1.002, p = 0.33; n = 10–14), whereas A2aR-Gpr88 mice, like CMV-Gpr88, showed reduced number of buried marbles (t18 = 4.01, p < 0.001; n = 10 genotype). Also, in the presence of a naive, wild-type, congener (Fig. 3D–F), CMV-Gpr88 (t14 = 2.88, p = 0.012; n = 8 genotype), and A2aR-Gpr88 mice (t16 = 2.06, p = 0.01; n = 10 genotype) showed increased numbers of nose contacts but D1R-Gpr88 mice displayed similar numbers of contacts than their control littermates (t20 = 2.57, p = 0.018; n = 10–14).

The present results confirm previous findings (Meirsman et al., 2016a) that Gpr88 deletion in D2R-neurons is sufficient to recapitulate emotional and social phenotypes observed in CMV-Gpr88 mice and reveal that deletion of Gpr88 in D1R-neurons does not alter neither defensive marble burying or social approach.
**A**<sub>2a</sub>-R-Gpr88 mice show hyperlocomotion, whereas D1R-Gpr88 mice show lack of habituation in a novel environment

Previous studies showed that mice lacking Gpr88 display increased spontaneous locomotor activity as well as lack of habituation to a novel environment (Quintana et al., 2012; Meirsman et al., 2016b; Maroteaux et al., 2018). Deletion of Gpr88 in D2R expressing neurons was further shown sufficient to recapitulate the hyperlocomotion phenotype observed in CMV-Gpr88 mice (Meirsman et al., 2016a). Here, we tested whether Gpr88 in D1R and D2R MSNs play a differential role in the regulation of locomotor and exploratory behavior. To do this, CMV-Gpr88, A<sub>2a</sub>R-Gpr88, and D1R-Gpr88 mice and their corresponding controls were individually placed in a dimly lit open field chambers during five successive daily 30-min sessions. Analysis of total locomotion confirmed a significantly increased locomotor activity for CMV-Gpr88 mice (two-way RM ANOVA; genotype: F<sub>1,46</sub> = 5.98, p = 0.0189; day: F<sub>4,180</sub> = 4.42; p = 0.002; interaction: F<sub>4,180</sub> = 7.19; p < 0.0001; n = 21/genotype; Fig. 4A, left panel). Further, while control animals decreased their general locomotion between the first and last session (see Fig. 4A, right panel), CMV-Gpr88 mice showed rather increased locomotion in the last compared to the first session (two-way ANOVA; genotype: F<sub>1,46</sub> = 4.93, p = 0.029; day: F<sub>4,180</sub> = 1.25, p = 0.27; interaction: F<sub>4,180</sub> = 8.94, p = 0.0037). D1R-Gpr88 mice (Fig. 4B, left panel) presented similar levels of general locomotor activity when compared to their littermates (two-way RM ANOVA; genotype: F<sub>1,23</sub> = 1.11, p = 0.30; day: F<sub>4,92</sub> = 31.03; p < 0.0001; interaction: F<sub>4,92</sub> = 11.82; p < 0.0001; n = 12–13) but, similar to CMV-Gpr88 mice, showed lack of locomotor habituation to the open field environment (two-way ANOVA; genotype: F<sub>1,46</sub> = 0.78, p = 0.38; day: F<sub>4,46</sub> = 26.75, p < 0.0001; interaction: F<sub>4,46</sub> = 11.01, p = 0.0018; Fig. 4B, right panel). Similar to CMV-Gpr88, A<sub>2a</sub>R-Gpr88 mice (Fig. 4C, left panel) significantly increased their locomotion when compared to control littermates (two-way RM ANOVA; genotype: F<sub>1,29</sub> = 8.0, p = 0.009; day: F<sub>4,100</sub> = 43.28; p < 0.0001; interaction: F<sub>4,100</sub> = 3.94; p = 0.005; n = 10–17). These mice, however, showed equal locomotor habituation profile than their littermates with decreased locomotion in the last compared to the first open field session (right panel; two-way ANOVA; genotype: F<sub>1,50</sub> = 8.17, p = 0.006; day: F<sub>5,150</sub> = 18.71, p < 0.0001; interaction: F<sub>5,150</sub> = 0.15, p = 0.70).

