



Validating Gpr88 as a new therapeutic target in models of the neuropsychiatric symptoms of Parkinson's Disease

Benjamin Galet

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Validating Gpr88 as a new therapeutic target in models of the neuropsychiatric symptoms of Parkinson's Disease.

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PhD thesis in Neurosciences

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List of abbreviations

3'UTR	3' Untranslated Region	DAT	Dopamine Transporter
5HT	5-Hydroxytryptamine (Serotonin)	DBS	Deep Brain Stimulation
5HT1A/1D/2A	Serotonin Receptors 1A/1D/2A	DLS	Dorsolateral Striatum
6OHDA	6-Hydroxydopamine	DMN	Default Mode Network
α-syn	Alpha-Synuclein	DMS	Dorsomedial Striatum
A2AR	Adenosine A2A Receptor	dMSN	direct/indirect pathway
AADC	Aromatic L-Amino Acid Decarboxylase	iMSN	Medium Spiny Neurons
AAV	Adeno-Associated Virus	DOR	Delta Opioid Receptor
ACh/AChE	Acetylcholine/Acetylcholinesterase	DRY	Aspartate–Arginine–Tyrosine
AIM	Abnormal Involuntary Movements	emGFP	Emerald Green Fluorescent Protein
AMPA	Ionotropic Glutamate Receptor (A-Amino-3-Hydroxy-5-Methyl-4-Isoxazolepropionic Acid)	F-DOPA	Fluorodopa
Amph	Amphetamine	FDA	Food and Drug Administration
ANOVA	Analysis Of Variance	fMRI	Functional Magnetic Resonance Imaging
ATP	Adenosine Triphosphate	FSI	Fast-Spiking Interneurons
AUD	Alcohol Use Disorder	FST	Forced Swim Test
BDNF	Brain-Derived Neurotrophic Factor	Gi/o	Gi / Go Alpha Subunit
BG	Basal Ganglia	GABA	Gamma-Aminobutyric Acid
cAMP	Cyclic Adenosine Monophosphate	GABAA	Type A GABA Receptor
CB1	Cannabinoid Receptor Type 1	Gad67	Glutamate Decarboxylase 67
CNS	Central Nervous System	GBA	Glucocerebrosidase
D1/2/3	Dopamine Receptor Type 1/2/3	GLU	Glutamate (-ergic)
D2DR	Gene Encoding The Dopamine Receptor Type 2	GluR1	Glutamate AMPA Receptor Type 1
DA	Dopamine (-ergic)	GPCR	G-Protein Coupled Receptor
DARPP32	Dopamine and cAMP Regulated Phosphoprotein	GPi / GPe	Internal/External Globus Pallidus
		GPR88	G-Protein Coupled Receptor 88
		HIV-1	Human Immunodeficiency Virus Type 1

IBZM	Iodobenzamide	ORF	Open Reading Frame
ICD	Impulse-Control Disorders	PBS	Phosphate-Buffered Saline
IN	Interneuron	PD	Parkinson's Disease
KD/KO	Knock-Down/Knock-Out	<i>Pdyn</i>	Prodynorphin gene
L-DOPA	L-3,4-Dihydroxyphenylalanine	<i>Penk</i>	Proenkephalin gene
LC	Locus Coeruleus	PET	Positron Emission Tomography
LID	L-Dopa Induced Dyskinesia	PGK	Phosphoglycerate Kinase
LRRK2	Leucine-Rich Repeat Kinase 2	PIGD	Postural-Instability-Gait Disorder
LV	Lentivirus	PINK1	PTEN-Induced Kinase 1
M1/M4	Muscarinic Acetylcholine Receptor Type 1 and 4	Pol II	RNA Polymerase II
MCI	Mild Cognitive Impairment	PPI	Prepulse Inhibition
MD	Motor-Dominant	PPN	Pedunculopontine Nucleus
MFB	Medial Forebrain Bundle	RBD	REM sleep Behaviour Disorder
miR-Gpr88 / miR-neg	Engineered miRNA directed against <i>Gpr88</i> / no target	REM	Rapid Eye Movement
miRNA	MicroRNA	RGS4	Regulator Of G-Protein Signalling 4
MOR	Mu-Opioid Receptor	RNA	Ribonucleic Acid
MPTP	1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine	RNAi	RNA Interference
mRNA	Messenger RNA	SERT	Serotonin transporter
MSN	Medium Spiny Neuron	shRNA	Short Hairpin RNA
NA	Noradrenalin / Norepinephrine	SNCA	Synuclein Gene
NAcc	Nucleus Accumbens	SND	Social Novelty Discrimination
NINDS	National Institute of Neurological Disorders And Stroke	SPECT	Single-Photon Emission Computed Tomography
NIMH	National Institute of Mental Health	STN	Subthalamic Nucleus
NMDA	Ionotropic Glutamate Receptor (N-Methyl-D-Aspartic Acid)	TAN	Tonically Active Interneurons
NMS	Non-Motor Symptoms	TH	Tyrosine Hydroxylase
		VS	Ventral Striatum
		VTA	Ventral Tegmental Area

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Résumé en français

La Maladie de Parkinson (MP) est une maladie neurodégénérative chronique, caractérisée par une perte précoce et progressive de l'innervation dopaminergique provenant de la Substance Noire, et qui s'accompagne également de l'altération d'autres systèmes de neurotransmetteurs.

Bien que longtemps considérée comme étant purement motrice, la MP est en réalité une maladie qui se déclare d'abord par ses symptômes neurovégétatifs (tels que des troubles sensoriels et du sommeil) et psychiatriques (dont la dépression, l'apathie, et des déficits cognitifs). Ces aspects sont d'autant plus importants qu'ils ont le plus lourd impact sur la qualité de vie des patients, particulièrement au cours des stades avancés de la maladie, lors desquels le développement de démences et de psychoses sont des facteurs majeurs de perte d'autonomie.

Depuis une cinquantaine d'années, le traitement de la MP repose essentiellement sur des stratégies de remplacement de la dopamine. Bien qu'étant très utiles lors des premières phases de traitement, elles sont limitées dans le temps par leur perte d'efficacité et le développement de complications sévères telles que des dyskinésies et des troubles de l'impulsivité. De plus, leur action restreinte au système dopaminergique nécessite fréquemment l'apport de médicaments ciblant d'autres systèmes de neurotransmetteurs, qui ont une efficacité variable et qui s'accompagnent d'effets secondaires supplémentaires. Il y a donc un réel besoin médical de cibles thérapeutiques aux mécanismes novateurs pour le traitement de l'ensemble des symptômes de la MP.

Dans ce contexte, le récepteur orphelin GPR88 constitue une cible particulièrement adaptée. Notre laboratoire s'est intéressé au potentiel de ce récepteur dans le traitement des désordres des ganglions de la base suite à notre identification de son association génétique au trouble bipolaire et à la schizophrénie. Plus récemment, des mutations invalidantes de *GPR88* ont également été associées à des déficits comportementaux sévères, dont une hypermotricité et des altérations de l'apprentissage.

Ce récepteur est présent essentiellement sur les neurones épineux moyens (NEM) du striatum, où son expression est particulièrement enrichie dans la région la plus affectée par la perte de dopamine lors de la MP. Au niveau subcellulaire, Gpr88 est préférentiellement localisé aux synapses corticostriatales, où il exerce une activité inhibitrice sur la neurotransmission (notamment dopaminergique, glutamatergique et opioïdergique). Ces caractéristiques lui confèrent un rôle important dans la régulation des comportements dépendants du striatum, qui sont particulièrement affectés lors de la MP.

Au cours de cette thèse, je me suis donc intéressé à évaluer le potentiel de GPR88 comme cible thérapeutique pour les symptômes moteurs et psychiatriques de la MP. Sur la base des caractéristiques de ce récepteur, nous avons émis l'hypothèse que son inhibition localisée permettrait de potentialiser la neurotransmission endogène, et ainsi d'avoir un effet bénéfique sur les déficits comportementaux.

Pour tester cette hypothèse, nous avons développé deux modèles de la MP chez le rat grâce à des injections stéréotaxiques de 6-hydroxydopamine (6-OHDA), une toxine induisant la dégénérescence des neurones dopaminergiques. Un premier modèle classique de lésion unilatérale totale a été mis en place pour évaluer les effets sur les symptômes moteurs. Afin de modéliser les symptômes psychiatriques sans interférences motrices, nous avons aussi développé un modèle des stades précoces de la MP, en reproduisant la perte initiale de dopamine observée chez les patients. Dans une démarche de thérapie génique, nous avons ensuite utilisé des vecteurs lentiviraux afin d'inactiver l'expression de *Gpr88* de manière précise dans différentes régions du striatum dorsal. Enfin, les effets ont été évalués grâce à des batteries de tests comportementaux, ainsi que des analyses moléculaires afin d'établir les mécanismes sous-jacents.

Nos résultats indiquent que l'inactivation localisée de *Gpr88* permet de réduire les déficits moteurs, motivationnels et cognitifs induits par les lésions dopaminergiques. De plus, les analyses moléculaires démontrent que ces effets sont portés par une réduction de l'hyperactivité des NEM de la voie indirecte, et une forte modulation du facteur de transcription Δ FosB. Enfin, nous avons également identifié dans le modèle des stades précoces de la MP des dynamiques striatales représentatives de celles observées chez les patients, et qui sont impliquées dans d'autres pathologies neuropsychiatriques.

Ces résultats permettent donc de valider l'intérêt de Gpr88 comme cible thérapeutique pour les symptômes neuropsychiatriques de la MP. De futures expériences nous aideront notamment à raffiner notre compréhension des mécanismes moléculaires impliqués. Ce travail s'inscrit dans la continuité d'une étude précédente du laboratoire, qui démontrait un effet thérapeutique de l'inactivation de *Gpr88* dans le *Nucleus Accumbens* d'un modèle animal de schizophrénie. Sur la base de ces travaux, nous proposons que le mode d'action de Gpr88 en fasse également une cible pertinente pour d'autres désordres des ganglions de la base, tels que l'addiction.

Introduction

Chapter 1 - Parkinson's Disease

Discovery

Parkinson's disease (PD) was first described medically 200 years ago by the physician and geologist James Parkinson as "the Shaking Palsy"¹, resulting from the association of resting tremor and slow or absent voluntary movements (bradykinesia/akinesia). 50 years later, Jean-Martin Charcot at the Salpêtrière hospital was the first to provide detailed descriptions of the clinical manifestations of the disorder, and separated tremor and bradykinesia as cardinal features of PD, that do not necessary coexist². The iconic biological feature of PD - degeneration of neurons from the Substantia Nigra - was first suggested by Brissaud in 1899³, and later supported by work from Trétiakoff in 1919⁴, who observed neuronal loss in that structure. Hornykiewicz was however the first to officially link dopamine to PD in 1960⁵, and to suggest the use of L-DOPA as a treatment⁶. These discoveries paved the way to decades of research and therapeutic development, that have seen PD evolve from a purely motor and dopaminergic phenomenon, to a vastly more complex and heterogeneous neuropsychiatric disorder.

Epidemiology

PD is the second most frequent neurodegenerative disorder after Alzheimer's Disease, affecting over 10 million people worldwide⁷, and over 200 000 in France⁸. The estimated yearly total cost (direct + indirect) of the disease in the United States alone is of 25 billion dollars⁷. A study found that for US citizens over 40 years old, their lifetime risk of developing PD is of 2% for men, and 1,3% for women⁹. As implied by this metric, prevalence is higher in men than in women, by a 3:2 ratio⁹. Life expectancy is shortened by 5 to 10 years for patients with PD compared to the general population, depending on the age of onset¹⁰. Causes of death are also strongly influenced by PD pathology, with for instance frequent cases of fatal pneumonia due to swallowing problems and aspiration of food in the lungs¹¹. The main risk factor for developing PD is ageing, as the prevalence rapidly increases after 50 years of age, to peak around 80. Indeed, while it affects an average of 14 in 100 000 people, this number goes up to 160 when considering people aged 65 or older⁹. Because of the global ageing of the human population, the number of people who develop the disease is expected to increase by over 50% in the next ten years¹².

A. Symptomatology

In this following section, the symptoms of untreated *de novo* Parkinson's Disease will be described. The symptoms that are linked to medical treatments will be addressed further in the introduction.

1. Motor symptoms

PD has historically been defined as a motor disorder. The main motor symptoms consist in bradykinesia (slowness in the initiation and execution of movements), as well as resting tremor and rigidity, which all strongly impair the performance of daily tasks¹². These symptoms usually manifest themselves initially as unilateral, or asymmetrical. As the disease progresses, there is an overall worsening of motor skills, leading to postural reflex disturbances (flexed postures and instability), as well as freezing of gait and frequent falls. The motor impairments may also affect facial muscles, leading to hypomimia (masked facies) and reduced blinking, which confer a blank facial expression to patients¹³.

The prevalence of tremor in the clinical profile of patients has led to motor sub-typing of PD: "tremor-dominant", "non-tremor-dominant", and intermediate profiles. The "non-tremor-dominant" PD seems to progress at a faster rate, and is associated with a worse prognosis¹³. Efforts to organise the heterogeneity of Parkinsonian clinical profiles will be discussed later in the introduction.

2. Psychiatric symptoms

While motor impairment is considered the core feature of PD, it is in most cases accompanied by non-motor symptoms (NMS) which are a great burden on the quality of life of patients¹⁴ (**Figure 1**). Interestingly, many of these non-motor symptoms may appear before the onset of the motor disability - sometimes over 10 years ahead of time¹⁵ - and could thus constitute good prodromal markers of PD.

i. Mood and motivation

Alterations in mood and motivation are common in PD, and often encompass depression, apathy, anhedonia and anxiety. Although depression in PD seems to be milder than non-PD depression¹⁶, it is typically reported in around 40 % of patients^{16,17}, and is one of the symptoms with the greatest negative impact on quality of life¹⁴. The depressive syndrome can frequently involve apathy and anhedonia as part of its forms of expression, and is often associated with anxiety.

However, while apathy in PD has long been considered as a symptom of the depressive state, its high prevalence (reports ranging from 17 to 60%)^{18,19} and occurrence without other depressive symptoms has led clinicians to consider it as a separate core feature of PD. Apathy is defined as a reduction in voluntary, goal-directed behaviour, and has been characterised as originating from multiple sub-components: emotional, cognitive and auto-activation apathy²⁰. While all subtypes can be observed in PD¹⁹, the frequent co-occurrence of cognitive impairment and apathy most resembles the “cognitive” subtype²¹. Furthermore, apathy constitutes a risk factor for the development of cognitive decline and dementia in PD patients²².

Anhedonia, defined as a reduced ability to experience pleasure²³, has also been reported at high frequencies in PD (47% according to a study)²⁴. However, it may be a challenging symptom to evaluate in patients as its lack a clear definition, has no evaluation scale validated for PD, and is common to the emotional expression of both apathy and depression^{18,23}.

Finally, anxiety has also been reported in up to 60% of PD patients¹⁶, and may further influence depressive and apathetic symptoms. Indeed, anxiety has a strong co-morbidity for depression, and can also impair goal-directed behaviours by inducing avoidance¹⁹.

As with other non-motor symptoms, the appearance of mood-related alterations often predates the onset of motor symptoms, suggesting that their presence may not be due to a psychological reaction to the PD state²⁵. At the time of diagnosis, a study found that the most frequent mood-related symptoms were depression (37 %), apathy (27 %) and anxiety (17%)²⁶.

ii. Cognition

Cognitive dysfunction is also frequent in PD, manifesting itself in earlier stages as mild cognitive impairments (MCI), which can frequently evolve into dementia. The Rotterdam prospective study, following almost 8000 participants over two decades, found that those who went on to develop PD presented some first signs of cognitive decline as early as 7 years before diagnosis²⁷. These alterations may however become prevalent only in the last couple of years before the onset of motor symptoms¹⁵. At the time of PD diagnosis, up to 40% of patients exhibit impairment of executive function²⁸, a domain responsible for the cognitive control of behaviour. Such deficits are reflected in alterations spanning attentional processing, action and sequence planning, decision making, inhibitory control of action, abstract thinking and cognitive flexibility²⁹⁻³¹. Interestingly, these cognitive deficits have been linked to impairment of sensorimotor filtering³². Alterations in memory have also been observed in patients³³.

The rate of cognitive decline is rather slow at first, and is often followed by an "inflexion point", after which the rate is increased³⁴. In later stages of the disease, up to 80% of patients may develop dementia, which is characterised by further deficits in memory and visuospatial function³⁴. The development of PD dementia also comes with a high comorbidity for Alzheimer's Disease, which may affect up to one third of demented patients¹³.

iii. Hallucinations and psychosis

PD patients may also suffer from psychotic symptoms, which manifest themselves at first as visual illusions and hallucinations, and at later stages as delusions and paranoid ideation^{17,35}. Although these symptoms were previously thought to reflect different symptom classes, a work group composed of the NINDS & NIMH (National Institute of Neurological Disorders and Stroke and National Institute of Mental Health) concluded in 2007 that they should rather be considered as forming a continuum that evolves over PD progression³⁵. PD psychosis has also commonly been viewed as a potential consequence of DA treatment. Although DA medication does increase the risk of developing psychotic symptoms³⁵, recent evidence has shown that such symptoms may appear at the earliest stages of PD, before the use of medication. For instance, 42 % of patients with early PD were found to have already experienced minor hallucinations³⁶, out of which one third also experienced such symptoms in the pre-motor phase, up to 8 years before the

onset of motor symptoms. These minor hallucinations mainly consist in passage and presence effects³⁵. The prevalence of PD psychosis increases with time, with as many as 83% of advanced PD patients experiencing symptoms on the psychosis spectrum, or 60% when excluding minor hallucinations³⁵. The presence of hallucinations has also been found to be a risk factor for the development of dementia⁵.

iv. Fatigue

Fatigue is a very common complaint, its prevalence ranging from 33 to 58% of PD patients³⁷. It can be distinguished as mental and physical fatigue, and both were found to be present in PD. It is considered as one of the most disabling symptoms of the disease, and a majority of patients express that this type of fatigue is qualitatively different from that experienced before the onset of PD³⁷. Interestingly, while PD fatigue has been associated with depression and anxiety, it was not found to correlate with motor disability³⁷. Fatigue is also prevalent in prodromal stages¹⁵, affects around one third of patients with early PD, and over 55% of patients after 8 years³⁷.

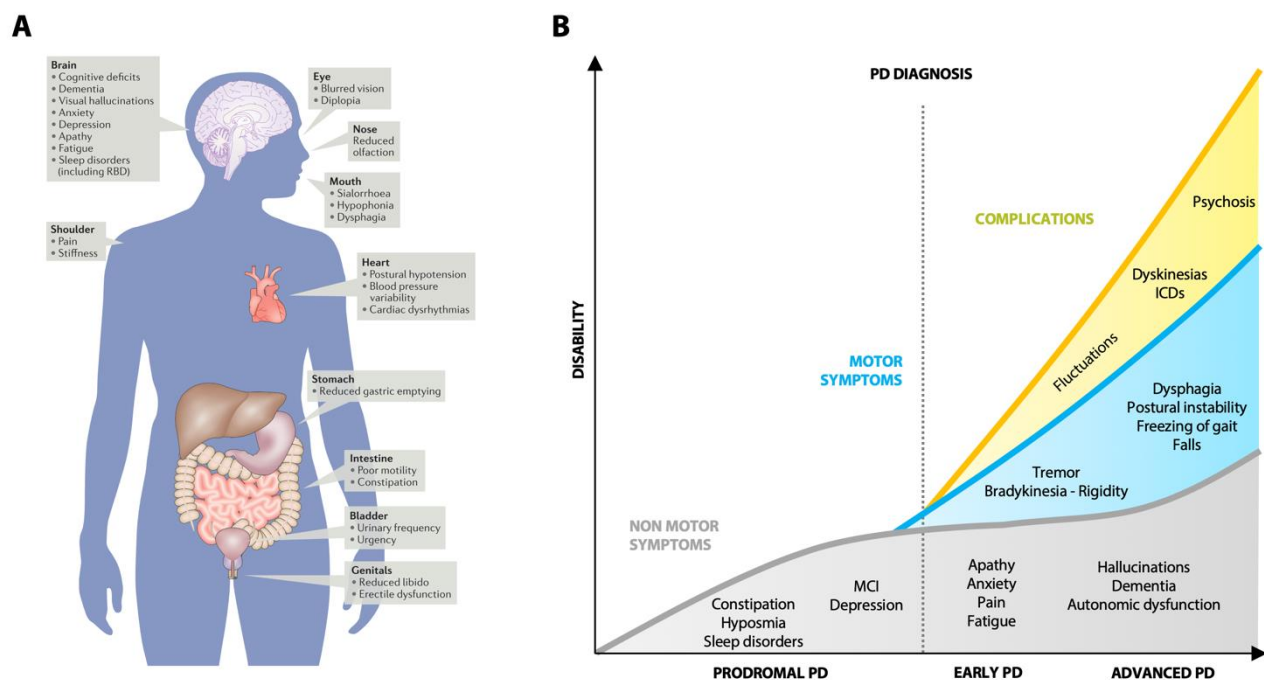


Figure 1 – Parkinson's disease symptomatology and time-course.

A: Visual representation of the various non-motor symptoms of PD

B: Patterns of symptom onset in prodromal, early and advanced PD.

RBD, REM sleep behaviour disorder; MCI, mild cognitive impairment; ICD, impulse control disorders

Adapted from Schapira et al., 2017, and Kalia et al., 2015^{12,16}

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3. Other non-motor symptoms

i. Sensory

Virtually all PD patients suffer from at least one impairment in sensory features¹⁶, typically affecting olfaction, vision and pain sensitivity. For instance, hyposmia (or anosmia), indicating impaired olfaction, may constitute one of the main components of pre-motor PD symptoms, and affects over 90% of patients¹⁶. As mentioned earlier, visual disturbances such as passage and presence hallucinations, as well as diplopia (double vision) are frequent. The sensory dysfunction also affects pain threshold, with up to 83% of patients suffering from muscular and/or visceral pain^{17,38}.

ii. Sleep

Sleep is almost always disrupted in PD, affecting both day and night-time functioning. For instance, patients report increased daytime somnolence (up to 50%) as well as insomnia, frequent awakenings, akathisia (restlessness), and RBD (REM sleep Behaviour Disorder)¹⁷. RBD occurs in up to a third of patients, and is caused by the loss of motor atonia during REM sleep, resulting in the appearance of strong abnormal movements and vocalisations¹⁷.

iii. Autonomic dysfunction

Autonomic dysfunction is very common, and affects bladder, bowel, heart and sexual function. For instance, while patients have an increased need and urgency to pass urine, they are very often also constipated and have deficient bowel emptying¹⁷. Alterations in cardiac function such as increased heart rate, and orthostatic hypotension are also common (30-58%)¹⁷. Finally, sexual dysfunction is also highly prevalent in PD patients, with around 80% of patients (female and male) reporting decreases in arousal, as well as erectile dysfunctions³⁹. While these dysautonomic symptoms often appear before the onset of motor impairment, their effect on quality of life is most important in later stages of PD, with high incidences of incontinence and choking⁴⁰.

4. Heterogeneity and subtypes

PD is a very heterogeneous disease, with strong differences in the clinical phenotype, response to treatments and prognosis. For instance, while the average duration of PD from the beginning of motor impairment to death is of 17 years, this period can be shortened to around 7 years in some of the patients. Similarly, while many patients suffer from cognitive impairment from early stages of the disease, others will be relatively unaffected until much later⁴¹. To tackle this issue, efforts have been made to split patients into subtypes, based on symptoms that tend to cluster together. Patient stratification is one of the main current frontiers of PD clinical research, the hope being that it will allow to identify specific biomarkers and devise personalised therapeutic approaches⁴¹.

The most longstanding classification has been on the basis of the prevalence of tremor in the behavioural phenotype, delineating “tremor-dominant” from “non-tremor-dominant” and intermediate groups⁴¹. “Tremor-dominant” patients are characterised by slower progression of the disease, relative absence of other types of symptoms and lower functional disability. By contrast, “non-tremor-dominant”, also known as “akinetic-rigid” or “postural instability-gait disorder” (PIGD) patients display a globally more severe phenotype, and a higher prevalence of apathy, cognitive decline and dementia^{13,42}.

It has however been argued that the proposed subtypes could simply reflect different stages of the disorder¹³. For this reason, recent studies have focused on the classification of patients in earlier stages of the disease, using insight from data-driven approaches. For instance, a clustering study of *de novo* PD patients using information based on motor and non-motor symptoms identified four subtypes of patients depending on the relative prevalence of motor vs non-motor symptoms⁴³ (**Figure 2**). Interestingly, one of the subgroups was very similar to PIGD patients (“Motor dominant”, MD), characterised by faster disease progression and worse impairment of psychiatric symptoms. Other subtypes were affected by a stronger involvement of non-psychiatric NMS, suggesting different biological underpinnings. Furthermore, clustering efforts based solely on NMS support this classification. A recent review of such studies summarised their findings into at least 6 different NMS subtypes of PD, amongst which: cognitive, apathetic, depression/anxiety, REM sleep disorder, pain and weight subtypes⁴⁴. The three first subtypes,

relating to psychiatric components, were all associated with stronger motor and gait impairment, characteristic of the PIGD/MD subtypes⁴⁵.

The proportion of PIGD-like patients in these cohorts ranged in between 17-27%^{43,45-47}. Biological differences in disease progression seem to be the basis for the observed PD subtypes. Indeed, differences in genetics, monoaminergic signalling, cerebral blood flow and functional connectivity have been observed between the main subgroups⁴⁸, as will be described in the next chapter.

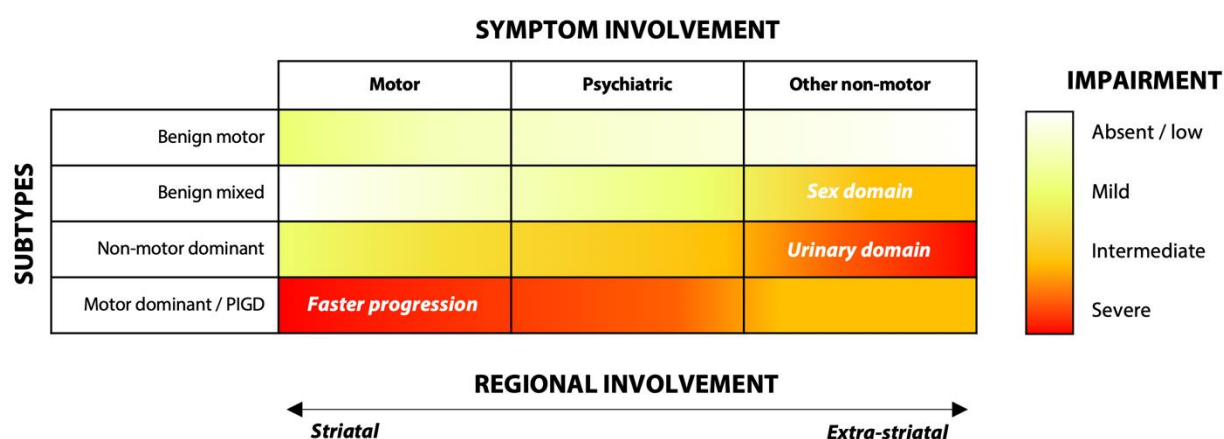


Figure 2 – Clustering of *de novo* Parkinson's disease patients into subtypes.

Patients in the “motor dominant” subtype (similar to PIGD) have the strongest prevalence of motor and psychiatric symptoms, later age of onset, and worse prognosis.

PIGD, postural instability-gait disorder

*Adapted from Erro et al., 2013*⁴³

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B. Biological bases

1. Genetic and environmental factors

i. Genetic risk factors

Population studies have allowed to quantify the relative influence of genetics factors (heritability) on the PD phenotype. For instance, a recent study analysing the family history information of over 1.5 million individuals⁴⁹ found a PD heritability value of 0.26, leaving a majority of the variance up to environmental interactions or stochastic processes.

