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GPR88 in A_{2A}receptor-expressing neurons modulates locomotor response to dopamine agonists but not sensorimotor gating

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Abstract

The orphan receptor, GPR88, is emerging as a key player in the pathophysiology of several neuropsychiatric diseases, including psychotic disorders. Knockout (KO) mice lacking GPR88 throughout the brain exhibit many abnormalities relevant to schizophrenia including locomotor hyperactivity, behavioral hypersensitivity to dopaminergic psychostimulants and deficient sensorimotor gating. Here, we used conditional knockout (cKO) mice lacking GPR88 selectively in striatal medium spiny neurons expressing A_{2A}receptor to determine neuronal circuits underlying these phenotypes. We first studied locomotor responses of A_{2A}R-*Gpr88* KO mice and their control littermates to psychotomimetic, amphetamine, and to selective D1 and D2 receptor agonists, SKF-81297 and quinpirole, respectively. To assess sensorimotor gating performance, mice were submitted to acoustic and visual prepulse inhibition (PPI) paradigms. Total knockout GPR88 mice were also studied for comparison. Like total GPR88 KO mice, A_{2A}R-*Gpr88* KO mice displayed a heighted sensitivity to locomotor stimulant effects of amphetamine and

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A.C.M., A.M.O. and B.L.K. designed the experiments. A.C.M. performed and analyzed behavioral experiments. A. K. E. provided the *Adora2a*-Cre mice. A.C.M., A.M.O. and B.L.K interpreted the results and wrote the article. All authors discussed the results, commented and edited on the manuscript.

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All raw data will be fully available upon contact to authors

SKF-81297. They also exhibited enhanced locomotor activity to quinpirole, which tended to suppress locomotion in control mice. By contrast, they had normal acoustic and visual PPI, unlike total GPR88 KO mice that show impairments across different sensory modalities. Finally, none of the genetic manipulations altered central auditory temporal processing assessed by gap-PPI. Together these findings support the role of GPR88 in the pathophysiology of schizophrenia and show that GPR88 in A_{2A}receptor expressing neurons modulates psychomotor behavior but not sensorimotor gating.

Keywords

Cross-modal PPI; schizophrenia; D2R-medium spiny neurons; GAP detection; striatum

Introduction

GPR88 is an orphan G protein coupled receptor (GPCR) that is highly enriched **in medium spiny neurons** of the neostriatum (caudate-putamen and nucleus accumbens) and the olfactory tubercle (Logue *et al.*, 2009; Massart *et al.*, 2009; Quintana *et al.*, 2012). The distinctive pattern of GPR88 expression has generated considerable excitement regarding the physiological role of this orphan receptor and its implication in brain diseases associated to striatal dysfunction. Human genetic studies revealed a positive association between GPR88 and schizophrenia as well as bipolar disorder (Ogden *et al.*, 2004; Del Zompo *et al.*, 2014). Deleterious mutation in GPR88 was also linked to a familial developmental disorder characterized by a childhood chorea (hyperkinetic movement disorder), learning disabilities and speech retardation (Alkufri *et al.*, 2016). Accordingly, studies conducted with knockout (KO) mice showed that GPR88 gene deletion leads to a wide range of behavioral abnormalities, including locomotor hyperactivity, stereotypic behavior, motor coordination deficits, altered emotional processing and impaired associative and procedural learning (Logue *et al.*, 2009; Quintana *et al.*, 2012; Meirsman, *et al.*, 2016a; Meirsman *et al.*, 2016b). Interestingly, local re-expression of GPR88 in the dorsal striatum (caudate-putamen) counteracted the observed locomotor hyperactivity and learning deficits demonstrating causal link between GPR88 loss in the dorsal striatum and the behavioral phenotypes of KO mice (Quintana *et al.*, 2012). GPR88 KO mice were also reported to display an enhanced sensitivity to psychomotor effects of dopaminergic agonists (e.g., apomorphine and amphetamine) and deficient sensorimotor gating mechanisms (prepulse inhibition of acoustic startle reflex, PPI), behavioral abnormalities of relevance to schizophrenia and related psychotic disorders. Accordingly, both PPI deficits and apomorphine-induced stereotypies could be reverted by typical (haloperidol) and atypical (risperidone) neuroleptic treatments suggesting that altered GPR88 signaling may contribute to some aspects of schizophrenia syndrome (Logue *et al.*, 2009). By contrast, Ingallinesi *et al.*, (2015) showed that local silencing of GPR88 in the ventral striatum (nucleus accumbens) produces no behavioral alterations in normal rats, but attenuates the schizophrenia-related phenotypes (amphetamine-induced locomotor hyperactivity and social novelty discrimination deficit) elicited by neonatal exposure to phencyclidine. Together, these findings highlight the complex role of GPR88 in the control of striatal function and suggest that dysfunction of

GPR88 signaling may contribute to a range of neuropsychiatric disorders that involve abnormal motor, cognitive and emotional behavior.

Despite major advance, the neuronal circuit underlying GPR88 function in the brain remains poorly understood. GPR88 expression in the striatum is confined to medium spiny projection neurons (MSNs) that form the vast majority of striatal neurons (Logue *et al.*, 2009, Massart *et al.*, 2009, Quintana *et al.*, 2012). MSNs are commonly segregated into two subpopulations based on receptor expression and projection targets. MSNs of the direct pathway express dopamine D1 receptors (D1R, D1R-MSNs) and project to the substantia nigra *pars reticulata* and the internal segment of the globus pallidus (entopeduncular nucleus in rodents), whereas MSNs of the indirect pathway contain dopamine D2 (D2R, D2R-MSNs) and adenosine A_{2A} receptors ($A_{2A}R$) and innervate the substantia nigra *pars reticulata* via the external segment of the globus pallidus (GPe) and subthalamic nucleus (Albin *et al.*, 1989). These two populations of MSNs are known to differentially participate to striatal functions and behavioral output as they are coupled to output pathways with opposing properties (Bateup *et al.*, 2010; Kravitz *et al.*, 2010; Lobo *et al.*, 2010; Durieux *et al.*, 2012; Durieux *et al.*, 2009). Recently, we generated conditional knockout (cKO) mice lacking GPR88 receptor selectively in $A_{2A}R$-expressing neurons ($A_{2A}R$-*Gpr88* KO, Meirsman *et al.*, 2016b). We showed that $A_{2A}R$-*Gpr88* KO mice recapitulate many of the phenotypes of total *Gpr88* KO mice, including the locomotor hyperactivity and the abnormal emotional reactivity and sociability, thus revealing the role of GPR88 in $A_{2A}R$-expressing neurons in the modulation of these complex behavioral traits.