These results first confirm that deletion of Gpr88 increases general locomotion and simultaneously abolishes locomotor habituation to a novel environment.
Further, our results suggest that deletion of D1R-Gpr88 does not impact general locomotion but abolishes locomotor habituation to a novel environment. In contrast, deletion of Gpr88 in D2R-MSNs increases locomotor activity without altering habituation to a novel environment.

A2AR-Gpr88 but not D1R-Gpr88 mice show increased stereotypies in the open field

Previous studies indicate increased repetitive motor behaviors or stereotypies (Logue et al., 2009; Meirsman et al., 2016b), as well as increased perseverative behavior (Maroteaux et al., 2018) in CMV-Gpr88 mice. To examine...
Figure 4. Locomotor activity is increased in A2AR-Gpr88 mice whereas D1R-Gpr88 mice show lack of locomotor habituation. When placed individually in a dimly lit open field for 30-min daily sessions during 5 d, both CMV-Gpr88 (A) and A2A-R-Gpr88 (C) but not D1R-R-Gpr88 (B) mice traveled a longer distance then their control littermates. D1R-Gpr88 mice, however, present similar total locomotion when compared to their control littermates (B). When comparing locomotion between the first (1) and last session (5), CMV-Gpr88 mice, in contrast to CMV-CTL, traveled a longer distance in the last compared to the first day. In contrast to their control littersmates, D1R-Gpr88 mice show similar locomotion in the first and last open field session. Regardless of their hyperlocomotion, A2A-R-Gpr88 mice habituated to the open field presenting decreased overall locomotion in the last test session. Line graphs show the distance traveled (cm) in 5-min bins over a 30-min session. Bar graphs show the average total distance traveled (cm) over the 30-min sessions period. Data are represented as mean ± SEM. A, n = 21 CMV-CTL; n = 21 CMV-Gpr88. B, N = 13 D1R-CTL, N = 12 D1R-Gpr88. C, n = 17 A2A-R-CTL; n = 10 A2A-R-Gpr88. Open stars: one star p < 0.05; two stars p < 0.01 (Student’s t test).

Figure 5. CMV-Gpr88 and A2A-R-Gpr88 gene deletion increases stereotypies. When placed in an open field for 30 min (day 1), CMV-Gpr88 (A) and A2A-R-Gpr88 (C) present increased number and duration of stereotypies. D1R-Gpr88 mice (B), however, show no difference in the number or time spent in stereotypies when compared to their control littermates. Data are represented as mean ± SEM. A, n = 21 CMV-CTL; n = 21 CMV-Gpr88. B, N = 13 D1R-CTL, N = 12 D1R-Gpr88. C, n = 17 A2A-R-CTL; n = 10 A2A-R-Gpr88. Black stars: one star p < 0.05; two stars p < 0.01 (Student’s t test).

whether this phenotype results from Gpr88 deletion in D1R- and/or D2R-MSNs, we analyzed stereotypies scores in the first open field session (30 min). Results indicate that both CMV-Gpr88 (Fig. 5A) and A2A-R-Gpr88 mice (Fig. 5C) presented higher stereotypies score (t_{40} = 2.23; p = 0.031; n = 21/genotype and t_{25} = 2.29; p = 0.031; n = 10–17, respectively) and increased stereotypy time (t_{40} = 2.82; p = 0.007; and t_{25} = 2.32; p = 0.029, respectively). On the contrary, D1R-Gpr88 mice (Fig. 5B) presented no altered stereotyped behavior when compared to control animals.

These results show that GPR88 in D2R-expressing but not D1R-expressing neurons regulates motor stereotypies.

