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

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GPR88 in D1R- and D2R-type medium spiny neurons differentially regulates affective and motor behaviors

Keywords: orphan GPCR, Medium-spiny neurons; striatum, dopamine receptors, motor coordination, locomotion, anxiety

Abstract

The orphan receptor GPR88 is highly expressed in D1R- and D2R-medium spiny neurons (MSNs) and has been associated to striatum-dependent functions in rodents. The total deletion of *Gpr88* 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 *Gpr88* gene knockout (KO) in D1R-MSNs (D1R-*Gpr88* mice) or D2R-MSNs (A_{2A}R-*Gpr88* mice) with effects of the total *Gpr88* KO (CMV-*Gpr88* mice). Overall, most phenotypes of CMV-*Gpr88* mice were recapitulated in A_{2A}R-*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 A_{2A}R-*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.

Significance statement

GPR88, an orphan G-protein-coupled receptor, has been implicated in the regulation of striatum-dependent behaviors. In the striatum, GPR88 is most abundant in both medium spiny neurons expressing dopamine D1 and D2 receptors. We compared effects of a conditional *Gpr88* gene knockout in 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- and D2R-type neurons function and the related behaviors.

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 knockout animals, thus providing a direct link between GPR88 loss in the dorsal striatum and the locomotor phenotype of knockout mice (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-D1R and substance P, D1R-MSNs) and indirect (co-expressing dopamine D2 receptors-D2R and adenosine A_{2A} receptor-A_{2A}R, 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; Bateup et al., 2010; Durieux et al., 2012). 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 knockout (KO) mouse line for GPR88 in D2R-MSNs was developed in a previous study, using a A_{2A}R-Cre driver line (A_{2A}R-*Gpr88* mice), and mutant mice showed hyperactive behavior, decreased anxiety-like behaviors and increased locomotor response to dopaminergic agonists (Meirsman et al., 2016a; Meirsman et al., 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 A_{2A}R-*Gpr88* mice and total knockout (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 3-5 animals *per* cage. Animals where maintained on a 12hr 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 A_{2A}R-*Gpr88* mice were produced as previously described (Meirsman et al., 2016b; Meirsman et al., 2016a). 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 (A_{2A}R-*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 (Meirsman et al., 2016b). First generation animals expressing the Cre under the control of A_{2A}R or D1R promotor (*Gpr88* $^{A2AR\text{-}Cre/+}$ and *Gpr88* $^{D1R\text{-}Cre/+}$) 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 (A_{2A}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 (Meirsman et al., 2016a). Mice (n= 3 D1R-CTL; 3 D1R-*Gpr88*) were sacrificed by cervical dislocation and fresh brains were extracted and embedded in OCT (Optimal Cutting Temperature medium, Thermo scientific, Waltham, MA, USA) frozen and kept at -80°C. Frozen brains were coronally sliced into 20 µm serial sections by using cryostat (CM3050 Leica, Wetzar,

Germany), placed in superfrost slides (Thermo scientific, Waltham, MA, USA) 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, Japan). Regions of interest were selected using Olyvia software (Olympus) and saved as PNG files. Three brain regions where analyzed: Rostral Caudate-Putamen (CPu; from 1.42mm to 0.98mm from Bregma); Caudal CPu (from 0.98mm to -0.58mm from Bregma) and Nucleus Accumbens (from 1.42mm to 1.10mm from Bregma).

For the CPu (rostral and caudal), at least 4 regions of interest (ROI) 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 (Image J) 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 GPR88 expressing cells co-expressing D1R or D2R x 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 lateral and medial CPu, relative percentage of each was pooled for graphical representation and statistical analysis.

[S35]-GTPγS binding assay. [S35]-GTPγS assays were performed on membrane preparations as described in previous report (Pradhan et al., 2009). To perform [S35]-GTPγS assays on

whole striatum mice were sacrificed 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 3 animals (males and females). Results are expressed by meaning 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, 0.2 mM EGTA, following which they were centrifuged at 23 000 g for 30 min. The pellets were homogenized in 800µL ice-cold sucrose solution (0.32 M) and kept at - 80°C. For each [35S]GTPγS binding assay 2µg of protein per well was used. Samples were incubated with and without ligands, for 1 hour at 25°C in assay buffer containing 30 mM GDP and 0.1 nM [35S]GTPγS. Bound radioactivity was quantified using a liquid scintillation counter. Bmax and Kd values were calculated. Non-specific binding was defined as binding in the presence of 10 µM GTPγS and binding in the absence of agonist was defined as the basal binding.