In the present follow-up study, we investigated the contribution of GPR88 in $A_{2A}R$-expressing neurons to the modulation of sensorimotor gating and susceptibility to psychomotor effect to dopaminergic drugs, behavioral traits relevant to psychotic disorders. We first compared the susceptibility of $A_{2A}R$-*Gpr88* KO mice and their control littermates to psychomotor effects of amphetamine. The selective D1R agonist, SKF-81287, and D2R agonist, quinpirole, were also used to evaluate the locomotor responses of mice to direct dopamine receptor stimulation. To gain a better insight on the role of GPR88 in the modulation of sensorimotor gating, $A_{2A}R$-*Gpr88* KO and control mice were assessed in acoustic and visual PPI paradigms. Finally, the impact of GPR88 ablations on auditory temporal processing was also evaluated using gap detection based on recent evidence implicating GPR88 in hearing loss (Marley *et al.*, 2013) and developmental delay of speech (Alkufri *et al.*, 2016). Total GPR88 KO mice were included in all studies for comparison.

Materials and Methods

Animals

Mice of both genders aged between 10–15 weeks were used in the present study. Mice were bred in house and group housed 3–5 animals *per* cage. They were maintained on a 12hr light/dark cycle at controlled temperature (22±1°C). All experiments were conducted during the light phase. Food and water were available *ad libitum* throughout all experiments. All experiments where approved by the local ethic comity (CREMEAS, 2003-10-08-[1]-58). For total ($Gpr88^{-/-}$; background: 13.96% C57B1/6; 60.94% C57B1/6J; 0.05% FVB/N; 25% 129/SvPas; 0.05% SJL/J) and $A_{2A}R$-*Gpr88* KO mice ($Gpr88^{A2AR\text{-}Cre}$; background: 1.08%

C57B1/6; 16.78% C57B1/6J; 0.01% FVB/N; 53.17% 129/SvPas; 0.01% SJL/J; 29.54% C57B1/6N) construction as well as conditional deletion were described previously (Meirsman *et al.*, 2016a, Meirsman *et al.*, 2016b). Also, we previously showed that introduction of loxP sites in the mouse *Gpr88* gene had no impact on GPR88 receptor agonist-induced activation in homozygous floxed mice (*Gpr88*$^{flx/flx}$) compared to wild type animals (*Gpr88* $^{+/+}$) (Meirsman *et al.*, 2016b). For all experiments *Gpr88*$^{A2A\text{-}Cre}$ mice were compared to their control littermates (*Gpr88*$^{flx/flx}$) and *Gpr88*$^{-/-}$ mice were compared to wild type *Gpr88*$^{+/+}$ mice.

Mice were genotyped using PCR-based genotyping with the following primers: 5′GAAGAGTGA AACCACAGGTGTGTACAC 3′, 5′ GTT TGT TTC CTC ACT GGC TGA GAG TC 3′ for *Gpr88* $^{+/+}$ and 5′ GTC CTA GGT GTG GAT ATG ACC TTA G 3′, 5′ GTT TGT TTC CTC ACT GGC TGA GAG TC 3′ for *Gpr88* $^{-/-}$ and *Gpr88*$^{\text{A2AR-Cre}}$. To verify the presence of Cre and Myosine (as a positive control) the following primers were used respectively: 5′ GAT CGC TGC CAG GAT ATA CG 3′, 5′CAT CGC CAT CTT CCA GCA G 3′ and 5′ TTA CGT CCA TCG TGG ACA GC 3′, 5′TGG GCT GGG TGT TAG CCT TA 3′.

Behavioral procedures

Independent cohorts of naïve *Gpr88*$^{A2AR\text{-}Cre}$ and control mice (*Gpr88* $^{Flx/Flx}$) were used for amphetamine (n=12 for *each genotype*), dopamine receptor **direct** agonists (n=32 for *Gpr88* $^{Flx/Flx}$ and 34 for *Gpr88* $^{A2AR\text{-}Cre}$) and PPI studies (n=8 *for Gpr88* $^{flx/flx}$ and 10 for *Gpr88* $^{A2A\text{-}Cre}$). For comparison, three independent cohorts of naive *Gpr88* $^{-/-}$ and wildtype mice (*Gpr88* $^{+/+}$) were included for amphetamine (n=18 *per* genotype), dopamine receptor agonists (n=24 *per* genotype) and PPI studies (n=19 for *Gpr88* $^{+/+}$ and 17 for *Gpr88* $^{-/-}$). All mice were tested for PPI in the following order: acoustic PPI, visual PPI and gap detection paradigms. Resting periods of at least 48h were used between two successive PPI testing.

Locomotor response to dopaminergic drugs

Testing was carried in transparent single cages (21 X 11 X 17 cm) under dim light (10Lux). Locomotor activity was monitored via an automated videotracking system (View Point, Lyon, France). For amphetamine-induced locomotion control and mutant mice were first placed in the unfamiliar cages for one hour to habituate to the novel environment. At the end of this first testing period, mice were allocated to 2.5 mg/kg of amphetamine (n=7 *Gpr88* $^{Flx/Flx}$, 6 *Gpr88* $^{A2AR\text{-}Cre}$; n=9 *Gpr88* $^{+/+}$, 9 *Gpr88* $^{-/-}$) or saline treatment (n= 5 *Gpr88* $^{Flx/Flx}$, 6 *Gpr88* $^{A2AR\text{-}Cre}$; n=9 *Gpr88* $^{+/+}$, 9 *Gpr88* $^{-/-}$) and their locomotor activity was immediately assessed for one hour. A similar protocol was used for dopamine receptor agonist studies. Upon habituation session, *Gpr88* $^{Flx/Flx}$ and *Gpr88* $^{A2AR\text{-}Cre}$ mice were allocated to 2.5 mg/kg SKF-81297 (n=8 and 10, respectively); 0.1 mg/kg quinpirole (n=12 *per* genotype) or saline treatment (n=12 *per* genotype*).* Similarly, *Gpr88* $^{+/+}$ and *Gpr88* $^{-/-}$ mice were allocated to SKF-81297 (N=6 *and* 9 respectively), quinpirole (N=6 *per* genotype) and saline treatment (N=12 and 9 respectively). D-Amphetamine sulfate (Sigma, USA), SKF-81297 and quinpirole hydrochloride (Tocris, France) were dissolved in isotonic saline solution (NaCl 0.9%) and injected subcutaneously in a volume of 10 ml/kg.