A2A-R-Gpr88 mice show impaired motor coordination, whereas D1R-Gpr88 mice show lack of motor skill learning

CMV-Gpr88 mice have been previously shown to present initial motor coordination deficits coupled with abolished motor skill learning throughout the rotarod tasks (Quintana et al., 2012; Meirism et al., 2016b). We therefore compared motor coordination and motor skill learning performances of CMV-, D1R-and A2A-R-Gpr88 mice by testing them in a rotating rod for six consecutive daily sessions. As depicted in Figure 6A, left panel, two-way RM ANOVA confirmed an impaired motor coordination for CMV-Gpr88 mice (genotype: F_{1,40} = 17.73, p < 0.0001; day: F_{23,920} = 13.49, p < 0.0001; n = 21/genotype) as well as a significant genotype × time effect (F_{23,920} = 3.16; p < 0.0001) confirming the lack of motor skill learn-
ing as previously published (Quintana et al., 2012; Meirsmann et al., 2016b). In fact, CMV-Gpr88 mice show decreased motor coordination in day 1 and maintained poor performance until the end of the task (day 6; right panel; two-way ANOVA; genotype: $F_{(1,80)} = 32.62, p < 0.0001$, day: $F_{(1,80)} = 17.67, p < 0.0001$; interaction: $F_{(1,80)} = 4.52, p = 0.0367$). Post hoc analysis revealed significant differences between CMV-Gpr88 mice and control animals during day 1 ($p < 0.05$) and 6 ($p < 0.0001$). In addition, only control animals showed an improved motor performance between days 1 and 6 ($p < 0.0001$). Similarly, D1R-Gpr88 mice (Fig. 6B, left panel) presented significantly decreased motor learning performance (two-way RM ANOVA; genotype: $F_{(1,23)} = 8.76, p = 0.007$; day: $F_{(23,529)} = 10.09, p < 0.0001$; n = 12–13) and significant genotype $\times$ time effect ($F_{(23,529)} = 7.61; p < 0.0001$). Despite similar motor coordination than their control littermates on day 1, D1R-Gpr88 mice failed to learn the
Table 2. Summary of behavioral phenotypes observed in CMV-Gpr88, D1R-Gpr88, and A2AR-Gpr88 mice

| Phenotype                  | CMV-Gpr88 | D1R-Gpr88 | A2AR-Gpr88 |
|----------------------------|-----------|-----------|------------|
| Marble burying             | ↓         | ↔         | ↓          |
| Social interaction         | ↓         | ↓         | ↓          |
| Open field                 | ↓         | ↓         | ↑          |
| Motor coordination         | ↓         | ↑         | ↓          |
| Motor skill learning       | ↓         | ↓         | ↓          |

Discussion

Results from the comparison of total versus conditional mouse lines are summarized in Table 2. In sum, data from marble burying and social interaction tests reveal a D2R cell-specific function of GPR88 in anxiety-related and social behavior (De Boer and Koolhaas, 2003), as modifications are detected in CMV-Gpr88 and A2AR-Gpr88 KO, but not D1R-Gpr88 KO, mice. With regards to open field results, we observe differential roles of GPR88 in D1R- and D2R-MSNs, suggesting that GPR88 in D1R-MSNs has no role on general locomotion or stereotypes but regulates locomotor habituation to a novel environment, whereas deletion of this receptor in D2R-MSNs increases spontaneous locomotion and stereotypes while preserving locomotor habituation. In the rotarod also, we show differential roles of GPR88 in D1R- and D2R-MSNs, indicating that GPR88 in D1R-MSNs contributes to motor skill learning, whereas the receptor in D2R-MSNs contributes to motor coordination but not learning in the task. Overall therefore, our study demonstrates that GPR88 modulates the function of both D1R- and D2R-MSNs and that GPR88 activity in these two neuron populations has very different and dissociable impacts on behavior.