Gene expression analysis Mice were sacrificed by cervical dislocation. Brains structures (nucleus accumbens n=8 D1R-CTL; 7 D1R-*Gpr88* and caudate putamen n=9 D1R-CTL; 9 D1R-*Gpr88,* hippocampus: n=9 D1R-CTL; 7 D1R-*Gpr88* and amygdala: n=6 D1R-CTL; 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, Cergy Pontoise, France) following the manufacturer's instructions. cDNA was synthetized 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 sec at 95°C, 15 sec at 60°C and 30 sec at 72°C. Relative expression ratios were normalized to the level of actin and the 2−ΔΔCt method was applied to assess differential expression level of GPR88.

Behavioral experiments. For all behavioral measures mice from different mouse lines and genotypes were tested in a random order and data were analyzed blind to genotype. However, to avoid order-related variability between mouse lines and genotypes, mouse lines were stratified so that each session included both genotypes from all lines. One mouse cohort (N=8 CMV-CTL, 10 CMV-*Gpr88;* N=10 A_{2A}R-*Gpr88*, 10 A_{2A}R-CTL; N=10 D1R-*Gpr88*, 14 D1R-CTL) was used to measure marble burying and social interaction. Independent cohorts of mice (N=21 CMV-CTL, 21 CMV-*Gpr88;* N=10 A_{2A}R-*Gpr88*, 17 A_{2A}R-CTL; N=13 D1R-*Gpr88*, 12 D1R-CTL) underwent five days of open field locomotion followed by a 48h resting period before seven days of rotarod motor skill learning.

Marble burying: Defensive burying was measured as previously described (Meirsman et al., 2016b) using the marble burying test carried out with 20 small glass marbles (15 mm) evenly spaced in a transparent single cage (21 X 11 X 17 cm) over 4cm sawdust bedding. The cage was covered by a plastic lid in a room illuminated at 40 Lux. Mice were left in the cage for 10 minutes and the number of marbles buried more than half in sawdust was counted.

Social interaction test: Social interaction was assessed, as previously described (Meirsman et al., 2016a), in an open field (50 × 50 cm) dimly lit (<10 Lux) using naïve wild type mice of the same age and weight as interactors. On the first day, all mice were individually placed in the open field arena and left for a 10 min period of habituation. The next day mice were placed in the open field arena with a wild type naive interactor and a 10 min session was recorder. Nose

contacts were measured manually using video recordings. If an interactor failed to engage in any interaction data from the respective mice were exclude from analysis.

Open field locomotion: To assess basal locomotion and habituation to novel environment mice were placed in a dimly lit open field (Accuscan Instruments, USA) for 30 minutes daily session. The experiment lasted 5 days and mice were placed in the same open field for all sessions tested. Open field where cleaned with water and 70% ethanol between trials. Total distance traveled, stereotypy counts and durations were automatically recorded.

Rotarod: The first day, mice were placed on the rod (30 mm plastic roller, Panlab Harvard) at constant speed of 4 r.p.m. until achieving 90sec without falling from the rod (habituation, data not shown). On the six next consecutive days mice were placed on the rod accelerating from 4 to 40 rpm in 5 min and remaining at maximum speed for the next 5 min for four trials every day. Light intensity in the room was inferior to 10 Lux. Mice rested a minimum of 1min between trials to avoid fatigue and exhaustion. When mice hang on the rod instead of running they were left for one complete turn but the timer was stopped if the mice engaged in a second consecutive turn. Animals were scored for their latency to fall (in seconds) in each trial. Mean values of four daily trials were used for statistical 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 two-way ANOVA was used to analyze global open field and rotarod results with genotypes as the between-subject factor and time as the repeated measures. One way ANOVA was used for open field habituation analysis (Day 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. Stereotypies, marble burying and social interaction contacts were