Acoustic startle reflex and PPI

Testing was carried in eight startle reflex devices (SRLAB, San Diego, CA, USA). Each device consisted of a ventilated sound-attenuated cubicle equipped with an animal enclosure (a Plexiglas cylinder with 5.1 cm outside diameter mounted on a Plexiglas platform). A high-frequency loudspeaker, placed 28 cm above the animal enclosure produces both a continuous background noise (65 dB) and the various acoustic stimuli. A piezoelectric accelerometer attached to the Plexiglas platform detects and transduces the movements of the animals within the cylinder. The visual stimuli (flashes of lights) were provided by a visual kit consisting of 10 white LEDs (5mm in diameter/5600 m.c.d.; Marl International Optosource, Cumbria, Los Angeles, CA) and mounted on the top of the cylinder. Before each PPI session, piezo accelerometer sensitivity, as well as acoustic and visual stimuli levels were calibrated. Startle amplitude were obtained from the recording of 65 readings of 1ms beginning at the stimulus onset.

Acoustic PPI procedure

The session started by a 5 min acclimation period followed by 5 consecutive startling pulses (white-noise 110-dB/40 ms) that were excluded from the analysis. Ten different trial types were then presented in a random order: startling pulse alone; eight different prepulse trials in which either 10 ms long 70, 80, 85, or 90 dB stimuli were presented alone or preceded the startling pulse by 50 ms, and finally one trial in which only the background noise (BN) was presented to measure the baseline movement in the Plexiglas cylinder. Inter-trial intervals lasted 20 sec in average (15–25 sec).

Visual PPI procedure

The session started by a 5 min acclimation period followed by 5 consecutive startling pulses (white-noise 110-dB/40 ms) that were excluded from the statistical analysis. Eleven different trial types were then presented: startling pulse alone, visual prepulse (1000 Lux/20 ms) presented alone or at various intervals (2, 10, 20, 50, 100, 200, 500, and 2000 ms between prepulse offset and pulse onset) before the startling pulse, and finally a trial in which only the BN was presented. All trials were applied 10 times and presented in random order with an inter-trial interval of 20 sec in average (15–25 sec).

Gap-PPI procedure

The session started by a 5 min acclimation period followed by 5 consecutive startling pulses (white-noise 120-dB/40 ms) that were excluded from the statistical analysis. Ten different trial types were then presented: startling pulse alone, a brief silent gap of various durations (5, 10, 15, 20, 25, 30, 35 and 40 ms) inserted immediately before the startling pulse, and a trial in which only the BN was presented. All trials were applied 10 times and presented in random order with an inter-trial interval of 20 sec in average (15–25 sec).

Statistics

Drug-induced locomotion data were analyzed using two-way ANOVA with genotypes as the between-subject factor and treatment as the within-subject. *Post-hoc* comparisons analyses were carried out using Bonferroni’s multiple comparisons test whenever the ANOVA

showed significant effects. PPI performance was expressed as percentage decrease in the amplitude of basal startle reflex caused by presentation of the prepulse (% PPI) according to the following formula: % PPI =100 * [(mean startle responses to the pulse-alone) - (mean startle responses to the prepulse + pulse)]/mean startle responses to the pulse-alone. Global acoustic and visual PPI performances (mean % PPI scores), were pooled across all prepulse intensities and intervals, respectively. Global gap-PPI performances were pooled across all gap durations. PPI and gap-PPI data were analyzed by RM two-way ANOVA with genotypes as the between-subject factor and the stimuli parameters (prepulse intensities, prepulse-pulse intervals and gap durations) as the repeated measures. *Post-hoc* comparisons analysis were carried out using Bonferroni's multiple comparisons test whenever the two-way ANOVAs indicated statistically significant main or interaction effects. All statistics were performed using GraphPad Prism 6 (GraphPad Software, Inc, USA) and the accepted level of significance was $p<0.05$.

Results

Locomotor activity response to dopamine agonists

As depicted in Figure 1A $Gpr88^{A2AR\text{-}Cre}$ mice were more sensitive to the locomotor stimulant effect of an acute (2.5 mg/kg) amphetamine treatment. Two-way ANOVA revealed a significant effect of genotype ($F_{1, 20} = 6.702$, $P = 0.0175$) and pharmacological treatment ($F_{1, 20} = 12.86$, $P = 0.0018$). *Post-hoc* comparisons show a significant difference between amphetamine treated mice ($P < 0.01$, Bonferroni's multiple comparisons test) but not saline injected mice. Likewise, (Figure 1B), acute treatment with D1R agonist (SKF-81297, 2,5mg/kg) significantly increased locomotion in both control and mutant mice when compared to saline treatment ($P < 0.001$; Bonferroni's multiple comparison test), but had an exacerbated effect in $Gpr88^{A2AR\text{-}Cre}$ when compared to control mice ($P < 0.001$; Bonferroni's multiple comparisons test). Two-way ANOVA indicates a genotype ($F_{1, 60} = 21.24$, $P < 0.0001$) and treatment effect ($F_{2, 60} = 67.35$, $P < 0.0001$). Analysis also revealed a significant genotype x treatment interaction ($F_{2, 60} = 4.76$, $P = 0.0121$). In agreement, the D2R agonist (quinpirole, 0.1mg/kg) decreased locomotion in control animals compared to saline treated mice (not significant) but had the opposite effect on $Gpr88^{A2AR\text{-}Cre}$ mice. *Post-hoc* comparison indicates a significant difference between genotypes for D2R-agonist treated animals ($P < 0.01$; Bonferroni's multiple comparisons test) but not saline treated mice.