We then show a differential effect of Gpr88 gene KO in D1R- or D2R-MSNs on general locomotion, with hyperlocomotor activity observed after D2R-Gpr88 deletion only. Converging data show that disruption of D2R-MSNs activity results in hyperlocomotor behavior (Durieux et al., 2012; Révy et al., 2014) while ablation of D1R-MSNs decreases locomotion (Durieux et al., 2012; Révy et al., 2014). Therefore, the increased locomotion observed in CMV-Gpr88 and A2AR-Gpr88 mice could simply result from decreased D2R-MSNs driven inhibition of locomotor output. Although deletion of Gpr88 in D1R-neurons did not alter overall locomotion throughout the five sessions, D1R-Gpr88 mice displayed decreased acute locomotor activity during the first open field session which would suggests impaired D1R-MSNs activity. Overall, locomotion results suggest that lack A2AR-Gpr88 mimics D2R-MSNs ablation (Durieux et al., 2009, 2012; Bateup et al., 2010; Révy et al., 2014). The question of how Gpr88 cell-specific deletion affects MSNs firing activity and...
basal ganglia output remains open, and future electrophysiological studies should measure basal ganglia output in D2R-Gpr88 and D1R-Gpr88 mice.

Another interesting locomotor phenotype in the open field is the lack of intersession habituation to the environment selectively observed in D1R-Gpr88 mice. Open field habituation is described as an adaptive process in which rodents decrease their locomotion with increasing exposure to the same environment and is taken as an index of memory (Tomaz et al., 1990; Cerbone and Sadile, 1994). A previous study showed that total deletion of Gpr88 improved spatial learning and memory tasks performances, thus suggesting that the non-habituating phenotype is not linked to spatial memory functions (Meirsman et al., 2016b). Surprisingly, our results contrast with the lack of open field habituation previously observed after ablation of D2R-MSNs (but not D1R-MSNs; Durieux et al., 2012). Therefore, in opposite to locomotion results, deletion of GPR88 in D1R-MSNs matches results observed after D2R-MSNs ablation, suggesting either MSNs cross talk or alteration of a common network shaping locomotor habituation. In fact, data show (Sanguedo et al., 2016) that locomotor habituation to novel environments is accompanied by activation of striatal and extra-striatal regions such as amygdala and frontal cortex. Accordingly, CMV-Gpr88 mice have been shown to have altered transcriptional profiles in these structures where both GPR88 and D1R are expressed (Meirsman et al., 2016b). Most importantly, recent studies using CMV-Gpr88 mice have shown impaired multisensory processing (Ehrlich et al., 2018) and sensorimotor gating (Meirsman et al., 2017) that, coupled with altered sensorimotor and cortico-striatal functional connectivity (Arefin et al., 2017), suggest a role of this receptor in the integration and processing of sensory information. Interestingly, it has also been suggested that modifications of the striato-cortical circuitry may underlie the hyperactivity observed in CMV-Gpr88 mice (Arefin et al., 2017). As such, future studies measuring functional connectivity in D2R-Gpr88 and D1R-Gpr88 mice will elucidate how cell-specific deletion of Gpr88 reshapes brain connectome leading to persistent changes in behavior.

Finally, the open field observations also reveal that A2aR-Gpr88 but not D1R-Gpr88 mice present increased number of stereotropies in the open field. Animal and clinical data indicate that dysregulation of cortico-striato-thalamo-cortical circuitry are associated with stereotropies (Lewis and Kim, 2009). Further, one study linked decreased D2R-MSNs activity with enhances stereotropies (Tanimuura et al., 2010, 2011) and a recent report indicates that increasing D2R-MSNs activity is sufficient to rescue repetitive behaviors observed in a genetic model of autism (Wang et al., 2017). The increased stereotropies of A2aR-Gpr88 mice may therefore result from diminished D2R-MSNs inhibitory projection. As for locomotion result, the electrophysiological impact of Gpr88 specific deletion should be assessed in future studies. On the other hand, stereotropies have been linked to dopaminergic overstimulation (Katherine, 2018), which could also cause the phenotype observed in A2aR-Gpr88 mice. In fact, we have previously reported altered DA levels in the CPu and midbrain nuclei of CMV-Gpr88 mice, and future studies should verify DA levels in conditional Gpr88 KO mice.