297 analyzed using t test (unpaired with Welch's correction). All statistical analysis were realized
298 using GraphPad Prism 7 (GraphPad Software, Inc, USA) and the accepted level of significance
299 was $p<0.05$. All the statistical methods are summarized in Table 1.
300
301 ## Results
302
303 ### *D1R-Gpr88* mice show *Gpr88* mRNA deletion in D1R-expressing neurons
304 To conditionally delete *Gpr88* exon 2 in cells expressing D1R, mice carrying two LoxP sites
305 flanking the second exon of the *Gpr88* gene (Meirsman et al., 2016b) were crossed with mice
306 expressing the Cre recombinase under the control of the *Drd1a* gene promoter (Gensat). We
307 first tested whether GPR88 transcript and protein are reduced in the striatum. We quantified
308 *Gpr88* mRNA levels by RT-qPCR for CPu and Nucleus Accumbens (Nacc) from D1R-*Gpr88* and
309 their control littermates. As shown in Figure 1A, *Gpr88* expression was significantly decreased in
310 striatal regions of conditional KO compared to controls (CPu: $t_{(16)} = 3.01$, $p = 0.008$; Nacc: $t_{(13)} =$
311 4.19, $p = 0.001$, Table 1). Testing *Gpr88* mRNA levels in the hippocampus and amygdala
312 showed a milder but significant reduction in hippocampus but not amygdala (Hipp: $t_{(14)} = 2.7$, $p =$
313 0.017; Amy $t_{(11)} = 0.53$, $p = 0.6$), indicating that GPR88 KO may also have occurred in some
314 extrastriatal regions containing D1R-type neurons (see discussion). There was no significant
315 difference in D1R expression levels across genotypes (not shown). To establish whether
316 reduced mRNA level translates into lower protein level, we tested GPR88 signalling in the
317 striatum. To this aim, we performed GPR88 agonist-induced [S35]-GTPγS binding assays
318 (Figure 1B) with membranes prepared from whole striatum (CPu and Nacc) of D1R-*Gpr88* mice
319 and their controls, as well as with total knockout CMV-*Gpr88* mice (negative control) and their
320 wild type control mice (positive control). Two-way Repeated-Measures (RM) ANOVA revealed a
321 significant genotype effect ($F_{(3, 66)} = 185.2$, $p<0.0001$) and interaction effect ($F_{(30, 66)} = 23.19$,
322 $p<0.0001$). Post hoc analysis (Tukey 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 co-localize with both *Drd1a* (left panel), and *Drd2* mRNA-expressing cells (right panel). In D1R-*Gpr88* mice however cells expressing *Gpr88* do not co-localize with *Drd1*-expressing cells (left panel), but still co-localize with *Drd2*- (right panel). Quantitative analysis (Figure 2B) in the CPu and Nacc confirmed that, in control animals, *Gpr88* mRNA is found in both *Drd1a*- (CPu: 43.96±1.54% and Nacc: 45.13±3.57%) and *Drd2*- (CPu: 62.11±1.95% and Nacc: 59.03±4.47%) positive 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: $F_{(1, 134)}$ = 0.42; p=0.52; Cell type: $F_{(1, 134)}$ = 957.2; p<0.0001; Interaction: $F_{(1, 134)}$ = 387.8, p<0.0001; Nacc; Genotype: $F_{(1, 36)}$ = 0,26, p=0,6134; Cell type: $F_{(1, 36)}$ = 204,3, p<0,0001; Interaction : $F_{(1, 36)}$ = 97.83, p<0.0001; n=3/genotype), indicating that the *Gpr88* deletion had occurred mostly in D1R-type MSNs.

A_{2A}R-*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* ($t_{(17)}$ =

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 ($t_{(22)}$ = 1.002, p=0.33; n=10-14) whereas A_{2A}R-*Gpr88* mice, like CMV-*Gpr88*, showed reduced number of buried marbles ($t_{(18)}$ = 4.01, p<0.001; n=10/genotype). Also, in the presence of a naïve, wild type, congener (Figure 3 D-F), CMV-*Gpr88* ($t_{(14)}$ =2.88, p=0.012; n=8/genotype) and A_{2A}R-*Gpr88* mice ($t_{(18)}$ = 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 ($t_{(20)}$ = 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_{2A}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, locomotor behavior of CMV-, A_{2A}R-and D1R-*Gpr88* mice 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 (Figure 4A, left panel) (Two-way RM ANOVA; Genotype: $F_{(1, 40)}$ = 5.98, p=0.0189; Day: $F_{(4, 180)}$ = 4.42; p=0.002; Interaction: $F_{(4, 180)}$ = 7.19; p<0.0001; n=21/genotype). Further, while control animals decreased their general

locomotion between the first and last session (see right panel) CMV-*Gpr88* mice showed rather increased locomotion in the last compared to the first session (Two-way ANOVA; Genotype: $F_{(1, 80)} = 4.93$, p=0.029; Day: $F_{(1, 80)} = 1.25$, p=0.27; Interaction: $F_{(1, 80)} = 8.94$, p=0.0037). D1R-*Gpr88* mice (Figure 4B, left panel) presented similar levels of general locomotor activity when compared to their littermates (Two-way RM ANOVA; Genotype: $F_{(1, 23)} = 1.11$, p=0.30; Day: $F_{(4, 92)} = 31.03$; p<0.0001; Interaction: $F_{(4, 92)} = 11.82$; p<0.0001; n=12-13) but, similar to CMV-*Gpr88* mice, showed lack of locomotor habituation to the open field environment (right panel; Two-way ANOVA; Genotype: $F_{(1, 46)} = 0.78$, p=0.38; Day: $F_{(1, 46)} = 26.75$, p<0.0001; Interaction: $F_{(1, 46)} = 11.01$, p=0.0018). Similar to CMV-*Gpr88*, A_{2A}R-*Gpr88* mice (Figure 4C, left panel) significantly increased their locomotion when compared to control littermates (Two-way RM ANOVA; Genotype: $F_{(1, 25)} = 8.0$, p=0.009; Day: $F_{(4, 100)} = 43.28$; *p*<0.0001; Interaction: $F_{(4, 100)} = 3.94$; *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: $F_{(1, 50)} = 8.17$, *p*=0.006; Day: $F_{(1, 50)} = 18.71$, *p*<0.0001; Interaction: $F_{(1, 50)} = 0.15$, *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.

