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Role of the orphan receptor GPR88 in psychiatric and motor disorders

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UNIVERSITÉ DE STRASBOURG



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INSERM U-964, CNRS UMR-7104

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Rôle du récepteur orphelin GPR88 dans les pathologies psychiatriques et motrices

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Abbreviations

AC : Adenylate Cyclase	GABA: γ -aminobutyric acid
ADHD : Attention deficit/hyperactive disorder	GAD: generalized Anxiety disorder
ANOVA : Analysis of variance	GDP: Guanosine diphosphate
ATP : Adenosine triphosphate	GTP: Guanosine triphosphate
BAC: bacterial artificial chromosome	GPCR: G protein-coupled receptor
BLA: Basolateral amygdala	HD: Huntington disease
BPD: Bipolar disease	Hipp : Hippocampus
cAMP : Cyclic adenosine monophosphate	HPC:Hippocampus
CeA : Central nucleus of the amygdala	<i>i.p.</i> : intraperitoneal
cKO : conditional knockout	KO: knockout
CPP: conditional place preference	LD: light-dark test
CPu: Caudate-putamen nucleus	LGP: Lateral globus pallidus
D1R: Dopamine receptor 1	MGP: medial globus pallidus
D2R: Dopamine receptor 2	MOR: Mu opioid receptor
DA: Dopamine	MPH: methylphenidate
DNA : Deoxyribonucleic acid	MSNs: medium spiny neurons
DLS : Dorsolateral striatum	Nac: Nucleus accumbens
DMS : dorsomedian striatum	NSF: Novelty suppressed feeding
DOR: Delta opioid receptor	SNc: substantia nigra compacta
DS : dorsla striatum	SNr: substantia nigra pars reticulate
eGFP : enhanced green fluorescent protein	OF: open field
Enk/PENK : Enkephaline/ Enkephaline gene	<i>p.o.</i> : <i>per os</i>
	PCR: polymerase chain reaction
	PD: Parkinson's disease

PFC: Prefrontal cortex

PKA : protein kinase A

PKC : protein Kinase C

PPI: prepulse inhibition

qPCR: quantitative polymerase chain
reaction

RNA: Ribonucleic acid

SP: Substance P

STN: subthalamic nucleus

vmHPC: Ventromedian Hippocampus

VS : ventral striatum

VTA: ventral tegmental area

WT: wild type

Introduction

In the next pages I recapitulate the most important fundamentals to understand the main results of my thesis.

In chapter 1 I describe the functional anatomy of the striatum and basal ganglia nuclei system. I also review the functions of medium spiny projection neurons including classical as well as recent literature.

Chapter 2 describes the semiology, animal models and treatments of some of the best known motor and neuropsychiatric basal ganglia disorders.

Chapter 3 defines G-protein coupled receptors (GPCRs) exemplifying some of the related intracellular mechanisms. Most importantly, this section summarizes the literature regarding the orphan GPCR GPR88.

Chapter 1:

The striatum & basal ganglia nuclei

The Striatum is the main input structure of the basal ganglia, a group of subcortical nuclei that integrate information about context, actions and outcomes to shape adaptive behavior (Ena et al., 2011, Macpherson et al., 2014). It receives glutamatergic projection from the cortex, hippocampus and thalamus and projects to output basal ganglia nuclei that regulate thalamo-cortical projections. Also, modulatory dopaminergic neurons from the ventral tegmental area and substantia nigra *pars compacta* projects to the striatum which in turn regulates these midbrain nuclei activity through feedback projections. The striatum is well known for its role in motor control and pathophysiology of motor disorders such as Parkinson's or Huntington diseases (DeLong, 1990, Wichmann and DeLong, 1996, Gittis and Kreitzer, 2012). Furthermore, this structure has also been shown to control cognitive functions such as learning and memory, attention and motivation, and is particularly known to regulate reward processing. In fact, striatal dysfunctions are found in several neuropsychiatric disorders such as schizophrenia and hyperactive disorder/Attention deficit (Heimer, 2003b, Heimer, 2003a, Kravitz et al., 2015)

Functional anatomy of the striatum

Based on its afferent and efferent cortical projections, the striatum was initially divided into dorsal (caudate-putamen in rodents) and ventral (Nucleus Accumbens and Olfactory tubercles) striatum (DS and VS respectively) sharing the same cytoarchitecture and biochemical composition (Heimer, 2003b, Heimer, 2003a, Devan et al., 2011) (Figure 1). The term "limbic striatum" has been used to refer to the VS as it receives projections from Allocortical (including entorhinal and piriform cortex, hippocampus and amygdala) and mesocortical (including prefrontal cortex) areas (Heimer, 2003b, Heimer, 2003a, Lynd-Balta and Haber, 1994, Alexander et al., 1986).

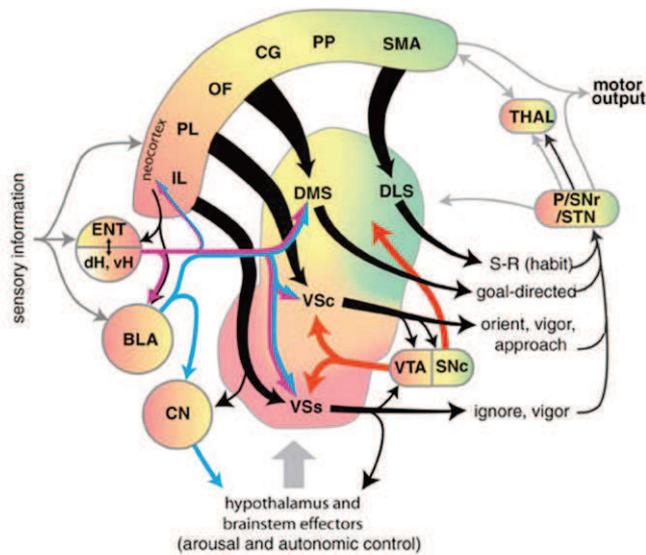


Figure 1: Schematic representation of striatal connectivity and main functions of striatum sub-regions in rodents. Four regions of rodent striatum are indicated by label and color; the color gradient approximates the gradient of afferent projections (Voorn et al., 2004). Corresponding colors in other structures represent general projections topography. Projections from dopamine neurons in the ventral tegmental area (VTA) and substantia nigra pars compacta (SNc) are shown in red. Abbreviations: DLS, dorsolateral striatum; DMS, dorsomedian striatum; VSc: core of the ventral striatum; VSs, shell of the ventral striatum; SNr, substantia nigra pars reticulata; P, pallidum; STN, subthalamic nucleus; dH, dorsal hippocampus; vH, ventral hippocampus; ENT, entorhinal cortex; BLA, basolateral nucleus of the amygdala; CN, central nucleus of the amygdala; IL, infralimbic; PL, prelimbic; OF, orbitofrontal; CG, cingulate; PP, parietal, SMA, sensorimotor. Adapted from (Gruber and McDonald, 2012)

Furthermore, the Nucleus accumbens (Nac) can be segregated into a central and dorsal part_ the *Core*_ and a surrounding lateral, ventral and medial part_ the *shell*_ regions. This distinction is made on a neurochemical (stronger immunoreactivity for calcium-binding protein, calbindin D28Kc within the core) as well as anatomical basis (distinct afferent projections) (for detailed review please refer to (Groenewegen et al., 1999)). The VS is thought to be involved in motivational processes and has been shown to encode the prediction of rewarding stimuli. For instance, lesions of the Nac impair previously acquired conditional response to food-paired conditional stimuli (Liljeholm and O'Doherty, 2012, Blaiss and Janak, 2009, Parkinson et al., 1999, Kelley, 2004, McBride et al., 1999). On the other hand, the dorsal region of the striatum is mainly known for its role in motor and

instrumental learning (Balleine et al., 2009, Jankowski et al., 2009, Atallah et al., 2007, Liljeholm and O'Doherty, 2012). Additionally, the DS can be further divided into dorsolateral Striatum (DLS) receiving projection from sensorimotor cortex, whereas association cortices project to dorsomedial striatum (DMS). The DMS seems required for initial stages of motor skill and goal directed (instrumental) learning, whereas the DLS is more engaged in habit acquisition (Grafton et al., 1995, Miyachi et al., 2002, Yin et al., 2009, Liljeholm and O'Doherty, 2012, Balleine et al., 2009).

In addition to these topographically organized cortical afferences, the striatum also receives ascending glutamatergic projection from the thalamus, dopaminergic modulation from the Substantia nigra *pars compacta* (SNc) and ventral tegmental area (VTA), as well as serotonergic release from the Dorsal Raphe. Moreover, dopaminergic release is regulated by feedback projection of striatal neurons to dopaminergic cell groups in the VTA and SNc (Heimer, 2003b, Heimer, 2003a, Devan et al., 2011, Liljeholm and O'Doherty, 2012, Gerfen and Surmeier, 2011, Do et al., 2012, Voorn et al., 2004).

Although not discussed in detail here, another interesting striatal compartmentalization is the Path-Matrix distinction. The striatal “patches” (or “striosome”) are interconnected zones embedded in the surrounding striatal matrix. At a neurochemical level, patches are differentiated by their μ -opioid receptors enrichment as well as their weak acetylcholinesterase staining (among others, for detailed review refer to (Crittenden and Graybiel, 2011)). Interestingly, while both compartments contain Medium Spiny Neurons (MSNs_ see below) projecting to both main output structures of the striatum, the patch compartment is thought to contain the only striatal neurons projecting directly to the substantia nigra *pars compacta* thus providing a direct loop to control dopamine release (Gerfen, 1992, Crittenden and Graybiel, 2011, Lopez-Huerta et al., 2015).

Cell population and the classical basal ganglia model

In rodents, the striatum is composed of about 90-95% of medium sized perikaryon densely spined cells called Medium spiny neurons (MSNs) and 5-10% of medium to large aspiny GABAergic and cholinergic interneurons. The MSNs integrate cortical and modulatory information and send GABAergic projections to downstream basal ganglia nuclei. According to their projection sites and the proteins they express these neurons form the striatonigral *direct* (co-expressing dopamine D₁

receptors_D1R, Dynorphin and Substance P) or the Striatopallidal *indirect* pathway (co-expressing dopamine D₂ receptors_D2R, Enkephalin and adenosine A_{2A} receptor_A2AR). Striatonigral pathway MSNs project monosynaptically to the medial globus pallidus (MGP) and substantia nigra *pars reticulata* (SNr). Striatopallidal MSNs project to the lateral globus pallidus (LGP) and reach the SNr and MGP by synaptic relay through the subthalamic nucleus (STN) (Figure 2) (Gerfen and Surmeier, 2011, Lobo et al., 2006, Lobo and Nestler, 2011, Ena et al., 2011, Schiffmann et al., 1991, Gerfen et al., 1990).

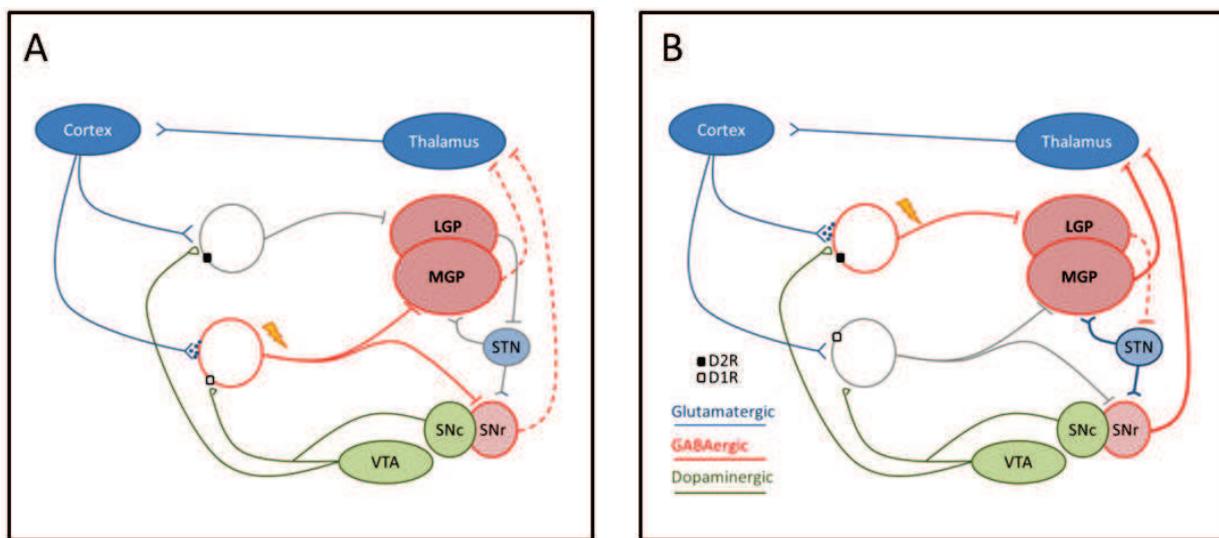


Figure 2: Schematic representation of the activation of Direct (A) and Indirect (B) projection pathways in the rodent dorsal striatum. (A) Direct pathway neurons promote movement decreasing inhibition of the thalamus. (B) Activation of the indirect projection pathway leads to the inhibition of the thalamus and consequent thalamo-cortical pathway. Excitatory (glutamatergic) synapses are represented in « arrow » shape. Inhibitory synapses (GABAergic) are represented by a bar. Modulatory (dopaminergic) synapses have round shape. Dashed lines indicate inactivity of the projection while bolded lines express increased activity. Abbreviations: LGP, MGP: lateral and medial globus pallidus respectively; STN: subthalamic nucleus; SNc, SNr: Substantia Nigra pars compacta and reticulata respectively; VTA: ventral tegmental area. Schematic representation based on Voorn *et al.*, 2004; Gerfen and Surmeier, 2011; Albin *et al.*, 1989; Calabresi *et al.*, 2014.

Fifteen years ago, researchers hypothesized a dual organization of the striatum and basal ganglia output. According to this classical model the direct and indirect projection pathways have opposite but balancing roles in the control of motor behavior. Activation of striatonigral MSNs

would inhibit GABAergic neurons of the SNr and MGP leading to a disinhibition of the thalamocortical glutamatergic neurons. Thus, activation of the striatonigral pathway is thought to promote movement (Figure 2A). Conversely, inhibition of the LGP by striatopallidal GABAergic neurons would disinhibit the glutamatergic neurons of the STN. Consequent activation of the MGP and SNr GABAergic neurons would hence inhibit the thalamus and therefore reduce locomotor activity. (Fig 2B)(Albin et al., 1989, Ena et al., 2011, Calabresi et al., 2014, Valjent et al., 2009). However, the clear identification of the differential function of the two MSNs subpopulations has been limited by their morphological similarities and heterogeneous distribution in the striatum.

The emergence of recent cell-type specific methodology including Bacterial artificial chromosomes (BAC) transgenesis, optogenetics and viral transgenesis has allowed to specifically target striatonigral and/or striatopallidal neurons. These techniques have allowed a better understanding on MSNs subtypes specific functions. For instance, the use of BAC reporter mouse lines in which EGFP or tdTomato (green and red fluorescent proteins) are expressed under the control of D₁R or D₂R promoters (*Drd1a* and *Drd2* respectively) has allowed to confirm the projection pattern of the basal ganglia model. Also, these mice have finally permitted the establishment of the proportion of MSNs expressing D₁R, D₂R or both (table1) (Valjent et al., 2009). Despite the general confirmation of the neuronal segregation previously proposed, these new methodologies have also allowed some interesting new insights.

Table1: Estimation of cell populations in the striatum of EGFP-expressing BAC transgenic mice (Valjent et al., 2009) (drd1a: Dopamine receptor 1 promotor; drd2: dopamine receptor 2 promotor)

For instance, in *Drd2*-eGFP mice, labeling was not only found in LGP but also in cholinergic interneurons as well as in neurons of the SNc and VTA, the latter corresponding to autoreceptors

	Dorsal striatum	NAc core	NAc shell
Neurons (% of total cells)	70	80	75
MSNs (% of total cells)	68	78	73
% MSNs with active <i>drd1a</i> only	52	53	47
% MSNs with active <i>drd2</i> only	43	41	36
% MSNs with active <i>drd1a</i> and <i>drd2</i>	5	6	17

located in dopaminergic neurons (Gong et al., 2003, Shuen et al., 2008). Moreover, while all MSNs projecting to the SNr expressed D₁R (and very few D₂R) some D₁R-EGFP-expressing fibers projected

to the LGP (Matamales et al., 2009). This result could either imply that neurons co-expressing D₁R and D₂R project to the LGP or that striatonigral MSNs extend collateral fibers to the LGP. This latter hypothesis is in agreement with the report of Cazorla *et al* (Cazorla et al., 2014) in which they found that increased excitability of the indirect pathway induced the growth of direct pathway bridging collaterals in the LGP. This possible interaction between direct and indirect pathway has important implication when interpreting behavioral regulation elicited by cell-type specific MSNs manipulation (Cazorla et al, 2015)

It is important to notice that these subpopulations of MSNs projections were originally characterized in dorsal striatum and projections from the Nac may not segregate in a similar manner (Figure 3). In fact, both D₁R and D₂R-expressing MSNs in the Nac (which have similar co-expression pattern then DS MSNs) project to the ventral pallidum (VP) which has intra and extra-basal ganglia projections (Kupchik et al., 2015). As such, projections to the VP are thought to functionally correspond to both direct and indirect pathway as they may oppositely regulate reward-seeking behavior (discussed below) (Gerfen, 1992, Nicola, 2007, Smith et al., 2013).

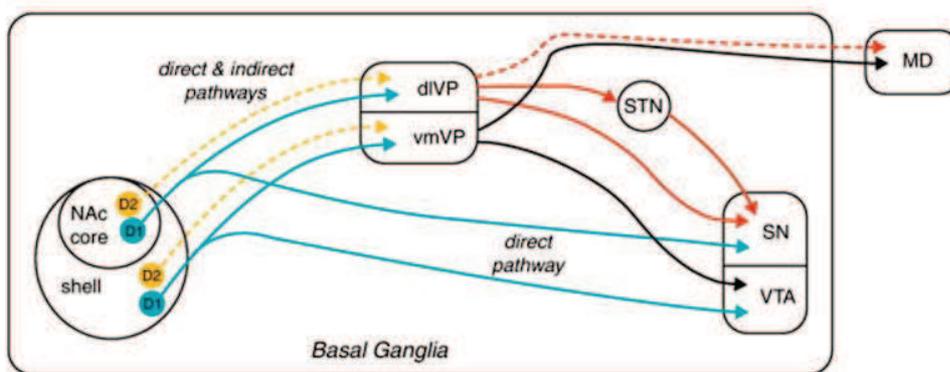


Figure 3: Schematic representation of Nac output pathways. MSNs from the Nac core project to the dorsolateral ventral pallidum (dIVP) while Naccshell project to the ventromedial part of the VP (vmVP). The dIVP sends direct projections to the Substantia nigra (SN) or through the subthalamic nucleus (STN, solid red lines) and, to a smaller extend also projects to the mediodorsal thalamus (MD, dashed lines). Neurons from the vmVP also project to the MD and the ventral tegmental area (VTA). D1-expressing MSNs (but not D2-MSNs) from both core and shell project to the VTA. (Smith, R.J. et al, 2013)

Modulation of glutamatergic inputs onto direct and indirect MSNs by dopaminergic projections

Electrophysiology and anatomical reports revealed distinct physiological properties of MSNs subtypes and show that striatopallidal cells are intrinsically more excitable and present smaller somatodendritic trees than striatonigral neurons (Kreitzer and Malenka, 2007, Gertler et al., 2008, Do et al., 2012, Gerfen and Surmeier, 2011). At rest, both MSNs subtypes are generally inhibited (“down state”) until membrane depolarization by excitatory glutamatergic release (switching to an “up state”). Dopamine is thought to oppositely regulate glutamatergic transmission in distinct MSNs subtypes based on their differential enrichment in excitatory D₁R or inhibitory D₂R. Briefly, activation of D₁R (acting through G_s and G_{oif}) facilitates glutamatergic transmission by somatic depolarization through increased L-type Ca²⁺ channels flow and decreased K⁺ currents. Oppositely, D₂R stimulation increases outward hyperpolarizing K⁺ channels and decreases dendritic Ca²⁺ currents reducing the excitability of striatopallidal neurons (Hiroi et al., 2002, Grace et al., 2007, Gertler et al., 2008, Gerfen and Surmeier, 2011, Do et al., 2012, Surmeier et al., 2007). Additionally, D₂R stimulation diminishes glutamate presynaptic release and AMPA receptor currents on MSNs (Hernandez-Echeagaray et al., 2004, Bamford et al., 2004, Gerfen and Surmeier, 2011, Do et al., 2012). Also, dopamine receptors are believed to differentially respond to phasic or tonic dopamine neurons firing. In fact, DA phasic firing modulates low-affinity D₁R and is assumed to be crucial in reward related behavior. Conversely, tonic DA firing stimulation of high-affinity D₂R has been shown to be suppressed by aversive stimuli (Grace et al., 2007, Mirenowicz and Schultz, 1994, Mirenowicz and Schultz, 1996, Ungless, 2004, Ungless et al., 2004). Also, DA striatal depletion has been found to selectively decrease spine density and glutamatergic synapses in striatopallidal MSNs (Day et al., 2006). Thus, changes in DA levels in the striatum differentially alter activity in the direct and indirect striatal output pathways.

Striatal projection neurons in motor control and motivational processes

Motor control: Converging evidence support the opposing influence of striatopallidal and striatonigral neurons in motor output systems. While bilateral optogenetic excitation of the striatopallidal pathway decreased locomotor initiation (Kravitz et al., 2010), ablation or disruption

of these neurons increased motor activity (Durieux PF, 2012, Bateup et al., 2010, Durieux PF, 2009) thus confirming the inhibitory functions of the indirect pathway. In contrast, optical stimulation of striatonigral MSNs increased locomotion while disruption or ablation of these neurons had the opposite effect. Evidence using inducible cell-target injection of diphtheria toxin (DT) further suggests a differential role of striatopallidal and striatonigral neurons in acquisition and expression of motor skill learning (Durieux PF, 2012). Inducible ablation of striatopallidal neurons delayed the acquisition of rotarod task but had no effect in a previously acquired motor skill. However, injection of DT in striatonigral neurons impaired motor skill learning regardless of the training extension and also disrupted performance of a previously learned motor sequence. Interestingly, a recent report (Cui et al., 2013) shows that direct and indirect pathway MSNs are concurrently activated when animals initiate an action. However, rather than refuting the classical model, these recent findings propose a more comprehensive view of basal ganglia function. According to this updated model co-activation of both pathways is necessary for action selection and initiation as well as inhibition of competing motor programs. In this case, hyperactive behavior or early motor skill impairments observed after indirect pathway inactivation could result from the lack of inhibition of unwanted motor program (Cui et al., 2013, Macpherson et al., 2014).

Motivation and goal-directed behavior: Recent findings also support a distinct role of direct/indirect pathway in mediating reinforcement and punishment processes. While optogenetic activation of the direct pathway was shown to increase reinforcement (mice persistently activated a trigger that elicited further direct pathway bilateral illumination), inactivation of these neurons by reversible neurotransmission blockage decreased conditional place preference (CPP) to appetitive stimuli (cocaine and chocolate). In contrast, activation of indirect MSNs induced transient punishment (mice transiently avoid the trigger that would illuminate the indirect pathway) while transmission blockage of this pathway impaired aversive behavior having no effect on reinforcement (Kravitz et al., 2012, Hikida et al., 2010). Interestingly, it was also shown that D₁R inhibition (D₁R antagonist SCH23390) and D₂R activation (D₂R agonist quinpirole) in the Nac mimics the deficit in reinforcement and punishment (respectively) caused by neurons transmission blocking (Hikida et al., 2013). Accordingly, silencing of VTA DA neurons evoked aversive responses to preferred dark room, and this response was abolished by D₂R but not D₁R knockdown (Danjo et al., 2014).

In contrast to the studies reported above in which inactivation of the striatopallidal neurons had no effect on CPP, silencing of this pathway in rats (Ferguson et al., 2011) and mice (Durieux et al., 2009) led to an increased amphetamine induced sensitization and CPP respectively. Similarly, Lobo *et al* (2010) demonstrated that activation of the indirect pathway decreased cocaine conditioned CPP while direct pathway MSNs activation had the opposite effect (Lobo et al., 2010). In Summary, data suggest that striatonigral transmission promotes reinforcement whereas striatopallidal inhibits drug reinforcement. Also, striatopallidal transmission is critical for aversive learning and avoidance behavior.

Interestingly, a distinct role of Accumbens projection neurons was also demonstrated in social interaction behavior also considered as a form of reward. Recent studies in mice demonstrate that intra-Nac infusion of D₁R but not D₂R antagonist blocked the pro-social effect elicited by increased DA levels in the Nac. Most importantly, optical activation of striatonigral MSNs was sufficient to increase social behavior and sucrose preference or reversed social avoidance induced by social defeat stress. Conversely, striatopallidal MSNs activity induced social avoidance after subthreshold social defeat stress. (Francis et al., 2015, Gunaydin et al., 2014) This data have important implications by unveiling new cell-specific functions of MSNs and extending this field to affective disorders research.

Chapter 2:

Basal Ganglia disorders

Basal ganglia disorders englobe several neurological diseases characterized by abnormalities in one or more components of basal ganglia circuit consequently displaying alteration in motor behavior, psychiatric signs and, in some cases, cognitive impairment. Although dysfunction in other brain regions are often also present, basal ganglia malfunctions are central to the pathophysiology of such diseases. The term “basal ganglia disorders” classically refer to motor disorders such as Parkinson and Huntington diseases that clearly arise from neurodegeneration in one or several basal ganglia structures (Albin et al., 1989, Wichmann and DeLong, 1996). However, with the development of new research tools and the gain in understanding of basal ganglia function and malfunction, a growing number of researchers include neuropsychiatric diseases in the family of basal ganglia disorders. Also, although not listed as cardinal symptoms, the majority of patient affected by motor disorders such as Parkinson or Huntington’s disease exhibit psychiatric symptoms (Ring and Serra-Mestres, 2002, Crittenden and Graybiel, 2011, Cazorla et al., 2015, Lobo and Nestler, 2011).

In the present section we will review some of the most common and well-studied striatal-related neuropsychiatric and motor disorders. A focus will be made on rodent research especially on mouse behavioral phenotypes related to such disorders. Animal models of human diseases are designed to (1) test hypothesis about the mechanism of the disease and (2) predict treatment response in human patients. To be considered as valid, an animal model should fulfill three main standard criteria: construct, face and predictive validity. Construct validity postulate that the method by which the model is constructed should be based on the known etiology of a disease. This criterion is especially difficult to satisfy as for most diseases the etiology is largely unknown. Models that best achieve this criteria use known gene variants or risk factors associated with diseased human populations. Face validity refers to the ability of an animal model to reflect anatomical, biochemical, neuropathological or behavioral features of the human disease. Given the difficulties

in diagnosing psychiatric disorders in humans, added to the absence of specific markers and the subjective and heterogeneous nature of symptoms, most of the animal models of psychiatric disorders capture specific aspects rather than recapitulate all components of the syndrome studied. Predictive validity indicates that a treatment currently used in a human disease should have the same efficacy in an animal model of such disease and, for some authors, the model's performance should help predict human phenomenon (Nestler and Hyman, 2010, Razafsha et al., 2013, Fernando and Robbins, 2011, Wong and Josselyn, 2015, Crawley, 2000, Belzung and Lemoine, 2011).

Parkinson's disease

Parkinson's disease (PD) is a neurodegenerative disorder characterized by the triad rigidity, tremor at rest, and bradykinesia (slowness in the performance of voluntary movements). This chronic disease has a progressive course and postural instability is also seen at a later stage of the disease. All patients with PD do not experience all three cardinal symptoms and two major subtypes have been defined: akinetic-rigid and tremor-dominant. PD is rarely seen in people under 40 years old and the American prevalence for people over 65 years old is of 1,6% (Xia and Mao, 2012, Beitz, 2014). Non-motor-related symptoms are often experienced at any stage of the disease and include anxiety, sleep disturbance, depression, constipation, hallucinations and a progressive decline of cognitive functions (sometimes leading to dementia)(Chaudhuri and Naidu, 2008).

The main pathological process giving rise to PD's symptoms is a massive loss of nigrostriatal dopaminergic (DA) neurons and the development of lewy bodies mainly composed of α -synuclein. Motor symptoms do not become apparent until 60-80 % loss of striatal dopamine. Also, neurodegeneration and α -synuclein are also found in the locus ceruleus, hypothalamus, cerebral cortex and central and peripheral components of the autonomic nervous system (Goedert et al., 2013, Le et al., 2014). Degeneration of nigral DA neurons is thought to create an imbalance between direct and indirect pathways of the basal ganglia resulting in a shift of the balance to the indirect

pathway with increased excitation of the Medial globus pallidus (also referred as internal globus pallidus _GPI) and substantia nigra pars *reticulata* (SNr) (Wichmann and DeLong, 1996) (Figure 4)

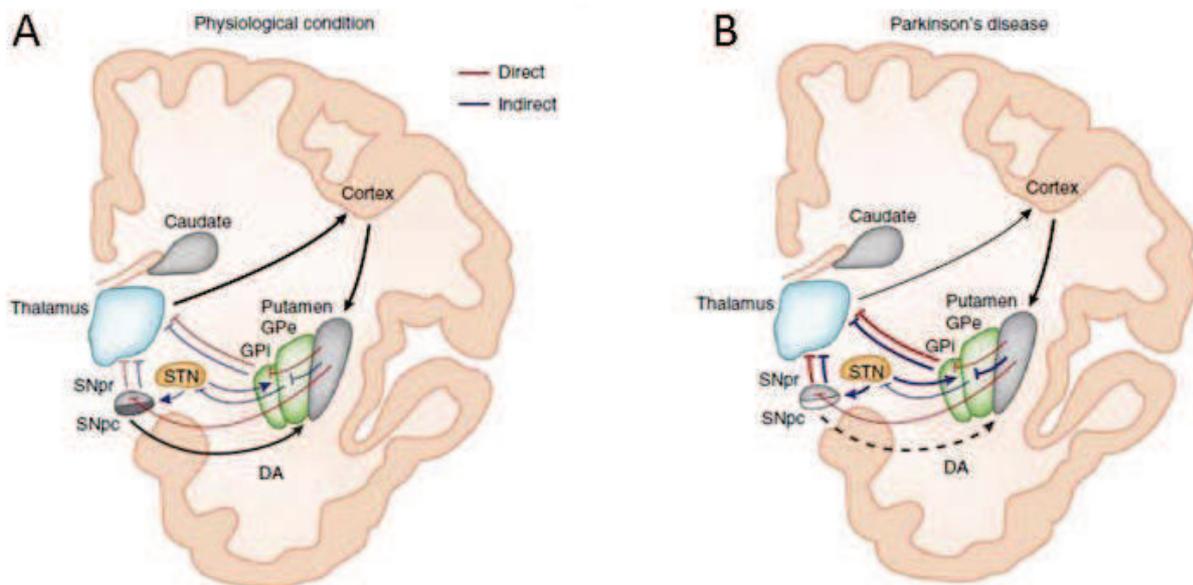


Figure 4: Schematic representation of the direct/indirect pathway classical model in the physiological condition and in Parkinson's disease. (A) In the physiological condition, DA arising from Substantia nigra pars compacta (SNpc) is thought to activate D1-expressing MSNs of the direct pathway (red lines) and to inhibit D2-expressing MSNs of the indirect pathway (blue lines). The output nuclei internal globus pallidus (GPi) and Substantia nigra pars reticulata (SNpr) project to the thalamus which in turn sends efferents that complete the cortico-basal ganglia-thalamo-cortical loop. (B) In Parkinson's disease, degeneration of nigral neurons reduces DA receptor stimulation in striatal MSNs. The imbalance between direct and indirect pathways results into abnormal activation of output nuclei and over inhibition of thalamic neurons projecting to the cortex. (Calabresi et al., 2014)

Heritable genetic factors are responsible for more than 10 % of PD cases ("familial PD") and while several genes including α -synuclein, parkin and VPS35 have been identified, the mechanisms through which these genetic defects cause neurodegeneration are still largely unknowns (Klein and Westenberger, 2012) . Also, various environmental risk factors such as exposure to manganese, solvents and some pesticides have been identified and associated with hallmarks of PD such as mitochondrial dysfunction and aggregation of α -synuclein (Chin-Chan et al., 2015).

There are mainly two kinds of animal models of PD: genetic models based on the known genetic variants found in PD patients and neurotoxic models that used either neurotoxin with known mechanisms (e.g. 6-OHDA and MPTP) or pesticides/herbicides (e.g. rotenone, paraquate) identified as risk factors for PD (Le et al., 2014). Behaviorally, animal models of Parkinson disease should at least present deficient motor coordination and balance as measured in the accelerating rotarod and balanced beam; decreased locomotion and grip strength as well as catalepsy. Striatal concentration of dopamine and its metabolites 3, 4-Dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) should be decreased and DA neurons loss should be verified. Finally, the presence of Lewy bodies can be measured by α -synuclein immunoreactivity (Valadas et al., 2015, Baptista et al., 2013).

Using 6-OHDA-mediated depletion of DA nigrostriatal neurons, Day and colleagues showed that striatal DA depletion leads to a selective decrease of indirect pathway's spines and glutamatergic synapses (Day et al., 2006). Interestingly, these authors also found that indirect pathway neurons are intrinsically more excitable than direct pathway neurons and that DA depletion increases this asymmetry (Day et al., 2008). Accordingly, optogenetic studies indicate that bilateral excitation of indirect pathway neurons elicits a parkinsonian state (increased freezing, bradykinesia and decreased locomotor initiation) (Kravitz et al., 2010). However, data indicating concurrent activation of both pathways during action initiation and the presence of axonal bridging collateral functionally linking both pathways should be considered when interpreting new data regarding Parkinson disease (Cazorla et al., 2015, Cui et al., 2013, Calabresi et al., 2014).

The gold standard in the treatment of PD consists of a DA replacement using the DA precursor L-DOPA. However, this treatment does not prevent the progression of the disease and is often associated with motor fluctuations and dyskinesia (involuntary movements). This treatment is often accompanied by DOPA decarboxylase inhibitors (reduce conversion of L-DOPA to dopamine in peripheral tissue thus reducing undesirable adverse effects) or catechol-O-methyl transferase (COMT) inhibitors. Other pharmacotherapies include dopamine agonists targeting dopamine post-synaptic D₂ receptors. Deep brain stimulations of the subthalamic nucleus (STN) or GPi are realized in patients with persistent motor symptoms despite optimal pharmacological therapy (Tarazi et al., 2014). Viral vector gene therapies aiming at restoring normal functioning of the STN also represent new promising therapeutic leads (Kaplitt et al., 2007)

Huntington's disease

Huntington's disease (HD) is an autosomal dominantly inherited progressive neurodegenerative disease caused by an instable CAG repeat expansion in the huntingtin gene (*HTT*). This disease leads to a prominent loss of GABAergic medium spiny neurons in the striatum accompanied by cortical projection neuronal loss in the deep layer cortex (Chang et al., 2015). Clinically, HD is described by the triad: motor dysfunctions, psychiatric symptoms and cognitive decline. While the age of onset of motor symptoms (between the ages of 30-50 years) is inversely correlated with the length of CAG repeats, this is not the case for psychiatric and cognitive decline which can develop sooner than motor dysfunctions (Stine et al., 1993, Vinther-Jensen et al., 2015).

Motor abnormalities in HD include chorea (a dance-like involuntary movement) which is the major motor symptom of this disease, bradykinesia and rigidity. In later stages of the disease, symptoms such as dystonia (involuntary muscles contractions), abnormal postures or dysphagia (difficulties swallowing) may also arise (Folstein et al., 1986). In rodents, HD's motor dysfunctions may be assessed by evaluation of spontaneous activity and general locomotion. Also, the use of rotarod, balance beam test, climbing test, and footprint or gait analysis may reveal information about motor coordination of the rodent model (Brooks and Dunnett, 2009, Pouladi et al., 2013). Cognitive deficits may be evident in the early stages of HD and include memory decline (visuospatial, procedural and episodic) measured in animal models of HD through a number of behavioral test such as the Morris water maze, Barnes circular maze and novel object recognition. Associative learning has also been studied through use of classical and operant conditioning (Pouladi et al., 2013). Lastly, sensorimotor gating deficits were also found in HD patients (Geyer, 2006). Sensorimotor gating refers to the inability of filtering non-relevant information and to allocate selective attentional resources to salient stimulus. Deficient sensorimotor gating is thought to underlie attentional and information processing problems. This process can be evaluated in humans and rodents in a very similar way using the prepulse inhibition test (PPI). In rodents, PPI test is used both as a tool to validate animal models of psychiatric diseases characterized by sensorimotor gating deficits and as a way to better understand key elements of the pathophysiology of such disorders. (Swerdlow and Geyer, 1998, Swerdlow et al., 2000, Powell et al., 2012, Geyer, 2008) Patients

suffering from HD may also present psychiatric symptoms such as depression, anxiety, impulsivity and aggressive behavior. Moreover, hallucinations, delusions and psychosis may occur in advanced stages of the disease (Rosenblatt, 2007) . Depression like behavior has been demonstrated in transgenic mice models of HD using the forced swim test and the sucrose preference test (Pang et al., 2009).

Despite the known etiology of the disease and the development in understanding the pathophysiology of neuronal death, HD remains without a cure and treatment are directed toward symptoms of the patients and aim at improving their life quality (Kumar et al., 2015). For instance, clonazepam (benzodiazepine) might be prescribed for symptoms such as chorea, dystonia or rigidity while antipsychotic drugs may attenuate psychosis and irritability. SSRI's are also frequently prescribed to control anxiety and depression (Ross and Tabrizi, 2011) .

Schizophrenia

Schizophrenia is a complex and disabling disorder that affects more than 1% of the worldwide population. The disorder often onsets in late adolescence or early adulthood and typically persists throughout life (Nestler and Hyman, 2010, Tsai and Coyle, 2002). Symptoms are classified in three major clusters: negative, positive and cognitive symptoms.

Negative symptoms include social withdrawal and diminished motivation. Hypodopaminergic states in the frontal cortical terminals as well as mesolimbic dopamine hypofunction resulting in abnormal ventral striatum function are thought to contribute to these symptoms (Davis et al., 1991, Murray et al., 2008, Bolkan et al., 2015). In animal models, one way to assess these symptoms is a social interaction test reflecting social withdrawal and diminished motivation to social reward as observed in schizophrenic patients (Razafsha et al., 2013, Crawley, 2000, Duncan et al., 1999, Bubenikova-Valesova et al., 2008, Samsom and Wong, 2015). Positive symptoms are psychotic behaviors characterized by the presence of abnormal processes such as hallucinations, delusion and stereotyped behavior. These symptoms are thought to be related to excessive mesolimbic dopaminergic release in ventral as well as dorsal striatum (Davis et al., 1991, Duncan et al., 1999). In healthy subjects, increased dopamine release induced by amphetamine

intake can mimic psychotic states. Moreover, in schizophrenic patients low doses of psychostimulants can exacerbate psychotic symptoms suggesting a sensitization of dopamine systems (Bell, 1965, Davis et al., 1991). In rodents, increased basal or amphetamine induced locomotion and motor stereotypies are considered as models of positive symptoms as they are correlated to hyperdopaminergic states (Carlsson et al., 2001, Wong and Josselyn, 2015, Bubenikova-Valesova et al., 2008). Cognitive symptoms such as working memory and sustained attention deficits are thought to result from cortical dysfunction. More precisely, abnormal function of the prefrontal cortex during the performance of executive function tasks in fMRI studies have been suggested to contribute to such symptoms (Karlsgodt et al., 2010). In rodents, cognitive impairments can be measured through the use of a delayed nonmatch to place task or a maze task measuring working or spatial memory (*e.g.* morris water maze, T-maze) (Wong and Josselyn, 2015, Bubenikova-Valesova et al., 2008, Razafsha et al., 2013, Samsom and Wong, 2015). Another common symptom in schizophrenia is the deficit in sensorimotor gating.

Schizophrenia is thought to result from the interplay between genetic risk factors and environmental influences. Many studies suggest a high heritability of this disease and a variety of genetic (*e.g.* *DRD2*) and environmental risk factors (*e.g.* prenatal viral exposure or autoimmune reactions) have been identified (Schizophrenia Working Group of the Psychiatric Genomics, 2014, Gottesman and Erlenmeyer-Kimling, 2001, Dean and Murray, 2005). Finally, at a neurochemical level, schizophrenia symptoms are thought to result from interplay of altered monoaminergic, Glutamatergic and GABAergic transmission (Lillrank et al., 1995, Lipska, 2004, Carlsson et al., 2001, Tsai and Coyle, 2002, Duncan et al., 1999, Wong and Josselyn, 2015, Nestler and Hyman, 2010).

Typical and atypical antipsychotics are the most commonly used pharmacological treatments for schizophrenic patients. Typical antipsychotics refer to substances such as haloperidol that directly acts on blocking Dopamine 2 like receptors (D2, D3 and D4 dopamine receptors) while atypical (second generation) treatments such as risperidone and clozapine also blocks 5-HT_{2A} serotonin receptors. However, in addition to their unwanted side effects, these medications have been reported to majorly act on positive symptoms being ineffective in cognitive and negative deficits (Fenton et al., 2003, Geyer, 2008, Miyamoto et al., 2012)

Attention deficit/Hyperactive disorder (ADHD)

Attention deficit/hyperactivity disorder (ADHD) is among the most common neuropsychiatric disorders (5-10% of children worldwide) with average age of onset at 7 years old and persisting through adolescence into adulthood for a great majority of patients. Clinically, symptoms are characterized by various combinations and degrees of the triad: Hyperactivity, Impulsivity and Attentional deficits (Faraone et al., 2003, Russell, 2007, Leo and Gainetdinov, 2013, Fernando and Robbins, 2011, Rader et al., 2009, Dopheide and Pliszka, 2009).

The existences of various subtypes of ADHD (predominantly inattentive subtype, predominantly hyperactive/impulsive subtype, and combined subtype) added with the high variability between subjects have limited the establishment of a theoretical rationale for the neurobiology underlying ADHD. Models often propose deficient inhibitory control in interaction with executive function but also altered reward sensitivity and motor control (Barkley, 1997, Dopheide and Pliszka, 2009, Sagvolden et al., 2005, Tripp and Wickens, 2012). Neuroimaging studies show reduced volume and decreased activation of the prefrontal and anterior cingulate cortices, as well as right caudate in ADHD patients during a response inhibition task (Dickstein et al., 2006, Valera et al., 2007). These abnormalities are thought to result in altered inhibitory control and reward processing respectively. Also, in a monetary delayed incentive task, adults and adolescents with ADHD show reduced activation of the ventral striatum in response to a cue predicting reinforcement (Scheres et al., 2007, Strohle et al., 2008). Furthermore, impulsive rats were shown to have reduced D2/D3 dopamine receptors binding in the ventral striatum (Dalley et al., 2007).

In rodents, hyperactivity is typically measured in a homecage or in an open field although the latter is a more valid model of hyperactivity since it involves a novel environment. As this symptom in children develops gradually when a situation becomes familiar, rodent models should not display hyperactivity in a novel environment but rather develop it over time (Sagvolden et al., 2005, Samsom and Wong, 2015). Motor Impulsivity is measured in rodents using operant tasks such as the five choice serial reaction time tests (5-CSRTT) and is operationalized as bursts of response not resulting in increased number of reinforcement (Fernando and Robbins, 2011, Sagvolden, 2000,

Sagvolden et al., 2005). Like hyperactivity, motor impulsivity is not present in novel situation or environments (Sagvolden et al., 2005). Likewise, the most common and well validated test to measure sustained attention is the 5-CSRTT in which accuracy of response is considered as an operational measure of sustained attention (Zimmermann et al., 2014, Sagvolden et al., 2005, Samsom and Wong, 2015, Sagvolden, 2000, Russell, 2007, Leo and Gainetdinov, 2013). Finally, PPI is also disrupted in ADHD subjects and is therefore used as a tool in the research and validation of animal models of ADHD (Kohl et al., 2013, Feifel et al., 2009, Woo et al., 2014)

Clinical studies indicate that ADHD has a significant genetic component involving several genes implicated in dopamine (dopamine receptor 4, *Drd4*; dopamine receptor 5, *Drd5*; and dopamine transporter, DAT) Norepinephrine (Norepinephrine transporter, NET) Glutamatergic (SNAP-25: protein required for transmitter release) and serotonergic (serotonin transporter, SERT) transmission (Gizer et al., 2009, Kim et al., 2008, Faraone and Khan, 2006, Purper-Ouakil et al., 2011). The etiology of this disorder most likely involves several genes in interaction with environmental factors. Several environmental risk factors have been identified and include prenatal exposure to tobacco, alcohol and other drugs or pregnancy/birth complications (Purper-Ouakil et al., 2011, Pineda et al., 2007, Thapar et al., 2003).

Pharmacological treatment of ADHD normally requires monoaminergic psychostimulants such as Methylphenidate (MPH) and Amphetamine (AMPH) or the catecholaminergic nonstimulant atomoxetine (Leo and Gainetdinov, 2013, Sagvolden et al., 2005, Rader et al., 2009)

Bipolar Disorder (BPD)

Bipolar disorder (BPD), also known as manic-depressive illness, is a chronic disease affecting 1-4% (depending on the subtype, see below) of the world's population and is characterized by episodes of mania with or without depression. This disease is classified into Bipolar I (one or more manic episodes) and Bipolar II (one or more hypomanic episodes and at least one depressive episode). Bipolar I is frequently accompanied by depressive episodes; in bipolar II, hypomanic episodes are defined as less severe forms of mania (Gould and Einat, 2007, Angst, 2013). The age of onset is not

clearly defined as some people have their first symptoms during childhood, while others may develop symptoms late in life (Young, 2005, Youngstrom et al., 2005).

This illness has a progressive and accelerating course with worsening of the clinical symptoms, reductions of inter-episode duration and response to treatment with the progression of the disease (Berk et al., 2011, Strojilovich et al., 2015). Furthermore, enlargement of the lateral ventricles with the recurrence of episodes has also been reported (Strakowski et al., 2002, Brambilla et al., 2001). BPD is known for its high heritability (~ 90%) and recent genome-wide association studies have identified several new robust risk variants which are currently being studied (Shinozaki and Potash, 2014, Kennedy et al., 2015). Neuroimaging studies have identified several volumetric and functional abnormalities in networks involving the prefrontal cortex, the anterior cingulate cortex, the striatum, the pallidum, the thalamus and the amygdala. It is hypothesized that altered prefrontal cortical regulation of limbic areas result in maladaptive emotional response and cognitive impairments (Chen et al., 2011, Maletic and Raison, 2014). Additionally, changes in oligodendroglia levels and alterations in GABA, Glutamate and monoamine transmission were also reported with glutamatergic alterations being the most consistent evidence (Kondo et al., 2014, Jun et al., 2014, Maletic and Raison, 2014).

Given the cyclic nature of the episodes and the inconclusive knowledge about the pathophysiology of this disease, the development of animal models of BPD has been very limited. Some investigators have attempted to generate cycling models but with incomplete validity with most studies working on symptom-based models. Symptoms of mania are operationalized in rodents as basal hyperactivity, supersensitivity to the locomotor effects of psychostimulants, increased aggressive behavior, changes in sleep pattern or increased goal directed and risk-taking behavior (Gould and Einat, 2007, Nestler and Hyman, 2010, Geyer, 2008). Excessive dopamine neurotransmission has been associated with the development of manic symptoms and the most often used rodent models of mania involve psychostimulant treatments (Berk et al., 2007, Nestler and Hyman, 2010). BPD patients also present cognitive impairments such as deficits in sustained attention and sensorimotor gating, both measurable symptoms in rodents (see sections above) (Perry et al., 2001, Bora et al., 2009). Moreover, patients affected with BPD often suffer from medical comorbidities such as cardio-and cerebrovascular disease and metabolic and endocrine

disorders which, added to increased suicide risk, severely reduces life expectancy of these patients (McIntyre et al., 2004, McIntyre et al., 2008, Chang et al., 2011).

Pharmacological treatment of BPD is generally episode-driven and polypharmacology is a common practice. Although mood stabilizers such as lithium or valproate are standard treatments in BPD, antipsychotics and antidepressants are frequently used. These treatments' efficiency is however very limited especially when it comes to cognitive impairments and several new treatments are currently being assessed (Konstantakopoulos et al., 2015).

Anxiety disorders

While fear is an adaptive reaction to an immediate and real danger, anxiety is a response to a threat or a potential danger accompanied by a heightened state of arousal and vigilance. Normal anxiety has a preservative function mediating avoidance behavior towards potentially harmful stimuli. This process becomes pathological when there are quantitative (excessive response to threatening stimuli) or qualitative (anxiety response to non-threatening stimuli) variations. Anxiety disorders are the most common psychiatric disorders (up to 29% of lifetime prevalence rates) being characterized by a sustained state of apprehension in the absence of immediate fear frequently accompanied by cognitive impairments (Newby et al., 2015). Clinically, anxiety disorders include generalized anxiety disorder (GAD), phobias, post-traumatic stress disorder (PTSD), panic and obsessive-compulsive disorder (OCD) (although, in the fifth edition of the *Diagnostic and statistical manual of American psychiatric association*, the latter two disorders have been removed from the anxiety disorder category) (Belzung and Griebel, 2001, Craske et al., 2009, Aupperle and Paulus, 2010).

Pavlovian fear conditioning has been extensively used to understand the etiological basis of anxiety disorders (Craske et al., 2009, Mineka and Zinbarg, 2006). The amygdala has been repeatedly shown to regulate the acquisition and expression of conditioned fear (Davis, 1992, Wilensky et al., 2006) and patients suffering from anxiety show enhanced amygdala activation that positively correlates with the severity of the pathology (Stein et al., 2007). Furthermore, functional neuroimaging in human indicate that, in patients suffering from GAD, the activation pattern of structures such as the insula, caudate nucleus, anterior cingulate cortex or ventromedial prefrontal

cortex (vmPFC) fail to discriminate between conditioned stimulus paired with an unconditioned stimulus and a non-paired similar stimulus, suggestive of excessive fear generalization (Cha et al., 2014, Dunsmoor and Paz, 2015). Finally, based on human neuroimaging data, a model of the neurocircuitry underlying PTSD postulates that hyperresponsivity of the amygdala and insufficient inhibitory control of the vmPFC over the amygdala contribute to deficient extinction of the conditioned fear response. (Rauch et al., 2006).

Avoidance behavior of a perceived threatening stimulus can be measured in rodents using several well validated anxiety models of innate (unconditioned) anxiety (more frequently used in transgenic mice). This is the case of the open field (OF), the elevated plus-maze (EPM) and the light-dark test. These “ethological tests” use the natural approach/avoidance conflict in which a rodent tendency to explore novel environment is confronted to the innate drive to avoid brightly illuminated and expose areas (Bailey and Crawley, 2009, Razafsha et al., 2013, Nestler and Hyman, 2010, Aupperle and Paulus, 2010). Optogenetic studies applied to the EPM and OF showed that stimulation of basolateral amygdala (BLA) neurons projecting to the central nucleus of the amygdala (CeA) has anxiolytic effects while inhibition of these neurons has the opposite (anxiogenic) effect (Tye et al., 2011, Tye and Deisseroth, 2012). Furthermore, using the same strategy, it was also shown that stimulation of BLA neurons projecting to the ventral hippocampus (vHPC) increased anxiety in the OF and EPM tests (Felix-Ortiz et al., 2013). These authors also show that the activation of BLA-vHPC similarly reduces social behavior suggesting a common neural mechanism. Indeed, patients suffering from GAD present social dysfunction and, in some cases, anxiety may be directed to social functioning (social anxiety disorder) (Schneier, 2006). As such, social interaction tests such as the intruder-resident or the Crawley test should be included in research on anxiety related processes (Allsop et al., 2014). Other, more complex, conflict tests such as the novelty suppressed feeding or Vogel test use natural biological needs and anxiogenic conditions to create a conflict (Bodnoff et al., 1989, Dulawa et al., 2004, Razafsha et al., 2013).

Current treatments for anxiety disorder include selective serotonin reuptake inhibitors (SSRIs) and serotonin and norepinephrine reuptake inhibitors (SNRIs). These drugs, also used in the treatment of depression, are effective for some anxiety disorders but some patients are resistant to these treatments. Despite their severe side effects, benzodiazepines remain an effective alternative

to SSRIs. Also, compounds acting on glutamatergic transmission have been shown to be effective in the treatment of some types of anxiety disorders. Also, functional imaging studies are revealing brain areas affected in specific anxiety disorders and providing new direction for “disorder-specific” treatments (Farb and Ratner, 2014).

Chapter 3:

The orphan receptor GPR88

G coupled protein receptors

G-coupled protein receptors (GPCRs) are a family of seven-transmembrane domain proteins that activate through various extracellular ligands including nucleotides, peptides, biogenic amines, light or glycoprotein. On the basis of their sequence and structure, GPCRs are currently classified into 5 families with unique ligand binding properties: Glutamate, Rhodopsin, *Adhesion*, Frizzled/Taste2, Secretin. (Huang and Thathiah, 2015, Oldham and Hamm, 2008, Lagerstrom and Schioth, 2008)

GPCRs are associated with G proteins in the plasma membrane that form heterotrimeric composed of three subunits: alpha (α), beta (β) and gamma (γ) (Figure 5). In the absence of signaling, all subunits form a heterodimer and the α subunit is bound to the nucleotide guanosine diphosphate (GDP). When activated by an extracellular signaling molecule, GPCRs change their conformation triggering an interaction between the receptor and a nearby α subunit which dissociate from the $\beta\gamma$ subunit complex and replace the GDP by a guanosine triphosphate (GTP) nucleotide. At this point, both subunits groups can interact with other membrane proteins involved in signal transduction. G proteins can interact with various enzymes affecting the production of hundreds or even thousands of second messenger molecules. Based on the sequence and function of their α subunits, mammalian heterotrimers are typically divided into four main classes: G_s , $G_{i/o}$, G_q and G_{12} . Although not detailed here, G protein can interact with multiple effectors leading to a variety of cellular responses including growth, death, transcription, cellular depolarization or hyperpolarization as well as retrograde message to regulate pre-synaptic neurotransmitter release (Figure 6). (Huang and Thathiah, 2015, Galandrin et al., 2007, Lodish, 2004, Kristiansen, 2004) In the present section only few of the best known G proteins actions are presented.

Regulation of cAMP levels

The best study intracellular mechanism drive by G proteins is the regulation of intracellular cAMP levels. G_s and G_i/G_o respectively activate and inhibit adenylyl cyclase (AC) enzyme which, when stimulated, catalyzes the synthesis of the second messenger cyclic adenosine monophosphate (cAMP) from molecules of adenosine triphosphate (ATP). cAMP in turn disinhibit the catalytic subunit of the protein kinase A (PKA) which phosphorylates a number of proteins involved in signal transduction and regulation of gene expression. In addition to the α subunit free $\beta\gamma$ -subunit also interacts with AC either increasing or decreasing the enzyme's activity, depending on the particular $\beta\gamma$ complex and the isoform of the cyclase.

Activation of Phospholipase C

G_q subunit can activates the enzyme Phospholipase C that in turn catalyzes the synthesis of two second messengers: diacylglycerol (DAG) and inositol triphosphate (IP3). IP3 elicit Ca^{2+} release from the endoplasmic reticulum, while DAG diffuses along the plasma membrane where it may activate protein kinase C (PKC). Once activated, PKC phosphorylates target proteins which may result in the facilitation of neuronal transmission.

Direct interaction with ion channels

By interacting with ions channels, G proteins directly modulate the membrane potential and neuronal excitability. Inhibitory GPCRs are thought to decrease the probability of membrane depolarization by inhibition of voltage-gated Ca^{2+} channels and activation of an inward rectifying K^+ channel. Although some subtypes of $\alpha_{i/o}$ can mediate this direct interaction, $\beta\gamma$ complex is the more important mechanism in this process. G_s proteins can also stimulate L-type Ca^{2+} channels thereby increasing their probability of opening in response to membrane depolarization.

Additionally, GPCRs can also act independently of G-protein involvement and thus modulate different signaling pathways, desensitization, internalization, recycling and degradation; all of which can contribute to the biological actions of ligand bound receptors (Gether, 2000, Maudsley et al., 2007, Kelly et al., 2008) . Further complexity is added by the discovery that some GPCRs can form homo or heterodimers which may result in unique signaling complexes (Oldham and Hamm, 2008, Ghanemi, 2015, Park et al., 2008).

Given their structure and function, more than 36% of currently marketed drugs target GPCRs (Rask-Andersen et al., 2011, Oldham and Hamm, 2008). This receptor superfamily is composed of approximately 1000 members among which more than 140 have unknown endogenous ligands (Levoye et al., 2006). These so-called orphans GPCR offer great promise as they may not only provide novel therapeutic target but also elucidate signal transduction pathways allowing for new strategies in drug design (Ghanemi, 2015, Rask-Andersen et al., 2011, Levoye et al., 2006).

The orphan receptor Gpr88

When the orphan G-protein coupled receptor Gpr88 was identified in 2000, Mizushima *et al* noted its “*remarkable evolutionary conservation in primary structure*”. Indeed, 95% of this orphan receptor’s genes is identical between humans and rodents (Mizushima K, 2000). Likewise, the expression pattern of this receptor is very similar across species. While never detected outside of brain tissue, *Gpr88* expression was systematically higher in the striatal region (caudate-putamen and nucleus accumbens) of human, non-human primates and rodent (Mizushima K, 2000, Massart R, 2009, Becker et al., 2008, Ghate et al., 2007, Logue et al., 2009, Quintana A, 2012) (see Figure 7 for expression in mice). Although less extensively, transcripts were also present in the olfactory tubercle, amygdala and lower levels were found in the neocortex of non-human primates and rodents (Logue et al., 2009, Massart R, 2009, Mizushima K, 2000, Quintana A, 2012). Additionally, in mice, the amygdalar expression was identified as central but not basolateral amygdala (Becker et al., 2008) and *Gpr88* mRNA was also present in the inferior olive nucleus

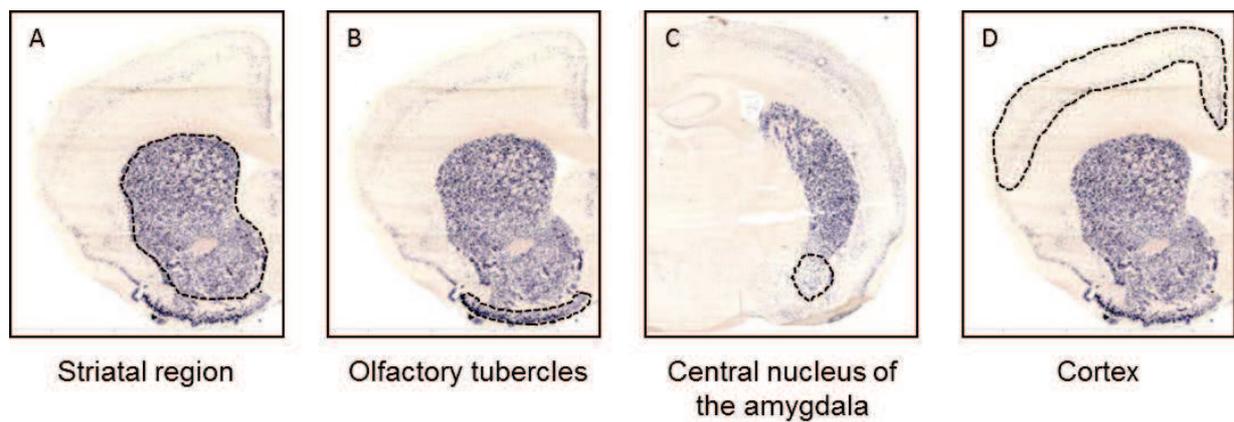


Figure 7: *Gpr88* expression in adult (10 weeks old) wild type mice. *In situ* hybridization (ISH) with nonradioactive Dig-dUTP-labeled antisense *GPR88* riboprobes in coronal sections. (A) High labelling of *Gpr88* in caudate-putamen (striatal region) and (B) olfactory tubercles. (C) Weaker expression in the central amygdalar nucleus. (D) Sparse detection of *Gpr88* mRNA in the neocortex. (ISH and image acquisition by Anne Robé, unpublished data)

(Mizushima K, 2000, Logue et al., 2009, Quintana A, 2012). Within the striatum, *Gpr88* transcripts were enriched in neurons expressing *Penk* (marker for indirect pathway medium spiny neurons) and *Pdyn* (marker for direct pathway medium spiny neurons) but absent in non-neuronal cells of mice and rat (Quintana A, 2012, Massart R, 2009, Komatsu et al., 2014).