As for the open field experiments, rotarod testing also reveals differential D1R- versus D2R-MSNs activities of GPR88. Mutants lacking Gpr88 in D1R-neurons present similar initial rotarod performance than control animals but show absence of motor skill learning throughout 6 d of task. On the contrary, mice lacking Gpr88 in D2R-neurons show decreased latency to fall in the first day but learned the task and increased their motor performances across days. Interestingly, as for the locomotor phenotype, results are comparable to those obtained after inducible ablation of D1R-MSNs and D2R-MSNs (Durieux et al., 2012). Worth noting, previous reports indicate that Gpr88 deletion does not alter striatal cell population or cytoarchitectural organization (Logue et al., 2009; Quintana et al., 2012) but increased levels of striatal pDARPP-32 Thr-34 and the ratio of pDARPP-32 Thr-34/DARPP-32 suggesting compromised MSNs functioning (Logue et al., 2009). Also, mRNA levels of genes encoding neurotransmitter receptors as well as GPCRs activation were found altered in the striatum of CMV-Gpr88 mice (Quintana et al., 2012; Meirsman et al., 2016b). In particular, Gpr88 deletion increased mu opioid and delta opioid receptors activation in the striatum. These receptors are known to activate Gi/o pathways, and could therefore contribute to increase MSNs hyperpolarization in Gpr88 mutant mice (Le Merrer et al., 2013; Pellissier et al., 2018). Interestingly, a previous study deletion of GluR2 in D2R-MSNs regulates levels of anxiety, social behavior, stereotypes, locomotion, and motor coordination, this receptor in D1R-MSNs does not seem to impact affective behaviors but regulates habituation to novelty and motor skill learning. It is important to note that in the present study deletion of Gpr88 is not exclusively striatal. Thus, a new approach to restrict D1R-Gpr88 deletion to the striatum will determine if extra-striatal structures are involved in the phenotypes observed in mutants lacking Gpr88 in D1R-neurons. In addition, cellular mechanisms underlying phenotypes observed in this study remain to be clarified. Interestingly, behavioral analyses show that both the total ablation of D2R MSNs (Durieux et al., 2012) and the deletion of Gpr88 in D2R-neurons (our study) reduce motor coordination and induces hyperlocomotion, suggesting that GPR88 activity normally stimulates D2R-MSNs. This is counterintuitive, as GPR88 has been proposed to be an inhibitory GPCR (Jin et al., 2018). Also, Quintana et al. (2012) have previously shown that total Gpr88 ablation reduced tonic GABA current and enhanced glutamatergic signaling in MSNs. They also showed that deletion of Gpr88 similarly affect the re-
response to cortical excitatory input or the tonic GABA currents in D1R or D2R MSNs. We may, however, consider a strong differential effect of selective versus total deletion of GPR88 on MSNs intrinsic electrical properties. Therefore, electrophysiological studies using cell-specific Gpr88 deletion and also the precise anatomic localization of the receptor at presynaptic or postsynaptic levels should help clarifying how GPR88 modulates D1R- and D2R-MSNs activities. In addition, deficient long distance communication between brain structures observed in CMV-Gpr88 mice (Arefin et al., 2017) may explain some of the present results and upcoming studies should compare respective functional connectivity alterations in the two conditional Gpr88 KO mouse lines. Hence, further dissection of D1R versus D2R specific GPR88 activities is essential to explore the full potential of this receptor as a target for affective and motor disorders.

References

Alkufri F, Shaag A, Abu-Libdeh B, Elpeleg O (2016) Deleterious mutation in GPR88 is associated with chorea, speech delay, and learning disabilities. Neuront Genet 2:264.

Arefin TM, Mechling AE, Waisman AC, Benet T, Hübner NS, Lee HL, Ben Hamida S, Ehrlich A, Roquet D, Hennig E, John von Elverfeldt D, Kieffer BL, Harasan LA (2017) Remodeling of sensorimotor brain connectivity in Gpr88-deficient mice. Brain Connect 7:526–540.

Bateup HS, Santini E, Shen W, Birnbaum S, Valjent E, Surmeier DJ, Fisone G, Nestler EJ, Greengard P (2010) Distinct subcellulars of medium spiny neurons differentially regulate striatal motor behaviors. Proc Natl Acad Sci USA 107:14845–14850.