Indeed, only a minority (up to 10%) of PD patients have a familial form of the disease, i.e. with an identifiable genetic cause⁵⁰. Mutations in the *SNCA* gene, coding for alpha-synuclein, were the first to be linked to PD¹². It was later found that mutations in the *GBA* gene, which encodes β -glucocerebrosidase, were associated with the greatest odds ratio (over 5) in PD patients¹². Dominant (including *SNCA*, *LRRK2*) and recessive (including *Parkin*, *PINK1*) mutations have also been associated with inherited forms of the disease. So far, 15 genes have been causally linked to PD⁵⁰.

Nevertheless, these monogenic forms of PD only account for a minority of patients. Genome-wide association studies have allowed to identify additional genetic factors. For instance, a recent meta-analysis of GWAS studies of PD found 24 loci that were significantly associated with the disease, including genes mentioned above (*GBA*, *LRRK2*, *SNCA*)¹². The presence of multiple risk loci in patients, referred to as the “polygenic load”, has been shown to influence the age of disease onset, but not the rate of disease progression⁵¹. A study of genome-wide SNPs from over 7000 patients⁵² yielded a PD heritability value of 0.27, similarly to the study of family histories mentioned earlier. However, currently loci that have been associated with PD only account for a minor fraction of this heritability (3-5%)⁵³, indicating that there still is a significant part of “missing heritability” to uncover, notably in the non-coding genome⁵⁴.

Interestingly, several genetic factors have been linked to symptoms that are more prevalent in subtypes of PD. Indeed, *GBA*, *SNCA* and *LRRK2* variants have all been linked to worse cognitive impairment and an increased risk of dementia, as well as to other motor characteristics that are more prevalent in PIGD subtypes⁵⁵.

ii. Environmental risk factors

Nevertheless, the vast majority of cases remain sporadic (of unknown aetiology)⁹, for which environmental factors may play a significant role (**Figure 3**). For instance, exposure to pesticides, rural living, well water drinking and agricultural profession have all been associated with increased risks of developing PD¹². Strikingly, pesticides such as paraquat and rotenone have even been used to develop animal models of PD⁵⁶. Other risk factors include consumption of dairy products, methamphetamine use, traumatic brain injury and melanoma.

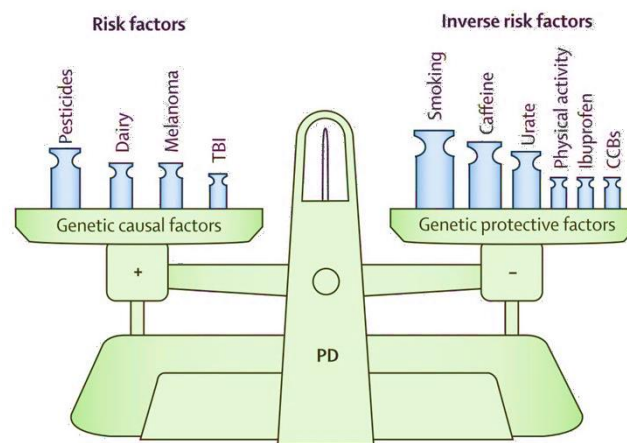


Figure 3 - Balance of genetic and environmental factors underlying Parkinson's disease occurrence.

Larger weights indicate stronger epidemiological evidence.

TBI: traumatic brain injury, CCB: calcium channel blockers.

Adapted from Ascherio et al., 2016 ⁹

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Several environmental factors have also been associated with decreases in the risk of developing PD. This is the case of tobacco use, coffee drinking and moderate alcohol intake, which could be linked to the neuroprotective effects of nicotine, caffeine and urate (which is elevated by alcoholic beverages) in animal models of PD⁹. Furthermore, physical activity, use of calcium channel blockers and non-steroidal anti-inflammatory drugs such as ibuprofen have also been linked to decreased risks of developing PD⁹.

2. Cellular pathology

The identification of SNCA mutations as a monogenic form of the disease led to the discovery that the protein it encodes, alpha-synuclein (α -syn), is centrally involved in PD pathology¹². Indeed, the abnormal aggregation of misfolded α -syn proteins has been extensively characterised in PD (**Figure 4**). These aggregations form intracellular inclusions, called Lewy bodies (in the cell body) or Lewy neurites (in dendrites and axons), that are collectively referred to as Lewy pathology or synucleinopathy.

Similar abnormal protein aggregation has also been characterised in other neurodegenerative diseases such as Alzheimer's disease (AD). Interestingly, β -amyloid plaques and tau-containing neurofibrillary tangles, which are typical of AD, have also been found in PD brains at high levels, and linked to an increased prevalence of dementia¹².

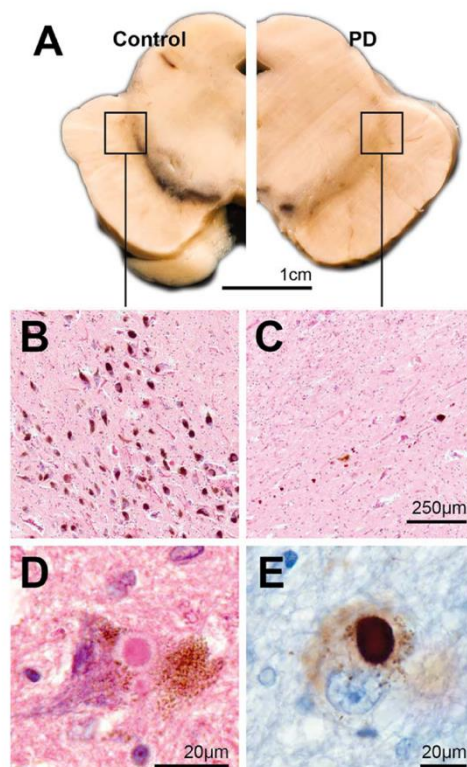


Figure 4 – Cell death and Lewy Bodies in the Substantia Nigra.

A: Transverse sections of control and PD midbrains, showing a strong reduction in neuromelanin-containing SN DA neurons.

B and C: Comparison of high magnification sections from control or PD tissues, stained with haematoxylin and eosin (H&E). A reduction in the number of stained nuclei is visible.

D and E: Intracellular Lewy Bodies in SN neurons of patients visualised with H&E staining (**D**) or with an α -synuclein antibody and cresyl violet counterstaining (**E**).

Adapted from Obeso et al., 2017¹³

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The α -syn protein is enriched in presynaptic compartments, where it is thought to regulate vesicle trafficking and neurotransmitter release⁵⁷. Models of α -syn over-expression have shown that its aggregation is necessary to induce neurodegeneration⁵⁷. However, the precise mechanisms by which the aggregations induce neuronal death have not been fully identified, although dysfunctional cellular degradation pathways seem to be involved both in the cause and consequence of the aggregation of α -syn misfolded proteins. For instance, α -syn accumulation significantly alters autophagic processes, as well as mitochondrial respiration and quality control, and induces Golgi fragmentation and endoplasmic reticulum stress response⁵⁷, inevitably leading to the death of vulnerable cells (**Figure 5**).

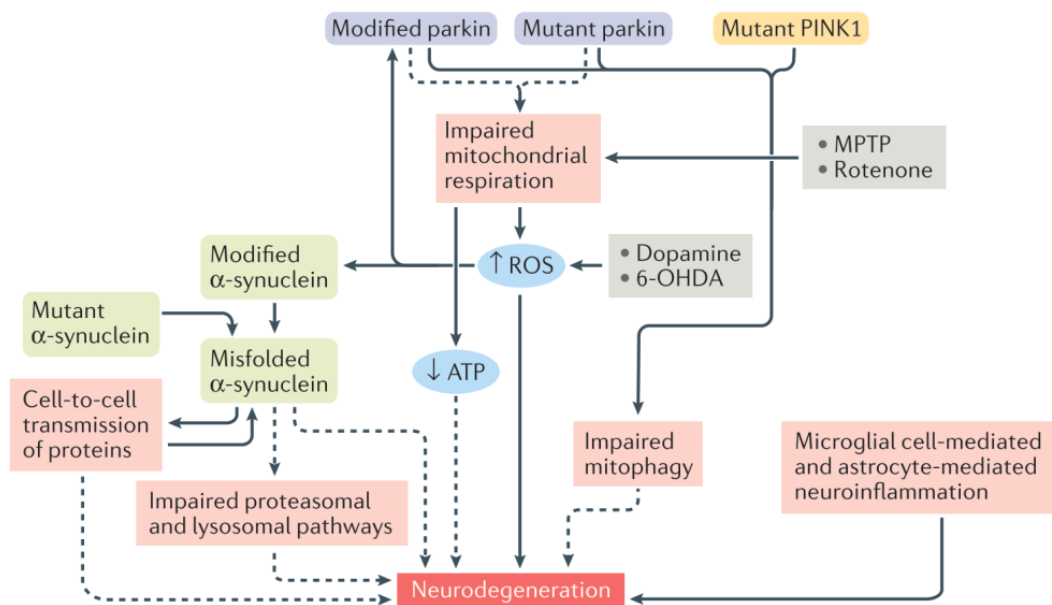


Figure 5 – Summary of potential cellular mechanisms involved in Parkinson's Disease pathology.

Continuous lines refer to established processes, whereas dashed lines correspond to hypothetical links. ROS: reactive oxygen species.

Adapted from Przedborski et al., 2017⁵⁸

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3. Progression mechanisms

i. At the cellular level

PD is a progressive disorder, with post-mortem histological analyses showing wide-spread accumulation of Lewy pathology in the brain, beyond its hallmark presence in midbrain nuclei such as the Substantia Nigra⁵⁹. Scenarios for the spreading of the synucleinopathy have been proposed, and will be described in the following section. The mechanisms by which α -syn may propagate are currently one of the main areas of focus in PD research. Two non-cell-autonomous mechanisms have been suggested so far, involving inflammatory processes and cell-to-cell propagation of α -syn.

Indeed, studies of neuroinflammation in PD have shown a clear activation of the innate and adaptive immune systems in patients, notably involving microglia⁶⁰. Although the mechanisms triggering microglial activation are not clearly known, the downstream effects are likely to contribute to the propagation of neuronal death, through production of cytokines, complement, and activation of phagocytes¹³. Targeting inflammatory processes may thus prove to be an effective disease-modifying approach.

The second mechanism refers to a prion-like propagation of α -syn. The initial finding that paved the way for this hypothesis was the observation that dopaminergic neurons grafted into the striatum of PD patients showed inclusions decades after the procedure⁶¹. More recently, *in vitro* studies showed that misfolded α -syn fibrils could enter neurons by endocytosis⁶², where they recruit endogenous α -syn into Lewy Body-like inclusions, leading to functional impairment and neuronal death. Although such mechanisms have yet to be observed in patients, they have been proposed as a cellular basis for the spreading of the synucleinopathy.

ii. Staging scenario of Parkinson's disease progression

This α -syn propagation has been suggested to follow a typical pattern over the course of PD. The popular 6-stage scenario proposed by Braak and colleagues in 2003⁵⁹ still holds a major influence on how PD is viewed (**Figure 6**). According to this scenario, the synucleinopathy would start in the peripheral nervous system, then gradually affect the central nervous system through brainstem nuclei. In particular, Braak hypothesised that PD may start in the gastrointestinal tract.

While some research may provide support to this hypothesis, it is still considered controversial and in need of further investigation⁶³.

This pattern particularly fits the symptomatology of PD, especially regarding the time-course of symptom onset. According to this model, in stages 1 and 2, the synucleinopathy would first affect the medulla oblongata, followed by higher brainstem areas including the raphe, reticular and coeruleus nuclei. These initial stages could for instance explain the high prevalence of non-motor dysfunctions in premotor PD¹⁷. In the following instances, the nigrostriatal system would be affected (stage 3), leading to the landmark motor and psychiatric features of PD. Finally, in advanced stages (4-6), the wide-spread cortical α -syn propagation provides a mechanism to explain the progressive development of dementia in patients¹².

This staging scenario should however be interpreted with caution. Indeed, only half of PD patients have a synucleinopathy pattern that aligns with the Braak staging model⁶⁴. Furthermore, the spreading of α -syn seems to be crucially linked to the vulnerability of specific populations of neurons. For instance, while the striatum is the main projection of substantia nigra neurons, striatal MSN do not seem to be affected by the α -syn aggregation⁶⁴. Thus, although the prion-like propagation hypothesis may provide a valid basis to explain the wide-spread distribution of α -syn aggregates, only neurons with specific features may go on to develop synucleinopathy. Proposed features for such neuronal vulnerability include 3 traits, that are found in substantia nigra DA neurons: complex arborisation of neurons with many vesicular release sites (enriched in α -syn), high intracellular calcium concentrations due to autonomous pacemaker activity, and high levels of basal mitochondrial oxidative stress⁶⁴.

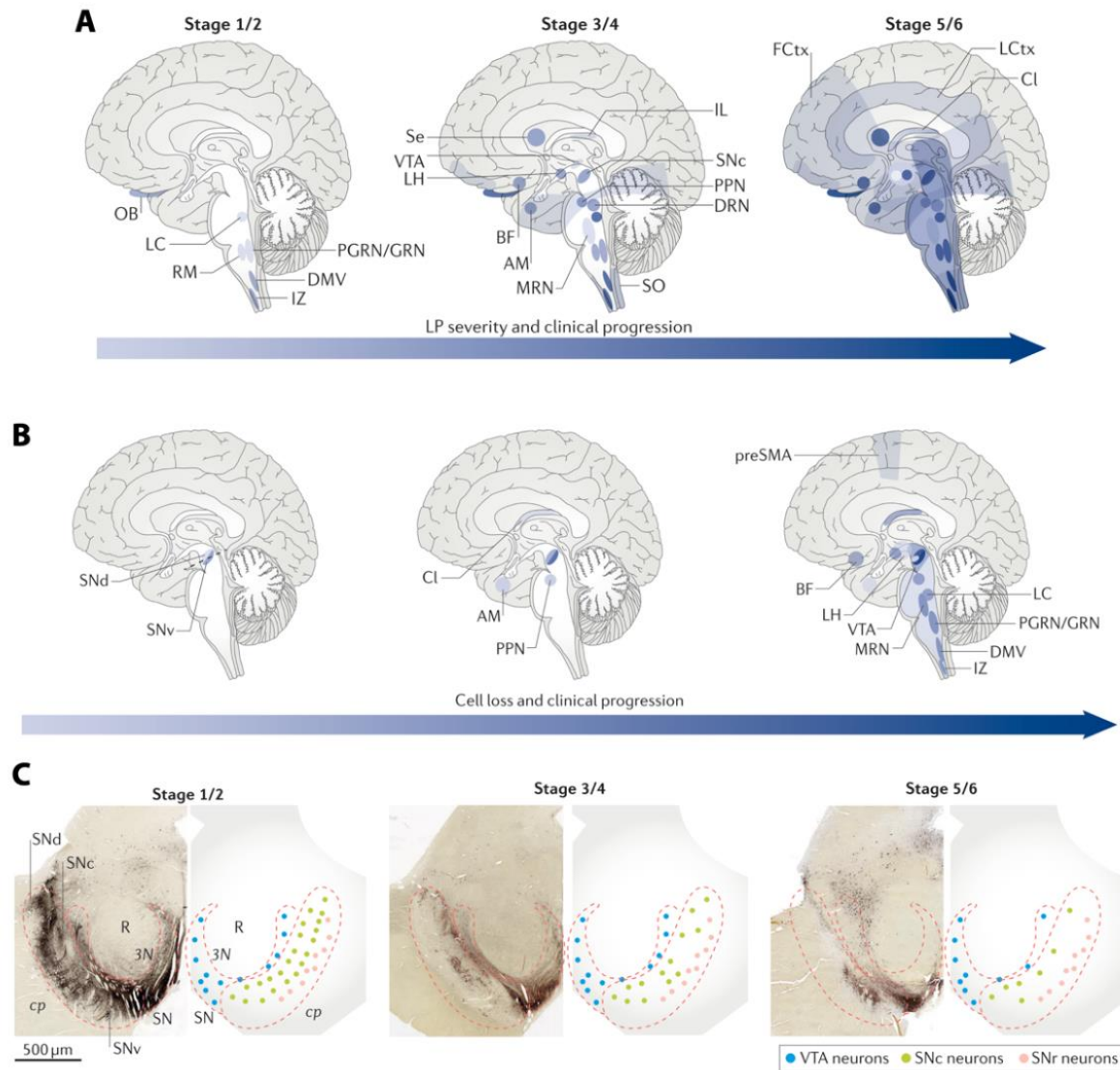


Figure 6 - Spreading of Lewy pathology and cell death in Parkinson's disease

A: Anatomical propagation of Lewy pathology (LP) in the brain according to the original study from Braak and colleagues⁵⁹. **B:** Evidence of neuronal death according to the literature. **C:** Midbrain transversal sections stained for tyrosine hydroxylase (left panels), along with a schematic delineation of the affected DA nuclei of the midbrain (right panels).

3N, third nerve; AM, amygdala; BF, magnocellular nuclei of the basal forebrain; Cl, claustrum; cp, cerebral peduncle; DMV, dorsal motor nucleus of the vagus; FCtx, frontal cortex; IL, intralaminar nuclei of the thalamus; IZ, intermediate reticular zone;

LC, locus coeruleus and sub-coeruleus; LCtx, limbic cortex; LH, lateral hypothalamus; MRN, median raphe nucleus; OB, olfactory bulb; PGRN/GRN, paragigantocellular and gigantocellular reticular nucleus; PPN, pedunculo-pontine nucleus; preSMA, presupplementary motor area; R, red nucleus; RM, raphe magnus; Se, septum; SNd, dorsal tier of the SNc; SNv, ventral tier of the SNc; SO, solitary tract nuclei; VTA, ventral tegmental area.

Adapted from Surmeier et al., 2017⁶⁴
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4. Brain-wide effects

The two most consistently reported biological alterations in idiopathic PD are the presence of synucleinopathy in the brain stem, and the loss of dopaminergic neurons in the ventrolateral part of the Substantia Nigra¹³. However, the effects of PD are not limited to these two findings. In the following sections, results from imaging and post-mortem studies will be summarised for the main degenerating neurotransmitter systems and other modalities that have been investigated in PD (**Figure 7**). As the focus of the thesis is on the psychiatric symptoms of PD, which typically emerge in earlier stages of the disease, a special emphasis has been put on the progressive nature of these alterations. The associations between these biological alterations and the psychiatric symptoms of PD will be addressed in the next chapter.

i. Dopaminergic system

Brain dopamine arises from multiple cells groups (A8 to A16), and form four major dopaminergic pathways, including (1) the nigrostriatal pathway, arising from A9 Substantia Nigra neurons and projecting mainly to the dorsal striatum (putamen and caudate nucleus) as well as some cortical areas⁶⁵, which is mainly affected in PD. (2) The mesolimbic and (3) mesocortical pathways, both arising from A10 Ventral Tegmental Area (VTA) neurons and innervating the ventral striatum (nucleus accumbens) and frontal cortical areas, respectively. (4) Finally, the tuberoinfundibular pathway is made up of neurons projecting from the A12 Arcuate nucleus to the median eminence⁶⁶.

PET (Positron Emission Tomography) and SPECT (Single Photon Emission Computed Tomography) have been consistently used to image changes in monoaminergic systems in PD patients, particularly regarding the dopaminergic system. The most-used markers have been 18-F-DOPA, which reflects activity of the presynaptic aromatic amino acid decarboxylase (AADC), and DAT ligands, which bind to the presynaptic dopamine transporter. It should however be noted that cellular changes both in AADC and DAT have been observed as compensatory mechanisms following loss of dopamine⁶⁷. Furthermore, they are not specific to dopamine, but rather to monoamines. Interpretation of clinical findings using these markers must thus be nuanced and consider those facts.

Nevertheless, F-DOPA and DAT have reliably been used to estimate the progression of dopaminergic degeneration in PD. Such imaging studies have consistently shown that the initial loss of dopamine-related markers mainly affects putaminal areas, specifically the posterior putamen. This preferential pattern is also associated with an increased rate of signal loss compared to caudate and ventral striatal areas⁶⁷. For instance, in early PD, landmark historical studies found strong decreases of F-DOPA uptake of 40-50% in the putamen, while lower values ranging from 10-20% were observed in the caudate nucleus⁶⁸⁻⁷⁰. Temporary increases in F-DOPA binding in the Anterior Cingulate Cortex and Dorsolateral Prefrontal Cortex have also been observed in early PD, suggesting potential compensatory mechanisms^{71,72}. Post-synaptic markers for D2 receptors (such as IBZM SPECT, or 11-C-Raclopride PET) have also been studied in PD, and show a typical increase in putaminal binding, likely reflecting compensatory responses to decreased synaptic dopamine^{67,69}. D2/D3 binding was also found to be altered in cortical areas and the thalamus of early⁷³ and advanced⁶⁷ PD patients.

These methods have also been used to attempt to detect preclinical dopamine dysfunction in prodromal stages of PD. For instance, studies in populations at risk of developing PD (reporting hyposmia for instance) found reduced DAT levels in the striatum⁷⁴, especially in the putamen⁷⁵. A longitudinal study estimated that PD motor symptom onset required a decrease in 18-F-DOPA uptake of 25% in the putamen, and of 9% in the caudate nucleus⁷⁶.

This preferential pattern of dopamine-related signal loss in the putaminal areas of the striatum is conserved throughout the disease, as evidenced by post-mortem histological analyses of patients' brains. Indeed, it was found that while putaminal levels of dopamine were reduced by 98% in patients, a slightly lower 81% of reduction was observed in the caudate head⁷⁷, and decreases in VTA DA cell counts were found in the 40-70% range⁷⁸. Complete loss of dopamine markers in the putamen may be found as early as 4 years post PD diagnosis⁷⁹. Strong reductions of dopamine have also been observed in extra-striatal areas, including frontal, entorhinal, cingulate cortical areas and in the hippocampus (-62% on average)⁸⁰.

ii. Serotonergic system

Brain serotonin arises from the Raphe nuclei of the brainstem, and innervates many regions, including cortical (medial) and subcortical areas (basal ganglia, hippocampus, hypothalamus, thalamus, amygdala), the caudal brainstem and the spinal cord⁸¹.

Studies of early PD have reported contrasting findings, ranging from decreases to increases in serotonergic markers in the raphe^{81–84}. The variability of these findings may however relate to PD subtype, as impairment in serotonergic function seems to be related to the tremulous subgroup⁸⁴. This aspect will be further developed in an upcoming section. Nevertheless, in advanced PD, wide-spread decreases in serotonin transporter (SERT) binding have been reported in cortical and subcortical areas^{81,85}. Interestingly, *in vivo* and post-mortem data show that the pattern of loss of serotonergic markers in the striatum preferentially affects the caudate nucleus⁸⁷, even in earlier stages of the disease⁸¹, opposite to the pattern of dopamine loss. For instance, a study found that while SERT binding decreased by 56% in the caudate nucleus of patients, it only decreased by 30% in their putamen⁸⁷.

Although the evidence of neuronal loss in the raphe nuclei is somewhat controversial⁸⁷, reductions in serotonergic markers have nevertheless been observed in post-mortem tissue, although not as pronounced as the loss of dopaminergic markers⁶⁷. These alterations affect several regions including the striatum, frontal cortex, hippocampus and hypothalamus⁶⁷.

iii. Noradrenergic system

The noradrenergic system in the brain is composed of two main pathways: (1) a caudal complex which sends projections to the locus coeruleus (LC), the hypothalamus and spinal cord, and a (2) rostral complex, composed of the LC and subcoeruleus, which have projections throughout the brain⁶⁷. Although the LC does innervate the striatum, the levels of noradrenaline (NA) in the striatum are at least 30 times lower than those of dopamine⁵, while they are present in similar ranges in cortical areas⁸⁰.

In studies of early PD, increased levels of 18-F-DOPA uptake and catecholaminergic transporter binding were observed in the LC, suggesting temporary compensatory mechanisms^{83,88}. In later stages however, F-DOPA uptake was found to be strongly decreased in the LC⁸³.

Post-mortem studies confirm this observation, reporting for instance a loss of 63% of LC cells in PD brains⁸⁹, and reduced levels of noradrenalin in the striatum⁵ and in cortical areas⁸⁰.

iv. Cholinergic system

Brain acetylcholine (ACh) is mainly produced by the nucleus basalis of Meynert and the Pedunculopontine nucleus (PPN), which project to cortical and subcortical areas, respectively⁶⁷. Although the striatum receives strong projections from the PPN⁹⁰, it has its own supply of local cholinergic interneurons, and expresses the highest levels of cholinergic markers in the brain⁹⁰.

PET studies have assessed acetylcholine esterase (AChE) activity as a proxy for cholinergic function. Deficits in cortical AChE activity have been observed in early, drug-naïve PD patients⁹¹, and throughout the cortex of advanced PD patients, with more severe reductions in those having developed dementia^{67,92}. However, in the striatum, animal models of PD have shown that the loss of striatal DA leads to an increase in local release of acetylcholine, which may worsen the motor impairment⁹³.

Post-mortem studies have shown alteration of cholinergic markers in the nucleus basalis of Meynert, the PPN, as well as in frontal cortical areas and in the striatum⁶⁷. The neuronal loss in both cholinergic nuclei was estimated at around -50%^{94,95}. In the striatum, a loss of ACh nicotinic receptor binding was observed⁹², likely reflecting increased cholinergic transmission.

v. Inflammatory markers

As mentioned previously, abnormal activation of microglia is thought to contribute to the progression of synucleinopathy in the PD brain. PET studies using a marker of activated microglia have found increased levels of binding in the midbrain of early PD patients, which inversely correlated with the level of DAT binding in the putamen⁹⁶. In more advanced PD

patients, the levels of activated microglia were found to be higher in the striatum, but also in extra-striatal (including cortical) areas⁹⁷. These results are consistent with the presence of activated microglial cells in the Substantia Nigra of post-mortem PD brains⁶⁰, and provide support for the hypothesis that neuro-inflammatory processes contribute to neurodegeneration.

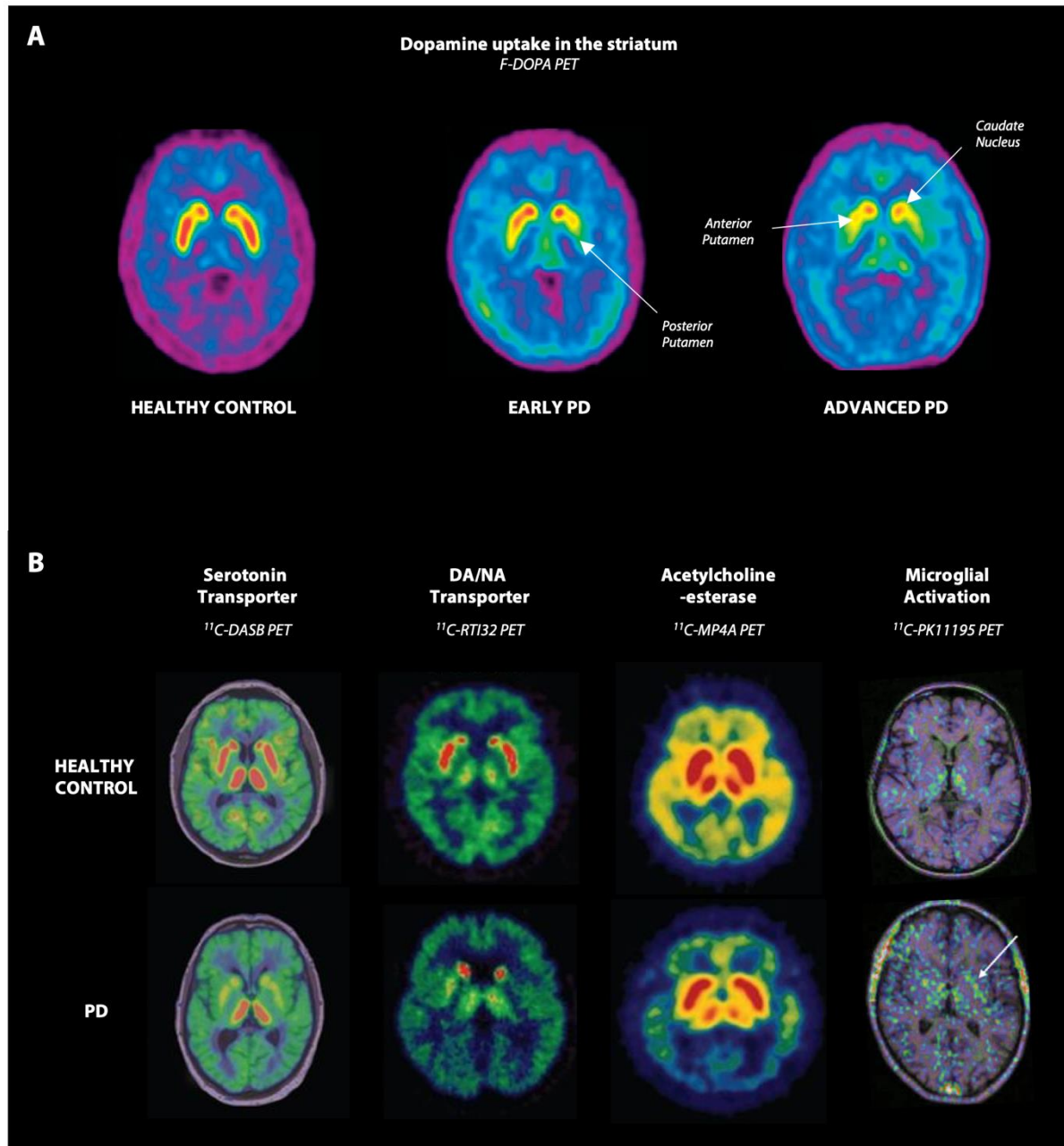


Figure 7 – Brain-wide alterations observed *in vivo* with PET imaging

The images were obtained from PET scans using a diversity of tracers, and represent transversal sections at the level of the striatum. **A:** Progressive loss of F-DOPA uptake in the striatum in early PD (at diagnosis) and in advanced stages (12 years after diagnosis). **B:** Alteration in other neurotransmitter systems and inflammatory processes as observed with a variety of PET tracers.