The presence of this receptor in interneurons is still uncertain given the lack of agreement between reports (Van Waes V, 2011, Massart R, 2009, Quintana A, 2012) but several authors currently use *Gpr88* promoter to specifically target MSNs (Naydenov et al., 2014, Zhang et al., 2014, Hisatsune et al., 2013). GPR88 immunosignal was detected in the somatodendritic neuronal compartment most frequently in asymmetrical synapses (Massart R, 2009). Overall, GPR88 is thought to be located in glutamatergic post-synaptic densities of medium spiny neurons (MSNs).

Gpr88 is expressed as soon as E18 (Allen brain atlas) displaying highest transcript levels in juvenile rats (post-natal day 25) and decreasing thereafter towards adult levels, suggestive of a role in the development of striatal physiology (Van Waes V, 2011). Indeed, phosphorylated DARPP-32 Thr-34 (Dopamine- and cAMP-regulated phosphoprotein essential for MSNs functioning (Gould and Manji, 2005, Svenningsson et al., 2004)) was enhanced in the striatum of *Gpr88* Knockout (KO)

animals, suggesting altered dopaminergic and glutamatergic transmission (Logue et al., 2009). Moreover, basal as well as amphetamine-induced *in vivo* dopamine levels were altered in mutant mice (Logue et al., 2009) while nigrostriatal dopamine depletion by 6-OHDA in wild type (WT) animals was shown to downregulate striatal *Gpr88* (Massart R, 2009). In addition to alteration of the dopaminergic system, deletion of this receptor was found to reduce *Rgs4* expression and $\beta 3$ GABA_A subunit levels in the striatum suggesting abnormal G α i/G α q GPCR and inhibitory signaling respectively. Lack of *Gpr88* also led to increased phosphorylation of GluR1 AMPA glutamate receptor subunit facilitating glutamatergic signaling. In agreement with these findings, electrophysiological data indicate reduced GABA-induced response as well as enhanced postsynaptic response to glutamatergic stimulation in mice lacking *Gpr88*, indicative of increased MSNs excitability (Quintana A, 2012). However, the precise mechanism by which GPR88 regulates MSNs transmission is still largely unknown. Moreover, extrastriatal GPR88 function has never been addressed.

Behaviorally, lack of *Gpr88* was shown to increase locomotor activity and impair motor coordination and balance in mice (Quintana A, 2012). Moreover, *Gpr88* KO mice present cognitive deficits displayed by impaired learning of a Morris water maze and active avoidance task (Quintana A, 2012) as well as deficient acoustic prepulse inhibition reversed by antipsychotic drugs (Logue et al., 2009). In agreement with an altered dopaminergic transmission, reports indicate enhanced locomotor response to amphetamine (Logue et al., 2009, Quintana A, 2012) and increased stereotypies after apomorphine administration (Logue et al., 2009). Conversely, a GPR88 agonist 2-PCCA was shown to decrease Amphetamine-induced hyperactivity (Jun-Xu et al., 2013). Additionally, while haloperidol (D₂R antagonist) and SKF-82197 (D₁R agonist) were less potent in mutant mice, quinpirole (D₂R agonist) increased (instead of decreasing) locomotion in these mice (Quintana A, 2012, Logue et al., 2009). Finally, deleting *Gpr88* in the Nacc of a neurodevelopment rat model of schizophrenia (neonatal phencyclidine treatment) abolished amphetamine-induced hyperlocomotion (Ingallinesi et al., 2014). This result further supports the relevance of GPR88 and in the regulation of basal ganglia-related behaviors and dopaminergic transmission.

In animal models of substance abuse, chronic morphine treatment as well as protracted abstinence from distinct drugs of abuse altered *Gpr88* expression (Le Merrer et al., 2012a, Befort et al., 2008). *Gpr88* mRNA levels in rodents was also shown to be regulated by chronic restraint stress (Ubaldi et al., 2015) lithium and valproate (mood stabilizers used in bipolar disease treatment) (Brandish et al., 2005, Ogden et al., 2004) as well as several antidepressant treatments (Bohm et al., 2006, Conti et al., 2007).

In sum, *Gpr88* KO animals present hallmark behavioral phenotypes of several neuropsychiatric disorders such as schizophrenia and Attention/hyperactivity disorder (Hyperactivity, stereotypies, sensorimotor gating and learning deficits) as well as basal ganglia motor disorders (deficient motor coordination). Moreover, pharmacological studies in rodents also suggest a role of GPR88 in drug addictions, bipolar disorder and depression. Finally, in a genetic association study, Del Zompo *et al* found a positive associations between *Gpr88* and subpopulations of Schizophrenia and Bipolar disease patients and their relatives (Del Zompo et al., 2014).

References

- ALBIN, R. L., YOUNG, A. B. & PENNEY, J. B. 1989. The functional anatomy of basal ganglia disorders. *Trends Neurosci*, 12, 366-75.
- ALEXANDER, G. E., DELONG, M. R. & STRICK, P. L. 1986. Parallel organization of functionally segregated circuits linking basal ganglia and cortex. *Annu Rev Neurosci*, 9, 357-81.
- ALLSOP, S. A., VANDER WEELE, C. M., WICHMANN, R. & TYE, K. M. 2014. Optogenetic insights on the relationship between anxiety-related behaviors and social deficits. *Front Behav Neurosci*, 8, 241.
- ANGST, J. 2013. Bipolar disorders in DSM-5: strengths, problems and perspectives. *Int J Bipolar Disord*, 1, 12.
- ATALLAH, H. E., LOPEZ-PANIAGUA, D., RUDY, J. W. & O'REILLY, R. C. 2007. Separate neural substrates for skill learning and performance in the ventral and dorsal striatum. *Nat Neurosci*, 10, 126-31.
- AUPPERLE, R. L. & PAULUS, M. P. 2010. Neural systems underlying approach and avoidance in anxiety disorders. *Dialogues Clin Neurosci*, 12, 517-31.
- BAILEY, K. R. & CRAWLEY, J. N. 2009. Anxiety-Related Behaviors in Mice. In: BUCCAFUSCO, J. J. (ed.) *Methods of Behavior Analysis in Neuroscience*. 2nd ed. Boca Raton (FL).
- BALLEINE, B. W., LILJEHOLM, M. & OSTLUND, S. B. 2009. The integrative function of the basal ganglia in instrumental conditioning. *Behav Brain Res*, 199, 43-52.
- BAMFORD, N. S., ZHANG, H., SCHMITZ, Y., WU, N. P., CEPEDA, C., LEVINE, M. S., SCHMAUSS, C., ZAKHARENKO, S. S., ZABLOW, L. & SULZER, D. 2004. Heterosynaptic dopamine neurotransmission selects sets of corticostriatal terminals. *Neuron*, 42, 653-63.
- BAPTISTA, M. A., DAVE, K. D., SHETH, N. P., DE SILVA, S. N., CARLSON, K. M., AZIZ, Y. N., FISKE, B. K., SHERER, T. B. & FRASIER, M. A. 2013. A strategy for the generation, characterization and distribution of animal models by The Michael J. Fox Foundation for Parkinson's Research. *Dis Model Mech*, 6, 1316-24.
- BARKLEY, R. A. 1997. Behavioral inhibition, sustained attention, and executive functions: constructing a unifying theory of ADHD. *Psychol Bull*, 121, 65-94.
- BATEUP, H. S., SANTINI, E., SHEN, W., BIRNBAUM, S., VALJENT, E., SURMEIER, D. J., FISONE, G., NESTLER, E. J. & GREENGARD, P. 2010. Distinct subclasses of medium spiny neurons differentially regulate striatal motor behaviors. *Proc Natl Acad Sci U S A*, 107, 14845-50.
- BECKER, J. A., BEFORT, K., BLAD, C., FILLIOL, D., GHATE, A., DEMBELE, D., THIBAUT, C., KOCH, M., MULLER, J., LARDENOIS, A., POCH, O. & KIEFFER, B. L. 2008. Transcriptome analysis identifies genes with enriched expression in the mouse central extended amygdala. *Neuroscience*, 156, 950-65.
- BEFORT, K., FILLIOL, D., GHATE, A., DARCO, E., MATIFAS, A., MULLER, J., LARDENOIS, A., THIBAUT, C., DEMBELE, D., LE MERRER, J., BECKER, J. A., POCH, O. & KIEFFER, B. L. 2008. Mu-opioid receptor activation induces transcriptional plasticity in the central extended amygdala. *Eur J Neurosci*, 27, 2973-84.
- BEITZ, J. M. 2014. Parkinson's disease: a review. *Front Biosci (Schol Ed)*, 6, 65-74.

- BELL, D. S. 1965. Comparison of Amphetamine Psychosis and Schizophrenia. *Br J Psychiatry*, 111, 701-7.
- BELZUNG, C. & GRIEBEL, G. 2001. Measuring normal and pathological anxiety-like behaviour in mice: a review. *Behav Brain Res*, 125, 141-9.
- BELZUNG, C. & LEMOINE, M. 2011. Criteria of validity for animal models of psychiatric disorders: focus on anxiety disorders and depression. *Biol Mood Anxiety Disord*, 1, 9.
- BERK, M., DODD, S., KAUER-SANT'ANNA, M., MALHI, G. S., BOURIN, M., KAPCZINSKI, F. & NORMAN, T. 2007. Dopamine dysregulation syndrome: implications for a dopamine hypothesis of bipolar disorder. *Acta Psychiatr Scand Suppl*, 41-9.
- BERK, M., KAPCZINSKI, F., ANDREAZZA, A. C., DEAN, O. M., GIORLANDO, F., MAES, M., YUCEL, M., GAMA, C. S., DODD, S., DEAN, B., MAGALHAES, P. V., AMMINGER, P., MCGORRY, P. & MALHI, G. S. 2011. Pathways underlying neuroprogression in bipolar disorder: focus on inflammation, oxidative stress and neurotrophic factors. *Neurosci Biobehav Rev*, 35, 804-17.
- BLAISS, C. A. & JANAK, P. H. 2009. The nucleus accumbens core and shell are critical for the expression, but not the consolidation, of Pavlovian conditioned approach. *Behav Brain Res*, 200, 22-32.
- BODNOFF, S. R., SURANYI-CADOTTE, B., QUIRION, R. & MEANEY, M. J. 1989. A comparison of the effects of diazepam versus several typical and atypical anti-depressant drugs in an animal model of anxiety. *Psychopharmacology (Berl)*, 97, 277-9.
- BOHM, C., NEWRZELLA, D., HERBERGER, S., SCHRAMM, N., EISENHARDT, G., SCHENK, V., SONNTAG-BUCK, V. & SORGENFREI, O. 2006. Effects of antidepressant treatment on gene expression profile in mouse brain: cell type-specific transcription profiling using laser microdissection and microarray analysis. *J Neurochem*, 97 Suppl 1, 44-9.
- BOLKAN, S. S., DE CARVALHO, F. D. & KELLENDONK, C. 2015. Using human brain imaging studies as a guide toward animal models of schizophrenia. *Neuroscience*.
- BORA, E., YUCEL, M. & PANTELIS, C. 2009. Cognitive endophenotypes of bipolar disorder: a meta-analysis of neuropsychological deficits in euthymic patients and their first-degree relatives. *J Affect Disord*, 113, 1-20.
- BRAMBILLA, P., HARENSKI, K., NICOLETTI, M., MALLINGER, A. G., FRANK, E., KUPFER, D. J., KESHAVAN, M. S. & SOARES, J. C. 2001. MRI study of posterior fossa structures and brain ventricles in bipolar patients. *J Psychiatr Res*, 35, 313-22.
- BRANDISH, P. E., SU, M., HOLDER, D. J., HODOR, P., SZUMILOSKI, J., KLEINHANZ, R. R., FORBES, J. E., MCWHORTER, M. E., DUENWALD, S. J., PARRISH, M. L., NA, S., LIU, Y., PHILLIPS, R. L., RENGER, J. J., SANKARANARAYANAN, S., SIMON, A. J. & SCOLNICK, E. M. 2005. Regulation of gene expression by lithium and depletion of inositol in slices of adult rat cortex. *Neuron*, 45, 861-72.
- BROOKS, S. P. & DUNNETT, S. B. 2009. Tests to assess motor phenotype in mice: a user's guide. *Nat Rev Neurosci*, 10, 519-29.
- BUBENIKOVA-VALESOVA, V., HORACEK, J., VRAJOVA, M. & HOSCHL, C. 2008. Models of schizophrenia in humans and animals based on inhibition of NMDA receptors. *Neurosci Biobehav Rev*, 32, 1014-23.
- CALABRESI, P., PICCONI, B., TOZZI, A., GHIGLIERI, V. & DI FILIPPO, M. 2014. Direct and indirect pathways of basal ganglia: a critical reappraisal. *Nat Neurosci*, 17, 1022-30.

- CARLSSON, A., WATERS, N., HOLM-WATERS, S., TEDROFF, J., NILSSON, M. & CARLSSON, M. L. 2001. Interactions between monoamines, glutamate, and GABA in schizophrenia: new evidence. *Annu Rev Pharmacol Toxicol*, 41, 237-60.
- CAZORLA, M., DE CARVALHO, F. D., CHOCHAN, M. O., SHEGDA, M., CHUHMA, N., RAYPORT, S., AHMARI, S. E., MOORE, H. & KELLENDONK, C. 2014. Dopamine D2 receptors regulate the anatomical and functional balance of basal ganglia circuitry. *Neuron*, 81, 153-64.
- CAZORLA, M., KANG, U. J. & KELLENDONK, C. 2015. Balancing the basal ganglia circuitry: A possible new role for dopamine D2 receptors in health and disease. *Mov Disord*.
- CHA, J., GREENBERG, T., CARLSON, J. M., DEDORA, D. J., HAJCAK, G. & MUJICA-PARODI, L. R. 2014. Circuit-wide structural and functional measures predict ventromedial prefrontal cortex fear generalization: implications for generalized anxiety disorder. *J Neurosci*, 34, 4043-53.
- CHANG, C. K., HAYES, R. D., PERERA, G., BROADBENT, M. T., FERNANDES, A. C., LEE, W. E., HOTOPF, M. & STEWART, R. 2011. Life expectancy at birth for people with serious mental illness and other major disorders from a secondary mental health care case register in London. *PLoS One*, 6, e19590.
- CHANG, R., LIU, X., LI, S. & LI, X. J. 2015. Transgenic animal models for study of the pathogenesis of Huntington's disease and therapy. *Drug Des Devel Ther*, 9, 2179-2188.
- CHAUDHURI, K. R. & NAIDU, Y. 2008. Early Parkinson's disease and non-motor issues. *J Neurol*, 255 Suppl 5, 33-8.
- CHEN, C. H., SUCKLING, J., LENNOX, B. R., OOI, C. & BULLMORE, E. T. 2011. A quantitative meta-analysis of fMRI studies in bipolar disorder. *Bipolar Disord*, 13, 1-15.
- CHIN-CHAN, M., NAVARRO-YEPES, J. & QUINTANILLA-VEGA, B. 2015. Environmental pollutants as risk factors for neurodegenerative disorders: Alzheimer and Parkinson diseases. *Front Cell Neurosci*, 9, 124.
- CONTI, B., MAIER, R., BARR, A. M., MORALE, M. C., LU, X., SANNA, P. P., BILBE, G., HOYER, D. & BARTFAI, T. 2007. Region-specific transcriptional changes following the three antidepressant treatments electroconvulsive therapy, sleep deprivation and fluoxetine. *Mol Psychiatry*, 12, 167-89.
- CRASKE, M. G., RAUCH, S. L., URSANO, R., PRENOVEAU, J., PINE, D. S. & ZINBARG, R. E. 2009. What is an anxiety disorder? *Depress Anxiety*, 26, 1066-85.
- CRAWLEY, J. N. 2000. *What's wrong with my mouse?: behavioral phenotype of transgenic and knockout mice*, Wiley-Liss.
- CRITTENDEN, J. R. & GRAYBIEL, A. M. 2011. Basal Ganglia disorders associated with imbalances in the striatal striosome and matrix compartments. *Front Neuroanat*, 5, 59.
- CUI, G., JUN, S. B., JIN, X., PHAM, M. D., VOGEL, S. S., LOVINGER, D. M. & COSTA, R. M. 2013. Concurrent activation of striatal direct and indirect pathways during action initiation. *Nature*, 494, 238-42.
- DALLEY, J. W., FRYER, T. D., BRICHARD, L., ROBINSON, E. S., THEOBALD, D. E., LAANE, K., PENA, Y., MURPHY, E. R., SHAH, Y., PROBST, K., ABAKUMOVA, I., AIGBIRHIO, F. I., RICHARDS, H. K., HONG, Y., BARON, J. C., EVERITT, B. J. & ROBBINS, T. W. 2007. Nucleus accumbens D2/3 receptors predict trait impulsivity and cocaine reinforcement. *Science*, 315, 1267-70.

- DANJO, T., YOSHIMI, K., FUNABIKI, K., YAWATA, S. & NAKANISHI, S. 2014. Aversive behavior induced by optogenetic inactivation of ventral tegmental area dopamine neurons is mediated by dopamine D2 receptors in the nucleus accumbens. *Proc Natl Acad Sci U S A*, 111, 6455-60.
- DAVIS, K. L., KAHN, R. S., KO, G. & DAVIDSON, M. 1991. Dopamine in schizophrenia: a review and reconceptualization. *Am J Psychiatry*, 148, 1474-86.
- DAVIS, M. 1992. The role of the amygdala in fear and anxiety. *Annu Rev Neurosci*, 15, 353-75.
- DAY, M., WANG, Z., DING, J., AN, X., INGHAM, C. A., SHERING, A. F., WOKOSIN, D., ILIJIC, E., SUN, Z., SAMPSON, A. R., MUGNAINI, E., DEUTCH, A. Y., SESACK, S. R., ARBUTHNOTT, G. W. & SURMEIER, D. J. 2006. Selective elimination of glutamatergic synapses on striatopallidal neurons in Parkinson disease models. *Nat Neurosci*, 9, 251-9.
- DAY, M., WOKOSIN, D., PLOTKIN, J. L., TIAN, X. & SURMEIER, D. J. 2008. Differential excitability and modulation of striatal medium spiny neuron dendrites. *J Neurosci*, 28, 11603-14.
- DEAN, K. & MURRAY, R. M. 2005. Environmental risk factors for psychosis. *Dialogues Clin Neurosci*, 7, 69-80.
- DEL ZOMPO, M., DELEUZE, J. F., CHILLOTTI, C., COUSIN, E., NIEHAUS, D., EBSTEIN, R. P., ARDAU, R., MACE, S., WARNICH, L., MUJAHED, M., SEVERINO, G., DIB, C., JORDAAN, E., MURAD, I., SOUBIGOU, S., KOEN, L., BANNOURA, I., ROCHER, C., LAURENT, C., DEROCK, M., FAUCON BIGUET, N., MALLET, J. & MELONI, R. 2014. Association study in three different populations between the GPR88 gene and major psychoses. *Mol Genet Genomic Med*, 2, 152-9.
- DELONG, M. R. 1990. Primate models of movement disorders of basal ganglia origin. *Trends Neurosci*, 13, 281-5.
- DEVAN, B. D., HONG, N. S. & MCDONALD, R. J. 2011. Parallel associative processing in the dorsal striatum: segregation of stimulus-response and cognitive control subregions. *Neurobiol Learn Mem*, 96, 95-120.
- DICKSTEIN, S. G., BANNON, K., CASTELLANOS, F. X. & MILHAM, M. P. 2006. The neural correlates of attention deficit hyperactivity disorder: an ALE meta-analysis. *J Child Psychol Psychiatry*, 47, 1051-62.
- DO, J., KIM, J. I., BAKES, J., LEE, K. & KAANG, B. K. 2012. Functional roles of neurotransmitters and neuromodulators in the dorsal striatum. *Learn Mem*, 20, 21-8.
- DOPHEIDE, J. A. & PLISZKA, S. R. 2009. Attention-deficit-hyperactivity disorder: an update. *Pharmacotherapy*, 29, 656-79.
- DULAWA, S. C., HOLICK, K. A., GUNDERSEN, B. & HEN, R. 2004. Effects of chronic fluoxetine in animal models of anxiety and depression. *Neuropsychopharmacology*, 29, 1321-30.
- DUNCAN, G. E., SHEITMAN, B. B. & LIEBERMAN, J. A. 1999. An integrated view of pathophysiological models of schizophrenia. *Brain Res Brain Res Rev*, 29, 250-64.
- DUNSMOOR, J. E. & PAZ, R. 2015. Fear Generalization and Anxiety: Behavioral and Neural Mechanisms. *Biol Psychiatry*.
- DURIEUX PF, B. B., GUIDUCCI S, BUCH T, WAISMAN A, ZOLI M, ET AL 2009. D2R striatopallidal neurons inhibit both locomotor and drug reward processes. *Nat Neurosci*, :393-395.

- DURIEUX PF, S. S., DE KERCHOVE D'EXAERDE A 2012. Differential regulation of motor control and response to dopaminergic drugs by D1R and D2R neurons in distinct dorsal striatum subregions. 640-653.
- DURIEUX, P. F. B., B. GUIDUCCI, S. BUCH, T., WAISMAN, A., ZOLI, M., SCHIFFMANN, S. N. & DE KERCHOVE D'EXAERDE, A. 2009. D2R striatopallidal neurons inhibit both locomotor and drug reward processes. *Nat Neurosci*, 12, 393-5.
- ENA, S., DE KERCHOVE D'EXAERDE, A. & SCHIFFMANN, S. N. 2011. Unraveling the differential functions and regulation of striatal neuron sub-populations in motor control, reward, and motivational processes. *Front Behav Neurosci*, 5, 47.
- FARAONE, S. V. & KHAN, S. A. 2006. Candidate gene studies of attention-deficit/hyperactivity disorder. *J Clin Psychiatry*, 67 Suppl 8, 13-20.
- FARAONE, S. V., SERGEANT, J., GILLBERG, C. & BIEDERMAN, J. 2003. The worldwide prevalence of ADHD: is it an American condition? *World Psychiatry*, 2, 104-13.
- FARB, D. H. & RATNER, M. H. 2014. Targeting the modulation of neural circuitry for the treatment of anxiety disorders. *Pharmacol Rev*, 66, 1002-32.
- FEIFEL, D., MINASSIAN, A. & PERRY, W. 2009. Prepulse inhibition of startle in adults with ADHD. *J Psychiatr Res*, 43, 484-9.
- FELIX-ORTIZ, A. C., BEYELER, A., SEO, C., LEPLA, C. A., WILDES, C. P. & TYE, K. M. 2013. BLA to vHPC inputs modulate anxiety-related behaviors. *Neuron*, 79, 658-64.
- FENTON, W. S., STOVER, E. L. & INSEL, T. R. 2003. Breaking the log-jam in treatment development for cognition in schizophrenia: NIMH perspective. *Psychopharmacology (Berl)*, 169, 365-6.
- FERGUSON, S. M., ESKENAZI, D., ISHIKAWA, M., WANAT, M. J., PHILLIPS, P. E., DONG, Y., ROTH, B. L. & NEUMAIER, J. F. 2011. Transient neuronal inhibition reveals opposing roles of indirect and direct pathways in sensitization. *Nat Neurosci*, 14, 22-4.
- FERNANDO, A. B. & ROBBINS, T. W. 2011. Animal models of neuropsychiatric disorders. *Annu Rev Clin Psychol*, 7, 39-61.
- FOLSTEIN, S. E., LEIGH, R. J., PARHAD, I. M. & FOLSTEIN, M. F. 1986. The diagnosis of Huntington's disease. *Neurology*, 36, 1279-83.
- FRANCIS, T. C., CHANDRA, R., FRIEND, D. M., FINKEL, E., DAYRIT, G., MIRANDA, J., BROOKS, J. M., INIGUEZ, S. D., O'DONNELL, P., KRAVITZ, A. & LOBO, M. K. 2015. Nucleus accumbens medium spiny neuron subtypes mediate depression-related outcomes to social defeat stress. *Biol Psychiatry*, 77, 212-22.
- GALANDRIN, S., OLIGNY-LONGPRE, G. & BOUVIER, M. 2007. The evasive nature of drug efficacy: implications for drug discovery. *Trends Pharmacol Sci*, 28, 423-30.
- GERFEN, C. R. 1992. The neostriatal mosaic: multiple levels of compartmental organization. *Trends Neurosci*, 15, 133-9.
- GERFEN, C. R., ENGBER, T. M., MAHAN, L. C., SUSEL, Z., CHASE, T. N., MONSMA, F. J., JR. & SIBLEY, D. R. 1990. D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. *Science*, 250, 1429-32.

- GERFEN, C. R. & SURMEIER, D. J. 2011. Modulation of striatal projection systems by dopamine. *Annu Rev Neurosci*, 34, 441-66.
- GERTLER, T. S., CHAN, C. S. & SURMEIER, D. J. 2008. Dichotomous anatomical properties of adult striatal medium spiny neurons. *J Neurosci*, 28, 10814-24.
- GETHER, U. 2000. Uncovering molecular mechanisms involved in activation of G protein-coupled receptors. *Endocr Rev*, 21, 90-113.
- GEYER, M. A. 2006. The family of sensorimotor gating disorders: comorbidities or diagnostic overlaps? *Neurotox Res*, 10, 211-20.
- GEYER, M. A. 2008. Developing translational animal models for symptoms of schizophrenia or bipolar mania. *Neurotox Res*, 14, 71-8.
- GHANEMI, A. 2015. Targeting G protein coupled receptor-related pathways as emerging molecular therapies. *Saudi Pharm J*, 23, 115-29.
- GHATE, A., BEFORT, K., BECKER, J. A., FILLIOL, D., BOLE-FEYSOT, C., DEMEBELE, D., JOST, B., KOCH, M. & KIEFFER, B. L. 2007. Identification of novel striatal genes by expression profiling in adult mouse brain. *Neuroscience*, 146, 1182-92.
- GITTIS, A. H. & KREITZER, A. C. 2012. Striatal microcircuitry and movement disorders. *Trends Neurosci*, 35, 557-64.
- GIZER, I. R., FICKS, C. & WALDMAN, I. D. 2009. Candidate gene studies of ADHD: a meta-analytic review. *Hum Genet*, 126, 51-90.
- GOEDERT, M., SPILLANTINI, M. G., DEL TREDICI, K. & BRAAK, H. 2013. 100 years of Lewy pathology. *Nat Rev Neurol*, 9, 13-24.
- GONG, S., ZHENG, C., DOUGHTY, M. L., LOSOS, K., DIDKOVSKY, N., SCHAMBRA, U. B., NOWAK, N. J., JOYNER, A., LEBLANC, G., HATTEN, M. E. & HEINTZ, N. 2003. A gene expression atlas of the central nervous system based on bacterial artificial chromosomes. *Nature*, 425, 917-25.
- GOTTESMAN, II & ERLLENMEYER-KIMLING, L. 2001. Family and twin strategies as a head start in defining prodromes and endophenotypes for hypothetical early-interventions in schizophrenia. *Schizophr Res*, 51, 93-102.
- GOULD, T. D. & EINAT, H. 2007. Animal models of bipolar disorder and mood stabilizer efficacy: a critical need for improvement. *Neurosci Biobehav Rev*, 31, 825-31.
- GOULD, T. D. & MANJI, H. K. 2005. DARPP-32: A molecular switch at the nexus of reward pathway plasticity. *Proc Natl Acad Sci U S A*, 102, 253-4.
- GRACE, A. A., FLORESCO, S. B., GOTO, Y. & LODGE, D. J. 2007. Regulation of firing of dopaminergic neurons and control of goal-directed behaviors. *Trends Neurosci*, 30, 220-7.
- GRAFTON, S. T., HAZELTINE, E. & IVRY, R. 1995. Functional mapping of sequence learning in normal humans. *J Cogn Neurosci*, 7, 497-510.
- GROENEWEGEN, H. J., WRIGHT, C. I., BEIJER, A. V. & VOORN, P. 1999. Convergence and segregation of ventral striatal inputs and outputs. *Ann NY Acad Sci*, 877, 49-63.

GUNAYDIN, L. A., GROSENICK, L., FINKELSTEIN, J. C., KAUVAR, I. V., FENNO, L. E., ADHIKARI, A., LAMMEL, S., MIRZABEKOV, J. J., AIRAN, R. D., ZALOCUSKY, K. A., TYE, K. M., ANIKEEVA, P., MALENKA, R. C. & DEISSEROTH, K. 2014. Natural neural projection dynamics underlying social behavior. *Cell*, 157, 1535-51.

HEIMER, L. 2003a. The legacy of the silver methods and the new anatomy of the basal forebrain: implications for neuropsychiatry and drug abuse. *Scand J Psychol*, 44, 189-201.

HEIMER, L. 2003b. A new anatomical framework for neuropsychiatric disorders and drug abuse. *Am J Psychiatry*, 160, 1726-39.

HERNANDEZ-ECHEAGARAY, E., STARLING, A. J., CEPEDA, C. & LEVINE, M. S. 2004. Modulation of AMPA currents by D2 dopamine receptors in striatal medium-sized spiny neurons: are dendrites necessary? *Eur J Neurosci*, 19, 2455-63.

HIKIDA, T., KIMURA, K., WADA, N., FUNABIKI, K. & NAKANISHI, S. 2010. Distinct roles of synaptic transmission in direct and indirect striatal pathways to reward and aversive behavior. *Neuron*, 66, 896-907.

HIKIDA, T., YAWATA, S., YAMAGUCHI, T., DANJO, T., SASAOKA, T., WANG, Y. & NAKANISHI, S. 2013. Pathway-specific modulation of nucleus accumbens in reward and aversive behavior via selective transmitter receptors. *Proc Natl Acad Sci U S A*, 110, 342-7.

HIROI, N., MARTIN, A. B., GRANDE, C., ALBERTI, I., RIVERA, A. & MORATALLA, R. 2002. Molecular dissection of dopamine receptor signaling. *J Chem Neuroanat*, 23, 237-42.

HISATSUNE, C., OGAWA, N. & MIKOSHIBA, K. 2013. Striatum-specific expression of Cre recombinase using the Gpr88 promoter in mice. *Transgenic Res*, 22, 1241-7.

HUANG, Y. & THATHIAH, A. 2015. Regulation of neuronal communication by G protein-coupled receptors. *FEBS Lett*.

INGALLINESI, M., LE BOUIL, L., FAUCON BIGUET, N., DO THI, A., MANNOURY LA COUR, C., MILLAN, M. J., RAVASSARD, P., MALLET, J. & MELONI, R. 2014. Local inactivation of Gpr88 in the nucleus accumbens attenuates behavioral deficits elicited by the neonatal administration of phencyclidine in rats. *Mol Psychiatry*.

JANKOWSKI, J., SCHEEF, L., HUPPE, C. & BOECKER, H. 2009. Distinct striatal regions for planning and executing novel and automated movement sequences. *Neuroimage*, 44, 1369-79.

JUN-XU, L., A., T. D. & CHUNYANG, J. 2013. The Gpr88 receptor agonist 2-PCCA does not alter the behavioral effects of methamphetamine in rats. *European Journal of pharmacology*, 272-277.

JUN, C., CHOI, Y., LIM, S. M., BAE, S., HONG, Y. S., KIM, J. E. & LYOO, I. K. 2014. Disturbance of the glutamatergic system in mood disorders. *Exp Neurobiol*, 23, 28-35.

KAPLITT, M. G., FEIGIN, A., TANG, C., FITZSIMONS, H. L., MATTIS, P., LAWLOR, P. A., BLAND, R. J., YOUNG, D., STRYBING, K., EIDELBERG, D. & DURING, M. J. 2007. Safety and tolerability of gene therapy with an adeno-associated virus (AAV) borne GAD gene for Parkinson's disease: an open label, phase I trial. *Lancet*, 369, 2097-105.

KARLSGODT, K. H., SUN, D. & CANNON, T. D. 2010. Structural and Functional Brain Abnormalities in Schizophrenia. *Curr Dir Psychol Sci*, 19, 226-231.

- KELLEY, A. E. 2004. Ventral striatal control of appetitive motivation: role in ingestive behavior and reward-related learning. *Neurosci Biobehav Rev*, 27, 765-76.
- KELLY, E., BAILEY, C. P. & HENDERSON, G. 2008. Agonist-selective mechanisms of GPCR desensitization. *Br J Pharmacol*, 153 Suppl 1, S379-88.
- KENNEDY, K. P., CULLEN, K. R., DEYOUNG, C. G. & KLIMES-DOUGAN, B. 2015. The genetics of early-onset bipolar disorder: A systematic review. *J Affect Disord*, 184, 1-12.
- KIM, C. H., WALDMAN, I. D., BLAKELY, R. D. & KIM, K. S. 2008. Functional gene variation in the human norepinephrine transporter: association with attention deficit hyperactivity disorder. *Ann NY Acad Sci*, 1129, 256-60.
- KLEIN, C. & WESTENBERGER, A. 2012. Genetics of Parkinson's disease. *Cold Spring Harb Perspect Med*, 2, a008888.
- KOHL, S., HEEKEREN, K., KLOSTERKOTTER, J. & KUHN, J. 2013. Prepulse inhibition in psychiatric disorders--apart from schizophrenia. *J Psychiatr Res*, 47, 445-52.
- KOMATSU, H., MARUYAMA, M., YAO, S., SHINOHARA, T., SAKUMA, K., IMAICHI, S., CHIKATSU, T., KUNIYEDA, K., SIU, F. K., PENG, L. S., ZHUO, K., MUN, L. S., HAN, T. M., MATSUMOTO, Y., HASHIMOTO, T., MIYAJIMA, N., ITOH, Y., OGI, K., HABATA, Y. & MORI, M. 2014. Anatomical transcriptome of G protein-coupled receptors leads to the identification of a novel therapeutic candidate GPR52 for psychiatric disorders. *PLoS One*, 9, e90134.
- KONDO, D. G., HELLEM, T. L., SHI, X. F., SUNG, Y. H., PRESCOT, A. P., KIM, T. S., HUBER, R. S., FORREST, L. N. & RENSHAW, P. F. 2014. A review of MR spectroscopy studies of pediatric bipolar disorder. *AJNR Am J Neuroradiol*, 35, S64-80.
- KONSTANTAKOPOULOS, G., DIMITRAKOPOULOS, S. & MICHALOPOULOU, P. G. 2015. Drugs under early investigation for the treatment of bipolar disorder. *Expert Opin Investig Drugs*, 24, 477-90.
- KRAVITZ, A. V., FREEZE, B. S., PARKER, P. R., KAY, K., THWIN, M. T., DEISSEROTH, K. & KREITZER, A. C. 2010. Regulation of parkinsonian motor behaviours by optogenetic control of basal ganglia circuitry. *Nature*, 466, 622-6.
- KRAVITZ, A. V., TOMASI, D., LEBLANC, K. H., BALER, R., VOLKOW, N. D., BONCI, A. & FERRE, S. 2015. Cortico-striatal circuits: Novel therapeutic targets for substance use disorders. *Brain Res*.
- KRAVITZ, A. V., TYE, L. D. & KREITZER, A. C. 2012. Distinct roles for direct and indirect pathway striatal neurons in reinforcement. *Nat Neurosci*, 15, 816-8.
- KREITZER, A. C. & MALENKA, R. C. 2007. Endocannabinoid-mediated rescue of striatal LTD and motor deficits in Parkinson's disease models. *Nature*, 445, 643-7.
- KRISTIANSEN, K. 2004. Molecular mechanisms of ligand binding, signaling, and regulation within the superfamily of G-protein-coupled receptors: molecular modeling and mutagenesis approaches to receptor structure and function. *Pharmacol Ther*, 103, 21-80.
- KUMAR, A., KUMAR SINGH, S., KUMAR, V., KUMAR, D., AGARWAL, S. & RANA, M. K. 2015. Huntington's disease: an update of therapeutic strategies. *Gene*, 556, 91-7.

- KUPCHIK, Y. M., BROWN, R. M., HEINSBROEK, J. A., LOBO, M. K., SCHWARTZ, D. J. & KALIVAS, P. W. 2015. Coding the direct/indirect pathways by D1 and D2 receptors is not valid for accumbens projections. *Nat Neurosci*.
- LAGERSTROM, M. C. & SCHIOTH, H. B. 2008. Structural diversity of G protein-coupled receptors and significance for drug discovery. *Nat Rev Drug Discov*, 7, 339-57.
- LE MERRER, J., BEFORT, K., GARDON, O., FILLIOL, D., DARCO, E., DEMBELE, D., BECKER, J. A. & KIEFFER, B. L. 2012. Protracted abstinence from distinct drugs of abuse shows regulation of a common gene network. *Addict Biol*, 17, 1-12.
- LE, W., SAYANA, P. & JANKOVIC, J. 2014. Animal models of Parkinson's disease: a gateway to therapeutics? *Neurotherapeutics*, 11, 92-110.
- LEO, D. & GAINETDINOV, R. R. 2013. Transgenic mouse models for ADHD. *Cell Tissue Res*, 354, 259-71.
- LEVOYE, A., DAM, J., AYOUB, M. A., GUILLAUME, J. L. & JOCKERS, R. 2006. Do orphan G-protein-coupled receptors have ligand-independent functions? New insights from receptor heterodimers. *EMBO Rep*, 7, 1094-8.
- LILJEHOLM, M. & O'DOHERTY, J. P. 2012. Contributions of the striatum to learning, motivation, and performance: an associative account. *Trends Cogn Sci*, 16, 467-75.
- LILLRANK, S. M., LIPSKA, B. K. & WEINBERGER, D. R. 1995. Neurodevelopmental animal models of schizophrenia. *Clin Neurosci*, 3, 98-104.
- LIPSKA, B. K. 2004. Using animal models to test a neurodevelopmental hypothesis of schizophrenia. *J Psychiatry Neurosci*, 29, 282-6.
- LOBO, M. K., COVINGTON, H. E., 3RD, CHAUDHURY, D., FRIEDMAN, A. K., SUN, H., DAMEZ-WERNO, D., DIETZ, D. M., ZAMAN, S., KOO, J. W., KENNEDY, P. J., MOUZON, E., MOGRI, M., NEVE, R. L., DEISSEROTH, K., HAN, M. H. & NESTLER, E. J. 2010. Cell type-specific loss of BDNF signaling mimics optogenetic control of cocaine reward. *Science*, 330, 385-90.
- LOBO, M. K., KARSTEN, S. L., GRAY, M., GESCHWIND, D. H. & YANG, X. W. 2006. FACS-array profiling of striatal projection neuron subtypes in juvenile and adult mouse brains. *Nat Neurosci*, 9, 443-52.
- LOBO, M. K. & NESTLER, E. J. 2011. The striatal balancing act in drug addiction: distinct roles of direct and indirect pathway medium spiny neurons. *Front Neuroanat*, 5, 41.
- LODISH, H. 2004. *Molecular Cell Biology*, W. H. Freeman.
- LOGUE, S. F., GRAUER, S. M., PAULSEN, J., GRAF, R., TAYLOR, N., SUNG, M. A., ZHANG, L., HUGHES, Z., PULITO, V. L., LIU, F., ROSENZWEIG-LIPSON, S., BRANDON, N. J., MARQUIS, K. L., BATES, B. & PAUSCH, M. 2009. The orphan GPCR, GPR88, modulates function of the striatal dopamine system: a possible therapeutic target for psychiatric disorders? *Mol Cell Neurosci*, 42, 438-47.
- LOPEZ-HUERTA, V. G., NAKANO, Y., BAUSENWEIN, J., JAIDAR, O., LAZARUS, M., CHERASSE, Y., GARCIA-MUNOZ, M. & ARBUTHNOTT, G. 2015. The neostriatum: two entities, one structure? *Brain Struct Funct*.
- LYND-BALTA, E. & HABER, S. N. 1994. The organization of midbrain projections to the striatum in the primate: sensorimotor-related striatum versus ventral striatum. *Neuroscience*, 59, 625-40.

- MACPHERSON, T., MORITA, M. & HIKIDA, T. 2014. Striatal direct and indirect pathways control decision-making behavior. *Front Psychol*, 5, 1301.
- MALETIC, V. & RAISON, C. 2014. Integrated neurobiology of bipolar disorder. *Front Psychiatry*, 5, 98.
- MASSART R, G. J., MIGNON V, SOKOLOFF P, DIAZ J 2009. Striatal GPR88 expression is confined to the whole projection neuron population and is regulated by dopaminergic and glutamatergic afferents. *Eur J Neurosci*, 397-414.
- MATAMALES, M., BERTRAN-GONZALEZ, J., SALOMON, L., DEGOS, B., DENIAU, J. M., VALJENT, E., HERVE, D. & GIRAULT, J. A. 2009. Striatal medium-sized spiny neurons: identification by nuclear staining and study of neuronal subpopulations in BAC transgenic mice. *PLoS One*, 4, e4770.
- MAUDSLEY, S., MARTIN, B. & LUTTRELL, L. M. 2007. G protein-coupled receptor signaling complexity in neuronal tissue: implications for novel therapeutics. *Curr Alzheimer Res*, 4, 3-19.
- MCBRIDE, W. J., MURPHY, J. M. & IKEMOTO, S. 1999. Localization of brain reinforcement mechanisms: intracranial self-administration and intracranial place-conditioning studies. *Behav Brain Res*, 101, 129-52.
- MCINTYRE, R. S., KONARSKI, J. Z. & YATHAM, L. N. 2004. Comorbidity in bipolar disorder: a framework for rational treatment selection. *Hum Psychopharmacol*, 19, 369-86.
- MCINTYRE, R. S., MUZINA, D. J., KEMP, D. E., BLANK, D., WOLDEYOHANNES, H. O., LOFCHY, J., SOCZYNSKA, J. K., BANIK, S. & KONARSKI, J. Z. 2008. Bipolar disorder and suicide: research synthesis and clinical translation. *Curr Psychiatry Rep*, 10, 66-72.
- MINEKA, S. & ZINBARG, R. 2006. A contemporary learning theory perspective on the etiology of anxiety disorders: it's not what you thought it was. *Am Psychol*, 61, 10-26.
- MIRENOWICZ, J. & SCHULTZ, W. 1994. Importance of unpredictability for reward responses in primate dopamine neurons. *J Neurophysiol*, 72, 1024-7.
- MIRENOWICZ, J. & SCHULTZ, W. 1996. Preferential activation of midbrain dopamine neurons by appetitive rather than aversive stimuli. *Nature*, 379, 449-51.
- MIYACHI, S., HIKOSAKA, O. & LU, X. 2002. Differential activation of monkey striatal neurons in the early and late stages of procedural learning. *Exp Brain Res*, 146, 122-6.
- MIYAMOTO, S., MIYAKE, N., JARSKOG, L. F., FLEISCHHACKER, W. W. & LIEBERMAN, J. A. 2012. Pharmacological treatment of schizophrenia: a critical review of the pharmacology and clinical effects of current and future therapeutic agents. *Mol Psychiatry*, 17, 1206-27.
- MIZUSHIMA K, M. Y., TSUKAHARA F, HIRAI M, SAKAKI Y, ITO T 2000. A novel G-protein-coupled receptor gene expressed in striatum. *Genomics*, 314-321.
- MURRAY, G. K., CORLETT, P. R., CLARK, L., PESSIGLIONE, M., BLACKWELL, A. D., HONEY, G., JONES, P. B., BULLMORE, E. T., ROBBINS, T. W. & FLETCHER, P. C. 2008. Substantia nigra/ventral tegmental reward prediction error disruption in psychosis. *Mol Psychiatry*, 13, 239, 267-76.
- NAYDENOV, A. V., SEPERS, M. D., SWINNEY, K., RAYMOND, L. A., PALMITER, R. D. & STELLA, N. 2014. Genetic rescue of CB1 receptors on medium spiny neurons prevents loss of excitatory striatal synapses but not motor impairment in HD mice. *Neurobiol Dis*, 71, 140-50.

- NESTLER, E. J. & HYMAN, S. E. 2010. Animal models of neuropsychiatric disorders. *Nat Neurosci*, 13, 1161-9.
- NEWBY, J. M., MCKINNON, A., KUYKEN, W., GILBODY, S. & DALGLEISH, T. 2015. Systematic review and meta-analysis of transdiagnostic psychological treatments for anxiety and depressive disorders in adulthood. *Clin Psychol Rev*, 40, 91-110.
- NICOLA, S. M. 2007. The nucleus accumbens as part of a basal ganglia action selection circuit. *Psychopharmacology (Berl)*, 191, 521-50.
- OGDEN, C. A., RICH, M. E., SCHORK, N. J., PAULUS, M. P., GEYER, M. A., LOHR, J. B., KUCZENSKI, R. & NICULESCU, A. B. 2004. Candidate genes, pathways and mechanisms for bipolar (manic-depressive) and related disorders: an expanded convergent functional genomics approach. *Mol Psychiatry*, 9, 1007-29.
- OLDHAM, W. M. & HAMM, H. E. 2008. Heterotrimeric G protein activation by G-protein-coupled receptors. *Nat Rev Mol Cell Biol*, 9, 60-71.
- PANG, T. Y., DU, X., ZAJAC, M. S., HOWARD, M. L. & HANNAN, A. J. 2009. Altered serotonin receptor expression is associated with depression-related behavior in the R6/1 transgenic mouse model of Huntington's disease. *Hum Mol Genet*, 18, 753-66.
- PARK, P. S., LODOWSKI, D. T. & PALCZEWSKI, K. 2008. Activation of G protein-coupled receptors: beyond two-state models and tertiary conformational changes. *Annu Rev Pharmacol Toxicol*, 48, 107-41.
- PARKINSON, J. A., OLMSTEAD, M. C., BURNS, L. H., ROBBINS, T. W. & EVERITT, B. J. 1999. Dissociation in effects of lesions of the nucleus accumbens core and shell on appetitive pavlovian approach behavior and the potentiation of conditioned reinforcement and locomotor activity by D-amphetamine. *J Neurosci*, 19, 2401-11.
- PERRY, W., MINASSIAN, A., FEIFEL, D. & BRAFF, D. L. 2001. Sensorimotor gating deficits in bipolar disorder patients with acute psychotic mania. *Biol Psychiatry*, 50, 418-24.
- PINEDA, D. A., PALACIO, L. G., PUERTA, I. C., MERCHAN, V., ARANGO, C. P., GALVIS, A. Y., GOMEZ, M., AGUIRRE, D. C., LOPERA, F. & ARCOS-BURGOS, M. 2007. Environmental influences that affect attention deficit/hyperactivity disorder: study of a genetic isolate. *Eur Child Adolesc Psychiatry*, 16, 337-46.
- POULADI, M. A., MORTON, A. J. & HAYDEN, M. R. 2013. Choosing an animal model for the study of Huntington's disease. *Nat Rev Neurosci*, 14, 708-21.
- POWELL, S. B., WEBER, M. & GEYER, M. A. 2012. Genetic models of sensorimotor gating: relevance to neuropsychiatric disorders. *Curr Top Behav Neurosci*, 12, 251-318.
- PURPER-OUAKIL, D., RAMOZ, N., LEPAGNOL-BESTEL, A. M., GORWOOD, P. & SIMONNEAU, M. 2011. Neurobiology of attention deficit/hyperactivity disorder. *Pediatr Res*, 69, 69R-76R.
- QUINTANA A, S. E., WANG W, STOREY GP, GULER AD, WANAT MJ, ET AL. 2012. Lack of GPR88 enhances medium spiny neuron activity and alters motor- and cue-dependent behaviors. *Nat Neurosci*, 1547-1555.
- RADER, R., MCCAULEY, L. & CALLEN, E. C. 2009. Current strategies in the diagnosis and treatment of childhood attention-deficit/hyperactivity disorder. *Am Fam Physician*, 79, 657-65.
- RASK-ANDERSEN, M., ALMEN, M. S. & SCHIOTH, H. B. 2011. Trends in the exploitation of novel drug targets. *Nat Rev Drug Discov*, 10, 579-90.

- RAUCH, S. L., SHIN, L. M. & PHELPS, E. A. 2006. Neurocircuitry models of posttraumatic stress disorder and extinction: human neuroimaging research--past, present, and future. *Biol Psychiatry*, 60, 376-82.
- RAZAFSHA, M., BEHFORUZI, H., HARATI, H., WAFAI, R. A., KHAKU, A., MONDELLO, S., GOLD, M. S. & KOBEISSY, F. H. 2013. An updated overview of animal models in neuropsychiatry. *Neuroscience*, 240, 204-18.
- RING, H. A. & SERRA-MESTRES, J. 2002. Neuropsychiatry of the basal ganglia. *J Neurol Neurosurg Psychiatry*, 72, 12-21.
- ROSENBLATT, A. 2007. Neuropsychiatry of Huntington's disease. *Dialogues Clin Neurosci*, 9, 191-7.
- ROSS, C. A. & TABRIZI, S. J. 2011. Huntington's disease: from molecular pathogenesis to clinical treatment. *Lancet Neurol*, 10, 83-98.
- RUSSELL, V. A. 2007. Neurobiology of animal models of attention-deficit hyperactivity disorder. *J Neurosci Methods*, 161, 185-98.
- SAGVOLDEN, T. 2000. Behavioral validation of the spontaneously hypertensive rat (SHR) as an animal model of attention-deficit/hyperactivity disorder (AD/HD). *Neurosci Biobehav Rev*, 24, 31-9.
- SAGVOLDEN, T., RUSSELL, V. A., AASE, H., JOHANSEN, E. B. & FARSHBAF, M. 2005. Rodent models of attention-deficit/hyperactivity disorder. *Biol Psychiatry*, 57, 1239-47.
- SAMSOM, J. N. & WONG, A. H. 2015. Schizophrenia and Depression Co-Morbidity: What We have Learned from Animal Models. *Front Psychiatry*, 6, 13.
- SCHERES, A., MILHAM, M. P., KNUTSON, B. & CASTELLANOS, F. X. 2007. Ventral striatal hyporesponsiveness during reward anticipation in attention-deficit/hyperactivity disorder. *Biol Psychiatry*, 61, 720-4.
- SCHIFFMANN, S. N., JACOBS, O. & VANDERHAEGHEN, J. J. 1991. Striatal restricted adenosine A2 receptor (RDC8) is expressed by enkephalin but not by substance P neurons: an in situ hybridization histochemistry study. *J Neurochem*, 57, 1062-7.
- SCHIZOPHRENIA WORKING GROUP OF THE PSYCHIATRIC GENOMICS, C. 2014. Biological insights from 108 schizophrenia-associated genetic loci. *Nature*, 511, 421-7.
- SCHNEIER, F. R. 2006. Clinical practice. Social anxiety disorder. *N Engl J Med*, 355, 1029-36.
- SHINOZAKI, G. & POTASH, J. B. 2014. New developments in the genetics of bipolar disorder. *Curr Psychiatry Rep*, 16, 493.
- SHUEN, J. A., CHEN, M., GLOSS, B. & CALAKOS, N. 2008. Drd1a-tdTomato BAC transgenic mice for simultaneous visualization of medium spiny neurons in the direct and indirect pathways of the basal ganglia. *J Neurosci*, 28, 2681-5.
- SMITH, R. J., LOBO, M. K., SPENCER, S. & KALIVAS, P. W. 2013. Cocaine-induced adaptations in D1 and D2 accumbens projection neurons (a dichotomy not necessarily synonymous with direct and indirect pathways). *Curr Opin Neurobiol*, 23, 546-52.
- STEIN, M. B., SIMMONS, A. N., FEINSTEIN, J. S. & PAULUS, M. P. 2007. Increased amygdala and insula activation during emotion processing in anxiety-prone subjects. *Am J Psychiatry*, 164, 318-27.

- STINE, O. C., PLEASANT, N., FRANZ, M. L., ABBOTT, M. H., FOLSTEIN, S. E. & ROSS, C. A. 1993. Correlation between the onset age of Huntington's disease and length of the trinucleotide repeat in IT-15. *Hum Mol Genet*, 2, 1547-9.
- STRAKOWSKI, S. M., DELBELLO, M. P., ZIMMERMAN, M. E., GETZ, G. E., MILLS, N. P., RET, J., SHEAR, P. & ADLER, C. M. 2002. Ventricular and periventricular structural volumes in first- versus multiple-episode bipolar disorder. *Am J Psychiatry*, 159, 1841-7.
- STREJILEVICH, S. A., SAMAME, C. & MARTINO, D. J. 2015. The trajectory of neuropsychological dysfunctions in bipolar disorders: a critical examination of a hypothesis. *J Affect Disord*, 175, 396-402.
- STROHLE, A., STOY, M., WRASE, J., SCHWARZER, S., SCHLAGENHAUF, F., HUSS, M., HEIN, J., NEDDERHUT, A., NEUMANN, B., GREGOR, A., JUCKEL, G., KNUTSON, B., LEHMKUHL, U., BAUER, M. & HEINZ, A. 2008. Reward anticipation and outcomes in adult males with attention-deficit/hyperactivity disorder. *Neuroimage*, 39, 966-72.
- SURMEIER, D. J., DING, J., DAY, M., WANG, Z. & SHEN, W. 2007. D1 and D2 dopamine-receptor modulation of striatal glutamatergic signaling in striatal medium spiny neurons. *Trends Neurosci*, 30, 228-35.
- SVENNINGSSON, P., NISHI, A., FISONE, G., GIRAULT, J. A., NAIRN, A. C. & GREENGARD, P. 2004. DARPP-32: an integrator of neurotransmission. *Annu Rev Pharmacol Toxicol*, 44, 269-96.
- SWERDLOW, N. R., BRAFF, D. L. & GEYER, M. A. 2000. Animal models of deficient sensorimotor gating: what we know, what we think we know, and what we hope to know soon. *Behav Pharmacol*, 11, 185-204.
- SWERDLOW, N. R. & GEYER, M. A. 1998. Using an animal model of deficient sensorimotor gating to study the pathophysiology and new treatments of schizophrenia. *Schizophr Bull*, 24, 285-301.
- TARAZI, F. I., SAHLI, Z. T., WOLNY, M. & MOUSA, S. A. 2014. Emerging therapies for Parkinson's disease: from bench to bedside. *Pharmacol Ther*, 144, 123-33.
- THAPAR, A., FOWLER, T., RICE, F., SCOURFIELD, J., VAN DEN BREE, M., THOMAS, H., HAROLD, G. & HAY, D. 2003. Maternal smoking during pregnancy and attention deficit hyperactivity disorder symptoms in offspring. *Am J Psychiatry*, 160, 1985-9.
- TRIPP, G. & WICKENS, J. 2012. Reinforcement, dopamine and rodent models in drug development for ADHD. *Neurotherapeutics*, 9, 622-34.
- TSAI, G. & COYLE, J. T. 2002. Glutamatergic mechanisms in schizophrenia. *Annu Rev Pharmacol Toxicol*, 42, 165-79.
- TYE, K. M. & DEISSEROTH, K. 2012. Optogenetic investigation of neural circuits underlying brain disease in animal models. *Nat Rev Neurosci*, 13, 251-66.
- TYE, K. M., PRAKASH, R., KIM, S. Y., FENNO, L. E., GROSENICK, L., ZARABI, H., THOMPSON, K. R., GRADINARU, V., RAMAKRISHNAN, C. & DEISSEROTH, K. 2011. Amygdala circuitry mediating reversible and bidirectional control of anxiety. *Nature*, 471, 358-62.
- UBALDI, M., RICCIARDELLI, E., PASQUALINI, L., SANNINO, G., SOVERCHIA, L., RUGGERI, B., FALCINELLI, S., RENZI, A., LUDKA, C., CICCOCIOPPO, R. & HARDIMAN, G. 2015. Biomarkers of hippocampal gene expression in a mouse restraint chronic stress model. *Pharmacogenomics*, 16, 471-82.

- UNGLESS, M. A. 2004. Dopamine: the salient issue. *Trends Neurosci*, 27, 702-6.
- UNGLESS, M. A., MAGILL, P. J. & BOLAM, J. P. 2004. Uniform inhibition of dopamine neurons in the ventral tegmental area by aversive stimuli. *Science*, 303, 2040-2.
- VALADAS, J. S., VOS, M. & VERSTREKEN, P. 2015. Therapeutic strategies in Parkinson's disease: what we have learned from animal models. *Ann NY Acad Sci*, 1338, 16-37.
- VALERA, E. M., FARAONE, S. V., MURRAY, K. E. & SEIDMAN, L. J. 2007. Meta-analysis of structural imaging findings in attention-deficit/hyperactivity disorder. *Biol Psychiatry*, 61, 1361-9.
- VALJENT, E., BERTRAN-GONZALEZ, J., HERVE, D., FISONE, G. & GIRAULT, J. A. 2009. Looking BAC at striatal signaling: cell-specific analysis in new transgenic mice. *Trends Neurosci*, 32, 538-47.
- VAN WAES V, T. K., STEINER H 2011. GPR88 - a putative signaling molecule predominantly expressed in the striatum: Cellular localization and developmental regulation. *Basal Ganglia*, 83-89.
- VINTHER-JENSEN, T., NIELSEN, T. T., BUDTZ-JORGENSEN, E., LARSEN, I. U., HANSEN, M. M., HASHOLT, L., HJERMIND, L. E., NIELSEN, J. E. & NORREMOLLE, A. 2015. Psychiatric and cognitive symptoms in Huntington's disease are modified by polymorphisms in catecholamine regulating enzyme genes. *Clin Genet*.
- VOORN, P., VANDERSCHUREN, L. J., GROENEWEGEN, H. J., ROBBINS, T. W. & PENNARTZ, C. M. 2004. Putting a spin on the dorsal-ventral divide of the striatum. *Trends Neurosci*, 27, 468-74.
- WICHMANN, T. & DELONG, M. R. 1996. Functional and pathophysiological models of the basal ganglia. *Curr Opin Neurobiol*, 6, 751-8.
- WILENSKY, A. E., SCHAFF, G. E., KRISTENSEN, M. P. & LEDOUX, J. E. 2006. Rethinking the fear circuit: the central nucleus of the amygdala is required for the acquisition, consolidation, and expression of Pavlovian fear conditioning. *J Neurosci*, 26, 12387-96.
- WONG, A. H. & JOSSELYN, S. A. 2015. Caution When Diagnosing Your Mouse with Schizophrenia: The Use and Misuse of Model Animals for Understanding Psychiatric Disorders. *Biol Psychiatry*.
- WOO, H., PARK, S. J., LEE, Y., KWON, G., GAO, Q., LEE, H. E., AHN, Y. J., SHIN, C. Y., CHEONG, J. H. & RYU, J. H. 2014. The effects of atomoxetine and methylphenidate on the prepulse inhibition of the acoustic startle response in mice. *Prog Neuropsychopharmacol Biol Psychiatry*, 54, 206-15.
- XIA, R. & MAO, Z. H. 2012. Progression of motor symptoms in Parkinson's disease. *Neurosci Bull*, 28, 39-48.
- YIN, H. H., MULCARE, S. P., HILARIO, M. R., CLOUSE, E., HOLLOWAY, T., DAVIS, M. I., HANSSON, A. C., LOVINGER, D. M. & COSTA, R. M. 2009. Dynamic reorganization of striatal circuits during the acquisition and consolidation of a skill. *Nat Neurosci*, 12, 333-41.
- YOUNG, R. C. 2005. Bipolar disorder in older persons: perspectives and new findings. *Am J Geriatr Psychiatry*, 13, 265-7.
- YOUNGSTROM, E. A., FINDLING, R. L., YOUNGSTROM, J. K. & CALABRESE, J. R. 2005. Toward an evidence-based assessment of pediatric bipolar disorder. *J Clin Child Adolesc Psychol*, 34, 433-48.

ZHANG, K., HILL, K., LABAK, S., BLATT, G. J. & SOGHOMONIAN, J. J. 2014. Loss of glutamic acid decarboxylase (Gad67) in Gpr88-expressing neurons induces learning and social behavior deficits in mice. *Neuroscience*, 275, 238-47.

ZIMMERMANN, A. M., JENE, T., WOLF, M., GORLICH, A., GURNIAK, C. B., SASSOE-POGNETTO, M., WITKE, W., FRIAUF, E. & RUST, M. B. 2014. Attention-Deficit/Hyperactivity Disorder-like Phenotype in a Mouse Model with Impaired Actin Dynamics. *Biol Psychiatry*.

Results

Chapter 1: Behavioral and biochemical alterations in mice lacking GPR88

The following chapter is divided in two parts:

- 1) Manuscript 1 (*published*: Meirsman *et al.*, 2015) describes the full *Gpr88* KO and shows, in these mice, biochemical and structural alteration in the striatum and other brain regions. Also, we show behavioral alterations in a large set of tests and a partial deficit reversal by a delta opioid receptor antagonist.
- 2) Section 2 briefly describes a collaboration with the laboratory of Dr. Laura Harsan in Freiburg. In this experiment we examined whether the hyperactivity phenotype of *Gpr88* KO mice is reversed by the gold standard treatment in attention deficit/hyperactivity disorder (Methylphenidate).