Borsini F, Podhorza J, Marazziti D (2002) Do animal models of anxiety predict anxiolytic-like effects of antidepressants? Psychopharmacology (Berl) 163:121–141.

Cerbone A, Sadile AG (1994) Behavioral habituation to spatial novelty: interference and noninterference studies. Neurosci Biobehav Rev 18:497–518.

De Boer SF, Koolhaas JM (2003) Defensive burying in rodents: ethology, neurobiology and psychopharmacology. Eur J Pharmacol 463:145–161.

Durieux PF, Bearzatto B, Guiducci S, Buch T, Waisman A, Zoli M, Schifflmann SN, de Kerchove d’Exaerde A (2009) D2R striatopallidal neurons inhibit both locomotor and drug reward processes. Nat Neurosci 12:393–395.

Durieux PF, Schifflmann SN, de Kerchove d’Exaerde A (2012) Differential regulation of motor control and response to dopaminergic drugs by D1R and D2R neurons in distinct dorsal striatum subregions. EMBO J 31:640–653.

Ehrlich AT, Semache M, Bailly J, Wojcik S, Arefin TM, Colley C, Le Gouill C, Gross F, Lukasheva V, Hogue M, Darceq E, Harasan LA, Bouvier M, Kieffer BL (2018) Mapping GPR88-Venus illuminates a novel role for GPR88 in sensory processing. Brain Struct Funct 223:1275–1296.

Francis TC, Lobo MK (2017) Emerging role for nucleus accumbens medium spiny neuron subtypes in depression. Biol Psychiatry 81:645–653.

Francis TC, Chandra R, Friend DM, Finkel E, Dayrit G, Miranda J, Brooks JM, Ilguzexe SD, O’Donnell P, Kravitz A, Lobo MK (2015) Nucleus accumbens medium spiny neuron subtypes mediate depression-related outcomes to social defeat stress. Biol Psychiatry 77:212–222.

Gunaydin LA, Grosenick L, Finkelstein JC, Kaufiv AR, Fennno LE, Adhikari A, Lammel S, Mitzabekov JJ, Airan RD, Zoladzuska KA, Tye KM, Anikeeva P, Malenka RC, Deisseroth K (2014) Natural neural projection dynamics underlying social behavior. Cell 157: 1535–1551.

Hikida T, Yawata S, Yamaguchi T, Danjo T, Sasaoka T, Wang Y, Nakashido S (2013) Pathway-specific modulation of nucleus accumbens in reward and aversive behavior via selective transmitter receptors. Proc Natl Acad Sci USA 110:342–347.

Jin C, Decker AM, Makhijani VH, Besheer J, Darceq E, Kieffer BL, Mastra R (2018) Discovery of a potent, selective, and brain-penetrant small molecule that activates the orphan receptor GPR88 and reduces alcohol intake. J Med Chem 61:6748–6758.

Katherine M (2018) Stereotypic movement disorders. Semin Pediatr Neurol 25:19–24.

Kratzv AV, Freeze BS, Parker PR, Kay K, Trwin MT, Deisseroth K, Kreitzer AC (2010) Regulation of parkinsonian motor behaviors by optogenetic control of basal ganglia circuitry. Nature 466:622–626.

Kratzv AV, Tye LD, Kreitzer AC (2012) Distinct roles for direct and indirect pathway striatal neurons in reinforcement. Nat Neurosci 15:816–818.

Le Merer J, Rezae X, Scherrer G, Becker JA, Kieffer BL (2013) Impaired hippocampus-dependent and facilitated striatum-dependent behaviors in mice lacking the delta opioid receptor. Neuropsychopharmacology 38:1050–1059.

Lewis M, Kim SJ (2009) The pathophysiology of restricted repetitive behavior. J Neurol Dev Disord 1:114–132.

Liljeholm M, O’Doherty JP (2012) Contributions of the striatum to learning, motivation, and performance: an associative account. Trends Cogn Sci 16:477–475.