A_{2A}R-*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 behaviour (Maroteaux et al., 2018) in CMV-*Gpr88* mice. In order to examine whether this phenotype results from *Gpr88* deletion in D1R- and/or D2R-MSNs, we analysed stereotypies scores in the first open field

session (30min). Results indicate that both CMV-*Gpr88* (Figure 5A) and A_{2A}R-*Gpr88* mice (Figure 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 (Figure 5B) presented no altered stereotyped behavior when compared to control animals.

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

A_{2A}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; Meirsman et al., 2016b). We therefore compared motor coordination and motor skill learning performances of CMV-, D1R-and A_{2A}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 x time effect ($F_{(23, 920)}$ = 3.16; p<0.0001) confirming the lack of motor skill learning as previously published (Quintana et al., 2012; Meirsman 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 day 1 and 6 (p<0.0001). Similarly, D1R-*Gpr88* mice (Figure 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 x 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 task presenting decreased time on the rod on day 6 compared to their littermates (right panel; Two-way ANOVA; Genotype: $F_{(1, 46)}$ = 13.62, p=0.0006; Day: $F_{(1. 46)}$ = 11.15, p=0.0017; Interaction: $F_{(1. 46)}$ = 7.64, p=0.008). Post-hoc analysis revealed significant differences between D1R-*Gpr88* mice and control animals only during day 6 (p<0.0001). Moreover, only control animals showed an improved motor performance between day 1 and 6 (p<0.001). A_{2A}R-*Gpr88* mice (Figure 6C, left panel) on the other hand also presented significantly decreased global performance (Two-way RM ANOVA; Genotype: $F_{(1, 25)}$ = 8.01, p=0.0091; Day: $F_{(23, 575)}$ = 13.74; p<0.0001; Interaction: $F_{(23, 575)}$ =1.02; p=0.44; n=10-17) but show motor learning skills over days of experiment. In fact, when comparing day 1 and day 6 (right panel), two-way ANOVA showed significant Genotype ($F_{(1, 50)}$ = 8.07, p=0.0065) and Day ($F_{(1. 50)}$ = 16.31, p=0.0002) effect but not Interaction: $F_{(1, 50)}$ = 1.29, p=0.2598). Subsequent analyses using the method of contrasts showed that both genotypes improved their performance in the accelerating rotarod between day 1 and day 6 (A_{2A}R-CTL: p=0.001; A2AR-*Gpr88* p=0.018).

These data first confirm that lack of GPR88 abolishes both motor coordination and motor skill learning and further shows that the deletion of A_{2A}R-*Gpr88* alters initial motor coordination while preserving motor skill learning, whereas D1R-*Gpr88* deletion selectively impairs 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 A_{2A}R-*Gpr88* KO, but not D1R-*Gpr88* KO mice. With regards to

453 open field results, we observe differential roles of GPR88 in D1R- and D2R-MSNs, suggesting
454 that GPR88 in D1R-MSNs has no role on general locomotion or stereotypies but regulates
455 locomotor habituation to a novel environment, whereas deletion of this receptor in D2R-MSNs
456 increases spontaneous locomotion and stereotypies while preserving locomotor habituation. In
457 the rotarod also, we show differential roles of GPR88 in D1R- and D2R-MSNs, indicating that
458 GPR88 in D1R-MSNs contributes to motor skill learning, whereas the receptor in D2R-MSNs
459 contributes to motor coordination but not learning in the task. Overall therefore, our study
460 demonstrates that GPR88 modulates the function of both D1R- and D2R-MSNs, and that
461 GPR88 activity in these two neuron populations has very different and dissociable impacts on
462 behavior.