1) Mice lacking GPR88 show motor deficit, improved spatial learning and low anxiety reversed by delta opioid antagonist

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2) Hyperactivity of Gpr88 KO mice is reversed by methylphenidate

In the following pages I present preliminary data that are part of a collaboration with the laboratory of Dr. Laura Harsan in Freiburg (University Medical Center Freiburg). *Gpr88* KO animals show locomotor hyperactivity, with failure to habituate to a novel environment, a behavioral hallmark of animal models for Attention-Deficit/Hyperactive Disorder (ADHD). The knockout mice also present neurobiological alterations in both striatum (caudate-putamen and nucleus accumbens) and prefrontal cortex (Meirsman et al., 2015), both structures highly implicated in the pathophysiology of Attention-Deficit/Hyperactive Disorder (ADHD) (Russell, 2007). As such, the project addresses functional connectivity in live mutant animals using resting state fMRI and the potential link with ADHD neuroimaging data. As a complement, and to further validate *Gpr88* KO as a potential genetic model for ADHD, I tested whether Methylphenidate (“ritaline”), the gold standard treatment in ADHD, is able to reverse the hyperactive phenotype of *Gpr88* KO animals (Rader et al., 2009, Sagvolden et al., 2005).

Hyperactivity of Gpr88 KO mice is reversed by methylphenidate

Materials and Methods

Animals

For open field locomotor activity recording *Gpr88*^{-/-} young adults (8-10-weeks) male (N=13 WT, N=10 KO) and female (N=8 WT, N=4 KO) were used. 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*^{-/-}.

Mice were housed in groups of three to five in a temperature and humidity controlled animal facility (21±2°C, 45±5% humidity) on a 12 hr light/12 hr dark cycle (lights on at 7:00 h) with *ad libitum* access to food and water. All animals used were bred in-house.

Open Field (OF) locomotion and drug

Open Field locomotor activity was measured in four equal square arenas (50 × 50 cm) separated by 35 cm-high opaque grey Plexiglas walls. Activity was measured during five daily sessions of 30 min. Methylphenidate hydrochloride (Sigma, France) (MPH) was dissolved in sterile isotonic saline solution (NaCl 0.9%) and injected in a volume of 10 ml/kg at a dose of 3mg/kg. Mice received a single intraperitoneal (*i.p.*) injection of either saline solution or MPH 15 min before being placed in a dimly lighted (10lux) open field in each daily session. Locomotor activity was automatically recorded via an automated tracking system (videotrack; View Point, Lyon, France). Only movements which speed was over 6 cm/s were taken into account for this measure.

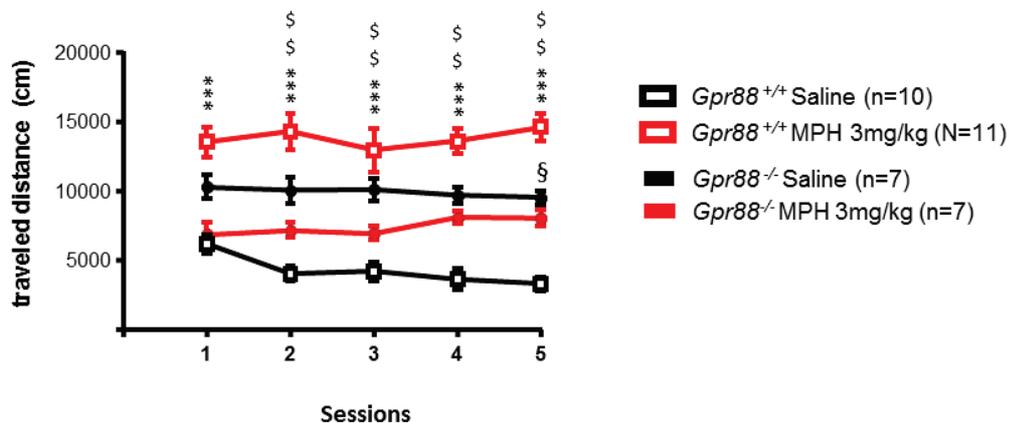


Figure 1: Hyperactivity of *Gpr88*^{-/-} mice is reversed by acute methylphenidate (MPH) administration. When placed for a daily 30 min session in the open field saline treated *Gpr88*^{-/-} mice traveled a longer distance than control saline animals. Upon MPH administration 15min before open field locomotion WT animals present an increased forward locomotion while this treatment decreased locomotion in KO animals. Line graphs represent the mean distance traveled (cm) over each 30 min session. Data are represented as mean \pm SEM. *Gpr88*^{+/+} Saline vs *Gpr88*^{+/+} MPH (*): three stars $p < 0.001$. *Gpr88*^{+/+} Saline vs *Gpr88*^{-/-} Saline (\$) two stars $p < 0, 01$; *Gpr88*^{-/-} Saline vs *Gpr88*^{+/+}MPH (§) (Sidak's multiple comparison test)

Results and discussion

Gpr88^{-/-} hyperactive behavior is reversed by Methylphenidate

To verify whether acute methylphenidate (MPH) treatment could reverse the hyperactive phenotype of *Gpr88*^{-/-} animals we started with low MPH doses based on previous reports in mice (Zimmermann et al., 2014, Humby et al., 2013). Pilot experiments using 1 or 2 mg/kg of MPH indicated that these concentrations do not alter locomotor activity of *Gpr88*^{-/-} animals while causing a slight locomotion increase in wild type littermates (not shown). When tested with 3mg/kg *Gpr88*^{-/-} mice displayed decreased locomotion in the open field 15 min after drug injection while 4mg/kg slightly increased these mice locomotion (not shown). Consequently, we treated *Gpr88*^{-/-} mice (n=7 for saline, n=7 for MPH) and wild-type controls (n=10 for saline, n=11 for MPH) with a 3mg/kg

injection, and measured locomotor activity (see method, [Figure 1](#)). Two-way ANOVA revealed a significant treatment ($F_{(9, 155)} = 8,244; p < 0, 0001$) and Genotype x treatment interaction effect ($F_{(9, 155)} = 22,19; p < 0,0001$). The spontaneous hyperactive behavior of *Gpr88*^{-/-} mice in a familiar environment is shown by *post hoc* Sidak multiple comparison test, indicating significant differences between saline WT and KO mice from the second to the last session day (for detailed statistical information please refer to [table 1](#)). This is consistent with the described hyperactive phenotype of these mutant mice (Quintana A, 2012, Meirsmann et al., 2015). Also, during the first four sessions, KO saline animals traveled the same distance as WT mice treated with 3mg/kg of MPH, demonstrating the stimulatory effect of MPH on WT animals. MPH-treated KO mice, on the other hand, showed a trend to lower activity compared to KO saline mice (not significant), and did not significantly differ from WT saline animals. The latter finding shows reversal of the hyperactive phenotype of KO animal by methylphenidate, at least partially. It is possible that chronic MPH treatment, as recently performed by D'Andrea et al (2015) would fully reverse the Gpr88 KO mice phenotype (D'Andrea et al., 2015). We conclude that *Gpr88*^{-/-} mice present both face and predictive validity to model the hyperactivity dimension of ADHD.

References

- D'ANDREA, I., FARDELLA, V., FARDELLA, S., PALLANTE, F., GHIGO, A., IACOBUCCI, R., MAFFEI, A., HIRSCH, E., LEMBO, G. & CARNEVALE, D. 2015. Lack of kinase-independent activity of PI3Kgamma in locus coeruleus induces ADHD symptoms through increased CREB signaling. *EMBO Mol Med*, 7, 904-17.
- HUMBY, T., EDDY, J. B., GOOD, M. A., REICHEL, A. C. & WILKINSON, L. S. 2013. A novel translational assay of response inhibition and impulsivity: effects of prefrontal cortex lesions, drugs used in ADHD, and serotonin 2C receptor antagonism. *Neuropsychopharmacology*, 38, 2150-9.
- MEIRSMAN, A. C., LE MERRER, J., PELLISSIER, L. P., DIAZ, J., CLESSE, D., KIEFFER, B. L. & BECKER, J. A. 2015. Mice Lacking GPR88 Show Motor Deficit, Improved Spatial Learning, and Low Anxiety Reversed by Delta Opioid Antagonist. *Biol Psychiatry*.
- QUINTANA A, S. E., WANG W, STOREY GP, GULER AD, WANAT MJ, ET AL. 2012. Lack of GPR88 enhances medium spiny neuron activity and alters motor- and cue-dependent behaviors. *Nat Neurosci*, 1547-1555.
- RADER, R., MCCAULEY, L. & CALLEN, E. C. 2009. Current strategies in the diagnosis and treatment of childhood attention-deficit/hyperactivity disorder. *Am Fam Physician*, 79, 657-65.
- RUSSELL, V. A. 2007. Neurobiology of animal models of attention-deficit hyperactivity disorder. *J Neurosci Methods*, 161, 185-98.

- SAGVOLDEN, T., RUSSELL, V. A., AASE, H., JOHANSEN, E. B. & FARSHBAF, M. 2005. Rodent models of attention-deficit/hyperactivity disorder. *Biol Psychiatry*, 57, 1239-47.
- ZIMMERMANN, A. M., JENE, T., WOLF, M., GORLICH, A., GURNIAK, C. B., SASSOE-POGNETTO, M., WITKE, W., FRIAUF, E. & RUST, M. B. 2014. Attention-Deficit/Hyperactivity Disorder-like Phenotype in a Mouse Model with Impaired Actin Dynamics. *Biol Psychiatry*.

Table 1. Statistical analysis: behavioral effects of Methylphenidate (3 mg/kg) or saline treatment in *Gpr88*^{-/-} and *Gpr88*^{+/-} animals

Groups				Two way ANOVA			Sidak Multiple comparison test					
<i>Gpr88</i> ^{+/-} Saline	<i>Gpr88</i> ^{-/-} Saline	<i>Gpr88</i> ^{+/-} MPH	<i>Gpr88</i> ^{-/-} MPH	Genotype	Treatment	Genotype x Treatment	Comparisation	Session 1	Session 2	Session 3	Session 4	Session 5
6M,4F	6M, 1F	7M,4F	4M, 3F	F (1, 155) = 0,6803 p=0,41	F (9, 155) = 8,244 p<0,0001	F (9, 155) = 22,19 p<0,0001	Gpr88 ^{+/-} Saline vs Gpr88 ^{-/-} Saline (\$)	t (155)= 2,998; N.S.	t(155)= 4,410; p<0,01	t(155)=4,310; p<0,01	t(155)=4,441; p<0,01	t(155)=4,541; p<0,01
							Gpr88 ^{+/-} Saline vs Gpr88 ^{+/-} MPH (*)	t(155)=6,048; p<0,0001	t(155)=8,473; p<0,0001	t(155)=7,214; p<0,0001	t(155)=8,249; p<0,0001	t(155)=9,316; p<0,0001
							Gpr88 ^{+/-} Saline vs Gpr88 ^{-/-} MPH	t (155)=0,5046 ; N.S.	t (155)=2,293 ; N.S.	t (155)=2,016; N.S.	t (155)=3,284 ; N.S.	t (155)=3,461 ; N.S.
							Gpr88 ^{-/-} Saline vs Gpr88 ^{-/-} MPH	t (155)=2,299 ; N.S.	t (155)=1,952 ; N.S.	t (155)=2,115 ; N.S.	t (155)=1,067 ; N.S.	t (155)=0,9957 ; N.S.
							Gpr88 ^{-/-} Saline vs Gpr88 ^{+/-} MPH (§)	t (155)=2,409 ; N.S.	t (155)=3,162 ; N.S.	t (155)=2,126; N.S.	t (155)=2,928; N.S.	t (155)=3,790; p<0,05

Chapter 2: Cell-specific and time controlled knockout of *Gpr88*

The following chapter is divided in three parts:

- 1) Manuscript 2 (*in preparation*) describes the successful conditional striatopallidal-specific *Gpr88* knockout (KO). In this report we compared emotional phenotypes of full and conditional *Gpr88* KO. Results show decreased anxiety-like behavior in both mice line tested but increased novelty approach and impaired fear conditioning in full but not conditional KO.
- 2) Section 2 focuses on GPR88 regulation of motor functions. In this report we tested motor coordination as well as locomotor responses in full *Gpr88* KO mice, conditional striatopallidal *Gpr88* KO mice and in a knock-down of *Gpr88* in the striatum of adult mice. We show that motor coordination is regulated by GPR88 in the adult brain. Also, results indicate that striatopallidal GPR88 regulates motor coordination, hyperactivity and dopamine-driven locomotion.
- 3) In section 3 I describe several attempts to induce the deletion of *Gpr88* in the adult mouse whole brain using CreER^{T2} approaches. Unfortunately, despite several mouse lines and protocols tested we were unable to reach satisfactory results. The results discussed in this chapter should nevertheless be useful for future experiments aiming at knocking out any gene of interest in a time-controlled manner.

1) GPR88 in striatopallidal neurons enhances anxiety-like behaviors

(In preparation)

GPR88 in striatopallidal neurons enhances anxiety-like behaviors

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Short title: *Gpr88*^{A2A-Cre} knockout and emotional responses.

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Abstract

Background. GPR88 is an orphan G protein-coupled receptor (GPCR) highly expressed in striatal medium spiny neurons of both striatopallidal and striatonigral pathways. Previous studies show that total brain deletion of this receptor decreases anxiety-like behaviors, demonstrating that GPR88 modulates emotional responses. Here we used a conditional gene knockout approach to test whether GPR88 in striatopallidal neurons contributes to this phenotype.

Methods. We generated conditional *Gpr88* knockout mice in striatopallidal neurons using *A_{2A}R-Cre*-driven recombination. We compared emotional responses of conditional knockout mice with full *Gpr88* KO mice, and evaluated signaling properties of several striatal Gi/o-coupled GPCRs.

Results. *Gpr88* mRNA was selectively decreased in D₂R neurons of the striatum and central amygdala. Accordingly, GPR88 signaling was decreased by 40% in the striatum. Both total and striatopallidal *Gpr88* KO mice showed similar increased locomotor activity, and decreased anxiety levels in light-dark, elevated plus-maze, marble burying tests, and in a social interaction test. In contrast, total but not conditional KO mice showed enhanced preference for a novel environment and decreased novelty-suppressed feeding, as well as impaired conditioned fear responses. We also found that delta and mu opioid, metabotropic glutamate and D₂ dopamine receptor signaling was modified in the striatum of conditional KO mice.

Conclusion. We conclude that the previously reported anxiogenic activity of GPR88 operates at the level of striatopallidal neurons, possibly through modulation of other GPCRs. We also show for the first time that GPR88 activity regulates approach behaviors and conditional fear, however these behaviors are not mediated by striatopallidal receptors.

Keywords: striatopallidal medium spiny neurons; G-protein coupled receptors; anxiety-like behaviors; fear

Introduction

G protein-coupled receptors (GPCRs) are the target for about 40% marketed drugs, and are major players in biomedicine (Rask-Andersen et al., 2011). Orphans GPCRs, whose ligands remain unknown and functions have been little studied, offer great promise (Ghanemi, 2015, Rask-Andersen et al., 2011, Levoye et al., 2006). The orphan GPCR GPR88 has been implicated in a number of behaviors related to psychiatric disorders. Mice lacking *Gpr88* present a complex behavioral phenotype that includes motor coordination deficits, reduced PPI, stereotypies and altered cue-based learning (Logue et al., 2009, Massart R, 2009, Quintana A, 2012, Ingallinesi et al., 2014). These behaviors can all be related to the strong enrichment of GPR88 in the striatum (Liljeholm and O'Doherty, 2012, Lewis and Kim, 2009, Swerdlow et al., 2001). In humans, the *Gpr88* gene was associated with bipolar disorders and schizophrenia (Del Zompo et al., 2014). Recently, we found that *Gpr88* deletion in mice also decreases anxiety-like behavior, implicating this receptor in emotional processing and in evaluation of environmental stimuli value. Concordant with this finding, *Gpr88* expression was shown regulated by antidepressant and mood stabilizer treatments in both rodent models and humans (Brandish et al., 2005, Ogden et al., 2004, Bohm et al., 2006, Conti et al., 2007).

Several lines of evidence suggest that GPR88 alters behavior by modulating striatal transmission. In the striatum (dorsal and ventral), *Gpr88* expression is limited to striatonigral (co-expressing dopamine D1 receptors_D1R and Substance P) and striatopallidal (co-expressing dopamine D2 receptors_D2R and adenosine A_{2A} receptor_A_{2A}R) medium spiny neurons (MSNs) and regulates excitability of these neurons possibly by acting on glutamatergic, GABAergic and dopaminergic receptors activity (Logue et al., 2009, Quintana A, 2012). Conversely, glutamatergic and dopaminergic depletion differentially alters *Gpr88* expression in the striatopallidal versus striatonigral pathways (Logue et al., 2009). However, the precise mechanism by which GPR88 regulates MSNs transmission to alter behavior is still largely unknown. 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. For instance, it has been suggested that altered striatopallidal pathway (D₂R-MSNs) transmission may disrupt inhibitory

controls and avoidance in a decision conflict task (Hikida et al., 2010, Hikida et al., 2013). Moreover, studies in humans and rodents suggest that the dopamine D₂R modulates reward and emotional processing (Pecina et al., 2013, Brandao et al., 2015, Hranilovic et al., 2008) while activation of D₂R-expressing striatopallidal neurons induced depressive like behavior (Francis et al., 2015).

To gain better understanding of how GPR88 in striatopallidal MSNs regulates emotional processing, we generated a conditional knockout (cKO) of *Gpr88* in neurons expressing A₂AR, also known to express D₂R. We compared behavioral responses of mice lacking *Gpr88* in striatopallidal neurons with full *Gpr88* Knockout (KO) animals using behavioral tests measuring anxiety-like behaviors and fear responses. We show that the conditional deletion of *Gpr88* decreases anxiety-like behaviors without affecting fear responses. Also, and as reported in full KO mice, deletion of *Gpr88* in striatopallidal neurons was sufficient to increase delta opioid receptor activation, possibly contributing to the low anxiety phenotype (Meirsmann et al., 2015). Finally, we report decreased metabotropic glutamatergic and D₂R activation, which were absent after complete deletion of *Gpr88*.

Materials and Methods

Animals:

Gpr88-floxed mice (*Gpr88*^{flx/flx}), full *Gpr88* KO (*Gpr88*^{-/-}) (Meirsmann et al., 2015) and *Adora2a-Cre* mice (Durieux PF, 2009) were produced as previously described. Briefly, *Gpr88*^{flx/flx} mice, in which exon 2 is flanked by a loxP site (upstream) and a Lox-FRT neomycin-resistance cassette (downstream) were crossed with CMV-Cre mice expressing Cre recombinase under the cytomegalovirus promoter. This led to germ-line deletion of *Gpr88* exon 2.

To generate a conditional KO of *Gpr88* in Striatopallidal medium spiny neurons (*Gpr88*^{A2A-Cre}) *Adora2a-Cre* mice were crossed with *Gpr88*^{flx/flx} mice (Durieux PF, 2009). First generation animals expressing the Cre under the control of A_{2A} promoter (*Gpr88*^{A2A-Cre/+}) were crossed a second time to eliminate the wild-type *Gpr88* gene (*Gpr88*^{A2A-Cre}). All mice were generated at Institut Clinique de la Souris-Institut de Génétique et Biologie Moléculaire et Cellulaire.

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*^{A2A-Cre}. To verify the presence of Cre and Myosine (the latter as a positive control) the following primers were used: 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'.

Mice (male and female) aged 9-15 weeks were bred in house and grouped-house 3-5 animals per cage. Animals were maintained on a 12hr light/dark cycle at controlled temperature (22±1°C). Food and water were available *ad libitum* throughout all experiments. All experiments were approved by the local ethic committee (CREMEAS, 2003-10-08-[1]-58). For all experiments *Gpr88*^{A2A-Cre} mice were compared to their littermates (*Gpr88*^{flx/flx}) and *Gpr88*^{-/-} mice were compared to *Gpr88*^{+/+} mice. An independent cohort of naïve animals was used for each behavioral paradigm, except for the fear conditioning that was performed in the same cohort as the Light Dark test 48h after the latter. All behavioral testing was performed and analyzed blind to genotypes.

Tissue preparation and fluorescent in situ hybridization

Mice (n= 4 *Gpr88*^{flx/flx}; 4 *Gpr88*^{A2A-Cre}) 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 20µm serial sections by using cryostat (CM3050 Leica, Wetzlar, Germany) and placed in superfrost slides (Thermo scientific, Waltham, MA, USA). *In situ* hybridizations were performed using the RNAscope® Multiplex Fluorescent Assay. GPR88 probes were coupled to FITC while D₁R and D₂R probes were coupled with Tritc and Cy5 respectively.

Relative expression of Gpr88 in D1R and D2R positive cells

Image acquisition was performed with the slide scanner NanoZoomer 2 HT and fluorescence module L11600-21 (Hamamatsu Photonics, Japan). To verify the specific excision of *Gpr88* in striatopallidal neurons *In situ* hybridization images were analyzed using NDP viewer software. For each brain, 4

slices were selected: two slices for the Caudate-Putamen (CPu) (Rostral: + 0,98mm from Bregma; Caudal: -0,58mm from Bregma) one slice for the nucleus accumbens (Nac) (+ 1,34mm relative to Bregma) and one slice for the central nucleus of the Amygdala (CeA) (-1, 22 from Bregma). For each structure, regions of interest (ROIs) were determined by drawing two-dimensional boxes with defined surfaces. Counting was performed on one ROI with a surface of 1mm² for the Nac, 0,250 mm² for the CeA and two ROI of 0,5mm² for the each CPu slice (to include both Dorsomedian and dorsolateral Striatum, see [Figure 1](#)). Counting was balanced between right and left hemispheres. To evaluate expression of *Gpr88* in D₁R and D₂R expressing cells, counting was performed manually using the NDP view counting add-up. First, cells expressing D₁R but not D₂R were marked and counted. For each D₁R positive cell, co-expression of *Gpr88* was verified and counted separately. This process was repeated for D₂R mRNA positive cells. Relative *Gpr88* expression is represented as a percentage of total D₁R or D₂R positive cells counted [(number D₁R or D₂R expressing cells co-expressing *Gpr88* x 100)/ total number of D₁R or D₂R expressing cells]. Given the lack of difference in *Gpr88* expression between lateral and medial CPu ([supplementary table 1](#)), relative percentage of each was pooled for graphical representation and statistical analysis.

[³⁵S]-GTP_γS binding assay

[³⁵S]-GTP_γS assays were performed as previously described (Pradhan et al., 2009). Briefly, to evaluate the activation of GPR88 in Prefrontal cortex (PFC), Caudate-Putamen (CPu), Nucleus Accumbens (Nac), central nucleus of the amygdala (CeA) and Hippocampus (HPC), structures were punched in 6 animals of each genotype (3 males 3 females) as previously described (Le Merrer et al., 2012a) and pooled for membrane preparation. To perform [³⁵S]-GTP_γS 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. Three membrane preparations were used per genotype, gathering tissue from three animals each (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 800 μ L ice-cold sucrose solution (0.32 M) and kept at -80°C. For each [³⁵S]GTP γ S binding assay 2 μ g 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]GTP γ S. Bound radioactivity was quantified using a liquid scintillation counter. B_{max} and K_d values were calculated. Non-specific binding was defined as binding in the presence of 10 μ M GTP γ S and binding in the absence of agonist was defined as the basal binding.

Drugs

The GPR88 agonist compound 19 (Meirsman et al., 2015) was kindly synthesized by Prestwick Chemicals (Illkirch, France) and dissolved in water (-)-Quinpirole hydrochloride (D2/3 agonist) and SNC-80 (delta opioid receptor agonist) were obtained from Tocris (Bristol, UK) and dissolved in isotonic saline solution (NaCl 0.9%) and 100% DMSO respectively. Carbamylcholine chloride (carbachol, non-selective cholinergic agonist) DAMGO (mu opioid receptor agonist) and Glutamate were obtained from Sigma (St Louis, USA) and dissolved in water.

Behavioral analysis

Open field locomotion: Mice were placed in a dimly lit (15 Lux) open field arena placed over a white Plexiglas infrared-lit platform. Locomotor activity was recorded during 30 minutes via an automated tracking system (videotrack; View Point, Lyon, France). Only movements which speed exceed 6 cm/s were taken into account for this measure.

Fear conditioning: Experiments were conducted in four dimly lighted operant chambers (28 x 21 x 22 cm, Coulbourn Instruments, Allentown, US), with a Plexiglas door and a metal bar floor connected to a shocker (Coulbourn Instruments). Chambers had a permanent house-light and were equipped with a speaker for tone delivery. An infrared activity monitor, used to assess animal motion, was placed on the ceiling of each chamber. The activity/inactivity behavior was monitored continuously during 100 ms period. Data are expressed in duration of inactivity per sec and the total time of inactivity displayed by each subject during training and testing sessions was counted. The procedure was similar as previously described (Goeldner et al., 2009). Briefly, the first day animals underwent one conditioning session and, the second day, contextual and cued fear conditioning were tested.

The conditioning session was initiated with a 4-min habituation period followed by a 20 s long tone of 20 KHz/75 dB (conditional stimulus, CS) coupled with a 0.4 mA footshock (unconditional stimulus, US) during the last second. Two minutes later, a similar CS-US pairing was presented and the mice were removed from the apparatus 2 min after the footshock. The following day, mice were exposed again to the conditioning chamber and immobility was measured during 4 min to assess contextual fear conditioning. The same day, 5 h after context fear was measured; cued fear conditioning was assessed in modified chambers.

Light-Dark Test. The light/dark apparatus is composed of two rectangular compartments (20 x 20 x 14 cm) separated by a tunnel (5 x 7 x 10 cm) (Imetronic, Pessac, France). One compartment is constituted of black floor and walls dimly lit (5 lux), whereas the other is made of a white floor and walls intensely lit (1000 lux). The apparatus is equipped with infrared beams and sensors. Mice were placed in the dark compartment and behavior was automatically recorded for 5 min.

Elevated plus-maze (EPM). The EPM was a plus-shaped maze elevated 52 cm from base, with black Plexiglas floor, consisting of two open and two closed arms (37 × 6 cm each) connected by a central platform (6 × 6 cm). The experiments were conducted under low-intensity light (15 lux). Movement and location of the mice were analyzed by an automated tracking system (Videotrack; View Point, Lyon, France). Each mouse was placed on the central platform facing a closed arm and observed for 5 min. Anxiety-like behavior was assessed by measures of the time spent and number of entries in closed and open arms of the maze, and related time and activity ratios (time spent or distance traveled in open arms/total time spent or distance in arms). Risk-taking behavior was evaluated by analyzing the time spent in the distal part of the open arms (time spent in the last 1/3 of the open arm) and the number of head dips (total number of head dips and head dips from the distal part of the open arms). Finally, the distance traveled in the maze was used as measures of locomotor activity.

Social interaction test. Social anxiety was assessed on 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 an interactor and a 10 min session was recorder. Nose

and paw contacts as well as following and grooming were measured. If an interactor failed to engage in any interaction data from the respective mice were excluded from analysis.

Marble burying. The marble burying test was carried out using 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. The mice were left in the cage for 10 minutes and the number of unburied marbles was counted.

Novelty preference. Novelty preference was assessed in unbiased computerized boxes (Imetronic, Pessac, France) previously described (Le Merrer et al., 2012b). Briefly, apparatus was composed of two chambers separated by a central alley. Two sliding doors separated the compartments from the central alley. Chambers differed in global shape (but same total surface), and floor texture. Mice were confined to one of the chambers (familiar chamber) for 15 min before being placed in the central corridor for 5 min. Then, both sliding doors were opened and mice were allowed to freely explore the apparatus. Time spent in each chamber was recorded and novelty preference was calculated as the percentage of time spent in the unfamiliar compartment.

Novelty suppressed feeding test. All mice were subjected to fasting 24 h before the beginning of the test but water was provided *ad libitum*. 30min before the beginning of the test, mice were isolated to increase anxiogenic conditions. During the test, 3 food pellets (regular chow) were placed on a square piece of white filter paper positioned in the center of a brightly illuminated (60Lux) open field (50 × 50 cm) filled with approximately 2 cm of sawdust bedding. Each mouse was placed in a corner of the open field facing the open field wall. The latency to the first bite of the food pellet was recorded (defined as the mouse sitting on its haunches and biting the pellet with the use of its forepaws). The Cut off time was defined as 15 min. After the test was over the animal was placed in his homecage and left alone for 5min. The food intake during this period was scored.

Statistics: All data are expressed as mean group value ± standard error of the mean (SEM) and analyzed using Student's test or two-way ANOVA whenever it was appropriate. When relevant, data were submitted to Sidak's or Turkey's multiple comparison post-hoc analysis. The criterion for statistical significance was $p < 0.05$. All statistics were performed using GraphPad Prism 6 (GraphPad Software, Inc, USA).

Results

***Gpr88*^{A2A-Cre} mice show decreased *Gpr88* mRNA levels in D₂R neurons of Caudate-Putamen, Nucleus Accumbens and Central Amygdala.**

To conditionally delete *Gpr88* exon 2 in striatopallidal D₂R-MSNs we crossed mice carrying two LoxP sites flanking the second exon of the *Gpr88* gene with mice expressing the Cre recombinase under the control of the striatopallidal specific *Adora2a* gene promoter (see methods). Then, we quantified *Gpr88*-expressing D₁R-positive and D₂R-positive neurons in *Gpr88*^{flx/flx} (control) and *Gpr88*^{A2A-Cre} (conditional KO) mice using triple *in situ* hybridization. Quantitative analysis was performed in four brain regions, including rostral Caudate-Putamen (CPu), caudal CPu, Nucleus accumbens (Nac) and Central nucleus of the amygdala (CeA) (Figure 1 A , supplementary Figure 1 and supplementary table 1 for detailed statistical analyses). In control animals, GPR88 was present in virtually all striatal D₁R (96,76 %±0,28) and D₂R (96,44%±0,74) expressing cells, as well as in the few cells co-expressing the two dopaminergic receptors. The latter greatly varied across structures and slices and were difficult to quantify (data not shown). In the CeA of control animals, *Gpr88* was expressed in fewer D₁R-positive (68, 31%±13,24) and D₂R-positive (78,26%±11,77) cells compared to striatum, and did not significantly differ in D₁R and D₂R expressing cells ($t_{(6)}=0,56$; $p=0,59$). Expression of the Cre in A_{2A}R expressing neurons had no effect on *Gpr88* expression in D₁R expressing neurons in any of the structures analyzed. In contrast, the number of *Gpr88*-positive cells was strongly reduced in D₂R expressing MSNs of rostral ((25,89%±7,86); $t_{(6)}=9,004$; $p<0,001$) and caudal CPu ((40,24%±2,68); $t_{(6)}=19,68$; $p<0,0001$) as well as in Nac ((40,08%±11,66); $t_{(6)}=4,736$; $p=0,0032$). Also, mice expressing the Cre in A_{2A}R expressing neurons had significantly lower number of *Gpr88*-positive cells in D₂R-MSNs of the CeA (34,33%±8,2) compared to control animals ($t_{(6)}=3,06$; $p=0,02$). Together, the data indicate that conditional *Gpr88*^{A2A-Cre} mice show a selective decrease of *Gpr88* transcript levels in D₂R MSNs of striatum and CeA.

***Gpr88*^{A2A-Cre} mice show decreased GPR88 agonist-induced [S35]-GTPγS binding**

To measure the consequences of *Gpr88* gene knockout at protein level, we performed GPR88 agonist-induced [S35]-GTPγS binding assays in *Gpr88*^{A2A-Cre} mice and their controls, as well as in total *Gpr88*^{-/-} (negative control) (Figure 1B). Structures were chosen based on A_{2A}R expression (Schiffmann et al., 1991, Schiffmann and Vanderhaeghen, 1993). We found a significant genotype effect in the CPu (*Gpr88*^{flx/flx}: 427%±22,61; *Gpr88*^{A2A-Cre}: 282,2%±15,46 ; $F_{(2,30)}=61,56$; $p<0,0001$), Nac (*Gpr88*^{flx/flx}: 342,4%±9,13; *Gpr88*^{A2A-Cre}: 205,1%±5,54; $F_{(2,30)}=152,9$; $p<0,0001$) and CeA (*Gpr88*^{flx/flx}: 170,3%±5,90; *Gpr88*^{A2A-Cre}: 141,2%±6,18; $F_{(2,30)}=37,48$; $p<0,0001$) (see Supplementary Table 2). *Post hoc* analysis (Turkey multiple comparisons) revealed significant differences between *Gpr88*^{A2A-Cre} and *Gpr88*^{flx/flx} for the two highest agonist concentrations (10⁻⁵ M and 10⁻⁶M) in the CPu, Nac and CeA. This result demonstrates that the selective *Gpr88* gene KO in D₂R-expressing cells, observed at mRNA level, translates into a significant reduction of protein levels in all regions of high GPR88 expression. Although the approach does not discriminate GPR88 signaling in D₁R and D₂R-expressing cells, the approximately 40% reduction on CPu membranes likely reflects the specific and almost complete *Gpr88* gene KO in D₂R cells, which represent approximately 40% of the dorsal striatum population of dopamine receptor-expressing neurons (Valjent et al., 2009)

Our previous study of full *Gpr88* KO mice showed that signaling properties of several Gi/o-coupled GPCRs was modified upon *Gpr88* deletion (Meirman et al., 2015). To test whether *Gpr88* deletion from striatopallidal neurons also altered striatal Gi/Go-coupled receptors neurotransmission we performed [S35]-GTPγS binding assays on whole striatum of *Gpr88*^{A2A-Cre} and their control (Figure 1C). As in total *Gpr88* KO mice, the conditional *Gpr88* deletion increased delta (*Gpr88*^{flx/flx}: 140,3%±5,29; *Gpr88*^{A2A-Cre}: 215%±9,7; $F_{(1,24)}=22,56$; $p<0,0001$) and mu (*Gpr88*^{flx/flx}: 157,4%±5,79; *Gpr88*^{A2A-Cre}: 192,5%±6,49; $F_{(1,24)}=83,84$; $p<0,0001$) opioid receptor activation. Interestingly, conditional *Gpr88* KO mice showed decreased activation of metabotropic glutamatergic receptors (*Gpr88*^{flx/flx}: 188,5%±12,6; *Gpr88*^{A2A-Cre}: 152,5%±8,57; $F_{(1,24)}=22,59$; $p<0,0001$) and Quinpirol (D₂/D₃ receptor agonist) induced activation (*Gpr88*^{flx/flx}: 143,3±7,04; *Gpr88*^{A2A-Cre}: 131,9±3,23; $F_{(1,24)}=6,361$; $p=0,0187$) as compared to control littermates, which was not detected in total *Gpr88* KO mice (Meirman et al., 2015). Carbachol (Muscarinic m₂/m₄ agonist)-induced dose dependent response was not affected by *Gpr88* specific deletion. (Detailed analysis in Supplementary 3) (19). Together, these data indicate that the striatopallidal *Gpr88* deletion impacts

GPCR signaling differently from the entire striatopallidal/striatonigral deletion, suggesting distinct modulatory roles of GPR88 in the two main MSNs populations.

Both total and A2A-conditional *Gpr88* gene deletion increase basal locomotor activity

Previous studies have demonstrated increased basal locomotor activity in total *Gpr88* KO mice (Quintana A, 2012, Meirsman et al., 2015). We compared general locomotor activity in total and conditional KO mice. Animals were individually placed in a dimly lit open field and analysis of mean forward locomotion revealed a significant increased activity for *Gpr88*^{-/-} as well as *Gpr88*^{A2A-Cre} mice (Figure 2 and detailed statistical analysis of behavioral tests in supplementary table 4 and 5). Deletion of *Gpr88* in striatopallidal neurons is therefore sufficient to recapitulate the hyperlocomotor phenotype of GPR88 KO mice.

Both total and A2A-conditional *Gpr88* gene deletion decrease anxiety-like behavior

Complete deletion of *Gpr88* in mice decreased anxiety levels in several models of anxiety-like behavior (Meirsman et al., 2015). To examine whether this behavior is dependent on GPR88 in striatopallidal MSNs we evaluated anxiety-like behaviors of *Gpr88*^{A2A-Cre} and *Gpr88*^{-/-} mice in the standard light-dark and elevated plus maze test. Results indicate that both full and conditional KO entered more frequently and spent more time exploring the aversive illuminated compartment of the light-dark apparatus (Figure 3A-D, Table S4 and S5). In the elevated plus maze, total and conditional deletion of *Gpr88* increased the percentage of distance and time traveled in the open arms (Figure 3E-J, Table S4 and S5). Also, both mutants spent more time in the distal part of the open arm and displayed higher number of total and distal head dips. There was no total distance traveled difference between mutants and control mice.

Anxiety can affect social behavior and the social test is considered as measure of social anxiety (Toth and Neumann, 2013). When scored for social interaction (Figure 3 K-R), both mutant lines show increased nose and paw contact and followed their interactor more frequently than control littermates. Also, *Gpr88*^{-/-} displayed decreased number of grooming episodes. Together, these data confirm our previous report indicating decreased anxiety-like behaviors in *Gpr88*^{-/-} mice (Meirsman et al., 2015) and show for the first time a similar phenotype in *Gpr88*^{A2A-Cre} mice.

A2A-conditional *Gpr88* gene deletion increases avoidance but does not regulate approach behaviors

Altered anxiety can be explained by an imbalance between approach-related and avoidance-related drives (Aupperle and Paulus, 2010). To further investigate the role of GPR88 in approach-avoidance conflict based anxiety tests we decided to measure avoidance and approach behaviors separately in both mice lines. Marble burying constitutes an unconditioned defensive behavior aiming to avoid threats, reversed by anxiolytic drugs (De Boer and Koolhaas, 2003). When placed in a novel caged with 20 marbles full and conditional KO buried significantly less marbles than their control littermates (Figure 4 A, B). When evaluating the motivation of mice for the exploration of novel instead of familiar environments (Figure 4 C and F, table S4 and S5) we observed a significant increase in novel environment exploration for *Gpr88*^{-/-} mice but not *Gpr88*^{A2A-Cre} mice. Moreover, in the novelty suppressed feeding test (Figure 4 D, E, G, H) *Gpr88*^{-/-} but not *Gpr88*^{A2A-Cre} show decreased latency to feed. This data suggest that total but not conditional *Gpr88* deletion regulates both approach and avoidance behaviors.

Total but not conditional *Gpr88* gene deletion impairs fear conditioning

Fear and anxiety are tightly interconnected processes and fear circuitry has been shown to be altered in anxiety disorders (Stein et al., 2007). To evaluate whether *Gpr88* deletion affects fear learning and expression we tested mice in a fear conditioning paradigm (Figure 5 A-F, Table S4 and S5). During conditioning (Figure 5 A, D), post-shock immobility increased regardless of the genotype suggestive of successful US-CS conditioning. When tested for contextual fear (Figure 5 B, E), *Gpr88*^{-/-} displayed significantly less immobility than control animal while *Gpr88*^{A2A-Cre} immobility scores were similar to *Gpr88*^{flx/flx} mice. A similar profile was found when mice were presented with a US-paired tone (Figure 5 C, F). In fact, ANOVA indicates a significant genotype effect for *Gpr88*^{-/-} cue testing and *post hoc* analysis (Sidak's multiple comparisons test) indicates a significant immobility

decrease for both cues presented. Like context fear measure, cue presentation led to similar immobility score between *Gpr88*^{A2A-Cre} and their control. Overall, these data indicate that complete but not striatopallidal deletion of *Gpr88* impairs fear expression in mice.

Discussion

In the present study we show that GPR88 in the striatopallidal pathway regulates anxiety-like behavior without affecting fear responses and drive toward novelty or food. *In situ* hybridization and GTPγS binding data indicate that *Gpr88* was efficiently deleted in neurons expressing the D₂R of *Gpr88*^{A2A-Cre} animals in dorsal as well as ventral striatum and, to a smaller extent, in the central nucleus of the amygdala. Importantly, GPR88 levels in D₁R-MSNs were intact in *Gpr88*^{A2A-Cre} mice when compared to control mice not expressing the Cre recombinase. The selective, although not fully complete deletion of *Gpr88* in D₂R MSNs of *Gpr88*^{A2A-Cre} mice is consistent with the previous characterization of *Adora2a*-cre mice showing specific expression of Cre recombinase in most but not all D₂R-MSNs (Durieux PF, 2009, Durieux PF, 2012). Furthermore we confirm previous data indicating that GPR88 is present in virtually all D₁R and D₂R expressing MSNs (Quintana A, 2012).

Converging evidence support the inhibitory function of striatopallidal pathway neurons in motor output systems. Optogenetic bilateral excitation of these neurons was shown to decrease the initiation of locomotor activity (Kravitz et al., 2010), while ablation or disruption of these neurons increased motor activity (Durieux PF, 2012, Bateup et al., 2010, Durieux PF, 2009). Several reports (Logue et al., 2009, Quintana A, 2012) including our own work (Meirman et al., 2015) indicate that *Gpr88* gene deletion leads to general hyperactive behavior. In the present report we show that deletion of striatopallidal *Gpr88* increases locomotor activity in a novel environment. This observation suggests that GPR88 in striatopallidal neurons normally acts as a brake on animal exploration, likely by facilitating the demonstrated inhibitory function of striatopallidal neurons on locomotor activity. Mechanisms underlying the positive modulatory role of GPR88 on striatopallidal neurons remain to be clarified. Another observation is that total and conditional KO mice show a

similar magnitude of hyperactivity, when tested under the same experimental conditions. Genetic inactivation of striatopallidal GPR88 is therefore sufficient to recapitulate the entire hyperactivity phenotype, suggesting that GPR88 receptors in the striatonigral pathway poorly contribute to this behavior.

In a previous report we showed that *Gpr88*^{-/-} mice present decreased anxiety-like behaviors (Meirsman et al., 2015). Also, recent reports suggest that D₂R and striatopallidal MSNs regulate emotional processing and goal directed behavior (Pecina et al., 2013, Brandao et al., 2015, Hranilovic et al., 2008, Kravitz et al., 2012, Francis et al., 2015). Here, we confirm the low anxiety of *Gpr88*^{-/-} mice and further extend the phenotype to social anxiety. Furthermore, we show that deletion of striatopallidal *Gpr88* is sufficient to induce an emotional phenotype similar to the low anxiety observed in full KO animals. In the light-dark and elevated plus maze both *Gpr88*^{-/-} and *Gpr88*^{A2A-Cre} mice displayed similar decreased anxiety-like behaviors with increased exploration of the light compartment/open arm of the apparatus. Note this anxiety phenotype cannot be explained by their overall hyperactive behavior since the total distance traveled did not differ from control animals. Further, both total and conditional deletion of *Gpr88* seemed to decrease social anxiety in the social interaction tests. Given the comparable emotional phenotype of full and conditional *Gpr88* KO animals in these behavioral assays, our data suggest that GPR88 in striatopallidal but not striatonigral neurons regulate anxiety-like behaviors.

In these anxiety tests the tendency to avoid threatening stimuli (bright light/exposed arms) is confronted with the inner driver toward exploration and this conflict is thought to inhibit exploration (36). As such, the low anxiety phenotype of mice lacking GPR88 could result from increased drive toward novelty exploration, decreased avoidance of a threatening environment, or both factors. We therefore evaluated avoidance behavior in the marble burying test that measures ethological defensive burying (De Boer and Koolhaas, 2003, Borsini et al., 2002). Both *Gpr88*^{-/-} as well as *Gpr88*^{A2A-Cre} mice buried less marbles than control littermates, showing decreased defensive burying consistent with reduced threat avoidance in these mice. To tackle approach behavior, we assessed novelty preference in both KO lines. Total but not conditional KO showed enhanced preference for the novel compartment, when presented a choice for novel or familiar environment. Similarly, in the novelty suppressed feeding test, total KO but not conditional animals showed lower

latency to start eating. In this test, both approach and avoidance components are enhanced by starving and neophobia respectively. The absence of phenotype of *Gpr88*^{A2A-Cre} mice in these two tests could therefore be explained by unaltered motivation towards new environment or food reinforcement. Taken together, these results suggest that GPR88 in striatopallidal neurons regulates avoidance rather than approach behavior.

MSNs from both dorsal striatum and NAc have been implicated in reward and aversive behavior (Durieux PF, 2009, Hikida et al., 2010, Hikida et al., 2013). In line with our hypothesis, recent reports suggesting that D₁R-MSNs encode predictive reward and mediate approach behavior, while D₂R-MSNs mediate aversive and defensive behavior (Hikida et al., 2010, Hikida et al., 2013, Durieux PF, 2009, Calabresi et al., 2014, Hranilovic et al., 2008, Kravitz and Bonci, 2013, Kravitz and Kreitzer, 2012, Kravitz et al., 2013, Kravitz et al., 2012). For instance, D₂R-MSNs transmission blockage in the Nac was found to disrupt aversive but not appetitive learning (Hikida et al., 2010, Hikida et al., 2013). Interestingly, in a previous report we show that *Gpr88* deletion decreases striatal dopamine levels and downregulates D₂R-MSNs gene markers (e.g. *Drd2*, *Adora2*, *Cnr1*, *Foxp1*, *Rgs4*, *Gpr6*, *Nr4a1*, *Penk*) (Meirsmann et al., 2015) while, in the present report, we show decreased D₂R and glutamatergic signaling in mice lacking GPR88 in D₂R-MSNs. Additionally, pharmacological blockage of D₂R was shown to decrease striatopallidal neurons excitability while its upregulation has the opposite effect through the down regulation of Kir channels (Cazorla et al., 2014, Cazorla et al., 2012, Chan et al., 2012). Based on these findings, we may therefore hypothesize that *Gpr88* in D₂R-MSNs normally increases neuronal transmission thereby enhancing avoidance behavior. Also, results from *Gpr88*^{-/-} showing both decreased avoidance behavior and increased novelty and food approach suggest that GPR88 in D₁R-MSNs neurons normally regulates approach behavior.

Finally, we tested fear responses of *Gpr88* total and conditional mice for the first time. Total but not selective deletion of *Gpr88* in striatopallidal neurons impaired both context and cue fear responses. Reduced fear responses in total KO mice is in agreement with altered cue-based learning previously reported in *Gpr88* KO animals. The lack of phenotype in *Gpr88*^{A2A-Cre} mice may be due to D₁R MSN-, rather than D₂R MSN-mediated mechanisms contributing to these behaviors. Alternatively, and because central amygdala functioning is essential in acquisition and expression of

fear conditioning (Wilensky et al., 2006), the partial GPR88 deletion at the level of the amygdala may be insufficient to alter fear responses. Further studies using viral approaches will define the precise role of GPR88 function in amygdala-mediated fear responses.

In sum, our results confirm previous reports indicating that GPR88 modulates MSNs functioning and regulates anxiety behaviors, and further identify modulatory roles of GPR88 in the striatopallidal pathway. The mutant *Gpr88*^{A2A-Cre} mouse line is a first step towards understanding circuit mechanisms underlying GPR88 function in the brain. Future studies will evaluate the role of GPR88 in D₁R-MSNs, and how this receptor regulates the striatonigral/striatopallidal balance. Finally, further demonstration of GPR88 implication in anxiety-related behaviors and threat evaluation definitely posit GPR88 blockade as a new target for treatment of anxiety-related disorders (Aupperle and Paulus, 2010).

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Disclosure/conflict of interest

The authors report no biomedical financial interests or potential conflicts of interest.

Author contributions

A.C.M., J.A.J.B and B.L.K. designed the experiments. A.C.M. and J.A.J.B. performed and analyzed behavioral experiments. A.C.M. performed and analyzed *In situ* hybridization and [³⁵S]-GTPγS binding experiments. A.C.M. and B.L.K. interpreted the results and wrote the article. All authors discussed the results and commented on the manuscript.

Legends to figures:

Figure 1: Molecular characterization of conditional *Gpr88*^{A2A-Cre} mice. (A) Cell counting on triple fluorescent *in situ* hybridization. (a-d) Quantification of GPR88 mRNA decrease in D₂R (red) but not D₁R (blue) expressing neurons in the CPu (a, b) Nac (c) and CeA (d) of mice expressing the Cre under the control of A_{2A} receptor. Percentage of *Gpr88* expression was calculated based on the total number of D₁R or D₂R positive cells counted [(number D₁R or D₂R-expressing cells co-expressing *Gpr88* x 100)/ total number of D₁R or D₂R expressing cells]. See more details in [Suppl Figure 1](#). (B) Decreased activation of GPR88 in several brain regions: GPR88-mediated [³⁵S]-GTPγS binding is decreased in the CPu, Nac and central amygdala of mutant animals. (C) Altered striatal GPCRs signaling in mutant mice: [³⁵S]-GTPγS binding assay shows facilitated delta (SNC80) and mu (DAMGO) opioid receptor function in striatal membranes of *Gpr88*^{A2A-Cre} mice, whereas signaling properties of metabotropic glutamate (glutamate) and D2 dopamine receptors (quinpirole) is decreased in these animals. Data are presented as mean ± SEM. (A) n=4 *Gpr88*^{flx/flx}; 4 *Gpr88*^{A2A-Cre}; solid stars: one star p < 0.05, two stars p < 0.01, three stars p < 0.001 (Student t test). (B) n=3 *Gpr88*^{flx/flx}; 3 *Gpr88*^{A2A-Cre}; 3 *Gpr88*^{-/-}; Text stars: one star p < 0.05, two stars p < 0.01, three stars p < 0.001 (*post-hoc*: Tukey's multiple comparisons test). (C) n=3 *Gpr88*^{flx/flx}; 3 *Gpr88*^{A2A-Cre}; Open stars: one star p < 0.05, three stars p < 0.001 (genotype effect on two-way ANOVA)

Figure 2: Increased Locomotor activity in both total and conditional *Gpr88* KO mice. When placed individually in a dimly lit open field for 30 min, both total and conditional knockout traveled a longer distance than their control littermates. (A and C) Line graphs show the distance traveled (cm) in 5 min bins over a 30 min session. (B and D) Bar graphs show the average total distance traveled (cm) over the 30 min sessions period. Data are represented as mean ± SEM. (A) n= 10 *Gpr88*^{flx/flx}; 10 *Gpr88*^{A2A-Cre} (C) n=12 *Gpr88*^{+/+}; 12 *Gpr88*^{-/-}; Open stars: three stars p<0.001. (B and D) Solid stars: one star p < 0.05, two stars p < 0.01, three stars p < 0.001 (Student t test).

Figure 3: Anxiety-related responses are similarly decreased in total and conditional *Gpr88* KO mice. Total and conditional *Gpr88* KO mice enter more frequently (A and C) and spent more time (B and D) in the light compartment of the light-dark test. In the elevated plus-maze *Gpr88*^{-/-} and *Gpr88*^{A2A-Cre} mice present higher open arms exploration ratios (E and H), more frequent total and distal head dips (F and I) and increased time spent in the distal zone of the open arms (G and J) when compared to their control littermates. Social interactions were evaluated in a dimly lighted open field with wild type naïve mice of the same age and gender (K-R). Both mutant animals display increased number of nose (K, O), and paw (L, P) contacts, as well as increased following behaviors (M and Q). *Gpr88*^{-/-} but not *Gpr88*^{A2A-Cre} engaged less frequently in grooming episodes than control animals (N and R). Data are presented as mean ± SEM. (A and B) n=11 *Gpr88*^{+/+}; 10 *Gpr88*^{-/-}; (C and D) n=9 *Gpr88*^{flx/flx}; 11 *Gpr88*^{A2A-Cre}; (E-G) n=9 *Gpr88*^{+/+}; 11 *Gpr88*^{-/-}; (H-J) n=13 *Gpr88*^{flx/flx}; 12 *Gpr88*^{A2A-Cre}; (K-N) n=6 *Gpr88*^{+/+}; 6 *Gpr88*^{-/-}; (O-R) n=9 *Gpr88*^{flx/flx}; 10 *Gpr88*^{A2A-Cre}; Solid stars: one star $p < 0.05$, two stars $p < 0.01$, three stars $p < 0.001$ (Student *t* test).

Figure 4: A2A-conditional *Gpr88* gene deletion increases avoidance but does not regulate approach behaviors. In the marble burying test (A and B) both KO mice bury less marbles than controls. When assessing novelty preference total (C) but not conditional (F) KO spent more time in the novel compartment when compared to their littermates. In the novelty-suppressed feeding test, *Gpr88*^{-/-} mice display shorter latencies to start eating in the center of the arena compared to *Gpr88*^{+/+} animals (D), eating normally when placed back in their home cage (E). *Gpr88*^{A2A-Cre} exhibit similar latencies to start eating (G) and home cage food intake (H) than *Gpr88*^{flx/flx} mice. Data are presented as mean ± SEM. (A) n=7 *Gpr88*^{+/+}; 7 *Gpr88*^{-/-}; (B) n=9 *Gpr88*^{flx/flx}; 7 *Gpr88*^{A2A-Cre} (C) n=7 *Gpr88*^{+/+}; 7 *Gpr88*^{-/-}; (F) n=6 *Gpr88*^{flx/flx}; 8 *Gpr88*^{A2A-Cre}; (D) n=7 *Gpr88*^{+/+}; 7 *Gpr88*^{-/-}; (G) n=11 *Gpr88*^{flx/flx}; 11 *Gpr88*^{A2A-Cre}; Solid stars: one star $p < 0.05$, two stars $p < 0.01$ (Student *t* test)

Figure 5: Total but not conditional *Gpr88* gene deletion impairs fear conditioning. To assess whether *Gpr88* deletion affect fear response we tested mice in a fear conditioning test. During the conditioning session (A, D), mutant and control animals displayed similar levels of immobility before and after tone-shock pairing when compared to control mice. 24h later, *Gpr88*^{-/-} mice displayed significantly lower context fear than *Gpr88*^{+/+} mice (B). The percentage of immobility of *Gpr88*^{-/-} was also decreased when tested for Cue fear memory (C). Deletion of *Gpr88* in striatopallidal neurons

didn't affect context (E) or Cue (F) fear memories; (A-C) n=10 *Gpr88*^{+/+}; 10 *Gpr88*^{-/-}; (D-F) n=11 *Gpr88*^{flx/flx}; 11 *Gpr88*^{A2A-Cre}; Solid stars: one star $p < 0.05$ (Student *t* test). Text stars (*): one star $p < 0.05$, two stars $p < 0.01$ (*post-hoc*: Sidak's multiple comparisons test)

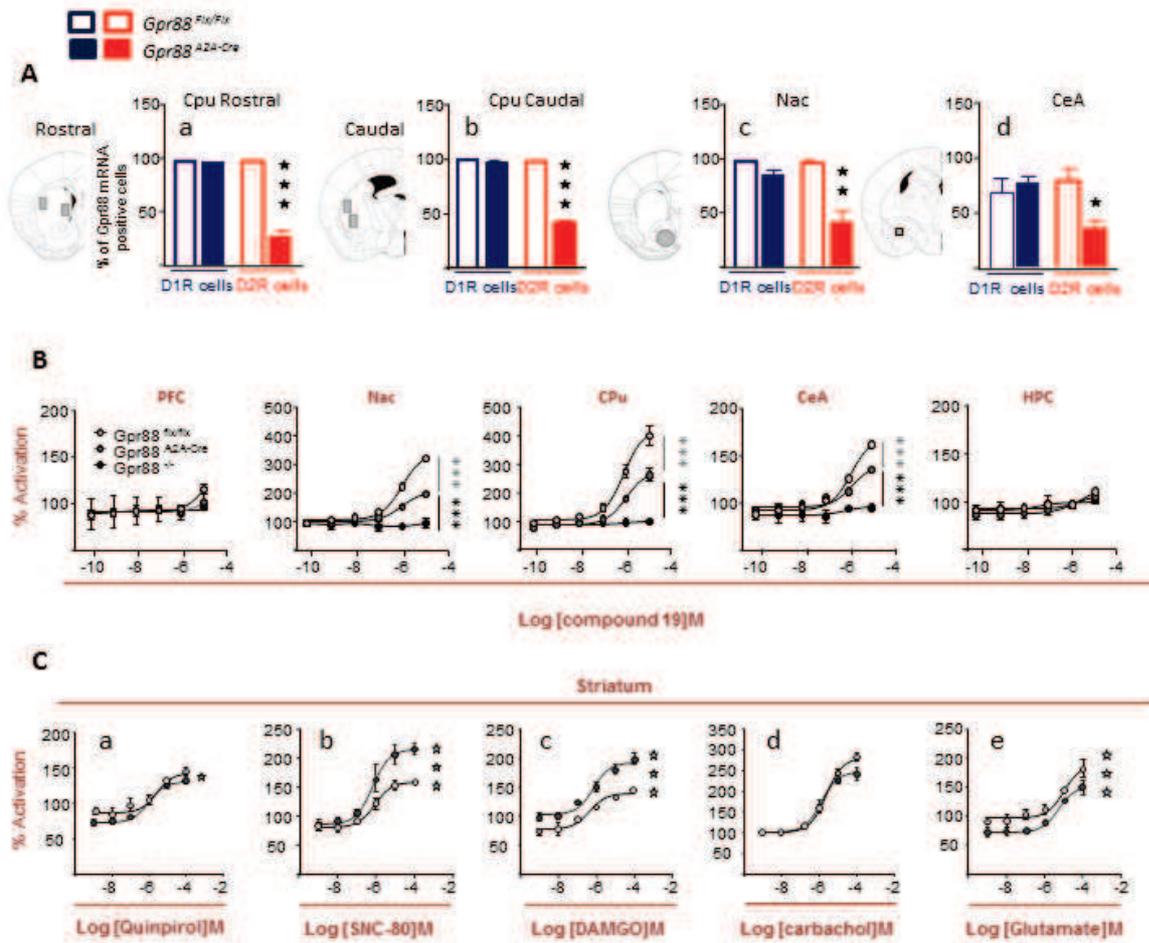
References

1. Rask-Andersen M, Almen MS, Schiøth HB. Trends in the exploitation of novel drug targets. *Nature reviews Drug discovery*. 2011;10(8):579-90.
2. Ghanemi A. Targeting G protein coupled receptor-related pathways as emerging molecular therapies. *Saudi pharmaceutical journal : SPJ : the official publication of the Saudi Pharmaceutical Society*. 2015;23(2):115-29.
3. Levoye A, Dam J, Ayoub MA, Guillaume JL, Jockers R. Do orphan G-protein-coupled receptors have ligand-independent functions? New insights from receptor heterodimers. *EMBO reports*. 2006;7(11):1094-8.
4. Logue SF, Grauer SM, Paulsen J, Graf R, Taylor N, Sung MA, et al. The orphan GPCR, GPR88, modulates function of the striatal dopamine system: a possible therapeutic target for psychiatric disorders? *Mol Cell Neurosci*. 2009;42(4):438-47.
5. Massart R GJ, Mignon V, Sokoloff P, Diaz J. Striatal GPR88 expression is confined to the whole projection neuron population and is regulated by dopaminergic and glutamatergic afferents. *Eur J Neurosci*. 2009(30):397-414.
6. Quintana ASE, Wang W, Storey GP, Guler AD, Wanat MJ, et al. Lack of GPR88 enhances medium spiny neuron activity and alters motor- and cue-dependent behaviors. *Nat Neurosci*. 2012(15):1547-55.
7. Ingallinesi M, Le Bouil L, Faucon Biguet N, Do Thi A, Mannoury la Cour C, Millan MJ, et al. Local inactivation of *Gpr88* in the nucleus accumbens attenuates behavioral deficits elicited by the neonatal administration of phencyclidine in rats. *Mol Psychiatry*. 2014.