Logue SF, Grauer SM, Paulsen J, Graf R, Taylor N, Sung MA, Zhang L, Hughes Z, Pulito VL, Liu F, Rosenzweig-Lipson S, Brandon NJ, Marcelius KL, Bates B, Pausch M (2009) The orphan GPCR, GPR88, modulates function of the striatal dopamine system: a possible therapeutic target for psychiatric disorders? Mol Cell Neurosci 42:438–447.

Maroteaux G, Arefin TM, Harasan LA, Darceq E, Ben Hamida S, Kieffer BL (2018) Lack of anticipatory behavior in Gpr88 knockout mice showed by automated home cage phenotyping. Genes Brain Behav 17:e12473.

Meirsman AC, Robe A, de Kerchove d’Exaerde A, Kieffer BL (2016a) GPR88 in A2AR neurons enhances anxiety-like behaviors. eNeuro 3.

Meirsman AC, Le Merer J, Pellissier LP, Diaz J, Clesse D, Kieffer BL, Becker JA (2016b) Mice lacking GPR88 show motor deficit, improved spatial learning, and low anxiety reversed by delta opioid antagonist. Biol Psychiatry 79:917–927.

Meirsman AC, de Kerchove d’Exaerde A, Kieffer BL, Ouagazzal AM (2017) GPR88 in A2a receptor-expressing neurons modulates locomotor response to dopamine agonists but not sensorimotor gating. Eur J Neurosci 46:2026–2034.

Pellissier LP, Pujol CN, Becker JAJ, Le Merer J (2018) Delta opioid receptors: learning and motivation. Handb Exp Pharmacol 247: 227–260.

Pradhan AA, Becker JA, Scherrer G, Tryoen-toth P, Filliod D, Mattias A, Massotte D, Gavériaux-Ruff C, Kieffer BL (2009) In vivo delta opioid receptor internalization controls behavioral effects of agonists. PLoS One 4:e45425.

Quintana A, Sanz E, Wang W, Storey GP, Güler AD, Wanat MJ, Roller BA, La Torre A, Amieux PS, McGinty KS, Barnford NS, Palmer RD (2012) Lack of GPR88 enhances medium spiny neuron activity and alters motor- and cue-dependent behaviors. Nat Neurosci 15:1547–1555.

Révy D, Jaouen F, Salin P, Melon C, Chabbert D, Tafi E, Concetta L, Révy D, Jaouen F, Salin P, Melon C, Chabbert D, Tafi E, Concetta L, Pelissier LP, Gavériaux-Ruff C, Kieffer BL (2009) In vivo delta opioid receptor internalization controls behavioral effects of agonists. PLoS One 4:e45425.

Sanguedo VF, Dias CV, Dias FR, Samuels RI, Carey RJ, Carrera MP (2016) Reciprocal activation/inactivation of ERK in the amygdala and frontal cortex is correlated with the degree of novelty of an open-field environment. Psychopharmacology (Berl) 233:841–850.

Tanimura Y, Vaziri S, Lewis MH (2010) Indirect basal ganglia pathway mediation of repetitive behavior: attenuation by adenosine receptor agonists. Behav Brain Res 210:116–122.

Tanimura Y, King MA, Williams DK, Lewis MH (2011) Development of repetitive behavior in a mouse model: roles of indirect and
Striosomal basal ganglia pathways. Int J Dev Neurosci 29:461–467.

Tomaz C, Aguiar MS, Nogueira PJ (1990) Facilitation of memory by peripheral administration of substance P and naloxone using avoidance and habituation learning tasks. Neurosci Biobehav Rev 14:447–453.

Wang W, Li C, Chen Q, van der Goes MS, Hawrot J, Yao AY, Gao X, Lu C, Zang Y, Zhang Q, Lyman K, Wang D, Guo B, Wu S, Gerfen CR, Fu Z, Feng G (2017) Striatopallidal dysfunction underlies repetitive behavior in Shank3-deficient model of autism. J Clin Invest 127:1978–1990.