Similar to conditional KO mice, $Gpr88^{-/-}$ KO mice displayed an increased amphetamine-induced locomotion when compared to wild type animals (Figure 1C). Two-way ANOVA showed a significant genotype ($F_{1, 32} = 30.61$, $P < 0.0001$) and treatment ($F_{1, 32} = 183.2$, $P < 0.0001$) effect. *Post-hoc* comparisons show that while locomotion after saline injection did not differ between genotypes, when injected with 2.5mg/kg of amphetamine total KO mice travelled a significantly increased distance ($P < 0.0001$, Bonferroni's multiple comparisons test). Similarly, acute injection of D1R agonist significantly increased locomotion in both wild type and total KO mice when compared to saline treatment ($P < 0.001$; Bonferroni's multiple comparisons test) with enhanced locomotion in mutants compared to control animals ($P < 0.05$, Bonferroni's multiple comparisons test) (Figure 1D). Analysis of

variance indicate a significant genotype ($F_{(1, 42)} = 33.69$; $p < 0.0001$) and treatment ($F_{2, 42} = 46.59$, $P < 0.0001$) effect. As for conditional KO mice, ANOVA also revealed a significant genotype x treatment interaction ($F_{2, 42} = 8.54$, $P = 0.0008$) probably associated with an opposite locomotor response of wildtypes and KO animals to D2R agonist treatment. Also, *post-hoc* comparisons indicate a significant difference between genotypes after D2R injection ($P < 0.0001$, Bonferroni's multiple comparisons test).

Auditory sensorimotor gating in *Gpr88* $^{A2AR\text{-}Cre}$ and *Gpr88* $^{-/-}$ KO mice

Figure 2A, illustrates acoustic PPI performances of *Gpr88*$^{A2AR\text{-}Cre}$ and *Gpr88*$^{Flx/Flx}$ control mice as function of the prepulse intensity. PPI level increased similarly in both genotypes when the prepulse intensity was raised from 70 to 90 dB. Two-way ANOVA showed a significant effect of prepulse intensity ($F_{3, 48} = 135.6$, $P < 0.0001$), but failed to reveal a significant effect of genotype ($F_{1, 16} = 1.12$, $P = 0.3057$) or a significant genotype x prepulse intensity interaction ($F_{3, 48} = 0.878$, $P = 0.4589$). Mean PPI scores pooled across all prepulse intensities were also comparable between genotypes ($t_{16} = 1.026$, $P = 0.32$; Student *t* test, Figure 2B). From Table 1 it can be seen that *Gpr88*$^{A2AR\text{-}Cre}$ mice had normal baseline startle response compared to their littermates ($t_{16} = 0.14$, $P = 0.89$; Student *t* test, Table 1). Presentation of acoustic prepulse alone tended to evoke a slight reaction at the highest intensities (≥ 85 dB), but no difference was detected between genotypes (Table 1).

Figures 2C illustrates acoustic PPI performances of *Gpr88*$^{-/-}$ *KO* mice and their wild type littermates. PPI level increased progressively in both genotypes with increasing prepulse intensity ($F_{3, 102} = 258.7$, $P < 0.0001$), but KO mice had overall a poor performance. Accordingly, two-way ANOVA showed a significant effect of genotype ($F_{1, 34} = 11.96$, $P = 0.0015$) and *post-hoc* comparisons indicated that total KO mice had significantly lower scores than wild types at 80 and 85dB prepulse intensities ($P < 0.05$ and $P < 0.01$ respectively, Bonferroni's multiple comparisons test, Figure 2C). Inspection of global PPI scores confirmed the poor performance of KO mice ($t_{34} = 3.44$, $P = 0.0016$; Student *t* test, Figure 2D). No differences in baseline startle response or reactivity to acoustic prepulses were detected between genotypes ($t_{34} = 0.42$, $P = 0.68$; Student *t* test Table 1).

Visual sensorimotor gating in *Gpr88*$^{A2AR\text{-}Cre}$ and *Gpr88*$^{-/-}$ KO mice

Figure 3A illustrates visual PPI of *Gpr88*$^{A2AR\text{-}Cre}$ and *Gpr88*$^{Flx/Flx}$ mice as function of the prepulse-pulse interval. The time function of visual PPI was similar between genotypes, a bell-shaped curve with a peak of inhibition at 20-ms lead time interval. Two-way ANOVA showed a significant effect of interval ($F_{7,112} = 9.47$, $P < 0.0001$), but failed to reveal a significant effect of genotype ($F_{1, 16} = 0.69$, $P = 0.4198$) or a significant genotype x interval interaction ($F_{7,112} = 0.37$, $P = 0.9178$). Global PPI scores pooled across all prepulse-pulse intervals were also comparable between genotypes ($t_{16} = 0.84$, $P = 0.41$ Figure 3B). No difference in baseline startle response was detected between *Gpr88*$^{A2AR\text{-}Cre}$ and control mice (Table 2). Presentation of the visual prepulse alone did not produce any overt reactions in mice, unlike acoustic prepulses (Table 2).

Figure 3C illustrates the temporal profile of visual PPI for *Gpr88* $^{-/-}$ and wild type mice. Both genotypes showed a bell-shaped curve with a maximal level of inhibition at 20 ms

prepulse-pulse interval, but KO mice had again a poor PPI performance (Figure 3C). There was a significant effect of genotype ($F_{1, 34}$ = 4.13, P = 0.0499) and *post-hoc* comparisons indicated that KO mice had a lower PPI scores than wild types at 20 ms lead time interval (P < 0.01, Bonferroni's multiple comparisons test, Figure 3C). Significant difference between genotypes was also found for global PPI scores (t_{34} = 2.048, P = 0.048; Student t test, Figure 3D). Baseline startle responses were comparable between KO and wild type mice (t_{34} = 0.14, P = 0.89; Student t test, Table 2).

Gap detection in Gpr88 $^{A2AR\text{-}Cre}$ and Gpr88$^{-/-}$ mice

Figure 4A shows that *Gpr88*$^{A2AR\text{-}Cre}$ mice had a normal gap detection compared to *Gpr88*$^{Flx/Flx}$ control animals. Two-way ANOVA showed a significant effect of gap duration ($F_{7, 112}$ = 129.5, P < 0.0001), but failed to reveal a significant effect of genotype ($F_{1, 16}$ = 1.36, P = 0.2613). Global scores pooled across all gap durations (Figure 4B) and baseline startle responses (Table 3) were also comparable between genotypes (t_{16} = 0.26, P = 0.80; Student t test).