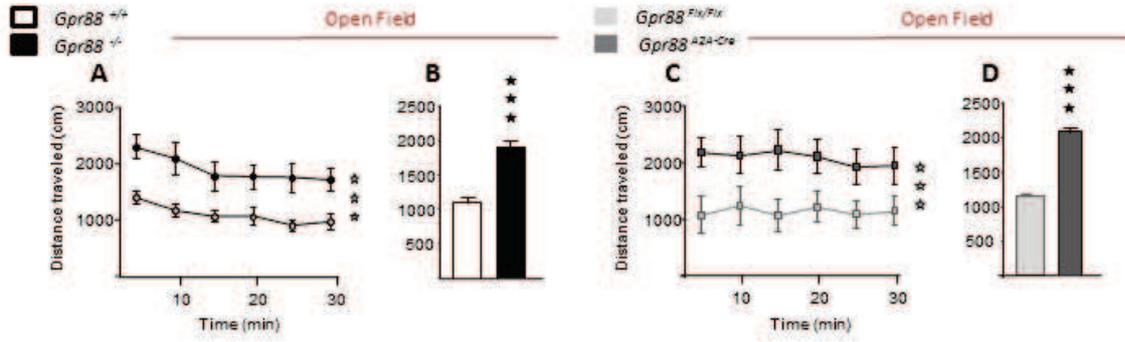
8. Liljeholm M, O'Doherty JP. Contributions of the striatum to learning, motivation, and performance: an associative account. *Trends in cognitive sciences*. 2012;16(9):467-75.
9. Lewis M, Kim SJ. The pathophysiology of restricted repetitive behavior. *Journal of neurodevelopmental disorders*. 2009;1(2):114-32.
10. Swerdlow NR, Geyer MA, Braff DL. Neural circuit regulation of prepulse inhibition of startle in the rat: current knowledge and future challenges. *Psychopharmacology*. 2001;156(2-3):194-215.
11. Del Zompo M, Deleuze JF, Chillotti C, Cousin E, Niehaus D, Ebstein RP, et al. Association study in three different populations between the GPR88 gene and major psychoses. *Mol Genet Genomic Med*. 2014;2(2):152-9.
12. Brandish PE, Su M, Holder DJ, Hodor P, Szumiloski J, Kleinhanz RR, et al. Regulation of gene expression by lithium and depletion of inositol in slices of adult rat cortex. *Neuron*. 2005;45(6):861-72.
13. Ogden CA, Rich ME, Schork NJ, Paulus MP, Geyer MA, Lohr JB, et al. Candidate genes, pathways and mechanisms for bipolar (manic-depressive) and related disorders: an expanded convergent functional genomics approach. *Mol Psychiatry*. 2004;9(11):1007-29.
14. Bohm C, Newrzella D, Herberger S, Schramm N, Eisenhardt G, Schenk V, et al. Effects of antidepressant treatment on gene expression profile in mouse brain: cell type-specific transcription profiling using laser microdissection and microarray analysis. *Journal of neurochemistry*. 2006;97 Suppl 1:44-9.
15. Conti B, Maier R, Barr AM, Morale MC, Lu X, Sanna PP, et al. Region-specific transcriptional changes following the three antidepressant treatments electroconvulsive therapy, sleep deprivation and fluoxetine. *Mol Psychiatry*. 2007;12(2):167-89.
16. Hikida T, Kimura K, Wada N, Funabiki K, Nakanishi S. Distinct roles of synaptic transmission in direct and indirect striatal pathways to reward and aversive behavior. *Neuron*. 2010;66(6):896-907.
17. Hikida T, Yawata S, Yamaguchi T, Danjo T, Sasaoka T, Wang Y, et al. Pathway-specific modulation of nucleus accumbens in reward and aversive behavior via selective transmitter receptors. *Proceedings of the National Academy of Sciences of the United States of America*. 2013;110(1):342-7.
18. Pecina M, Mickey BJ, Love T, Wang H, Langenecker SA, Hodgkinson C, et al. DRD2 polymorphisms modulate reward and emotion processing, dopamine neurotransmission and openness to experience. *Cortex; a journal devoted to the study of the nervous system and behavior*. 2013;49(3):877-90.
19. Brandao ML, de Oliveira AR, Muthuraju S, Colombo AC, Saito VM, Talbot T. Dual role of dopamine D-like receptors in the mediation of conditioned and unconditioned fear. *FEBS letters*. 2015.
20. Hranilovic D, Bucan M, Wang Y. Emotional response in dopamine D2L receptor-deficient mice. *Behavioural brain research*. 2008;195(2):246-50.
21. Francis TC, Chandra R, Friend DM, Finkel E, Dayrit G, Miranda J, et al. Nucleus accumbens medium spiny neuron subtypes mediate depression-related outcomes to social defeat stress. *Biological psychiatry*. 2015;77(3):212-22.
22. Meirsmann AC, Le Merrer J, Pellissier LP, Diaz J, Clesse D, Kieffer BL, et al. Mice Lacking GPR88 Show Motor Deficit, Improved Spatial Learning, and Low Anxiety Reversed by Delta Opioid Antagonist. *Biological psychiatry*. 2015.
23. Durieux PF, Guiducci S, Buch T, Waisman A, Zoli M, et al. D2R striatopallidal neurons inhibit both locomotor and drug reward processes. *Nat Neurosci*. 2009(12)::393-5.
24. Pradhan AA, Becker JA, Scherrer G, Tryoen-Toth P, Filliol D, Matifas A, et al. In vivo delta opioid receptor internalization controls behavioral effects of agonists. *PLoS one*. 2009;4(5):e5425.
25. Le Merrer J, Befort K, Gardon O, Filliol D, Darcq E, Dembele D, et al. Protracted abstinence from distinct drugs of abuse shows regulation of a common gene network. *Addiction biology*. 2012;17(1):1-12.
26. Goeldner C, Reiss D, Wichmann J, Kieffer BL, Ouagazzal AM. Activation of nociceptin opioid peptide (NOP) receptor impairs contextual fear learning in mice through glutamatergic mechanisms. *Neurobiology of learning and memory*. 2009;91(4):393-401.

27. Le Merrer J, Faget L, Matifas A, Kieffer BL. Cues predicting drug or food reward restore morphine-induced place conditioning in mice lacking delta opioid receptors. *Psychopharmacology*. 2012;223(1):99-106.
28. Schiffmann SN, Jacobs O, Vanderhaeghen JJ. Striatal restricted adenosine A2 receptor (RDC8) is expressed by enkephalin but not by substance P neurons: an in situ hybridization histochemistry study. *Journal of neurochemistry*. 1991;57(3):1062-7.
29. Schiffmann SN, Vanderhaeghen JJ. Adenosine A2 receptors regulate the gene expression of striatopallidal and striatonigral neurons. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 1993;13(3):1080-7.
30. Valjent E, Bertran-Gonzalez J, Herve D, Fisone G, Girault JA. Looking BAC at striatal signaling: cell-specific analysis in new transgenic mice. *Trends in neurosciences*. 2009;32(10):538-47.
31. Toth I, Neumann ID. Animal models of social avoidance and social fear. *Cell and tissue research*. 2013;354(1):107-18.
32. De Boer SF, Koolhaas JM. Defensive burying in rodents: ethology, neurobiology and psychopharmacology. *Eur J Pharmacol*. 2003;463(1-3):145-61.
33. Aupperle RL, Paulus MP. Neural systems underlying approach and avoidance in anxiety disorders. *Dialogues in clinical neuroscience*. 2010;12(4):517-31.
34. Stein MB, Simmons AN, Feinstein JS, Paulus MP. Increased amygdala and insula activation during emotion processing in anxiety-prone subjects. *The American journal of psychiatry*. 2007;164(2):318-27.
35. Durieux PF, de Kerchove d'Exaerde A. Differential regulation of motor control and response to dopaminergic drugs by D1R and D2R neurons in distinct dorsal striatum subregions. 2012(31):640-53.
36. Kravitz AV, Freeze BS, Parker PR, Kay K, Thwin MT, Deisseroth K, et al. Regulation of parkinsonian motor behaviours by optogenetic control of basal ganglia circuitry. *Nature*. 2010;466(7306):622-6.
37. Bateup HS, Santini E, Shen W, Birnbaum S, Valjent E, Surmeier DJ, et al. Distinct subclasses of medium spiny neurons differentially regulate striatal motor behaviors. *Proceedings of the National Academy of Sciences of the United States of America*. 2010;107(33):14845-50.
38. Kravitz AV, Tye LD, Kreitzer AC. Distinct roles for direct and indirect pathway striatal neurons in reinforcement. *Nat Neurosci*. 2012;15(6):816-8.
39. Borsini F, Podhorna J, Marazziti D. Do animal models of anxiety predict anxiolytic-like effects of antidepressants? *Psychopharmacology*. 2002;163(2):121-41.
40. Bailey KR, Crawley JN. Anxiety-Related Behaviors in Mice. In: Buccafusco JJ, editor. *Methods of Behavior Analysis in Neuroscience*. Frontiers in Neuroscience. 2nd ed. Boca Raton (FL) 2009.
41. Calabresi P, Picconi B, Tozzi A, Ghiglieri V, Di Filippo M. Direct and indirect pathways of basal ganglia: a critical reappraisal. *Nat Neurosci*. 2014;17(8):1022-30.
42. Kravitz AV, Bonci A. Optogenetics, physiology, and emotions. *Frontiers in behavioral neuroscience*. 2013;7:169.
43. Kravitz AV, Kreitzer AC. Striatal mechanisms underlying movement, reinforcement, and punishment. *Physiology*. 2012;27(3):167-77.
44. Kravitz AV, Owen SF, Kreitzer AC. Optogenetic identification of striatal projection neuron subtypes during in vivo recordings. *Brain research*. 2013;1511:21-32.
45. Cazorla M, de Carvalho FD, Chohan MO, Shegda M, Chuhma N, Rayport S, et al. Dopamine D2 receptors regulate the anatomical and functional balance of basal ganglia circuitry. *Neuron*. 2014;81(1):153-64.
46. Cazorla M, Shegda M, Ramesh B, Harrison NL, Kellendonk C. Striatal D2 receptors regulate dendritic morphology of medium spiny neurons via Kir2 channels. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2012;32(7):2398-409.

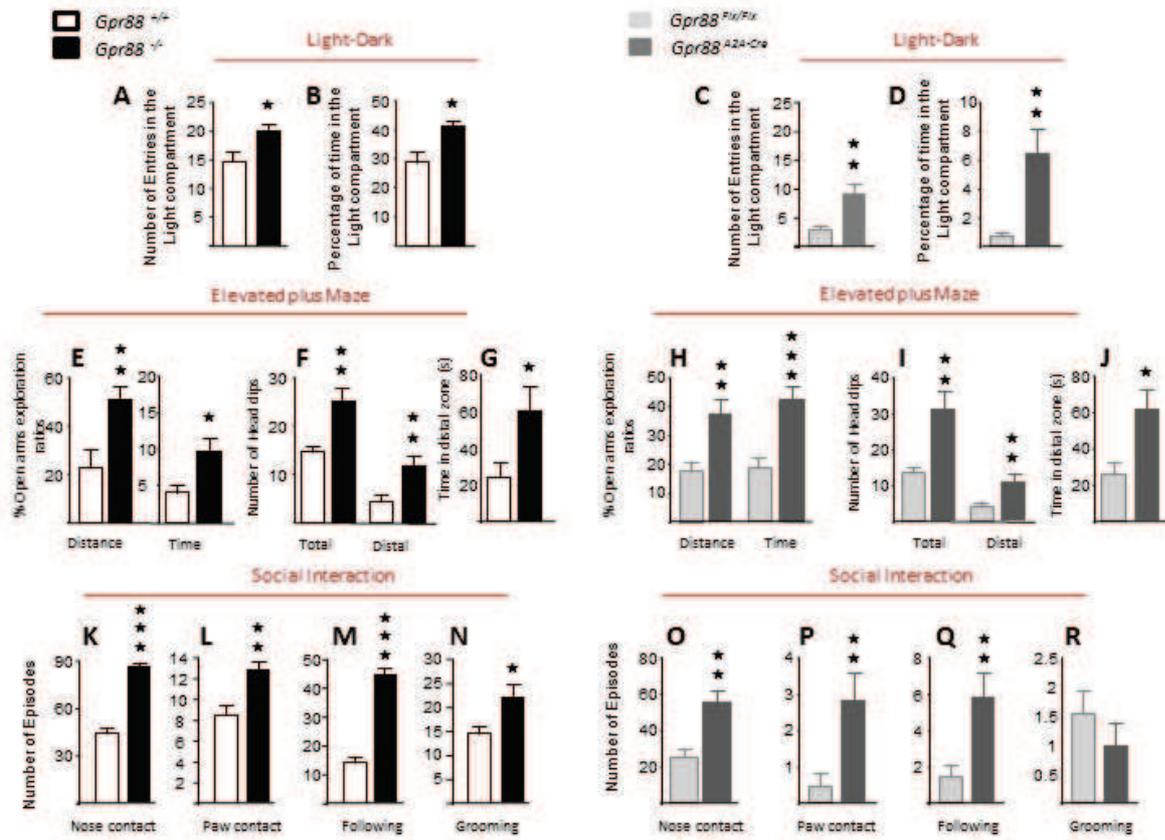
47. Chan CS, Peterson JD, Gertler TS, Glajch KE, Quintana RE, Cui Q, et al. Strain-specific regulation of striatal phenotype in *Drd2*-eGFP BAC transgenic mice. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2012;32(27):9124-32.
48. Kupchik YM, Brown RM, Heinsbroek JA, Lobo MK, Schwartz DJ, Kalivas PW. Coding the direct/indirect pathways by D1 and D2 receptors is not valid for accumbens projections. *Nat Neurosci*. 2015.
49. Smith RJ, Lobo MK, Spencer S, Kalivas PW. Cocaine-induced adaptations in D1 and D2 accumbens projection neurons (a dichotomy not necessarily synonymous with direct and indirect pathways). *Current opinion in neurobiology*. 2013;23(4):546-52.
50. Wilensky AE, Schafe GE, Kristensen MP, LeDoux JE. Rethinking the fear circuit: the central nucleus of the amygdala is required for the acquisition, consolidation, and expression of Pavlovian fear conditioning. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2006;26(48):12387-96.



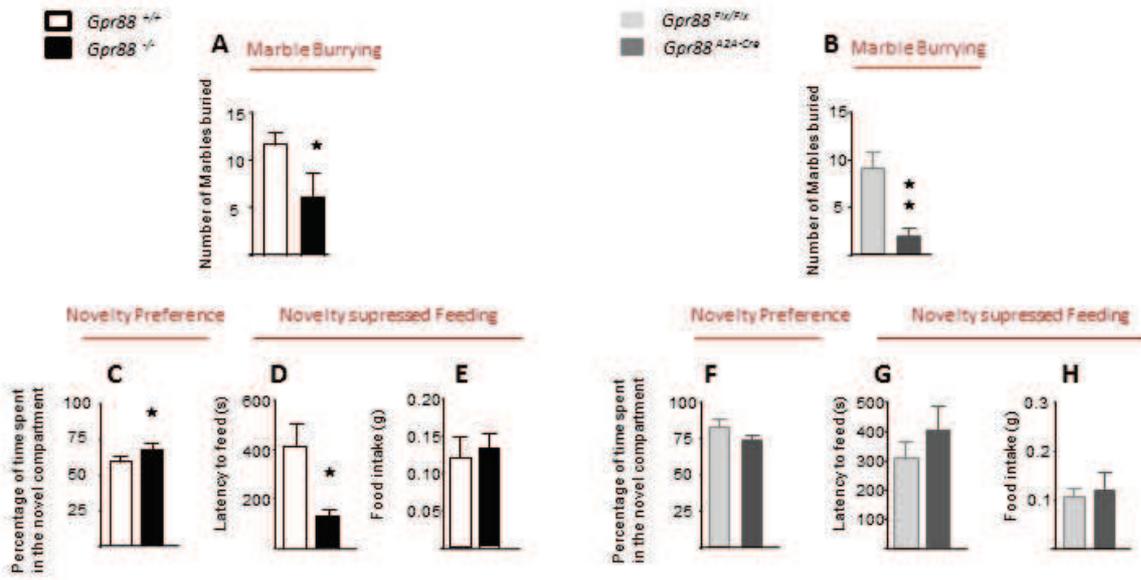
Meirsman et al Figure 1



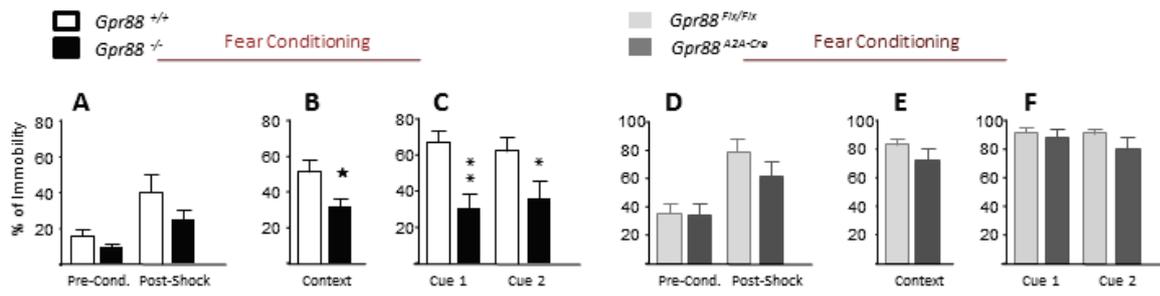
Meirsman et al Figure 2



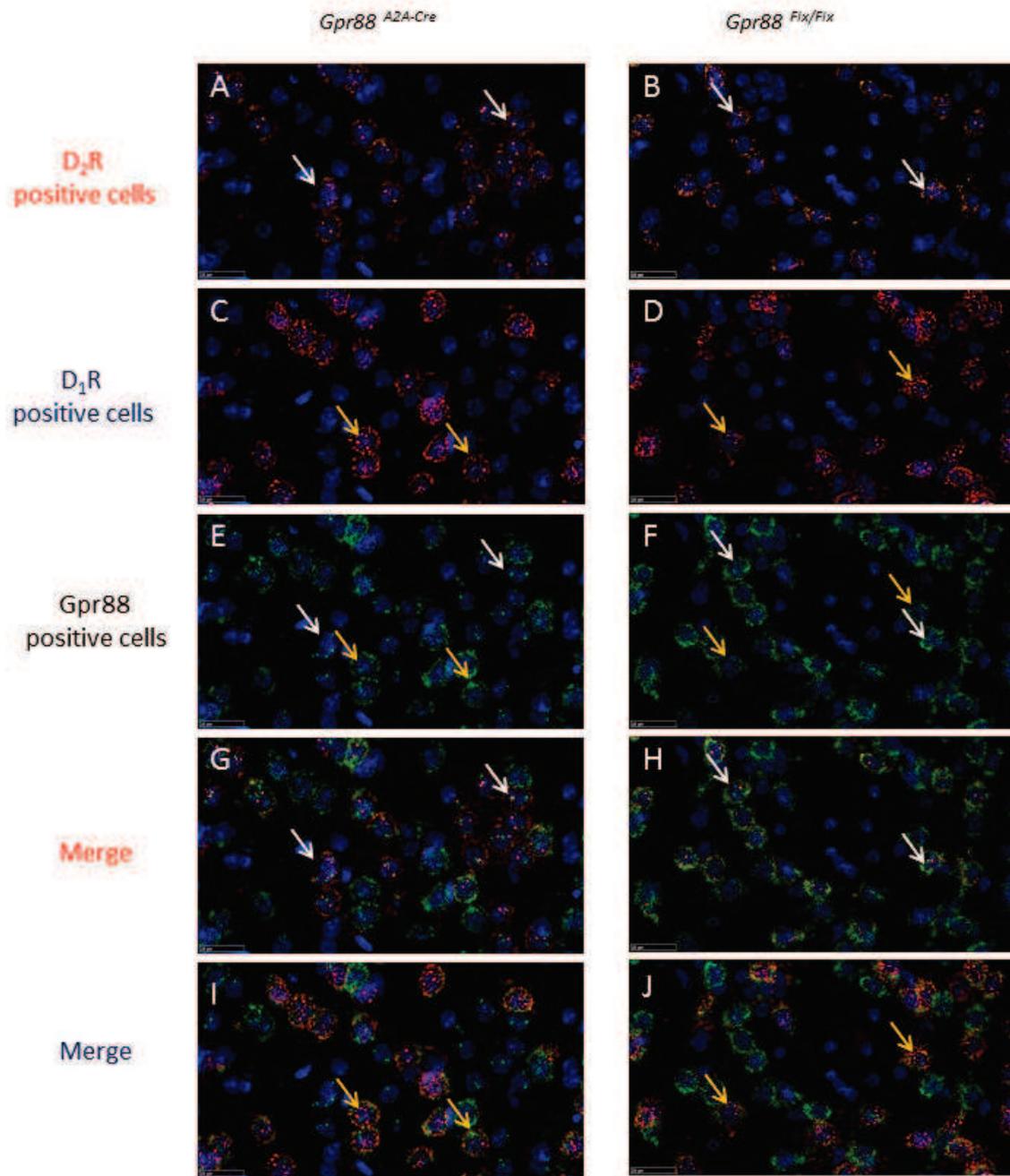
Meirsman et al Figure 3



Meirsman et al Figure 4



Meirsman et al Figure 5



Supplementary Figure 1: Selective deletion of *Gpr88* in D₂R expressing MSNs of the rostromedial CPu of *Gpr88^{A2A-Cre}* mice (left panel) but not control animals (right panel). Triple fluorescent *in situ* hybridization probing D₂R (A and B, probe labelling in orange) D₁R (red; C and D) and *Gpr88* (green; E and F). In *Gpr88^{Flx/Flx}* animals *Gpr88* co-localizes with both D₂R (merge: H) and D₁R mRNA (merge: J). In contrast only D₁R (merge: I) but not D₂R (G) co-localize with *Gpr88* mRNA in *Gpr88^{A2A-Cre}* mice. White and yellow arrows indicate examples of D₂R and D₁R positive cells respectively. Dapi staining (blue) was used to label all cells nuclei. (Scale bar: 25µm).

Table S1: Cell counting and statistical analysis on triple *in situ* hybridization in the Cpu, Nac and CeA of *Gpr88^{flx/flx}* (n=4) and *Gpr88^{AZA-Cre}* (n=4)

% of Gpr88 in	mouse line	CPU								Nacc	t test	CeA	t test
		Rostral				Caudal							
		Lateral	Median	Mean	t test	Lateral	Median	Mean	t test				
D1R positive cells	<i>Gpr88^{flx/flx}</i>	96,69±0,33	96,86±0,94	96,78±0,58	t(6)=1,02; p=0,35	97,21±0,89	97,19±0,70	97,2±0,4	t(6)=1,955; p=0,09	96,30±0,48	t(6)=2,41; p=0,0525	68,31±13,24	t(6)=0,556; p=0,5977
	<i>Gpr88^{AZA-Cre}</i>	93,50±3,01	96,95±0,99	95,23±1,40		98,20±0,23	94,19±0,86	96,2±0,32		83,59±5,25		76,67±7,06	
D2R positive cells	<i>Gpr88^{flx/flx}</i>	96,58±1,22	97,07±1,36	96,83±1,21	t(6)=9,004; p=0,0001	96,83±0,92	96,21±1,12	96,52±0,99	t(6)=19,68; p<0,0001	95,98±1,85	t(6)=4,736; p=0,0032	78,26±11,77	t(6)=3,06; p=0,0221
	<i>Gpr88^{AZA-Cre}</i>	24,38±5,58	27,41±11,09	25,89±7,86		40,22±3,41	40,25±3,74	40,24±2,68		40,08±11,66		34,33±8,2	

Table S2. Statistical analysis: GPR88 agonist-induced [35S]-GTPγS binding of *Gpr88*^{Flx/Flx} versus *Gpr88*^{A2A-Cre} and *Gpr88*^{-/-} mice

Structure	Bmax			EC50			Two-way ANOVA		
	<i>Gpr88</i> ^{flx/flx}	<i>Gpr88</i> ^{A2A-Cre}	% of decrease	<i>Gpr88</i> ^{-/-}	<i>Gpr88</i> ^{flx/flx}	<i>Gpr88</i> ^{A2A-Cre}	<i>Gpr88</i> ^{-/-}	Genotype effect	Agonist concentration effect
PFC	141,2±1,5	125,4±2,68	11,19	93,93±14,2	9,90E-05	0,00002076	1,29E-09	F (2, 30) = 0,3714 p = 0,6929	F (5, 30) = 2,328 p = 0,0617
CPu	427±22,61	282,2±15,46	40,10	103,7±3,4	8,33E-06	1,05E-05	7,829E-07	F (2, 30) = 61,56 p < 0,0001	F (5, 30) = 59,68 p < 0,0001
Nac	342,4±9,13	205,1±5,54	33,91	88,03±7,6	9,91E-06	9,17E-06	1,654E-08	F (2, 30) = 152,9 p < 0,0001	F (5, 30) = 107,1 p < 0,0001
CeA	170,3±5,90	141,2±6,18	17,09	96,56±6,4	1,27E-05	1,45E-05	6,712E-07	F (2,30) = 37,48 p < 0,0001	F (5, 30) = 43,02 p < 0,0001
HPC	116,8±6,86	106,6±6,29	8,73	99,83±3,4	3,90E-05	1,03E-05	4,325E-08	F (2,30) = 1,90 p = 0,1669	F (5, 30) = 6,30 p = 0,0004

Table S3. Statistical analysis: agonist-induced [³⁵S]-GTPγS binding of *Gpr88*^{Flx/Flx} versus *Gpr88*^{A2A-Cre} in the Str

Agonist	Bmax (%)		EC50		Two-way ANOVA	
	<i>Gpr88</i> ^{flx/flx}	<i>Gpr88</i> ^{A2A-Cre}	<i>Gpr88</i> ^{flx/flx}	<i>Gpr88</i> ^{A2A-Cre}	Genotype effect	Agonist concentration effect
Quinpirol	143,3±7,04	131,9±3,23	2,47E-06	8,946E-07	F (1, 24) = 6,361; p = 0,0187	F (5, 24) = 43,98; p < 0,0001
SNC-80	157,4±5,79	215±9,7	8,548E-07	6,585E-07	F (1, 24) = 22,56; p < 0,0001	F (5, 24) = 35,87; p < 0,0001
DAMGO	140,3±5,29	192,5±6,49	4,858E-07	7,597E-07	F (1, 24) = 83,84; p < 0,0001	F (5, 24) = 57,31; p < 0,0001
Carbachol	280,8±8,74	244,9±8,56	2,39E-06	1,31E-06	F (1, 24) = 1,037; p = 0,3186	F (5, 24) = 108,3; p < 0,0001
Glutamate	188,5±12,6	152,5±8,57	9,31E-06	5,45E-06	F (1, 24) = 22,59; p < 0,0001	F (5, 24) = 32,76; p < 0,0001

Table S4. Statistical analysis: Behavioral analysis of Gpr88^{A2A-Cre} versus Gpr88^{Fib/Fib}

Test	Animals	Parametres	Student t-test	Two-way ANOVA		
				Genotype	intra-session	Interaction
Fear conditioning	11WT (6M,5F) 11cKO (6M,5F)	Conditioning		F(1,20)=1 p=0,33	F(1,20)=23,25 p=0,0001	F(1,20)=1,19 p=0,29
		Context testing	t(20)=1,14; p=0,27			
		Cue testing		F(1,20)=1,06 p=0,31	F(1,20)=2 p=0,17	F(1,20)=1,85 p=0,19
Open Field	10WT (7M,3F) 10 cKO (6M, 4F)	Foward locomotion	t(10)=15,60; p<0,0001	F (1, 108) = 28,93 p<0,0001	F (5, 108) = 0,1184 p=0,98	F (5, 108) = 0,1194 p=0,98
Light-Dark	9WT (4M;5F) 11 cKO (6M;5F)	Lentency to enter light compartment (LC)	t (18)=1,90; p=0,07			
		Number of entries in the LC	t (18)= 3,01; p=0,008			
		% of time in the LC	t (18)= 3,11; p=0,006			
Elevated Plus-Maze	13 WT(8M,5F) 12 cKO (6M,6F)	Number (nb) of open arms (OA) entries	t (23)= 2,37; p=0,026			
		% time in the OA	t (23)= 4,13; p=0,0004			
		% of distance traveled in the OA	t (23)= 3,43; p=0,0023			
		Nb of total head dips (HD)	t (23)= 3,54; p=0,0017			
		Nb of HD in the distal zone of the OA	t (23)= 2,95; p=0,0072			
		Time in the distal zone of the OA	t (23)= 2,76; p=0,011			
		Total arm entries	t (23)= 0,09; p=0,925			
		Total distance traveled	t (23)= 0,14; p=0,886			
Social interaction test	9WT(6M,3F) 10cKO (4M,6F)	Nose contact	t (17)= 3,39; p=0,004			
		Paw contact	t (17)= 2,73; p=0,014			
		Following	t (17)= 2,72; p=0,014			
		Grooming	t (17)= 1,01; p=0,325			
Novelty preference	6WT(4M,2F) 8cKO(5M3F)	% time in the novel compartment	t(12)=1,43; p=0,17			
Marble burrying	9WT(7M,2F) 7cKO(2M5F)	Nb of marbles burried	t(14)=3,28; p=0,005			
Novelty supressed feeding	11WT (7M,4F) 11cKO (6M,5F)	Latency to feed	t(20)=0,07 p=0,29			
		Food ingestion in home cage	t(20)=0,43; p=0,67			

Table S5. Statistical analysis: Behavioral analysis of Gpr88^{-/-} versus Gpr88^{+/+}

Test	Animals	Parametres	Student t-test	Two-way ANOVA		
				Genotype	intra-session	Interaction
Fear conditioning	10WT (7M,3F) 10KO (6M,4F)	Conditioning		F(1,18)=2,95 <i>p</i> =0,10	F(1,18)=17,05 <i>p</i> =0,0006	F(1,18)=0,99 <i>p</i> =0,33
		Context testing	t(18)=2,36; <i>p</i> =0,029			
		Cue testing		F(1,18)=8,89 <i>p</i> =0,008	F(1,18)=0,017 <i>p</i> =0,89	F(1,18)=1,67 <i>p</i> =0,21
Open Field	12WT (7M,4F) 12 KO (6M, 6F)	Foward locomotion	t(10)=6,65; <i>p</i> <0,0001	F(1, 132) = 52,72 <i>p</i> <0,0001	F(5, 132) = 2,250 <i>p</i> = 0,0530	F(5, 132)=0,1312 <i>p</i> =0,98
Light-Dark	11WT (7M;4F) 10 KO (7M;3F)	Lentency to enter light compartment (LC)	t (19)=1,17; <i>p</i> =0,26			
		Number of entries in the LC	t (19)= 2,46; <i>p</i> =0,023			
		% of time in the LC	t (19)= 2,84; <i>p</i> =0,01			
Elevated Plus-Maze	9 WT(5M,4F) 11 KO (7M,4F)	Number (nb) of open arms (OA) entries	t (18)= 2,96; <i>p</i> =0,008			
		% time in the OA	t (18)= 2,71; <i>p</i> =0,015			
		% of distance traveled in the OA	t (18)= 3,05; <i>p</i> =0,007			
		Nb of total head dips (HD)	t (18)= 3,05; <i>p</i> =0,007			
		Nb of HD in the distal zone of the OA	t (18)= 2,81; <i>p</i> =0,012			
		Time in the distal zone of the OA	t (18)= 2,17; <i>p</i> =0,043			
		Total arm entries	t (18)= 0,65; <i>p</i> =0,52			
Social interaction test	6WT(3M,3F) 6KO (3M,3F)	Nose contact	t (10)= 10,12; <i>p</i> <0,0001			
		Paw contact	t (10)= 3,94; <i>p</i> =0,0028			
		Following	t (10)= 12,03; <i>p</i> <0,0001			
		Grooming	t (10)= 2,28; <i>p</i> =0,045			
Novelty preference	7WT(4M3F) 7KO(3M4F)	% time in the novel compartment	t(12)=2,31; <i>p</i> =0,039			
Marble burying	7WT(2M,5F) 7KO(4M3F)	Nb of marbles burried	t(12)=2,42; <i>p</i> =0,032			
Novelty supressed feeding	7WT (3M,4F) 7KO (4M,3F)	Latency to feed	t(12)=2,92; <i>p</i> =0,013			
		Food ingestion in home cage	t(12)=0,39; <i>p</i> =0,702			

2) Role of GPR88 in the regulation of motor function

Role of GPR88 in the regulation of motor function

Introduction

Among brain orphan G protein coupled receptors (GPCRs), GPR88 shows highest and most restricted expression in the striatum, a crucial region in motor control (Liljeholm and O'Doherty, 2012, Quintana A, 2012). Within the striatum, *Gpr88* is expressed specifically in the vast majority of medium spiny neurons (MSNs) of both the striatonigral (co-expressing dopamine D1 receptors_D1R and Substance P) and striatopallidal (co-expressing dopamine D2 receptors_D2R and adenosine A2A receptor_A2AR) pathways. Previous reports show decreased basal dopamine (DA) levels (Logue et al., 2009) and motor coordination deficit in *Gpr88*^{-/-} animals (Quintana A, 2012, Meirsman et al., 2015) suggesting that this receptor may be implicated in development of Parkinson's disease symptoms. In an animal model of this disease, DA depletion was shown to decrease dendritic spines and glutamatergic synapses selectively on striatopallidal but not on striatonigral MSNs (Day et al., 2006). Interestingly, DA depletion was also shown to increase striatopallidal *Penk* expression (Asselin et al., 1994, Salin et al., 1997, Murer et al., 2000) and dendritic excitability (Day et al., 2008) while *Gpr88* expression in these neurons was decreased by DA depletion (Massart R, 2009). Likewise, while the D2R agonist quinpirol decreased locomotion in wild type animals, *Gpr88*^{-/-} mice showed an opposite increased locomotor response (Quintana A, 2012) as well as a decreased sensitivity to haloperidol (Logue et al., 2009) overall suggesting altered D2R transmission in mice lacking GPR88. However, to date, no report has focused on the specific role of GPR88 within each MSNs subpopulation in the regulation of associated behaviors. To examine GPR88-mediated motor functions specifically in striatopallidal MSNs, we created conditional *Gpr88*^{A2A-Cre} mice and compared their motor performance to those of *Gpr88*^{-/-} animals (manuscript 2). Noteworthy, similar experiments are underway to test GPR88 control over motor function at the level of striatonigral MSNs.

Another important behavioral consequence of *Gpr88* deletion is the hyperactivity trait displayed in the open field (Meirsman et al., 2015) and activity chambers (Quintana A, 2012). In

addition to the hyperactive phenotype, *Gpr88*^{-/-} mice also display increased amphetamine-induced locomotor responses, both hallmark phenotypes for schizophrenia (Geyer, 2008, Duncan et al., 1999, Bell, 1965). While Parkinson's disease is a late onset neurodegenerative disorder, schizophrenia is thought to result from neurodevelopmental dysfunctions (Rapoport et al., 2005). Accordingly, in mice, *Gpr88* is expressed as soon as E18 (Allen brain atlas <http://mouse.brain-map.org/>) displaying highest striatal transcript levels in juvenile rats (post-natal day 25) and decreasing thereafter towards adult levels, suggestive of a role in the development of striatal physiology (Van Waes V, 2011). To elucidate whether the motor phenotypes of *Gpr88*^{-/-} mice result from a tonic or a developmental role of GPR88, we induced the *Gpr88* gene knock-down specifically in the striatum of adult mice using a viral approach and compared behavioral performance of total knockout animals (*Gpr88*^{-/-}) with those of striatal adult knock-down animals (AAV-Cre).

The present chapter addresses two distinct questions: first, we tested the contribution of developmental/tonic roles of striatal GPR88 in motor phenotypes, which are observed in the total GPR88 animals. Second, we addressed the specific role of striatopallidal GPR88 in these motor phenotypes, independent on whether the mechanism involves developmental or tonic GPR88 functions.

Motor coordination deficits were found in all three mouse lines, although to different extents. Our results suggest that motor coordination is regulated by striatopallidal GPR88, and that this behavior is controlled by tonic GPR88 activity in the adult brain. In contrast, the hyperactive behavior observed in *Gpr88*^{-/-} and *Gpr88*^{A2A-Cre}, but not AAV-Cre mice, seems to be a developmental phenotype of GPR88 in striatopallidal neurons. Finally, we found strikingly similar altered D₁R- and D₂R-agonist induced locomotion in full and striatopallidal *Gpr88* KO mice suggesting that GPR88 deletion in one MSN population alters DA transmission in both indirect and direct pathways.

Materials and methods

Animals

Mice (Male and female) were bred in house as described previously (please refer to manuscript 2 of the present section). Briefly, we generated both total and conditional mice using Cre-LoxP strategy. The *Gpr88*^{-/-} line was obtained by crossing *Gpr88*-floxed mice (*Gpr88*^{flx/flx}) with CMV-Cre mice. The conditional KO (cKO) of GPR88 in striatopallidal MSNs (*Gpr88*^{A2A-Cre}) was obtained by crossing *Gpr88*^{flx/flx} mice with *Adora2a*-Cre mice (Durieux et al., 2009). *Gpr88* gene deletion in a region and time-specific manner was achieved using bilateral stereotaxic injections of AAV-Cre-eGFP (AAV-Cre mice) or AAV-eGFP (control mice) in the striatum of adult *Gpr88*-floxed mice. Animals were group housed (3-5 animals/cage) in a 12h dark/light cycle (lights on at 7am) at controlled temperature (22±1°C). Water and food were available *ad libitum*. Animals were aged 10-15 weeks when tested

Three successive cohorts were prepared by stereotaxic AAV-Cre injection in order to test stereotypies then the rotarod, with a resting period of 48h. For open field assessment, only one of the three cohorts was used and locomotion was measured 48h after rotarod assessment. For *Gpr88*^{-/-} and *Gpr88*^{A2A-Cre}, one and two cohorts of animals respectively were used to test stereotypies, rotarod “easy” and open field locomotion in this order and separated by a resting period of 48h. Two separated cohorts of these animals underwent rotarod “hard” and dopaminergic agonist-induced locomotion in this order and separated by a resting period of 48h. For basal and haloperidol-induced catalepsy, naïve mice from both *Gpr88*^{-/-} and *Gpr88*^{A2A-Cre} mouse lines were used.

Generation of viral vectors

Recombinant adenoassociated virus serotype 1 (AAV1) viral vectors were generated expressing enhanced green fluorescent protein (eGFP) and Cre (AAV1-Cre-eGFP) under the control of CMV promoters. Control vectors encode for eGFP alone (AAV1-eGFP). AAV1 vectors were generated by triple transfection of AAV-293 cell line (Stratagene) using (i) either pAAVeGFP or pAAV-Cre-eGFP, (ii) pAAV-RC (Stratagene) containing rep and cap genes of the AAV1 and (iii) pHelper (Stratagene) encoding for the adenoviral helping functions. Following 2 days cells were collected, lysed and treated with Benzonase (50 U/ml, sigma, 30 nm, 37°C). Viral vectors were purified by iodixanol gradient ultracentrifugation (Zolotukhin et al., 2002) followed by dialysis and concentration against Dulbecco phosphate buffered saline (PBS) using centrifugal filters (Amicon Ultra-15 Centrifugal Filter

Devices 50 K). Viral particles were quantified by real time PCR using a plasmid standard pAAV-eGFP. To achieve comparable working concentrations, viruses were diluted in Dulbecco-PBS buffer to a final concentration of 3×10^{11} viral genomes per ml (vg/ml) and finally stored at -80°C until use.

Stereotaxic surgery

Gpr88^{flx/flx} mice were anesthetized with an intraperitoneal (10 ml/kg, i.p.) injection of ketamine/xylazine (Virbac/Bayer, 100/10 mg/kg) dissolved in sterile isotonic saline (NaCl 0.9%) and mounted onto a stereotaxic frame (Unimecanique, France). AAV1-Cre-GFP vectors and control AAV1-eGFP vectors (with titers of $4, 9 \times 10^{12}$ and $2, 6 \times 10^{12}$ of infectious units/ml respectively) were injected bilaterally into the striatum (Coordinates relative to bregma: Anterior-Posterior = + 0.7 mm; Medial-Lateral = ± 2 mm; Dorsal-Ventral = -4, 5 mm and -4 mm) according to the mouse brain atlas (Paxinos and Franklin, 2001) (Figure 1A). A Volume of 1.5 μl of AAV vectors per site of injection was delivered (*i.e.* 3 μl in each striatum) bilaterally with a slow injection rate (0, 1 $\mu\text{l}/\text{min}$) through a 30-gauge stainless steel cannula attached to a stereotaxic arm and connected to an infusion pump. After the first injection was completed, the injector was left in place for 10 min before placing the cannula at the second point of injection. When the two injections were completed the cannula was left for an additional 5min to minimize backflow while withdrawing the injector. All of the behavioral experiments were conducted 4 weeks after AAV vector injections to allow sufficient time for viral vector transduction, Cre expression and GPR88 turnover. For verification of GPR88 reductions, the striatum of all experimental mice were dissected and stored at -80°C before [S35]-GTP γ S assays.

[35S]-GTP γ S binding assay

GTP γ S assays on membrane preparations were performed as previously described (Meirsmen et al., 2015). Briefly, mice were sacrificed by cervical dislocation and both striatum were rapidly removed, frozen in dry ice and stored at -80°C until use. Membranes were prepared by homogenizing the tissue in ice-cold 0.25 M sucrose solution 10 vol (ml/g wet weight of tissue), centrifuged at 2500 g for 10 min. Supernatants were diluted 10 times in buffer, following which they were centrifuged at 23 000 g for 30 min. The pellets were homogenized in 800 μL ice-cold sucrose solution (0.32 M), aliquoted and kept at -80°C until further use. For each [35S]GTP γ S binding assay 2 μg 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 using Microsoft Excel Software. 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.

Drugs

The D₁R agonist SKF81297 and D₂R agonist Quinpirole (Tocris, UK) were dissolved in isotonic saline solution (NaCl 0.9%) and injected subcutaneously in a volume of 10 ml/kg. Control animals received saline. Haloperidol (Haldol®, Janssen, Australia) was dissolved in isotonic saline solution and pH was adjusted to 5.5-6. Mice were injected intraperitoneally with 1,5mg/kg 30min before catalepsy assessment. The GPR88 agonist “compound 19” (Meirsmann et al., 2015) used in [S35]-GTPγS essays was kindly synthesized by Prestwick Chemicals (Illkirch, France).

Rotarod

In the present chapter we will present results from two different rotarod sessions: an “easy” version and a “hard” version. In both sessions mice were placed on a rod accelerating from 4 to 40 rpm in 5 min and remained at maximum speed for the next 5 min. 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.

Easy: In the “easy” condition, the rod was covered with insulation tubing, which external perimeter was 5 cm. On the first day mice were habituated to the apparatus and trained to run on the rod for a minimum of 150 sec at a constant speed of 4rpm. The next four days, mice were tested for three trials a day.

Hard: In this condition, tubing was removed and external perimeter of the rod was 3 cm. Unlike the “easy” condition, mice were tested for four trials every day during six consecutive days. The first day, mice were placed on the rod at constant speed of 4 r.p.m. until achieving 90sec without falling from the rod. Animals were scored for their latency to fall (in seconds) in each trial.

Open field locomotion

Mice were placed in a dimly lit (15 Lux) open field arena placed over a white Plexiglas infrared-lit platform. Locomotor activity was recorded during 30 minutes via an automated tracking system (videotrack; View Point, Lyon, France). Only movements which speed exceed 6 cm/s were taken into account for this measure.

Stereotypies

Mice were individually placed in clear standard cages (21×11×17 cm) filled with 3-cm deep fresh sawdust for 10min (15 lx). Numbers of rearing, burying, grooming, circling episodes and total time spent burying were scored by direct observation.

Basal and haloperidol induced catalepsy

Mice were placed individually so that their forelimbs rested on a wooden bar (0,2cm diameter) placed 4, 5 cm above the floor. Time recording started once the mice was placed and ended with the first forepaws movement. The assay was repeated three times and the average catalepsy time was calculated. 24h later, haloperidol (1,5mg/kg, *i.p.*) was injected 30 minutes before catalepsy assessment.

SKF and Quinpirole induced locomotion

To assess locomotor response to dopaminergic agonist mice were first placed in individual cages for one hour before being injected subcutaneously with either Dopamine 1 receptor (D₁R) agonist SKF (2,5mg/kg) or Dopamine 2 receptor (D₂R) Quinpirole (0,1 mg/kg). Locomotion was immediately after assessed for 1hour. Distance traveled was analyzed by an automated tracking system equipped with an infrared-sensitive camera (videotrack; View Point, Lyon, France).

Statistics

Student t test (mean open field locomotion, stereotypies, catalepsy scoring) and two-way ANOVA (rotarod, drug induced locomotion) were used whenever it was appropriate. All statistical analysis were realized using GraphPad Prism 6 (GraphPad Software, Inc, USA).

1. Role of adult striatal GPR88 in motor responses

Results

Virally-mediated GPR88 gene deletion in the adult striatum

Targeted deletion of GPR88 in a region and time-specific manner was achieved upon local AAV-mediated Cre recombinase delivery in the striatum of *Gpr88^{flx/flx}* mice. [Figure 1A](#) shows viral expression at the site of injection three weeks after surgery (no behavioral assessment in this mice). To quantify the decrease in GPR88 protein activity we used the agonist stimulated [35S]-GTPγS binding assays and measured GPR88 activation in all animals after completion of the behavioral experiments ([Figure 1B](#)). On average, GPR88 activity was significantly decreased in AAV-Cre mice compared to control AAV-eGFP-injected mice (Emax 513, 23 ± 10 , 03% AAV-eGFP; 347, 81 ± 10 , 67% AAV-Cre mice). For each AAV-Cre individual animal, percentage of maximal activation relative to mean maximum activation of control animals was calculated, and animals showing maximal GPR88 activation > 70% were removed from all statistical analysis. *In situ* hybridization performed on a separate group of virally-treated individuals showed a clear dorso-ventral reduction in *Gpr88* mRNA level in mice injected with AAV-Cre covering the entire lateral striatum ([Figure 1C](#)). Taken together, these observations demonstrate that the viral approach leads to efficient functional loss of about 50% striatal GPR88.

Rotarod

To investigate whether *Gpr88* deletion in the striatum of adult mice alters motor coordination we tested both *Gpr88^{-/-}* and AAV-Cre-treated mice in the rotarod for five consecutive days ([Figure 2A and B](#)). As total KO mice ($F_{(1, 168)} = 2,283$; $p < 0,0001$), AAV-Cre mice showed impaired performance in the rotarod. Performance increased across trials for both groups ($F_{(11, 312)} = 3,039$; $p = 0,0007$) and ANOVA indicates a significant treatment effect for AAV-injected mice ($F_{(1, 312)} = 70,57$; $p < 0,0001$), demonstrating that the striatal deletion of *Gpr88* in adult mice impairs motor coordination. Motor skill learning, which is also impaired in total KO mice (interaction: $F_{(11, 168)} = 14,48$; $p < 0,0001$), was not altered by the viral *Gpr88* knock-down under these conditions (interaction: $F_{(11, 312)} = 0,74$;

$p=0,699$) . In fact, *post hoc* comparison (Sidak's multiple comparisons test) indicates no significant difference in the last rotarod session for AAV-Cre mice suggesting that these mice learned the task. These results suggest that *Gpr88* knock-down in the adult striatum impairs motor coordination without affecting motor skill learning.

Open field locomotion

We then tested the previously reported hyperactive phenotype of *Gpr88*^{-/-} mice in the open field for 30 min, and examined whether AAV-Cre mice showed a similar behavior in a parallel experiment (Figure 2D-G). Total KO mice showed increased open field locomotion ($F_{(1, 132)}=52,72$; $p<0, 0001$), as expected. For virally-treated animals, ANOVA using treatment and time as factors (Figure 2 F) reveals a significant treatment effect ($F_{(1, 72)}=4,37$, $p=0,04$) while, contrarily to *Gpr88*^{-/-} mice, *post hoc* comparison indicates no significant differences for none of the 5 min beams. Analysis of mean forward locomotion throughout the 30 min session (Figure 2 G) reveals no difference between AAV-Cre treated mice and their control ($t(10) =1, 66$; $p=0, 13$) indicating no hyperactive phenotype in these mice. Together, results suggest that the GPR88 knock-down in the adult striatum does not induce the hyperactive behavior seen in *Gpr88*^{-/-} animals.

Stereotypies

Dysfunctions in striatal circuits are associated with the appearance of motor stereotypies (Lewis and Levitt, 2002). In this experiment, we show stereotypic behaviors that we had previously

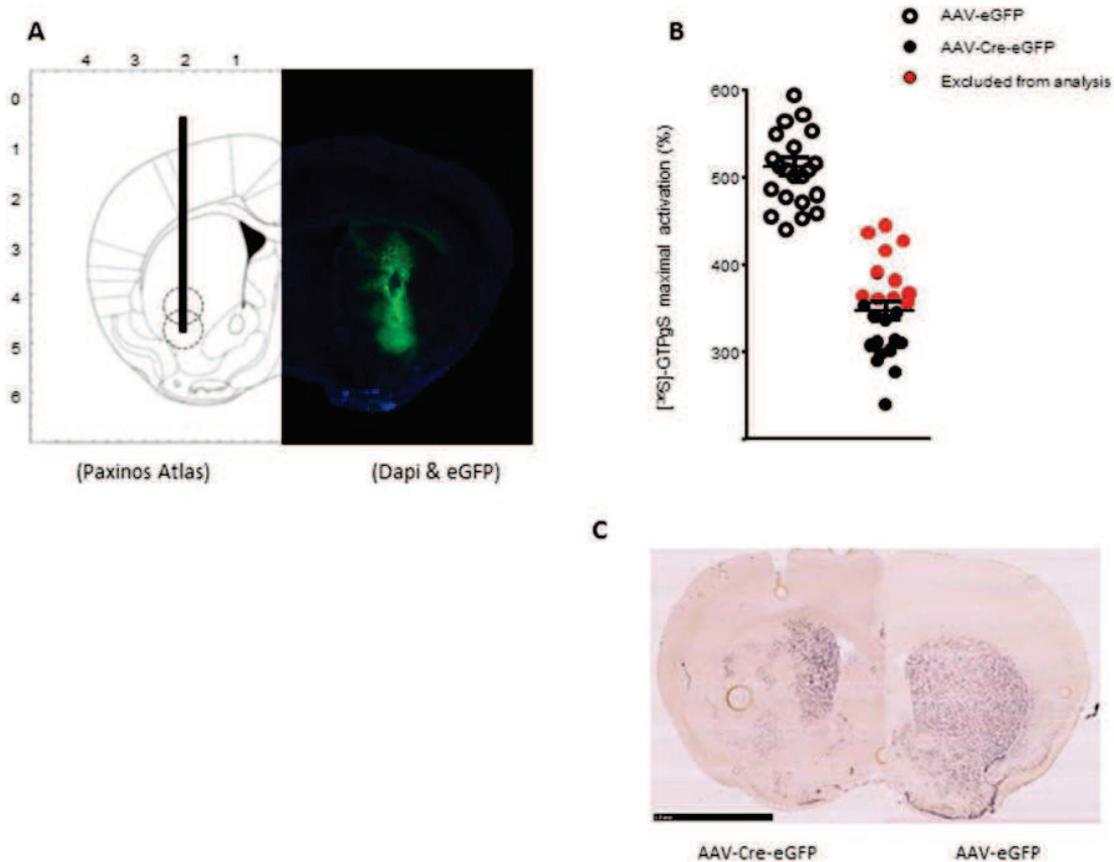


Figure 1: Conditional deletion of *Gpr88* in the Striatum of adult (8 weeks) mice. Figure 1A shows the injection sites (left panel) and the diffusion of the eGFP protein induced by AAV infection (Right panel). Dapi staining was used to label all nuclei. Agonist stimulated [³⁵S]-GTPγS binding assays (B) indicate that AAV-mediated Cre expression resulted in the decrease of GPR88 activation (black circles). Mice with receptor activation higher than 70% (red circles) were excluded from analysis. *In situ* hybridization (C) were realized in mice injected with AAV-eGFP (right panel) or AAV-Cre-eGFP (left panel) and shows a clear reduction of *Gpr88* mRNA throughout dorsal and ventral striatum, but higher decrease in lateral versus medial dorsal striatum. (Scale bar: 2,5mm)

reported including decreased rearing and burying episodes (Meersman et al., 2015)(Figure A-E). These behaviors, however, were not observed upon *Gpr88* deletion in the adult mice's striatum (Figure 3K-O)

In summary, the viral deletion of *Gpr88* in the striatum of adult mice induces motor coordination deficits observed in total KO mice, but does not induce detectable motor skill learning impairments, hyperlocomotion or stereotypies.

Discussion

To determine whether GPR88 plays a developmental or tonic role in striatal motor functions we created an AAV-mediated striatal *gpr88* knock-down and tested behavioral responses in these mice. *In situ* hybridization and [S35]-GTPγS essays indicate that *gpr88* was efficiently, although not totally, knocked-down throughout dorsal and ventral striatum. Notably also, i) approximately 50% of striatal *Gpr88* was deleted, ii) the remaining *Gpr88* mRNA was concentrated in the dorsomedian (DMS) striatum. Our behavioral data indicates that this partial deletion was sufficient to decrease motor coordination in the rotarod, suggesting that GPR88 activity in the adult mice tonically regulates motor coordination. This result has important implications, as newly developed GPR88 may indeed improve motor coordination with therapeutic potential for Parkinson disease (Tarazi et al., 2014)

AAV-Cre injected mice showed an initial impairment in the rotarod test, which reversed to control levels during the last trials, indicating that these animals ultimately learned the task. In contrast, the constitutive deletion of *Gpr88* not only impacts motor coordination as soon as the first trial, but also alters the ability to learn the task. The DMS is known to be required for initial stages of motor skill learning, whereas the dorsolateral striatum (DLS) is more engaged in habit acquisition (Yin et al., 2009, Liljeholm and O'Doherty, 2012, Luft and Buitrago, 2005). Our data are therefore consistent with the notion that *Gpr88* deletion in the DMS of AAV-Cre-treated adult mice was sufficient to impair motor coordination, as reflected by altered initial motor skill abilities, and that intact GPR88 in the DLS allowed learning the task along sessions.

When placed in an open field, AAV-Cre mice showed an increase in locomotor activity when compared to control animals, but this increase was not significant throughout the session. This result suggests that lack of GPR88 in the adult striatum does not cause basal hyperactivity as seen in *Gpr88*^{-/-} mice. Likewise, a previous report using lentiviral-mediated *Gpr88* silencing in the nucleus accumbens showed no alteration of basal locomotion (Ingallinesi et al., 2014). Altogether, these results suggest that spontaneous high locomotor activity in *Gpr88*^{-/-} mice is a developmental phenotype. GPR88 therefore may contribute to the development of neural wiring within locomotion-associated networks, most likely at the level of striatum (Van Waes V, 2011). Similarly, unchanged stereotypic behaviors in AAV-treated adult mice, contrasting with strongly enhanced stereotypies in total GPR88 KO mice, suggest that GPR88 contributes to the organization of neural networks subserving these behaviors during development, with minimal impact later in adulthood. Note that in both cases (hyperlocomotion and stereotypies), we cannot exclude that GPR88 has a tonic role but the receptor knock-down was insufficient to reveal a behavioral phenotype.

On the other hand, hyperactivity observed in the very first 10 minutes of the test suggest that deletion of *Gpr88* later at adulthood may alter striatal physiology leading specific modification of initial exploration behavior. This observation deserves further investigations, as a tonic GPR88 role in exploratory behavior may have implications in the development of GPR88 drugs for therapeutic purposes.

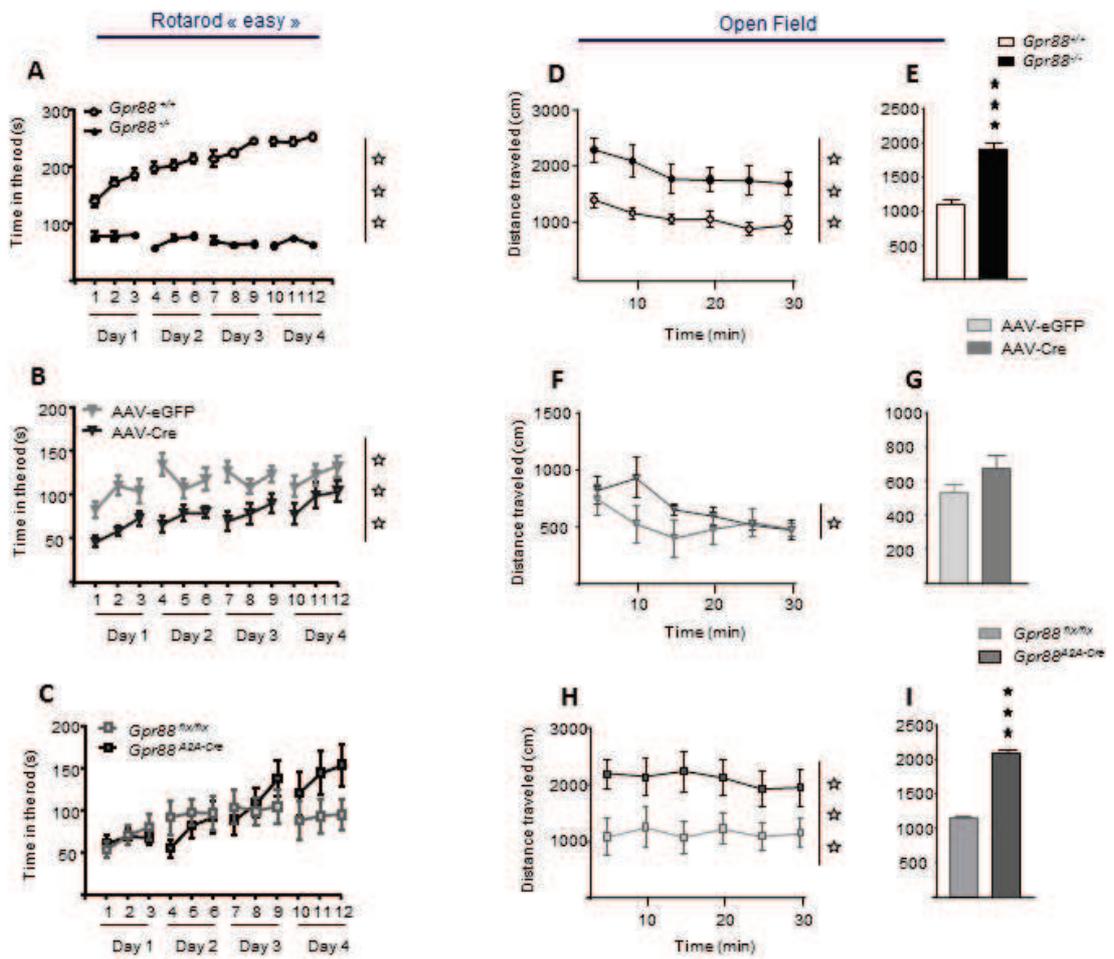


Figure 2: Motor coordination and open field locomotion in full KO (A, D, E) striatal knock-down (B, F, G) and conditional KO (C, H, I). Rotarod assessment (left part, A-C) show impaired motor coordination in full KO (N= 8 KO; 8 WT) and striatal knock-down (N= 11 AAV-eGFP; 13 AAV-Cre) but not conditional KO of *Gpr88* in striatopallidal neurons (N= 13 *Gpr88^{flx/flx}*, 12 *Gpr88^{A2A-Cre}*). When placed in an open field (D-I) both *Gpr88^{-/-}* (N= 12 KO; 12 WT) and *Gpr88^{A2A-Cre}* (N= 10 *Gpr88^{flx/flx}*, 10 *Gpr88^{A2A-Cre}*) displayed increased locomotion. AAV-Cre mice (N= 7 AAV-eGFP; 7 AAV-Cre) locomotion ANOVA also revealed a significant increased locomotion. However, when comparing mean forward locomotion throughout the session no significant difference was found. Line graphs show the distance traveled (cm) in 5 min bins over a 30 min session (D-H). Bar graphs show the average total distance traveled (cm) over the 30 min sessions period (E-I). Data are represented as mean \pm SEM. Solid stars: three stars $p < 0.001$ (Student t test). Open stars: one star $p < 0, 05$; three stars $p < 0.001$ (Genotype effect, Two-way ANOVA)

Stéréotypies

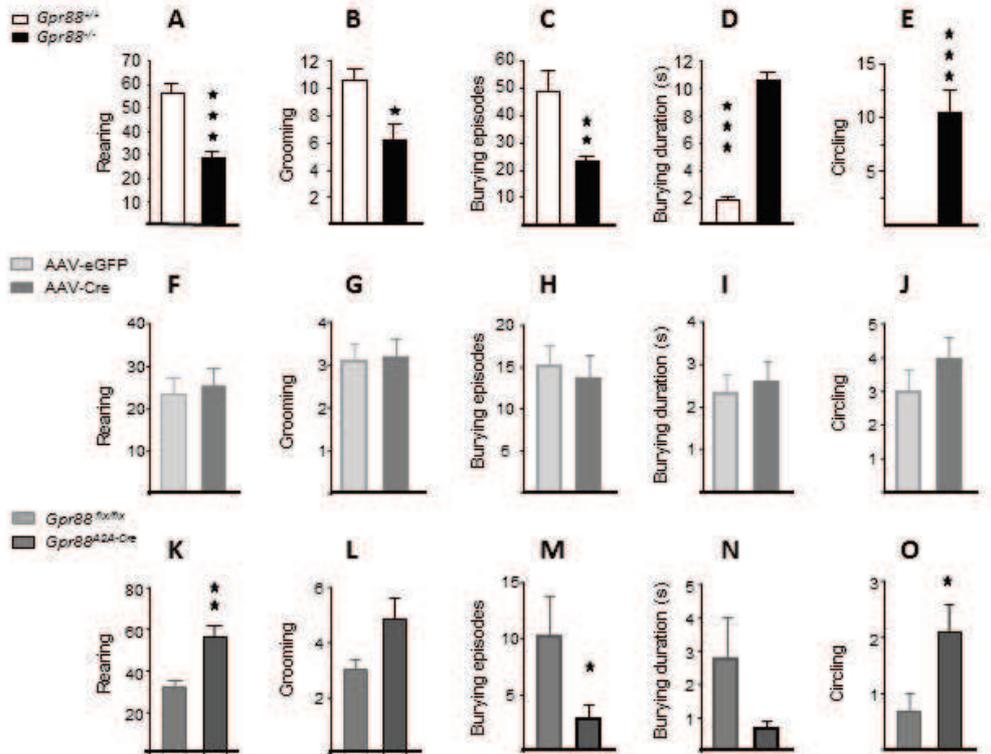


Figure 3: Spontaneous motor stereotypies. Full KO (A-E) display increased burying duration (D) and number of circling (E) but decreased number of rearing (A) and grooming (B) (N= 12 KO; 12WT). Deletion of *Gpr88* in the striatum of adult mice had no effect on spontaneous motor stereotypies (F-J) (N= 15 AAV-eGFP; 13 AAV-Cre). *Gpr88*^{A2A-Cre} mice also show decreased number of burying (M) but these mice present increased number of rearing (K) and circling (O) (N= 9 *Gpr88*^{flox/flox}, 11 *Gpr88*^{A2A-Cre}). Data are represented as mean ± SEM. Solid stars: one star p < 0.05, two stars p < 0.01, three stars p < 0.001 (Studentttest).