Adapted from Rodriguez-Oroz et al., 2009, Brooks and Pavese, 2011, Politis 2014 ^{28,67,98}
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vi. Morphology and connectivity

Imaging studies have also been very useful to visualise numerous changes in brain structure, function and connectivity¹³. For instance, cortical thinning, as well as shrinkage of the amygdala, hippocampus, nucleus accumbens and caudate nucleus head have all been reported in PD^{99–102}. Atrophy of cortical areas and of the nucleus accumbens have even been observed in newly diagnosed PD patients with mild cognitive impairment¹⁰². Furthermore, while cerebral blood flow was found to increase in pallidal and putaminal areas, decreases were observed in cortical areas, amongst which the dorsolateral prefrontal cortices⁶⁷.

Of particular interest to this thesis, resting state fMRI investigations have shown some level of re-organisation of cortico-striatal networks in PD. For instance, studies have found that while functional connectivity decreased in cortico-striatal sensorimotor networks, increases were observed in cortico-striatal associative networks^{103–104}. This effect seemed to be related to the extent of DA loss¹⁰³, and may thus reflect temporary compensatory mechanisms, as other studies have also reported decreases in the functional connectivity of associative circuits¹⁰⁶.

vii. Biological bases of PD subtypes

Differences in the patterns of neurodegeneration have been observed in PD subtypes. Indeed, *de novo* PIGD-subtypes exhibit significantly lower **dopamine** transporter binding in the putamen, but not the caudate nucleus, compared to tremor-dominant patients^{107,108}. This pattern of decreased putaminal binding was also replicated in a study looking at the evolution of PD subtypes over a period of 2.5 years¹⁰⁹. Post-mortem studies have also found differences in the patterns of DA neuron loss in the substantia nigra pars compacta, with PIGD patients having more severe loss in the ventrolateral part, whereas tremor-dominant patients showed increased loss in the medial part⁴⁸.

A recent large-scale SPECT study involving 345 early PD patients found that while PIGD patients were characterised by more severe striatal dopaminergic deficits, tremor-dominant patients showed stronger loss of **serotonin** transporter binding. Furthermore, they found that serotonin binding negatively correlated with tremor severity, but not with psychiatric symptoms⁸⁴. In

support of this finding, another study of early PD found that loss of DAT binding in the striatum correlated with hypokinesia and rigidity, but not with tremor¹⁰⁸.

Similarly, *in vivo* studies have found that PD tremor was associated with higher levels of **noradrenalin** transporter binding, suggesting that NA impairment may be prevalent in non-tremor dominant (PIGD) subtypes¹¹⁰. A post-mortem study also found increased cell loss in the LC of PIKD patients, compared to tremor-dominant patients¹¹¹. Loss of **cholinergic** neurons in the PPN has also been associated with the PIKD subtype¹¹², and a higher prevalence of cortical ACh deficits was found in patients with cognitive impairment and dementia⁶⁷. Finally, **functional connectivity** studies found that PIKD patients exhibited decreased sensorimotor and intrastriatal connectivity, along with increases in cortical frontoparietal and insular connectivity^{113,114}.

viii. Summary

In the very earliest stages of PD, the main evidence points to a loss of dopamine affecting the posterior putamen, and to a lesser extent the anterior putamen and caudate nucleus. Cortical cholinergic innervation also shows its first signs of impairment. Levels of noradrenergic and serotonergic markers may however remain stable depending on PD subtype, potentially reflecting the efficiency of compensatory mechanisms. On a global level, a shift may be observed in cortico-striatal connectivity, with increases in associative network activity potentially compensating for defective sensorimotor networks.

Nevertheless, such compensatory mechanisms may be overwhelmed by PD progression, as several neurotransmitter systems become impaired: dopamine loss gradually affects all of the striatum, including associative and limbic territories. Noradrenergic, serotonergic and cholinergic systems become severely impacted, in both striatal and extra-striatal territories, fuelled by abnormal inflammatory processes.

5. Associations with the psychiatric symptoms

Strikingly, **all of the PD psychiatric symptoms** mentioned earlier have been linked to dysfunctional dopaminergic transmission in the striatum, especially involving the caudate nucleus (CN)¹¹⁵. This is not surprising, as the CN is implicated in executive cognitive processes and goal-directed behaviours, directly relating to the dysexecutive and apathetic symptoms observed in patients¹¹⁶.

Nevertheless, despite this solid DA base, alterations in other neurotransmitter systems have also been associated with several psychiatric symptoms (**Figure 8**). For instance, regarding **depression**, while a substantial body of work has associated this symptom with serotonergic alterations⁸¹, other imaging studies did not replicate these findings^{82,84}, possibly due to subtype differences. Levels of inflammation may also play a role, as a study found that leukocyte levels of p11, an adaptor protein that is linked to inflammatory responses and to antidepressant treatments, correlated with depression scores in patients¹¹⁷. Finally, according to a small study, **depression, anxiety and apathy** may also be associated with alterations in noradrenalin dysfunction⁸⁶.

Although **cognitive impairment** has consistently been associated with DA loss in the CN, it has also been linked to alterations in dopaminergic and cholinergic markers in the cortex^{92,102,115,118–120}, to shrinkage of cortical and subcortical (amygdala, nucleus accumbens) areas, and to decreases in functional connectivity^{99,106,121}. It is also speculated to be associated with noradrenalin dysfunction¹²².

As associative striatal areas receive strong projections from visual cortices¹²³, it is not surprising that dopaminergic dysfunctions in the caudate nucleus may also be associated with **visual hallucinations** (VH)¹²⁴. Nevertheless, serotonergic and cholinergic alterations may also be involved in the PD psychosis spectrum¹¹⁵, as well as atrophy of brain areas processing visual stimuli¹³.

Finally, a study found that PD patients with **fatigue** had significantly lower levels of SERT binding in cortical and subcortical areas (including the striatum)¹²⁵. As is the case with other symptoms, this study also reported reduced F-DOPA uptake in the CN of fatigued patients.

Taken together, these findings indicate that while early (premotor) DA dysfunctions may be the *primum movens* driving the emergence of the psychiatric symptoms, loss of other neurotransmitter systems during PD progression may further influence the severity of the symptoms, eventually leading to the development of dementia and psychosis.

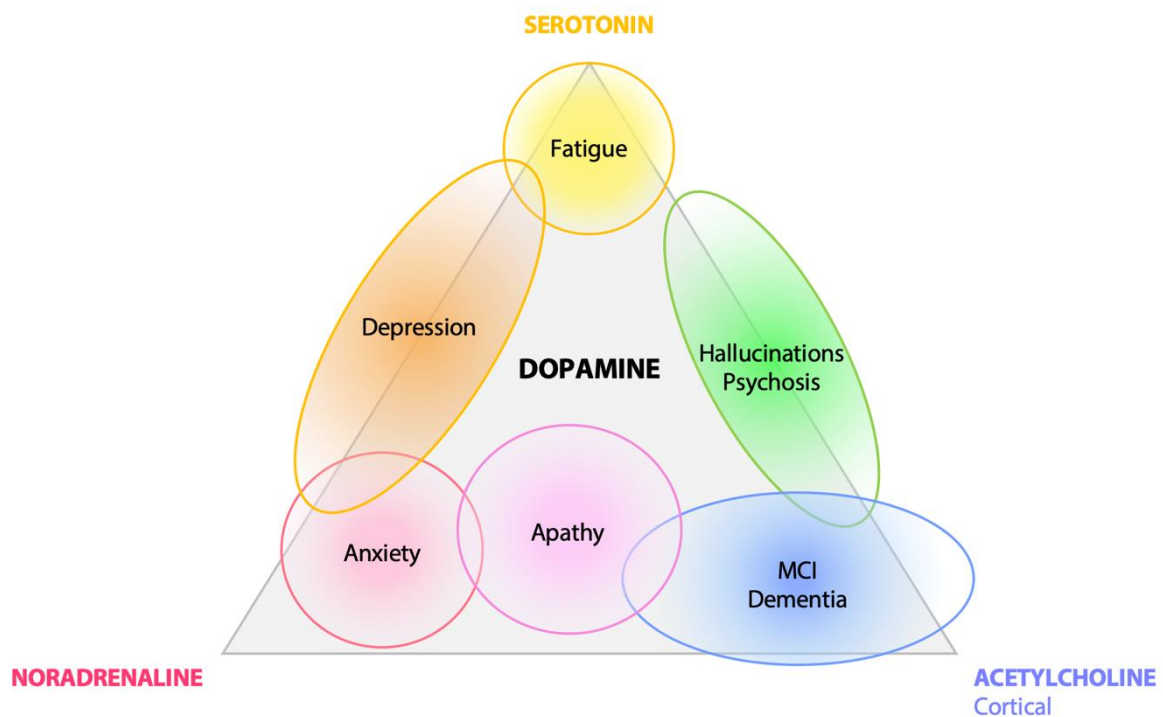


Figure 8 – Association of the psychiatric symptoms with alterations in neurotransmitter systems

Schematic representation of the reported association of PD psychiatric symptoms with alterations in neurotransmitter systems observed with *in vivo* PET/SPECT imaging.

MCI, mild cognitive impairment

6. A focus on the basal ganglia

As discussed earlier, the progressive loss of dopamine and other neurotransmitters in the basal ganglia (BG) is a central feature of PD pathology. Understanding the effects of PD pathology at the level of BG function provides crucial insights into the aetiology of symptoms, and may help devise new therapeutic strategies.

i. Basal ganglia organisation and function

The basal ganglia are a series of nuclei that process information from cortical inputs in relatively segregated parallel loops. According to classical BG models, the striatum (putamen, caudate nucleus, nucleus accumbens) constitutes the major hub. It receives the entering glutamatergic (GLU) cortical and thalamic inputs, and connects directly or indirectly to the two major output nuclei, the internal globus pallidus (GPi) and substantia nigra pars reticulata. These different pathways are mediated by striatal GABAergic medium spiny neurons (MSN), which project either directly to the output nuclei (striatonigral “direct” MSN, dMSN), or through the external globus pallidus (GPe) and subthalamic nucleus (STN) (striatopallidal “indirect” MSN, iMSN) (**Figure 9**)¹¹⁶. The output nuclei exert inhibitory control over thalamic and brainstem structures, which can be lifted through activation of the “direct” pathway. For this reason, the direct and indirect pathways have been classically associated with GO/NO-GO functions, respectively. By activating dMSN or indirect MSN, the cortico-striatal GLU input can thus drive (“GO”) or prevent (“NO-GO”) action.

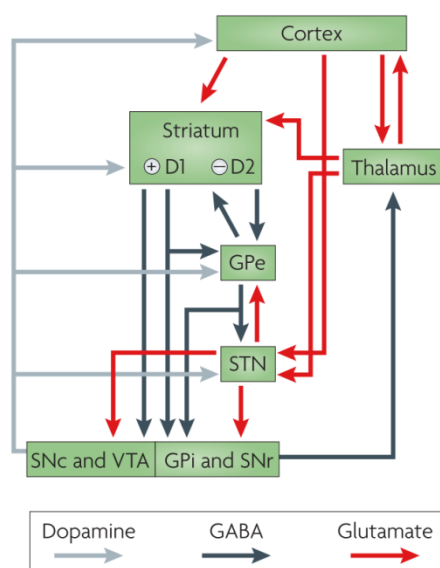


Figure 9 – Connectivity in the basal ganglia

Connectivity diagram of the BG, representing the direct and indirect MSN pathways (D1/D2) at the level of the striatum. D1/D2, dopamine receptor types 1/2

*Adapted from Redgrave et al., 2010*¹¹⁶

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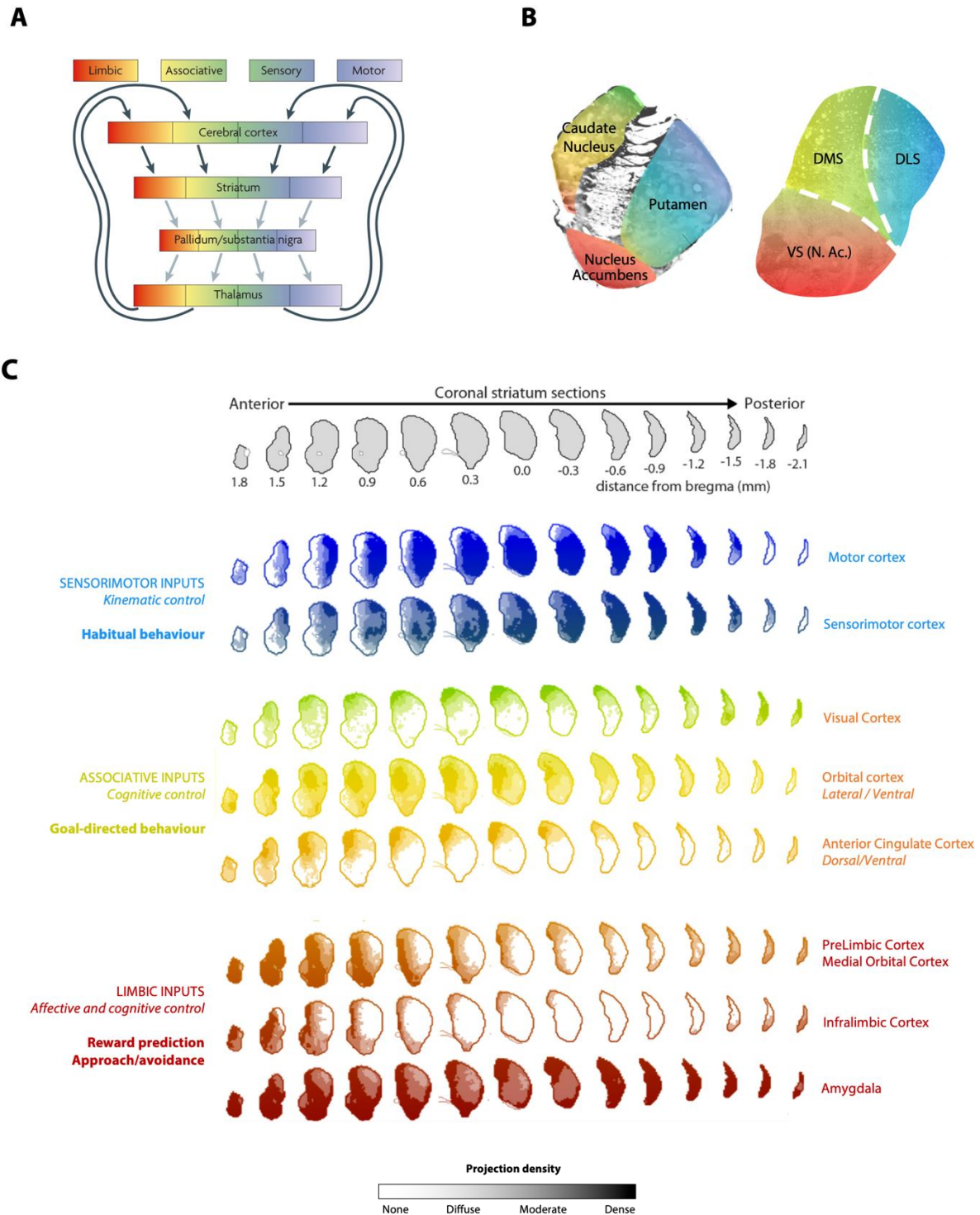


Figure 10 – Topography of the basal ganglia

A: Simplified organisation of basal ganglia loops. **B:** Human (left) and rat (right) right hemisphere striata compared. The colour-coding indicates the approximate anatomical equivalences between species. *DLS*: posterior putamen, *DMS*: anterior putamen, caudate nucleus, *VS*: Nucleus Accumbens (N. Ac.). **C:** Map of selected corticostriatal projections underlying the functional topography of the BG in mice.

Parts of this figure are adapted from Redgrave et al. 2010, Morigaki et al., 2016, Hunnicutt et al., 2016^{116,123,126}

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Models of the basal ganglia propose that the main function of this parallel loop organisation may be to select between competing options in order to drive behaviour¹²⁷, for which dMSN and iMSN may play complementary roles¹²⁸. These parallel BG loops have been roughly segregated in three major types: limbic, associative and sensorimotor¹¹⁶ (**Figure 10**). These segregations are based on the topography of cortical and subcortical inputs to the striatum, which follows a gradient from ventromedial (Nucleus Accumbens) to dorsomedial (caudate nucleus and anterior putamen) and dorsolateral territories (posterior putamen)^{116,123}.

The nigrostriatal DA pathway projects onto both associative and sensorimotor striatal territories, which have different roles in instrumental learning. Indeed, it has been shown that while associative networks drive goal-directed actions, the acquisition of habits depends on gradual recruitment of sensorimotor BG loops¹¹⁶. Although relatively segregated in their roles, these functional subdivisions cooperate for the execution of complex behaviours. The mesolimbic DA pathway, originating from the VTA, projects to limbic striatal areas, which have been implicated in many functions amongst which reward prediction and approach/avoidance behaviours¹²⁹.

ii. Complex modulation of the corticostriatal synapse

This rather simple model is however under tight control from a variety of “neuromodulators”, which serve to fine-tune the corticostriatal synapse, and provide multiple adaptive mechanisms (**Figure 11**). **Dopamine** afferences arguably provide the strongest modulation of this system. For instance, in the dorsal striatum, while D1 receptor stimulation on dMSN increases intracellular cAMP through activation of the adenylyl cyclase, D2 receptor stimulation on iMSN inhibits the activity of this enzyme⁶⁶. Hence, upon presentation of a salient stimulus, phasic DA release from striatonigral neurons onto MSN facilitates the activation of dMSN by GLU while preventing iMSN activation, thus favouring action execution. This mechanism provides the basis for instrumental learning in the striatum¹²⁷.

Furthermore, **serotonin** is known to facilitate DA release from nigrostriatal terminals¹³⁰. However, through its action on a multitude of receptors, striatal serotonin may have complex effects. Computational models have proposed that striatal serotonin may serve as a fine-tune mechanism to maintain a balance in DA release, and/or as a “high-pass” filter to improve action selection^{131,132}.

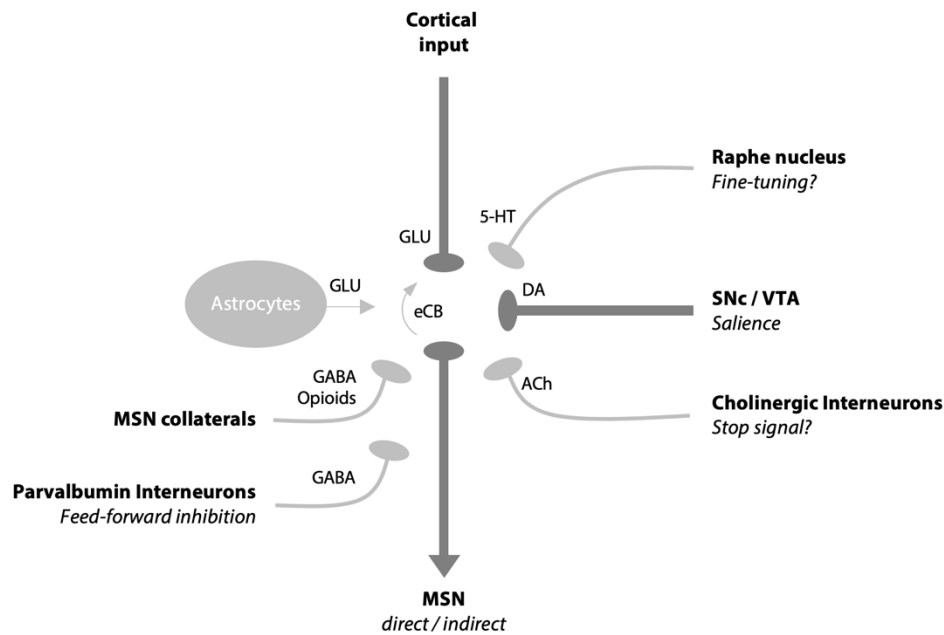


Figure 11 – The corticostriatal synapse

Simplified representation of main modulatory inputs at the level of the corticostriatal synapse.

Another source of modulation comes from the giant tonically active **cholinergic** interneurons (TAN). Through the activation of different receptor subtypes, ACh modulates glutamate and dopamine release, preferentially driving the activity of iMSN¹³³. It has been suggested that bursts of cholinergic release in the striatum, driven by thalamic input, may serve as a brake on ongoing action in response to environmental stimuli¹³³.

Additional compensatory mechanisms exist to modulate the cortico-striatal synapse in an activity-dependent manner. For instance, MSN overactivity leads to increased levels of **endogenous opiate** synthesis such as dynorphin and enkephalin, which in turn dampen the effects of the excessive stimulation on transcriptional mechanisms¹³⁴. **Endocannabinoids**, which are synthesised post-synaptically following activation of different signalling pathways, have the ability to induce long-term depression (LTD) by retroactively binding to pre-synaptic CB1 receptors and reducing neurotransmitter release onto MSN¹³⁵. **Adenosine**, a by-product of synaptic ATP which acts as a neurotransmitter and gliotransmitter, can modulate GLU and DA release (through presynaptic receptors) and the affinity of post synaptic DA receptors (through the formation of heteromers)¹³⁶. Additionally, these neurotransmitters and neuropeptides can also act on **astrocytes** to modulate glutamate release into the corticostriatal synaptic cleft¹³⁶.

Finally, **local GABA** release also affects corticostriatal activity. This GABAergic control of MSN arises both from the feed-forward inhibitory action of fast-spiking parvalbumin interneurons (under cortical control), as well as from MSN-to-MSN collaterals which together provide an additional layer of modulation and filtering^{137,138}.

ii. Basal ganglia alterations in Parkinson's disease

In the context of PD, the loss of dopamine induces a hyperactivity of iMSN and a hypoactivity of dMSN. Furthermore, this imbalance is accentuated by elevations in acetylcholine release and gradual loss of serotonin, which both favour iMSN activity^{132,133}. This leads to an excessively inhibitory BG output, and resulting difficulties in initiating and executing movements. An increase in striatal enkephalin expression may however help compensate for iMSN overactivity, and is thought to reduce motor impairment¹³⁴.

Furthermore, as described earlier, the cardinal loss of DA observed in PD preferentially affects the sensorimotor territories compared to the associative (and limbic) ones. Considering this well-established fact, it is thus not clear how the psychiatric symptoms - which have been related to associative and limbic dysfunction - may so often emerge before the onset of motor (sensorimotor-related) symptoms.

A hypothesis formulated by Redgrave and colleagues in 2010 provides a coherent basis to explain this observation¹¹⁶. They posit that with gradual loss of DA in sensorimotor territories, patients may have to increasingly rely on associative, goal-directed control of behaviour. As they point out, computational models of the BG suggest that while sensorimotor loops process information in a fast and parallel (but inflexible) way, associative loops are slower and serial (but more flexible), and require more computational resources. Due to these differences in information processing abilities, increased reliance on the costlier goal-directed mode of action may lead to an “**overload**” of computational processing. Furthermore, because the BG loops eventually converge onto a final common pathway to drive action (brainstem/motor cortex), the authors hypothesise that the loss of DA in sensorimotor territories may lead to a distorted inhibitory output. In order for goal-directed actions to be expressed, they would thus have to overcome the distorted signal, further increasing the burden on associative territories. According to this hypothesis, the

overload of associative networks would impair related behaviours, thus explaining the emergence of executive dysfunction and apathy in the earliest stages of PD (**Figure 12**).

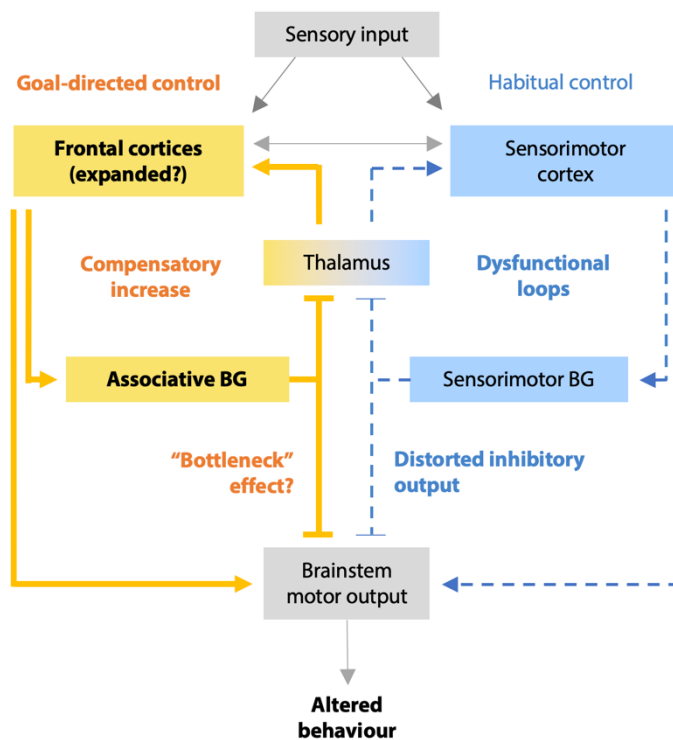


Figure 12 – The “bottleneck” hypothesis

Schematic representation of the “bottleneck” or “overload” hypothesis, as formulated by Redgrave and colleagues, and supported by some findings from clinical imaging research.

Adapted from Redgrave et al., 2010¹¹⁶

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This hypothesis has since gained some support from studies of functional connectivity in PD patients. As mentioned earlier, a couple of studies have found that while decreases in functional connectivity were observed in sensorimotor networks of patients, compensatory increases were observed in associative networks^{103,104}. This effect was mediated by changes in cortico-striatal connectivity, with sensorimotor cortices exhibiting increased coupling with associative striatal territories¹⁰³. These results led the authors of one of these studies to emit a very similar “**neural bottleneck**” hypothesis¹⁰³. Interestingly, they also found that the increase in functional connectivity was stronger in the least-affected hemisphere, suggesting that this may reflect a transient compensatory mechanism, dependent on the extent of dopamine loss.