Similarly, no difference in gap detection was observed between *Gpr88*$^{-/-}$ KO mice and their wild type counterparts ($F_{1, 34}$ = 0.69, P=0.4105, Figure 4C). Global scores (Figure 4D) and baseline startle response (Table 3) were also comparable between genotypes (t_{34} = 0.66, P = 0.52; Student t test).

Discussion

GPR88 is selectively and highly expressed in medium spiny neurons of the striatum and has been implicated in the pathophysiology of psychotic disorders. Previous studies showed that GPR88 gene deletion throughout the brain increases spontaneous locomotor activity and exacerbates locomotor responses of mice to amphetamine (Quintana *et al.*, 2012; Meirsman *et al.*, 2016a; Meirsman *et al.*, 2016b). Our findings extend these observations and demonstrate that striatal MSNs expressing $A_{2A}R$ play a central role in relaying the inhibitory influence of GPR88 on these behavioral traits. The hypersensitivity of *Gpr88*$^{A2AR\text{-}Cre}$ mice to psychomotor effect of amphetamine may likely involve post-synaptic D2R mechanisms since striatal $A_{2A}R$ are expressed in D2R-MSNs that give rise to the indirect pathway. To address this possibility, we tested the locomotor responses of *Gpr88*$^{A2AR\text{-}Cre}$ mice to selective D1R and D2R stimulation using SKF81297 and quinpirole, respectively. As demonstrated by numerous studies, D2R plays a dual role in the modulation of locomotor activity. In mice, DR2 agonists reduce locomotor activity over a wide dose range (Ralph & Caine, 2005; Li *et al.*, 2010), an effect attributed to presynaptic D2 autoreceptors that inhibit dopamine release (Usiello *et al.*, 2000; Wang *et al.*, 2000). However, the psychomotor stimulant actions of D2 agonists can be unmasked by pharmacological and genetic manipulations that lead to a hypersensitivity of post-synaptic D2R (Gomeza *et al.*, 1999; Gainetdinov *et al.*, 2003; Thompson *et al.*, 2010; Espinoza *et al.*, 2015). Accordingly, quinpirole reduced spontaneous locomotor activity in control mice while it produced a robust locomotor hyperactivity in *Gpr88*$^{A2AR\text{-}Cre}$ mice. Similar opposite locomotor effects of quinpirole were also seen in total GPR88 KO and their wildtype counterparts, as previously reported by Quintana et al., (2012). *Gpr88*$^{A2AR\text{-}Cre}$ mice were

also more sensitive to psyhomotor effects of the D1R agonist, SKF-81297, indicating that deletion of GPR88 in A_{2A}R-expressing MSNs alters striatal physiology beyond D2R-MSNs. Collectively, these observations suggest that enhanced functioning of post-synaptic D1R and D2R at striatal MSNs may underlie the locomotor phenotype of *Gpr88*$^{A2AR\text{-}Cre}$ mice. However, other mechanisms cannot be excluded since GPR88 ablation might also produce transcriptional and anatomical modifications as previously found in total KO mice (Quintana *et al.*, 2012; Meirsman *et al.*, 2016a). It should also be stressed that striatal cholinergic interneurons have been reported to express A_{2A}R transcripts. Although it is still unclear whether GPR88 is also present in the former neurons (Massart *et al.*, 2009; Van Waes *et al.*, 2011; Quintana *et al.*, 2012), the possibility that cholinergic mechanism may contribute to the locomotor phenotypes of *Gpr88*$^{A2AR\text{-}Cre}$ mice cannot be completely ruled out.

GPR88 was also implicated in the modulation of sensorimotor gating, a pre-attentive sensory filtering mechanism that is central to perceptual and mental integration (Logue *et al.*, 2009). In the present study we confirm the acoustic PPI impairment reported in total GPR88 KO mice and further demonstrate that sensorimotor gating deficit generalizes to visual stimuli. The presentation of the flash light at varying intervals before the pulse produced the typical temporal profile of visual PPI in wild type and KO mice: a bell-shaped curve with a sharp peak of inhibition occurring at short (20 ms) lead time interval (Aubert *et al.*, 2006; Ces *et al.*, 2012). Interestingly, KO mice displayed a poor performance at lead time intervals starting from 20–100 ms, a temporal window corresponding to the effective startle inhibition. PPI is considered to reflect a transient activation of a ‘protective gate’ triggered by detection (or perception) of the prepulse. The activation of this gating mechanism allows the processing of the prepulse to occur without disruption by the succeeding pulse (Geyer *et al.*, 2002). Several line of evidence indicates that PPI deficits of GPR88 KO mice reflect a disruption of the gating mechanisms rather than reduced detectability/temporal processing of the prepulses. Indeed, KO mice had normal motor reactions to acoustic prepulses (Table 1) and also normal gap detection, which rule out deleterious effects of the mutation on auditory function. The pattern of deficits obtained with visual PPI also argues against an impairment of the visual prepulse detection. As demonstrated by previous studies, decrement in visual sensitivity or visual prepulse strength causes a delay in the onset of PPI: a shift of the bell-shaped curve to the right (Aubert *et al.*, 2006; Ces *et al.*, 2012). However, no shift in the onset of PPI was detected in KO mice. The present findings clearly show that GPR88 loss causes a genuine disruption of the gating processes that is generalized across sensory modalities as reported in schizophrenia patients (Braff *et al.*, 2001).