2. Role of striatopallidal GPR88 in motor responses

Results

Conditional deletion of GPR88 in striatopallidal neurons.

The conditional *Gpr88*^{A2A-Cre} mice are described in manuscript 2. In these mice gene knockout is targeted to MSNs from the striatopallidal pathway, and occurs in embryonic stages when the A2AR promoter becomes active (Svenningsson et al., 1999, Schiffmann and Vanderhaeghen, 1993).

Rotarod

We compared motor coordination and motor skill learning performance of *Gpr88*^{-/-} and striatopallidal conditional (*Gpr88*^{A2A-Cre}) animals using the exact same conditions (Figure 2 A and C). For both mouse lines, performance increased across trials (*Gpr88*^{-/-}: $F_{(11, 168)} = 9,040$; $p < 0,0001$; *Gpr88*^{A2A-Cre}: $F_{(11, 276)} = 2,973$; $p < 0,0001$). Results indicate a difference between genotypes for *Gpr88*^{-/-} mice ($F_{(1, 168)} = 2,283$; $p < 0,0001$) but no genotype effect was detected the *Gpr88*^{A2A-Cre} line. Additionally, total but not conditional deletion of *Gpr88* also led to an interaction between genotype and trial effect ($F_{(11, 168)} = 14,48$; $p < 0,0001$). (Data from *Gpr88*^{-/-} published in (Meirman et al., 2015)). These data suggest that, in contrast to the total *Gpr88* knockout, the conditional deletion of striatopallidal *Gpr88* does not alter motor coordination, at least with standard experimental conditions.

Rotarod “hard”

To further confirm that deletion of *Gpr88* in striatopallidal neurons has no effect in motor coordination we tested *Gpr88*^{A2A-Cre} mice in a harder version of the rotarod test and compared the performance of these mice with *Gpr88*^{-/-} mice (Figure 4 A and B). Under these conditions, performance was reduced, and both *Gpr88*^{A2A-Cre} and *Gpr88*^{-/-} mice presented a clear motor coordination deficit (genotype effect; *Gpr88*^{-/-}: $F_{(1, 17)} = 22,39$; $p = 0,0002$; *Gpr88*^{A2A-Cre}: $F_{(1, 17)} = 22,48$; $p = 0,0002$) while control littermates seemed to correctly learn the task (trial effect; *Gpr88*^{-/-}: $F_{(23, 391)} = 1,77$; $p = 0,016$; *Gpr88*^{A2A-Cre}: $F_{(23, 391)} = 3,13$; $p < 0,0001$). Additionally, both mice lines failed to

learn the task (interaction: *Gpr88*^{-/-}: $F_{(23, 391)}=2,16; p=0,0017$; *Gpr88*^{A2A-Cre}: $F_{(23, 391)}=3,22; p<0,0001$) suggesting impaired motor skill learning. We conclude that the striatopallidal *Gpr88* deletion also impairs motor coordination and motor skill learning, although not as drastically as observed in *Gpr88*^{-/-} mice.

Open field locomotion

Gpr88^{A2A-Cre} and *Gpr88*^{-/-} were placed in an open field and forward locomotion was recorded for 30 min (Data from manuscript 2) (Figure 2 D, E, H and I). ANOVA using genotype and time as factors revealed a significant genotype effect for both mouse lines (*Gpr88*^{-/-}: $F_{(1, 132)}=52,72; p<0,0001$; *Gpr88*^{A2A-Cre}: $F_{(1, 108)}=28,93; p<0,0001$). Also, analysis of mean forward locomotion (Figure 2 E and I) revealed a significant genotype difference for *Gpr88*^{-/-} ($t(10) = 6,65; p<0,0001$) as well as *Gpr88*^{A2A-Cre} mice ($t(10) = 15,60; p < 0,0001$). In sum, full and conditional deletion of *Gpr88* in striatopallidal neurons both lead to similar hyperactive behavior in mice.

Stereotypies

When spontaneous stereotypies were recorded, both total and conditional KO mice showed abnormal behaviors compared to control littermates (Figure 3 A-E and K-O). As we had previously reported (Meirsman et al., 2015) *Gpr88*^{-/-} mice displayed decreased number of rearing ($t(22)=5; p<0,0001$), grooming ($t(22)=2,91; p=0,0082$), and burying episodes ($t(22)=3,49; p=0,0021$) and also spent more time burying ($t(9)=4,31; p=0,002$) due to markedly longer episodes than controls, as well as increased frequency of circling ($t(22)=5,22; p<0,0001$) (data published in Meirsman et al., 2015). *Gpr88*^{A2A-Cre} mice also presented increased number of circling ($t(18)=2,28; p=0,035$) and decreased number of burying episodes ($t(18) = 2,13; p=0,047$), the duration of these burying was not significantly different than their control littermates. In contrast with full KO animals, *Gpr88*^{A2A-Cre} mice displayed increased number of rearing ($t(18) = 3,51; p=0,0025$). Lack of *Gpr88* in striatopallidal neurons, therefore produces modifications of stereotypic behaviors that differ from those observed in total KO mice.

Rotarod « hard »

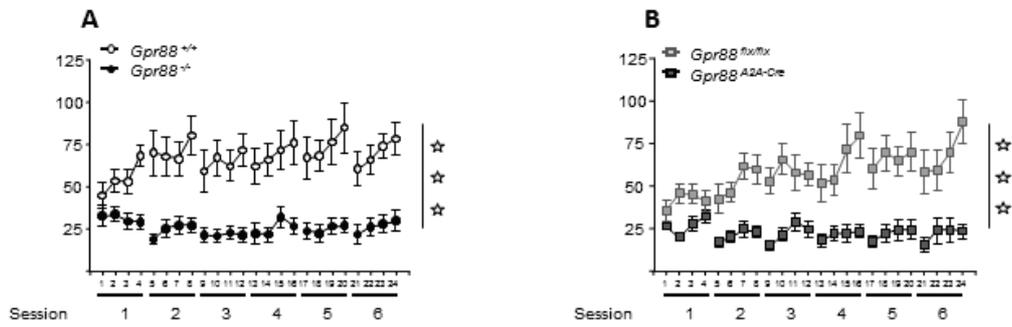


Figure 4: Motor coordination in full and conditional KO. When tested in a harder version of the rotarod both total KO (A) (N= 10 KO; 9 WT) and conditional KO of *Gpr88* in striatopallidal neurons (B) (N= 9 *Gpr88*^{flx/flx}, 10 *Gpr88*^{A2A-Cre}) show a clear impairment in motor coordination and a complete inability to learn the task. Data are represented as mean ± SEM. Open stars: three stars p < 0.001 (Genotype effect, Two-way ANOVA)

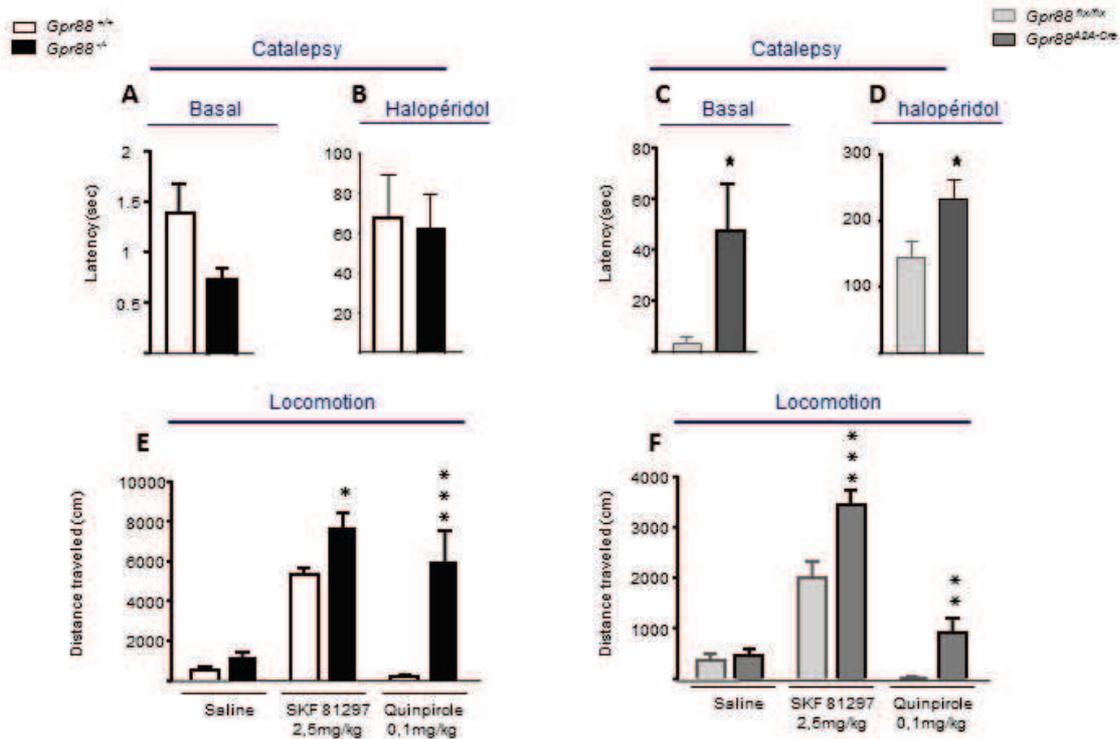


Figure 5: Responses to dopaminergic drugs in full and conditional KO. When tested for basal catalepsy conditional (C) but not full KO presented increased latency to move in the bar test. The same result was obtained when mice were injected with 1,5mg/kg (*i.p.*) of haloperidol 30 min before bar testing (B and D). While saline locomotion does not differ between genotypes (N= 12 WT, 9 KO; N= 12 *Gpr88*^{flx/flx}, 12 *Gpr88*^{A2A-Cre}), when injected with 2, 5 mg/kg of SKF 81297 both *Gpr88*^{-/-} (N= 6 WT, 9 KO) and *Gpr88*^{A2A-Cre} (N= 8 *Gpr88*^{flx/flx}, 10 *Gpr88*^{A2A-Cre}) traveled a longer distance than their control littermates (E and F). While quinpirole decreased locomotion in wild type animals, this drug increased locomotion of full (N= 6 WT, 6 KO) as well as conditional KO (N= 12 *Gpr88*^{flx/flx}, 12 *Gpr88*^{A2A-Cre}). Solid stars: one star $p < 0.05$, (Student t test). Text stars: one star $p < 0.05$, two stars $p < 0.01$, three stars $p < 0.001$ (Sidak's multiple comparison test).

In summary, deletion of *Gpr88* in striatopallidal neurons leads to a hyperactive phenotype similar to full KO animals, while motor coordination deficits and stereotypic behavior differ from *Gpr88*^{-/-} mice.

Basal and Haloperidol induced catalepsy

In order to determine whether *Gpr88* deletion in striatopallidal neurons alters response to haloperidol we tested *Gpr88*^{-/-} and *Gpr88*^{A2A-Cre}, and their control littermates, in basal as well as haloperidol-induced catalepsy (Figure 5 A-D). When assessed for basal catalepsy, *Gpr88*^{A2A-Cre} (t (27) =2,23; *p*=0,03) but not *Gpr88*^{-/-} (t (15) =2,03; *p*=0,06) mice showed significantly higher latency to move when compared to controls. A similar result was obtained when mice were injected with the D₂R agonist haloperidol (*Gpr88*^{A2A-Cre}: t (27) =2,28; *p*=0,03; *Gpr88*^{-/-} : t (15) =0,17; *p*=0,86), suggesting that GPR88 in striatopallidal neurons influences cataleptic responses in a manner that is undetectable when both striatonigral and striatopallidal receptors are deleted.

Locomotion under dopaminergic agonists

To investigate the effect of *Gpr88* deletion in striatopallidal neurons in the response to dopaminergic agents we compared locomotor responses of *Gpr88*^{-/-} and *Gpr88*^{A2A-Cre} mice after administration of D₁R-and D₂R-agonists (Figure 5 E and F). As previously reported (Quintana A, 2012, Logue et al., 2009) full *Gpr88* deletion altered response to dopaminergic agonist ($F_{(1, 42)}=33,69$; *p*<0,0001). When compared to control animals, *Gpr88*^{-/-} mice showed increased locomotion after SKF 81297 (t (42) = 2,63; *p*<0, 05) and quinpirol (t (42) = 5,91; *p*<0,0001) administration. Likewise, locomotor responses to both SKF 81297 (t (60) =4, 30, *p* <0,001) and quinpirole (t (60) =3,07; *p* <0,01) were significantly enhanced in *Gpr88*^{A2A-Cre} mice when compared to their littermates ($F_{(1, 60)}=21,24$; *p*<0,0001). These results show that the striatopallidal *Gpr88* deletion recapitulates behavioral deficits of the total GPR88 knockout, suggesting that this receptor population controls the locomotor effects of DA agonists.

Discussion

To examine the role of striatopallidal GPR88 in motor functions we used a conditional KO mice expressing the Cre recombinase under the control of the striatopallidal-specific *Adora2a* gene. In the previous section (Manuscript 2) we showed that *Gpr88* was selectively deleted in D₂R expressing neurons throughout DMS and DLS in rostral and caudal Caudate-Putamen. Interestingly, the conditional KO mice showed no motor coordination deficit in the “easy” rotarod condition. However, when the task was made more difficult (see methods) *Gpr88*^{A2A-Cre} mice showed both motor coordination and skill learning deficits, which were comparable to *Gpr88*^{-/-} animals. Together these tests revealed that *Gpr88*^{A2A-Cre} mice showed lower performance on the rotarod, although not as pronounced as in full KO animals, indicating that the striatonigral GPR88 deletion partly contributes to the overall rotarod deficit in *Gpr88*^{-/-} mice. A previous report (Durieux PF, 2012) shows that ablation of striatopallidal MSNs caused task impairment only during initial trials but did not affect later performance in the rotarod. On the other hand, the same report indicates that ablation of striatonigral MSNs lead to motor coordination and skill learning deficits, as seen in both total and *Gpr88*^{A2A-Cre} mice in our study. It is possible, therefore, that the GPR88 deletion in striatopallidal neurons partially mimics the deletion of striatonigral neurons in Durieux’s study, and involves a similar disruption of the striatopallidal/striatonigral balance.

Overall, our data showing that deletion of striatopallidal *Gpr88* induces a motor coordination and skill learning deficit, although not to the same extent as for *Gpr88*^{-/-} mice, suggest a possible role of striatonigral GPR88 in the observed phenotype. This hypothesis may be further tested using electrophysiological analysis of D₁R- and D₂R-type MSNs in *Gpr88*^{A2A-Cre} mice. Also, the analysis of mice lacking GPR88 in striatonigral neurons will further help deciphering the exact contribution of the two receptor population in motor coordination and motor skills learning.

When placed in an open field *Gpr88*^{A2A-Cre} mice displayed hyperactive behavior throughout the testing session with similar levels than full KO animals, suggesting that GPR88 activity in striatopallidal neurons fully accounts for the hyperactive phenotype. Several studies have reported that ablation (Durieux PF, 2009) or disruption (Bateup et al., 2010) of striatopallidal neurons caused open field hyperactivity in mice. Similar consequences of striatopallidal GPR88 ablation or entire

neuron ablation suggest that GPR88 is key to support striatopallidal neuron activity. It is likely that GPR88, expressed on most MSNs, modulates both striatonigral and striatopallidal pathways, and exerts a complex modulatory role on the D₁R/D₂R neuron balance, which in turn has distinct consequences on the different behavioral outcomes depending on striatum activity (see general discussion). Once again, it would be interesting to know if mice lacking *Gpr88* in D₁R-MSNs present a similar or opposite phenotype in the open field.

Finally, *Gpr88*^{A2A-Cre} mice also presented increased number of stereotypies namely rearing. While the neurobiological mechanism underlying stereotypies are still unknown, dysregulation of cortico-striato-thalamo-cortical circuitry are thought to be associated to these behaviors (Lewis and Kim, 2009). Interestingly, Tanimura et al (2010) reported that stereotypies may result from decreased striatopallidal transmission and that adenosine agonists reversed this pathological behavior (Tanimura et al., 2010, Tanimura et al., 2011). It is therefore possible that a similar mechanism occurred in *Gpr88*^{A2A-Cre} mice.

Catalepsy scoring results surprisingly showed that *Gpr88*^{A2A-Cre} mice presented higher catalepsy than control animals. When placed on the bar, some mutant mice freezed on the bar for long periods (more than 300sec for some trials) to, immediately after, jump from their place. Also, these mice presented higher sensitivity to haloperidol with higher catalepsy scores after administration of this D₂R antagonist. These results clearly suggest compromised striatopallidal transmission and altered dopaminergic sensitivity in mice lacking striatopallidal GPR88. However, further investigation are needed to fully explain the mechanisms underlying simultaneous presence of hyperactivity and increased catalepsy in these mice. In previous reports *Gpr88*^{-/-} animals (Meirman et al., 2015, Logue et al., 2009) were shown to have decreased dopaminergic basal levels in the striatum, which could result in supersensitization of dopamine receptors. Accordingly, when administered with either D₁R or D₂R agonists, both full and conditional KO presented an increased locomotor response. While basal dopaminergic levels were never measured in *Gpr88*^{A2A-Cre} mice, binding assays results (Manuscript 2) suggest altered D₂R receptor activation in these mice. Future studies using D₁R-Cre driven *Gpr88* deletion to study dopaminergic agonist-induced locomotion will help us gain a better understanding of the mechanisms underlying GPR88 modulation of dopamine receptor and medium spiny neurons transmission.

Conclusion

In sum, motor coordination deficits were found in all the mouse lines, including full GPR88 knockout, conditional GPR88 striatopallidal knockout and adult striatum GPR88 knock-down animals. We conclude that GPR88 likely plays a tonic role in motor coordination at the level of striatal networks. These findings have important implications in therapeutic approaches to striatal motor diseases such as Parkinson's or Huntington disease, and newly developed GPR88 agonists should be tested towards restoring impaired motor coordination.

In contrast, the hyperactivity detected in *Gpr88*^{-/-} and *Gpr88*^{A2A-Cre} mice was absent in AAV-Cre mice suggesting that GPR88 regulates this behavior through a developmental role. Hyperactivity in *Gpr88*^{A2A-Cre} mice suggest decreased striatopallidal transmission as a consequence of *Gpr88* deletion but further studies are needed to clarify this point. Also, as for *Gpr88*^{-/-}, *Gpr88*^{A2A-Cre} mice show altered D₁R (SKF) and D₂R (Quinpirole) agonist-induced locomotor response. As such, GPR88 in the striatopallidal pathway regulates locomotion and exploratory behavior, possibly by enhancing sensitivity to dopamine.

Overall, these results shows that GPR88 regulates the development of striatal circuitry, its function in the adult, and also shows cell-autonomous activities that strongly influence the complex striatopallidal/striatonigral interplay.

References

- ASSELIN, M. C., SOGHOMONIAN, J. J., COTE, P. Y. & PARENT, A. 1994. Striatal changes in preproenkephalin mRNA levels in parkinsonian monkeys. *Neuroreport*, 5, 2137-40.
- BATEUP, H. S., SANTINI, E., SHEN, W., BIRNBAUM, S., VALJENT, E., SURMEIER, D. J., FISIONE, G., NESTLER, E. J. & GREENGARD, P. 2010. Distinct subclasses of medium spiny neurons differentially regulate striatal motor behaviors. *Proc Natl Acad Sci U S A*, 107, 14845-50.
- BELL, D. S. 1965. Comparison of Amphetamine Psychosis and Schizophrenia. *Br J Psychiatry*, 111, 701-7.
- DAY, M., WANG, Z., DING, J., AN, X., INGHAM, C. A., SHERING, A. F., WOKOSIN, D., ILIJIC, E., SUN, Z., SAMPSON, A. R., MUGNAINI, E., DEUTCH, A. Y., SESACK, S. R., ARBUTHNOTT, G. W. & SURMEIER, D. J. 2006. Selective elimination of glutamatergic synapses on striatopallidal neurons in Parkinson disease models. *Nat Neurosci*, 9, 251-9.
- DAY, M., WOKOSIN, D., PLOTKIN, J. L., TIAN, X. & SURMEIER, D. J. 2008. Differential excitability and modulation of striatal medium spiny neuron dendrites. *J Neurosci*, 28, 11603-14.
- DUNCAN, G. E., SHEITMAN, B. B. & LIEBERMAN, J. A. 1999. An integrated view of pathophysiological models of schizophrenia. *Brain Res Brain Res Rev*, 29, 250-64.
- DURIEUX PF, B. B., GUIDUCCI S, BUCH T, WAISMAN A, ZOLI M, ET AL 2009. D2R striatopallidal neurons inhibit both locomotor and drug reward processes. *Nat Neurosci*, :393-395.
- DURIEUX PF, S. S., DE KERCHOVE D'EXAERDE A 2012. Differential regulation of motor control and response to dopaminergic drugs by D1R and D2R neurons in distinct dorsal striatum subregions. 640-653.
- DURIEUX, P. F. B., B. GUIDUCCI, S. BUCH, T., WAISMAN, A., ZOLI, M., SCHIFFMANN, S. N. & DE KERCHOVE D'EXAERDE, A. 2009. D2R striatopallidal neurons inhibit both locomotor and drug reward processes. *Nat Neurosci*, 12, 393-5.
- GEYER, M. A. 2008. Developing translational animal models for symptoms of schizophrenia or bipolar mania. *Neurotox Res*, 14, 71-8.
- INGALLINESI, M., LE BOUIL, L., FAUCON BIGUET, N., DO THI, A., MANNOURY LA COUR, C., MILLAN, M. J., RAVASSARD, P., MALLET, J. & MELONI, R. 2014. Local inactivation of Gpr88 in the nucleus accumbens attenuates behavioral deficits elicited by the neonatal administration of phencyclidine in rats. *Mol Psychiatry*.
- LEWIS, D. A. & LEVITT, P. 2002. Schizophrenia as a disorder of neurodevelopment. *Annu Rev Neurosci*, 25, 409-32.
- LEWIS, M. & KIM, S. J. 2009. The pathophysiology of restricted repetitive behavior. *J Neurodev Disord*, 1, 114-32.
- LILJEHOLM, M. & O'DOHERTY, J. P. 2012. Contributions of the striatum to learning, motivation, and performance: an associative account. *Trends Cogn Sci*, 16, 467-75.
- LOGUE, S. F., GRAUER, S. M., PAULSEN, J., GRAF, R., TAYLOR, N., SUNG, M. A., ZHANG, L., HUGHES, Z., PULITO, V. L., LIU, F., ROSENZWEIG-LIPSON, S., BRANDON, N. J., MARQUIS, K. L., BATES, B. & PAUSCH, M. 2009. The orphan GPCR, GPR88, modulates function of the striatal dopamine system: a possible therapeutic target for psychiatric disorders? *Mol Cell Neurosci*, 42, 438-47.
- LUFT, A. R. & BUITRAGO, M. M. 2005. Stages of motor skill learning. *Mol Neurobiol*, 32, 205-16.
- MASSART R, G. J., MIGNON V, SOKOLOFF P, DIAZ J 2009. Striatal GPR88 expression is confined to the whole projection neuron population and is regulated by dopaminergic and glutamatergic afferents. *Eur J Neurosci*, 397-414.

- MEIRSMAN, A. C., LE MERRER, J., PELLISSIER, L. P., DIAZ, J., CLESSE, D., KIEFFER, B. L. & BECKER, J. A. 2015. Mice Lacking GPR88 Show Motor Deficit, Improved Spatial Learning, and Low Anxiety Reversed by Delta Opioid Antagonist. *Biol Psychiatry*.
- MURER, M. G., DZIEWCZAPOLSKI, G., SALIN, P., VILA, M., TSENG, K. Y., RUBERG, M., RUBINSTEIN, M., KELLY, M. A., GRANDY, D. K., LOW, M. J., HIRSCH, E., RAISMAN-VOZARI, R. & GERSHNIK, O. 2000. The indirect basal ganglia pathway in dopamine D(2) receptor-deficient mice. *Neuroscience*, 99, 643-50.
- PAXINOS, G. & FRANKLIN, K. B. J. 2001. *The mouse brain in stereotaxic coordinates*, academic press.
- QUINTANA A, S. E., WANG W, STOREY GP, GULER AD, WANAT MJ, ET AL. 2012. Lack of GPR88 enhances medium spiny neuron activity and alters motor- and cue-dependent behaviors. *Nat Neurosci.*, 1547-1555.
- RAPOPORT, J. L., ADDINGTON, A. M., FRANGOU, S. & PSYCH, M. R. 2005. The neurodevelopmental model of schizophrenia: update 2005. *Mol Psychiatry*, 10, 434-49.
- SALIN, P., DZIEWCZAPOLSKI, G., GERSHNIK, O. S., NIEOULLON, A. & RAISMAN-VOZARI, R. 1997. Differential regional effects of long-term L-DOPA treatment on preproenkephalin and preprotachykinin gene expression in the striatum of 6-hydroxydopamine-lesioned rat. *Brain Res Mol Brain Res*, 47, 311-21.
- SCHIFFMANN, S. N. & VANDERHAEGHEN, J. J. 1993. Adenosine A2 receptors regulate the gene expression of striatopallidal and striatonigral neurons. *J Neurosci*, 13, 1080-7.
- SVENNINGSSON, P., LE MOINE, C., FISONE, G. & FREDHOLM, B. B. 1999. Distribution, biochemistry and function of striatal adenosine A2A receptors. *Prog Neurobiol*, 59, 355-96.
- TANIMURA, Y., KING, M. A., WILLIAMS, D. K. & LEWIS, M. H. 2011. Development of repetitive behavior in a mouse model: roles of indirect and striosomal basal ganglia pathways. *Int J Dev Neurosci*, 29, 461-7.
- TANIMURA, Y., VAZIRI, S. & LEWIS, M. H. 2010. Indirect basal ganglia pathway mediation of repetitive behavior: attenuation by adenosine receptor agonists. *Behav Brain Res*, 210, 116-22.
- TARAZI, F. I., SAHLI, Z. T., WOLNY, M. & MOUSA, S. A. 2014. Emerging therapies for Parkinson's disease: from bench to bedside. *Pharmacol Ther*, 144, 123-33.
- VAN WAES V, T. K., STEINER H 2011. GPR88 - a putative signaling molecule predominantly expressed in the striatum: Cellular localization and developmental regulation. *Basal Ganglia*, 83-89.
- YIN, H. H., MULCARE, S. P., HILARIO, M. R., CLOUSE, E., HOLLOWAY, T., DAVIS, M. I., HANSSON, A. C., LOVINGER, D. M. & COSTA, R. M. 2009. Dynamic reorganization of striatal circuits during the acquisition and consolidation of a skill. *Nat Neurosci*, 12, 333-41.
- ZOLOTUKHIN, S., POTTER, M., ZOLOTUKHIN, I., SAKAI, Y., LOILER, S., FRAITES, T. J., JR., CHIODO, V. A., PHILLIPSBERG, T., MUZYCZKA, N., HAUSWIRTH, W. W., FLOTTE, T. R., BYRNE, B. J. & SNYDER, R. O. 2002. Production and purification of serotype 1, 2, and 5 recombinant adeno-associated viral vectors. *Methods*, 28, 158-67.

3) *Gpr88*-CreER^{T2}: Inducible conditional knockout

Gpr88-CreERT2: Inducible conditional knockout

Introduction

According to the Allen Brain Atlas, *Gpr88* mRNA is found as soon as E18 (<http://mouse.brain-map.org/>). In the rat striatum, the receptor expression achieves highest transcript level at P25 and decrease thereafter toward adult levels (P70) (Van Waes V, 2011). GPR88 may therefore be involved in brain development, particularly at the level of corticostriatal circuits (Massart R, 2009). As a consequence, constitutive deletion of the *Gpr88* gene may have developmental consequences, whose contributions are indistinguishable from receptor function in the adult when interpreting phenotyping data.

In particular, the constitutive *Gpr88* deletion in mice (total *Gpr88* Knockout, KO: *Gpr88*^{-/-}) causes motor dysfunction such as increased locomotion and stereotyped behavior, as well motor coordination deficits. Constitutive absence of GPR88 also leads to deficits in sensorimotor integration and decreased levels of anxiety (Meirman et al., 2015, Logue et al., 2009). Interestingly, these behavioral deficits represent cardinal phenotypes of animal models of psychiatric disorder such as schizophrenia, attention deficit/hyperactivity disorder or bipolar disease. Accordingly, genetic association studies in humans show a positive association between *Gpr88* and bipolar as well as schizophrenia diseases (Del Zompo et al., 2014). Considering the recognized (Lewis and Levitt, 2002, Ronald et al., 2010, Rapoport et al., 2005) neurodevelopmental origin of these neuropsychiatric disorders it is essential to determine whether behavioral phenotypes observed in total *Gpr88*^{-/-} mice arise from developmental reorganization in the absence of receptor, tonic activity of the receptor in the adult, or both. In our previous study using AAV-Cre mediated *Gpr88* deletion in the striatum (see section 2 of the present chapter), we showed that a 50% GPR88 knock-down in the striatum of adult mice impairs motor coordination, highlighting a tonic function for GPR88. However, this result does not reveal other potential tonic activities of GPR88 in the striatum that require low receptor levels, and does not clarify whether GPR88 in other brain structures have a tonic function. To fully dissociate developmental from tonic GPR88 roles across the vast array of behaviors regulated by this receptor (Meirman et al., 2015), it would be necessary to delete the *Gpr88* gene in adult mice, and across all structures where the receptor is expressed. Given the

scattered and sometimes sparse localization of GPR88 in the brain, the AAV-mediated approach is not ideal. Development of the CreER^{T2} technology, however, has been instrumental for this purpose, and allows gene excision (i) at the time decided by the experimenter and (ii) in cells targeted by the Cre-ER^{T2} promoter (Metzger and Chambon, 2001, Feil et al., 1996).

The Cre recombinase is an enzyme derived from the P1 bacteriophage that mediates recombination between loxP sites. The Cre/LoxP-recombination system has been extensively used to generate conditional KO animals in target cells of interest, by placing the Cre recombinase under the control of a cell or tissue-specific promoter (Kuhn and Torres, 2002) in any part of the body, including the nervous system (Gaveriaux-Ruff and Kieffer, 2007). The Cre/LoxP system was made inducible by placing a fusion protein of the Cre recombinase with a mutated ligand-binding domain of the human estrogen-receptor (CreER^{T2}). The fused Cre enzyme is thus retained in the cell cytoplasm and remains inactive. (Metzger and Chambon, 2001, Feil et al., 1996). Cre-mediated recombination is then induced by administration of tamoxifen or its metabolite 4-hydroxitamoxifen, which binds to the estrogen receptor allowing CreER translocation to the nucleus. Additionally, when placed under the control of a cell-specific promoter, Cre-mediated deletion is both site and time controlled. This strategy has allowed inducible gene knockout in neurons (Erdmann et al., 2007, Weber et al., 2015), glia (Mori et al., 2006, Leone et al., 2003) as well as other somatic tissues (Sohal et al., 2001). Generally, efficient recombination requires a treatment of 1-2mg/day during at least 5 to 15 days (Brake et al., 2004, Weber et al., 2001, Imayoshi et al., 2008, Lau et al., 2011). Unfortunately, systemic tamoxifen administration has low brain penetration, and was shown to cause cell toxicity as well as deficient learning behavior (Higashi et al., 2009, Vogt et al., 2008). Tamoxifen delivery needs therefore to be optimized for efficient gene excision with minimal toxicity.

In the present study, we aimed to delete *Gpr88* throughout all brain regions of the adult mice using the CreER^{T2} system. Three novel neuron-specific inducible transgenic Cre-driver lines had been constructed at the mouse clinic institute, and had not been characterized as yet. We therefore bred floxed *Gpr88* mice with each line, in order to induce the gene KO in adult animals, and test whether behavioral deficits described in *Gpr88*^{-/-} (Logue et al., 2009, Quintana A, 2012, Meirsmann et al., 2015) are also detected when *Gpr88* is deleted during adulthood.

Materials and Methods

Generation of *Gpr88*-Cre-ERT₂ mice

Mice expressing the Cre-ER^{T2} under the control of brain specific SRY-box containing gene 2 (*Sox2*), Neurofilament light polypeptide (*NeFL*) and Enolase2 (*Eno 2*) promoters were obtained from the Institut Clinique de la souris (Illkirch, France; <http://www.ics-mci.fr/mousecre/index>). These mice resulting from BAC transgenesis were crossed with *Gpr88*-floxed mice to obtain *Gpr88*-*NeFL*-Cre-ER^{T2}, *Gpr88*-*Sox2*-Cre-ER^{T2} and *Gpr88*-*Eno2*-Cre-ER^{T2} (Figure 1).

Subjects and Treatment.

Male and female mice aged 8 weeks in the beginning of the treatment were bred in-house. Animals were group housed and maintained on a 12hr light/dark cycle (lights on at 7:00 AM) at controlled temperature (22±1°C). Food and water were available *ad libitum* throughout all experiments except during the novelty suppressed feeding test. All experiments were analyzed blind to genotypes. All experimental procedures were reviewed and approved by the local ethic comity.

Tamoxifen was dissolved in 10 % Ethanol absolute and 90% Sunflower oil. Control animals received vehicle solution (10% Ethanol, 90% sunflower oil). Protocols of tamoxifen administration used in each mouse line are detailed in Table 1. Given tamoxifen toxicity, treatments were tested as to minimize total tamoxifen administration. For each mouse line, PCR on genomic DNA was performed in order to verify treatment and site-specific *Gpr88* excision. Then, if the latter was confirmed, *Gpr88* expression and receptor activation were quantified 3 weeks after the last injection by qPCR and [S35]-GTPγS respectively.

Genomic PCR

All PCRs were run one week after the last injection to allow complete genomic excision. Tissue from tail, lungs and striatum were collected and PCR was run in order to test for deletion of *Gpr88* exon 2. We used one forward primer (5' GTC CTAGGT GTG GAT ATG ACC TTA G 3') and one reverse primer (5' GTT TGT TTC CTC ACT GGC TGA GAG TC 3') to detect the excision (216pb). Two to three mice per treatment were used for each PCR analysis.

Real-time quantitative PCR analysis

All RT-qPCR were run 3 weeks after the last injection of tamoxifen to allow RNA downregulation after *Gpr88* excision. Brains were removed and striata were manually dissected. Tissues were immediately frozen on dry ice and kept at -80°C until use. RNA was extracted and purified using the MIRNeasy mini-kit (Qiagen, Courtaboeuf, France). cDNA was synthesized using the first-strand Superscript II kit (Invitrogen®, Life Technologies, Saint Thomas, France). qRT-PCR was performed in triplicates on a LightCycler 480 Real-Time PCR (Roche, Mannheim, Germany) using iQ-SYBR Green supermix (Bio-Rad, Marnes-la-Coquette, France) kit with 0.25µl cDNA in a 12.5 µl final volume. Gene-specific primers were acquired from sigma Aldrich (Saint-Quentin Fallavier, France). Relative expression ratios were normalized to the level of HPRT and the $2^{-\Delta\Delta Ct}$ method was applied to evaluate differential expression level. For PCR analysis two to five mice per treatment were used.

[³⁵S]-GTPγS binding assays

All functional assays were run 3 weeks after the last injection of tamoxifen to permit degradation of pre-existing GPR88. To perform GTPγS assays mice were sacrificed by cervical dislocation and the whole striatum was bilaterally removed, frozen in dry ice and stored at -80°C until use. All assays were performed on membrane preparations.

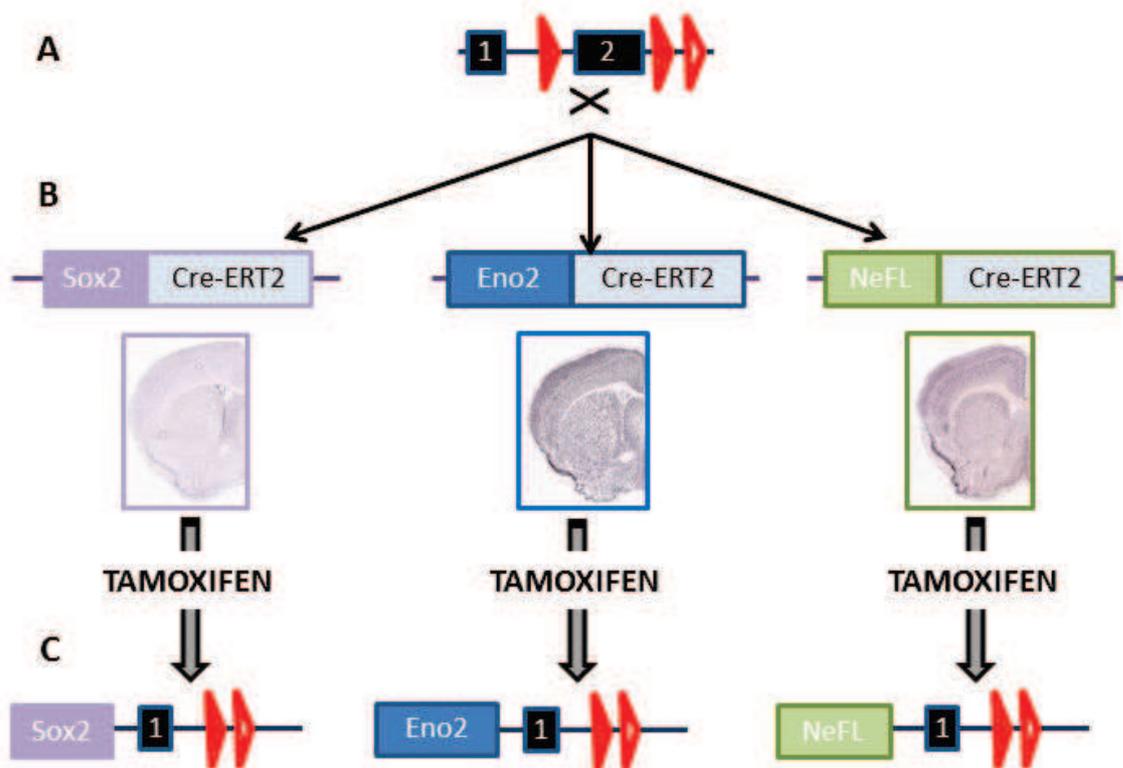


Figure 1: Schematic representation of *Gpr88* inducible mouse lines.

(A) Exon 2 of the *Gpr88* gene is flanked by LoxP sites in the *Gpr88*^{flx/flx} mouse line. (B) These mice were bred with 3 mouse lines expressing the Cre-ERT2 under the control of 3 strong brain specific promoters (Allen brain atlas <http://mouse.brain-map.org/>). (C) When Cre+ offspring is treated with tamoxifen, the Cre enzyme translocates to the nucleus and deletes the flanked exon 2.

Table 1: Summary of tamoxifen treatment administered to *Gpr88* inducible mouse lines.

i.p.: intraperitoneal; *p.o.*: per os.

Protocol	delivery	Quantity /injection	Volume /injection	Treatments /day	Total /day	nb of days	Gpr88-Sox 2-Cre-ERT2	Gpr88-Eno2-Cre-ERT2	Gpr88-NeFL-Cre-ERT2
Protocol 1	<i>i.p.</i>	1 mg	100 µL	2	2mg	5	x	x	
Protocol 2	<i>i.p.</i>	1 mg	100 µL	2	2mg	15		x	x
Protocol 3	<i>p.o.</i>	10mg	300µL	1	10mg	2			x
Protocol 4	<i>p.o.</i>	5 mg	150µL	2	10mg	5			x
Protocol 5*	<i>i.p.</i>	5mg	150µL	1	5mg	5			x
Protocol 6*	<i>p.o.</i>	5mg	150µL	1	5mg	5			x

* These protocols lead to tamoxifen-specific mortality and were immediately abandoned (not shown)

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), aliquoted and kept at - 80°C until further use. For each [35S]GTPγS binding assay 2µg of protein was used per well. Samples were incubated with and without ligand, 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. B_{max} and K_d values were calculated using

Microsoft Excel Software. Non-specific binding was defined as binding in the presence of 10 µM GTPγS and Basal binding was defined as binding in the absence of agonist.

Behavioral experiments

To assess behavioral responses mice expressing (Cre+) or not (Cre-) the CreER^{T2} were used. For each genotype, one group was treated with vehicle (oil) while the other group was treated with tamoxifen. Mice underwent subsequently elevated plus maze (Day1), habituation to the open field for 10 min (day 2), social interaction (day 3) stereotypies recording (day 4), marble burying (day 5), novelty suppressed feeding (day 8 to 10), accelerating rotarod (day 15-19), grip and string tests (day 22).

Elevated plus-maze (EPM). The EPM was a plus-shaped maze elevated 52 cm from base, with black Plexiglas floor, consisting of two open and two closed arms (37 × 6 cm each) connected by a central platform (6 × 6 cm). The experiments were conducted under low-intensity light (15 lux). Movement and location of the mice were analyzed by an automated tracking system (Videotrack; View Point, Lyon, France). Each mouse was placed on the central platform facing a closed arm and observed for 5 min. Anxiety-like behavior was assessed by measures of the time spent and number of entries in closed and open arms of the maze, and related time and activity ratios (time spent or number of entries in open arms/total time spent or number of entries in arms). Risk-taking behavior was

evaluated by the percentage of time spent in the distal part of the open arms (time spent in the last 1/3 of the open arm/total time in arms) and the number and duration of head dips (total number of head dips and head dips from the distal part of the open arms).

Social interaction test. Mice were placed in the open field under deem light condition (<10 Lux) with a naïve wild time control animal with the same genetic background. The session lasted 10min and was recorded by a videotrack system (Videotrack; View Point, Lyon, France). In total four behavioral parameters were analyzed: nose and paw contact, following and grooming.

Spontaneous motor stereotypies. For spontaneous motor stereotypies scoring, mice were gently placed in a single house cage filled with approximately 2 cm of animal bedding and observed for 10 min in a deem light environment. The following parameters were directly scored: number of rearings, burying, grooming, circling and head shakes episodes. The total duration of the burying episodes was also measured.

Marble burying test. The marble burying test was carried out using 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. The mice were left in the cage for 15 minutes and the number of unburied marbles was counted. The total number of marbles buried was considered as an index of anxiety levels.

Novelty suppressed feeding test. All mice were subjected to fasting for 24 h before the beginning of the test but water was provided *ad libitum*. 30min before the beginning of the test, mice were isolated to increase anxiogenic conditions.

During the test, 3 food pellets (regular chow) were placed on a square piece of white filter paper positioned in the center of an open field (50 × 50 cm) that was filled with approximately 2 cm of animal bedding. Each mouse was placed in a corner of the open field. The latency to the first bite of the food pellet was recorded (defined as the mouse sitting on its haunches and biting the pellet with the use of its forepaws). The cut off time was set at 15 min. After the test was over the animal was placed in his homecage and left alone for 5min. The food intake during this period was scored.

Accelerating rotating rod test (“easy”). Coordination, equilibrium, and motor skill acquisition were tested using an accelerated rotating rod (Panlab, Barcelona, Spain) test. Briefly, mice were placed on the rod covered with insulation tubing in three trials every day for a period of 4 days. The rod accelerated from 4 to 40 rpm in 5 min and remained at maximum speed for the next 5 min. Animals were scored for their latency to fall (in seconds) for each trial and rested a minimum of 1min between trials to avoid fatigue and exhaustion.

Muscular strength measurement: Grip test. Mice placed on the grid of a dynamometer (BioSeb, Chaville, France) and were pulled by their tails in the opposite direction. The maximal strength exerted by the mouse before losing grip was recorded. The mean of three measurements allowing 30 sec of recovery time between each of them was calculated.

String test. The apparatus was a length of string (50cm) pulled taut between two vertical supports and elevated 40 cm surface. The mouse was placed on the string on his forepaw at a point midway between the supports and the latency for the animal to place his hindpaws on the string was rated.

***In situ* mRNA hybridization**

In situ hybridization using nonradioactive Dig-dUTP-labeled antisense GPR88 riboprobes were performed on cryostat sections (25 µm) of Gpr88-Eno2-Cre-ERT₂ treated and not treated with 1mg of tamoxifen twice a day during 15 days (protocol 2). Mice were sacrificed 4 weeks after tamoxifen treatment. The GPR88 riboprobe (717 bp) encompassed the entire exon 2 and the 50 150-bp portion of exon 3.

The sections were fixed in 4 % PFA (in PBS, pH 7.4) for 10 min at room temperature, washed in PBS three times for 3 min each, acetylated (acetic anhydride 1%, triethanolamine 2%, Tween 3%, 1M HCl 5%) for 10min at room temperature and then washed in PBS three times for 3 min each. The slides were dehydrated in 1 min successive baths : 60%, 75%, 95%, 100% EtOH, 100% CHCl₃, 95%, 100% EtOH before an overnight hybridization at 70 °C with 1µL/150 µL labeled antisense probe in hybridization buffer (50 % deionized formamide , 20× SSC 2%, 50% Dextran,50X Denhardt, yeast tRNA 1%).

After two post-hybridization washes with 2× SSC Tween 20 (10 %) (1h at 70°C and 5 min at Room temperature) the sections were washed in PBS three times for 10 min each. The sections were then treated for 1 h with blocking solution [10 % Normal Goat serum (1M Tris pH7.5, 5M NaCl)] and incubated with Antidig Ab (1:2500 in blocking solution) for 2 hours. The sections were washed three times for 10 min each with 1M Tris pH7.5, 5M NaCl solution where they were stored at 4°C. The next day all section were washed in 5M NaCl, 1M MgCl₂, 1M Tris pH9.5, Tween 20 (10%) before visualized in dark with bromochloro-indolyl phosphate and nitrobluetetrazolium (Amresco). After they developed sufficient color, the reaction was stopped by a wather wash. The sections were stored at Room Temperature until ordered, and mounted on to pertex coated slides.

Results

Molecular analyses are shown in Figure 2 and behavioral analyses in Figure 3.

I. Gpr88-Sox2-Cre-ER^{T2}

A. Protocol 1: 2mg/day-5days *i.p.*

1) Gpr88 excision in Sox2-Cre-ER^{T2} mice is not treatment-specific

PCR results show, for both treatments, the presence of a 216 bp band (Figure 2A), indicating that exon 2 of the *Gpr88* gene was excised in both tamoxifen and vehicle groups. Experiments in this mouse line were therefore stopped.

II. Gpr88-Eno2-Cre-ER^{T2}

A. Protocol 1: 2mg/day-5days *i.p.*

1) Protocol 1 leads to treatment-specific *Gpr88* excision in Eno2-Cre-ER^{T2}

Genomic PCR results indicate the successful tamoxifen-specific *Gpr88* excision (Figure 2B). Intriguingly, *Gpr88* gene excision was also detected in lung tissue (data not shown). Since *Gpr88* expression is restricted to the brain, we proceeded to further analysis at protein level.

2) GPR88 receptor signaling is not altered by tamoxifen treatment in protocol 1

Agonist induced [35S]-GTPγS results (Figure 2C) indicate no difference in agonist-induced receptor activation between mice treated with tamoxifen or vehicle.

B. Protocol 2: 2mg/day-15days *i.p.*

1) Mice treated with protocol 2 show decreased *Gpr88* mRNA and receptor activation.

Three weeks after the last tamoxifen injection, mice were sacrificed and striata were removed for both RNA extraction and membrane preparation. Results indicated that this treatment leads to reduced mRNA levels by RT-qPCR ($t(9) = 2,25$, $p = 0,051$) (Figure 2D), although *in situ* hybridization analysis did not allow the detection of reduced mRNA signal on striatal slices of tamoxifen-treated animals (Figure 2E). We further tested GPR88 signaling and found a detectable reduction of the agonist-induced [35S]-GTPγS signal, which was variable across individuals (with average decrease of $\pm 24\%$, see Figure 2F). We therefore generated animal cohorts treated with this protocol for subsequent behavioral studies.

2) Deletion of *Gpr88* by protocol 2 does not produce behavioral deficits

The overall behavioral analysis, including rotarod, revealed no difference between mice treated with tamoxifen or vehicle (Figure 3). Experiments in this mouse line were therefore stopped.

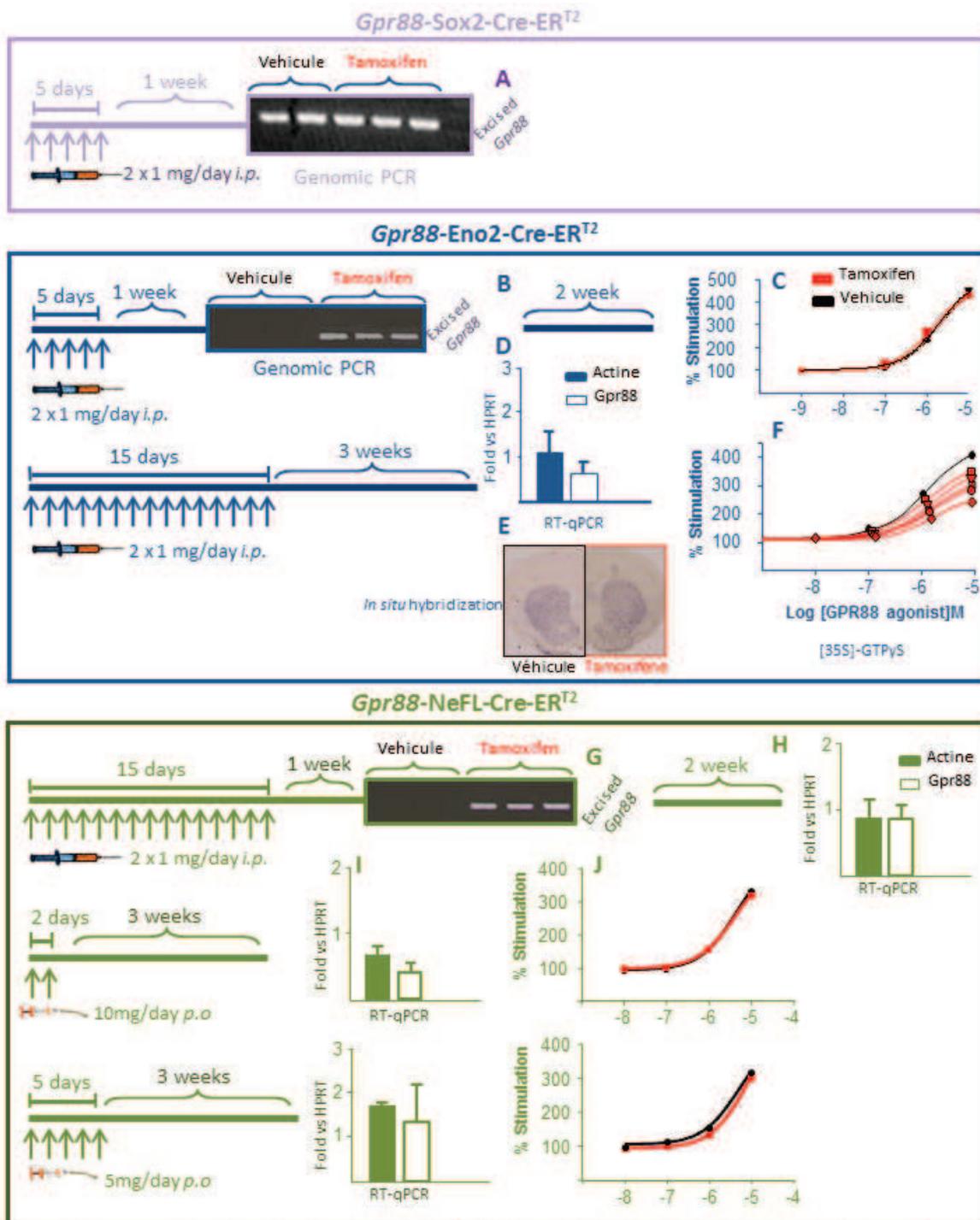


Figure 2: Summary of protocols and mouse lines tested for tamoxifen-induced *Gpr88* gene excision (genomic PCR) and reduction of GPR88 signaling (GTP γ S assay) in the striatum of adult mice. Protocol 1 in *Gpr88-Sox2-Cre-ER^{T2}* mice show tamoxifen-independent excision of *Gpr88* (A). In *Gpr88-Eno2-Cre-ER^{T2}* protocol 1 led to tamoxifen-dependent *Gpr88* gene excision (B) but no decrease in receptor signaling (C). On the contrary, protocol 2 successfully led to a partial decrease of GPR88 signaling (F) and small decrease in mRNA levels (D) although not detectable in *In situ* hybridization imaging (E). Protocol 2 also led to successful tamoxifen-dependent excision of *Gpr88* in *Gpr88-NeFL-Cre-ER^{T2}* (G) but the mRNA level remained unchanged (GTP γ S was not tested). In the case of *per os* tamoxifen administration (protocol 3 & 4) *Gpr88* transcript levels (H) and receptor activity (I) were unaltered

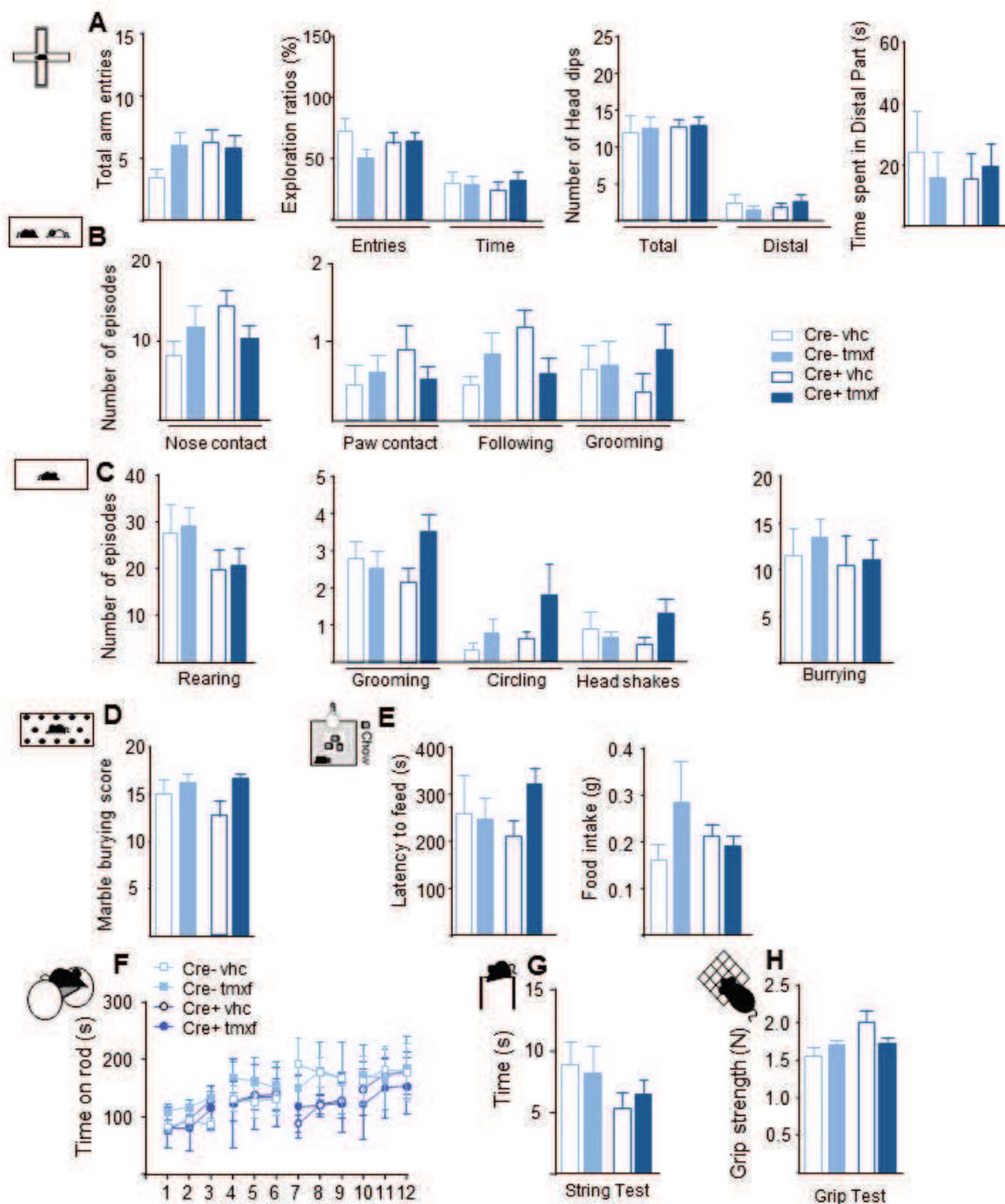


Figure 3: No behavioral consequences of tamoxifen-induced *Gpr88* KO in adult mice. The *Gpr88-Eno2-Cre-ERT²* line that responded to tamoxifen was tested in a five-test battery. The four groups of mice (*Cre-vhc* n=11, *Cre-tmx* n=13, *Cre+vhc* n=11; *Cre+tmx* n=17, treatment protocol 2) were tested for (A) exploration and anxiety levels in the elevated plus maze, and no difference was found between genotypes or treatments. (B) In the social interaction test, four behavioral parameters were evaluated, and no genotype or treatment effect was found. (C) No difference were found when scoring motor stereotypies. Testing animals in other anxiety tests, including (D) marble burying or (E) novelty suppressed feeding, indicates no effect of tamoxifen treatment or genotype. Finally, motor coordination and muscular strength were assessed in the (F) rotarod, (G) string and (H) grip tests revealing no significant difference across the groups (Two-Way ANOVA).

III. Gpr88-NeFL-Cre-ER^{T2}

A. Protocol 2: 2mg/day-15days i.p.

1) Protocol 2 leads to treatment- and site-specific Gpr88 excision in NeFL-Cre-ER^{T2}

PCR results depicted in [Figure 2G](#) show treatment specific excision of *Gpr88* in the striatum. Also, the deletion was tissue specific not affecting tissue from lungs (data not shown).

2) Gpr88 expression is not altered by tamoxifen treatment in protocol 2

Protocol 2 had no effect on *Gpr88* mRNA levels ([Figure 2H](#)).

B. Protocols 3 & 4: 10mg/day_2days & 5mg/day_5 days p.o

1) Gpr88 expression and receptor activation were not modified by tamoxifen treatment

None of the *per os* protocols tested in Gpr88-NeFL-Cre-ER^{T2} enabled *Gpr88* mRNA ([Figure 2I](#)) or protein decrease ([Figure 2J](#))

Discussion

In the present chapter we presented several attempts to induce the knock-down of *Gpr88* in the adult mouse brain, and none of them was successful. There are many possible explanations for failure in these experiments, and we will discuss some of them.