Combined with the biological alterations observed in early PD, this hypothesis provides a framework to explain the time-course of the psychiatric symptoms. For instance, in premotor stages, these symptoms may first emerge as an indirect consequence of dopamine loss in sensorimotor territories, and subsequent “overload” or “bottleneck” effect. With disease progression, the decreasing levels of DA in associative territories would gradually deplete compensatory resources, and result in a worsening of the psychiatric symptoms.

This “bottleneck” hypothesis has yet to be investigated in animal models of PD. Such studies could provide important insight into PD pathophysiology, and may open up therapeutic avenues. While the cellular mechanisms underlying such striatal dynamics are not known, research on animal models of other neuropsychiatric disorders may provide some valuable clues, which will be addressed in the discussion of this thesis, along with the findings from this project.

C. Treatments

Although a lot of efforts have been directed at identifying and testing disease-modifying approaches¹³⁹, the only therapies that are currently approved for PD aim at symptomatic relief. Specifically, the use of dopamine replacement therapies such as Levodopa (L-DOPA) and dopamine agonist administration constitute the main therapeutic approach in treating both motor and psychiatric symptoms of PD. While these approaches may help control some of the symptoms, they are associated with severe adverse effects. In the following section, an overview of currently approved therapeutic approaches will be given.

i. L-DOPA

L-DOPA administration is recommended in all stages of the disease. This approach relies on L-DOPA uptake by striatal monoaminergic terminals, where it is transformed into dopamine by the aromatic acid decarboxylase (AADC), and released in the synaptic cleft¹⁴⁰. The first few years of therapy are referred to as the “honeymoon period”, as the clinical response to L-DOPA (the “ON” phase) is constant. However, following this period, patients start experiencing “OFF” phases with reduced response to the drug, characterised at first by end-of-dose akinesia and re-emergence of other non-motor symptoms such as anxiety¹⁴¹. After 6-10 years of treatment, patients frequently suffer from excessive “OFF” phases, and in up to 80% of cases¹⁴², L-DOPA’s pulsatile effect leads to the development of dyskinesias (abnormal movements) during the “ON” phase. Furthermore, late-stage administration of L-DOPA has also been linked to the induction or worsening of hallucinations and psychosis¹⁴³.

Several developments have helped control some of these adverse effects. For instance, administration of inhibitors of central and peripheral L-DOPA degrading enzymes such as the MAO-B and COMT help to extend the absorption of L-DOPA and the duration of its action in the brain, and the pulsatile effects of L-DOPA administration can be somewhat reduced with slow-release formulations. Furthermore, the use of intra-jejunal infusion pumps helps avoid pulsatile levels of plasma L-DOPA and linked side effects. It is however an expensive and invasive approach, which requires expert skills¹³⁹.

While the precise mechanisms underlying the development of dyskinesias are for the most part unknown, they have nevertheless been linked to overstimulation of dMSN, as well as dysfunctions in serotonergic and adrenergic systems^{81,143}. Amantadine, a NMDA receptor antagonist, was the first pharmacological agent to be approved by the FDA in 2017 for the treatment of dyskinesias.

ii. DA agonists

Several dopaminergic agonists are used for the treatment of PD, and mainly exhibit D2/D3 preferential binding. They may be efficiently used as a monotherapy in early PD, and as an adjunct therapy in more advanced stages¹³⁹. Successful use of dopaminergic agonists has also been reported for the treatment of depression, anxiety and apathy¹⁴¹. Although their use may provide symptomatic relief, they however do not decrease the incidence of dyskinesias in later stages when used in combination with L-DOPA¹³⁹. Furthermore, their use is strongly limited by aggravating psychiatric adverse effects. The main risk consists in the development of a spectrum of hyperdopaminergic behaviours, including impulse control disorders (behavioural addictions, seen in up to 17% of patients)¹⁴², punding (abnormal fascination with non-goal-oriented activities) and dopamine dysregulation syndrome (resulting from excessive use of dopaminergic drugs as self-medication)¹⁴⁴. While ICDs are imputable to excessive D2/D3 tonic stimulation, punding may result from abnormal phasic stimulation of D1/D2 receptors^{144,145}. Interestingly, the loss of DA in *de novo* PD may favour impulsivity, a risk factor for the development of ICDs¹⁴⁶.

The administration of DA agonists in advanced stages is also associated with an increased risk of cognitive impairment and dementia, and they may induce or aggravate hallucinations in some patients¹³⁹. Finally, another limitation of dopamine agonists is their ability to bind to peripheral receptors, and induce adverse effects such as nausea, orthostatic dysregulation, and leg oedema¹³⁹.

iii. Other pharmacological approaches

Antidepressants have shown successful applications for the treatments of some of the psychiatric symptoms. For instance, serotonin and serotonin-noradrenalin reuptake inhibitors (SSRI/SNRI) are commonly prescribed for depression and anxiety in PD, as well as tricyclic antidepressants¹³⁹.

Regarding MCI and dementia, atomoxetine, a **NA reuptake inhibitor** commonly prescribed for ADHD, has also been found to improve executive function in patients¹³⁹. Furthermore, rivastigmine, an **AChE inhibitor**, can be effective for the treatment of dementia¹², although it is associated with a worsening of motor symptoms (tremor, falls)¹⁴⁷.

Finally, pimavenserin, a **5HT_{2A} receptor antagonist**, has been recently approved by the FDA for the treatment of PD psychotic symptoms³⁵.

iv. Deep brain stimulation

The use of deep brain stimulation (DBS) for the treatment of the motor symptoms of PD provides drastic symptomatic relief. DBS is believed to act by locally overriding disturbed neural network activity¹³. The targeted areas usually consist of the STN or GPi, but for treatment-resistant tremor, the ventral intermediate nucleus (VIM) may also be chosen¹³.

Post-operative apathy has however been observed in DBS patients. It may result from withdrawal of dopaminergic drugs, in which case the symptom can be reversed by reintroducing dopaminergic agonists. In later stages, postoperative apathy may nevertheless be linked to more widespread synucleinopathy, and cognitive impairment¹⁹.

v. A role for gene therapy?

Gene therapy approaches may provide considerable advantages, as they could help avoid off-target effects by transducing discrete brain areas, and ensure a continuous action, as opposed to pulsatile pharmacological stimulations.

Recent efforts from Palfi and colleagues have reported encouraging results using “ProSavin” gene therapy¹⁴⁸. The lentiviral construct they used encoded the rate limiting DA synthesis enzyme Tyrosine Hydroxylase (TH), as well as the AADC and cyclohydrolase 1. When injected in the putamen of advanced PD patients, moderate motor improvements were observed¹⁴⁸. These effects were preserved in time according to a follow-up study that assessed patients up to 8 years post-transduction, and the safety of the procedure was deemed satisfactory¹⁴⁹. The team is now

focusing on optimising the vectors to increase the efficiency of dopamine production, and targeting the Caudate Nucleus for the treatment of psychiatric symptoms.

vi. Unmet medical need

As of today, dopamine replacement therapy is still the cornerstone of PD treatment. As described earlier, it is however associated with important motor and non-motor complications, thus warranting the search for novel therapeutic targets.

Given the involvement of multiple neurotransmitter systems in PD, it is unlikely that purely dopaminergic approaches will provide sufficient symptomatic relief. There is thus a need for therapeutic targets with novel modes of action, which could act on several neurotransmitter systems. The identification and validation of such a target would thus be highly relevant in the treatment of the motor and psychiatric symptoms of PD.

Chapter 2 - GPR88

In this context, the orphan G-protein coupled receptor 88 (GPR88), which has been identified and investigated as a risk gene for neuropsychiatric diseases by our laboratory, stands out as a particularly suited target. Namely, our interest in this receptor stems from previous genetic studies by our group showing that *GPR88* is associated with Bipolar Disorder and Schizophrenia^{150,151}. Furthermore, another group recently reported cases of patients with deleterious mutations of *GPR88*, who suffered from profound learning deficits and a hyperkinetic movement disorder¹⁵². In the following section, an overview of the characteristics of the receptor and studies of its function will be provided, and its relevance to PD pathophysiology will be highlighted.

A. Studies of Gpr88

1. Characteristics

i. Gene and protein

GPR88 was first discovered by Mizushima and colleagues in 2000, and identified as an orphan G-protein coupled receptor (GPCR) with very high enrichment in the striatum¹⁵³. In humans, the *GPR88* gene is present on the 1p21.3 chromosome. Like other GPCR, the entire open read frame (ORF) of the gene is encoded by one exon¹⁵³.

The receptor is composed of 384 amino acids. Comparisons of the primary sequences of GPR88 between mice and humans revealed 95% of evolutionary conservation, suggesting a high functional importance of this receptor¹⁵³. In support of this finding, Gpr88 orthologs have also been reported in a number of vertebrate and invertebrate species according to the PANTHER classification system¹⁵⁴.

ii. Distribution

Gpr88 expression is exclusively neuronal, and limited to the CNS^{153,155,156} (**Figure 13**). While *Gpr88* mRNA and protein is most highly expressed in the striatum, it is also found in extra-striatal structures^{155–157}. Indeed, Gpr88 is also expressed in the olfactory tubercle, amygdala, inferior olive, cortex and hypothalamus. In the cortex, Gpr88 has been reported in layers II to IV, and is notably enriched in sensory cortex layer IV^{155,157}.

Although Gpr88 mRNA and protein distribution were found to match¹⁵⁶, the subcellular localisation of the Gpr88 protein varies depending on the structure^{155,156} (**Figure 13**). In the striatum, ultrastructural immunolabelling revealed that Gpr88 was preferentially expressed at post-synaptic dendritic areas of cortico-striatal (but not thalamo-striatal) afferences, suggesting a particular role in the integration of cortical information into the BG¹⁵⁶. It is also expressed on the cell body and in primary cilia¹⁵⁶. While virtually all MSN express high levels of *Gpr88*, interneuron expression seems to be lower, if not controversial^{156,158,159}. Interestingly, *Gpr88* expression and translation was found to be significantly higher in iMSN compared to dMSN^{156,160}.

Regarding regional distribution, *Gpr88* expression is highest in the sensorimotor areas of the striatum, followed by associative and limbic striatal areas¹⁵⁸, thus matching the distribution of other GPCRs such as the D2 and CB1 receptors.

By contrast, expression in the cortex and amygdala is limited to the cell nucleus and the nuclear membrane¹⁵⁷. This phenomenon has already been observed in other GPCRs, and suggests potential interaction with transcription elements¹⁵⁷. This topic is currently under investigation by Diaz and colleagues.

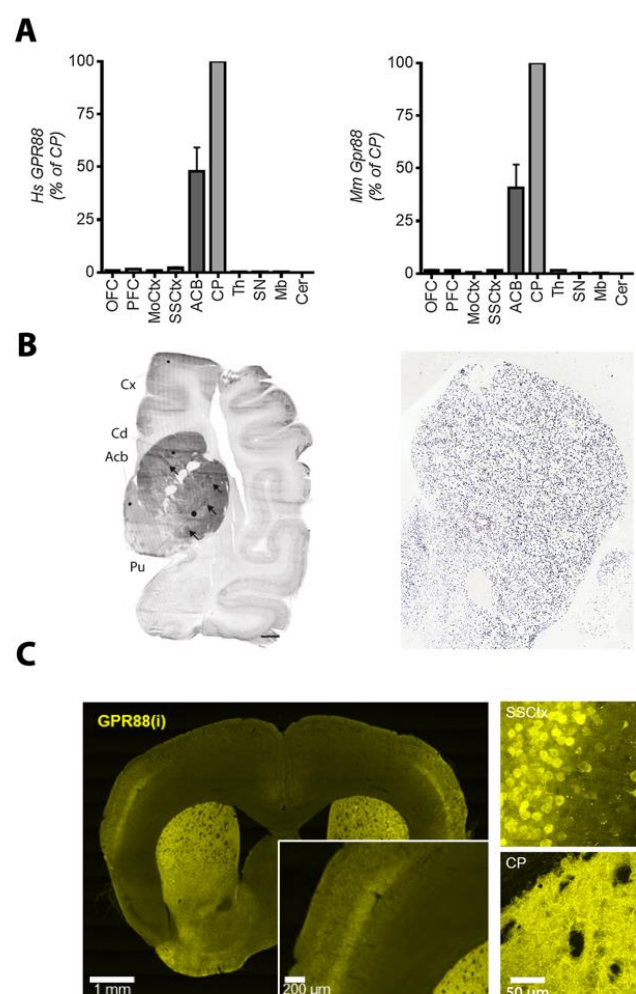


Figure 13 – GPR88 expression in the brain

A: *GPR88/Gpr88* mRNA expression in the human primate (left) and mouse (right) brain quantified by RT-qPCR, and normalised to the CP expression level. **B:** *In situ* hybridization of *GPR88/Gpr88* in the non-human primate (left) and rat (right) brain. **C:** Left - Localisation of the Gpr88 protein in a Gpr88-venus transgenic mouse (left).

Right – different subcellular expression in the CP (on fibers, bottom right) and SSCtX (nuclear, top right).

OFC: orbitofrontal cortex, PFC: prefrontal cortex, MoCtx: motor cortex, SSCtX: somatosensory cortex, ACB: nucleus accumbens, CP: caudo-putamen, Cd: caudate, Pu: putamen, Th: thalamys, SN: substantia nigra, Mb: midbrain, Cer: cerebellum.

Parts of the figure are adapted from Massart et al., 2009, Elrich et al., 2017^{155,156}

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iii. Developmental regulation

High expression of *Gpr88* can be detected counting from E16 in the structures mentioned above¹⁵⁷. During embryonic development, *Gpr88* is also transiently expressed in some peripheral tissues, but the levels become undetectable after P7¹⁵⁷.

Gpr88 expression in the dorsal striatum is highest in juvenile rats, and decreases until adulthood, similarly to the patterns of expression of D1 and D2 receptors. In the ventral striatum, the opposite pattern is however observed¹⁵⁸. BDNF may play a role in the modulation of *Gpr88* expression during development, as striatal *Gpr88* mRNA was found to be lower in heterozygote BDNF-deficient mice¹⁶¹.

iv. Modulation

Studies of changes in the expression of *Gpr88* following specific treatments provide clues about its regulation. For instance, in extrastriatal areas, cortical *Gpr88* mRNA was found to be modulated by methamphetamine, valproate and lithium^{162,163}. Hypothalamic *Gpr88* mRNA is also modulated by antidepressant treatments including fluoxetine (increase), electroconvulsive therapy and sleep deprivation (decrease)¹⁶⁴.

In the striatum, the patterns of *Gpr88* modulation depend on the neuronal type. In iMSN, where *Gpr88* is most expressed, loss of nigrostriatal dopaminergic or corticostriatal glutamatergic inputs both result in decreases in the expression of *Gpr88*¹⁵⁶. In support of this finding, local knockdown of *Drd2* in the ventral striatum leads to a decrease in *Gpr88* expression¹⁶⁵. By contrast, in dMSN, *Gpr88* expression increases following DA depletion but also cocaine administration, while loss of GLU afferences does not have a significant effect^{156,160}. Furthermore, while the effects of DA loss can be reversed in both cell types following L-DOPA treatment, the normalisation of dMSN *Gpr88* expression depends on D2 (iMSN) activation, just as the normalisation of iMSN *Gpr88* expression is mediated by D1 (dMSN) stimulation¹⁵⁶. This effect suggests that striatal *Gpr88* expression may be in part modulated by MSN crosstalk, possibly through collateral connections or interneurons.

2. Insights into Gpr88 function

i. Biochemical insights

GPR88's primary structure presents 18% homology with monoamine receptors 5HT1D and $\beta 3$ ¹⁵³. As other GPCRs, GPR88 contains seven putative transmembrane domains. However, its lacks of an intracellular DRY motif and of disulfide bonds between its extracellular loops suggest that GPR88 may belong to a novel type of GPCR¹⁵³.

Efforts to develop specific agonists have revealed likely coupling of GPR88 with Gi/o proteins, as the synthetic agonist 2-PCCA was found to inhibit cAMP accumulation¹⁶⁶. This characteristic suggests an inhibitory activity of GPR88 *in vivo*, which is supported by findings from functional studies described below.

ii. Transcriptional insights

Transcription studies of Gpr88 knock-out (KO) mice have shown an upregulation of numerous genes that negatively regulate cell signalling, hence supporting the inhibitory role of Gpr88 on neurotransmission¹⁵⁹. Furthermore, KO mice show downregulation of genes coding for neurotransmitter receptors (amongst which GLU and DA receptors)¹⁶⁷, potentially reflecting a compensatory response to increased signalling. Additionally, *Rgs4* is strongly downregulated in the striatum of KO mice^{159,167}. *Rgs4*, a GTPase accelerating protein, plays an essential role in neurotransmission by downregulating Gi/o-coupled receptors involved in DA, GLU, 5HT, ACh and opioidergic signalling^{159,168–171}.

iii. Regulation of neurotransmission

In vivo and *in vitro* data also confirm Gpr88's role as an inhibitor of neurotransmission. First of all, regarding MSN excitability, Quintana and colleagues¹⁵⁹ showed *in vitro* that both dMSN and iMSN from Gpr88 KO mice exhibited an increased sensitivity to corticostriatal GLU stimulation, and a decreased sensitivity to GABA (from interneurons or collaterals), which led to increased MSN firing *in vivo*. These effects were mediated by an increased phosphorylation of GluR1, which facilitates AMPA signalling, and a decrease in the $\beta 3$ subunit of GABA_A receptors. Furthermore, they found these alterations to be causally linked to the reduced expression of *Rgs4*.

Gpr88 KO mice also exhibit increased responses to amphetamine administration, which may be linked to hypersensitivity to D2 agonists apomorphine and quinpirole^{159,172,173}. Although *Gpr88* inhibition may alter the expression of *D2DR* mRNA depending on the striatal region^{165,167}, studies have failed to find differences in the density, affinity and efficacy of the D2 receptor in KO mice^{167,172}. This increased sensitivity to D2 agonists could nevertheless be mediated by *Rgs4*, which is known to modulate D2 signalling downstream of the receptor¹⁷⁰. It is however not clear if the KO mice have an altered sensitivity to D1 agonists. Studies have found opposing results depending on the dose of D1 agonist used, with only low doses yielding increased sensitivity in KO mice, potentially due to interactions with iMSN^{159,173}. Nevertheless, the fact that DARPP-32 Thr-34 phosphorylation was found to be elevated in the striatum of KO mice supports an increase in D1 signalling¹⁷⁴.

Additionally, Gpr88 KO mice were found to have increased signalling efficiency of muscarinic (M1/M4) and opioid (DOR, MOR) receptors¹⁶⁷.

iv. Brain-wide connectivity

In KO mice, studies of connectivity using MRI have reported significant reorganisation of brain networks. Alterations were observed in the default mode network (DMN), as well as in the functional connectivity of somatosensory and motor cortices, mesocorticolimbic circuitry, and of the dorsal striatum, hippocampus and amygdala^{175,176}. These alterations were associated with increased fractional anisotropy in the somatosensory cortex and dorsal striatum¹⁵⁵.

v. Behavioural regulation

Considering the wide-ranging effects of Gpr88 on neurotransmission, it is not surprising that KO mice have a strongly altered behavioural phenotype. Consistent with its preferential expression in sensorimotor territories of the striatum, KO mice exhibit motor hyperactivity in basal conditions and in response to dopaminergic drugs, altered sensorimotor gating, deficits in sensory tasks and in cue-based behaviours^{159,172,175}. Furthermore, they show deficits in some goal-directed and anticipatory behaviour, as well as reduced anxiety levels^{167,176–178}. Studies of conditional KO indicate that depletion of Gpr88 in A2AR-expressing cells (iMSN and cholinergic

interneurons) is sufficient to drive the motor phenotype as well as some of the anxiety-related behavioural alterations, but not the sensorimotor deficits^{173,179}.

KO mice also show behavioural and metabolic modifications linked to extra-striatal function, such as improved spatial learning (hippocampus-dependant), increased risk-taking behaviour (partly amygdala-dependant) and impaired regulation of energy homeostasis (hypothalamus-dependant)^{167,180}.

3. Gpr88 as a therapeutic target

The aforementioned characteristics of Gpr88 make it a relevant target for neuropsychiatric disorders of the basal ganglia. For instance, a study from our group showed that local inactivation of *Gpr88* in the Nucleus Accumbens reversed the cognitive and motor symptoms in a rat developmental model of schizophrenia¹⁶⁵.

More recently, Ben Hamida and colleagues¹⁷⁶ showed that Gpr88 KO mice demonstrated increased alcohol seeking and drinking, and alterations in functional connectivity that are reminiscent of people at risk of developing alcohol use disorders (AUD). The authors thus suggested that Gpr88 could represent a therapeutic target for AUD.

B. Relevance to Parkinson's disease

In PD, the loss of DA induces an imbalance at the level of the striatum: iMSN activation is facilitated, leading to overactivity of this pathway, while dMSNs are hypoactive. This imbalance is further accentuated by the increase in striatal acetylcholine release and the gradual loss of serotonin.

Therapeutic approaches have thus aimed at reinstating balance by inhibiting iMSNs and facilitating dMSN activity. While this can be temporarily achieved with DA replacement therapies using L-DOPA or dopamine agonists, they are limited by their eventual loss of efficacy or the development of severe adverse effects such as ICDs and dyskinesias. Furthermore, they frequently require the use of additional treatments targeting other neurotransmitter systems, which can bring on additional adverse effects.

Unlike current treatment options, inhibiting Gpr88 would allow to modulate the endogenous signalling of multiple neurotransmitters that are impaired in PD. For instance, by increasing DA and GLU signalling, Gpr88 downregulation may thus help facilitate dMSN activity. Furthermore, increased DOR efficiency may also potentiate the compensatory effects of enkephalin, and increased M4 auto-receptor signalling in striatal cholinergic interneurons (where Gpr88 may be expressed) could reduce ACh release and help alleviate motor deficits^{93,181}.

Another important consideration in the use of Gpr88 as a therapeutic target is that its distribution in the striatum precisely matches the pattern of dopamine loss in PD. Furthermore, its expression level in extrastriatal areas is much lower, thus minimising the risks of off-target effects in the case of pharmacological targeting.

However, due to the current lack of available antagonists or inverse agonists for Gpr88, current studies require alternative approaches to downregulate its activity, such as lentiviral-mediated knock-downs. Nevertheless, this type of approach has significant advantages, as it allows targeting of discrete brain regions while avoiding the limitations of systemic treatments.

Chapter 3 - The research project

A. Objectives

Considering the relevance of *Gpr88* as a novel therapeutic target for PD, our team has focused on assessing its validity in rat models of the disease. Two complementary projects were devised, focusing on:

- The motor symptoms of PD and L-DOPA induced dyskinesias (LID), a project I contributed to (article 1)
- The psychiatric symptoms of *de novo* PD, which was the focus of my thesis (article 2).

To do so, we developed two models of PD based on dopamine depletion. We then used lentiviral-mediated RNA interference to knock-down (KD) the expression of *Gpr88* in discrete striatal areas. Finally, the effects of *Gpr88*-KD were evaluated using behavioural testing as well as molecular investigations. The results are presented in the two articles that follow this introduction. This last section of the introduction contains a brief overview of the PD models and gene therapy approach we used.

B. Modeling Parkinson's disease in rats

Many animal models of PD have been developed, based either on the administration of neurotoxins, viral-mediated α -synuclein overexpression in the SNpc, or genetic mutations¹⁸².

Although limited by their construction validity, models using intracerebral injections of toxins such as 6-hydroxydopamine (6-OHDA) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) allow more flexibility in the induction of specific patterns of DA loss. Furthermore, they have been the most popular approach for modeling both the motor and psychiatric symptoms of PD in rats^{183,184}, hence providing an extensively characterised framework to evaluate the potential of symptomatic treatments. For our projects, we thus developed two 6-OHDA models of PD (**Figure 14**).

1. The 6-OHDA toxin

6OHDA is a molecule with a highly analogous chemical structure to catecholamines. Due to this property, 6OHDA can be up-taken by catecholamine transporters into DA/NA neurons, where it induces severe oxidative stress and destruction of the neuronal membrane, leading to cell death¹⁸⁵.

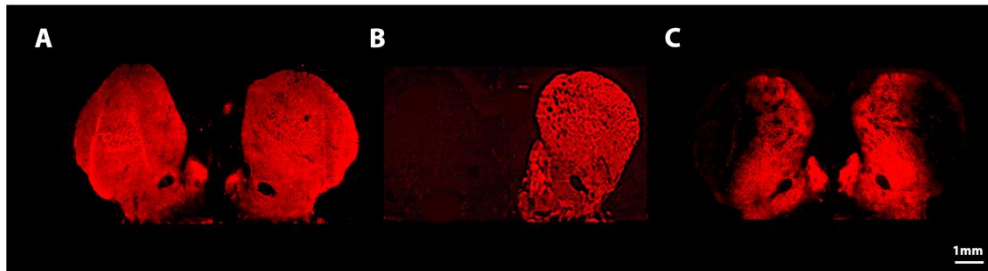


Figure 14 – 6-OHDA models of Parkinson’s disease
Tyrosine Hydroxylase immunofluorescence labelling.
A: un-lesioned rat striata, **B:** unilateral 6-OHDA lesion,
C: bilateral 6-OHDA lesion of the dorsolateral striatum (DLS).

2. Modeling the motor symptoms

To study the motor aspects of PD, we used the “hemiparkinsonian” DA depletion model, induced by a unilateral injection of 6OHDA in the medial forebrain bundle (**Figure 14**) (MFB). This lesion induces a motor imbalance that results in a turning behaviour following the administration of direct and indirect DA agonists such as apomorphine and amphetamine (Amph), respectively. Reductions of this turning behaviour can be used to assess the efficacy of experimental therapeutic approaches¹⁸³. Furthermore, chronic L-DOPA treatment in this model provokes the development of dyskinesias, thus allowing testing of experimental treatments for the control of this motor complication¹⁴³.

3. Modeling the psychiatric symptoms

Due to limitations inherent to rodent models, researchers have focused on developing models of early PD by inducing partial loss of DA in the striatum, in order to study the psychiatric symptoms without interferences from motor deficits. While such models do not recapitulate all of the neurotransmitter changes observed in PD, they have reasonable face validity as they

consistently reproduce behavioural deficits linked to the psychiatric symptoms¹⁸⁴. Importantly, Drui and colleagues demonstrated that the specific loss of nigrostriatal - but not mesolimbic - DA afferences was sufficient to induce behavioural deficits representative of apathy, depression and anxiety that many patients endure¹⁸⁶. In our project, we aimed at reproducing the putative loss of DA that happens during the earliest stages, which is mainly restricted to sensorimotor striatal areas (**Figure 14**).

C. Lentiviral-mediated *Gpr88* knockdown

1. The basis: engineered RNA interference

As no antagonists currently exist for *Gpr88*, we relied on a lentiviral-mediated knock-down approach that was previously designed and published by our group¹⁶⁵. The lentiviral construct contained an engineered microRNA (miR) sequence, consisting in a short hairpin RNA (shRNA) inserted within the miRNA 155 flanking sequences (**Figure 15**). The shRNA complementary sequence was designed to target either the *Gpr88* mRNA and prevent its translation (a process referred to as RNA interference)¹⁸⁷, or a sequence not expressed in the genome (negative control). The point of this engineered construct was to take advantage of the high specificity of shRNAs, while preserving the adaptability of a miR. Indeed, this construct allows the miR to be placed in the 3'UTR of the emGFP reporter gene to monitor transduction location, and under control of the PGK promoter, which is best suited for *in vivo* studies.

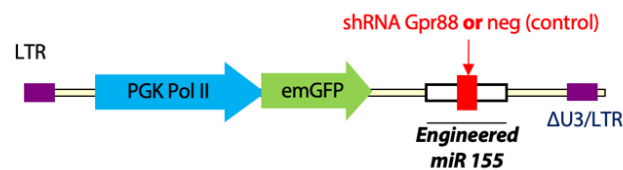


Figure 15 – Engineered miR construct

Simplified map of the vector used to knock-down *Gpr88* expression *in vivo*.

LTR: long terminal repeat, *PGK*: phosphoglycerate kinase, *Pol II*: Polymerase II, *emGFP*: emerald green fluorescent protein, *ΔU3/LTR*: deletion of part of the U3 region of the long terminal repeats (LTR), which leads to a self-inactivating vector.