An important result was that GPR88 deletion in A_{2A}R/D2R-expressing MSNs had no impact on sensorimotor gating. The absence of effect on acoustic PPI is somewhat not surprizing knowing that D1R rather than D2R play a prominent role in the modulation of acoustic PPI in mice. Indeed, several research groups, including our, showed that direct stimulation of D1R but not D2R produces disruption of acoustic PPI in mice, a pharmacological profile opposite to that obtained in rats (Ralph-Williams *et al.* 2002; Ralph & Caine 2005; Geyer 2006; Ces *et al.* 2012). Our data therefore extend these mouse studies by showing that genetic deletion of GPR88 activity in striatal D2R-MSNs is ineffective on acoustic PPI. To unravel a possible impact of the mutation on sensorimotor gating we used visual PPI, which is highly sensitive to D2R perturbations (Ces *et al.*, 2012). However, no deficit in visual PPI

was detected in *Gpr88*$^{A2AR\text{-}Cre}$ mice as compared to the control mice. It is worth noting that *Gpr88*$^{A2AR\text{-}Cre}$ mice display also normal performance in series of associative (contextual and cued fear conditioning, Meirsman *et al.*, 2016b) and non-associative learning tasks (habituation after repeated exposure to spatial context, data not shown), unlike total GPR88 KO mice that exhibit cognitive impairments (Logue *et al.*, 2009; Quintana *et al.*, 2012; Meirsman *et al.* 2016a;b). The lack of phenotype suggests that GPR88 modulation of mnemonic functions and pre-attentive filtering processes may not operate at the level of A_{2A}R/D2R-expressing MSNs. Future studies using conditional knockout mice should clarify whether such cognitive functions is subserved by GPR88 in D1R expressing MSNs.

Finally, the impact of GPR88 ablations on gap detection (a brief gap inserted in the background noise that acts as a prepulse) was also assessed based on recent studies pointing to the role of this orphan receptor in hearing loss (Marley *et al.*, 2013) and in developmental speech delay (Alkufri *et al.*, 2016). Gap detection is widely used as a measure of central auditory temporal processing, which is critical for speech perception and phonological processing (Phillips, 1999). For instance, impairments of gap detection have been directly linked to speech perception deficits in children with language learning disorders and in elderly adults (Phillips, 1999; Walton, 2010). Gap detection deficits have been also reported in patients with autism spectrum disorder that is linked to dysfunction of the cortico-basal ganglia-cortical loop (Bhatara *et al.*, 2013). As expected, presentation of the brief gap produced a robust inhibition of the startle response to the pulse as seen with the acoustic and the visual prepulses. However, no notable alteration was detected in either total GPR88 KO or conditional *Gpr88*$^{A2AR\text{-}Cre}$ mice. The differential effect of GPR88 ablation on PPI and gap detection is particularly interesting as it demonstrates the specific contribution of GPR88 to brain processes filtering incoming sensory stimuli.

In conclusion, our findings confirm the modulatory role of GPR88 on sensorimotor gating. More importantly, they shed a new light on the function of striatal GPR88. They show that GPR88 in A_{2A}R/D2R-expressing MSNs play a prominent role in the modulation of locomotor responses to dopaminergic agonists and suggest altered post-synaptic D1R and D2R sensitivity. By contrast, it does not contribute to sensorimotor gating. These findings complement our previous work showing that GPR88 in striatal A_{2A}R/D2R-expressing MSNs acts as an important modulator of risk taking behaviours **and social behaviour** (Meirsman *et al.*, 2016b). Recent human genetic studies have implicated GPR88 in both schizophrenia and bipolar disorder (Ogden *et al.*, 2004; Del Zompo *et al.*, 2014). Our work corroborates these studies and further suggests that alterations of GPR88 signaling in A_{2A}R-expressing neurons may contribute to some aspect of psychomotor agitation associated to these psychiatric diseases.

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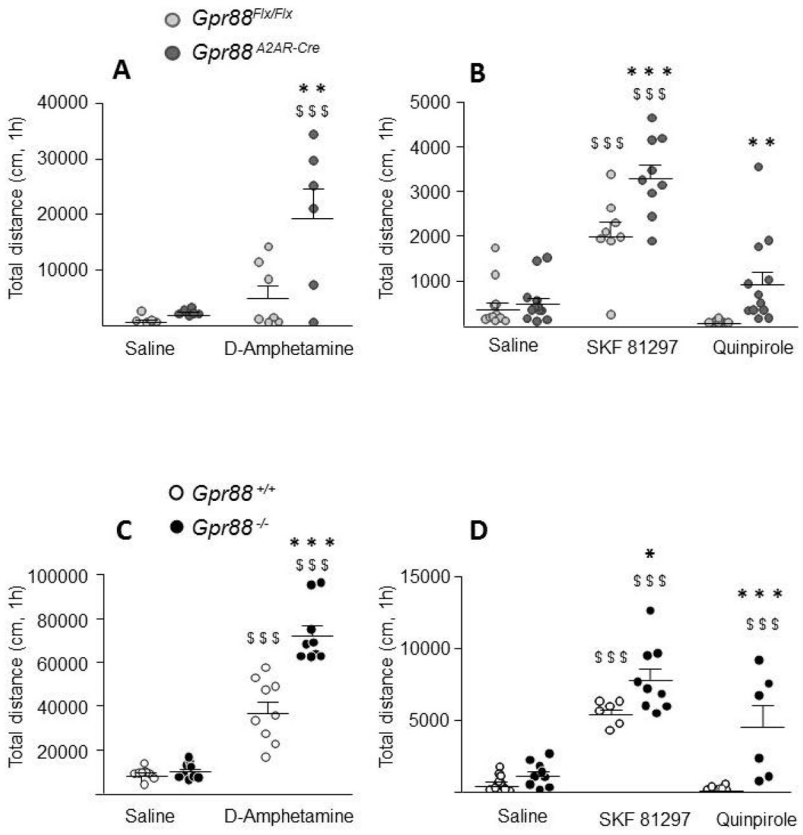

Figure 1. Locomotor effects of dopamine agonists in mice lacking *Gpr88*

After the habituation phase (data not shown) *Gpr88* $^{A2AR\text{-}Cre}$ mice (**A**) show exacerbated locomotor hyperactivity after 2.5 mg/kg amphetamine injection compared to *Gpr88* $^{flx/flx}$ control mice (n= 5–7 *per* treatment and genotype). (**B)** D1R agonist injection (SKF-81297 2.5mg/kg, n= 8–12 *per* treatment and genotype) increased locomotion in mutant and control animals with *Gpr88*$^{A2AR\text{-}Cre}$ mice presenting increased total distance travelled when compared to control mice. Conditional KO mice also present enhanced total distance traveled after D2R stimulation (quinpirole 0.1 mg/kg, n=12 *per* treatment and genotype) when compared to their control littermates. Similarly, total GPR88 KO mice (**C**) present a potentiated increase in locomotion after amphetamine (n= 9 *per* treatment and genotype $^{-}$) and D1R agonist (n= 6–12 *per* treatment and genotype) treatment (**D**), compared to *Gpr88* $^{+/+}$ mice. Like conditional KO, *Gpr88* $^{-/-}$ mice show enhanced locomotion compared to wildtype mice after D2R agonist injection (n= 6–12 *per* treatment and genotype). Lines represent mean and SEM, **and all animal are represented as data points**. Text stars (*): one star $p<0.05$; two stars $p < 0.01$; three stars $p < 0.001$ (*vs* control animals, Bonferroni's multiple comparisons test). Dollars symbol ($) three stars $p < 0.001$ (*vs* saline treatments, Bonferroni's multiple comparison test).