Sox2 is a transcription factor essential for the maintenance of pluripotency of undifferentiated embryonic stem cells. Sox2 signaling is crucial for development, notably the nervous system (Arnold et al., 2011) and was chosen to ensure excision in adult neurons although

it has been detected in placodal epithelium, gut endoderm and developing eye (Brazel et al., 2005, Wood and Episkopou, 1999). Despite its strong expression during embryogenesis, we hoped that the CreER^{T2} fusion would remain silent during development, and be induced under tamoxifen treatment at the adult stage only. It seems that, in this case, Cre expression was leaky, as is sometimes the case with strong promoters (Garcia-Otin and Guillou, 2006)

Enolase 2 (or neuron-specific enolase_ *NSE*) is a glycolytic enzyme found in most neurons and neuroendocrine cells (Fors-Petter et al., 1990, Rosenstein, 1993), and the Enolase 2 (*Eno2*) promoter was chosen on this basis. Using *Eno2*-Cre transgenic mouse lines, Cinato and collaborators (2001) showed that the Cre was highly expressed in the cerebellum and cortex with lower expression in other areas of the brain. Also, using a LacZ reporter mouse line, these authors demonstrate Cre activity (although low) in the caudate-putamen under the control of this promoter (Cinato et al., 2001). In accordance, our experiments show successful, although very partial, decrease of GPR88 signaling activity in tamoxifen-treated *Gpr88*-*Eno2*-CreER^{T2}. The limited efficacy of tamoxifen across individuals, even upon the strongest treatment protocol, may be due to low strength of this promoter in neurons of the striatum, either because of the promoter itself or due to unfavorable integration site of the transgene in this particular line. Alternatively, GPR88 mRNA and protein turnover may be unusually slow, and analysis at later time points may reveal a more complete *Gpr88* KO.

The *Gpr88* KO was partial but nevertheless detectable. We therefore underwent behavioral testing. When using AAV-Cre injection in the striatum of adult *Gpr88* floxed mice (see section 2 of the present chapter) we found that partial knockdown of *Gpr88* in the striatum was sufficient to impair motor coordination. As such, it was expected that tamoxifen treatment in *Gpr88*-*Eno2*-Cre-ER^{T2+} mice would, at the very least, lead to decreased latency to fall from the rotarod. Surprisingly, the overall behavioral analysis, including rotarod, reveals no difference between mice treated with tamoxifen or vehicle. One explanation could be that all of the tested behaviors have a developmental origin. This conclusion, however, would contradict our data from virally-mediated *Gpr88* knockdown, as well as our own observation that acute treatment with a selective GPR88 agonist enhances animal performance on the rotarod (unpublished). The most likely explanation is that *Gpr88* KO in the tamoxifen treated-GPR88-*Eno2*-Cre-ER^{T2} line is not sufficient to alter any

behavior. Interestingly a recent paper, published after these experiments were stopped, reported another *Eno2*-CreER^{T2} driver line, with Cre expression restricted to the cerebellum and dentate gyrus of the hippocampus, and no detectable signal in the striatum or other *Gpr88*-expressing areas (Pohlkamp et al., 2014). Although our *Eno2*-CreER^{T2} line is a different transgenic line, this study strengthens the notion that *Eno2* promoter activity is weak at the level of the striatum. The characterization of our line with reporter mice would be one way to confirm this hypothesis.

Neurofilament light polypeptide (NeFL) assembles along with neurofilament heavy and medium polypeptide into neurofilaments, the main structure of the cytoskeleton in mature neurons (Calmon et al., 2015). This promoter was selected on the basis of high expression of NeFL in adult neurons. However, despite successful genomic deletion of *Gpr88* in the striatum of tamoxifen-treated mice, *Gpr88* expression levels and receptor signaling remained unchanged, and this was the case for all three protocols tested. It is possible that the genomic site of transgene integration did not allow efficient CreER^{T2} expression. In fact, we tried more aggressive protocols leading to high lethality (Table 1) and *In situ* hybridization analysis of *Gpr88* mRNA in brain slices showed a detectable qualitative decrease of *Gpr88* expression (data not shown). However, these protocols were abandoned due to ethical issues.

In conclusion, attempts to characterize inducible neuronal Cre-driver lines, which would allow time-controlled *Gpr88* KO, were not continued. Obviously, the screening of more founder lines, and improvement of tamoxifen efficacy in the brain are required to pursue this goal. Should progress be done in the future, these lines may prove invaluable tools to tackle gene function in the developing animal, and the genetic bases of neurodevelopmental disorders.

References

- ARNOLD, K., SARKAR, A., YRAM, M. A., POLO, J. M., BRONSON, R., SENGUPTA, S., SEANDEL, M., GEIJSEN, N. & HOCHEDLINGER, K. 2011. Sox2(+) adult stem and progenitor cells are important for tissue regeneration and survival of mice. *Cell Stem Cell*, 9, 317-29.
- BRAKE, R. L., SIMMONS, P. J. & BEGLEY, C. G. 2004. Cross-contamination with tamoxifen induces transgene expression in non-exposed inducible transgenic mice. *Genet Mol Res*, 3, 456-62.
- BRAZEL, C. Y., LIMKE, T. L., OSBORNE, J. K., MIURA, T., CAI, J., PEVNY, L. & RAO, M. S. 2005. Sox2 expression defines a heterogeneous population of neurosphere-forming cells in the adult murine brain. *Aging Cell*, 4, 197-207.
- CALMON, M. F., JESCHKE, J., ZHANG, W., DHIR, M., SIEBENKAS, C., HERRERA, A., TSAI, H. C., O'HAGAN, H. M., PAPPOU, E. P., HOOKER, C. M., FU, T., SCHUEBEL, K. E., GABRIELSON, E., RAHAL, P., HERMAN, J. G., BAYLIN, S. B. & AHUJA, N. 2015. Epigenetic silencing of neurofilament genes promotes an aggressive phenotype in breast cancer. *Epigenetics*, 10, 622-32.
- CINATO, E., MIROTSOU, M. & SABLITZKY, F. 2001. Cre-mediated transgene activation in the developing and adult mouse brain. *Genesis*, 31, 118-25.
- DEL ZOMPO, M., DELEUZE, J. F., CHILLOTTI, C., COUSIN, E., NIEHAUS, D., EBSTEIN, R. P., ARDAU, R., MACE, S., WARNICH, L., MUJAHED, M., SEVERINO, G., DIB, C., JORDAAN, E., MURAD, I., SOUBIGOU, S., KOEN, L., BANNOURA, I., ROCHER, C., LAURENT, C., DEROCK, M., FAUCON BIGUET, N., MALLET, J. & MELONI, R. 2014. Association study in three different populations between the GPR88 gene and major psychoses. *Mol Genet Genomic Med*, 2, 152-9.
- ERDMANN, G., SCHUTZ, G. & BERGER, S. 2007. Inducible gene inactivation in neurons of the adult mouse forebrain. *BMC Neurosci*, 8, 63.
- FEIL, R., BROCARD, J., MASCREZ, B., LEMEURE, M., METZGER, D. & CHAMBON, P. 1996. Ligand-activated site-specific recombination in mice. *Proc Natl Acad Sci U S A*, 93, 10887-90.
- FORSS-PETTER, S., DANIELSON, P. E., CATSICAS, S., BATTENBERG, E., PRICE, J., NERENBERG, M. & SUTCLIFFE, J. G. 1990. Transgenic mice expressing beta-galactosidase in mature neurons under neuron-specific enolase promoter control. *Neuron*, 5, 187-97.
- GARCIA-OTIN, A. L. & GUILLOU, F. 2006. Mammalian genome targeting using site-specific recombinases. *Front Biosci*, 11, 1108-36.
- GAVERIAUX-RUFF, C. & KIEFFER, B. L. 2007. Conditional gene targeting in the mouse nervous system: Insights into brain function and diseases. *Pharmacol Ther*, 113, 619-34.
- HIGASHI, A. Y., IKAWA, T., MURAMATSU, M., ECONOMIDES, A. N., NIWA, A., OKUDA, T., MURPHY, A. J., ROJAS, J., HEIKE, T., NAKAHATA, T., KAWAMOTO, H., KITA, T. & YANAGITA, M. 2009. Direct hematological toxicity and illegitimate chromosomal recombination caused by the systemic activation of CreERT2. *J Immunol*, 182, 5633-40.
- IMAYOSHI, I., SAKAMOTO, M., OHTSUKA, T., TAKAO, K., MIYAKAWA, T., YAMAGUCHI, M., MORI, K., IKEDA, T., ITOHARA, S. & KAGEYAMA, R. 2008. Roles of continuous neurogenesis in the structural and functional integrity of the adult forebrain. *Nat Neurosci*, 11, 1153-61.
- KUHN, R. & TORRES, R. M. 2002. Cre/loxP recombination system and gene targeting. *Methods Mol Biol*, 180, 175-204.
- LAU, J., MINETT, M. S., ZHAO, J., DENNEHY, U., WANG, F., WOOD, J. N. & BOGDANOV, Y. D. 2011. Temporal control of gene deletion in sensory ganglia using a tamoxifen-inducible Advillin-Cre-ERT2 recombinase mouse. *Mol Pain*, 7, 100.
- LEONE, D. P., GENOUD, S., ATANASOSKI, S., GRAUSENBURGER, R., BERGER, P., METZGER, D., MACKLIN, W. B., CHAMBON, P. & SUTER, U. 2003. Tamoxifen-inducible glia-specific Cre mice for somatic mutagenesis in oligodendrocytes and Schwann cells. *Mol Cell Neurosci*, 22, 430-40.
- LEWIS, D. A. & LEVITT, P. 2002. Schizophrenia as a disorder of neurodevelopment. *Annu Rev Neurosci*, 25, 409-32.

- LOGUE, S. F., GRAUER, S. M., PAULSEN, J., GRAF, R., TAYLOR, N., SUNG, M. A., ZHANG, L., HUGHES, Z., PULITO, V. L., LIU, F., ROSENZWEIG-LIPSON, S., BRANDON, N. J., MARQUIS, K. L., BATES, B. & PAUSCH, M. 2009. The orphan GPCR, GPR88, modulates function of the striatal dopamine system: a possible therapeutic target for psychiatric disorders? *Mol Cell Neurosci*, 42, 438-47.
- MASSART R, G. J., MIGNON V, SOKOLOFF P, DIAZ J 2009. Striatal GPR88 expression is confined to the whole projection neuron population and is regulated by dopaminergic and glutamatergic afferents. *Eur J Neurosci*, 397-414.
- MEIRSMAN, A. C., LE MERRER, J., PELLISSIER, L. P., DIAZ, J., CLESSE, D., KIEFFER, B. L. & BECKER, J. A. 2015. Mice Lacking GPR88 Show Motor Deficit, Improved Spatial Learning, and Low Anxiety Reversed by Delta Opioid Antagonist. *Biol Psychiatry*.
- METZGER, D. & CHAMBON, P. 2001. Site- and time-specific gene targeting in the mouse. *Methods*, 24, 71-80.
- MORI, T., TANAKA, K., BUFFO, A., WURST, W., KUHN, R. & GOTZ, M. 2006. Inducible gene deletion in astroglia and radial glia--a valuable tool for functional and lineage analysis. *Glia*, 54, 21-34.
- POHLKAMP, T., STELLER, L., MAY, P., GUNTHER, T., SCHULE, R., FROTSCHER, M., HERZ, J. & BOCK, H. H. 2014. Generation and characterization of an Nse-CreERT2 transgenic line suitable for inducible gene manipulation in cerebellar granule cells. *PLoS One*, 9, e100384.
- QUINTANA A, S. E., WANG W, STOREY GP, GULER AD, WANAT MJ, ET AL. 2012. Lack of GPR88 enhances medium spiny neuron activity and alters motor- and cue-dependent behaviors. *Nat Neurosci*, 1547-1555.
- RAPOPORT, J. L., ADDINGTON, A. M., FRANGOU, S. & PSYCH, M. R. 2005. The neurodevelopmental model of schizophrenia: update 2005. *Mol Psychiatry*, 10, 434-49.
- RONALD, A., PENNELL, C. E. & WHITEHOUSE, A. J. 2010. Prenatal Maternal Stress Associated with ADHD and Autistic Traits in early Childhood. *Front Psychol*, 1, 223.
- ROSENSTEIN, J. M. 1993. Developmental expression of neuron-specific enolase immunoreactivity and cytochrome oxidase activity in neocortical transplants. *Exp Neurol*, 124, 208-18.
- SOHAL, D. S., NGHIEM, M., CRACKOWER, M. A., WITT, S. A., KIMBALL, T. R., TYMITZ, K. M., PENNINGER, J. M. & MOKKENTIN, J. D. 2001. Temporally regulated and tissue-specific gene manipulations in the adult and embryonic heart using a tamoxifen-inducible Cre protein. *Circ Res*, 89, 20-5.
- VAN WAES V, T. K., STEINER H 2011. GPR88 - a putative signaling molecule predominantly expressed in the striatum: Cellular localization and developmental regulation. *Basal Ganglia*, 83-89.
- VOGT, M. A., CHOURBAJI, S., BRANDWEIN, C., DORMANN, C., SPRENGEL, R. & GASS, P. 2008. Suitability of tamoxifen-induced mutagenesis for behavioral phenotyping. *Exp Neurol*, 211, 25-33.
- WEBER, P., METZGER, D. & CHAMBON, P. 2001. Temporally controlled targeted somatic mutagenesis in the mouse brain. *Eur J Neurosci*, 14, 1777-83.
- WEBER, T., VOGT, M. A., GARTSIDE, S. E., BERGER, S. M., LUJAN, R., LAU, T., HERRMANN, E., SPRENGEL, R., BARTSCH, D. & GASS, P. 2015. Adult AMPA GLUA1 receptor subunit loss in 5-HT neurons results in a specific anxiety-phenotype with evidence for dysregulation of 5-HT neuronal activity. *Neuropsychopharmacology*, 40, 1471-84.
- WOOD, H. B. & EPISKOPOU, V. 1999. Comparative expression of the mouse Sox1, Sox2 and Sox3 genes from pre-gastrulation to early somite stages. *Mech Dev*, 86, 197-201.

Chapter 3: Cross-modal PPI deficit in mice lacking GPR88 in whole brain but not in striatopallidal neurons

(in preparation)

Cross-modal PPI deficit in mice lacking GPR88 in whole brain but not in striatopallidal neurons

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Abstract

Background. GPR88 is a G protein-coupled receptor (GPCR) highly expressed in medium spiny projection neurons of the striatum, a brain region implicated in neuropsychiatric disorders characterized by deficient sensorimotor gating. In previous reports, mice lacking *Gpr88* were shown to present several behavioral abnormalities relevant to schizophrenia, including acoustic prepulse inhibition (PPI) deficits. In the present study we investigated whether the sensorimotor gating deficit observed in *Gpr88*^{-/-} animals extend to visual modalities, and further examined the role of striatopallidal GPR88 in the regulation of sensorimotor gating.

Methods. We tested both total *Gpr88* knockout mice and conditional *A_{2A}-Gpr88* knockout mice in striatopallidal neurons for performance in acoustic and visual PPI. Also, we evaluated general inhibitory process by testing GAP detection in the two mouse lines.

Results. We show that the full deletion of *Gpr88* impairs acoustic as well as visual sensorimotor gating. Also, we show that this deficit is not the result of a general inhibition deficit. Furthermore, we demonstrate that deletion of *Gpr88* in striatopallidal projection neurons is not sufficient to disrupt neither acoustic nor visual PPI, suggesting that these receptors are not implicated in the regulation of PPI.

Conclusion. Our results definitely implicate GPR88 in the pathophysiology of sensorimotor gating disorders such as schizophrenia. Also, our data suggest that GPR88 in striatopallidal neurons co-expressing the dopamine 2 receptor do not regulate this process, suggesting a possible role of striatonigral GPR88 in sensorimotor gating.

Keywords: Cross-modal PPI, schizophrenia, striatopallidal GPR88, GAP detection.

Introduction

GPR88 is an orphan G-protein coupled receptor (GPCR) highly enriched in the dorsal (caudate-putamen, CPU) and ventral striatum (nucleus accumbens, Nac and the olfactory tubercle) (Logue et al., 2009, Massart R, 2009, Quintana A, 2012). The distinctive pattern of *Gpr88* expression has generated considerable excitement regarding the physiological role of this receptor and its potential implication in brain diseases. Human genetic studies reported a positive association between *Gpr88* and schizophrenia as well as bipolar disorder, thus pointing to this receptor's gene as a strong candidate for these psychiatric conditions (Del Zompo et al., 2014). Accordingly, studies using full knockout (KO) mice for GPR88 showed that the lack of receptor produces behavioral phenotypes relevant to schizophrenia, such as sensorimotor gating deficits, psychomotor agitation, enhanced behavioral responses to dopamine psychostimulants (apomorphine and amphetamine) and impaired cue-based learning (Quintana A, 2012, Logue et al., 2009). The sensorimotor gating deficits as well as increased sensitivity to apomorphine-induced stereotypies could be prevented by pretreatment with the typical and the atypical neuroleptics, haloperidol and risperidone, respectively (Logue et al., 2009). More recently, Ingallinesi et al. (2014) showed that local ablation of GPR88 into the nucleus accumbens produces no behavioral alterations in normal rats, but could attenuate the schizophrenia-related phenotypes (the hyperlocomotion in response to amphetamine and the social novelty discrimination deficit) induced by neonatal administration of PCP (Ingallinesi et al., 2014). Altogether these studies suggest that GPR88 may play role in the pathophysiology of schizophrenia, but further studies are needed to substantiate GPR88 has a target for this complex disorder, and understand the underlying circuit mechanisms.

Prepulse inhibition of acoustic startle reflex (PPI) is considered one of the few schizophrenia-related behavioral measure that has been modeled in animal research. Acoustic PPI refers to the attenuation of a startle reflex response to a loud acoustic startling stimulus (pulse) when it is preceded shortly by a weak stimulus. PPI is a time-linked phenomenon that reflects a transient activation of a 'protective gate' triggered by the detection of the prepulse allowing the processing of the prepulse to occur without disruption by the succeeding pulse and thus prevent the organism for information overload (Geyer et al., 2002). PPI impairments have been reported in patients with schizophrenia and in several other neuropsychiatric disorders linked to dysfunction of the cortico-striato-pallido-thalamo circuitry, such as Tourette's syndrome, Huntington's disease and attention

deficit/hyperactivity disorder. (Powell et al., 2012, Swerdlow et al., 2000, Geyer, 2006, Geyer, 2008). Previous studies showed impaired sensorimotor gating in *Gpr88*^{-/-} mice (Logue et al., 2009), but data relied solely on the use acoustic PPI paradigms. PPI is a multimodal phenomenon where the prepulse and the startling stimulus can be presented in either the same or different sensory modalities (Swerdlow et al., 2000). As demonstrated by numerous studies, the neural substrates subserving PPI varies greatly with stimuli modality (Swerdlow et al., 2001, Ces et al., 2012). Also, the PPI deficits reported in schizophrenia patients are evident across multiple sensory modalities (Braff et al., 2001). Here we tested whether the acoustic prepulse inhibition deficit observed in *Gpr88*^{-/-} animals extends to visual PPI, and therefore generalizes across modalities. In addition, we tested KO animals in a GAP detection paradigm, involving a brief interruption of continuous background noise prior to presentation of the startling pulse, to examine whether the *Gpr88* KO produces a more general inhibitory dysfunction.

Gpr88 expression in the striatum is confined to medium spiny projection neurons (MSNs) commonly segregated into two subpopulations based on receptor expression and their projection targets. MSNs of the direct striatonigral pathway express dopamine D1 receptors (D₁R) and project to the substantia nigra *pars reticulata* and the internal segment of the globus pallidus, whereas MSNs of the indirect striatopallidal pathway contain dopamine D2 (D₂R) and adenosine A₂A receptors and innervate the substantia nigra *pars reticulata* via the external segment of the globus pallidus (GPe) and subthalamic nucleus (Calabresi et al., 2014, Quintana A, 2012, Logue et al., 2009, Massart R, 2009). These two populations of MSNs are known to differentially participate to striatal functions as they are coupled to output pathways with opposing properties. To tackle circuit mechanisms of GPR88 function, we recently developed conditional *Gpr88* knockout mice lacking the receptor selectively in D₂R-expressing neurons (*Gpr88*^{A2A-Cre}). We found that this mouse line recapitulates several phenotypes observed in the total *Gpr88* KO mice, indicating that GPR88 in striatopallidal neurons regulates these behaviors. These include increased locomotion and exploratory behavior (manuscript 2) as well as impaired motor coordination and altered locomotor response to dopaminergic agonists (*in preparation*). Here, we further investigated whether *Gpr88* deletion in D₂R-expressing MSNs also modulates sensorimotor gating processes.

Altogether, the goal of this study was to test total *Gpr88* KO mice (*Gpr88*^{-/-}) and *Gpr88*^{A2A-Cre} conditional KO mice, and their controls, in acoustic and visual PPIs, as well as in GAP detection. Our data indicate that *Gpr88*^{-/-} mice show a deficit in the two PPI modalities, but this is not observed for *Gpr88*^{A2A-Cre} KO. The two lines otherwise show normal GAP detection.

Materials and Methods

Animals

Mice (male and female) aged 10-15 weeks were bred in house and grouped house 3-5 animals per cage. Animals were maintained on a 12hr light/dark cycle at controlled temperature (22±1°C). Food and water were available ad libitum throughout all experiments. All experiments were approved by the local ethic committee (CREMEAS, 2003-10-08-[1]-58). For all mouse lines used construction as well as total or conditional knockout were described previously (Meirnsman et al., 2015)(manuscript 2).

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*^{A2A-Cre}. To verify the presence of Cre and Myosine (the latter as a positive control) the following primers were used: 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'.

Apparatus

Testing was carried in six 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, acoustic and visual stimuli levels were calibrated. Startle amplitude were obtained from the recording of 65 readings of 1ms beginning at the stimulus onset.

General procedure

Each mice cohort were submitted to PPI testing in the following order: acoustic PPI, visual PPI and GAP detection paradigms. Resting period of at least 2 days was used between two successive PPI testing.

Acoustic PPI procedure: The session starts 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 starts 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 detection 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).

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 * [(startle response for pulse-alone) - (startle response for prepulse + pulse)]/ startle response for pulse-alone. The magnitude of the acoustic startle response and the reactivity to the acoustic prepulses was calculated as the average response to all of the pulse-alone and prepulse-alone trials, respectively.

Statistics: For all PPI sessions data were analyzed by 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. Posthoc comparisons were carried out using Bonferroni's multiple comparisons test whenever the two-way ANOVAs indicated statistically significant main or interaction effects. For Basal startle reactivity and mean PPI percentage unpaired Student t test were used. All statistics were performed using GraphPad Prism 6 (GraphPad Software, Inc, USA) and the accepted level of significance was $p < 0.05$.

Results

Deficient acoustic prepulse inhibition in total *Gpr88*^{-/-} but not *Gpr88*^{A2A-Cre} KO mice

As depicted in [Figure 1 A](#) the prepulse inhibition percentage (PPI %) increased with increasing prepulse intensity for *Gpr88*^{-/-} as well as control littermates ($F_{(3,102)} = 258,7$; $p < 0,0001$) attaining higher levels when a prepulse of 90dB was presented (For detailed statistical analysis refer to

supplementary table 1). ANOVA indicates a significant genotype effect for *Gpr88*^{-/-} mice compared to wild type littermates ($F_{(1, 34)} = 11,96$; $p = 0,0015$). *Post hoc* comparison revealed significant differences for prepulse intensities of 80 and 85dB (80dB: $t(136) = 2,98$; $p < 0,05$; 85dB: $t(136) = 3,5$; $p < 0,01$) When comparing mean total PPI levels (Figure 1 B) there was also a significant difference between *Gpr88*^{-/-} animals and their control ($t(34) = 3,44$; $p = 0,0016$). This genotype differences were not due to an altered startle reactivity as *Gpr88*^{-/-} and control animals present the same startle reactivity ($t(34) = 0,42$; $p = 0,679$) (Table 1).

Similar to *Gpr88*^{-/-} mice, *Gpr88*^{A2A-Cre} and their control present increased levels of PPI % with increase in prepulse intensities ($F_{(3, 48)} = 135,6$; $p < 0,0001$) (Figure 1 C). However, deletion of *Gpr88* in striatopallidal neurons did not altered PPI levels in cKO mice for none of the prepulse intensities tested ($F_{(1, 16)} = 1,12$; $p = 0,305$). As depicted in Table 1 control and experimental animals from cKO mouse line present similar levels of basal startle reflex ($t(16) = 0,14$; $p = 0,89$).

Deficient visual prepulse inhibition in total *Gpr88*^{-/-} but not *Gpr88*^{A2A-Cre} KO mice

When tested for visual PPI, *Gpr88*^{-/-} mice and their control presented higher levels of inhibition for visual stimuli presented 20ms before the pulse, and decreasing thereafter (Figure 2A). ANOVA therefore revealed a significant prepulse-to-pulse interval effect ($F_{(7, 238)} = 18,17$; $p < 0,0001$) Two-way ANOVA also revealed a significant difference between *Gpr88*^{+/+} and *Gpr88*^{-/-} mice ($F_{(1, 34)} = 4,134$; $p = 0,0499$). Multiple comparison shown a significant difference for visual prepulse presented 20ms before the pulse ($t(272) = 3,67$; $p < 0,01$). When comparing global levels of PPI % regardless of the prepulse-to-pulse interval (Figure 2 B) Student *t* test also indicates a significant PPI % decrease for *Gpr88*^{-/-} mice ($t(34) = 2,048$; $p = 0,048$). Analysis of baseline startle reactivity (Table 1) show no significant difference between control and experimental animals ($t(34) = 0,14$; $p = 0,89$).

Conditional KO animals presented a visual PPI profile similar to full KO animals with higher PPI levels for visual stimuli presented 20ms before the pulse (Figure 2 C) ($F_{(7, 112)} = 9,47$; $p < 0,0001$). However, there was no genotype effect ($F_{(1, 16)} = 0,69$; $p = 0,419$) or global PPI % differences ($t(16)$

=0,84; $p=0,41$) for this mouse line (Figure 2 D). Similarly, *Gpr88*^{A2A-Cre} present similar startle reflex levels (Table 1) than their control animals ($t(16) = 0,64$; $p=0,53$).

Normal gap detection in total *Gpr88*^{-/-} and *Gpr88*^{A2A-Cre} KO mice

Figure 3A shows an increased GAP detection with increasing GAP duration for *Gpr88*^{-/-} mice and their control littermates ($F(7, 238) = 313,8$; $p < 0, 0001$). When compared to wild type animals, *Gpr88*^{-/-} mice present a systematic lower GAP detection for higher GAP duration (>20ms). Consistently, ANOVA show an interaction between GAP duration and genotype effect ($F(7, 238) = 2,111$; $p = 0,0432$). However, there was no genotype effect ($F(1, 34) = 0, 69$; $p = 0, 41$) nor significant difference in global GAP detection for *Gpr88*^{-/-} ($t(34) = 0,88$; $p=0,39$). As for acoustic and visual PPI there was no difference in startle reactivity ($t(34) = 0,66$; $p=0,52$)

As for *Gpr88*^{-/-} mice, conditional KO animals presented increased GAP detections for higher GAP durations (Figure 3B) ($F(7, 112) = 129,12$; $p < 0,0001$). However, genotype ($F(1, 16) = 1,36$; $p = 0,26$), interaction effect ($F(7, 112) = 0, 64$; $p = 0, 72$) or differences in global GAP detection ($t(16) = 1,26$; $p=0,23$) were absent for the *Gpr88*^{A2A-Cre} conditional KO (Figure 2C and Figure 2F). Once again, cKO and control animals displayed similar startle reflex levels ($t(16) = 0,26$; $p=0,799$).

Discussion

In the present report we confirm the acoustic prepulse inhibition deficit of *Gpr88*^{-/-} reported previously (Logue et al., 2009) and further demonstrate that the sensorimotor gating deficit extends to visual prepulse. Importantly, we show that these deficits do not require GPR88 in striatopallidal pathway MSNs.

Previous reports indicate that lack of GPR88 increases basal locomotor activity and alters responses to dopaminergic drugs (Quintana A, 2012). Furthermore, Logue *et al* report a deficit in acoustic PPI reversed by antipsychotic drugs in *Gpr88*^{-/-} mice, suggesting for the first time a role of this receptor in the pathophysiology of schizophrenia (Logue et al., 2009). In agreement, *Gpr88*^{-/-} mice in the present study showed a general decrease in acoustic PPI percentage significant for prepulses of 80 and 85 dB, despite a normal startle reflex and acoustic prepulse reactivity. When presented with a visual prepulse at different intervals from the startling pulse mice exhibit a prepulse facilitation (increased startle reactivity) for shorter intervals (2 and 10ms) but a decrease in startle reactivity for visual prepulses presented 20-2000ms before the pulse. General visual PPI is decreased in *Gpr88*^{-/-} animals with highest differences from control when the visual flash is presented 20ms before the acoustic startle. Once again, this impairment in sensorimotor gating cannot be attributed to changes in startle or prepulse reactivity as *Gpr88* deletion had no effect on this parameters. While background noise (BN) was shown to facilitate acoustic startle reflex (ASR), interruption of the BN is considered a form of GAP detection acting on inhibitory circuits in the brainstem to decrease startle reactivity (Ison et al., 1998, Ouagazzal et al., 2006). When testing mice in a GAP detection sessions, we corroborate previous findings showing that the presentation of a GAP in the background noise inhibits the startle reflex (Ison and Allen, 2003). This inhibition increased for higher GAP duration, with KO and control animals displaying similar levels of startle reactivity. This result thus indicates that the deficient acoustic and visual PPI observed in *Gpr88*^{-/-} animals result from a deficient sensorimotor gating rather than from a general inhibition deficit. The transcriptional, cellular, and neurochemical modifications, previously identified in the striatum of *Gpr88*^{-/-} mice and including downregulated dopamine D₂R-MSN and glutamate signaling marker genes, low dendritic spine density, and DA contents (Meirsmann et al., 2015), may contribute to sensorimotor gating processes involving GPR88.

In human as in rodents there is converging evidence supporting the importance of dopaminergic transmission acting via D₂R in the control of PPI (Swerdlow et al., 2000, Swerdlow and Geyer, 1998, Swerdlow et al., 2001, Powell et al., 2012). For instance, it has been shown that amphetamine does not disrupt PPI in mice lacking D₂R whereas D₁R deletion has no effect on amphetamine-mediated PPI disruption, suggesting a prominent role of D₂R and indirect pathway in

the regulation of PPI (Ralph et al., 1999). Another pharmacological study showed that administration of the D2-like receptor agonist quinlorane in mice disrupts visual PPI (Ces et al., 2012), and altogether, we anticipated that mice lacking striatopallidal GPR88 would show significant impairment of both acoustic and visual PPIs, as do total knockout animals. Our data, however, show no detectable deficit for these animals. It is possible that selective deletion of *Gpr88* in the striatopallidal projection pathway was not sufficient to impair sensorimotor gating regardless the sensory modality. Alternatively, the lack of sensorimotor gating deficit in *Gpr88*^{A2A-Cre} mice may indicate that GPR88 activity in D₂R neurons does not influence this particular function of striatopallidal neurons. Noteworthy is our observation of motor phenotypes in these conditional knockout mice (*in preparation*), strongly suggesting that GPR88 in this pathway does not modulate sensorimotor gating but regulates other aspects of D₂R MSN function.

We cannot exclude that GPR88 contributes to sensorimotor gating via receptor activity at the level of D₁R MSNs. In line with this hypothesis, pharmacological activation of D₁R-like receptor was shown to decrease both acoustic and visual PPI in mice (Ces et al., 2012, Ralph-Williams et al., 2002), demonstrating the possible implication of striatonigral neurons also. A final possibility is that lack of GPR88 in both MSNs is necessary to disrupt PPI. Future investigations using conditional KO of *Gpr88* in striatonigral pathway MSNs expressing D₁R will help us understanding how GPR88 in MSNs subpopulations regulates sensorimotor gating. Finally, and independently from MSN activities in adult animals, the contribution of GPR88 for the normal development of neural circuits underlying sensorimotor gating remains to be clarified, particularly when considering GPR88 as a target for drug design to effective treatments of sensory gating-related mental disorders.

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Disclosure/conflict of interest

The authors report no biomedical financial interests or potential conflicts of interest.

Legends to figures

Figure 1: Acoustic prepulse inhibition (PPI) in mice lacking *Gpr88*. Full (A) (N=19 *Gpr88*^{+/+}, 17 *Gpr88*^{-/-}) and conditional (C) KO animals (N=8 *Gpr88*^{flx/flx}, 10 *Gpr88*^{A2A-Cre}) present increased PPI levels with the increasing prepulse intensities. When compared to control littermates *Gpr88*^{-/-} show impaired general PPI (B) with significant decrease for prepulses of 80 and 85 dB (A). However, *Gpr88*^{A2A-Cre} mice present normal acoustic PPI (C and D). Data are represented as mean ± SEM. 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 2: Visual prepulse inhibition (PPI) in mice lacking *Gpr88*. Full (A) and conditional (C) KO animals present visual PPI for prepulses presented between 10 and 200ms. When compared to control littermates *Gpr88*^{-/-} (N=19 *Gpr88*^{+/+}, 17 *Gpr88*^{-/-}) show impaired visual PPI (B) with significant decrease for prepulses presented 20 ms before the pulse (A). In contrast, *Gpr88*^{A2A-Cre} mice (N=8 *Gpr88*^{flx/flx}, 10 *Gpr88*^{A2A-Cre}) display visual PPI levels similar to their control littermates (C and D). Data are represented as mean ± SEM. Solid stars: one star p < 0.05 (Student t test). Text stars: one star p < 0, 05 (Bonferroni's multiple comparisons test)

Figure 3: GAP detection in mice lacking *Gpr88*. Full (A) (N=19 *Gpr88*^{+/+}, 17 *Gpr88*^{-/-}) and conditional (C) KO animals (N=8 *Gpr88*^{flx/flx}, 10 *Gpr88*^{A2A-Cre}) present an increased inhibition of the startle reactivity for increasing duration of the background noise interruption. When compared to wild type animals there was no significant difference in the percentage of GAP detection for full (A and B) or conditional (C and D) KO animals. Data are represented as mean ± SEM.

Table 1. (Acoustic PPI): Baseline activity (background noise, BN), reactivity to the acoustic prepulse and startle reflex response of *Gpr88*^{-/-} and *Gpr88*^{A2A-Cre}.

Mouse line		Prepulse intensity (dB)				Pulse	
		<i>BN</i>	<i>70</i>	<i>80</i>	<i>85</i>	<i>90</i>	<i>110-dB/40 ms</i>
<i>Gpr88</i> ^{-/-}	WT	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
<i>Gpr88</i> ^{A2A-Cre}	WT	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

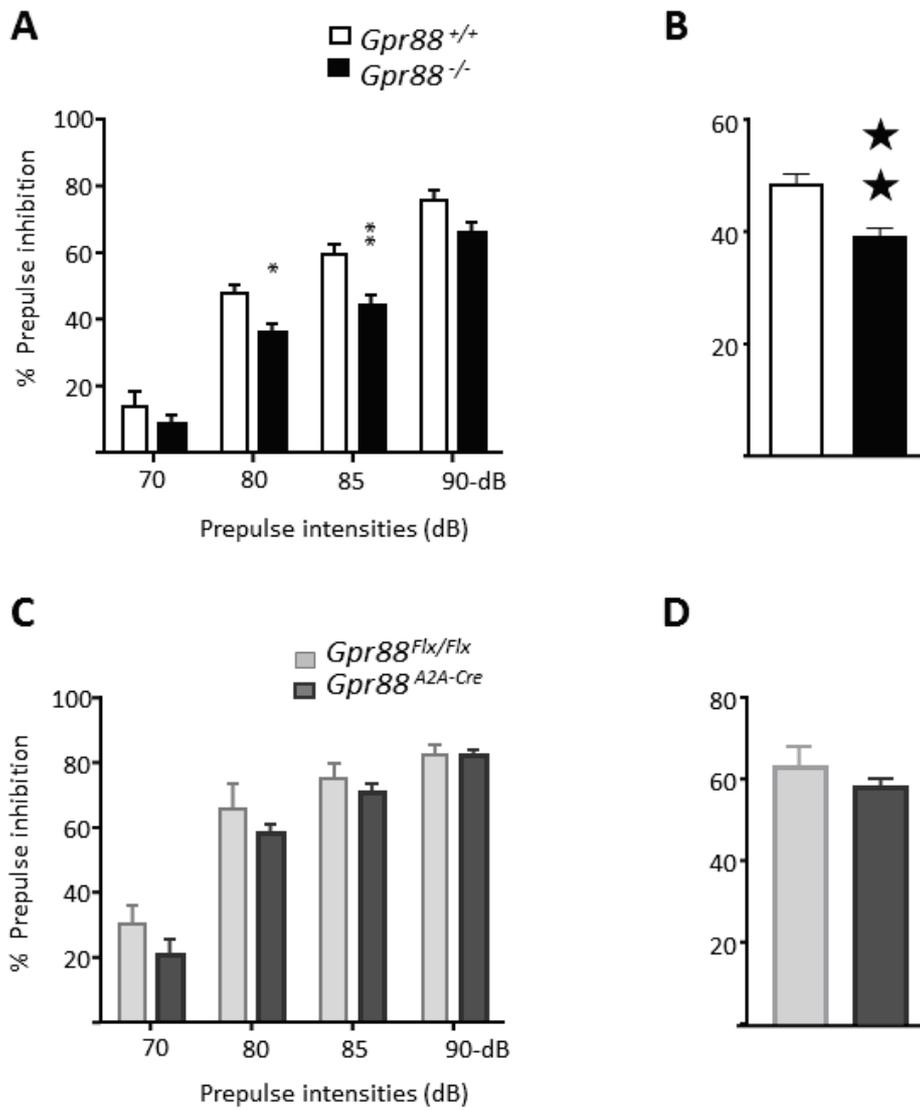
Table 2. (Visual PPI): Baseline activity (background noise, BN), reactivity to the visual prepulse and startle reflex response of *Gpr88*^{-/-} and *Gpr88*^{A2A-Cre}.

Mouse line		Visual Prepulse (1000Lux/20ms)		Pulse
		<i>BN</i>	<i>Prepulse alone</i>	<i>110-dB/40 ms</i>
<i>Gpr88</i> ^{-/-}	WT	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

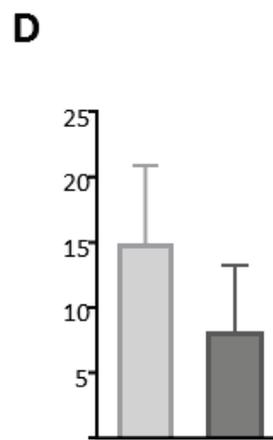
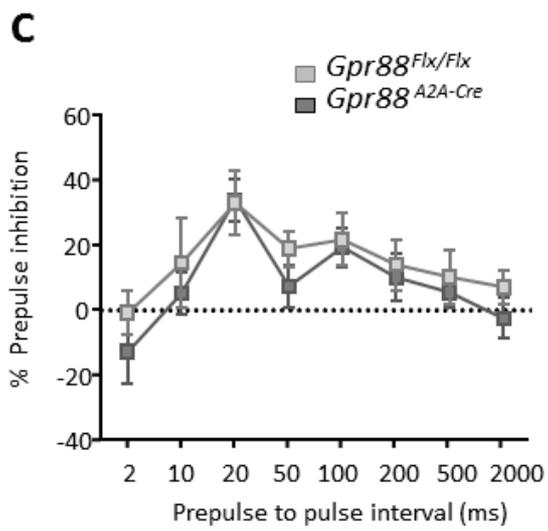
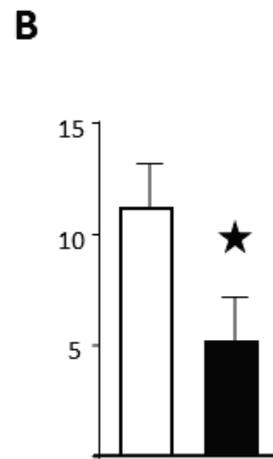
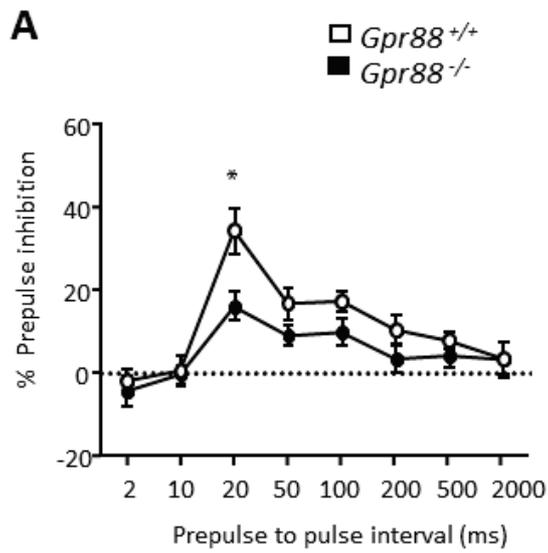
Gpr88^{A2A-Cre}	WT	6,4±1,0	5,9±1,1	190,2±44,2
	cKO	8,4±1,6	8,2±1,4	249,3±43,1

Table 3. (GAP detection): Baseline activity (background noise, BN) and startle reflex response to the pulse of Gpr88^{-/-} and Gpr88^{A2A-Cre}

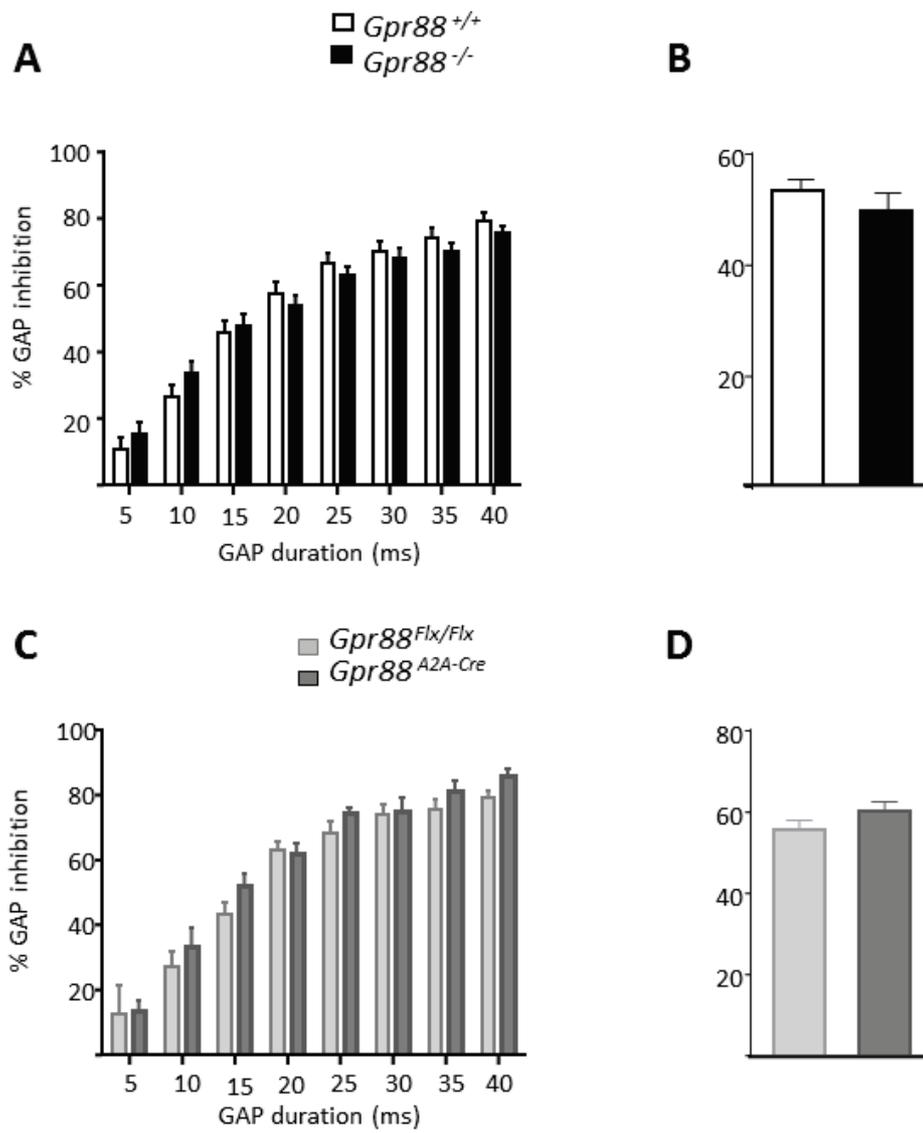
Mouse line		Pulse	
		<i>BN</i>	<i>120-dB/40 ms</i>
Gpr88^{-/-}	WT	4,48 ± 0,2	335,36 ± 6,3
	KO	5,59 ± 0,3	361,4 ± 6,9



Meirsmann et al Figure 1



Meirsman et al Figure 2



Meirsman et al Figure 3

Table S1: statistical analysis of PPI sessions in *Gpr88*^{-/-} and *Gpr88*^{A2A-Cre} mice

Mouse line	PPI Session	Student t-test	Repeated measures ANOVA			Student t-test
		Acoustic Startle Reactivity	Genotype/ treatment effect	prepulse effect	Interaction	Mean PPI
<i>Gpr88</i> ^{-/-} n= 19 WT, 17 KO	Acoustic	t(34) = 0,42 p = 0,68	F (1, 34) = 11,96 p = 0,0015	F (3, 102) = 258,7 p < 0,0001	F (3, 102) = 1,192 p = 0,3168	t (34) =3, 44 p=0, 0016
	Visual	t(34) = 0,14 p = 0,89	F (1, 34) = 4,134 p = 0,0499	F (7, 238) = 18,17 p < 0,0001	F (7, 238) = 1,820 p = 0,0841	t(34)= 2,048 p=0,048
	GAP	t(34) = 0,66 p = 0,52	F (1, 34) = 0,6944 p = 0,4105	F (7, 238) = 313,8 p < 0,0001	F (7, 238) = 2,111 p = 0,0432	t (34) = 0, 88 p=0, 39
<i>Gpr88</i> ^{A2A-Cre} n= 8 WT, 10 cKO	Acoustic	t(16) = 0,14 p = 0,89	F (1, 16) = 1,120 p = 0,3057	F (3, 48) = 135,6 p < 0,0001	F (3, 48) = 0,8784 p = 0,4589	t(16) = 1,026 p = 0,32
	Visual	t(16) = 0,64 p = 0,53	F (1, 16) = 0,6857 p = 0,4198	F (7, 112) = 9,469 p < 0,0001	F (7, 112) = 0,3704 p = 0,9178	t(16)=0, 84 p=0, 41
	GAP	t(16) = 0,26 p = 0,80	F (1, 16) = 1,356 p = 0,2613	F (7, 112) = 129,5 p < 0,0001	F (7, 112) = 0,6388 p = 0,7229	t(16) = 1, 26 p=0, 23

References

- BRAFF, D. L., GEYER, M. A. & SWERDLOW, N. R. 2001. Human studies of prepulse inhibition of startle: normal subjects, patient groups, and pharmacological studies. *Psychopharmacology (Berl)*, 156, 234-58.
- CALABRESI, P., PICCONI, B., TOZZI, A., GHIGLIERI, V. & DI FILIPPO, M. 2014. Direct and indirect pathways of basal ganglia: a critical reappraisal. *Nat Neurosci*, 17, 1022-30.
- CES, A., REISS, D., WALTER, O., WICHMANN, J., PRINSEN, E. P., KIEFFER, B. L. & OUAGAZZAL, A. M. 2012. Activation of nociceptin/orphanin FQ peptide receptors disrupts visual but not auditory sensorimotor gating in BALB/cByJ mice: comparison to dopamine receptor agonists. *Neuropsychopharmacology*, 37, 378-89.
- DEL ZOMPO, M., DELEUZE, J. F., CHILLOTTI, C., COUSIN, E., NIEHAUS, D., EBSTEIN, R. P., ARDAU, R., MACE, S., WARNICH, L., MUJAHED, M., SEVERINO, G., DIB, C., JORDAAN, E., MURAD, I., SOUBIGOU, S., KOEN, L., BANNOURA, I., ROCHER, C., LAURENT, C., DEROCK, M., FAUCON BIGUET, N., MALLET, J. & MELONI, R. 2014. Association study in three different populations between the GPR88 gene and major psychoses. *Mol Genet Genomic Med*, 2, 152-9.
- GEYER, M. A. 2006. The family of sensorimotor gating disorders: comorbidities or diagnostic overlaps? *Neurotox Res*, 10, 211-20.
- GEYER, M. A. 2008. Developing translational animal models for symptoms of schizophrenia or bipolar mania. *Neurotox Res*, 14, 71-8.
- GEYER, M. A., MCILWAIN, K. L. & PAYLOR, R. 2002. Mouse genetic models for prepulse inhibition: an early review. *Mol Psychiatry*, 7, 1039-53.
- INGALLINESI, M., LE BOUIL, L., FAUCON BIGUET, N., DO THI, A., MANNOURY LA COUR, C., MILLAN, M. J., RAVASSARD, P., MALLET, J. & MELONI, R. 2014. Local inactivation of Gpr88 in the nucleus accumbens attenuates behavioral deficits elicited by the neonatal administration of phencyclidine in rats. *Mol Psychiatry*.
- ISON, J. R., AGRAWAL, P., PAK, J. & VAUGHN, W. J. 1998. Changes in temporal acuity with age and with hearing impairment in the mouse: a study of the acoustic startle reflex and its inhibition by brief decrements in noise level. *J Acoust Soc Am*, 104, 1696-704.
- ISON, J. R. & ALLEN, P. 2003. A diminished rate of "physiological decay" at noise offset contributes to age-related changes in temporal acuity in the CBA mouse model of presbycusis. *J Acoust Soc Am*, 114, 522-8.
- LOGUE, S. F., GRAUER, S. M., PAULSEN, J., GRAF, R., TAYLOR, N., SUNG, M. A., ZHANG, L., HUGHES, Z., PULITO, V. L., LIU, F., ROSENZWEIG-LIPSON, S., BRANDON, N. J., MARQUIS, K. L., BATES, B. & PAUSCH, M. 2009. The orphan GPCR, GPR88, modulates function of the striatal dopamine system: a possible therapeutic target for psychiatric disorders? *Mol Cell Neurosci*, 42, 438-47.
- MASSART R, G. J., MIGNON V, SOKOLOFF P, DIAZ J 2009. Striatal GPR88 expression is confined to the whole projection neuron population and is regulated by dopaminergic and glutamatergic afferents. *Eur J Neurosci*, 397-414.
- MEIRSMAN, A. C., LE MERRER, J., PELLISSIER, L. P., DIAZ, J., CLESSE, D., KIEFFER, B. L. & BECKER, J. A. 2015. Mice Lacking GPR88 Show Motor Deficit, Improved Spatial Learning, and Low Anxiety Reversed by Delta Opioid Antagonist. *Biol Psychiatry*.
- OUAGAZZAL, A. M., REISS, D. & ROMAND, R. 2006. Effects of age-related hearing loss on startle reflex and prepulse inhibition in mice on pure and mixed C57BL and 129 genetic background. *Behav Brain Res*, 172, 307-15.
- POWELL, S. B., WEBER, M. & GEYER, M. A. 2012. Genetic models of sensorimotor gating: relevance to neuropsychiatric disorders. *Curr Top Behav Neurosci*, 12, 251-318.

- QUINTANA A, S. E., WANG W, STOREY GP, GULER AD, WANAT MJ, ET AL. 2012. Lack of GPR88 enhances medium spiny neuron activity and alters motor- and cue-dependent behaviors. *Nat Neurosci.*, 1547-1555.
- RALPH-WILLIAMS, R. J., LEHMANN-MASTEN, V., OTERO-CORCHON, V., LOW, M. J. & GEYER, M. A. 2002. Differential effects of direct and indirect dopamine agonists on prepulse inhibition: a study in D1 and D2 receptor knock-out mice. *J Neurosci*, 22, 9604-11.
- RALPH, R. J., VARTY, G. B., KELLY, M. A., WANG, Y. M., CARON, M. G., RUBINSTEIN, M., GRANDY, D. K., LOW, M. J. & GEYER, M. A. 1999. The dopamine D2, but not D3 or D4, receptor subtype is essential for the disruption of prepulse inhibition produced by amphetamine in mice. *J Neurosci*, 19, 4627-33.
- SWERDLOW, N. R., BRAFF, D. L. & GEYER, M. A. 2000. Animal models of deficient sensorimotor gating: what we know, what we think we know, and what we hope to know soon. *Behav Pharmacol*, 11, 185-204.
- SWERDLOW, N. R. & GEYER, M. A. 1998. Using an animal model of deficient sensorimotor gating to study the pathophysiology and new treatments of schizophrenia. *Schizophr Bull*, 24, 285-301.
- SWERDLOW, N. R., GEYER, M. A. & BRAFF, D. L. 2001. Neural circuit regulation of prepulse inhibition of startle in the rat: current knowledge and future challenges. *Psychopharmacology (Berl)*, 156, 194-215.

General Discussion

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In the present discussion I will overview the results presented throughout this thesis and put it in the context of the recent literature. Most importantly, I will discuss the relevance of these findings to the understanding of GPR88 function and future directions that may contribute to this knowledge. For this matter this section will be divided in three parts: (1) GPR88 KO mice and basal ganglia disorders (2) Tonic and developmental role of GPR88 and (3) GPR88 regulation of Medium Spiny Neurons (MSNs) activity.

1. GPR88 KO mice and basal ganglia disorders.

Functional abnormalities within the striatum and basal ganglia circuits have been shown in several neuropsychiatric and motor disorders (Ring and Serra-Mestres, 2002). Along with dopaminergic D₁R and D₂R, the adenosine A_{2a} receptor and the orphan receptors GPR6 and GPR52, GPR88 is one of the most abundant non-odorant G protein-coupled receptors in the rodent striatum (Komatsu, 2015). Given the high and almost specific expression of *Gpr88* throughout dorsal and ventral striatal regions it is not surprising that deletion of this receptor's gene results in abnormal behavioral outcomes characteristic of basal ganglia disorders.

The first behavioral report using GPR88 knockout (KO) animals postulates that these mice could constitute a valid animal model for schizophrenia disorder (Logue et al., 2009). In fact, in this report, the authors showed increased apomorphine-induced stereotypies as well as sensitization to the locomotor effect of d-amphetamine. Most importantly, they found prepulse inhibition (PPI) deficits reversed by both typical and atypical antipsychotics further demonstrating that lack of GPR88 induces phenotypes relevant to schizophrenia. Accordingly, another report confirmed the increased response to amphetamine-induced locomotion in *Gpr88* KO animals and further shows initial learning deficits in these animals (Quintana A, 2012). The association study indicating a positive correlation between *Gpr88* and schizophrenia subpopulations finally established the importance of GPR88 in the study of the pathophysiology of schizophrenia (Del Zompo et al., 2014). In Manuscript 1 we confirmed the increased basal locomotion of *Gpr88* KO mice and further demonstrated that these mice present stereotypic behavior. Furthermore, we showed for the first time alterations in the prefrontal cortex (PFC) of mutant animals (*e.g.* downregulation of *Drd2* and *Drd3*) further suggesting that these animals may constitute a valid animal model of schizophrenia disorder. Unlike schizophrenic patients, we found decrease and unaltered dopamine levels in the Caudate-

Putamen (CPu) and Nucleus Accumbens (Nac) respectively which could either result from different measurement techniques or different physiological alteration. Also, in Manuscript 2, we showed that *Gpr88* KO mice present increased drive toward social interaction, which contrasts with the social withdrawal observed in schizophrenic patients suggesting that lack of GPR88 does not model this symptomatic dimension.

In chapter 3, we confirmed the sensorimotor gating deficits shown by Logue and collaborators and further extend it to visual stimuli. PPI deficits are not exclusive to schizophrenic patients and are also seen, for instance, in attention deficit/hyperactive disorder (ADHD) patients (Feifel et al., 2009). Accordingly, as observed in ADHD patients, we also showed (see chapter 1 section 2) that mutant mice presented hyperactive behavior in familiar environments, reversed by Methylphenidate treatment. Accordingly, the dopamine transporter (DAT) gene (*Slc6a3*) was found to be down- and up-regulated in the CPu and Nac of *Gpr88*^{-/-} mice respectively (Manuscript 1). Unfortunately, to our knowledge, impulsive or attention deficit phenotypes were never assessed in these mice. Relevant to both schizophrenia and ADHD research, further studies could include a five choice serial reaction time test (5-CSRTT) assessing both impulsive behavior and sustained attention. Moreover, further investigation on PFC activity (*e.g.* c-fos counting) in *Gpr88* KO could also help understanding the mechanisms underlying the general behavior of these mice. In fact, it is possible that a PFC hypofunction underlies behavioral disinhibition observed in mutant animals (Valera et al., 2007, Maletic and Raison, 2014). These investigations on executive functions could also determine whether *Gpr88* KO mice represent a valid model of mania. Indeed, in addition to the genetic association found between *Gpr88* and bipolar disorder (BPD) patients, lack of this receptor causes general hyperactivity, supersensitivity to psychostimulants and increased exploratory behavior. So far, existing data are still poor to determine the relevance of this receptor in BPD research. Also, brains of GPR88 mutant mice show no gross cytoarchitectural abnormalities (Logue et al., 2009) as observed in advanced BPD patients. The low-anxiety phenotype found in several well validated tests suggests a susceptibility for risk-taking behaviors but more appropriate measures (*e.g.* Iowa Gambling Task) are needed to validate a model of risk-taking (Manuscript 1 and 2). This low-anxiety phenotype is actually quite puzzling when considering the overall behavioral phenotype of *Gpr88*^{-/-} mice. First, as shown above, these mice present several behavioral outcomes comorbid to various psychiatric diseases but high rather than low anxiety is also often reported in such pathologies. Also, while pharmacological activation of GPR88 could be considered as a potential target for the treatment of schizophrenia, ADHD or mania, anxiety disorder patients would benefit from pharmacological blockage (rather than activation) of GPR88. As such, before considering activation of GPR88

as a potential treatment for psychotic symptoms, possible side effects such as increased anxiety should be assessed.

Besides the psychiatric symptoms modeled by *Gpr88* deletion, mutant mice also presented motor coordination deficits observed in several reports and different rotarod sessions (Quintana A, 2012) (Manuscript 1 and chapter 2 section 2). Additionally, 6-OHDA-induced depletion of dopamine neurons (pharmacological model of Parkinson's disease) decreased and increased striatopallidal and striatonigral *Gpr88* expression respectively (Massart R, 2009). Consistently, deletion of *Gpr88* was found to decrease dopamine levels and spine density in the dorsal striatum further suggesting that this receptor's activation could constitute a relevant pharmacological target for Parkinson's disease (PD) (Manuscript 1) (Logue et al., 2009, Deutch et al., 2007). As such, despite the absence of neurodegeneration or catalepsy in GPR88 KO mice added to the increased (rather than decreased) locomotor initiation, data suggest that GPR88 may be involved in the cellular mechanisms arising from DA depletion in PD. Moreover, striatal *Gpr88* re-expression reversed the motor coordination deficit observed in full KO animals (Quintana A, 2012) and virally-mediated deletion of *Gpr88* in adult mice decreased motor coordination (chapter 2 section 2). Therefore, future investigations should verify whether GPR88 agonist administration in rodent models of Parkinson's disease improve motor coordination deficits.

2. Tonic and developmental role of GPR88

Many psychiatric disorders such as schizophrenia and ADHD are thought to originate from neurodevelopmental abnormalities (Lewis and Levitt, 2002, Ronald et al., 2010). On the contrary, Parkinson's or Huntington's disease have late onsets and are the consequence of neurodegeneration. *Gpr88* expression starts during embryonic phases and is highly regulated during post-natal development suggesting a role in [development](#) of striatal physiology (Van Waes V, 2011). As such, it is possible that phenotypes observed in constitutive KO animals are the consequence of GPR88 absence during development rather than during adulthood. Unrevealing the tonic vs developmental role of GPR88 would bring us a step closer to understanding its role in neuropsychiatric and motor disorders.

After constitutive and total deletion of *Gpr88*, Quintana et al (2012) showed that re-expression of *Gpr88* in adult mice's striatum reversed the hyperlocomotion and motor deficits thus indicating that this receptor regulates such functions in the adult striatum. In our lab, using a virally-mediated knockdown approach we deleted *Gpr88* in the adult mice striatum (chapter 2 section 2) and confirmed that this

receptor tonically regulates motor coordination. However, we tested other emotional phenotypes shown to be altered in full KO animals (e.g. Elevated plus maze, Open Field exploration, novelty suppressed feeding) but found no differences with control mice injected with AAV-eGFP. (Data not shown). It is possible that (1) GPR88 regulates these behaviors during developmental stages, (2) deletion of *Gpr88* ($\pm 50\%$) was not sufficient to induce such phenotypes, or (3) these behaviors are not regulated by GPR88 in the striatum. Considering this latter possibility we tried to develop an inducible deletion of *Gpr88* in the whole adult brain using CreER^{T2} approach. As described in section 3 of the Chapter 2 we were unfortunately not able to significantly delete *Gpr88* using this approach. However, a recent report suggest that GPR88 in the adult rat Nac regulates phenotypes modeling schizophrenic symptoms (PCP-induced hyperlocomotion and deficient social novelty discrimination) further confirming that GPR88 may represent a novel target addressing psychotic and cognitive symptoms (Ingallinesi et al., 2014). Future studies should consider the importance of clearly determining the function of striatal and extra-striatal GPR88 in the adult brain if we aim a comprehensive understanding of this receptor's functioning throughout the brain.