2. The delivery: lentiviral vectors

This construct was packaged into self-inactivating lentiviruses in order to transduce striatal neurons following stereotaxic injections. Compared to other viral vectors used for gene transfer (**Table 1**), the limited diffusion of lentiviral vectors allows the targeting of precise areas, and their genomic integration ensures a long-term effect of the Gpr88-KD. It should however be noted that this integration comes with a risk of insertional mutagenesis¹⁸⁸.

	Adenovirus	AAV	HSV-1	Lentivirus
GENETIC MATERIAL	dsDNA	ssDNA	dsDNA	RNA
PACKAGING CAPACITY	8kB* 30kB**	< 5kB	40kB * 150kB ***	8 kb
TROPISM	Broad	Broad	Strong for neurons	Broad
INFLAMMATORY POTENTIAL	High	Low	High	Low
VECTOR GENOME FORMS	Episomal	Episomal (>90%) Integrated (<10%)	Episomal	Integrated
MAIN LIMITATIONS	Strong inflammatory response	Small packaging capacity	Inflammatory response Transient expression in non-neuronal cell types	Possible insertional mutagenesis
MAIN ADVANTAGES	Extremely efficient transduction of most tissues	Non-inflammatory, Non-pathogenic	Large packaging capacity Strong neuronal tropism	Persistent gene transfer Non inflammatory / pathogenic

Table 1 – Main viral vector groups

Main characteristics of the different groups of viral vectors. Use of lentiviruses allows long-term expression of the transgene with low inflammatory responses.

* Replication defective, **Helper dependent, *** Amplicon, AAV: adeno-associated virus, HSV-1: herpes simplex virus 1, ssDNA: single-stranded DNA, dsDNA: double-stranded DNA.

Adapted from Thomas et al., 2003¹⁸⁹

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Finally, we took advantage of this approach to assess the effect of *Gpr88* knockdown in discrete striatal regions. For instance, as the sensorimotor and associative areas of the striatum are both crucially involved in the aetiology of the motor and psychiatric symptoms of PD, we compared the effect of Gpr88-KD in each area independently.

Results

Article 1

Summary

Ever since its first description by James Parkinson, PD has been mainly characterised by its motor symptoms, which include bradykinesia, rigidity and resting tremor. Despite decades of clinical research, the main treatment for PD still relies on dopamine replacement strategies. Although they are extremely useful during the first years of treatment, their use is eventually limited by the development of serious side effects such as L-DOPA induced dyskinesia (LID). There is thus a crucial need for new therapeutic strategies with novel modes of action.

GPR88, an orphan GPCR highly expressed in the striatum, may represent such a target. GPR88 is most enriched in the dorsal striatum, where it appears to exert an inhibitory control over motor behaviour. Indeed, individuals with loss of function mutations present a hyperkinetic disorder, and *Gpr88-KO* mice exhibit motor hyperactivity^{152,172}. This effect is mediated by an increased excitability of striatal MSN to GLU cortical inputs, as well as a hypersensitivity of D2 receptors¹⁵⁹.

Based on these findings, we thus aimed to evaluate the effects of *Gpr88* downregulation as a therapeutic target for the motor symptoms of PD as well as LIDs. To do so, we used the “hemiparkinsonian” rat model of PD, which presents a motor imbalance following stimulation with dopaminergic direct or indirect agonists. To assess the effect of *Gpr88* downregulation on this motor imbalance, rotational behaviour in 6OHDA rats was assessed before and after LV-mediated knock-down of *Gpr88* expression in the dorsal striatum. Rats were then chronically treated with L-DOPA in order to induce LIDs, and the frequency of development and severity of these abnormal involuntary movements (AIMs) were evaluated. Finally, molecular markers of MSN activity were quantified in the dorsal striatum, along with the expression of transcription factor Δ FosB, which is causally involved in the development of LIDs¹⁹⁰.

The main results were the following:

- As expected, the 6-OHDA unilateral lesion induced a strong turning behaviour in response to acute treatments. This motor imbalance was linked to an overactivity of iMSN in the lesioned striatum, as indicated by an increase in *Penk* expression.

- Gpr88-KD significantly reduced the motor imbalance following Amph/L-DOPA stimulation. This effect was mediated by a reduction of iMSN overactivity, and an increase in Δ FosB accumulation.
- Chronic L-DOPA treatment led to the development of LIDs in over half of the treated rats, and was associated with an upregulation of *Pdyn* expression. Despite inducing a strong increase in Δ FosB accumulation, Gpr88-KD did not affect the development or severity of LIDs, and prevented the upregulation of *Pdyn*. As Δ FosB accumulation is known to increase *Pdyn* expression and to correlate with LID severity, these results suggest that Gpr88-KD may in fact uncouple the Δ FosB-*Pdyn* association from LID development.

Taken together, these findings indicate that Gpr88 inhibition may indeed be a valid strategy to reduce motor impairment in *de novo* PD by reducing the hyperactivity of iMSN. Furthermore, by improving motor function and uncoupling Δ FosB accumulation from LID development and severity, Gpr88 inhibition may allow to delay and reduce the necessary doses of L-DOPA administration, and thus limit the development of LIDs.

TITLE: Knock-down of GPR88 alters the response of medium spiny neurons to loss of DA input and L-DOPA.

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ABSTRACT

Background

The orphan G protein-coupled receptor Gpr88 expressed in striatal output neurons is implicated in motor activity and is down-regulated by 6-OHDA lesions, an effect that is reversed by L-DOPA.

Objectives and methods

To evaluate Gpr88 as a potential target for the management of Parkinson's disease and L-DOPA-induced dyskinesia (LID), we investigated the effects of Gpr88 inactivation (KD-Gpr88) in the dorsal striatum on circling behavior and LID as well as on specific markers of striatal neurons activity in a 6-OHDA rat model of hemiparkinsonism.

Results

The KD-Gpr88 moderately increased acute L-DOPA-induced turning and normalized the expression of striatal *Gad67* and *Proenkephalin*. Moreover, despite promoting Δ FosB accumulation, KD-Gpr88 was associated neither to upregulation of prodynorphin nor to aggravation of LID following chronic L-DOPA.

Conclusions

These results justify further evaluation of Gpr88 as a novel target for the management of PD, and for improvement of the response to L-DOPA.

INTRODUCTION

The loss of dopamine (DA) input to the GABAergic striatal medium spiny neurons (MSN) induces an imbalance between the direct stimulatory striato-nigral and the indirect inhibitory striato-pallidal pathways responsible for movement initiation, thereby inducing the motor symptoms of Parkinson's disease (PD). The altered activation of these striatal outputs is associated with a net increase of the striatal Glutamic Acid Decarboxylase 67 (*Gad67*) expression, and the concomitant upregulation of Proenkephalin (*Penk*) in the indirect pathway as well as downregulation of Prodynorphin (*Pdyn*) in the direct pathway ¹. L-DOPA replacement therapy alleviates the motor symptoms by activating the direct stimulatory pathway but in the long term provokes L-DOPA-induced dyskinesia (LID) by a complex pattern of interaction with neural networks and cellular substrates ². LID appears to be causally linked to the accumulation of Δ FosB ³, which upregulates *Pdyn* ^{4, 5} in the MSN of the direct pathway. Thus, new antiparkinsonian targets offering alternative and/or complementary approaches to L-DOPA therapy, or moderating its untoward effects, are needed for the treatment of PD ⁶.

To this end we have investigated *Gpr88*, an orphan G protein-coupled receptor almost exclusively expressed in the MSN ⁷. *Gpr88* KO mice display DA hypersensitivity suggesting that *Gpr88* may have an inhibitory influence on DA-dependent MSN activity ^{8, 9}. Moreover, *Gpr88* expression is down-regulated by 6-hydroxydopamine (6-OHDA) lesions of the DAergic nigrostriatal pathway and is restored by L-DOPA ^{10, 11}. However, while the *Gpr88* KO results in increased locomotion in response to DA stimulation ⁸, we have previously shown that the local inactivation of *Gpr88* in the ventral striatum, decreases the motor hyperactivity induced by Amphetamine (Amph) ¹². Thus, to clarify the role of *Gpr88* in PD and its treatment, we locally inactivated *Gpr88* in the dorsal region of the striatum, a territory that is associated with motor regulation ¹³, that was DA-denervated by a unilateral 6-OHDA lesion. We then assessed the impact of *Gpr88* knock-down (KD-*Gpr88*) on (i) the turning behavior induced by Amph and L-DOPA, (ii) the development of LID and on (iii) the expression of markers of MSN activity that are known to be altered by the 6-OHDA lesion and chronic L-DOPA treatment.

MATERIALS AND METHODS

Animal studies authorized by “Ministère de la Recherche” (APAFIS#3669-2016011817516297 v6) were conducted in an approved animal facility (agreement # B75-13-19).

i. Experimental timeline

Male rats received unilateral injections of 6-OHDA (n = 54) or control solution (n = 10) as previously described¹⁴ followed by lentiviral (LV) vectors produced according to standard procedures^{15, 16} co-expressing the Emerald Green Fluorescent Protein (EmGFP) and an engineered miR containing a shRNA directed against Gpr88 (KD-Gpr88) or a control inactive sequence (KD-neg).

Turning behavior was assessed in response to Amph after each stereotaxic injection in miR-neg (n=9) and miR-Gpr88 (n = 11) and to L-DOPA after lentiviral injection only in miR-neg (n=22) and miR-Gpr88 (n = 17) rats. A group of sham- (n = 6) and 6-OHDA- (n = 32) either KD-Gpr88 or KD-neg rats were thereafter treated for 21 days with L-DOPA or saline solution. LID development was assessed by scoring abnormal involuntary movements (AIMs) at 9 -11-16-18 days of treatment (Fig. 1a).

ii. In situ hybridizations and immunohistochemistry studies

Brain sections from miR neg (n = 8) or miR Gpr88 (n = 7) rats were probed with antisense riboprobes as previously described¹² for Tyrosine hydroxylase (*Th*), *Gpr88*, *Gad67*, *Penk*, *Pdyn* and an anti-delta FosB (Abcam, 1/500) antibody.

iii. Statistical analyses

Inclusion criteria for statistical analyses using GRAPHPAD PRISM (GraphPad Software Inc., San Diego, CA) were a minimum of 5 turns/min during the first amphetamine challenge, (corresponding to a permanent reduction in striatal DA greater than 95%¹⁴), complete unilateral loss of *Th* expression in 6-OHDA-lesioned and detectable EmGFP signals in sham- and 6-OHDA-lesioned rats.

RESULTS

i. The KD-Gpr88 improves hemiparkinsonian turning behavior.

In KD-neg (n = 6) and KD-Gpr88 (n = 4) sham-lesioned rats no turning behavior was observed with drug treatments. In the 6-OHDA-lesioned rats, Amph elicited strong ipsilateral turning (11.7 ± 0.9 turns/min). After lentiviral vector injections the Amph-induced net turning behavior was significantly decreased in KD-Gpr88 as compared to KD-neg rats (Fig. 1b).

Acute L-DOPA administration induced an initial ipsilateral turning that switched to the contralateral side after half an hour, as previously reported in the literature¹⁷. The contralateral turning rate was significantly increased in the KD-Gpr88 as compared to KD-neg rats (Fig. 1c).

ii. KD-Gpr88 and L-DOPA-induced dyskinesia

The sham-lesioned rats chronically treated with saline or L-DOPA, as well as the 6-OHDA-lesioned rats chronically treated with saline did not develop LID, irrespective of their KD-neg or KD-Gpr88 status.

On the contrary and as expected according to the literature¹⁸, over 50% of the 6-OHDA-lesioned rats chronically treated with L-DOPA developed LID, in non-significantly different rates or proportions between the KD-neg and KD-Gpr88.

The total AIMs score in the LID-positive animals was of about 55% of the maximal theoretical score¹⁹ and was not significantly different between KD-neg ($222,7 \pm 27,9$; n = 8) and KD-Gpr88 ($206,4 \pm 17,3$; n = 12).

iii. The KD-Gpr88 normalizes the 6-OHDA-induced Gad67 and Penk overexpression and prevents LID-associated Pdyn upregulation.

As expected¹⁸, the 6-OHDA lesion significantly increased *Gad67* and *Penk* and decreased *Pdyn* expression in KD-neg rats. However, the KD-Gpr88 significantly reversed to baseline levels both *Gad67* and *Penk* but not *Pdyn* expression (Fig. 2a). LID is associated to *Gad67*, *Penk* and *Pdyn* over-

expression. Accordingly, in LID-displaying KD-neg rats the expression of *Gad67*, *Penk*, *Pdyn* were concomitantly increased. However, in the KD-Gpr88 rats with LID, while *Gad67* and *Penk* were upregulated to the same extent as their KD-neg counterparts, *Pdyn* expression remained inferior to the baseline and significantly different from *Pdyn* expression in the KD-neg rats (Fig. 2b).

iv. The KD-Gpr88 dissociates hyperactivation of Δ FosB from LID development.

LID-associated *Pdyn* up-regulation, which is positively correlated with AIMs scores, is promoted by Δ FosB activation¹⁸. Strikingly, the KD-Gpr88 also strongly and significantly increased Δ FosB in saline-treated animals (Fig. 2c) and prevented any further increase of Δ FosB following chronic L-DOPA treatment (Fig. 2d).

DISCUSSION

DA modulates MSN output by facilitating the activity of the D1-regulated striato-nigral pathway and inhibiting the activity of D2-regulated striato-pallidal pathway ²⁰. The DA loss following 6-OHDA lesions leads to a net MSN hyperactivation with an increase in the expression of *Gad67*, the rate-limiting enzyme in the synthesis of GABA, decreased expression of *Pdyn*, co-transmitter in the D1 striato-nigral pathway and increased expression of *Penk* co-transmitter in the D2 striato-pallidal pathway ¹⁸. The imbalance between these striatal output pathways following unilateral DA loss results in turning behavior in response to DA receptor stimulation and constitutes a model of PD motor function deficits ²¹.

We have found that the KD-*Gpr88* in the dorsal DA-deafferented striatum produced a significant effect on motor activity in such a model of PD by inhibiting the ipsilateral Amph- and enhancing the contralateral L-DOPA-induced turning behavior. This effect is consistent with an inhibitory role of *Gpr88* on DA-dependent MSN activity, since *Gpr88* KO mice display DA hypersensitivity, as shown by increased locomotion in basal conditions and in response to DA receptor stimulation with direct and indirect agonists ^{8,9}. Moreover, the KD-*Gpr88* normalized the expression of *Gad67* and *Penk* that were upregulated by the 6-OHDA lesion. However, it was unable to modify the decrease in *Pdyn* expression induced by the DA loss. Thus, under basal conditions, the KD-*Gpr88* appears to reduce the imbalance in motor responses to DA receptor stimulation essentially by normalizing the activity of the indirect inhibitory striato-pallidal pathway, without impact on the direct striato-nigral pathway that may remain hypersensitive to D1 receptor stimulation. This may explain the lack of effects of *Gpr88* inactivation on the development of LID, since the KD-*Gpr88* did not modify the intensity of AIMs that are associated with the chronic L-DOPA-induced hyperactivation of the direct pathway ²².

The L-DOPA replacement therapy for the treatment of PD facilitates motor function by enhancing the activity of the D1-regulated striato-nigral pathway, an effect that, in the long term, leads to the development of LID ²². Moreover, the development of LID is associated with an upregulation of *Gad67*, *Penk*, *Pdyn*, and the induction of Δ FosB in the direct pathway that is linked to the severity of LID ^{23, 24}. However, only *Gad67* and *Penk* were upregulated to the same extent both in KD-neg and KD-*Gpr88* rats with LID, while the increase of *Pdyn* in KD-neg was

not paralleled in KD-Gpr88 rats where it remained slightly below baseline. Furthermore, Δ FosB was significantly increased by about 3-fold both in saline- and L-DOPA-treated KD-Gpr88 as compared to KD-neg rats. Nevertheless, the sharp increase of Δ FosB in saline-treated KD-Gpr88 rats was not associated with the development of AIMs. Accordingly, it has been shown that high levels of Δ FosB expression obtained by viral vector transduction in the DA-denervated striatum do not *per se* induce dyskinesia. However, the overexpression of Δ FosB results in the abrupt appearance of high-score AIMs immediately after an acute L-DOPA challenge, which are significantly increased compared to the AIMs scores displayed after chronic L-DOPA treatment²⁵. This is not the case for the Δ FosB sharp increase induced by the KD-Gpr88, which is not associated with a significant increase in AIMs scores even after chronic L-DOPA. Thus, the lack of aggravation of AIMs by the KD-Gpr88 suggests that while it may act by hyperactivating the D1 direct pathway through the induction of Δ FosB, this effect is not directly coupled to dyskinetic effects of L-DOPA. Indeed, Δ FosB upregulates *Pdyn*²⁶, which is linked to both development and severity of LID^{4,5}. However, the KD-Gpr88, while increasing Δ FosB activation, prevented the upregulation of *Pdyn* and the consequent exacerbation of AIMs.

Taken together, our results indicate that the KD-Gpr88 is able to reduce the imbalance between the hyperactive indirect and hypoactive direct pathways following the 6-OHDA lesion. By virtue of this combined action, the KD-Gpr88 has an antiparkinsonian-like effect without a dyskinesia-associated upregulation of MSN activity markers. However, the development of specific pharmacological antagonists will be crucial for further establishing whether GPR88 receptors genuinely represent an alternative target for the treatment of PD - alone or in association with other classes of agents - with a lower propensity to provoke motor side-effects than long-term L-DOPA therapy.

FIGURES

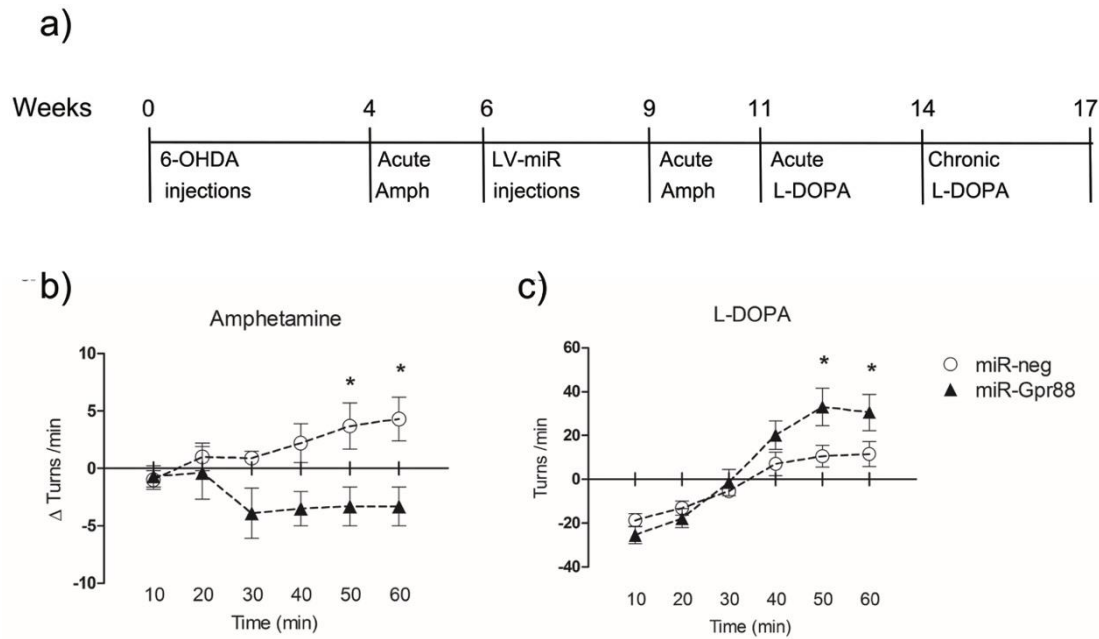


Figure 1

a) Experimental timeline

b) Amphetamine-induced net turning behavior

Two way ANOVA : $F_{1, 108} = 20.26$, $p = 0.0001$. * $P < 0.05$ (Bonferroni's post-test).

c) L-DOPA-induced turning behavior

Two way ANOVA : $F_{5, 270} = 14.09$; $p = 0.0001$. * $P < 0.05$ (Bonferroni's post-test).

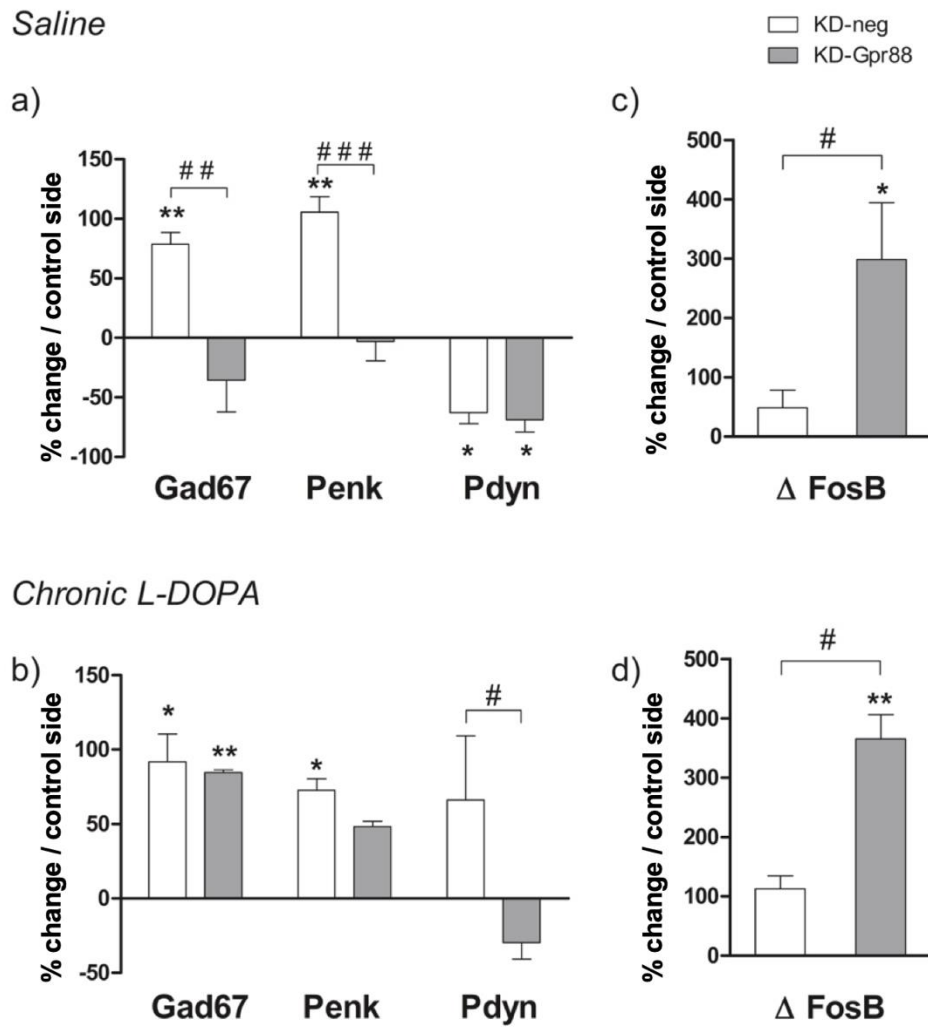


Figure 2

Gad67, Penk, Pdyn mRNA (a, b) and Δ FosB protein (c, d) expression.

Comparisons to the untreated control hemisphere (baseline):

Kruskal-Wallis tests followed by Dunn corrections for multiple comparisons.

P: * < 0.05; ** < 0.01; *** < 0.001

Effect of KD-Gpr88 vs KD-neg:

Multiple t-tests followed by Holm-Sidak corrections for multiple comparisons.

P: # < 0.05; ## < 0.01; ### < 0.0005

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Article 2

Summary

Beyond its motor symptomatology, *de novo* PD is also characterised by an array of psychiatric symptoms such as apathy, depression, anxiety and cognitive deficits, which often emerge in prodromal stages and are a heavy burden on patient quality of life. While current treatments may provide some level of symptomatic relief, their use can be limited by the development of adverse effects such as impulse-control disorders and psychotic symptoms. There is thus an unmet medical need for new targets to treat these aspects of PD.

In the past decade, researchers have developed models of early PD in order to better understand the aetiology of the psychiatric symptoms. As described earlier, such studies have notably established that a partial loss of nigrostriatal DA afferences is sufficient to induce behavioural alterations representative of the main psychiatric symptoms of the disorder¹⁸⁶, thus providing a valuable translational framework to test experimental therapies.

In this context, we aimed at evaluating the relevance of Gpr88 as a therapeutic target for the psychiatric symptoms of PD. Gpr88 is a Gi/o coupled orphan receptor that is highly enriched in striatal MSN, especially at the level of the corticostriatal synapse, where it exerts inhibitory control over neurotransmission^{156,159}. The receptor also seems to be an important modulator of striatal-dependant behaviours, as *Gpr88-KO* mice show alterations in goal-directed and sensorimotor function, and patients with loss of function mutations were found to have impaired learning abilities and speech delay^{152,172,173,177}. Furthermore, several studies from our team have established that *GPR88* is genetically associated with bipolar disorder and schizophrenia, and that inhibiting its expression in the ventral striatum was able to reverse the cognitive deficits in a developmental rat model of schizophrenia^{150,151,165}.

Given this receptor's properties, we hypothesised that inhibiting Gpr88 in a model of early PD may help potentiate endogenous DA neurotransmission, and thus reduce the behavioural deficits. To do so, we first reproduced early PD DA loss in rats by injecting 6-OHDA bilaterally

into the sensorimotor territory of the striatum (dorsolateral, DLS). Using LV-mediated gene transfer, we then knocked-down (KD) *Gpr88* expression in the DLS or in the associative, dorsomedial striatum (DMS), an area that has been consistently associated with the psychiatric symptoms in patients¹¹⁵. The effects of both procedures were assessed at the behavioural and molecular levels, in order to characterise the mechanisms involved.

Our results indicate that:

- the loss of DA limited to the DLS was sufficient to induce behavioural deficits that are representative of the most frequent psychiatric symptoms of early PD (cognitive impairment, apathy and depression), without however affecting motor function.
- this loss of DA increased the activity of MSN in the neighbouring DMS, reminiscent of functional connectivity changes that have been observed in patients. These compensatory changes were reflected by a decreased expression level of transcription factor Δ FosB.
- *Gpr88*-KD in the DMS, but not the DLS, reduced all of the behavioural deficits, and restored Δ FosB expression.

Taken together, these findings support the relevance of *Gpr88* as a therapeutic target for the treatment of the psychiatric symptoms of *de novo* PD, and identify Δ FosB as a key player in its effects. Furthermore, we revealed striatal dynamics that had not yet been characterised in rodent models of the disorder, and which provide support to a “bottleneck” hypothesis of the aetiology of the psychiatric symptoms of PD^{103–105,116}.

TITLE: Gpr88 knock-down in the associative striatum reduces the psychiatric symptoms in a model of Parkinson's Disease.

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ABSTRACT:

Beyond the motor disability, Parkinson's disease (PD) is also characterised by an early appearance of psychiatric symptoms such as apathy, depression, anxiety and cognitive deficits, which can develop into dementia and psychosis in later stages. While current treatments may provide some level of symptomatic relief, their use can be limited by the development of adverse effects such as impulse-control disorders and psychotic symptoms. There is thus a medical need for targets with novel modes of action to treat these aspects of PD.

In this regard, the orphan G-protein coupled receptor Gpr88 may be of interest. *GPR88* is associated with psychiatric disorders, and is highly expressed in the striatum where it exerts inhibitory control over neurotransmitter systems that are compromised in PD. To evaluate the potential of Gpr88 as a target for the treatment of the psychiatric symptoms of PD, we knocked-down (KD) its expression in sensorimotor (DLS) or associative (DMS) striatal areas in a rat model of early PD.

Our findings indicate that Gpr88-KD in the DMS, but not DLS, reduced the alterations in mood, motivation and cognition that characterised the model, and identify the transcription factor Δ FosB as a mediator of this effect. Furthermore, we report alterations in striatal dynamics that are reminiscent of those observed in patients, and which may provide insight into the aetiology of the symptoms.