463 We find that specific deletion of *Gpr88* in D2R-MSNs, but not in D1R-MSNs, decreases
464 anxiety-like behavior as shown by reduced defensive burying activity (Borsini et al., 2002; De
465 Boer and Koolhaas, 2003; Meirsman et al., 2016a). This result is in line with data showing that
466 blocking D2R-MSNs activity disrupts avoidance behavior and aversive learning (Hikida et al.,
467 2013). We also extend previous data (Meirsman et al., 2016a) by showing that *Gpr88* deletion in
468 D2R but not D1R-neurons increases social approach. Reports have shown that dopamine
469 signaling through D1Rs is necessary for mediating pro-social behavior (Gunaydin et al., 2014).
470 Also, it was shown that while D1R-MSNs display reduced mEPSC frequency after chronic social
471 defeat, optical stimulation of D1R-MSNs was sufficient to reverse social avoidance induced by
472 social defeat stress (Francis et al., 2015; Francis and Lobo, 2017). Therefore, although baseline
473 social approach is not affected by D1R-*Gpr88* deletion, different results may be obtained after
474 chronic social stress. Knowing that inducible ablation of D1R-MSNs also reduces anxiety
475 behaviors in mice (Revy et al., 2014), our result suggest that D1R-MSNs and D1R's-dependent
476 anxiety and social behaviors was not affected by D1R-*Gpr88* ablation. However, to fully
477 understand GPR88 function in affective and social behaviors future studies should compare

responses of D1R-*Gpr88* and A_{2A}R -*Gpr88* mice in reward and aversive learning paradigms and investigate the role of this orphan receptor in stress-induced social avoidance.

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; Revy et al., 2014) while ablation of D1R-MSNs decreases locomotion (Durieux et al., 2012; Revy et al., 2014). Therefore, the increased locomotion observed in CMV-*Gpr88* and A_{2A}R-*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 A_{2A}R-*Gpr88* mimics D2R-MSNs ablation (Durieux et al., 2009; Bateup et al., 2010; Durieux et al., 2012; Revy 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- and D1R-*Gpr88* mice.

Another interesting locomotor phenotype in the open field is the lack of inter-session 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 obtained 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- and D1R-*Gpr88* mice will elucidate how cell-specific deletion *of Gpr88* reshape brain connectome leading to persistent changes in behavior.

Finally, the open field observations also reveal that A_{2A}R-*Gpr88* but not D1R-*Gpr88* mice present increased number of stereotypies in the open field. Animal and clinical data indicate that dysregulation of cortico-striato-thalamo-cortical circuitry are associated with stereotypies (Lewis and Kim, 2009). Further, one study linked decreased D2R-MSNs activity with enhances stereotypies (Tanimura et al., 2010; Tanimura et al., 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 stereotypies of A_{2A}R-*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, stereotypies have been linked to dopaminergic overstimulation (Katherine, 2018), which could also cause the phenotype observed in A_{2A}R-*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-MSN 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 days 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 report showed that chronic administration of DOR antagonist (naltrindole) in CMV-*Gpr88* mice reversed their initial motor coordination impairment suggesting that increased DOR activity may underlie the deficit observed in A_{2A}R-*Gpr88.* Future studies pharmacologically tackling receptors known to interact with GPR88 will elucidate MSNs-specific GPR88 interactions with other receptors.

In conclusion, the present study demonstrates dissociable roles of GPR88 at the level of MSNs. While GPR88 in D2R-MSNs regulates levels of anxiety, social behavior, stereotypies, 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

555 to restrict D1R-Gpr88 deletion to the striatum will determine whether extra-striatal structures are
556 involved in the phenotypes observed in mutants lacking Gpr88 in D1R-neurons. In addition,
557 cellular mechanisms underlying phenotypes observed in this study remain to be clarified.
558 Interesting to note, behavioral analyses show that both the total ablation of D2R MSNs (Durieux
559 et al., 2012) and the deletion of *Gpr88* in D2R-neurons (our study) reduce motor coordination
560 and induces hyperlocomotion, suggesting that GPR88 activity normally stimulates D2R-MSNs.
561 This is counterintuitive, as GPR88 has been proposed to be an inhibitory G protein coupled
562 receptor (Jin et al., 2018). Also, Quintana and colleagues (2012) have previously shown that
563 total GPR88 ablation reduced tonic GABA current and enhanced glutamatergic signaling in
564 MSNs. They also showed that deletion of Gpr88 similarly affect the response to cortical
565 excitatory input or the tonic GABA currents in D1R or D2R MSNs. We may however consider a
566 strong differential effect of selective versus total deletion of GPR88 on MSNs intrinsic electrical
567 properties. Therefore electrophysiological studies using cell-specific *Gpr88* deletion and also the
568 precise anatomical localization of the receptor at pre- or postsynaptic levels should help
569 clarifying how GPR88 modulates D1R- and D2R MSN activities. In addition, deficient long
570 distance communication between brain structures observed in CMV-*Gpr88* mice (Arefin et al.,
571 2017) may explain some of the present results and upcoming studies should compare respective
572 functional connectivity alterations in the two conditional *Gpr88* KO mouse lines. Hence, further
573 dissection of D1R versus D2R specific GPR88 activities is essential to explore the full potential
574 of this receptor as a target for affective and motor disorders.
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577 **References**