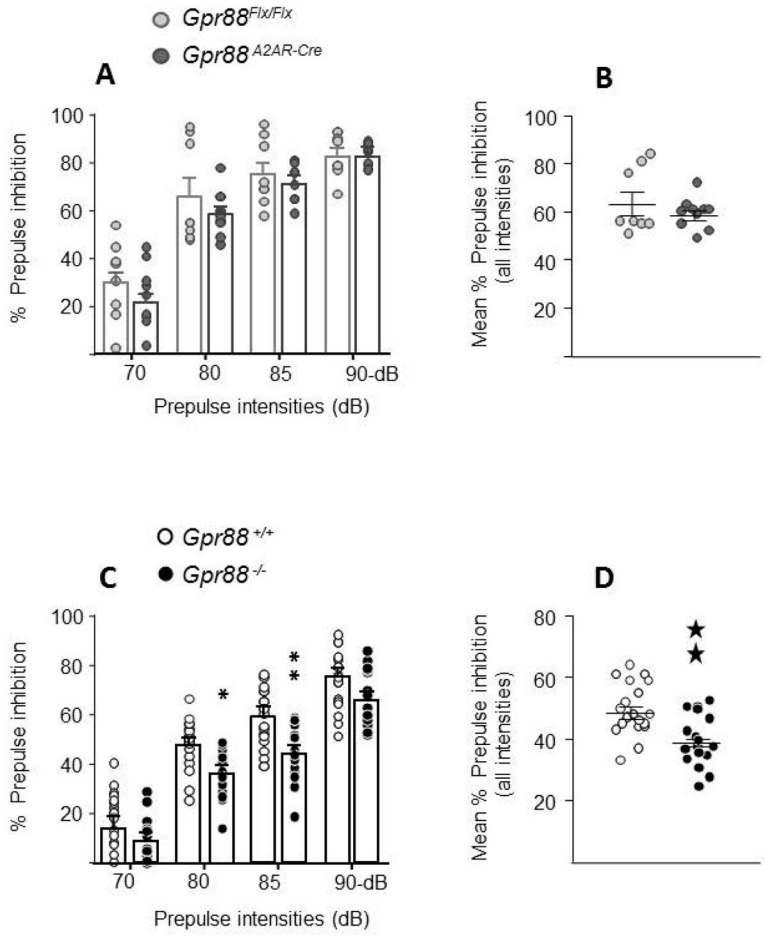

Figure 2. Acoustic prepulse inhibition (PPI) in mice lacking *Gpr88*

A_{2A}R-*Gpr88* (**A**) (N=8 *Gpr88* $^{flx/flx}$, 10 *Gpr88* $^{A2AR-Cre}$) and full (**C**) KO animals (N=19 *Gpr88* $^{+/+}$, 17 *Gpr88* $^{-/-}$) present increased PPI levels with the increasing prepulse intensities. When compared to control littermates *Gpr88* $^{A2A-Cre}$ mice present normal acoustic PPI (**A** and **B**) Conversely, *Gpr88* $^{-/-}$ show impaired general PPI (**D**) with significant decrease for prepulses of 80 and 85 dB (**C**). **Data are represented as mean ± SEM, and all animal are represented as data points**. Solid stars: two stars $p < 0.01$ (Student t test). Text stars (*): one star $p<0.05$; two stars $p < 0.01$ (Bonferroni's multiple comparisons test).