Taken together, these results thus highlight the relevance of Gpr88 as a therapeutic target for the psychiatric symptoms of PD.

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INTRODUCTION

While Parkinson's Disease is classically defined as a motor disorder, it also entails psychiatric symptoms that are a major burden on patients' quality of life (1). Many of these symptoms have been grouped as part of a "hypodopaminergic" syndrome resulting from the loss of nigrostriatal neurons, which includes apathy, depression, anxiety and cognitive impairment, and affects 35 to 85% of patients depending on the symptom (2, 3). Apathy in particular, defined as a decrease in motivational drive, has emerged as a core symptom of PD, and is present in up to 70% of patients (4). It is often associated with cognitive impairment, as apathetic patients are found to have worse executive dysfunction and an increased risk of developing dementia in later stages (4–6). Psychosis also develops in up to 60% of patients, and is an additional risk factor for dementia (5, 7–9).

A remarkable feature of these symptoms is that they very often emerge years before the onset of motor impairments (10, 11). At the time of diagnosis, studies have found that 37% of patients already suffered from depression, 27% from apathy, 17% from anxiety (12), 20-40% from cognitive dysfunction (13), and 42% reported minor psychotic phenomena, such as passage and presence hallucinations (14).

While dopaminergic medications can efficiently treat some of these aspects (3, 4, 15), their value is hindered by the frequent development of "hyperdopaminergic" symptoms such as impulse-control disorders, which can have disastrous consequences (2, 16). Furthermore, existing treatments for cognitive impairment and psychosis have variable efficacy and limiting side effects (7, 17). There is thus a crucial unmet medical need for the development of new symptomatic treatments to manage these symptoms.

In this context, the orphan G-protein Coupled Receptor 88 (GPR88) is emerging as a particularly suited target. *GPR88* has been associated with Bipolar Disorder and Schizophrenia (18, 19), and deleterious mutations were found to induce profound learning deficits and a hyperkinetic movement disorder in humans (20). The receptor is very highly expressed in the Medium Spiny Neurons (MSN) of the striatum, where it has been found to be modulated by psychotropic drugs (21–24). Functional studies in *Gpr88*-KO mice have shown that *Gpr88* regulates monoamine and neuropeptide neurotransmission through $G_{i/o}$ coupling (25–28). Given these characteristics, it is

not surprising that Gpr88-KO mice were found to have impaired affective, motivated, cognitive and sensorimotor behaviours (22, 27–32). At the striatal level, these effects are mediated by an increased excitability of MSN by cortical inputs and D2 hypersensitivity (22, 28).

Gpr88's restricted localization and wide-ranging effects have made it an interesting therapeutic target for disorders of the basal ganglia. Preclinical studies have already revealed promising results in a model of schizophrenia (33) and potential involvement in alcohol use disorder (34). Given that Gpr88 exerts inhibitory control over the key neurotransmitter systems that are altered in PD, we hypothesized that inhibiting Gpr88 in the striatum could have a therapeutic effect by potentiating endogenous neurotransmission.

In order to evaluate GPR88's potential as a therapeutic target for the psychiatric symptoms of PD, we first developed a translational model of early PD in rats by reproducing the loss of dopamine that is observed in the first stages of the disease, specifically affecting sensorimotor territories of the striatum (35–39). As no antagonists are currently available for this receptor, we then used a localized gene therapy approach to knock-down the expression of *Gpr88* (Gpr88-KD), which we have previously shown to be effective in a model of schizophrenia (40). This localised approach enabled us to assess the effects of Gpr88-KD in the sensorimotor and associative areas of the striatum independently (dorsolateral and dorsomedial striatum in rodents, respectively), the latter having been frequently associated with psychiatric symptoms in patients (41). Finally, the effects of both procedures were then assessed at the behavioural and molecular level.

MATERIALS AND METHODS

i. Animals

Animal studies were authorised by an ethical committee reporting to the French Ministry of Agriculture (APAFIS reference #3669-2016011817516297) and were conducted in the in-house SPF animal facility, which was approved by the Veterinary Inspection Office (agreement reference B75-13-19).

The experiments were performed with male Sprague-Dawley rats that weighed on average 300g at the time of the first surgery (7 weeks old). The animals were first housed in groups of four until the first surgery, after which they were housed in pairs. Animal well-being was checked daily by the experimenters or animal facility staff. The cages were ventilated and enriched with cardboard tunnels, and food and water were available *ad libitum*. The animal facility used a 12h day/night cycle starting at 8 AM each day, and controlled the temperature and the humidity levels daily.

ii. Study design

Two hypotheses were emitted at the beginning of the project, namely that (i) the partial lesion of the rat DLS would reproduce psychiatric symptoms of PD, and that (ii) inactivating Gpr88 in different striatal areas of the lesioned rats would affect different behavioural parameters.

In order to test each hypothesis, several experimental groups were designed. (i) To assess the effect of the loss of DA, two groups of animals were compared: 6-OHDA injected *vs* SHAM injected. Each group was also transduced with a control LV miR-neg sequence in the different striatal compartments. (ii) To determine the effects of Gpr88 inactivation in 6OHDA lesioned rats, three groups were initially designed, as the stereotaxic injections of lentiviruses were performed either in the dorsolateral, dorsomedial or ventral striatum (nucleus accumbens core). However, as experiments were underway, no major effects of Gpr88 inactivation in the ventral striatum were observed, while intriguing interactions were emerging between the dorsolateral and dorsomedial tiers of the striatum. For the sake of clarity, we thus decided to limit the scope of this article to the DLS-DMS interactions in both the effects of the lesion and of Gpr88-KD.

The data were accumulated over 17 replication batches. Each batch consisted of 6-10 animals that were randomly distributed across the different experimental conditions, making sure that no 2 rats of the same conditions were housed together. A schematic representation of the study design and timeline is presented in the supplementary figures (**Fig. S1**).

Sample size was calculated based on behavioural data from preliminary experiments, using the G*Power3 software (42). In order to reach statistical power of 0.9 with the alpha level set at 0.05, the recommended sample size was of 9-10 animals per condition, depending on the behavioural task. Replication batches were stopped once the recommended sample size was met.

Finally, we observed an important level of cumulative variability in the quality and extent of the lesions and lentiviral transductions. In order to limit the effect of this variability and enhance the reproducibility of our findings, strict inclusion criteria were applied. First of all, the DA loss had to affect at least 20% of the DLS, but no more than 15% of the neighbouring DMS and VS. Next, sufficient GFP fluorescence had to be present in the area targeted with LV miR-Gpr88. Furthermore, in the rare occurrences of adverse events (such as important weight loss or inflammatory reactions), the rats were excluded from the statistical analyses.

iii. Stereotaxic injections of 6-OHDA

To reproduce the DA loss observed in early PD, we aimed to affect the sensorimotor territories of the striatum in rodents. However, while striatal compartments are anatomically separated by the internal capsule in primates, it is not the case in rodents. Nevertheless, experiments have delineated the anatomical equivalences between the two orders, and their involvement in basal ganglia loops (42). For instance, (i) the sensorimotor loops involve the posterior putamen in primates, which is equivalent to the dorsolateral striatum (DLS) in rodents. (ii) The primate associative loops involve the anterior putamen and caudate nucleus, corresponding to the dorsomedial striatum (DMS) in rodents.

Before the beginning of the surgeries, the animals were first anaesthetised with 4% isoflurane (IsoVet, Osalia) in an induction chamber (Minerve Veterinary Equipment, Esternay, France) for 5 minutes before being placed into a Kopf stereotaxic surgery apparatus (Phymep, Paris, France). Anaesthesia was maintained throughout the surgery with an isoflurane pump (Univentor,

Zejtun, Malta). General and localised analgesia was induced with subcutaneous injections of buprenorphine (0,05mg/kg) and lidocaine (17,5mg/kg) (Ceva) before beginning the surgery. 4µl of a solution of 6OHDA (3µg/µl, 12µg total per hemisphere) in saline + 0.02% ascorbic acid (all chemicals from Sigma) or a control solution without 6OHDA were then injected bilaterally in the DLS using a 10µL Hamilton syringe (Phymep, Paris, France). The coordinates were as follow: anteroposterior (AP) + 0,7mm; mediolateral (ML) \pm 3,8mm; dorsoventral (DV) -5,5mm (2µl) and - 4,5mm (remaining 2µl) from bregma (43). The injection was performed at a rate of 0.5µl/min, and the syringe was left in place for 3 minutes after the end of the injections to allow for proper diffusion of the toxin. Following the surgeries, the wellbeing of the animals was checked daily. While some rats transiently lost weight, they typically recovered within 3-5 days.

iv. Lentivirus production and stereotaxic injection

The lentiviral vectors were generated at the in-house iVector platform, using the BLOCK-iT Pol II miR RNAi expression vector kit (Invitrogen). The vector construct contained an engineered miR sequence to drive *Gpr88* knockdown (**Fig. 4A**). The modified miR consisted of an shRNA inserted with the miRNA 155 flanking sequences. One of the shRNA complementary sequences was designed to target either the *Gpr88* mRNA ("miR-*Gpr88*"), or a control sequence that is not expressed in the genome ("miR-neg"). Use of shRNA allowed for high specificity of RNA interference, while preserving the advantages of a miR. Namely, the miR containing the shRNA was placed in the 3'UTR of the emGFP reporter gene, allowing to monitor transfection location, and under control of the PGK promoter, best suited for *in vivo* studies. The lentiviruses were stored in PBS, at an average of $1,6 \times 10^5$ transducing units (TU) /µL.

6µL of lentivirus solution was bilaterally injected in the DLS or DMS two weeks after the 6OHDA injections, following the same general surgery procedure. The coordinates were however different, as the lentiviruses were injected bilaterally at 4 different sub-sites (8 total) to insure sufficient knock-down of *Gpr88* expression. When targeting the DLS, the coordinates were the following: (1) AP +1,2 mm; ML \pm 3,6 mm; DV -5,5mm and -4,5mm (2) AP +0,2 mm, ML \pm 4mm, DV -5,5mm and -4,5mm from bregma. Regarding the DMS, the coordinates were: (1) AP +1,2 mm; ML \pm 2 mm; DV -5,5mm and -4,5mm (2) AP +0,2 mm, ML \pm 2,2mm, DV -5,5mm and -4,5mm from bregma.

v. Behavioural tests

All the rats followed the same sequence of behavioural tests, which is summarised in **Fig. S1**. Each experiment was performed by the same experimenter and around the same time of day for each batch of animals, in between 9 AM and 4 PM. The rats were brought in the testing rooms one hour before the beginning of the task in order for them to acclimate to the environment. The luminosity was controlled for every experiment (35 lux), and the testing apparatus were cleaned after each rat with a disinfectant solution (Aniospray, Dutscher).

Actimeter - General motor and exploratory behaviour was assessed for 15 minutes using the Panlab Infrared Actimeter (Harvard Apparatus, Holliston, MA, USA). Horizontal, stereotyped and vertical movements were automatically tracked and cumulated in 5-minute segments.

Sucrose preference - Rats were isolated in enriched individual cages for 72h, during which time they given access to two bottles containing either tap water, or tap water supplemented with 0,5% sucrose (Sigma). The first 24h were considered as an acclimation phase, and were not included in the analysis. After 48h, the position of the bottles was inverted to avoid side preference effects. Bottles were weighed daily in order to calculate the amount of consumed liquids. Sucrose preference was calculated as the percentage of sucrose intake / total intake. However, as the level of sucrose preference exhibited by control rats was lower than what we had observed during pilot experiments (at 0,25% sucrose), the concentration had to be re-evaluated after several batches of animals (0,5%). For this reason, the number of data points for this experiment is relatively lower. General consummatory behaviour was also tracked by weighing the food dispenser at the beginning and end of the isolation phase. Before ending the isolation phase, the social novelty discrimination task was performed.

Social Novelty Discrimination (SND) - Social interaction and selective attention were then evaluated using the social novelty discrimination task (SND) as previously described (40). As this behavioural paradigm requires a preliminary isolation phase, we performed it at the end of the sucrose preference test. Briefly, a first juvenile was placed into the home cage of the tested rat for a first presentation period (P1) of 30 min. The time spent by the tested rat investigating the juvenile (anogenital sniffing, pursuing, allogrooming) was timed manually for the first 5 min. At the end of P1, a second juvenile was introduced in the cage, and the time spent investigating the

novel vs familiar juvenile was timed by the experimenter (presentation period P2). The “discrimination ratio” was calculated as the time spent in P2 by the adult with the novel juvenile over the familiar juvenile.

Prepulse Inhibition (PPI) - Sensorimotor gating was assessed using an auditory Prepulse Inhibition apparatus (IMETRONIC, Pessac, France). The adapted protocol (44) contained three phases: a first acclimation period of 10 minutes, followed by a phase of habituation to the startle stimulus, and ending with the testing phase. During the acclimation phase, a background white noise of 60dB was played, which persisted throughout the whole testing session. The habituation phase consisted of 10 startle-inducing auditory “pulses” played at 110dB (7Khz, 100ms), and at random intervals between 15-30 seconds. During the testing phase, four different types of stimuli were presented: a pulse alone (110dB), a prepulse-pulse pairing, a prepulse alone, or no sound (to assess background movement). The prepulses (20ms, 70-80dB) preceded the startle pulses by 100ms. Each condition was presented 10 times in a pseudo-randomised order, at random intervals of 15-30 seconds. Prepulse inhibition (%PPI) was measured as the reduction of startle response during prepulse–pulse conditions compared with pulse-alone trials.

Forced Swim Test (FST) - The rats were placed in a transparent cylinder filled up to 35 cm with 24°C (± 1) water for 5 minutes, and recorded using a digital camera. A trained experimenter blinded to the conditions then analysed the behaviour of the animals using a previously described sampling method (45), calculating immobility, swimming, climbing and diving counts.

vi. Immunolabelling and in situ hybridisation

Following the end of the behavioural procedures (after 2-4 days), the rats were anaesthetised in an isoflurane chamber and decapitated. The brains were rapidly removed and snap-frozen for 90 seconds in isopentane at -55°C (Carlo Erba Reagents). Coronal sections (12 μ m) of the striatum were used to control for the presence and extent of GFP transduction in the targeted areas. The slices were then post-fixed in 4% PFA for 30 minutes in order to perform immunolabelling or *in situ* hybridisation experiments.

Immunolabelling procedures were adapted from previously published protocols (46, 47) using primary antibodies directed against Tyrosine Hydroxylase (TH) (Millipore MAB318, 1:400) or

Δ FosB (Abcam AB11959, 1:500). TH immunolabelling was completed using a fluorophore-coupled secondary antibody (Alexa Fluor 647, Invitrogen A21235, 1:1000) while Δ FosB required DAB revelation for best results (BA-2000 secondary antibody, 1:250, and PK6100 kit from Vector Laboratories) (**Fig.S2A,C**).

In situ hybridization (ISH) was performed with antisense digoxigenin-labeled complementary RNA probes designed to recognise *Gpr88*, 67-kDa glutamate decarboxylase (*Gad67*), proenkephaline (*Penk*) and prodynorphine (*Pdyn*) mRNAs as previously described (40) (**Fig. S2B, D**).

Digitisation and signal quantification - Slides were then digitised using the Axio Scan.Z1 and ZEN software (Zeiss, Oberkochen, Germany). The resulting images were exported for processing in Fiji (NIH, Bethesda, MD, USA) (48, 49). As fluorescence and colorimetric staining are not stoichiometrically related to biological content, the signal intensity was not quantified. Instead, a threshold was determined using control slides (secondary antibody alone/sense probe) or control areas within a slice (corpus callosum), and applied to all of the images from a same experiment. To evaluate the loss of dopaminergic terminals, the TH signal was then quantified in each striatal area (DLS, DMS, ventral striatum) according to previously published methods (50). Regarding the nuclear markers (Δ FosB and all of the ISH targets), a fixed-size region of interest was drawn in the DLS and DMS to quantify the positive signal in each area (**see Fig.S6E**). For each striatum, the signal was measured over at least 3 anteroposterior locations between AP +0,2 mm and +1,2 mm, and averaged. The values were then normalised to those obtained in control rats (SHAM + miR-neg). A Fiji macro script was written to automatise the process. As the lesion and transduction extent varied, each striatum (2/brain) was considered as a biological replicate for statistical analyses.

vii. Statistics

Data from the experiment were analysed using the Prism 6.0 software (GraphPad Software Inc, La Jolla, CA, USA). Different tests were performed depending on the nature of the data and the driving hypotheses that were exposed in the “study design” section. For instance, regarding behavioural data, to assess the effects of the lesion (hypothesis 1), the two groups (SHAM-neg *vs* 6OHDA-neg) were compared with two-tailed unpaired t-tests, followed by Welch corrections.

Then, to evaluate the effects of Gpr88-KD in 6OHDA lesioned animals (hypothesis 2), the groups were compared using one-way ANOVAs followed by Bonferroni multiple comparison tests, as the data distribution passed normality tests. However, some of the actimeter, PPI and SND data required the use of 2-way ANOVAs followed by Bonferroni corrections to assess interaction with independent variables (time, prepulse intensity, novelty status). Finally, as each striatal area was differentially affected by the 6OHDA lesion, the data from the immunolabelling and ISH experiments were analysed using multiple t-tests followed by Holm-Sidak corrections.

RESULTS

i. 6OHDA stereotaxic injections induce a dopaminergic denervation limited to the DLS.

In order to reproduce the loss of DA observed in early-stage Parkinsonian patients (51), 6-OHDA was stereotaxically injected into the dorsolateral striata (DLS) of adult male rats. Control animals were injected with a vehicle solution (SHAM). The toxin induced a partial loss of dopamine afferences to the DLS, as indicated by a mean reduction of Tyrosine Hydroxylase immunoreactivity of 48% over at least 1mm on the anterior-posterior axis. The neighbouring striatal regions (dorsomedial and ventral) were unaffected by the lesion (Fig. 1).

*ii. Efficient lentivirus-mediated knockdown of *Gpr88* expression in the DLS / DMS*

Lentiviral vectors containing a control miR (miR-neg) or a miR directed against *Gpr88* (miR-*Gpr88*) were stereotaxically injected either into the DLS or the DMS of 6-OHDA lesioned rats (Fig. 2 A, B). SHAM-lesioned rats were injected with miR-neg containing vectors. Expression of *Gpr88* mRNA was efficiently reduced by 92% and 64% in the transduced areas of the DLS or DMS respectively (Fig. 2 C, D).

iii. Partial dopaminergic loss in the DLS reproduces psychiatric symptoms of PD without inducing motor deficits

Motor behaviour was assessed at two and four weeks after 6-OHDA injections by measuring horizontal movements in the Actimeter. This parameter was unaffected by the lesion at both timepoints (Fig.3A, Fig. S3A), as expected with a limited loss of DA (50). Stereotyped behaviour in the Actimeter test was also preserved. However, at 4 weeks after the lesion, the number of rearings was reduced at the 15-minute timepoint, indicating a decrease in novelty exploration behaviour (Fig.3A).

Sensorimotor gating was then assessed using the Prepulse Inhibition (PPI) test. Although the 6-OHDA lesion increased the startle reaction in response to a loud auditory stimulus (110dB), the %PPI was preserved (Fig. 3B).

Affective parameters were then evaluated with the sucrose preference and forced swim (FST) tests. Whereas the 6-OHDA did not alter sucrose preference (Fig. 3C), it decreased latency to immobility and increased immobility time in the FST (Fig. 3D). These results indicate that the partial DA loss induces a depressive-like state, that is however not associated with anhedonia. Consummatory behaviour, measured by daily food and water intake, was not affected by the lesion (Fig. S3B).

Finally, social interaction and cognitive abilities relating to selective attention were assessed using the Social Novelty Discrimination task (52). In the first part of the test, the lesioned animals spent less time interacting with a juvenile than controls (Fig. 3E). In the discrimination task, the lesioned animals also failed to preferentially direct their attention to the novel juvenile, which resulted in a decreased discrimination ratio. This effect was however not due to a decrease in total social interaction during the discrimination task (Fig. S3C).

iv. Gpr88 knockdown in the DMS, but not the DLS, reverses the behavioural deficits.

No effect of *Gpr88*-KD on horizontal activity was observed, thus excluding potential motor interferences in the following tests. Silencing of *Gpr88* in the DMS reversed the deficit in rearing behaviour at the 15-minute timepoint (Fig. 4A). It also increased sensorimotor gating without affecting startle amplitude (Fig. 4B).

Whereas *Gpr88*-KD had no effect on sucrose preference, inactivation in the DMS had an antidepressant-like effect in the FST, by increasing latency and reducing immobility time (Fig. 4C). This reduction was mediated by an increase in swimming behaviour specifically (Fig. S4A) (53). No effect on consummatory behaviour was observed (Fig. S4B).

Gpr88-KD in the DMS also had a pro-motivational effect by increasing social interaction duration (Fig. 4E). Finally, whereas the inactivation in both the DMS and DLS increased preferential interaction with the novel juvenile in the social discrimination task, only the effect in the DMS was large enough to restore the discrimination ratio. This pro-cognitive effect was however not related to an increase in total interaction time during the discrimination task (Fig. S4C).

v. DA loss in the DLS induces molecular changes in the adjacent DMS

Molecular studies were undertaken to further characterize the model. *Glutamate decarboxylase 67* (*Gad67*), *Prodynorphin* (*Pdyn*) and *Proenkephalin* (*Penk*) mRNA expression were quantified by *in situ* hybridization, in order to evaluate the global activity of GABAergic neurons (and interneurons) of the striatum, as well as the activity of the direct (dMSN) and indirect (iMSN) pathway neurons respectively. Δ FosB, a key transcription factor regulating the striatal direct and indirect pathways, was chosen as the final molecular readout. *Gpr88* mRNA was also quantified to check for potential effects of the lesion on its expression.

Strikingly, despite the DA loss being strictly limited to the DLS, molecular alterations were also observed in the adjacent, intact DMS. For instance, regarding neuronal activity, the lesion upregulated *Gad67* expression in both areas (Fig. 5A). However, different patterns were observed for the direct and indirect pathways markers. For instance, the lesion decreased *Pdyn* and increased *Penk* expression levels in the DLS. By contrast, both *Pdyn* and *Penk* expression were upregulated in the un-lesioned DMS, indicating overactivity of both dMSN and iMSN (Fig. 5A). Regarding Δ FosB, its expression was increased locally by the loss of DA, but was decreased in the un-lesioned DMS (Fig. 5B). No effect of the 6-OHDA lesion was found on *Gpr88* expression in either striatal area (Fig. S5).

vi. Gpr88 inactivation modulates indirect pathway activity and transcription factor Δ FosB

Gpr88-KD in both areas reduced *Gad67* expression (Fig. 6A). *Gpr88-KD* also decreased the expression of *Penk* in the DLS, while *Pdyn* expression remained unchanged. *Gpr88-KD* had opposing effect on Δ FosB expression in the DLS and DMS, reverting in both cases the 6OHDA induced alterations (Fig. 6B). Taken together, these results suggest that *Gpr88-KD* acts preferentially on indirect pathway activity, and normalizes Δ FosB accumulation.

vii. Effect of Gpr88-KD on lateral ventricle size

During the histological processing of brain slices, it became evident that lateral ventricle size was strongly affected by *Gpr88-KD* (Fig. S6A). This effect was most pronounced when the miR-*Gpr88* was injected in the DMS, causing a 241% increase in ventricle size compared to miR-neg injected rats (Fig. S6B).

DISCUSSION

In our experiments, we showed that reproducing early Parkinson's DA loss in the DLS induced behavioural deficits that are representative of some psychiatric symptoms of the disease. For instance, the DA loss increased immobility time in the forced swim test, an indicator of behavioural despair linked to depression (45). It also induced a decrease in social interaction and rearing behaviour (related to novelty exploration (54)), two parameters that have been proposed as translational and ecological measures of apathy (55). These results are also coherent with the observation that behavioural and social aspects of apathy are the most affected in PD, and are associated with depression (56, 57).

Furthermore, the loss of DA also impaired performance in the social novelty discrimination task, which was designed to evaluate selective attention (52), a feature of executive functioning which is impaired in PD (58). It could be argued that the deficit in discrimination ratio that is present in the 6-OHDA rats may be due to a lack of motivational drive. However, during the discrimination task, no alteration in the total interaction time was observed between 6-OHDA and control rats, suggesting that the impairment may indeed be due to deficits in attentional processing. Finally, the lesion did not alter motor behaviour, thus excluding potential interferences in other behavioural tests.

Taken together, these results indicate that the loss of DA restricted to the DLS is thus sufficient to reproduce deficits relating to depression, apathy and cognitive impairment, which are three of the most prevalent symptoms at the time of PD diagnosis (59). However, we did not find previously reported deficits relating to anhedonia and sensorimotor gating in the sucrose preference and prepulse inhibition (PPI) tests (60–62). Anhedonia is frequently reported in PD, but has however been associated with the mesolimbic dopamine pathway, which we did not affect in our study (10, 63). Altered sensorimotor gating is also present in PD as in many basal ganglia disorders, and has been linked to PD cognitive dysfunction (64). In the PPI test, while the lesioned rats exhibited a significantly increased startle reaction, they maintained a normal level of %PPI, suggesting the presence of efficient compensatory mechanisms. Previous studies have found that extensive dopamine loss is required to induce PPI alterations (61). It is thus likely that the lack of PPI and sucrose preference alterations in our model is due to the restricted nature of the lesion.

We then showed that Gpr88-KD in the associative, but not the sensorimotor striatum, had pro-motivational, pro-cognitive and antidepressant effects, as it reversed all of the behavioural deficits induced by the restricted DA loss, without however affecting motor behaviour in the Actimeter test. Gpr88-KD in the DMS also increased sensorimotor gating in the PPI test, indicating an increased efficiency of pre-attentive information processing, which could underlie the pro-cognitive effect in the social novelty discrimination task. Interestingly, models of PD psychosis have linked the emergence of hallucinations to deficits in gating and attentional processing (7). Gpr88-KD may thus be of interest for the treatment of this aspect as well.

To better understand the mechanisms underlying the effects of the 6OHDA lesion and Gpr88-KD, we then quantified molecular markers of neuronal activity. *Prodynorphin* (*Pdyn*) and *Proenkephalin* (*Penk*) are expressed in the dMSN and iMSN respectively, where their expression level reflects neuronal activity (65). Furthermore, the neuropeptides they encode act a feedback mechanism to dampen the effects of overactivity, by modulating nigrostriatal dopamine release and MSN transcriptional mechanisms. We also quantified the expression of *Glutamate decarboxylase 67* (*Gad67*), an enzyme involved in the synthesis of GABA, that is considered a proxy for assessing the level GABAergic transmission in the striatum, notably arising from interneurons (66).

As, expected, in the DLS, the loss of DA induced a decrease in *Pdyn* and an increase in *Penk* and *Gad67* expression, reflecting the imbalance in MSN activity that has previously been characterised in PD models (65). However, this restricted loss of DA also induced molecular changes in the neighbouring associative territory of the striatum (DMS). Indeed, in the DMS, all of the aforementioned markers were significantly elevated, suggesting a compensatory overactivity of MSN in response to DA depletion of the DLS.

We found that the effects of Gpr88-KD in the DLS were mediated by a decrease in the expression of *Gad67* and *Penk*, but not of *Pdyn*. This result indicates that Gpr88-KD acts preferentially through iMSN, which is coherent with the receptor's relative enrichment in this neuronal type (23), and the hypersensitivity to D2 agonists that has been consistently reported in Gpr88-KO mice. As Gpr88-KO mice also exhibit increased signalling of the enkephalin receptor DOR (69), it could be expected that the reduced *Penk* expression following local KD results from potentiated compensatory mechanisms. Interestingly, Gpr88-KD in the DMS decreased *Gad67* expression

without however affecting *Penk* or *Pdyn*, suggesting a potential decrease in GABA interneuron activity (66), in which *Gpr88* expression has been reported (23).