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682 **Table 1: Detailed statistical analysis**

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				ANOVA			t-test
Assay	Mouse line	Number	Figure	Genotype effect	Cell type/Time/Treatment	Interaction	
RT-qPCR	D1R-Gpr88	N=9 D1R-CTL; 9 D1R-Gpr88	1A (Cpu)				t(16) = 3.01, p = 0.008
		N=8 D1R-CTL; 7 D1R-Gpr88	1A (Nacc)				t(13) = 4.19, p = 0.001
		N=9 D1R-CTL; 7 D1R-Gpr88	1A (Hipp)				t(14) = 2.7, p = 0.017
		N=6 D1R-CTL; 7 D1R-Gpr88	1A (Amy)				t(11) = 0.53, p = 0. 6
[35S]-GTPgS binding	CMV-Gpr88 ; D1R-Gpr88	N=3 D1R-CTL, D1R-Gpr88, CMV-CTL, CMV-Gpr88	1B	F (3. 66) = 185.2; P<0.0001	F (10. 66) = 95.64; P<0.0001	F (30. 66) = 23.19; P<0.0001	
In situ Hybridization / Cell counting	D1R-Gpr88	N=3 D1R-CTL, 3 D1R-Gpr88	2B (Cpu)	F (1. 134) = 0.4164; P=0.5198	F (1. 134) = 957.2; P<0.0001	F (1. 134) = 387.8; P<0.0001	
			2B (Nacc)	F (1. 36) = 0.2597; P=0.6134	F (1. 36) = 204.3; P<0.0001	F (1. 36) = 97.83; P<0.0001	
Marble burrying	CMV-Gpr88	N=9 CMV-CTL, 10 CMV-Gpr88	3A (left)				t (17) = 2.03, p=0.059
	D1R-Gpr88	N=14 D1R-CTL, 10 D1R-Gpr88	3B (left)				t (22) = 1.002, p=0.33
	A2AR-Gpr88	N=10 A2AR-CTL, 10 A2AR-Gpr88	3C (left)				t (18) = 4.01, p<0.001
Nose contact in social interaction	CMV-Gpr88	N=8 CMV-CTL, 8 CMV-Gpr88	3D (right)				t (14) =2.88, p=0.012
	D1R-Gpr88	N=14 D1R-CTL, 8 D1R-Gpr88	3E (right)				t (20) = 2.57, p=0.018
	A2AR-Gpr88	N=10 A2AR-CTL, 10 A2AR-Gpr88	3F (right)				t (18) = 2.06, p=0.01
Open Field (all sessions)	CMV-Gpr88	N=21 CMV-CTL, 21 CMV-Gpr88	4A (left)	F (1. 40) = 4.357; P=0.0425	F (4. 180) = 4.419; P=0.002	F (4. 180) = 7.189; P<0.0001	
	D1R-Gpr88	N=13 D1R-CTL, 12 D1R-Gpr88	4B (left)	F (1. 23) = 1.106; P=0.3038	F (4. 92) = 31.03; P<0.0001	F (4. 92) = 11.82; P<0.0001	
	A2AR-Gpr88	N=17 A2AR-CTL, 10 A2AR-Gpr88	4C (left)	F (1. 25) = 8.004; P=0.0091	F (4. 100) = 43.28; P<0.0001	F (4. 100) = 3.939; P=0.0052	
Open Field (sessions 1 & 5)	CMV-Gpr88	N=21 CMV-CTL, 21 CMV-Gpr88	4A (right)	F (1, 80) = 4.93; P=0.0292	F (1, 80) = 1.25; P=0.2679	F (1, 80) = 8.94; P=0.0037	
	D1R-Gpr88	N=13 D1R-CTL, 12 D1R-Gpr88	4B (right)	F (1, 46) = 0.78; P=0.3811	F (1, 46) = 26.75; P<0.0001	F (1, 46) = 11.01; P=0.0018	
	A2AR-Gpr88	N=17 A2AR-CTL, 10 A2AR-Gpr88	4C (right)	F (1, 50) = 8.17; P=0.0062	F (1, 50) = 18.71; P<0.0001	F (1, 50) = 0.1479; P=0.7021	
Stereotypies	CMV-Gpr88	N=21 CMV-CTL, 21 CMV-Gpr88	5A				Score: t (40) =2.228; p=0.0316
							Time: t (40) =2.818; p=0.0075
	D1R-Gpr88	N=13 D1R-CTL, 12 D1R-Gpr88	5B				Score: t (23) =1.156; p=0.2594
							Time: t (23) =0.7174; p=0.4803
	A2AR-Gpr88	N=17 A2AR-CTL, 10 A2AR-Gpr88	5C				Score: t (25) =2.291; p=0.0307
							Time: t (25) =2.317; p=0.0290
Rotarod (all sessions)	CMV-Gpr88	N=21 CMV-CTL, 21 CMV-Gpr88	6A (left)	F (1. 40) = 17.73; P=0.0001	F (23. 920) = 13.49; P<0.0001	F (23. 920) = 3.159; P<0.0001	
	D1R-Gpr88	N=13 D1R-CTL, 12 D1R-Gpr88	6B (left)	F (1. 23) = 8.759; P=0.0070	F (23. 529) = 10.09; P<0.0001	F (23. 529) = 7.607; P<0.0001	
	A2AR-Gpr88	N=17 A2AR-CTL, 10 A2AR-Gpr88	6C (left)	F (1. 25) = 8.008; P=0.0091	F (23. 575) = 13.74; P<0.0001	F (23. 575) = 1.017; P=0.4403	
Rotarod (sessions 1 and 6)	CMV-Gpr88	N=21 CMV-CTL, 21 CMV-Gpr88	6A (right)	F (1. 80) = 32.62; P<0.0001	F (1. 80) = 17.67; P<0.0001	F (1. 80) = 4.517; P=0.0367	
	D1R-Gpr88	N=13 D1R-CTL, 12 D1R-Gpr88	6B (right)	F (1. 46) = 13.62; P=0.0006	F (1. 46) = 11.15; P=0.0017	F (1. 46) = 7.643; P=0.0082	
	A2AR-Gpr88	N=17 A2AR-CTL, 10 A2AR-Gpr88	6C (right)	F (1. 50) = 8.067; P=0.0065	F (1. 50) = 16.31; P=0.0002	F (1. 50) = 1.299; P=0.2598	