3. GPR88 regulation of Medium Spiny Neurons (MSNs) activity

Figure 1 summarizes our hypothesis on the role of GPR88 in striatopallidal and striatonigral neurons based on our data from full and A2A-conditional *Gpr88* KO mice.

Within the striatum, GPR88 is found in both Enkephalin (Penk, selectively expressed in striatopallidal neurons co-expressing D₂R) and substance P (SP, selectively expressed in striatonigral neurons co-expressing D₁R) expressing cells, mainly in post-synaptic domains contacting asymmetrical synapses suggesting a potential role in modulating excitatory inputs on MSNs (Massart R, 2009, Quintana A, 2012). Electrophysiological studies on *Gpr88*^{-/-} animals revealed increased firing rates in MSNs of KO animals accompanied by increased and decreased Glutamatergic and GABAergic transmission respectively (Quintana A, 2012). Added to the decreased cyclic Adenosine monophosphate (cAMP) in *Gpr88*-expressing cells (data not shown) and the GPR88 agonist-induced dose-dependent [S35]-GTPγS response in striatal membranes (functional assay used to measure G_{i/o} but not G_s dependent GPCR activity), overall, data suggest that GPR88 is coupled to a G_{i/o} protein.

The precise mechanism underlying GPR88 regulation of MSNs function still remains to be understood. Within MSNs, dopamine (DA) afferent inputs acting on D₁R increases intracellular cAMP facilitating glutamatergic transmission therefore increasing striatonigral transmission and SP expression. On the contrary, dopamine acting on D₂R decreases membrane potential and presynaptic glutamatergic release therefore decreasing striatopallidal transmission and Penk expression. Consequently, DAergic depletion increases Penk expression and striatopallidal transmission while decreasing SP expression and striatonigral transmission (Murer et al., 2000). Intriguingly, dopamine depletion has the opposite effect on Gpr88 expression. In fact, this receptor is upregulated and downregulated in striatonigral and striatopallidal neurons respectively (Massart R, 2009). Gpr88 full deletion downregulated genes selectively expressed in striatopallidal neurons (Adora2a, Gpr6, Drd2 and Penk) while having no effect on striatonigral-specific Pdyn (Manuscript 1). This result suggests a possible decrease of striatopallidal activity in Gpr88^{-/-} mice. Accordingly, full KO animals show increased locomotion as observed in striatopallidal depleted mice (Durieux PF, 2009, Durieux PF, 2012, Bateup et al., 2010). Alternatively, this increased locomotion could be the result of increased striatonigral transmission. When Gpr88 is selectively decreased in striatopallidal neurons, mice display the same hyperactive phenotype than full GPR88 KO animals further suggesting decreased striatopallidal transmission. Also, in agreement with a decreased striatopallidal transmission, we showed that both full and conditional KO animals display decreased avoidance behavior and increased stereotypies previously suggested to be regulated by these neurons (Hikida et al., 2010, Tanimura et al., 2010, Tanimura et al., 2011). On the other hand only full KO show increased approach behavior shown to be mediated by activation of striatonigral neurons (Manuscript 2). A previous report has suggested that deletion of Gpr88 may increase the sensitivity of post synaptic D₂R (Logue et al., 2009). This could in fact explain the decreased expression of Adora2a, Gpr6, Drd2 and Penk found in full KO animals and the decreased glutamatergic transmission in Gpr88^{A2A-Cre} mice. Also, an increased sensitivity of post-synaptic dopaminergic receptors could result increased response to dopaminergic agents. In Gpr88^{A2A-Cre} mice, the increased SKF-induced (D₁R agonist) locomotion could be the consequence of a general locomotor disinhibition (mediated by decreased striatopallidal transmission) but a possible compensatory alteration in

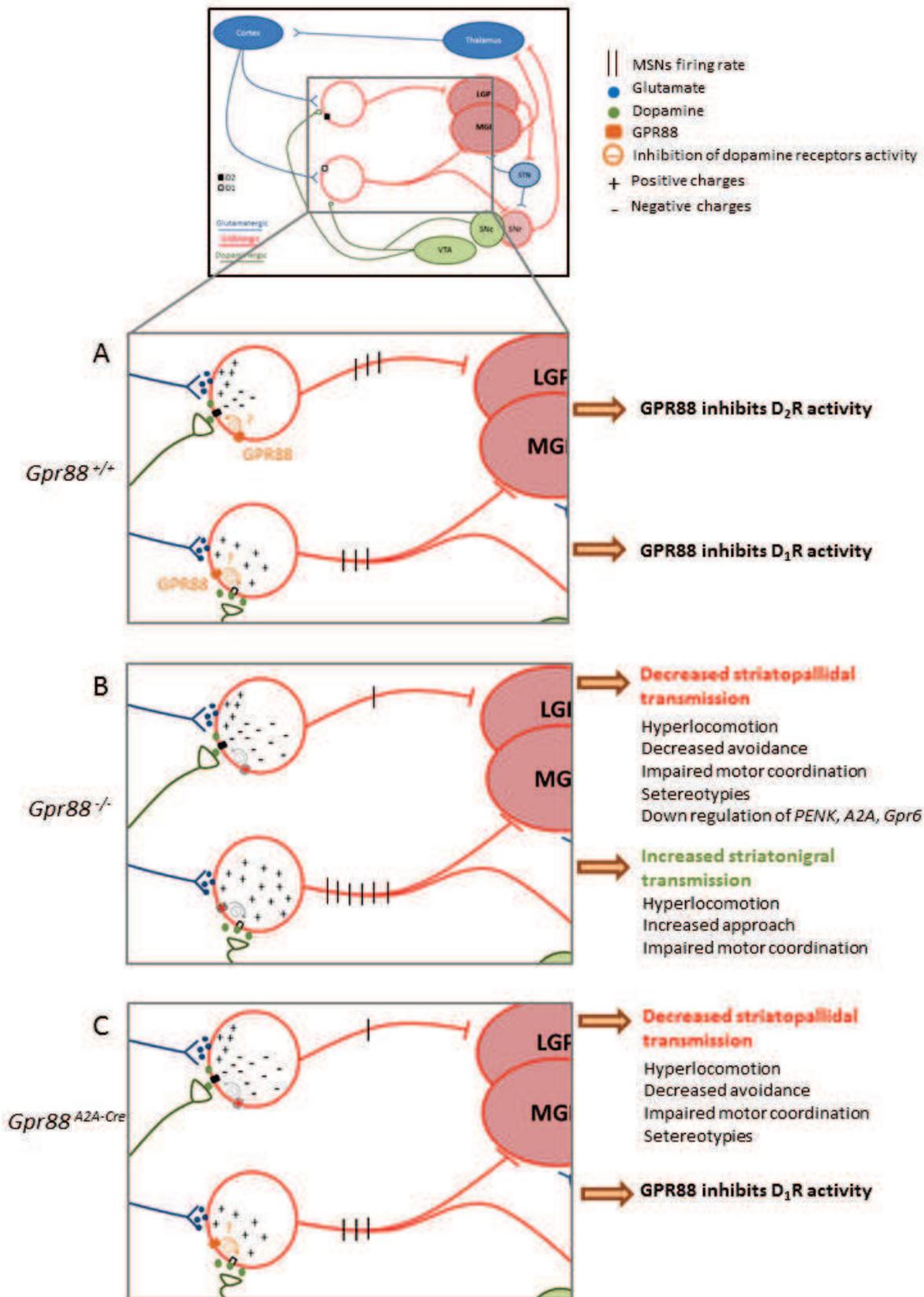


Figure 1. Schematic representation of a hypothetical mechanism underlying behavioral outcomes observed in mice lacking GPR88. If GPR88 directly or indirectly inhibits dopamine (DA) receptors activity (A), deletion of the receptor gene would increase their activity. In striatopallidal neurons increased D2R activity would decrease MSNs transmission leading to increased locomotion, decreased avoidance behavior and impaired motor coordination (B and C) (Durieux et al, 2012; Bateup et al 2010). In striatonigral MSNs (B), increased D1R activity would oppositely lead to increased activity of striatonigral transmission. This would (also) increase locomotion as well as goal-directed behavior (Bateup et al. 2010; Hikida et al. 2010). A conditional KO mice of GPR88 in striatonigral neurons would help confirm this model.

striatonigral MSNs in these mice should not be excluded. The lack of increased sensitivity to haloperidol in *Gpr88*^{-/-} mice could result from an increased striatonigral transmission counteracting D₂R mediated pro-cataleptic effects. It is also likely that GPR88 regulation of MSNs transmission involves more complex mechanisms that indirectly implicate dopamine receptors activity. In fact, further investigations are needed to validate the hypothesis of increased dopamine receptors activity in KO mice. For instance, if striatonigral transmission is enhanced by *Gpr88* deletion, then approach or goal-directed behaviors should also be increased. A more suitable way for assessing approach behaviors would include place preference for either cocaine or chocolate as measured in previous studies (Hikida et al., 2010, Hikida et al., 2013, Kravitz and Kreitzer, 2012, Kravitz et al., 2012) . Also, increased and decreased striatonigral and striatopallidal transmission respectively would ideally be measured with electrophysiological approaches.

In sum, data suggests that lack of GPR88 in striatopallidal neurons decreases transmission in this particular MSN population, but further investigations are needed to validate this hypothesis. This in progress project will include studying the effect of *Gpr88* deletion in D₁R-expressing striatonigral neurons.

Conclusion

In the present thesis we extend knowledge of GPR88 function not only in the striatum but in extrastriatal regions. Moreover, for the first time, we aim to elucidate the role of this receptor within different MSNs subpopulations.

Behavioral and pharmacological results confirmed the importance of GPR88 in basal ganglia disorder and show for the first time involvement of this receptor in anxiety-like behaviors. In fact, we clearly show that GPR88 regulates striatal and extrastriatal physiology and neurotransmitter release. Future studies should continue to investigate the role of GPR88 in extrastriatal areas and regulation of monoamine release and receptors activation. Also, we show that GPR88 in striatopallidal MSNs regulates anxiety-like behaviors and motor functions but has no impact on sensorimotor gating. Finally, the conditional KO approach suggests that this receptor may regulate MSNs function in opposite ways and, most importantly, open new avenues for further studies ultimately leading to a full and comprehensive understanding of GPR88 function.

References

- BATEUP, H. S., SANTINI, E., SHEN, W., BIRNBAUM, S., VALJENT, E., SURMEIER, D. J., FISONE, G., NESTLER, E. J. & GREENGARD, P. 2010. Distinct subclasses of medium spiny neurons differentially regulate striatal motor behaviors. *Proc Natl Acad Sci U S A*, 107, 14845-50.
- DEL ZOMPO, M., DELEUZE, J. F., CHILLOTTI, C., COUSIN, E., NIEHAUS, D., EBSTEIN, R. P., ARDAU, R., MACE, S., WARNICH, L., MUJAHED, M., SEVERINO, G., DIB, C., JORDAAN, E., MURAD, I., SOUBIGOU, S., KOEN, L., BANNOURA, I., ROCHER, C., LAURENT, C., DEROCK, M., FAUCON BIGUET, N., MALLET, J. & MELONI, R. 2014. Association study in three different populations between the GPR88 gene and major psychoses. *Mol Genet Genomic Med*, 2, 152-9.
- DEUTCH, A. Y., COLBRAN, R. J. & WINDER, D. J. 2007. Striatal plasticity and medium spiny neuron dendritic remodeling in parkinsonism. *Parkinsonism Relat Disord*, 13 Suppl 3, S251-8.
- DURIEUX PF, B. B., GUIDUCCI S, BUCH T, WAISMAN A, ZOLI M, ET AL 2009. D2R striatopallidal neurons inhibit both locomotor and drug reward processes. *Nat Neurosci*, :393-395.
- DURIEUX PF, S. S., DE KERCHOVE D'EXAERDE A 2012. Differential regulation of motor control and response to dopaminergic drugs by D1R and D2R neurons in distinct dorsal striatum subregions. 640-653.
- FEIFEL, D., MINASSIAN, A. & PERRY, W. 2009. Prepulse inhibition of startle in adults with ADHD. *J Psychiatr Res*, 43, 484-9.
- HIKIDA, T., KIMURA, K., WADA, N., FUNABIKI, K. & NAKANISHI, S. 2010. Distinct roles of synaptic transmission in direct and indirect striatal pathways to reward and aversive behavior. *Neuron*, 66, 896-907.

- HIKIDA, T., YAWATA, S., YAMAGUCHI, T., DANJO, T., SASAOKA, T., WANG, Y. & NAKANISHI, S. 2013. Pathway-specific modulation of nucleus accumbens in reward and aversive behavior via selective transmitter receptors. *Proc Natl Acad Sci U S A*, 110, 342-7.
- INGALLINESI, M., LE BOUIL, L., FAUCON BIGUET, N., DO THI, A., MANNOURY LA COUR, C., MILLAN, M. J., RAVASSARD, P., MALLET, J. & MELONI, R. 2014. Local inactivation of Gpr88 in the nucleus accumbens attenuates behavioral deficits elicited by the neonatal administration of phencyclidine in rats. *Mol Psychiatry*.
- KOMATSU, H. 2015. Novel Therapeutic GPCRs for Psychiatric Disorders. *Int J Mol Sci*, 16, 14109-21.
- KRAVITZ, A. V. & KREITZER, A. C. 2012. Striatal mechanisms underlying movement, reinforcement, and punishment. *Physiology (Bethesda)*, 27, 167-77.
- KRAVITZ, A. V., TYE, L. D. & KREITZER, A. C. 2012. Distinct roles for direct and indirect pathway striatal neurons in reinforcement. *Nat Neurosci*, 15, 816-8.
- LEWIS, D. A. & LEVITT, P. 2002. Schizophrenia as a disorder of neurodevelopment. *Annu Rev Neurosci*, 25, 409-32.
- LOGUE, S. F., GRAUER, S. M., PAULSEN, J., GRAF, R., TAYLOR, N., SUNG, M. A., ZHANG, L., HUGHES, Z., PULITO, V. L., LIU, F., ROSENZWEIG-LIPSON, S., BRANDON, N. J., MARQUIS, K. L., BATES, B. & PAUSCH, M. 2009. The orphan GPCR, GPR88, modulates function of the striatal dopamine system: a possible therapeutic target for psychiatric disorders? *Mol Cell Neurosci*, 42, 438-47.
- MALETIC, V. & RAISON, C. 2014. Integrated neurobiology of bipolar disorder. *Front Psychiatry*, 5, 98.
- MASSART R, G. J., MIGNON V, SOKOLOFF P, DIAZ J 2009. Striatal GPR88 expression is confined to the whole projection neuron population and is regulated by dopaminergic and glutamatergic afferents. *Eur J Neurosci*, 397-414.
- MURER, M. G., DZIEWCZAPOLSKI, G., SALIN, P., VILA, M., TSENG, K. Y., RUBERG, M., RUBINSTEIN, M., KELLY, M. A., GRANDY, D. K., LOW, M. J., HIRSCH, E., RAISMAN-VOZARI, R. & GERSHANI, O. 2000. The indirect basal ganglia pathway in dopamine D(2) receptor-deficient mice. *Neuroscience*, 99, 643-50.
- QUINTANA A, S. E., WANG W, STOREY GP, GULER AD, WANAT MJ, ET AL. 2012. Lack of GPR88 enhances medium spiny neuron activity and alters motor- and cue-dependent behaviors. *Nat Neurosci*, 1547-1555.
- RING, H. A. & SERRA-MESTRES, J. 2002. Neuropsychiatry of the basal ganglia. *J Neurol Neurosurg Psychiatry*, 72, 12-21.
- RONALD, A., PENNELL, C. E. & WHITEHOUSE, A. J. 2010. Prenatal Maternal Stress Associated with ADHD and Autistic Traits in early Childhood. *Front Psychol*, 1, 223.
- TANIMURA, Y., KING, M. A., WILLIAMS, D. K. & LEWIS, M. H. 2011. Development of repetitive behavior in a mouse model: roles of indirect and striosomal basal ganglia pathways. *Int J Dev Neurosci*, 29, 461-7.
- TANIMURA, Y., VAZIRI, S. & LEWIS, M. H. 2010. Indirect basal ganglia pathway mediation of repetitive behavior: attenuation by adenosine receptor agonists. *Behav Brain Res*, 210, 116-22.
- VALERA, E. M., FARAONE, S. V., MURRAY, K. E. & SEIDMAN, L. J. 2007. Meta-analysis of structural imaging findings in attention-deficit/hyperactivity disorder. *Biol Psychiatry*, 61, 1361-9.
- VAN WAES V, T. K., STEINER H 2011. GPR88 - a putative signaling molecule predominantly expressed in the striatum: Cellular localization and developmental regulation. *Basal Ganglia*, 83-89.

General References

- ALBIN, R. L., YOUNG, A. B. & PENNEY, J. B. 1989. The functional anatomy of basal ganglia disorders. *Trends Neurosci*, 12, 366-75.
- ALEXANDER, G. E., DELONG, M. R. & STRICK, P. L. 1986. Parallel organization of functionally segregated circuits linking basal ganglia and cortex. *Annu Rev Neurosci*, 9, 357-81.
- ALLSOP, S. A., VANDER WEELE, C. M., WICHMANN, R. & TYE, K. M. 2014. Optogenetic insights on the relationship between anxiety-related behaviors and social deficits. *Front Behav Neurosci*, 8, 241.
- ANGST, J. 2013. Bipolar disorders in DSM-5: strengths, problems and perspectives. *Int J Bipolar Disord*, 1, 12.
- ARNOLD, K., SARKAR, A., YRAM, M. A., POLO, J. M., BRONSON, R., SENGUPTA, S., SEANDEL, M., GEIJSEN, N. & HOCHEDLINGER, K. 2011. Sox2(+) adult stem and progenitor cells are important for tissue regeneration and survival of mice. *Cell Stem Cell*, 9, 317-29.
- ASSELIN, M. C., SOGHOMONIAN, J. J., COTE, P. Y. & PARENT, A. 1994. Striatal changes in preproenkephalin mRNA levels in parkinsonian monkeys. *Neuroreport*, 5, 2137-40.
- ATALLAH, H. E., LOPEZ-PANIAGUA, D., RUDY, J. W. & O'REILLY, R. C. 2007. Separate neural substrates for skill learning and performance in the ventral and dorsal striatum. *Nat Neurosci*, 10, 126-31.
- AUPPERLE, R. L. & PAULUS, M. P. 2010. Neural systems underlying approach and avoidance in anxiety disorders. *Dialogues Clin Neurosci*, 12, 517-31.
- BAILEY, K. R. & CRAWLEY, J. N. 2009. Anxiety-Related Behaviors in Mice. In: BUCCAFUSCO, J. J. (ed.) *Methods of Behavior Analysis in Neuroscience*. 2nd ed. Boca Raton (FL).
- BALLEINE, B. W., LILJEHOLM, M. & OSTLUND, S. B. 2009. The integrative function of the basal ganglia in instrumental conditioning. *Behav Brain Res*, 199, 43-52.
- BAMFORD, N. S., ZHANG, H., SCHMITZ, Y., WU, N. P., CEPEDA, C., LEVINE, M. S., SCHMAUSS, C., ZAKHARENKO, S. S., ZABLOW, L. & SULZER, D. 2004. Heterosynaptic dopamine neurotransmission selects sets of corticostriatal terminals. *Neuron*, 42, 653-63.
- BAPTISTA, M. A., DAVE, K. D., SHETH, N. P., DE SILVA, S. N., CARLSON, K. M., AZIZ, Y. N., FISKE, B. K., SHERER, T. B. & FRASIER, M. A. 2013. A strategy for the generation, characterization and distribution of animal models by The Michael J. Fox Foundation for Parkinson's Research. *Dis Model Mech*, 6, 1316-24.
- BARKLEY, R. A. 1997. Behavioral inhibition, sustained attention, and executive functions: constructing a unifying theory of ADHD. *Psychol Bull*, 121, 65-94.
- BATEUP, H. S., SANTINI, E., SHEN, W., BIRNBAUM, S., VALJENT, E., SURMEIER, D. J., FISONE, G., NESTLER, E. J. & GREENGARD, P. 2010. Distinct subclasses of medium spiny neurons differentially regulate striatal motor behaviors. *Proc Natl Acad Sci U S A*, 107, 14845-50.
- BECKER, J. A., BEFORT, K., BLAD, C., FILLIOL, D., GHATE, A., DEMBELE, D., THIBAUT, C., KOCH, M., MULLER, J., LARDENOIS, A., POCH, O. & KIEFFER, B. L. 2008. Transcriptome analysis identifies genes with enriched expression in the mouse central extended amygdala. *Neuroscience*, 156, 950-65.
- BEFORT, K., FILLIOL, D., GHATE, A., DARCO, E., MATIFAS, A., MULLER, J., LARDENOIS, A., THIBAUT, C., DEMBELE, D., LE MERRER, J., BECKER, J. A., POCH, O. & KIEFFER, B. L. 2008. Mu-opioid receptor activation induces transcriptional plasticity in the central extended amygdala. *Eur J Neurosci*, 27, 2973-84.
- BEITZ, J. M. 2014. Parkinson's disease: a review. *Front Biosci (Schol Ed)*, 6, 65-74.
- BELL, D. S. 1965. Comparison of Amphetamine Psychosis and Schizophrenia. *Br J Psychiatry*, 111, 701-7.
- BELZUNG, C. & GRIEBEL, G. 2001. Measuring normal and pathological anxiety-like behaviour in mice: a review. *Behav Brain Res*, 125, 141-9.

- BELZUNG, C. & LEMOINE, M. 2011. Criteria of validity for animal models of psychiatric disorders: focus on anxiety disorders and depression. *Biol Mood Anxiety Disord*, 1, 9.
- BERK, M., DODD, S., KAUER-SANT'ANNA, M., MALHI, G. S., BOURIN, M., KAPCZINSKI, F. & NORMAN, T. 2007. Dopamine dysregulation syndrome: implications for a dopamine hypothesis of bipolar disorder. *Acta Psychiatr Scand Suppl*, 41-9.
- BERK, M., KAPCZINSKI, F., ANDREAZZA, A. C., DEAN, O. M., GIORLANDO, F., MAES, M., YUCEL, M., GAMA, C. S., DODD, S., DEAN, B., MAGALHAES, P. V., AMMINGER, P., MCGORRY, P. & MALHI, G. S. 2011. Pathways underlying neuroprogression in bipolar disorder: focus on inflammation, oxidative stress and neurotrophic factors. *Neurosci Biobehav Rev*, 35, 804-17.
- BLAISS, C. A. & JANAK, P. H. 2009. The nucleus accumbens core and shell are critical for the expression, but not the consolidation, of Pavlovian conditioned approach. *Behav Brain Res*, 200, 22-32.
- BODNOFF, S. R., SURANYI-CADOTTE, B., QUIRION, R. & MEANEY, M. J. 1989. A comparison of the effects of diazepam versus several typical and atypical anti-depressant drugs in an animal model of anxiety. *Psychopharmacology (Berl)*, 97, 277-9.
- BOHM, C., NEWRZELLA, D., HERBERGER, S., SCHRAMM, N., EISENHARDT, G., SCHENK, V., SONNTAG-BUCK, V. & SORGENFREI, O. 2006. Effects of antidepressant treatment on gene expression profile in mouse brain: cell type-specific transcription profiling using laser microdissection and microarray analysis. *J Neurochem*, 97 Suppl 1, 44-9.
- BOLKAN, S. S., DE CARVALHO, F. D. & KELLENDONK, C. 2015. Using human brain imaging studies as a guide toward animal models of schizophrenia. *Neuroscience*.
- BORA, E., YUCEL, M. & PANTELIS, C. 2009. Cognitive endophenotypes of bipolar disorder: a meta-analysis of neuropsychological deficits in euthymic patients and their first-degree relatives. *J Affect Disord*, 113, 1-20.
- BORSINI, F., PODHORNA, J. & MARAZZITI, D. 2002. Do animal models of anxiety predict anxiolytic-like effects of antidepressants? *Psychopharmacology (Berl)*, 163, 121-41.
- BRAFF, D. L., GEYER, M. A. & SWERDLOW, N. R. 2001. Human studies of prepulse inhibition of startle: normal subjects, patient groups, and pharmacological studies. *Psychopharmacology (Berl)*, 156, 234-58.
- BRAKE, R. L., SIMMONS, P. J. & BEGLEY, C. G. 2004. Cross-contamination with tamoxifen induces transgene expression in non-exposed inducible transgenic mice. *Genet Mol Res*, 3, 456-62.
- BRAMBILLA, P., HARENSKI, K., NICOLETTI, M., MALLINGER, A. G., FRANK, E., KUPFER, D. J., KESHAVAN, M. S. & SOARES, J. C. 2001. MRI study of posterior fossa structures and brain ventricles in bipolar patients. *J Psychiatr Res*, 35, 313-22.
- BRANDAO, M. L., DE OLIVEIRA, A. R., MUTHURAJU, S., COLOMBO, A. C., SAITO, V. M. & TALBOT, T. 2015. Dual role of dopamine D-like receptors in the mediation of conditioned and unconditioned fear. *FEBS Lett*.
- BRANDISH, P. E., SU, M., HOLDER, D. J., HODOR, P., SZUMILOSKI, J., KLEINHANZ, R. R., FORBES, J. E., MCWHORTER, M. E., DUENWALD, S. J., PARRISH, M. L., NA, S., LIU, Y., PHILLIPS, R. L., RENGER, J. J., SANKARANARAYANAN, S., SIMON, A. J. & SCOLNICK, E. M. 2005. Regulation of gene expression by lithium and depletion of inositol in slices of adult rat cortex. *Neuron*, 45, 861-72.
- BRAZEL, C. Y., LIMKE, T. L., OSBORNE, J. K., MIURA, T., CAI, J., PEVNY, L. & RAO, M. S. 2005. Sox2 expression defines a heterogeneous population of neurosphere-forming cells in the adult murine brain. *Aging Cell*, 4, 197-207.
- BROOKS, S. P. & DUNNETT, S. B. 2009. Tests to assess motor phenotype in mice: a user's guide. *Nat Rev Neurosci*, 10, 519-29.
- BUBENIKOVA-VALESOVA, V., HORACEK, J., VRAJOVA, M. & HOSCHL, C. 2008. Models of schizophrenia in humans and animals based on inhibition of NMDA receptors. *Neurosci Biobehav Rev*, 32, 1014-23.

- CALABRESI, P., PICCONI, B., TOZZI, A., GHIGLIERI, V. & DI FILIPPO, M. 2014. Direct and indirect pathways of basal ganglia: a critical reappraisal. *Nat Neurosci*, 17, 1022-30.
- CALMON, M. F., JESCHKE, J., ZHANG, W., DHIR, M., SIEBENKAS, C., HERRERA, A., TSAI, H. C., O'HAGAN, H. M., PAPPOU, E. P., HOOKER, C. M., FU, T., SCHUEBEL, K. E., GABRIELSON, E., RAHAL, P., HERMAN, J. G., BAYLIN, S. B. & AHUJA, N. 2015. Epigenetic silencing of neurofilament genes promotes an aggressive phenotype in breast cancer. *Epigenetics*, 10, 622-32.
- CARLSSON, A., WATERS, N., HOLM-WATERS, S., TEDROFF, J., NILSSON, M. & CARLSSON, M. L. 2001. Interactions between monoamines, glutamate, and GABA in schizophrenia: new evidence. *Annu Rev Pharmacol Toxicol*, 41, 237-60.
- CAZORLA, M., DE CARVALHO, F. D., CHOCHAN, M. O., SHEGDA, M., CHUHMA, N., RAYPORT, S., AHMARI, S. E., MOORE, H. & KELLENDONK, C. 2014. Dopamine D2 receptors regulate the anatomical and functional balance of basal ganglia circuitry. *Neuron*, 81, 153-64.
- CAZORLA, M., KANG, U. J. & KELLENDONK, C. 2015. Balancing the basal ganglia circuitry: A possible new role for dopamine D2 receptors in health and disease. *Mov Disord*.
- CAZORLA, M., SHEGDA, M., RAMESH, B., HARRISON, N. L. & KELLENDONK, C. 2012. Striatal D2 receptors regulate dendritic morphology of medium spiny neurons via Kir2 channels. *J Neurosci*, 32, 2398-409.
- CES, A., REISS, D., WALTER, O., WICHMANN, J., PRINSEN, E. P., KIEFFER, B. L. & OUAGAZZAL, A. M. 2012. Activation of nociceptin/orphanin FQ peptide receptors disrupts visual but not auditory sensorimotor gating in BALB/cByJ mice: comparison to dopamine receptor agonists. *Neuropsychopharmacology*, 37, 378-89.
- CHA, J., GREENBERG, T., CARLSON, J. M., DEDORA, D. J., HAJCAK, G. & MUJICA-PARODI, L. R. 2014. Circuit-wide structural and functional measures predict ventromedial prefrontal cortex fear generalization: implications for generalized anxiety disorder. *J Neurosci*, 34, 4043-53.
- CHAN, C. S., PETERSON, J. D., GERTLER, T. S., GLAJCH, K. E., QUINTANA, R. E., CUI, Q., SEBEL, L. E., PLOTKIN, J. L., SHEN, W., HEIMAN, M., HEINTZ, N., GREENGARD, P. & SURMEIER, D. J. 2012. Strain-specific regulation of striatal phenotype in *Drd2-eGFP* BAC transgenic mice. *J Neurosci*, 32, 9124-32.
- CHANG, C. K., HAYES, R. D., PERERA, G., BROADBENT, M. T., FERNANDES, A. C., LEE, W. E., HOTOPF, M. & STEWART, R. 2011. Life expectancy at birth for people with serious mental illness and other major disorders from a secondary mental health care case register in London. *PLoS One*, 6, e19590.
- CHANG, R., LIU, X., LI, S. & LI, X. J. 2015. Transgenic animal models for study of the pathogenesis of Huntington's disease and therapy. *Drug Des Devel Ther*, 9, 2179-2188.
- CHAUDHURI, K. R. & NAIDU, Y. 2008. Early Parkinson's disease and non-motor issues. *J Neurol*, 255 Suppl 5, 33-8.
- CHEN, C. H., SUCKLING, J., LENNOX, B. R., OOI, C. & BULLMORE, E. T. 2011. A quantitative meta-analysis of fMRI studies in bipolar disorder. *Bipolar Disord*, 13, 1-15.
- CHIN-CHAN, M., NAVARRO-YEPES, J. & QUINTANILLA-VEGA, B. 2015. Environmental pollutants as risk factors for neurodegenerative disorders: Alzheimer and Parkinson diseases. *Front Cell Neurosci*, 9, 124.
- CINATO, E., MIROTSOU, M. & SABLITZKY, F. 2001. Cre-mediated transgene activation in the developing and adult mouse brain. *Genesis*, 31, 118-25.
- CONTI, B., MAIER, R., BARR, A. M., MORALE, M. C., LU, X., SANNA, P. P., BILBE, G., HOYER, D. & BARTFAI, T. 2007. Region-specific transcriptional changes following the three antidepressant treatments electroconvulsive therapy, sleep deprivation and fluoxetine. *Mol Psychiatry*, 12, 167-89.
- CRASKE, M. G., RAUCH, S. L., URSANO, R., PRENOVEAU, J., PINE, D. S. & ZINBARG, R. E. 2009. What is an anxiety disorder? *Depress Anxiety*, 26, 1066-85.
- CRAWLEY, J. N. 2000. *What's wrong with my mouse?: behavioral phenotype of transgenic and knockout mice*, Wiley-Liss.

- CRITTENDEN, J. R. & GRAYBIEL, A. M. 2011. Basal Ganglia disorders associated with imbalances in the striatal striosome and matrix compartments. *Front Neuroanat*, 5, 59.
- CUI, G., JUN, S. B., JIN, X., PHAM, M. D., VOGEL, S. S., LOVINGER, D. M. & COSTA, R. M. 2013. Concurrent activation of striatal direct and indirect pathways during action initiation. *Nature*, 494, 238-42.
- D'ANDREA, I., FARDELLA, V., FARDELLA, S., PALLANTE, F., GHIGO, A., IACOBUCCI, R., MAFFEI, A., HIRSCH, E., LEMBO, G. & CARNEVALE, D. 2015. Lack of kinase-independent activity of PI3Kgamma in locus coeruleus induces ADHD symptoms through increased CREB signaling. *EMBO Mol Med*, 7, 904-17.
- DALLEY, J. W., FRYER, T. D., BRICHARD, L., ROBINSON, E. S., THEOBALD, D. E., LAANE, K., PENA, Y., MURPHY, E. R., SHAH, Y., PROBST, K., ABAKUMOVA, I., AIGBIRHIO, F. I., RICHARDS, H. K., HONG, Y., BARON, J. C., EVERITT, B. J. & ROBBINS, T. W. 2007. Nucleus accumbens D2/3 receptors predict trait impulsivity and cocaine reinforcement. *Science*, 315, 1267-70.
- DANJO, T., YOSHIMI, K., FUNABIKI, K., YAWATA, S. & NAKANISHI, S. 2014. Aversive behavior induced by optogenetic inactivation of ventral tegmental area dopamine neurons is mediated by dopamine D2 receptors in the nucleus accumbens. *Proc Natl Acad Sci U S A*, 111, 6455-60.
- DAVIS, K. L., KAHN, R. S., KO, G. & DAVIDSON, M. 1991. Dopamine in schizophrenia: a review and reconceptualization. *Am J Psychiatry*, 148, 1474-86.
- DAVIS, M. 1992. The role of the amygdala in fear and anxiety. *Annu Rev Neurosci*, 15, 353-75.
- DAY, M., WANG, Z., DING, J., AN, X., INGHAM, C. A., SHERING, A. F., WOKOSIN, D., ILIJIC, E., SUN, Z., SAMPSON, A. R., MUGNAINI, E., DEUTCH, A. Y., SESACK, S. R., ARBUTHNOTT, G. W. & SURMEIER, D. J. 2006. Selective elimination of glutamatergic synapses on striatopallidal neurons in Parkinson disease models. *Nat Neurosci*, 9, 251-9.
- DAY, M., WOKOSIN, D., PLOTKIN, J. L., TIAN, X. & SURMEIER, D. J. 2008. Differential excitability and modulation of striatal medium spiny neuron dendrites. *J Neurosci*, 28, 11603-14.
- DE BOER, S. F. & KOOLHAAS, J. M. 2003. Defensive burying in rodents: ethology, neurobiology and psychopharmacology. *Eur J Pharmacol*, 463, 145-61.
- DEAN, K. & MURRAY, R. M. 2005. Environmental risk factors for psychosis. *Dialogues Clin Neurosci*, 7, 69-80.
- DEL ZOMPO, M., DELEUZE, J. F., CHILLOTTI, C., COUSIN, E., NIEHAUS, D., EBSTEIN, R. P., ARDAU, R., MACE, S., WARNICH, L., MUJAHED, M., SEVERINO, G., DIB, C., JORDAAN, E., MURAD, I., SOUBIGOU, S., KOEN, L., BANNOURA, I., ROCHER, C., LAURENT, C., DEROCK, M., FAUCON BIGUET, N., MALLET, J. & MELONI, R. 2014. Association study in three different populations between the GPR88 gene and major psychoses. *Mol Genet Genomic Med*, 2, 152-9.
- DELONG, M. R. 1990. Primate models of movement disorders of basal ganglia origin. *Trends Neurosci*, 13, 281-5.
- DEUTCH, A. Y., COLBRAN, R. J. & WINDER, D. J. 2007. Striatal plasticity and medium spiny neuron dendritic remodeling in parkinsonism. *Parkinsonism Relat Disord*, 13 Suppl 3, S251-8.
- DEVAN, B. D., HONG, N. S. & MCDONALD, R. J. 2011. Parallel associative processing in the dorsal striatum: segregation of stimulus-response and cognitive control subregions. *Neurobiol Learn Mem*, 96, 95-120.
- DICKSTEIN, S. G., BANNON, K., CASTELLANOS, F. X. & MILHAM, M. P. 2006. The neural correlates of attention deficit hyperactivity disorder: an ALE meta-analysis. *J Child Psychol Psychiatry*, 47, 1051-62.
- DO, J., KIM, J. I., BAKES, J., LEE, K. & KAANG, B. K. 2012. Functional roles of neurotransmitters and neuromodulators in the dorsal striatum. *Learn Mem*, 20, 21-8.
- DOPHEIDE, J. A. & PLISZKA, S. R. 2009. Attention-deficit-hyperactivity disorder: an update. *Pharmacotherapy*, 29, 656-79.
- DULAWA, S. C., HOLICK, K. A., GUNDERSEN, B. & HEN, R. 2004. Effects of chronic fluoxetine in animal models of anxiety and depression. *Neuropsychopharmacology*, 29, 1321-30.

- DUNCAN, G. E., SHEITMAN, B. B. & LIEBERMAN, J. A. 1999. An integrated view of pathophysiological models of schizophrenia. *Brain Res Brain Res Rev*, 29, 250-64.
- DUNSMOOR, J. E. & PAZ, R. 2015. Fear Generalization and Anxiety: Behavioral and Neural Mechanisms. *Biol Psychiatry*.
- DURIEUX PF, B. B., GUIDUCCI S, BUCH T, WAISMAN A, ZOLI M, ET AL 2009. D2R striatopallidal neurons inhibit both locomotor and drug reward processes. *Nat Neurosci*, :393-395.
- DURIEUX PF, S. S., DE KERCHOVE D'EXAERDE A 2012. Differential regulation of motor control and response to dopaminergic drugs by D1R and D2R neurons in distinct dorsal striatum subregions. 640-653.
- DURIEUX, P. F. B., B. GUIDUCCI, S. BUCH, T., WAISMAN, A., ZOLI, M., SCHIFFMANN, S. N. & DE KERCHOVE D'EXAERDE, A. 2009. D2R striatopallidal neurons inhibit both locomotor and drug reward processes. *Nat Neurosci*, 12, 393-5.
- ENA, S., DE KERCHOVE D'EXAERDE, A. & SCHIFFMANN, S. N. 2011. Unraveling the differential functions and regulation of striatal neuron sub-populations in motor control, reward, and motivational processes. *Front Behav Neurosci*, 5, 47.
- ERDMANN, G., SCHUTZ, G. & BERGER, S. 2007. Inducible gene inactivation in neurons of the adult mouse forebrain. *BMC Neurosci*, 8, 63.
- FARAONE, S. V. & KHAN, S. A. 2006. Candidate gene studies of attention-deficit/hyperactivity disorder. *J Clin Psychiatry*, 67 Suppl 8, 13-20.
- FARAONE, S. V., SERGEANT, J., GILLBERG, C. & BIEDERMAN, J. 2003. The worldwide prevalence of ADHD: is it an American condition? *World Psychiatry*, 2, 104-13.
- FARB, D. H. & RATNER, M. H. 2014. Targeting the modulation of neural circuitry for the treatment of anxiety disorders. *Pharmacol Rev*, 66, 1002-32.
- FEIFEL, D., MINASSIAN, A. & PERRY, W. 2009. Prepulse inhibition of startle in adults with ADHD. *J Psychiatr Res*, 43, 484-9.
- FEIL, R., BROCARD, J., MASCREZ, B., LEMEURE, M., METZGER, D. & CHAMBON, P. 1996. Ligand-activated site-specific recombination in mice. *Proc Natl Acad Sci U S A*, 93, 10887-90.
- FELIX-ORTIZ, A. C., BEYELER, A., SEO, C., LEPLA, C. A., WILDES, C. P. & TYE, K. M. 2013. BLA to vHPC inputs modulate anxiety-related behaviors. *Neuron*, 79, 658-64.
- FENTON, W. S., STOVER, E. L. & INSEL, T. R. 2003. Breaking the log-jam in treatment development for cognition in schizophrenia: NIMH perspective. *Psychopharmacology (Berl)*, 169, 365-6.
- FERGUSON, S. M., ESKENAZI, D., ISHIKAWA, M., WANAT, M. J., PHILLIPS, P. E., DONG, Y., ROTH, B. L. & NEUMAIER, J. F. 2011. Transient neuronal inhibition reveals opposing roles of indirect and direct pathways in sensitization. *Nat Neurosci*, 14, 22-4.
- FERNANDO, A. B. & ROBBINS, T. W. 2011. Animal models of neuropsychiatric disorders. *Annu Rev Clin Psychol*, 7, 39-61.
- FOLSTEIN, S. E., LEIGH, R. J., PARHAD, I. M. & FOLSTEIN, M. F. 1986. The diagnosis of Huntington's disease. *Neurology*, 36, 1279-83.
- FORSS-PETTER, S., DANIELSON, P. E., CATSICAS, S., BATTENBERG, E., PRICE, J., NERENBERG, M. & SUTCLIFFE, J. G. 1990. Transgenic mice expressing beta-galactosidase in mature neurons under neuron-specific enolase promoter control. *Neuron*, 5, 187-97.
- FRANCIS, T. C., CHANDRA, R., FRIEND, D. M., FINKEL, E., DAYRIT, G., MIRANDA, J., BROOKS, J. M., INIGUEZ, S. D., O'DONNELL, P., KRAVITZ, A. & LOBO, M. K. 2015. Nucleus accumbens medium spiny neuron subtypes mediate depression-related outcomes to social defeat stress. *Biol Psychiatry*, 77, 212-22.
- GALANDRIN, S., OLIGNY-LONGPRE, G. & BOUVIER, M. 2007. The evasive nature of drug efficacy: implications for drug discovery. *Trends Pharmacol Sci*, 28, 423-30.
- GARCIA-OTIN, A. L. & GUILLOU, F. 2006. Mammalian genome targeting using site-specific recombinases. *Front Biosci*, 11, 1108-36.

- GAVERIAUX-RUFF, C. & KIEFFER, B. L. 2007. Conditional gene targeting in the mouse nervous system: Insights into brain function and diseases. *Pharmacol Ther*, 113, 619-34.
- GERFEN, C. R. 1992. The neostriatal mosaic: multiple levels of compartmental organization. *Trends Neurosci*, 15, 133-9.
- GERFEN, C. R., ENGBER, T. M., MAHAN, L. C., SUSEL, Z., CHASE, T. N., MONSMA, F. J., JR. & SIBLEY, D. R. 1990. D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. *Science*, 250, 1429-32.
- GERFEN, C. R. & SURMEIER, D. J. 2011. Modulation of striatal projection systems by dopamine. *Annu Rev Neurosci*, 34, 441-66.
- GERTLER, T. S., CHAN, C. S. & SURMEIER, D. J. 2008. Dichotomous anatomical properties of adult striatal medium spiny neurons. *J Neurosci*, 28, 10814-24.
- GETHER, U. 2000. Uncovering molecular mechanisms involved in activation of G protein-coupled receptors. *Endocr Rev*, 21, 90-113.
- GEYER, M. A. 2006. The family of sensorimotor gating disorders: comorbidities or diagnostic overlaps? *Neurotox Res*, 10, 211-20.
- GEYER, M. A. 2008. Developing translational animal models for symptoms of schizophrenia or bipolar mania. *Neurotox Res*, 14, 71-8.
- GEYER, M. A., MCILWAIN, K. L. & PAYLOR, R. 2002. Mouse genetic models for prepulse inhibition: an early review. *Mol Psychiatry*, 7, 1039-53.
- GHANEMI, A. 2015. Targeting G protein coupled receptor-related pathways as emerging molecular therapies. *Saudi Pharm J*, 23, 115-29.
- GHATE, A., BEFORT, K., BECKER, J. A., FILLIOL, D., BOLE-FEYSOT, C., DEMEBELE, D., JOST, B., KOCH, M. & KIEFFER, B. L. 2007. Identification of novel striatal genes by expression profiling in adult mouse brain. *Neuroscience*, 146, 1182-92.
- GITTIS, A. H. & KREITZER, A. C. 2012. Striatal microcircuitry and movement disorders. *Trends Neurosci*, 35, 557-64.
- GIZER, I. R., FICKS, C. & WALDMAN, I. D. 2009. Candidate gene studies of ADHD: a meta-analytic review. *Hum Genet*, 126, 51-90.
- GOEDERT, M., SPILLANTINI, M. G., DEL TREDICI, K. & BRAAK, H. 2013. 100 years of Lewy pathology. *Nat Rev Neurol*, 9, 13-24.
- GOELDNER, C., REISS, D., WICHMANN, J., KIEFFER, B. L. & OUAGAZZAL, A. M. 2009. Activation of nociceptin opioid peptide (NOP) receptor impairs contextual fear learning in mice through glutamatergic mechanisms. *Neurobiol Learn Mem*, 91, 393-401.
- GONG, S., ZHENG, C., DOUGHTY, M. L., LOSOS, K., DIDKOVSKY, N., SCHAMBRA, U. B., NOWAK, N. J., JOYNER, A., LEBLANC, G., HATTEN, M. E. & HEINTZ, N. 2003. A gene expression atlas of the central nervous system based on bacterial artificial chromosomes. *Nature*, 425, 917-25.
- GOTTESMAN, II & ERLNMEYER-KIMLING, L. 2001. Family and twin strategies as a head start in defining prodromes and endophenotypes for hypothetical early-interventions in schizophrenia. *Schizophr Res*, 51, 93-102.
- GOULD, T. D. & EINAT, H. 2007. Animal models of bipolar disorder and mood stabilizer efficacy: a critical need for improvement. *Neurosci Biobehav Rev*, 31, 825-31.
- GOULD, T. D. & MANJI, H. K. 2005. DARPP-32: A molecular switch at the nexus of reward pathway plasticity. *Proc Natl Acad Sci U S A*, 102, 253-4.
- GRACE, A. A., FLORESCO, S. B., GOTO, Y. & LODGE, D. J. 2007. Regulation of firing of dopaminergic neurons and control of goal-directed behaviors. *Trends Neurosci*, 30, 220-7.
- GRAFTON, S. T., HAZELTINE, E. & IVRY, R. 1995. Functional mapping of sequence learning in normal humans. *J Cogn Neurosci*, 7, 497-510.

- GROENEWEGEN, H. J., WRIGHT, C. I., BEIJER, A. V. & VOORN, P. 1999. Convergence and segregation of ventral striatal inputs and outputs. *Ann NY Acad Sci*, 877, 49-63.
- GUNAYDIN, L. A., GROSENICK, L., FINKELSTEIN, J. C., KAUVAR, I. V., FENNO, L. E., ADHIKARI, A., LAMMEL, S., MIRZABEKOV, J. J., AIRAN, R. D., ZALOCUSKY, K. A., TYE, K. M., ANIKEEVA, P., MALENKA, R. C. & DEISSEROTH, K. 2014. Natural neural projection dynamics underlying social behavior. *Cell*, 157, 1535-51.
- HEIMER, L. 2003a. The legacy of the silver methods and the new anatomy of the basal forebrain: implications for neuropsychiatry and drug abuse. *Scand J Psychol*, 44, 189-201.
- HEIMER, L. 2003b. A new anatomical framework for neuropsychiatric disorders and drug abuse. *Am J Psychiatry*, 160, 1726-39.
- HERNANDEZ-ECHEAGARAY, E., STARLING, A. J., CEPEDA, C. & LEVINE, M. S. 2004. Modulation of AMPA currents by D2 dopamine receptors in striatal medium-sized spiny neurons: are dendrites necessary? *Eur J Neurosci*, 19, 2455-63.
- HIGASHI, A. Y., IKAWA, T., MURAMATSU, M., ECONOMIDES, A. N., NIWA, A., OKUDA, T., MURPHY, A. J., ROJAS, J., HEIKE, T., NAKAHATA, T., KAWAMOTO, H., KITA, T. & YANAGITA, M. 2009. Direct hematological toxicity and illegitimate chromosomal recombination caused by the systemic activation of CreERT2. *J Immunol*, 182, 5633-40.
- HIKIDA, T., KIMURA, K., WADA, N., FUNABIKI, K. & NAKANISHI, S. 2010. Distinct roles of synaptic transmission in direct and indirect striatal pathways to reward and aversive behavior. *Neuron*, 66, 896-907.
- HIKIDA, T., YAWATA, S., YAMAGUCHI, T., DANJO, T., SASAOKA, T., WANG, Y. & NAKANISHI, S. 2013. Pathway-specific modulation of nucleus accumbens in reward and aversive behavior via selective transmitter receptors. *Proc Natl Acad Sci U S A*, 110, 342-7.
- HIROI, N., MARTIN, A. B., GRANDE, C., ALBERTI, I., RIVERA, A. & MORATALLA, R. 2002. Molecular dissection of dopamine receptor signaling. *J Chem Neuroanat*, 23, 237-42.
- HISATSUNE, C., OGAWA, N. & MIKOSHIBA, K. 2013. Striatum-specific expression of Cre recombinase using the Gpr88 promoter in mice. *Transgenic Res*, 22, 1241-7.
- HRANILOVIC, D., BUCAN, M. & WANG, Y. 2008. Emotional response in dopamine D2L receptor-deficient mice. *Behav Brain Res*, 195, 246-50.
- HUANG, Y. & THATHIAH, A. 2015. Regulation of neuronal communication by G protein-coupled receptors. *FEBS Lett*.
- HUMBY, T., EDDY, J. B., GOOD, M. A., REICHEL, A. C. & WILKINSON, L. S. 2013. A novel translational assay of response inhibition and impulsivity: effects of prefrontal cortex lesions, drugs used in ADHD, and serotonin 2C receptor antagonism. *Neuropsychopharmacology*, 38, 2150-9.
- IMAYOSHI, I., SAKAMOTO, M., OHTSUKA, T., TAKAO, K., MIYAKAWA, T., YAMAGUCHI, M., MORI, K., IKEDA, T., ITOHARA, S. & KAGEYAMA, R. 2008. Roles of continuous neurogenesis in the structural and functional integrity of the adult forebrain. *Nat Neurosci*, 11, 1153-61.
- INGALLINESI, M., LE BOUIL, L., FAUCON BIGUET, N., DO THI, A., MANNOURY LA COUR, C., MILLAN, M. J., RAVASSARD, P., MALLETT, J. & MELONI, R. 2014. Local inactivation of Gpr88 in the nucleus accumbens attenuates behavioral deficits elicited by the neonatal administration of phencyclidine in rats. *Mol Psychiatry*.
- ISON, J. R., AGRAWAL, P., PAK, J. & VAUGHN, W. J. 1998. Changes in temporal acuity with age and with hearing impairment in the mouse: a study of the acoustic startle reflex and its inhibition by brief decrements in noise level. *J Acoust Soc Am*, 104, 1696-704.
- ISON, J. R. & ALLEN, P. 2003. A diminished rate of "physiological decay" at noise offset contributes to age-related changes in temporal acuity in the CBA mouse model of presbycusis. *J Acoust Soc Am*, 114, 522-8.

- JANKOWSKI, J., SCHEEF, L., HUPPE, C. & BOECKER, H. 2009. Distinct striatal regions for planning and executing novel and automated movement sequences. *Neuroimage*, 44, 1369-79.
- JUN-XU, L., A., T. D. & CHUNYANG, J. 2013. The Gpr88 receptor agonist 2-PCCA does not alter the behavioral effects of methamphetamine in rats. *European Journal of pharmacology*, 272-277.
- JUN, C., CHOI, Y., LIM, S. M., BAE, S., HONG, Y. S., KIM, J. E. & LYOO, I. K. 2014. Disturbance of the glutamatergic system in mood disorders. *Exp Neurobiol*, 23, 28-35.
- KAPLITT, M. G., FEIGIN, A., TANG, C., FITZSIMONS, H. L., MATTIS, P., LAWLOR, P. A., BLAND, R. J., YOUNG, D., STRYBING, K., EIDELBERG, D. & DURING, M. J. 2007. Safety and tolerability of gene therapy with an adeno-associated virus (AAV) borne GAD gene for Parkinson's disease: an open label, phase I trial. *Lancet*, 369, 2097-105.
- KARLSGODT, K. H., SUN, D. & CANNON, T. D. 2010. Structural and Functional Brain Abnormalities in Schizophrenia. *Curr Dir Psychol Sci*, 19, 226-231.
- KELLEY, A. E. 2004. Ventral striatal control of appetitive motivation: role in ingestive behavior and reward-related learning. *Neurosci Biobehav Rev*, 27, 765-76.
- KELLY, E., BAILEY, C. P. & HENDERSON, G. 2008. Agonist-selective mechanisms of GPCR desensitization. *Br J Pharmacol*, 153 Suppl 1, S379-88.
- KENNEDY, K. P., CULLEN, K. R., DEYOUNG, C. G. & KLIMES-DOUGAN, B. 2015. The genetics of early-onset bipolar disorder: A systematic review. *J Affect Disord*, 184, 1-12.
- KIM, C. H., WALDMAN, I. D., BLAKELY, R. D. & KIM, K. S. 2008. Functional gene variation in the human norepinephrine transporter: association with attention deficit hyperactivity disorder. *Ann NY Acad Sci*, 1129, 256-60.
- KLEIN, C. & WESTENBERGER, A. 2012. Genetics of Parkinson's disease. *Cold Spring Harb Perspect Med*, 2, a008888.
- KOHL, S., HEEKEREN, K., KLOSTERKOTTER, J. & KUHN, J. 2013. Prepulse inhibition in psychiatric disorders-- apart from schizophrenia. *J Psychiatr Res*, 47, 445-52.
- KOMATSU, H. 2015. Novel Therapeutic GPCRs for Psychiatric Disorders. *Int J Mol Sci*, 16, 14109-21.
- KOMATSU, H., MARUYAMA, M., YAO, S., SHINOHARA, T., SAKUMA, K., IMAICHI, S., CHIKATSU, T., KUNIYEDA, K., SIU, F. K., PENG, L. S., ZHUO, K., MUN, L. S., HAN, T. M., MATSUMOTO, Y., HASHIMOTO, T., MIYAJIMA, N., ITOH, Y., OGI, K., HABATA, Y. & MORI, M. 2014. Anatomical transcriptome of G protein-coupled receptors leads to the identification of a novel therapeutic candidate GPR52 for psychiatric disorders. *PLoS One*, 9, e90134.
- KONDO, D. G., HELLEM, T. L., SHI, X. F., SUNG, Y. H., PRESCOT, A. P., KIM, T. S., HUBER, R. S., FORREST, L. N. & RENSHAW, P. F. 2014. A review of MR spectroscopy studies of pediatric bipolar disorder. *AJNR Am J Neuroradiol*, 35, S64-80.
- KONSTANTAKOPOULOS, G., DIMITRAKOPOULOS, S. & MICHALOPOULOU, P. G. 2015. Drugs under early investigation for the treatment of bipolar disorder. *Expert Opin Investig Drugs*, 24, 477-90.
- KRAVITZ, A. V. & BONCI, A. 2013. Optogenetics, physiology, and emotions. *Front Behav Neurosci*, 7, 169.
- KRAVITZ, A. V., FREEZE, B. S., PARKER, P. R., KAY, K., THWIN, M. T., DEISSEROTH, K. & KREITZER, A. C. 2010. Regulation of parkinsonian motor behaviours by optogenetic control of basal ganglia circuitry. *Nature*, 466, 622-6.
- KRAVITZ, A. V. & KREITZER, A. C. 2012. Striatal mechanisms underlying movement, reinforcement, and punishment. *Physiology (Bethesda)*, 27, 167-77.
- KRAVITZ, A. V., OWEN, S. F. & KREITZER, A. C. 2013. Optogenetic identification of striatal projection neuron subtypes during in vivo recordings. *Brain Res*, 1511, 21-32.
- KRAVITZ, A. V., TOMASI, D., LEBLANC, K. H., BALER, R., VOLKOW, N. D., BONCI, A. & FERRE, S. 2015. Cortico-striatal circuits: Novel therapeutic targets for substance use disorders. *Brain Res*.
- KRAVITZ, A. V., TYE, L. D. & KREITZER, A. C. 2012. Distinct roles for direct and indirect pathway striatal neurons in reinforcement. *Nat Neurosci*, 15, 816-8.

- KREITZER, A. C. & MALENKA, R. C. 2007. Endocannabinoid-mediated rescue of striatal LTD and motor deficits in Parkinson's disease models. *Nature*, 445, 643-7.
- KRISTIANSEN, K. 2004. Molecular mechanisms of ligand binding, signaling, and regulation within the superfamily of G-protein-coupled receptors: molecular modeling and mutagenesis approaches to receptor structure and function. *Pharmacol Ther*, 103, 21-80.
- KUHN, R. & TORRES, R. M. 2002. Cre/loxP recombination system and gene targeting. *Methods Mol Biol*, 180, 175-204.
- KUMAR, A., KUMAR SINGH, S., KUMAR, V., KUMAR, D., AGARWAL, S. & RANA, M. K. 2015. Huntington's disease: an update of therapeutic strategies. *Gene*, 556, 91-7.
- KUPCHIK, Y. M., BROWN, R. M., HEINSBROEK, J. A., LOBO, M. K., SCHWARTZ, D. J. & KALIVAS, P. W. 2015. Coding the direct/indirect pathways by D1 and D2 receptors is not valid for accumbens projections. *Nat Neurosci*.
- LAGERSTROM, M. C. & SCHIOTH, H. B. 2008. Structural diversity of G protein-coupled receptors and significance for drug discovery. *Nat Rev Drug Discov*, 7, 339-57.
- LAU, J., MINETT, M. S., ZHAO, J., DENNEHY, U., WANG, F., WOOD, J. N. & BOGDANOV, Y. D. 2011. Temporal control of gene deletion in sensory ganglia using a tamoxifen-inducible Advillin-Cre-ERT2 recombinase mouse. *Mol Pain*, 7, 100.
- LE MERRER, J., BEFORT, K., GARDON, O., FILLIOL, D., DARCO, E., DEMBELE, D., BECKER, J. A. & KIEFFER, B. L. 2012a. Protracted abstinence from distinct drugs of abuse shows regulation of a common gene network. *Addict Biol*, 17, 1-12.
- LE MERRER, J., FAGET, L., MATIFAS, A. & KIEFFER, B. L. 2012b. Cues predicting drug or food reward restore morphine-induced place conditioning in mice lacking delta opioid receptors. *Psychopharmacology (Berl)*, 223, 99-106.
- LE, W., SAYANA, P. & JANKOVIC, J. 2014. Animal models of Parkinson's disease: a gateway to therapeutics? *Neurotherapeutics*, 11, 92-110.
- LEO, D. & GAINETDINOV, R. R. 2013. Transgenic mouse models for ADHD. *Cell Tissue Res*, 354, 259-71.
- LEONE, D. P., GENOUD, S., ATANASOSKI, S., GRAUSENBURGER, R., BERGER, P., METZGER, D., MACKLIN, W. B., CHAMBON, P. & SUTER, U. 2003. Tamoxifen-inducible glia-specific Cre mice for somatic mutagenesis in oligodendrocytes and Schwann cells. *Mol Cell Neurosci*, 22, 430-40.
- LEVOYE, A., DAM, J., AYOUB, M. A., GUILLAUME, J. L. & JOCKERS, R. 2006. Do orphan G-protein-coupled receptors have ligand-independent functions? New insights from receptor heterodimers. *EMBO Rep*, 7, 1094-8.
- LEWIS, D. A. & LEVITT, P. 2002. Schizophrenia as a disorder of neurodevelopment. *Annu Rev Neurosci*, 25, 409-32.
- LEWIS, M. & KIM, S. J. 2009. The pathophysiology of restricted repetitive behavior. *J Neurodev Disord*, 1, 114-32.
- LILJEHOLM, M. & O'DOHERTY, J. P. 2012. Contributions of the striatum to learning, motivation, and performance: an associative account. *Trends Cogn Sci*, 16, 467-75.
- LILLRANK, S. M., LIPSKA, B. K. & WEINBERGER, D. R. 1995. Neurodevelopmental animal models of schizophrenia. *Clin Neurosci*, 3, 98-104.
- LIPSKA, B. K. 2004. Using animal models to test a neurodevelopmental hypothesis of schizophrenia. *J Psychiatry Neurosci*, 29, 282-6.
- LOBO, M. K., COVINGTON, H. E., 3RD, CHAUDHURY, D., FRIEDMAN, A. K., SUN, H., DAMEZ-WERNO, D., DIETZ, D. M., ZAMAN, S., KOO, J. W., KENNEDY, P. J., MOUZON, E., MOGRI, M., NEVE, R. L., DEISSEROTH, K., HAN, M. H. & NESTLER, E. J. 2010. Cell type-specific loss of BDNF signaling mimics optogenetic control of cocaine reward. *Science*, 330, 385-90.
- LOBO, M. K., KARSTEN, S. L., GRAY, M., GESCHWIND, D. H. & YANG, X. W. 2006. FACS-array profiling of striatal projection neuron subtypes in juvenile and adult mouse brains. *Nat Neurosci*, 9, 443-52.