Δ FosB, a transcription factor involved in PD, depression and addiction, was chosen as a final molecular readout, as its unique accumulation profile confers it a long-lasting effect on the regulation of striatal gene networks (70–72). Furthermore, Δ FosB is known to interact with DA signalling. Indeed, in the dorsal striatum, Δ FosB accumulation leads to sensitisation of the D1 receptor, an effect which is potentiated by D2 stimulation (73–75).

While Δ FosB accumulation in PD is linked to the effects of L-DOPA treatment (76), we also observed an increase in the DLS of the lesioned rats, without pharmacological stimulation. This unexpected effect could be due to stimulation of hypersensitive D1 receptors in the DLS by DA released by sprouted afferences (77), or resulting from a “volume transmission” effect from the un-lesioned striatum (78). In the DMS however, there was a striking decrease in Δ FosB accumulation. This could in part result from *Pdyn* overexpression, which by dampening the effects of dMSN overactivity on immediate-early gene (IEG) induction (65), may prevent Δ FosB accumulation.

Gpr88-KD normalized Δ FosB expression in both the DLS and DMS, without however affecting *Pdyn* expression. This suggests that while the activity level of dMSN remains unchanged, its effect on Δ FosB accumulation is reduced. Although our results suggest that this effect may be mediated by changes in iMSN or interneuron activity, the underlying mechanism is not clear and requires further investigation. Nevertheless, as Δ FosB accumulation sensitizes D1 receptor, the increase observed in the DMS following *Gpr88-KD* may thus potentiate dMSN signalling, and help promote goal-directed behaviour. For instance, the antidepressant effect of *Gpr88-KD* observed in the FST may be due to its modulation of Δ FosB, as fluoxetine was also found to increase the accumulation of this transcription factor in the DMS (53).

To conclude, our results support the relevance of *Gpr88* as a therapeutic target for the psychiatric symptoms of PD, as we demonstrate that knocking-down its expression in the associative striatum reduces the behavioural deficits in a rat model of the disease. Furthermore, we identified that *Gpr88-KD* strongly modulated the expression of Δ FosB, a transcription factor that has previously been linked to PD pathophysiology (72, 76, 79).

Beyond validating *Gpr88* as a therapeutic target, this study also provides insights into PD pathophysiology. During the design of the experiments, special attention was given to the translational value of the model. As PD is a highly heterogeneous disease, recent efforts have focused on stratification of PD patients starting from *de novo* stages. Such approaches have enabled the identification of patient clusters based on differences in symptomatology (80) and biological markers. For instance, subtypes with the highest prevalence of psychiatric symptoms are associated with stronger dopamine loss in the striatum, whereas the prevalence of other non-motor symptoms (such as sleep and autonomic impairment for instance) has been associated with non-dopaminergic alterations (81–83). These findings suggest that the loss of dopamine itself may be the driving force in the emergence of the psychiatric symptoms. For this reason, we chose to adapt the 6OHDA model of PD, by reproducing the early loss of DA which is observed in sensorimotor territories (DLS).

While previous models of the psychiatric symptoms of PD induced a loss of DA in both the DLS and DMS (50, 84), our results show that loss of dopamine in the DLS alone is sufficient to induce behavioural impairments representative of frequent psychiatric symptoms of PD. Such symptoms have however been typically associated with DA dysfunctions in associative networks (DMS) (41), which were not affected by the DA loss in our experiments. Nevertheless, we observed an overactivity of MSN in the DMS, suggesting possible interactions between striatal areas. This observation is reminiscent of findings from fMRI studies of PD patients. Indeed, a couple of studies have found that whereas functional connectivity decreased in the dopamine-deprived fronto-striatal sensorimotor networks, an increase was observed in associative networks (67, 68). This increase is thought to reflect system-wide adaptive mechanisms, whereby associative networks are found to expand their cortical connectivity to compensate for the loss of DA in sensorimotor territories. However, due to the resulting overlap of networks within limited striatal computational resources, it has been suggested that this compensatory mechanism could lead to a “neural bottleneck” or “overload” of associative networks, and impairment in related behaviours (67, 68, 85). This hypothesis could thus explain the emergence of psychiatric deficits relating to cognition and motivation during the earliest stages of PD and in our model of the disease, before the loss of DA affects associative networks.

In this context, the results from our molecular investigations may provide a cellular mechanism for the “bottleneck” hypothesis. Indeed, as dynorphin downregulates both nigrostriatal

dopamine release and dMSN output (65), its overexpression in the DMS of 6-OHDA lesioned rats may act as a throttle on information processing. Furthermore, the increased activity of iMSN we observed in the DMS might also contribute to the “bottleneck” effect, by antagonizing dMSN function.

Although similar alterations in striatal dynamics have been reported in models of other basal ganglia disorders such as addiction (86), OCD (87) and Huntington’s disease (88), this study is the first to our knowledge to investigate this aspect in a model of PD. Taken together, these results thus confer both face and construct validity to the model of PD we used to evaluate the therapeutic potential of Gpr88-KD.

Several aspects nevertheless need to be further investigated. For instance, behavioural tests assessing specific cognitive and motivational parameters would be welcome, to further characterize the effects of Gpr88-KD. As the model we used recapitulates aspects of early stage PD, it will also be necessary to assess the effects of Gpr88-KD in models of advanced PD, presenting more extensive DA loss and alterations in other neurotransmitter systems. Furthermore, while our molecular experiments have provided some clues regarding the mechanisms involved, additional in-depth studies are required as very little is known about the signalling pathways regulated by Gpr88. Finally, although no adverse effects of Gpr88-KD on behaviour were observed, we noticed that when Gpr88 was inactivated in the areas closest to the lateral ventricles (DMS), their volume increased. Further studies are thus required to determine the mechanism behind this increase, and any potential detrimental effects.

Provided that these questions can be answered, there are still a few steps to consider in order to advance the translational process to the clinic. Indeed, while Gpr88’s restricted expression makes it an ideal therapeutic target, no antagonist has been developed so far for this orphan receptor. It is thus crucial to develop lead identification efforts. Nevertheless, gene therapy approaches have showed successful applications for PD in recent years (89, 90). Based on the presented results and previous findings from our laboratory, Gpr88 could thus constitute a highly relevant gene therapy target for the treatment of the psychiatric symptoms of PD and of other basal ganglia disorders (40).

ACKNOWLEDGMENTS

The lentiviral vectors were produced by the in-house iVECTOR facility. *In vivo* experiments were carried out in the Pheno-ICMice animal facility, and the histological procedures were performed at the HISTOMICS platform, both also situated at ICM.

FIGURES

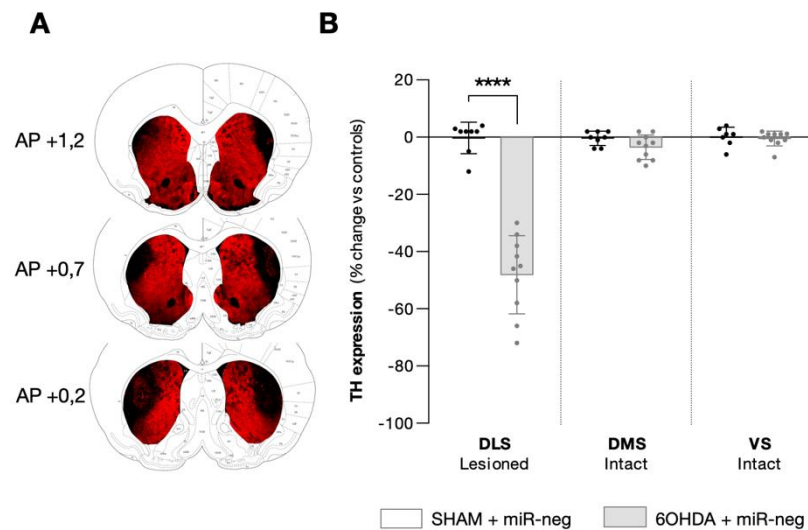


Fig. 1 – Bilateral 6-OHDA injections induce a dopaminergic loss restricted to the DLS. (A) Representative images of 6-OHDA lesioned striata. Coronal sections stained for Tyrosine Hydroxylase (TH) by immunofluorescence (+1,2 to +0,2 anterior to bregma), superimposed on the Paxinos and Watson rat brain atlas (43) for anatomical reference. (B) Quantification of the extent of TH signal loss in the dorsolateral (DLS), dorsomedial (DMS), and ventral striatum (VS). Compared to SHAM-injected rats, the 6-OHDA induced a mean loss of 48% of the TH signal in the DLS, without affecting the DMS and VS. Data are presented as mean \pm SD and compared with Holm-Sidak corrected multiple t-tests. **** $p < 0.0001$.

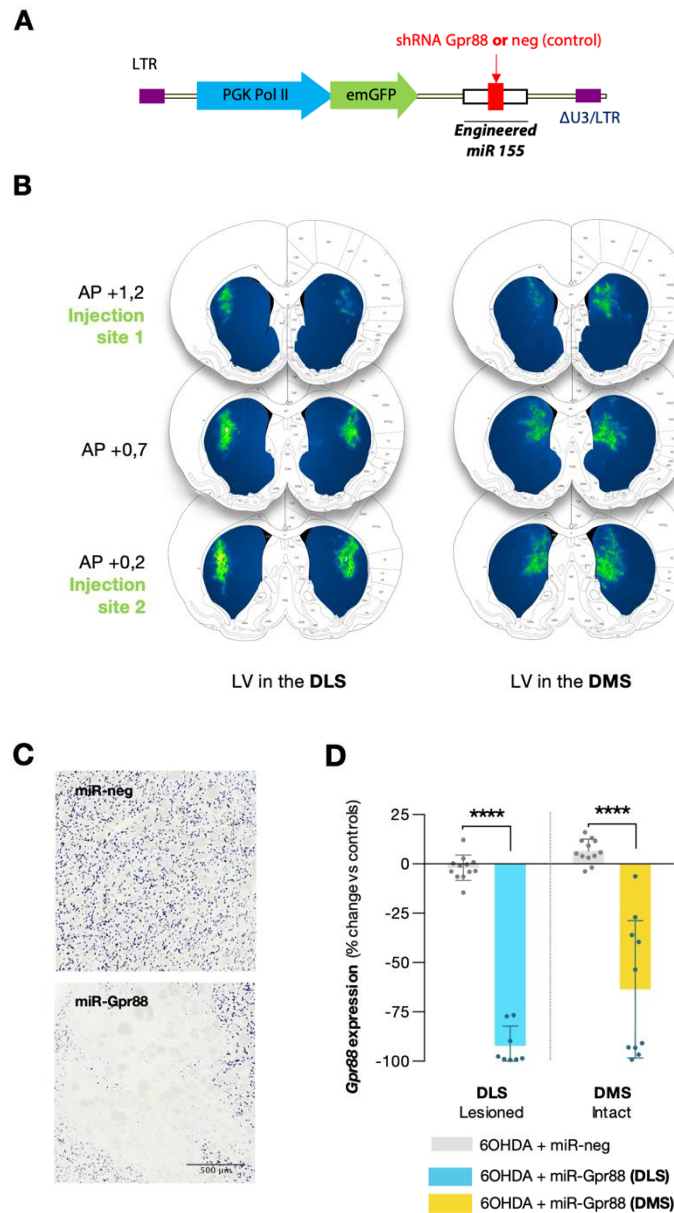


Fig. 2 – Efficient bilateral knock-down of *Gpr88* expression in the DLS/DMS by lentiviral vector injections. (A) Schematic representation of the lentiviral construct used to transduce the targeted striatal regions. (B) Representative images of DLS or DMS transduced striata. The lentiviruses were injected at two coordinates anterior to bregma: +1,2 and +0,2mm. For visibility purposes, a LUT was applied to the images in ImageJ in order to visualize the GFP fluorescence on a blue background. The photographs were then superimposed on the Paxinos and Watson rat brain atlas (43) for anatomical reference. (B) *In situ* hybridization shows a strong suppression of *Gpr88* expression induced by miR-Gpr88, but not miR-neg. (C) Quantification of the *in situ* hybridization signal for *Gpr88*. miR-Gpr88 induced a loss of 92% (DLS) and 64% (DMS) of its expression in the transduced areas. The values were normalized to those of the control group (SHAM + miR-neg). They are presented as mean \pm SD and compared with Holm-Sidak corrected multiple t-tests. **** $p < 0.0001$.

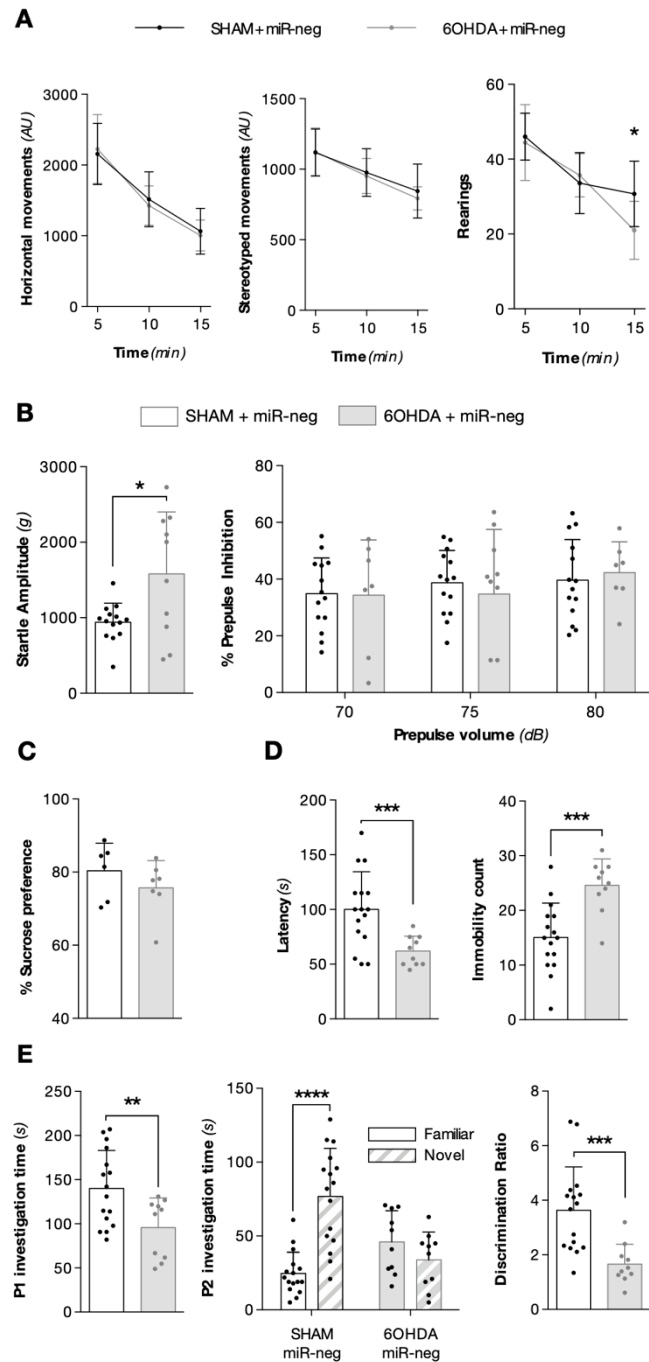


Fig. 3 – Dopamine loss in the DLS reproduces psychiatric symptoms of PD. Data are presented as mean \pm SD. When two groups were compared, two-tailed, Welch-corrected t-tests were performed. In the case of interaction with additional factors, two-way ANOVAs, followed by Bonferroni's multiple comparison test were used (Actimeter data, %PPI, and P2 investigation time). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. **(A)** Motor, sensorimotor and rearing behavior measured were measured with the Actimeter. The 6OHDA lesion only affected rearing at the 15-minute timepoint. **(B)** The lesion increased the startle amplitude in response to a loud stimulus (110dB), however without affecting % Prepulse Inhibition at any of the tested prepulse intensities. **(C)** Sucrose preference was not affected by the 6-OHDA lesion. **(D)** The lesion however induced a depressive like behavior, reflected by an decrease in latency and an increase in the immobility count. **(E)** 6-OHDA reduced social interaction duration, abolished preferential interaction with a novel juvenile, and impaired social discrimination ratio.

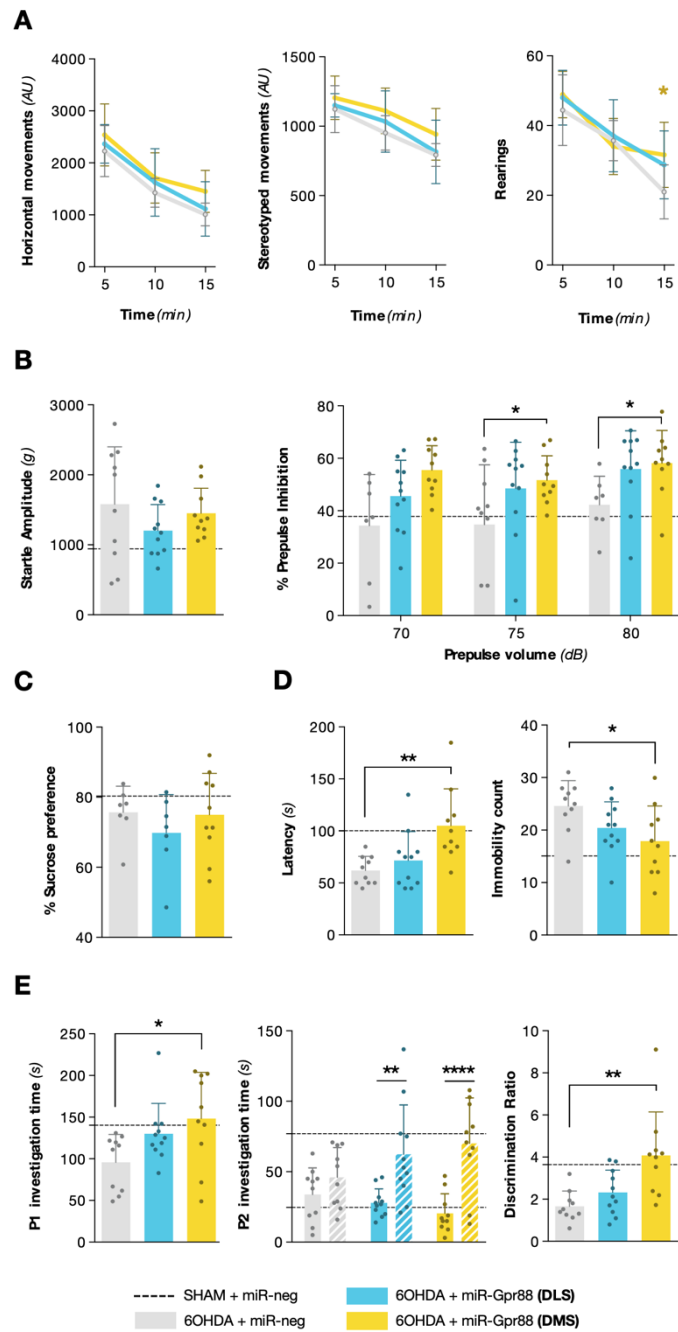


Fig. 4 – Gpr88 knockdown in the DMS but not the DLS reverses the behavioural deficits. Data are presented as mean \pm SD. For reference, a dashed horizontal line indicates the values from the control group (SHAM miR-neg), that were presented in Fig. 3. When the three 6OHDA groups were compared, one-way ANOVAs followed by Bonferroni's multiple comparisons test were performed. In the case of interaction with additional factors, two-way ANOVAs, followed by the Bonferroni multiple comparisons test were used (Actimeter data, %PPI, and P2 investigation time). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. **(A)** Gpr88-KD in the DMS reversed the rearing deficit at the 15-minute timepoint, without affecting horizontal or stereotyped behaviour. **(B)** While Gpr88-KD did not affect startle amplitude, it increased % Prepulse inhibition at the higher prepulse volumes. **(C)** Sucrose preference was not affected by Gpr88-KD. **(D)** Gpr88-KD in the DMS had an antidepressant effect by increasing latency and decreasing the immobility count. **(E)** Gpr88-KD in the DMS increased social interaction duration, and restored novelty preference and discrimination ratio. Gpr88-KD in the DLS selectively increased the novelty preference, but the effect was not strong enough to restore the discrimination ratio.

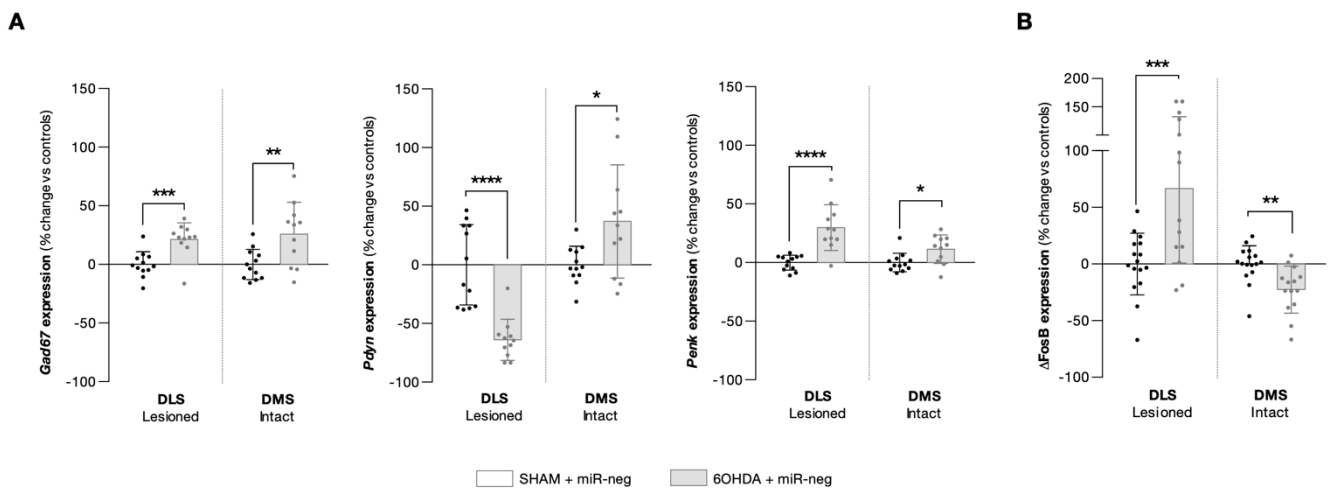


Fig. 5 – Molecular changes induced by DA loss in the DLS also affect the adjacent DMS. The values were normalized to those of the control group (SHAM + miR-neg). Data are presented as mean \pm SD, and compared using multiple t-tests with Holm-Sidak correction. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. **(A)** The lesioned rats had an increased expression of *Gad67* and *Penk* in both DLS and DMS. However, whereas *Pdyn* expression was strongly decreased in the lesioned DLS, it was increased in the intact DMS. **(B)** The 6-OHDA lesion induced a local increase in Δ FosB expression, but a decrease in the un-lesioned DMS.

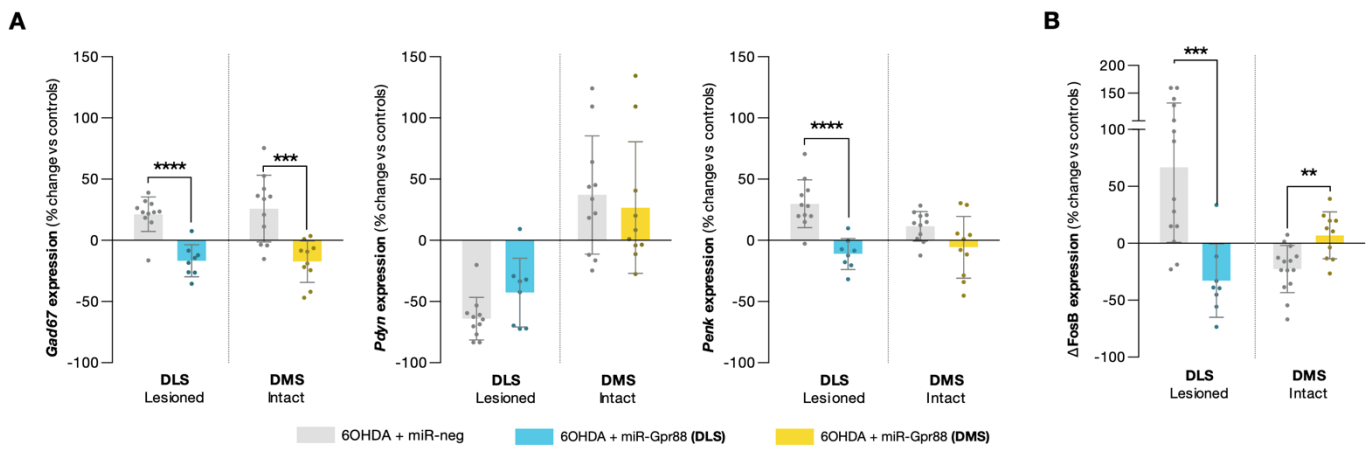
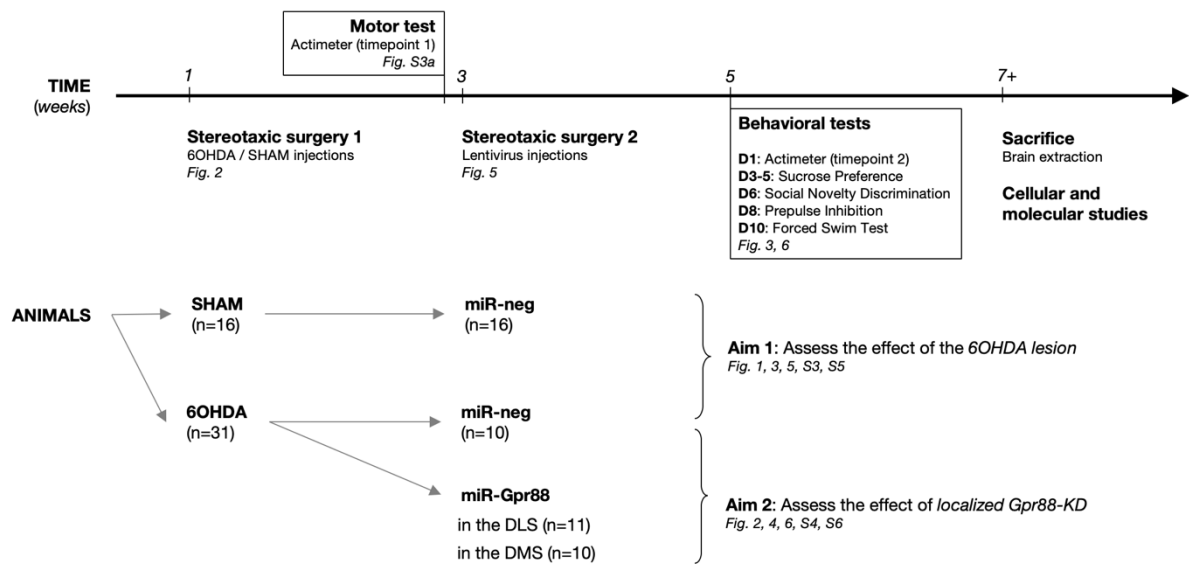
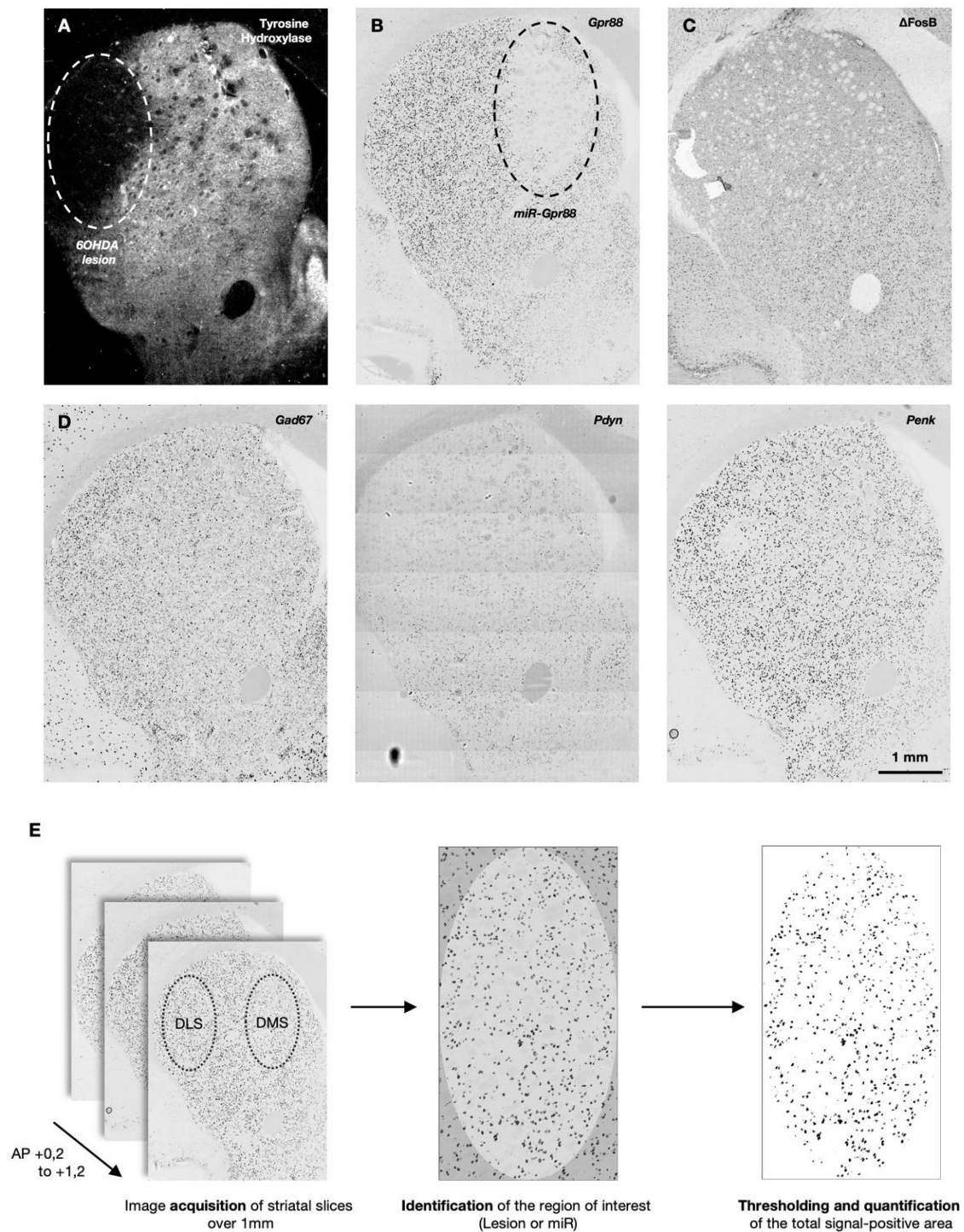


Fig. 6 – Gpr88 knockdown modulates indirect pathway function and Δ FosB expression. The values were normalized to those of the control group (SHAM + miR-neg). They are presented as mean \pm SD, and compared using multiple t-tests with Holm-Sidak correction. **p<0.01, ***p<0.001, ****p<0.0001. **(A)** Gpr88-KD decreased *Gad67* and *Penk* expression independently of dopamine status. It however did not affect *Pdyn* expression. **(B)** Gpr88-KD reversed Δ FosB expression in a dopamine-dependent manner.