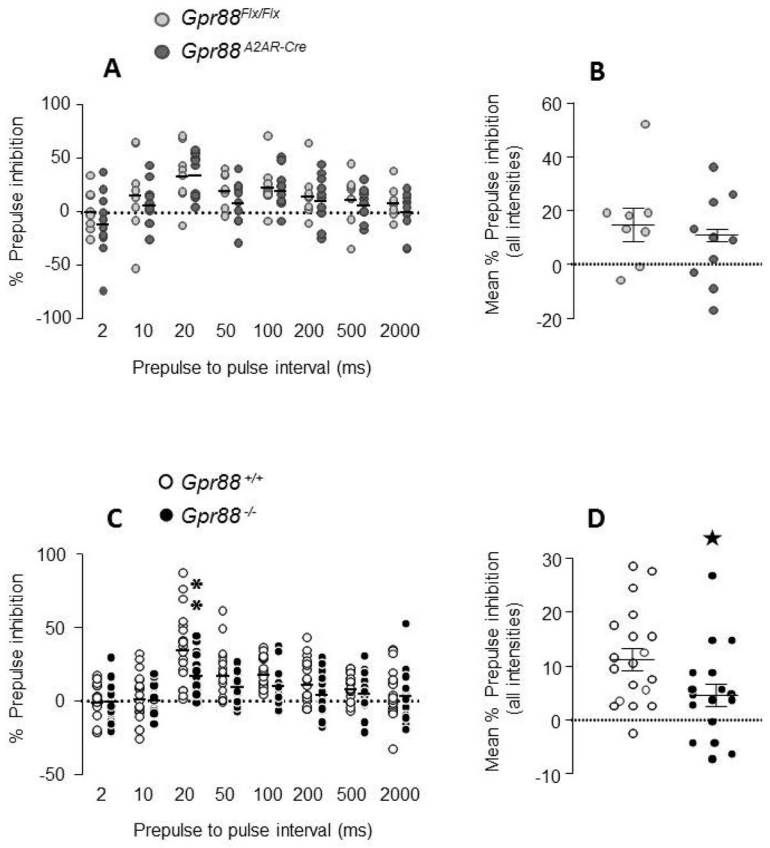

Figure 3. Visual prepulse inhibition (PPI) in mice lacking *Gpr88*

$A_{2A}R$-*Gpr88* (**A**) and full (**C**) KO animals present visual PPI for prepulses presented between 10 and 200ms. *Gpr88* $^{A2AR\text{-}Cre}$ mice (N=8 *Gpr88* $^{flx/flx}$, 10 *Gpr88* $^{A2AR\text{-}Cre}$) display visual PPI levels similar to their control littermates (**A** and **B**). In contrast, *Gpr88* $^{-/-}$ (N=19 *Gpr88* $^{+/+}$, 17 *Gpr88* $^{-/-}$) show impaired visual PPI (**D**) with significant decrease for prepulses presented 20 ms before the pulse (**C**).). **Data are represented as mean, and all animal are represented as data points**. Solid stars: one star $p < 0.05$ (Student t test). Text stars (*): one star $p < 0.05$ (Bonferroni's multiple comparisons test).

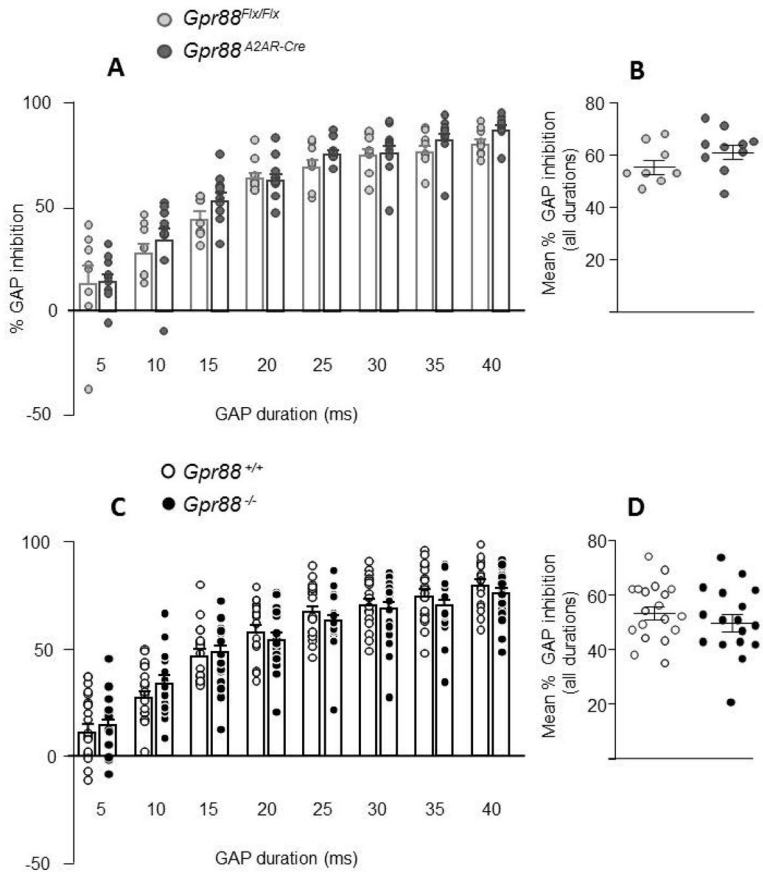

Figure 4. GAP detection in mice lacking *Gpr88*

$A_{2A}R$-*Gpr88* (**A**) (N=8 *Gpr88* $^{flx/flx}$; 10 *Gpr88* $^{A2AR-Cre}$) and full (**C**) KO animals (N=19 *Gpr88* $^{+/+}$, 17 *Gpr88* $^{-/-}$) present an increased inhibition of the startle reactivity for increasing duration of the background noise interruption. When compared to control animals, there was no significant difference in the percentage of GAP detection for full (**C** and **D**) or conditional (**A** and **B**) KO animals.). **Data are represented as mean ± SEM, and all animal are represented as data points**.

Table 1

(Acoustic PPI): Baseline activity (background noise, BN), reactivity to the acoustic prepulse and startle reflex response of *Gpr88*$^{A2AR\ -Cre}$ and *Gpr88*$^{-/-}$.

Mouse line		Prepulse intensity (dB)					Pulse
		BN	***70***	***80***	***85***	***90***	***110-dB/40 ms***
Gpr88$^{A2AR\ -Cre}$	Control	2.2±0.5	2.6±0.4	9.9±3.1	20.1±6.0	70.8±14.4	318.4±67.7
	cKO	2.6±0.3	2.8±0.4	4.4±0.9	10.9±4.2	31.0±12.9	330.4±54.1
Gpr88$^{-/-}$	Control	4.2±0.6	4.5±0.7	6.3±0.6	8.3±0.7	22.8±4.0	283.7±23.0
	KO	4.1±0.6	4.4±0.7	7.4±0.8	11.6±1.6	38.0±5.5	297.2±22.6

Table 2

(Visual PPI): Baseline activity (background noise, BN), reactivity to the visual prepulse and startle reflex response of *Gpr88*$^{A2AR\ -Cre}$ and *Gpr88*$^{-/-}$.

Mouse line		Visual Prepulse (1000Lux/20ms)		Pulse
		BN	***Prepulse alone***	***110-dB/40 ms***
Gpr88$^{A2AR\ -Cre}$	Control	6.4±1.0	5.9±1.1	190.2±44.2
	cKO	8.4±1.6	8.2±1.4	249.3±43.1
Gpr88$^{-/-}$	Control	8.2±0.8	8.1±0.9	292.3±24.6
	KO	7.1±0.6	7.4±0.7	296.9±23.0

Table 3

(GAP detection): Baseline activity (background noise, BN) and startle reflex response to the pulse of *Gpr88*$^{A2AR\ -Cre}$ and *Gpr88*$^{-/-}$.

Mouse line		Pulse	
		BN	***120-dB/40 ms***
Gpr88$^{A2AR\ -Cre}$	Control	4.41±1.6	309.35±68.2
	cKO	4.65±0.8	316.82±47.2
Gpr88$^{-/-}$	Control	4.48 ± 0.2	335.36 ± 6.3
	KO	5.59 ± 0.3	361.4 ± 6.9