- LOBO, M. K. & NESTLER, E. J. 2011. The striatal balancing act in drug addiction: distinct roles of direct and indirect pathway medium spiny neurons. *Front Neuroanat*, 5, 41.
- LODISH, H. 2004. *Molecular Cell Biology*, W. H. Freeman.
- LOGUE, S. F., GRAUER, S. M., PAULSEN, J., GRAF, R., TAYLOR, N., SUNG, M. A., ZHANG, L., HUGHES, Z., PULITO, V. L., LIU, F., ROSENZWEIG-LIPSON, S., BRANDON, N. J., MARQUIS, K. L., BATES, B. & PAUSCH, M. 2009. The orphan GPCR, GPR88, modulates function of the striatal dopamine system: a possible therapeutic target for psychiatric disorders? *Mol Cell Neurosci*, 42, 438-47.
- LOPEZ-HUERTA, V. G., NAKANO, Y., BAUSENWEIN, J., JAIDAR, O., LAZARUS, M., CHERASSE, Y., GARCIA-MUNOZ, M. & ARBUTHNOTT, G. 2015. The neostriatum: two entities, one structure? *Brain Struct Funct*.
- LUFT, A. R. & BUITRAGO, M. M. 2005. Stages of motor skill learning. *Mol Neurobiol*, 32, 205-16.
- LYND-BALTA, E. & HABER, S. N. 1994. The organization of midbrain projections to the striatum in the primate: sensorimotor-related striatum versus ventral striatum. *Neuroscience*, 59, 625-40.
- MACPHERSON, T., MORITA, M. & HIKIDA, T. 2014. Striatal direct and indirect pathways control decision-making behavior. *Front Psychol*, 5, 1301.
- MALETIC, V. & RAISON, C. 2014. Integrated neurobiology of bipolar disorder. *Front Psychiatry*, 5, 98.
- MASSART, G. J., MIGNON, V., SOKOLOFF, P., DIAZ, J. 2009. Striatal GPR88 expression is confined to the whole projection neuron population and is regulated by dopaminergic and glutamatergic afferents. *Eur J Neurosci*, 397-414.
- MATAMALES, M., BERTRAN-GONZALEZ, J., SALOMON, L., DEGOS, B., DENIAU, J. M., VALJENT, E., HERVE, D. & GIRAULT, J. A. 2009. Striatal medium-sized spiny neurons: identification by nuclear staining and study of neuronal subpopulations in BAC transgenic mice. *PLoS One*, 4, e4770.
- MAUDSLEY, S., MARTIN, B. & LUTTRELL, L. M. 2007. G protein-coupled receptor signaling complexity in neuronal tissue: implications for novel therapeutics. *Curr Alzheimer Res*, 4, 3-19.
- MCBRIDE, W. J., MURPHY, J. M. & IKEMOTO, S. 1999. Localization of brain reinforcement mechanisms: intracranial self-administration and intracranial place-conditioning studies. *Behav Brain Res*, 101, 129-52.
- MCINTYRE, R. S., KONARSKI, J. Z. & YATHAM, L. N. 2004. Comorbidity in bipolar disorder: a framework for rational treatment selection. *Hum Psychopharmacol*, 19, 369-86.
- MCINTYRE, R. S., MUZINA, D. J., KEMP, D. E., BLANK, D., WOLDEYOHANNES, H. O., LOFCHY, J., SOCZYNSKA, J. K., BANIK, S. & KONARSKI, J. Z. 2008. Bipolar disorder and suicide: research synthesis and clinical translation. *Curr Psychiatry Rep*, 10, 66-72.
- MEIRSMAN, A. C., LE MERRER, J., PELLISSIER, L. P., DIAZ, J., CLESSE, D., KIEFFER, B. L. & BECKER, J. A. 2015. Mice Lacking GPR88 Show Motor Deficit, Improved Spatial Learning, and Low Anxiety Reversed by Delta Opioid Antagonist. *Biol Psychiatry*.
- METZGER, D. & CHAMBON, P. 2001. Site- and time-specific gene targeting in the mouse. *Methods*, 24, 71-80.
- MINEKA, S. & ZINBARG, R. 2006. A contemporary learning theory perspective on the etiology of anxiety disorders: it's not what you thought it was. *Am Psychol*, 61, 10-26.
- MIRENOWICZ, J. & SCHULTZ, W. 1994. Importance of unpredictability for reward responses in primate dopamine neurons. *J Neurophysiol*, 72, 1024-7.
- MIRENOWICZ, J. & SCHULTZ, W. 1996. Preferential activation of midbrain dopamine neurons by appetitive rather than aversive stimuli. *Nature*, 379, 449-51.
- MIYACHI, S., HIKOSAKA, O. & LU, X. 2002. Differential activation of monkey striatal neurons in the early and late stages of procedural learning. *Exp Brain Res*, 146, 122-6.
- MIYAMOTO, S., MIYAKE, N., JARSKOG, L. F., FLEISCHHACKER, W. W. & LIEBERMAN, J. A. 2012. Pharmacological treatment of schizophrenia: a critical review of the pharmacology and clinical effects of current and future therapeutic agents. *Mol Psychiatry*, 17, 1206-27.

- MIZUSHIMA K, M. Y., TSUKAHARA F, HIRAI M, SAKAKI Y, ITO T 2000. A novel G-protein-coupled receptor gene expressed in striatum. *Genomics*, 314-321.
- MORI, T., TANAKA, K., BUFFO, A., WURST, W., KUHN, R. & GOTZ, M. 2006. Inducible gene deletion in astroglia and radial glia--a valuable tool for functional and lineage analysis. *Glia*, 54, 21-34.
- MURER, M. G., DZIEWCZAPOLSKI, G., SALIN, P., VILA, M., TSENG, K. Y., RUBERG, M., RUBINSTEIN, M., KELLY, M. A., GRANDY, D. K., LOW, M. J., HIRSCH, E., RAISMAN-VOZARI, R. & GERSHANI, O. 2000. The indirect basal ganglia pathway in dopamine D(2) receptor-deficient mice. *Neuroscience*, 99, 643-50.
- MURRAY, G. K., CORLETT, P. R., CLARK, L., PESSIGLIONE, M., BLACKWELL, A. D., HONEY, G., JONES, P. B., BULLMORE, E. T., ROBBINS, T. W. & FLETCHER, P. C. 2008. Substantia nigra/ventral tegmental reward prediction error disruption in psychosis. *Mol Psychiatry*, 13, 239, 267-76.
- NAYDENOV, A. V., SEPER, M. D., SWINNEY, K., RAYMOND, L. A., PALMITER, R. D. & STELLA, N. 2014. Genetic rescue of CB1 receptors on medium spiny neurons prevents loss of excitatory striatal synapses but not motor impairment in HD mice. *Neurobiol Dis*, 71, 140-50.
- NESTLER, E. J. & HYMAN, S. E. 2010. Animal models of neuropsychiatric disorders. *Nat Neurosci*, 13, 1161-9.
- NEWBY, J. M., MCKINNON, A., KUYKEN, W., GILBODY, S. & DALGLEISH, T. 2015. Systematic review and meta-analysis of transdiagnostic psychological treatments for anxiety and depressive disorders in adulthood. *Clin Psychol Rev*, 40, 91-110.
- NICOLA, S. M. 2007. The nucleus accumbens as part of a basal ganglia action selection circuit. *Psychopharmacology (Berl)*, 191, 521-50.
- OGDEN, C. A., RICH, M. E., SCHORK, N. J., PAULUS, M. P., GEYER, M. A., LOHR, J. B., KUCZENSKI, R. & NICULESCU, A. B. 2004. Candidate genes, pathways and mechanisms for bipolar (manic-depressive) and related disorders: an expanded convergent functional genomics approach. *Mol Psychiatry*, 9, 1007-29.
- OLDHAM, W. M. & HAMM, H. E. 2008. Heterotrimeric G protein activation by G-protein-coupled receptors. *Nat Rev Mol Cell Biol*, 9, 60-71.
- OUAGAZZAL, A. M., REISS, D. & ROMAND, R. 2006. Effects of age-related hearing loss on startle reflex and prepulse inhibition in mice on pure and mixed C57BL and 129 genetic background. *Behav Brain Res*, 172, 307-15.
- PANG, T. Y., DU, X., ZAJAC, M. S., HOWARD, M. L. & HANNAN, A. J. 2009. Altered serotonin receptor expression is associated with depression-related behavior in the R6/1 transgenic mouse model of Huntington's disease. *Hum Mol Genet*, 18, 753-66.
- PARK, P. S., LODOWSKI, D. T. & PALCZEWSKI, K. 2008. Activation of G protein-coupled receptors: beyond two-state models and tertiary conformational changes. *Annu Rev Pharmacol Toxicol*, 48, 107-41.
- PARKINSON, J. A., OLMSTEAD, M. C., BURNS, L. H., ROBBINS, T. W. & EVERITT, B. J. 1999. Dissociation in effects of lesions of the nucleus accumbens core and shell on appetitive pavlovian approach behavior and the potentiation of conditioned reinforcement and locomotor activity by D-amphetamine. *J Neurosci*, 19, 2401-11.
- PAXINOS, G. & FRANKLIN, K. B. J. 2001. *The mouse brain in stereotaxic coordinates*, academic press.
- PECINA, M., MICKEY, B. J., LOVE, T., WANG, H., LANGENECKER, S. A., HODGKINSON, C., SHEN, P. H., VILLAFUERTE, S., HSU, D., WEISENBACH, S. L., STOHLER, C. S., GOLDMAN, D. & ZUBIETA, J. K. 2013. DRD2 polymorphisms modulate reward and emotion processing, dopamine neurotransmission and openness to experience. *Cortex*, 49, 877-90.
- PERRY, W., MINASSIAN, A., FEIFEL, D. & BRAFF, D. L. 2001. Sensorimotor gating deficits in bipolar disorder patients with acute psychotic mania. *Biol Psychiatry*, 50, 418-24.
- PINEDA, D. A., PALACIO, L. G., PUERTA, I. C., MERCHAN, V., ARANGO, C. P., GALVIS, A. Y., GOMEZ, M., AGUIRRE, D. C., LOPERA, F. & ARCOS-BURGOS, M. 2007. Environmental influences that affect attention deficit/hyperactivity disorder: study of a genetic isolate. *Eur Child Adolesc Psychiatry*, 16, 337-46.

- POHLKAMP, T., STELLER, L., MAY, P., GUNTHER, T., SCHULE, R., FROTSCHER, M., HERZ, J. & BOCK, H. H. 2014. Generation and characterization of an Nse-CreERT2 transgenic line suitable for inducible gene manipulation in cerebellar granule cells. *PLoS One*, 9, e100384.
- POULADI, M. A., MORTON, A. J. & HAYDEN, M. R. 2013. Choosing an animal model for the study of Huntington's disease. *Nat Rev Neurosci*, 14, 708-21.
- POWELL, S. B., WEBER, M. & GEYER, M. A. 2012. Genetic models of sensorimotor gating: relevance to neuropsychiatric disorders. *Curr Top Behav Neurosci*, 12, 251-318.
- PRADHAN, A. A., BECKER, J. A., SCHERRER, G., TRYOEN-TOTH, P., FILLIOL, D., MATIFAS, A., MASSOTTE, D., GAVERIAUX-RUFF, C. & KIEFFER, B. L. 2009. In vivo delta opioid receptor internalization controls behavioral effects of agonists. *PLoS One*, 4, e5425.
- PURPER-OUAKIL, D., RAMOZ, N., LEPAGNOL-BESTEL, A. M., GORWOOD, P. & SIMONNEAU, M. 2011. Neurobiology of attention deficit/hyperactivity disorder. *Pediatr Res*, 69, 69R-76R.
- QUINTANA A, S. E., WANG W, STOREY GP, GULER AD, WANAT MJ, ET AL. 2012. Lack of GPR88 enhances medium spiny neuron activity and alters motor- and cue-dependent behaviors. *Nat Neurosci.*, 1547-1555.
- RADER, R., MCCAULEY, L. & CALLEN, E. C. 2009. Current strategies in the diagnosis and treatment of childhood attention-deficit/hyperactivity disorder. *Am Fam Physician*, 79, 657-65.
- RALPH-WILLIAMS, R. J., LEHMANN-MASTEN, V., OTERO-CORCHON, V., LOW, M. J. & GEYER, M. A. 2002. Differential effects of direct and indirect dopamine agonists on prepulse inhibition: a study in D1 and D2 receptor knock-out mice. *J Neurosci*, 22, 9604-11.
- RALPH, R. J., VARTY, G. B., KELLY, M. A., WANG, Y. M., CARON, M. G., RUBINSTEIN, M., GRANDY, D. K., LOW, M. J. & GEYER, M. A. 1999. The dopamine D2, but not D3 or D4, receptor subtype is essential for the disruption of prepulse inhibition produced by amphetamine in mice. *J Neurosci*, 19, 4627-33.
- RAPOPORT, J. L., ADDINGTON, A. M., FRANGOU, S. & PSYCH, M. R. 2005. The neurodevelopmental model of schizophrenia: update 2005. *Mol Psychiatry*, 10, 434-49.
- RASK-ANDERSEN, M., ALMEN, M. S. & SCHIOTH, H. B. 2011. Trends in the exploitation of novel drug targets. *Nat Rev Drug Discov*, 10, 579-90.
- RAUCH, S. L., SHIN, L. M. & PHELPS, E. A. 2006. Neurocircuitry models of posttraumatic stress disorder and extinction: human neuroimaging research--past, present, and future. *Biol Psychiatry*, 60, 376-82.
- RAZAFSHA, M., BEHFORUZI, H., HARATI, H., WAFAI, R. A., KHAKU, A., MONDELLO, S., GOLD, M. S. & KOBEISSY, F. H. 2013. An updated overview of animal models in neuropsychiatry. *Neuroscience*, 240, 204-18.
- RING, H. A. & SERRA-MESTRES, J. 2002. Neuropsychiatry of the basal ganglia. *J Neurol Neurosurg Psychiatry*, 72, 12-21.
- RONALD, A., PENNELL, C. E. & WHITEHOUSE, A. J. 2010. Prenatal Maternal Stress Associated with ADHD and Autistic Traits in early Childhood. *Front Psychol*, 1, 223.
- ROSENBLATT, A. 2007. Neuropsychiatry of Huntington's disease. *Dialogues Clin Neurosci*, 9, 191-7.
- ROSENSTEIN, J. M. 1993. Developmental expression of neuron-specific enolase immunoreactivity and cytochrome oxidase activity in neocortical transplants. *Exp Neurol*, 124, 208-18.
- ROSS, C. A. & TABRIZI, S. J. 2011. Huntington's disease: from molecular pathogenesis to clinical treatment. *Lancet Neurol*, 10, 83-98.
- RUSSELL, V. A. 2007. Neurobiology of animal models of attention-deficit hyperactivity disorder. *J Neurosci Methods*, 161, 185-98.
- SAGVOLDEN, T. 2000. Behavioral validation of the spontaneously hypertensive rat (SHR) as an animal model of attention-deficit/hyperactivity disorder (AD/HD). *Neurosci Biobehav Rev*, 24, 31-9.
- SAGVOLDEN, T., RUSSELL, V. A., AASE, H., JOHANSEN, E. B. & FARSHBAF, M. 2005. Rodent models of attention-deficit/hyperactivity disorder. *Biol Psychiatry*, 57, 1239-47.

- SALIN, P., DZIEWCZAPOLSKI, G., GERSHANI, O. S., NIEOULLON, A. & RAISMAN-VOZARI, R. 1997. Differential regional effects of long-term L-DOPA treatment on preproenkephalin and preprotachykinin gene expression in the striatum of 6-hydroxydopamine-lesioned rat. *Brain Res Mol Brain Res*, 47, 311-21.
- SAMSOM, J. N. & WONG, A. H. 2015. Schizophrenia and Depression Co-Morbidity: What We have Learned from Animal Models. *Front Psychiatry*, 6, 13.
- SCHERES, A., MILHAM, M. P., KNUTSON, B. & CASTELLANOS, F. X. 2007. Ventral striatal hypo-responsiveness during reward anticipation in attention-deficit/hyperactivity disorder. *Biol Psychiatry*, 61, 720-4.
- SCHIFFMANN, S. N., JACOBS, O. & VANDERHAEGHEN, J. J. 1991. Striatal restricted adenosine A2 receptor (RDC8) is expressed by enkephalin but not by substance P neurons: an in situ hybridization histochemistry study. *J Neurochem*, 57, 1062-7.
- SCHIFFMANN, S. N. & VANDERHAEGHEN, J. J. 1993. Adenosine A2 receptors regulate the gene expression of striatopallidal and striatonigral neurons. *J Neurosci*, 13, 1080-7.
- SCHIZOPHRENIA WORKING GROUP OF THE PSYCHIATRIC GENOMICS, C. 2014. Biological insights from 108 schizophrenia-associated genetic loci. *Nature*, 511, 421-7.
- SCHNEIER, F. R. 2006. Clinical practice. Social anxiety disorder. *N Engl J Med*, 355, 1029-36.
- SHINOZAKI, G. & POTASH, J. B. 2014. New developments in the genetics of bipolar disorder. *Curr Psychiatry Rep*, 16, 493.
- SHUEN, J. A., CHEN, M., GLOSS, B. & CALAKOS, N. 2008. Drd1a-tdTomato BAC transgenic mice for simultaneous visualization of medium spiny neurons in the direct and indirect pathways of the basal ganglia. *J Neurosci*, 28, 2681-5.
- SMITH, R. J., LOBO, M. K., SPENCER, S. & KALIVAS, P. W. 2013. Cocaine-induced adaptations in D1 and D2 accumbens projection neurons (a dichotomy not necessarily synonymous with direct and indirect pathways). *Curr Opin Neurobiol*, 23, 546-52.
- SOHAL, D. S., NGHIEM, M., CRACKOWER, M. A., WITT, S. A., KIMBALL, T. R., TYMITZ, K. M., PENNINGER, J. M. & MOKKENTIN, J. D. 2001. Temporally regulated and tissue-specific gene manipulations in the adult and embryonic heart using a tamoxifen-inducible Cre protein. *Circ Res*, 89, 20-5.
- STEIN, M. B., SIMMONS, A. N., FEINSTEIN, J. S. & PAULUS, M. P. 2007. Increased amygdala and insula activation during emotion processing in anxiety-prone subjects. *Am J Psychiatry*, 164, 318-27.
- STINE, O. C., PLEASANT, N., FRANZ, M. L., ABBOTT, M. H., FOLSTEIN, S. E. & ROSS, C. A. 1993. Correlation between the onset age of Huntington's disease and length of the trinucleotide repeat in IT-15. *Hum Mol Genet*, 2, 1547-9.
- STRAKOWSKI, S. M., DELBELLO, M. P., ZIMMERMAN, M. E., GETZ, G. E., MILLS, N. P., RET, J., SHEAR, P. & ADLER, C. M. 2002. Ventricular and periventricular structural volumes in first- versus multiple-episode bipolar disorder. *Am J Psychiatry*, 159, 1841-7.
- STREJILEVICH, S. A., SAMAME, C. & MARTINO, D. J. 2015. The trajectory of neuropsychological dysfunctions in bipolar disorders: a critical examination of a hypothesis. *J Affect Disord*, 175, 396-402.
- STROHLE, A., STOY, M., WRASE, J., SCHWARZER, S., SCHLAGENHAUF, F., HUSS, M., HEIN, J., NEDDERHUT, A., NEUMANN, B., GREGOR, A., JUCKEL, G., KNUTSON, B., LEHMKUHL, U., BAUER, M. & HEINZ, A. 2008. Reward anticipation and outcomes in adult males with attention-deficit/hyperactivity disorder. *Neuroimage*, 39, 966-72.
- SURMEIER, D. J., DING, J., DAY, M., WANG, Z. & SHEN, W. 2007. D1 and D2 dopamine-receptor modulation of striatal glutamatergic signaling in striatal medium spiny neurons. *Trends Neurosci*, 30, 228-35.
- SVENNINGSSON, P., LEMOINE, C., FISONE, G. & FREDHOLM, B. B. 1999. Distribution, biochemistry and function of striatal adenosine A2A receptors. *Prog Neurobiol*, 59, 355-96.
- SVENNINGSSON, P., NISHI, A., FISONE, G., GIRAULT, J. A., NAIRN, A. C. & GREENGARD, P. 2004. DARPP-32: an integrator of neurotransmission. *Annu Rev Pharmacol Toxicol*, 44, 269-96.

- SWERDLOW, N. R., BRAFF, D. L. & GEYER, M. A. 2000. Animal models of deficient sensorimotor gating: what we know, what we think we know, and what we hope to know soon. *Behav Pharmacol*, 11, 185-204.
- SWERDLOW, N. R. & GEYER, M. A. 1998. Using an animal model of deficient sensorimotor gating to study the pathophysiology and new treatments of schizophrenia. *Schizophr Bull*, 24, 285-301.
- SWERDLOW, N. R., GEYER, M. A. & BRAFF, D. L. 2001. Neural circuit regulation of prepulse inhibition of startle in the rat: current knowledge and future challenges. *Psychopharmacology (Berl)*, 156, 194-215.
- TANIMURA, Y., KING, M. A., WILLIAMS, D. K. & LEWIS, M. H. 2011. Development of repetitive behavior in a mouse model: roles of indirect and striosomal basal ganglia pathways. *Int J Dev Neurosci*, 29, 461-7.
- TANIMURA, Y., VAZIRI, S. & LEWIS, M. H. 2010. Indirect basal ganglia pathway mediation of repetitive behavior: attenuation by adenosine receptor agonists. *Behav Brain Res*, 210, 116-22.
- TARAZI, F. I., SAHLI, Z. T., WOLNY, M. & MOUSA, S. A. 2014. Emerging therapies for Parkinson's disease: from bench to bedside. *Pharmacol Ther*, 144, 123-33.
- THAPAR, A., FOWLER, T., RICE, F., SCOURFIELD, J., VAN DEN BREE, M., THOMAS, H., HAROLD, G. & HAY, D. 2003. Maternal smoking during pregnancy and attention deficit hyperactivity disorder symptoms in offspring. *Am J Psychiatry*, 160, 1985-9.
- TOTH, I. & NEUMANN, I. D. 2013. Animal models of social avoidance and social fear. *Cell Tissue Res*, 354, 107-18.
- TRIPP, G. & WICKENS, J. 2012. Reinforcement, dopamine and rodent models in drug development for ADHD. *Neurotherapeutics*, 9, 622-34.
- TSAI, G. & COYLE, J. T. 2002. Glutamatergic mechanisms in schizophrenia. *Annu Rev Pharmacol Toxicol*, 42, 165-79.
- TYE, K. M. & DEISSEROTH, K. 2012. Optogenetic investigation of neural circuits underlying brain disease in animal models. *Nat Rev Neurosci*, 13, 251-66.
- TYE, K. M., PRAKASH, R., KIM, S. Y., FENNO, L. E., GROSENICK, L., ZARABI, H., THOMPSON, K. R., GRADINARU, V., RAMAKRISHNAN, C. & DEISSEROTH, K. 2011. Amygdala circuitry mediating reversible and bidirectional control of anxiety. *Nature*, 471, 358-62.
- UBALDI, M., RICCIARDELLI, E., PASQUALINI, L., SANNINO, G., SOVERCHIA, L., RUGGERI, B., FALCINELLI, S., RENZI, A., LUDKA, C., CICCOCIOPPO, R. & HARDIMAN, G. 2015. Biomarkers of hippocampal gene expression in a mouse restraint chronic stress model. *Pharmacogenomics*, 16, 471-82.
- UNGLESS, M. A. 2004. Dopamine: the salient issue. *Trends Neurosci*, 27, 702-6.
- UNGLESS, M. A., MAGILL, P. J. & BOLAM, J. P. 2004. Uniform inhibition of dopamine neurons in the ventral tegmental area by aversive stimuli. *Science*, 303, 2040-2.
- VALADAS, J. S., VOS, M. & VERSTREKEN, P. 2015. Therapeutic strategies in Parkinson's disease: what we have learned from animal models. *Ann NY Acad Sci*, 1338, 16-37.
- VALERA, E. M., FARAONE, S. V., MURRAY, K. E. & SEIDMAN, L. J. 2007. Meta-analysis of structural imaging findings in attention-deficit/hyperactivity disorder. *Biol Psychiatry*, 61, 1361-9.
- VALJENT, E., BERTRAN-GONZALEZ, J., HERVE, D., FISIONE, G. & GIRAULT, J. A. 2009. Looking BAC at striatal signaling: cell-specific analysis in new transgenic mice. *Trends Neurosci*, 32, 538-47.
- VAN WAES V, T. K., STEINER H 2011. GPR88 - a putative signaling molecule predominantly expressed in the striatum: Cellular localization and developmental regulation. *Basal Ganglia*, 83-89.
- VINTHER-JENSEN, T., NIELSEN, T. T., BUDTZ-JORGENSEN, E., LARSEN, I. U., HANSEN, M. M., HASHOLT, L., HJERMIND, L. E., NIELSEN, J. E. & NORREMOLLE, A. 2015. Psychiatric and cognitive symptoms in Huntington's disease are modified by polymorphisms in catecholamine regulating enzyme genes. *Clin Genet*.
- VOGT, M. A., CHOURBAJI, S., BRANDWEIN, C., DORMANN, C., SPRENGEL, R. & GASS, P. 2008. Suitability of tamoxifen-induced mutagenesis for behavioral phenotyping. *Exp Neurol*, 211, 25-33.

- VOORN, P., VANDERSCHUREN, L. J., GROENEWEGEN, H. J., ROBBINS, T. W. & PENNARTZ, C. M. 2004. Putting a spin on the dorsal-ventral divide of the striatum. *Trends Neurosci*, 27, 468-74.
- WEBER, P., METZGER, D. & CHAMBON, P. 2001. Temporally controlled targeted somatic mutagenesis in the mouse brain. *Eur J Neurosci*, 14, 1777-83.
- WEBER, T., VOGT, M. A., GARTSIDE, S. E., BERGER, S. M., LUJAN, R., LAU, T., HERRMANN, E., SPRENGEL, R., BARTSCH, D. & GASS, P. 2015. Adult AMPA GLUA1 receptor subunit loss in 5-HT neurons results in a specific anxiety-phenotype with evidence for dysregulation of 5-HT neuronal activity. *Neuropsychopharmacology*, 40, 1471-84.
- WICHMANN, T. & DELONG, M. R. 1996. Functional and pathophysiological models of the basal ganglia. *Curr Opin Neurobiol*, 6, 751-8.
- WILENSKY, A. E., SCHAFE, G. E., KRISTENSEN, M. P. & LEDOUX, J. E. 2006. Rethinking the fear circuit: the central nucleus of the amygdala is required for the acquisition, consolidation, and expression of Pavlovian fear conditioning. *J Neurosci*, 26, 12387-96.
- WONG, A. H. & JOSSELYN, S. A. 2015. Caution When Diagnosing Your Mouse with Schizophrenia: The Use and Misuse of Model Animals for Understanding Psychiatric Disorders. *Biol Psychiatry*.
- WOO, H., PARK, S. J., LEE, Y., KWON, G., GAO, Q., LEE, H. E., AHN, Y. J., SHIN, C. Y., CHEONG, J. H. & RYU, J. H. 2014. The effects of atomoxetine and methylphenidate on the prepulse inhibition of the acoustic startle response in mice. *Prog Neuropsychopharmacol Biol Psychiatry*, 54, 206-15.
- WOOD, H. B. & EPISKOPOU, V. 1999. Comparative expression of the mouse Sox1, Sox2 and Sox3 genes from pre-gastrulation to early somite stages. *Mech Dev*, 86, 197-201.
- XIA, R. & MAO, Z. H. 2012. Progression of motor symptoms in Parkinson's disease. *Neurosci Bull*, 28, 39-48.
- YIN, H. H., MULCARE, S. P., HILARIO, M. R., CLOUSE, E., HOLLOWAY, T., DAVIS, M. I., HANSSON, A. C., LOVINGER, D. M. & COSTA, R. M. 2009. Dynamic reorganization of striatal circuits during the acquisition and consolidation of a skill. *Nat Neurosci*, 12, 333-41.
- YOUNG, R. C. 2005. Bipolar disorder in older persons: perspectives and new findings. *Am J Geriatr Psychiatry*, 13, 265-7.
- YOUNGSTROM, E. A., FINDLING, R. L., YOUNGSTROM, J. K. & CALABRESE, J. R. 2005. Toward an evidence-based assessment of pediatric bipolar disorder. *J Clin Child Adolesc Psychol*, 34, 433-48.
- ZHANG, K., HILL, K., LABAK, S., BLATT, G. J. & SOGHOMONIAN, J. J. 2014. Loss of glutamic acid decarboxylase (Gad67) in Gpr88-expressing neurons induces learning and social behavior deficits in mice. *Neuroscience*, 275, 238-47.
- ZIMMERMANN, A. M., JENE, T., WOLF, M., GORLICH, A., GURNIAK, C. B., SASSOE-POGNETTO, M., WITKE, W., FRIAUF, E. & RUST, M. B. 2014. Attention-Deficit/Hyperactivity Disorder-like Phenotype in a Mouse Model with Impaired Actin Dynamics. *Biol Psychiatry*.
- ZOLOTUKHIN, S., POTTER, M., ZOLOTUKHIN, I., SAKAI, Y., LOILER, S., FRAITES, T. J., JR., CHIODO, V. A., PHILLIPSBERG, T., MUZYCZKA, N., HAUSWIRTH, W. W., FLOTTE, T. R., BYRNE, B. J. & SNYDER, R. O. 2002. Production and purification of serotype 1, 2, and 5 recombinant adeno-associated viral vectors. *Methods*, 28, 158-67.

Résumé étendu de Thèse

GPR88 est un récepteur couplé à protéine G (RCPG) orphelin exprimé dans le système nerveux central principalement au niveau du striatum (caudé-putamen, Cpu et noyau Accumbens, Nac) et de l'amygdale centrale (CeA). Actuellement peu d'études adressent le rôle de GPR88 *in vivo*. Des études montrent une régulation de l'expression de GPR88 suite à des traitements antidépresseurs et des régulateurs d'humeurs. De plus Logue et collaborateur (2009) propose que l'inactivation de l'expression de GPR88 pourrait conduire à une pathologie de type schizophrénique. L'ensemble de ces données suggère que GPR88 pourrait jouer un rôle important dans le développement et/ou l'expression de pathologies psychiatriques. Ces troubles atteignent une population jeune dont les soins représentent d'énormes investissements de la part des familles et de l'État. Le DSMIV compte aujourd'hui plus d'une dizaine de catégories de troubles psychiatriques dont les causes restent à élucider. La recherche dans le domaine des pathologies psychiatriques représente ainsi un des grands axes de recherche scientifique en France et dans le monde.

Mon projet de thèse a pour but d'étudier dans le temps et dans l'espace la fonction de GPR88 dans le développement et/ou l'expression de troubles psychiatrique (*e.g.* schizophrénie, trouble obsessionnel compulsif, trouble de l'attention/hyperactivité) et moteurs (*e.g.* Parkinson).

Notre laboratoire a créé une lignée de souris knockout (KO) constitutive pour GPR88 (*GPR88*^{-/-}) et montré que celles-ci présentent des phénotypes caractéristiques de modèles de pathologies psychiatriques comme la schizophrénie (déficit du filtre sensorimoteur, hyperactivité motrice, exacerbation de l'effet locomoteur des amphétamines, stéréotypies, diminution de l'anxiété avec augmentation de la prise de risque), ainsi que de pathologies motrices comme la maladie de Parkinson (déficit de coordination motrice).

J'ai contribué à cette étude en examinant si GPR88 interagit avec d'autres RCPG inhibiteurs. Nous avons évalué les réponses [S35]-GTPγS induites par des agonistes spécifiques sur des membranes de striatum de souris *GPR88*^{-/-}. Nous avons testé l'activation des récepteurs à la dopamine D2, mGluR glutamatérique, m2/m4 muscariniques ainsi que les récepteurs opioïdes Delta et Mu. L'analyse du profil d'activation de ces RCPG inhibiteurs nous indique que la délétion de *Gpr88* mène

à une forte augmentation de l'activation des récepteurs opioïdes Delta et Mu. Cette information nous a permis de montrer que la diminution d'anxiété et le déficit de coordination motrice présentée par les souris *Gpr88*^{-/-} sont restaurés par l'administration d'un antagoniste du récepteur opioïde Delta (Naltrindole) (Figure 1).

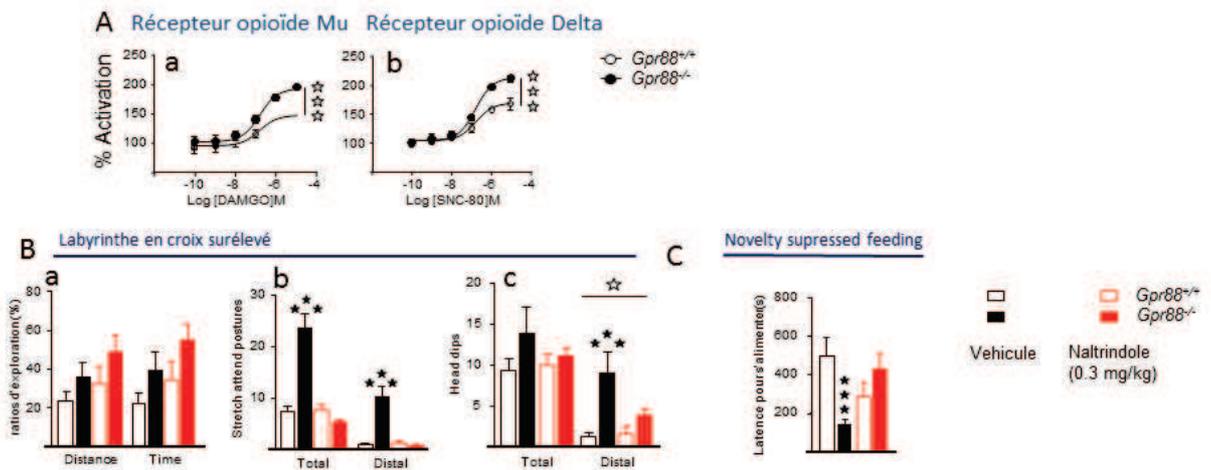


Figure 1: La délétion de *Gpr88* cause une augmentation de l'activation du récepteur opioïde Delta partiellement responsable d'une diminution des comportement du type anxieux. (A) Les essais [S35]-GTPγS sur des membranes de striatum de souris mutantes et sauvages indiquent une augmentation de la transmission Mu (a) et Delta (b) chez les souris *Gpr88*^{-/-}. (B) Certains phénotypes du labyrinthe en croix surélevé comme l'augmentation des « stretch attend postures » (b) et des « head dips » (c) effectués dans la zone distale de l'appareil sont contrecarrés par l'administration chronique d'un antagoniste du récepteur Delta (Naltrindol). De même, la diminution de neophagie des souris *Gpr88*^{-/-} est contrecarrée par l'administration de l'antagoniste Delta (C). Les données sont représentées en moyenne ± SEM. Les étoiles ouvertes représentent les ANOVA à 2 facteurs et les étoiles noires représentent l'analyse t de Student. Une étoile: $p < 0.05$; 2 étoiles: $p < 0.01$; 3 étoiles: $p < 0.0001$.

Nous avons ensuite décidé de nous pencher sur les mécanismes *cellulaires* de GPR88 dans le striatum. Cette région est la structure d'entrée des ganglions de la base, un système de noyaux sous-corticaux fortement impliqués dans le contrôle moteur et les processus motivationnels, et dont le fonctionnement est altéré dans des pathologies aussi bien motrices que psychiatriques.

Les neurones de projection du striatum peuvent être divisés en deux populations selon la structure de projection et les récepteurs exprimés. On y distingue ainsi la voie Directe (Striato-Nigral) qui co-exprime le récepteur à la dopamine D1 (D1R) et à la substance P ; et la voie Indirecte

(striato-pallidale) qui exprime le récepteur D2 (D2R), A_{2A} et Enképhaline. L'Ensemble des données de la littérature suggère un rôle antagoniste de ces deux voies de projection ; cependant, plusieurs études récentes mettent le modèle classique en cause et soulignent l'importance des possibles interactions entre ces deux voies pour le bon fonctionnement des circuits des ganglions de la base. De façon intéressante, et contrairement à la plupart des récepteurs exprimés dans le striatum, GPR88 est fortement exprimé dans les deux voies de projections.

Afin de mieux comprendre le rôle de ce récepteur au sein du striatum nous avons développé un knockout du gène dans chacune des deux populations principales du striatum (voies *directe* et *indirecte*) par une approche Cre/Lox. Nous avons commencé par croisé des souris *Gpr88*^{flox/flox} avec des souris exprimant la cre-recombinase sous contrôle du promoteur du récepteur A_{2A} (*Gpr88*^{ff} X Adora2-Cre_ cKO de la voie *Indirecte*). La vérification, par marquage d'hybridation *in situ*, de la délétion du gène du récepteur GPR88 (A_{2A}-GPR88), nous a montré une diminution de l'ARNm de ce gène spécifiquement dans les neurones exprimant le récepteur D2R dans le striatum (dorsal et ventral) ainsi que dans le noyau centrale de l'amygdale (Figure 2). J'ai caractérisé le knockout du gène dans cette lignée et ai procédé à l'étude du profil d'activation des RCPG inhibiteurs sur ces souris knockout conditionnelles. Nous avons testé l'activation des récepteurs à la dopamine D2, mGluR glutamatèrgique, m2/m4 muscariniques ainsi que les récepteurs opioïdes Delta et Mu. Les résultats montrent que la délétion conditionnelle de *Gpr88* mène aussi à une forte augmentation de l'activation des récepteurs opioïdes Delta et Mu ainsi qu'à une altération de l'activation du récepteur à la dopamine D2 et une diminution de l'activation des RCPG du glutamate (mGluR). La caractérisation comportementale de cette lignée nous a permis de montrer que la délétion de *Gpr88* dans la voie indirecte est suffisante pour causer une forte diminution de l'anxiété, sans affecter les réponses physiologiques de peur.

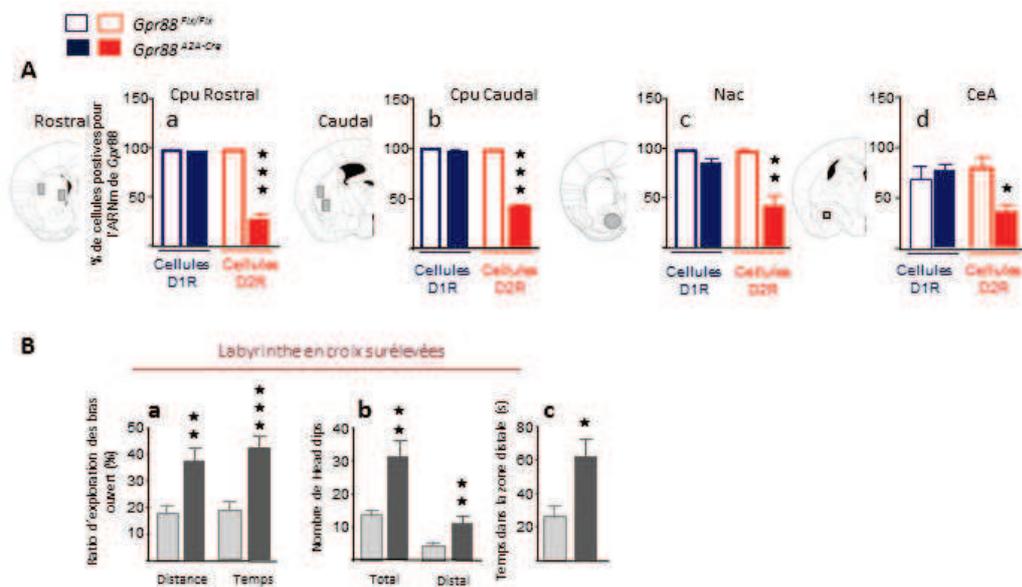


Figure 2: La délétion sélective de *Gpr88* dans les neurones exprimant le récepteur à la dopamine D2 (A) mène à une diminution du comportement du type anxieux. (A) comptage cellulaire sur triple hybridation *in situ* avec sonde fluorescente. (a-d) La quantification de l'ARNm de *Gpr88* montre une diminution de celui-ci dans les neurones exprimant D2R (rouge) mais pas D1R (bleu) dans le caudé-putamen (Cpu: a,b) noyau accumbens (Nac: c) et amygdale central (CeA: d) des souris exprimant la Cre sous le contrôle du promoteur du gène *Adora2a* (*Gpr88* *A2A-Cre*). Le pourcentage d'expression de *Gpr88* a été calculé par rapport au nombre total de cellules comptés exprimant D1R ou D2R [(nombre de cellules exprimant D1R ou D2R co-exprimant *Gpr88* x 100)/ nombre totale de cellules exprimant D1R or D2R]. Quand les souris *Gpr88* *A2A-Cre* sont placés dans un labyrinthe en croix surélevées elles montrent un pourcentage supérieur de distance et temps passé en bras ouvert que leurs congénères (a). Aussi, ces souris mutantes plongent leurs têtes dans le vide (« head dips ») plus souvent (b) et passent plus de temps que les souris contrôles dans la zone distale des bras ouvert (c). Les données sont représentées en moyenne \pm SEM. Les étoiles noires représentent l'analyses t de student. Une étoile: $p < 0.05$; 2 étoiles: $p < 0.01$; 3 étoiles: $p < 0.0001$

Lors de la caractérisation de la lignée *Gpr88*^{-/-} nous avons aussi vérifié que ces souris présentent des déficits sévères de **coordination motrice**. Afin de vérifier les mécanismes par lesquels GPR88 régule la coordination motrice nous avons réalisé le knockout conditionnel (cKO) inducible de *Gpr88* chez l'adulte.

Pour ce faire j'ai réalisé des injections stéréotaxiques de virus recombinant associés à l'adénovirus (AAV) codant pour la Cre recombinase dans le striatum de souris *GPR88* floxés adultes. Cette approche consiste à réaliser des injections stéréotaxiques de virus recombinant associés à l'adénovirus (AAV) codant pour la Cre recombinase sur des souris *GPR88* floxés adultes.

Au long de ma première année de thèse j'avais déterminé les coordonnées stéréotaxiques, la souche virale, et le temps nécessaire à l'invalidation génomique et fonctionnelle du récepteur GPR88 dans le striatum. Les expériences fonctionnelles sur des échantillons de striatum nous ont permis de montrer une diminution de 43% de l'expression du récepteur 4 semaines après l'injection du virus.

Ainsi, j'ai généré des cohortes expérimentales composé de souris injectées avec de l'AAV-Cre-eGFP (groupe expérimentale, N= 24) ou de l'AAV-eGFP (souris contrôles, N=22). Chaque cohorte a été soumise à une batterie de tests comportementaux, s'étendant sur 4 semaines, incluant des tests émotionnels (ex : Elevated plus maze) et moteurs (ex : Rotarod). A la fin de chaque batterie de tests les striatum de chaque souris étaient prélevés pour effectuer des essais GTPγS permettant de quantifié le pourcentage de diminution de l'activité du récepteur GPR88.

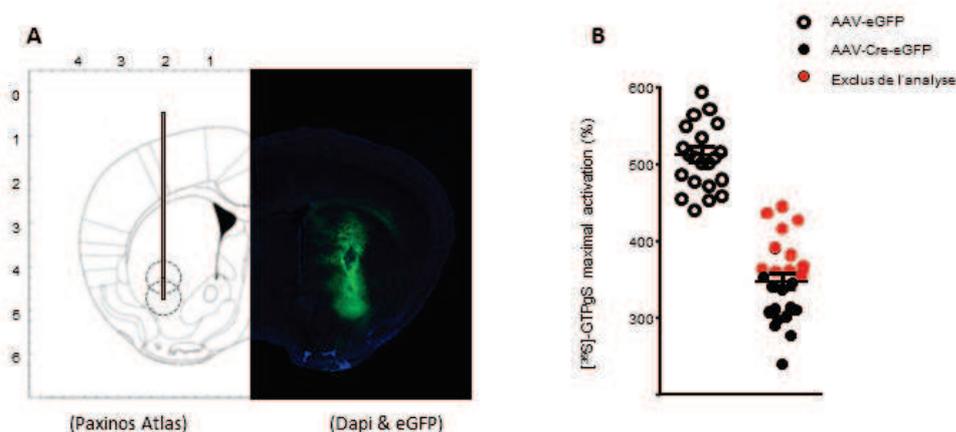


Figure 3: Excision conditionnelle de *Gpr88* dans le striatum de souris adulte (8 semaines). La figure 1A montre le site d'injection basé sur l'atlas paxinos (panel de gauche) et le signal eGFP suite à l'injection du virus associé à l'adenovirus (AAV). Les noyaux cellulaires ont été marqués au DAPI. Les essais [³⁵S]-GTPγS réalisés suite aux tests comportementaux montrent que l'expression de la Cre par l'injection d'AAV a mené à une forte diminution de l'activation de GPR88 (cercles noirs). Toutefois, les souris dont l'activation de GPR88 était égale ou supérieure à 70% de l'activation des souris contrôle ont été exclus de toute analyses statistique (cercles rouges).

Nous avons ainsi montré que si la délétion de *Gpr88* dans le striatum de la souris adulte cause un déficit de coordination motrice, cette délétion n'affecte pas les phénotypes émotionnels de ces souris. Le premier résultat nous permet d'affirmer que GPR88 possède une fonction tonique qui régule les fonctions motrices chez le rongeur. Quant au dernier, différentes explications peuvent surgir : soit GPR88 ne possède pas de fonction tonique sur les processus émotionnels, soit cette fonction (tonique) est exercée par des récepteurs GPR88 exprimés dans d'autres structures cérébrales.

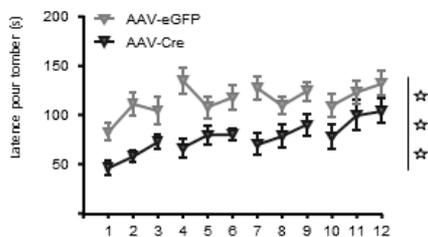


Figure 4: Diminution de la coordination motrice suite à l'injection d'AAV-Cre chez la souris. Quatre semaines après les injections stéréotaxiques les souris expérimentales (AAV-Cre) et contrôles (AAV-Cre-eGFP) ont été placées sur un rotarod et la latence pour tomber de celui-ci a été mesurée. L'ANOVA montre une diminution significative du temps passé sur l'appareil pour les souris AAV-Cre. Les données sont représentées en moyenne \pm SEM. Les étoiles ouvertes représentent les ANOVA à 2 facteurs. Une étoile: $p < 0.05$; 2 étoiles: $p < 0.01$; 3 étoiles: $p < 0.0001$

Afin de vérifier si GPR88 possède un rôle tonique dans d'autres structures cérébrales nous avons généré des animaux GPR88 cKO inducible en croisant les animaux GPR88 floxés avec une souris transgénique exprimant une Cre-recombinase inducible par le tamoxifène (CreER^{T2}) sous le contrôle d'un promoteur neuronal : l'Enolase 2 (ENO2-CreERT2). Dans un premier temps, j'avais déterminé que 15 jours d'exposition au tamoxifène entraînait non seulement une excision robuste et reproductible du gène codant pour GPR88, mais aussi une diminution de 41% de la réponse GTP γ S induite par un agoniste spécifique du récepteur.

Ainsi, j'ai générée des cohortes expérimentales et soumis les souris à une batterie de tests incluant des tests émotionnels et moteurs (ex : Elevated plus maze, Rotarod). Considérant nos résultats indiquant un rôle tonique de GPR88 dans le striatum sur la coordination motrice, l'absence totale

de phénotype par l'approche Cre-ERT2 suggère que l'excision générée par cette technique est insuffisante pour révéler un phénotype. Ainsi, j'ai testé différentes doses et voies d'administration de tamoxifène afin d'aboutir à une excision supérieure à celle que nous avons obtenue précédemment (Table 1). Malheureusement, nous ne sommes jamais aboutis à des niveaux satisfaisants d'excision et ce projet a été abandonné.

Table 1: Résumé des traitements au tamoxifène administrés à différentes lignées de souris

i.p.: intrapéritonéale; *p.o.*: per os.

Protocole	voie	Quantité /injection	Volume /injection	Traitements /jour	Total /jour	nb de jours	Gpr88-Sox 2-Cre-ERT ²	Gpr88-Eno2-Cre-ERT ²	Gpr88-NeFL-Cre-ERT ²
Protocole 1	<i>i.p.</i>	1 mg	100 µL	2	2mg	5	x	x	
Protocole 2	<i>i.p.</i>	1 mg	100 µL	2	2mg	15		x	x
Protocole 3	<i>p.o.</i>	10mg	300µL	1	10mg	2			x
Protocole 4	<i>p.o.</i>	5 mg	150µL	2	10mg	5			x
Protocole 5*	<i>i.p.</i>	5mg	150µL	1	5mg	5			x
Protocole 6*	<i>p.o.</i>	5mg	150µL	1	5mg	5			x

* Ces protocoles ont mené à une toxicité excessive et ont été immédiatement abandonnés pour des raisons éthiques

Finalement, nous avons aussi voulu vérifier si l'excision constitutive de GPR88 dans la voie *Indirecte* du striatum suffisait pour causer un déficit moteur. J'ai comparé les souris *GPR88*^{-/-} et *A_{2A}-GPR88* dans un test de coordination motrice (rotarod) avec deux degrés de difficultés. Les résultats indiquent que si les souris *GPR88*^{-/-} présentent des déficits de coordination motrice dans les deux conditions et indépendamment de la difficulté de la tâche, les souris *A_{2A}-GPR88* présentent un déficit uniquement dans les conditions de difficulté accrue. Ce résultat suggère que GPR88 dans la voie indirecte du striatum participe à la régulation de la coordination motrice en interaction avec GPR88 exprimé dans la voie directe du striatum.

Pour vérifier cette hypothèse il est impératif d'évaluer l'impact de l'absence de GPR88 dans la voie directe sur la coordination motrice. Pour ce faire, nous développons couramment une souris

knockout constitutive conditionnelle de GPR88 dans les neurones de la voie directe du striatum ($GPR88^{f/f}$ X D1-Cre).

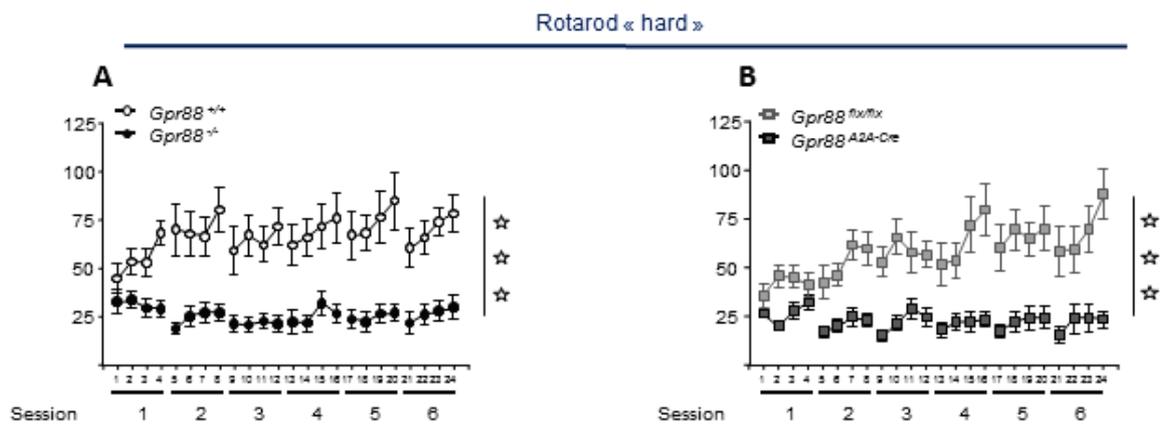


Figure 5: coordination motrice chez les souris KO total et conditionnels . Les deux lignées de souris ont été testées pour une version plus difficile du rotarod où le cylindre, plus fin, est plus difficile à accrocher. Avec ce protocole les KO total (A) (N=10 KO et 9 WT) et conditionnels (B) (N= 9 $Gpr88^{flx/flx}$, 10 $Gpr88^{A2A-Cre}$) présentent un déficit de coordination motrice. Les données sont représentées en moyenne \pm SEM. 3 étoiles: $p < 0.001$ (effet genotype, Two-way ANOVA)

Aussi, nous avons testé les effets locomoteurs des agonistes des récepteurs D1 (D1R, spécifiquement exprimé au niveau de la voie directe) et D2 (D2R, spécifiquement exprimé au niveau de la voie indirecte) à la dopamine sur les souris $GPR88^{-/-}$ et A_{2A} -GPR88. Nous avons observé que, en absence de GPR88 dans la voie indirecte du striatum, les souris présentent une sensibilité augmentée aux effets locomoteurs de l'agoniste des récepteurs D2 à la dopamine mais aussi de l'agoniste des récepteurs D1 à la dopamine. Ce résultat suggère que la délétion de $Gpr88$ au sein de la voie indirecte peut avoir causé des altérations au niveau de la voie directe. Un résultat similaire a

été obtenue avec les souris *GPR88*^{-/-}. Aussi, nous avons testé la catalepsie induite par l'antagoniste du récepteur 2 à la dopamine (halopéridol) sur les souris *GPR88*^{-/-} et A_{2A}-GPR88. Les résultats de cette analyse montrent que la délétion de *Gpr88* dans la voie indirecte augmente la sensibilité aux effets cataleptiques de l'administration de l'antipsychotique halopéridol. Ce résultats n'a pas été observé sur les souris *GPR88*^{-/-} qui présentent la même catalepsie que les souris contrôles. Ensemble ces résultats montrent que la délétion de *Gpr88* sur les deux voies ou uniquement sur la voie indirecte du striatum n'a pas le même effet sur le fonctionnement des récepteurs à la dopamine D2.

Le rôle de GPR88 ne se limite pas au processus moteurs et émotionnel. En effet, j'ai confirmé que l'absence ubiquitaire de GPR88 menait à un **déficit du filtre sensorimoteur** (mesuré par le test du Prépulse inhibition_PPI) et ceci sur plusieurs modalités sensorielles (auditif, visuel et gap détection). Der plus nous avons montré que ce déficit n'est pas lié à un déficit général d'inhibition. Le test du PPI est utilisé chez l'humain pour le diagnostic de plusieurs pathologies psychiatriques. Chez le rongeur, ce test est utilisé sous des conditions quasiment identiques et constitue un outil primordial dans l'étude de pathologies neuropsychiatriques. Nous avons donc voulu vérifier si GPR88 dans la voie indirecte du striatum jouaient un rôle dans le phénomène d'inhibition du prépulse. Nos études nous ont permis de montrer que la délétion constitutive de *Gpr88* dans la voie indirecte du striatum ne suffisent pas à causer un déficit d'inhibition du prépulse, indépendamment de la modalité sensorielle.

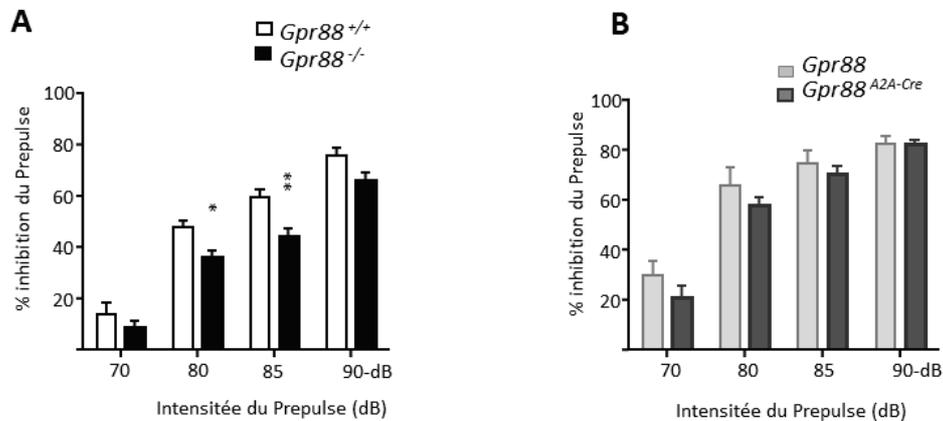


Figure 5: Déficit d'inhibition du prepulse (PPI) acoustique chez les souris *Gpr88*^{-/-}. (A) En accord avec des données publiées, les souris *Gpr88*^{-/-} présentent un déficit de PPI significatif pour les prépulse de 80 et 85 dB. (B) Contrairement au KO total, les souris *Gpr88*^{A2A-Cre} présentent les mêmes valeurs de PPI que leurs congénères suggérant un filtre sensorimoteur intact. Les données sont représentées en moyenne \pm SEM. Les étoiles de texte représentent l'analyse post hoc suite à l'ANOVA. Une étoile: $p < 0.05$; 2 étoiles: $p < 0.01$; 3 étoiles: $p < 0.0001$.

Par ailleurs, j'ai aussi montré que l'administration aiguë de méthylphénidate (traitement pharmacologique du trouble de déficit de l'attention avec hyperactivité, TDAH) réduit l'hyperactivité motrice des souris *GPR88*^{-/-} confirmant l'importance de ce récepteur dans l'étude des mécanismes liés au TDAH (non publié).

En conclusion, mon projet de thèse a permis de positionner de façon décisive le récepteur GPR88 comme une nouvelle cible pour le traitement de plusieurs troubles. Nous avons pour la première fois montré que ce récepteur joue un rôle dans les processus liés à l'anxiété. Aussi, nos données montrent que ce rôle serait joué par les récepteurs situés dans les neurones striataux exprimant le récepteur D2R. Aussi nous avons montré que GPR88 interagit fonctionnellement avec le récepteur opiacé Delta et que l'excision du gène de GPR88 dans les neurones D2R du striatum suffit à augmenter l'activation du récepteur Delta. Au niveau moteur, nous avons prouvé qu'une altération de ce récepteur chez l'adulte peut être liée aux déficits de coordination motrice observés

en clinique. Aussi, nos résultats suggèrent que GPR88 régule la coordination motrice par une action combinée dans les neurones striato-pallidaux et striato-nigraux. . Cependant, la délétion de ce récepteur uniquement au niveau striatopallidal suffit pour altérer les réponses aux agents dopaminergiques. Ces résultats innovants permettront non seulement d'approfondir nos connaissances sur les processus liés à l'anxiété mais aussi l'envisagement de nouveaux traitements pour les troubles associés.



Aura Callia Carole MEIRSMAN

**Rôle du récepteur orphelin
GPR88 dans les pathologies
psychiatriques et motrices**

Résumé

GPR88 est un récepteur couplé aux protéines G orphelin exprimé principalement au niveau du striatum spécifiquement dans les neurones moyens épineux de la voie striato-nigrale et de la voie striato-pallidale.

Premièrement nous avons étudié les souris Gpr88 KO et montré des altérations biochimiques, structurales et comportementales. Aussi les résultats montrent que l'hyperactivité des souris Gpr88 KO est diminuée par l'administration de méthylphénidate.

Deuxièmement nous avons montré que la diminution des comportements liés à l'anxiété dépend de GPR88 dans la voie striato-pallidale et que la coordination motrice est régulée par GPR88 dans le striatum adulte (injection AAV-Cre) et dans la voie striato-pallidale.

Dernièrement, nous avons confirmé un déficit d'inhibition du prépulse chez les souris Gpr88 KO, mais aussi montré que celui-ci s'étend à la modalité visuelle et n'est pas lié à un déficit général d'inhibition ou à la délétion de Gpr88 dans les neurones striato-pallidaux.

Mots clefs: récepteur couplé à protéine G; Anxiété; coordination motrice; inhibition du prépulse; striatum; neurones moyen épineux.

Résumé en anglais

Among brain orphan G protein-coupled receptors, GPR88 shows high expression mainly in the striatum specifically in medium spiny neurons of both the striatonigral and striatopallidal pathways

First, we examine full Gpr88 KO mice and show biochemical, structural and behavioral alterations. Results also show that the hyperactivity phenotype of Gpr88 KO mice is reversed by Methylphenidate.

Second, we show that Gpr88 in striatopallidal neurons (cKO approach) exerts anxiogenic activity and that motor coordination is regulated by GPR88 in the adult brain (AAV-Cre approach) and in the striatopallidal pathway.

Finally, we confirmed previous data showing impaired acoustic prepulse inhibition in Gpr88 KO mice and further show that this deficit is not the result of a general inhibition deficit or of the lack of GPR88 in striatopallidal neurons.

Keywords: G protein coupled receptor; Anxiety; motor coordination; prepulse inhibition; striatum; medium spiny neurons.