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692 **Table 2: Summary of behavioral phenotypes observed in CMV-; D1R- and A2AR-*Gpr88***
693 **mice.**
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		CMV-Gpr88	D1R-Gpr88	A_{2A}R-Gpr88
Marble burying		↓	↔	↓
Social interaction		↑	↔	↑
Open Field	Locomotion	↑	↔	↑
	Habituation	↓	↓	↔
	Stereotypies	↑	↔	↑
Rotarod	Motor coordination	↓	↔	↓
	Motor skill learning	↓	↓	↔

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698 **Figure legends**

699 **Figure 1. GPR88 agonist-induced activation and mRNA levels in D1R-*Gpr88* mice**

700 We measured levels of *Gpr88* mRNA in D1R-CTL and D1R-*Gpr88* mice (A) and show a
701 significant reduction of GPR88 expression in the caudate putamen (CPu), nucleus accumbens
702 (Nacc), hippocampus (Hipp) and amygdala (Amy). We also performed GPR88-mediated [35S]-
703 GTPγS assay (B) and show that protein activation was totally and partially abolished in the
704 striatum of CMV-*Gpr88* and D1R-*Gpr88* mice respectively. Two (CMV-*Gpr88* and control mice)
705 and three (D1R-*Gpr88* and control mice) membrane preparations were used per genotype. Data
706 are presented as mean ± SEM. (A) CPu: n=9 D1R-CTL; 9 D1R-*Gpr88*; Nacc: n=8 D1R-CTL; 7
707 D1R-*Gpr88*; Hipp: n=9 D1R-CTL; 7 D1R-*Gpr88;* Amy: n=6 D1R-CTL; 7 D1R-*Gpr88*. Stars: two
708 black stars p<0.01; three black stars p < 0.001 (Welch's t-test), (B) n=3 D1R-CTL; 3 D1R-*Gpr88*;
709 2 CMV-Gpr88 and 2 CMV-CTL. Three text stars p < 0.001 Tukey's multiple comparisons of
710 D1R-CTL or CMV-CTL vs D1R-*Gpr88* and CMV-*Gpr88* vs D1R-*Gpr88*).