SUPPLEMENTARY FIGURES



Supplementary methods - Fig. S1 – Experimental design and timeline



Supplementary methods – Fig. S2 – Histological characterization. (A-D) High resolution scans of coronal slices from the same animal (6OHDA + miR-*Gpr88* in the DMS), stained for (A) Tyrosine Hydroxylase, (B) *Gpr88*, (C) Δ FosB, (D) *Gad67*, *Pdyn* and *Penk*. (E) Summary of the image processing and quantification workflow, detailed in the materials and methods section.

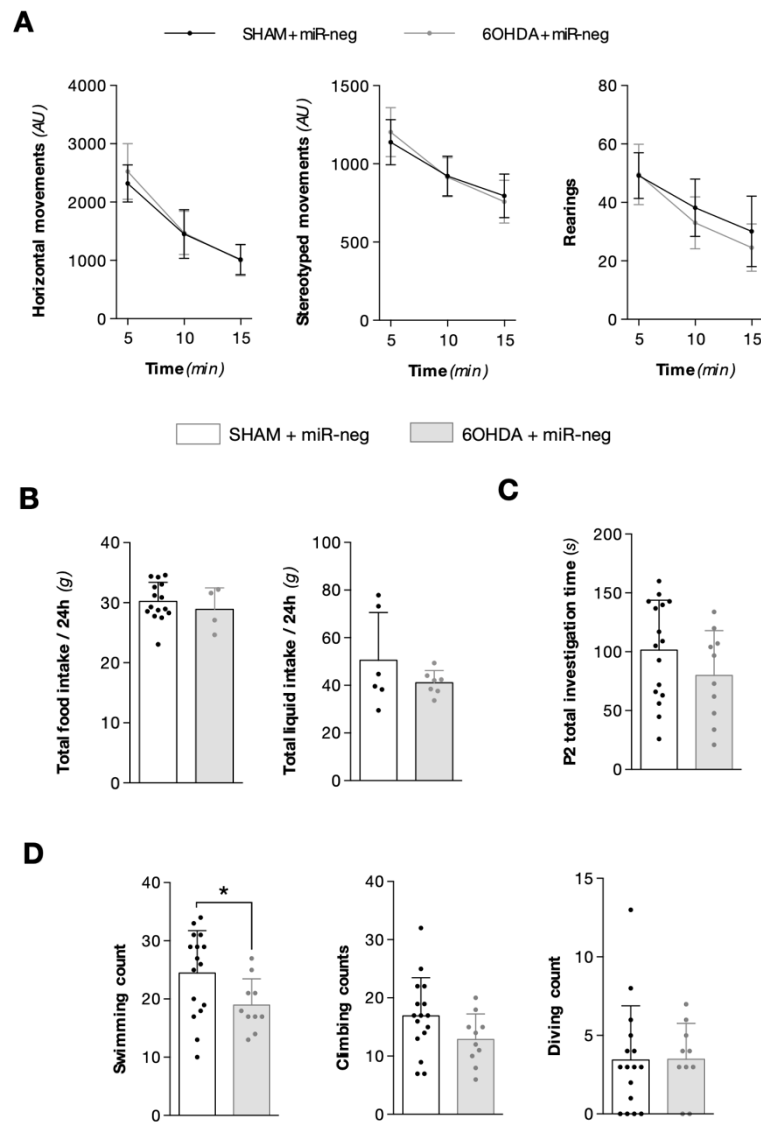


Fig. S3 – Behavioral effects of the 6-OHDA lesion: additional data. Data are presented as mean \pm SD. When two groups were compared, two-tailed, Welch-corrected t-tests were performed (B and C). In the case of interaction with additional factors, two-way ANOVAs, followed by Bonferroni's multiple comparison test were used (A). * $p < 0.05$ (A) The 6-OHDA had no effect on horizontal, stereotyped and rearing behavior at 2 weeks after injection. (B) The lesion did not affect consummatory behavior, (C) or total social investigation time during the discrimination task (P2). (D) Swimming, climbing and diving behaviors were also quantified during the FST. The increase in immobility count induced by the lesion (Fig. 3D) was mediated by a decrease in swimming.

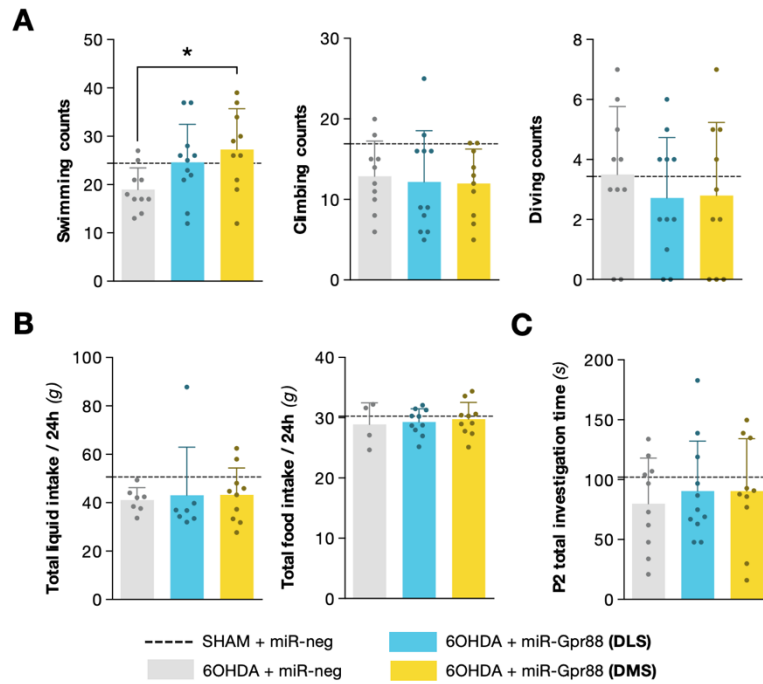


Fig. S4 – Behavioural effects of *Gpr88* knockdown: additional data.

Data are presented as mean \pm SD. For reference, a dashed horizontal line indicates the values from the control group (SHAM miR-neg), that were presented in Fig. 3. One-way ANOVAs followed by Bonferroni's multiple comparisons test were performed. * $p < 0.05$. **(A)** *Gpr88*-KD in the DMS reduced the immobility count (Fig. 4D) by specifically increasing swimming behavior. **(B)** *Gpr88*-KD did not affect consummatory behavior, **(C)** or total social interaction duration in the discrimination task.

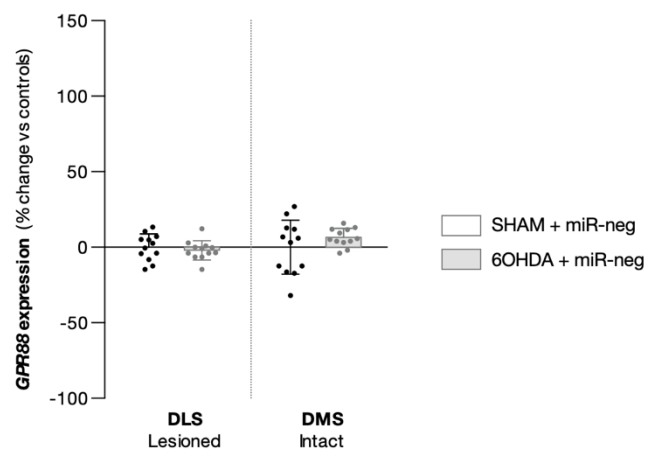


Fig. S5 – The 6OHDA lesion does not affect *Gpr88* expression. The values were normalized to those of the control group (SHAM + miR-neg). Data are presented as mean \pm SD, and compared using multiple t-tests with Holm-Sidak correction.

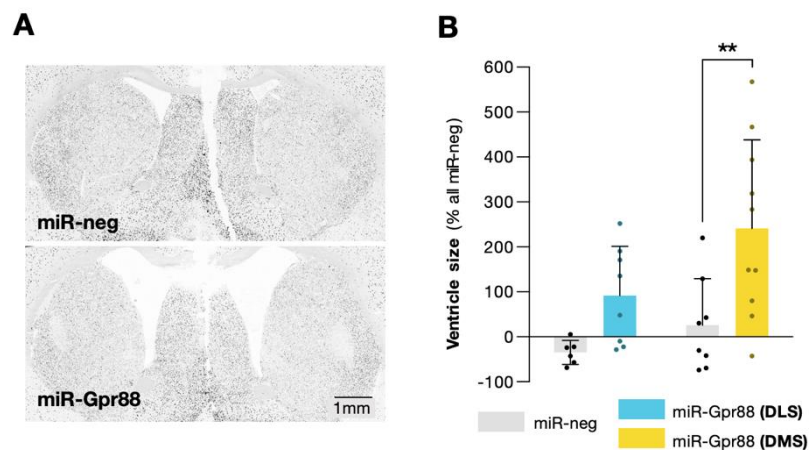


Fig. S6 – *Gpr88* knockdown in the DMS increases lateral ventricle size. (A) Representative pictures showing the effect of miR-Gpr88 on ventricle size. These coronal slice images were taken at similar anterior-posterior sites. (B) Quantification of ventricle size at 3 different antero-posterior sites. *Gpr88*-KD in the DMS increased the size of the lateral ventricles by 241%. The values were normalized to those of miR-neg transduced striata. They are presented as mean \pm SD, and compared using multiple t-tests with Holm-Sidak correction. ** $p < 0.01$.

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General discussion

A. Validating Gpr88 as a therapeutic target for Parkinson's disease

1. Main findings

i. Behaviour

We hypothesised that by potentiating endogenous neurotransmission (notably dopaminergic), Gpr88-KD would have a positive effect on the behavioural alterations in models of the neuropsychiatric symptoms of PD. The findings exposed in both articles support this hypothesis. Indeed, in the absence of chronic pharmacological treatments, Gpr88-KD reduced some of the motor and psychiatric deficits that were induced by the 6OHDA lesions.

Furthermore, our targeted approach using LV vectors allowed us to demonstrate region-dependant effects of Gpr88-KD. For instance, in the model of the psychiatric symptoms, while Gpr88-KD in the DLS had no effect on the behavioural deficits induced by the lesion, all of these deficits were alleviated when targeting the DMS. This localised effect also supports the role of the associative striatum (Caudate Nucleus/Anterior Putamen in primates) in the aetiology of the psychiatric symptoms.

ii. Molecular

A significant part of the results from our molecular investigations in each model show similar effects of the 6OHDA lesions and Gpr88-KD. First of all, the loss of DA induces in both models an expected increase in *Penk* expression (indicating iMSN overactivity), and a decrease in *Pdyn* expression (dMSN hypoactivity).

We also show that in basal conditions (without pharmacological stimulation), the Gpr88-KD preferentially acts on iMSN activity, as evidenced by decreased expression of *Penk* in both models, consistent with findings from Gpr88-KO mice.

Furthermore, in both models, we observed drastic effects of Gpr88-KD on Δ FosB accumulation. Previous studies have shown that both acute and chronic D2 receptor stimulations (with

quinpirole or apomorphine) potentiate Δ FosB accumulation, which in turn sensitises the D1 receptor^{191–193}. Thus, by increasing D2 sensitivity, Gpr88 downregulation may promote the accumulation of Δ FosB in dMSN. Indeed, in both of our models, we observe that in the presence of DA (endogenous or through L-DOPA administration), Gpr88-KD increases Δ FosB accumulation.

Interestingly, Δ FosB is also known to up-regulate the expression of *Pdyn* in the context of L-DOPA administration, and to promote the development of dyskinesia¹⁹⁰. In our experiments however, Δ FosB accumulation in Gpr88-KD rats did not increase *Pdyn* expression or dyskinesia severity, suggesting an uncoupling of Δ FosB and its effect on LID severity.

2. Limitations and future directions

Nevertheless, the precise mechanisms underlying Gpr88-KD's effects are not clear, and require more in-depth investigation, in order to establish the “proof of mechanism”, an essential requirement of translational development.

For instance, findings from KO mice suggest that Gpr88 may act in part through Rgs4^{159,167}. Rgs4 negatively modulates an array of Gi/o coupled receptors (involved in DA, GLU, 5-HT, ACh, and opioid signalling) that are implicated in PD^{93,159,169,170,194–197}. Furthermore, Rgs4 downregulation has shown antiparkinsonian effects in animal models of the disorder by reinstating iMSN LTD and reducing ACh release from cholinergic interneurons^{93,170}, both favouring dMSN activity.

In the future, combining localised inactivations with -omics approaches (transcriptomics, proteomics, signalomics) would allow to identify the precise mechanisms involved in Gpr88's function. Such an approach could be carried out in *in vivo* models of disorders, as well as in cellular models. For instance, recent developments in microfluidics have allowed modeling of the cortico-striatal synapse *in vitro*^{198,199}. Although simplified, such tools provide greater flexibility in the design of experiments and in the ability to test a variety of conditions.

Additionally, the findings we report from the animal models require more in-depth characterisation. For instance, regarding the psychiatric symptoms of PD, more sophisticated behavioural tests such as operant conditioning and attentional set shifting tasks would help assess precise motivational and executive function parameters^{186,200}.

Furthermore, while our model of the psychiatric symptoms provides support for the “bottleneck” hypothesis, it represents a very early stage of PD. As DA dysfunction in associative and limbic territories worsens with PD progression, assessing the effects of Gpr88-KD in models presenting increased DA loss is thus necessary to further validate Gpr88 as a therapeutic target.

Provided that these endeavours can be met with satisfactory answers and that pharmacological antagonists can be developed, our preliminary results prompt us to speculate that Gpr88 inhibition could constitute a relevant monotherapy for the motor and psychiatric symptoms in early-stage PD, and could help delay the use of dopamine replacement therapy. In later stages, its use as an adjuvant could potentially allow to reduce the required doses of L-DOPA/DA agonists, and thus limit the development of adverse effects. Considering gene therapy approaches, adding a *Gpr88* RNA interference sequence to vectors stimulating DA production (ProSavin-like) could for example strongly potentiate their effect.

Furthermore, if further investigations reveal significant effects of Gpr88 modulation on other neurotransmitter systems that are involved in PD, targeting this receptor may also help limit the use of additional medications such as SSRI or AChE inhibitors.

B. Striatal dynamics in basal ganglia disorders

Aside from highlighting the validity of Gpr88 as a therapeutic target, the model of the psychiatric symptoms of PD we developed revealed striatal dynamics that are reminiscent of those observed in patients, and provides support for the “bottleneck” hypothesis as discussed in the article.

Similar striatal dynamics are emerging as key players in other disorders of the basal ganglia. As the closing section of this thesis, I will briefly address how research on this theme is providing insight into the pathophysiology of several neuropsychiatric disorders, and opening up new therapeutic avenues for which Gpr88 targeting may be of interest.

1. Striatal imbalances

As described in the introduction, the BG are organised in parallel loops that treat information both as parallel and integrative processing networks. While associative loops (DMS dependent) are believed to drive goal-directed behaviour, sensorimotor loops (DLS dependent) are involved in the execution of habitual actions. Both systems however cooperate in order to execute complex tasks, and are fundamentally dynamic. Indeed, as an action becomes gradually habitual with overtraining, a “shift” is observed in the striatum: the behaviour becomes controlled by sensorimotor territories¹¹⁶. This process is thought to free up attentional resources by providing a faster and more energy-efficient mode of action, but at the cost of decreased flexibility.

While shifts in the striatal control of behaviour are the normal process of instrumental learning, they can however be biased in pathological contexts, resulting in an imbalance in DMS/DLS control of behaviour. Such bias may for instance reflect adaptive responses to neurodegenerative disorder such as in PD, where an increased reliance on a goal-directed mode of action may impair normal motivated and executive processing. A recent study reported similar changes in a transgenic model of Huntington’s Disease (HD). Indeed, in the pre-degenerative state, the mice exhibited an increased activation of MSN in the DMS²⁰¹. As in PD, cognitive dysfunction is also one of the main signs of prodromal HD, and can be detected over a decade ahead of HD diagnosis²⁰². Although the neurodegenerative processes are different, both disorders may thus share this pathological overactivity of the DMS in early stages.

Most strikingly however, bias in striatal dynamics may also result from a dysregulation of the normal learning system, as has been extensively studied in the case of addiction. Indeed, several studies have revealed that repeated use of a rewarding substance such as cocaine induces a “maladaptive” shift in the striatal control of drug intake, from goal-directed (DMS-dependent) to habitual and compulsive (DLS-dependent)²⁰³.

Similar processes may also be at play in obsessive-compulsive disorders (OCD) and Tourette’s syndrome, in which overactivity of the DMS has been linked to the deficits in behavioural flexibility and executive functioning that characterise patients²⁰⁴.

Furthermore, the frequent development of ICDs in PD following the use of dopamine replacement therapy may result from similar alterations in striatal dynamics, depending on patient personality traits and neurodegenerative patterns¹⁴⁶.

2. Biological bases of striatal shifts

Recent studies have provided some clues regarding the biological bases of such striatal shifts. At the cortical level, it was found that during the early stages of learning, both DMS and DLS cortico-striatal projections are active²⁰⁵. Indeed, while prefrontal DMS inputs may provide cognitive and attentional control of the behaviour, sensorimotor DLS connections seem to hold a particular role in the kinematic execution of the behaviour²⁰⁶. However, with training, cortico-striatal projections to the DMS gradually disengage, while DLS inputs potentiate^{205,207}. Gremel and colleagues²⁰⁸ proposed that this shift may result from gradual inhibition of the DMS through increased Gi/o signalling, and identified endocannabinoids as a key mediator of this effect.

This shift in cortico-striatal networks may also be mirrored by changes in nigro-striatal DA connectivity. Indeed, DMS and DLS have been found to be interconnected through striato-nigro-striatal spiralling projections, which may provide an additional route for lateral transfer of information in the striatum^{209,210}. Work from Belin and colleagues²⁰³ has also suggested an important involvement of the nucleus accumbens and amygdala in the gradual recruitment of the DLS in the context of compulsive drug use.

Finally, fast-spiking interneurons (FSI), which provide feedforward inhibition of MSN activity, have also been implicated in habit formation²⁰⁶, as well as in pathological states such as OCD. Indeed, Burguière and colleagues have shown that in a mouse model of the disorder, pathological overactivity of the DMS was associated with decreased firing of FSI²¹¹.

3. Therapeutic targeting of the DMS/DLS imbalance

These findings have led to the development of new therapeutic strategies, aiming to reduce the DMS/DLS imbalance in experimental models of OCD and addiction.

For instance, regarding OCD, optogenetic stimulation of the lateral orbitofrontal cortex (lOFC) was found to increase the firing of FSI in the DMS, thus reducing MSN overactivity and eliminating compulsive grooming in a mouse model of the disorder²¹¹. By contrast, in models of addiction, increasing goal-directed control of behaviour has proven to be an efficient strategy²¹². For example, in a rat model of methamphetamine addiction, pharmacological inhibition of iMSN in the DMS restored the animals' sensitivity to negative feedback and inhibitory control over the maladaptive habit²¹³.

In the context of addiction, Gpr88-KD in the DMS may thus constitute a relevant target. Indeed, we showed in our models of PD that *Gpr88* downregulation decreased iMSN activity, and favoured goal-directed behaviours when targeting the DMS. Furthermore, if *Rgs4* is indeed a mediator of Gpr88's effect, lifting its inhibition of Gi/o signalling through Gpr88-KD may constitute a powerful way to potentiate cortico-striatal connectivity in the DMS, and increase behavioural control over such compulsive behaviours.

General conclusion

To conclude, the experiments that were carried out for this thesis provide evidence supporting the validity of Gpr88 as a therapeutic target for the neuropsychiatric symptoms of Parkinson's Disease, and identify Δ FosB as a key mediator of Gpr88 signalling.

We also report the presence of adaptive striatal dynamics in a translational model of early PD that are reminiscent of those observed in patients and in other neuropsychiatric disorders, and show that targeting the DMS/DLS imbalance can constitute a successful therapeutic strategy.

Finally, this work is in the continuity of previous findings from our team supporting the relevance of Gpr88 as a target for schizophrenia, and we propose that it may also be of interest for the treatment of other basal ganglia disorders such as addiction.

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Curriculum Vitae

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EDUCATION

PhD Candidate	Sorbonne University <i>Brain, Cognition and Behaviour Doctoral School (ED3C)</i>	10/2015 – 12/2018
D.I.U.	Sorbonne University – Paris Sud University <i>Training course in translational research and therapeutic innovation for disorders of the CNS.</i> "D.I.U.": Inter-University Degree.	01/2018 – 05/2018
Master's Degree	University of California, San Diego University of Bordeaux <i>Neuroscience and Neuropsychopharmacology</i>	2012 - 2014
Bachelor's Degree	University Bordeaux 2 Victor Ségalen <i>Physiology, Cellular and Molecular Biology</i>	2009 - 2012

RESEARCH EXPERIENCE

PhD Candidate	Brain and Spine Institute (ICM) – Paris, France <i>Biotechnology and Biotherapy Team (Dr Philippe Ravassard)</i> Project: Validation of Gpr88 as a therapeutic target for the psychiatric symptoms of Parkinson's disease. Supervisor: Dr Rolando Meloni.	10/2015 – 12/2018
Visiting Worker	Imperial College London, MRC CSC – London, UK <i>Neurophysiology Group (Dr Mark Ungless)</i> Project: Molecular investigation of VTA dopamine neurons.	2015 (5 months)
Intern	Salk Institute for Biological Studies – San Diego, US <i>Laboratory of Genetics, Gage (Dr Fred Gage)</i> Project: iPSC modelling of Parkinson Disease (Master's Thesis).	2014 (5 months)
Intern	University Bordeaux Ségalen – Bordeaux, France <i>Human Factors and Cognition Laboratory (Dr Bernard N'Kaoua)</i> Project: Assessing electrophysiological (EEG) correlates of musical stimuli.	2010 (3 months)

AWARDS

Brain Booster Challenge	Brain and Spine Institute <i>Recipient of awards to fund year-long independent research projects.</i> 2015 Award: testing a novel animal model of schizophrenia. 2018 Award: LuSciD, a platform for collaborations between storytellers and scientists.	2015, 2018
ICM Poster Prize	Brain and Spine Institute Best poster presentation in the "final-years" category.	2018
PhD Scholarship	ED3C Doctoral School, Sorbonne University Government funded competitive scholarship scheme (ranking: 3/50).	2015
International Scholarship	FidEx, University of Bordeaux Scholarship awarded to fund the MSc internship at the Salk Institute.	2014

PRESENTATIONS AND PUBLICATIONS

Presentations *Study of Gpr88 as a therapeutic target for the psychiatric symptoms of Parkinson's Disease.*
B. Galet, M. Ingallinesi, J. Pegon, A. Do Thi, N. Faucon Biguet, R. Meloni

- Oral presentation, ICM-IoN Workshop, Paris, 2018
- Poster, FENS Meeting, Berlin, 2018
Supported by a travel grant from the French Neuroscience Society
- Poster, ECNP Junior Scientists Workshop, Nice, 2018
- Poster, ICM-UCL Institute of Neurology Workshop, London, 2017
- Poster, Neurofrance, Bordeaux, 2017
- Poster, ICM – Florey Institute Workshop, Paris, 2016
- Oral presentation, ICM Days, Paris, 2016
- Poster, ED3C Doctoral School Days, Paris, 2015-2018

Publications C. Bardy, M. Van Den Hurk, T. Eames, C. Marchand, R. Hernandez, M. Kellogg, M. Gorris, **B. Galet**, V. Palomares, J. Brown, A. Bang, J. Mertens, L. Boehnke, L. Boyer, S. Simon, F.H. Gage (2015). Neuronal medium that supports basic synaptic function and activity of human neurons in vitro.
Proceedings of the National Academy of Sciences.

TEACHING AND RELATED EXPERIENCE

Teaching Assistant **Language Department, Sorbonne University** 10/2015 – 12/2018
English conversation workshops and assistance for students in the language department.
Covering professors for their English classes.

Academic Tutor **University of Bordeaux** 2013
Went over course material, worked on exercises and assignments with groups of undergraduate students during weekly discussion sessions. Tutored for 2 courses: Genetics, and Plant Physiology.

Scientific Mediator **Cap Sciences (Science Exhibition Centre) – Bordeaux** 2013
Worked for a bilingual exhibition about the brain ("Cervorama"), guiding groups, primary and secondary schools, general public in French and in English.

SKILLS

Scientific

- Translational research: in-depth understanding of key concepts and challenges.
- Animal experimentation: stereotaxic surgery and behavioural tests. *French certificates: concepteur de projet, initiation à la chirurgie expérimentale.*
- Cell culture: dopaminergic iPS cells derived from Parkinsonian patients.
- Cellular and molecular: immunostainings, *in situ* hybridization, RT-qPCR.
- Microscopy: wide-field, confocal, spinning disk and image analysis (ImageJ/Fiji, CellProfiler).
- Data processing and statistical analyses: Graphpad Prism, G*Power, Excel.

Linguistic

- English: native (British and French citizen).
- Spanish: intermediate.