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712 **Figure 2. Molecular characterization of conditional D1R-*Gpr88* mice.**

713 GPR88, D1R and D2R mRNA expression in the Caudate Putamen (CPu) of D1R-CTL (left
714 panels) and D1R-*Gpr88* (right panels) mice using triple fluorescent *in situ* hybridization (A).
715 GPR88 is labelled in green (FITC), D1R (left panels) in red (TRITC) and D2R (right panels) in
716 red (Cy5). In D1R-CTL animals, GPR88 mRNA co-localizes with both D2R and D1R mRNA. In
717 contrast, D2R but not D1R co-localize with GPR88 mRNA in D1R-*Gpr88* mice. White and yellow
718 arrows indicate examples of D1R and D2R positive cells respectively. Dapi staining (blue) was
719 used to label all cells nuclei. Quantification of GPR88/D2R (red) and GPR88/D1R (blue) mRNA
720 co-expression in the CPu and nucleus accumbens (Nacc) (B) of D1R-*Gpr88* and control mice
721 (n=3/genotype). Colocalization of GPR88 and D1R mRNA was significantly decreased in the
722 CPu and Nacc of D1R-*Gpr88* mice compared to control littermates (Sidak's multiple comparison;
723 p<0.0001). Percentage of co-expression was calculated based on the total number of GPR88-

positive cells counted [(number GPR88-expressing cells co- expressing DR1 or DR2 x 100)/ total number of GPR88-expressing cells]. Data are presented as mean ± SEM. (B) n=3 D1R-CTL; 3 D1R-*Gpr88*. Text Stars: three stars $p < 0.001$ (Sidak's multiple comparison of GPR88/D1R co-expression between genotypes).

Figure 3. CMV- and A_{2A}R-*Gpr88* but not D1R-*Gpr88 mice* show altered defensive burying and social behavior

When placed in the presence of 20 marbles CMV- (A) and A_{2A}R-*Gpr88* mice (C) buried less marbles than control animals. D1R-*Gpr88* mice (B) show similar numbers of buried marbles compared to control animals. To test social behaviors all mice where left in the presence of a naïve wild type congener and nose contact was counted. Once again, both CMV- (D) and A_{2A}R-*Gpr88* mice (F) but not D1R-*Gpr88* mice (E) showed increased number of nose contacts compared to their littermates. Data are represented as mean ± SEM. (A,D) n=8 CMV-CTL, 10 CMV-*Gpr88* (B,E) N=14 D1R-CTL, 10 D1R-*Gpr88*; (C,F) n= 10 A_{2A}R-CTL; 10 A_{2A}R-*Gpr88*. Black stars: one star p <0.05 (Welch's t-test).

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 five days, both CMV-*Gpr88* (A) and A_{2A}R-*Gpr88* (C) but not D1R-*Gpr88* mice (B) 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 with CMV-CTL, travelled a longer distance in the last compared to the first day. In contrast with their control littermates, D1R-*Gpr88* mice show similar locomotion in the first and last open field session. Regardless of their hyperlocomotion, A_{2A}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; 21 CMV-*Gpr88*; (B) N=13 D1R-CTL, 12 D1R-*Gpr88*; (C) n= 17 A_{2A}R-CTL; 10 A_{2A}R-*Gpr88*. Open stars: one star p <0.05; two stars p <0.01 (RM Two-way ANOVA).

Figure 5. CMV- and A_{2A}R-*Gpr88* gene deletion increases stereotypies. When placed in an open field for 30 min (day 1) CMV-*Gpr88* (A) and A_{2A}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; 21 CMV-*Gpr88* (B) N=13 D1R-CTL, 12 D1R-*Gpr88* (C) n= 17 A_{2A}R-CTL; 10 A_{2A}R-*Gpr88*. black stars: one star p <0.05; two star p< 0.01 (Student t test).

Figure 6. Motor coordination deficits in A_{2A}R-*Gpr88* mice and motor skill learning deficits in D1R-Gpr88 KO mice. Mice where tested on a rotating rod for four daily trials lasting six days. Overall (Left panel), CMV-*Gpr88* (A), D1R-*Gpr88* (B) and A_{2A}R- (C) mice show decreased latency to fall form the rod. When selectively analyzing the first and last training session (right panel) we observe that CMV-*Gpr88* and A_{2A}R-*Gpr88* mice presented motor coordination deficits as soon as the first session which is not present in D1R-*Gpr88*. In the last session, however, CMV-*Gpr88* and D1R-*Gpr88* both present a significantly decreased time on the rod when compared to control mice whereas A_{2A}R-*Gpr88* mice present no significant difference in the time spent on the rod when compared to their littermates. Data are represented as mean ± SEM. (A) n=21 CMV-CTL; 21 CMV-*Gpr88*; (B) N=13 D1R-CTL, 12 D1R-*Gpr88*; (C) n= 17 A_{2A}R-CTL; 10 A_{2A}R-*Gpr88*. Open stars: one star p <0.05; Two star p <0.01; three stars p <0.001 (RM Two-way ANOVA). Text Stars: three stars p < 0.001; one star p<0.05 (Sidak's multiple comparison).