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Mechanisms of motor and non-motor complications in experimental parkinsonism

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Mechanisms of motor and non-motor complications in experimental parkinsonism

Thesis for Doctoral Degree (Ph.D.)

By

Carina Plewnia

The thesis will be defended in public at Eva & Georg Klein lecture room, Solnavägen 9, on Friday, October 27th, 2023 at 9.30 am

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To my Family

ABSTRACT

Parkinson's disease (PD), one of the most common neurodegenerative disorders, is classically characterized by the progressive loss of midbrain dopaminergic neurons and by the emergence of cardinal motor symptoms including rigidity, tremor and bradykinesia. PD treatment relies on pharmacological dopamine replacement, which is the most effective therapy to alleviate motor symptoms. However, the use of L-Dopa and dopamine receptor agonists is associated with the development of motor and non-motor complications, such as dyskinesia and neuropsychiatric disorders, which can be even more debilitating than the cardinal symptoms of PD and represent a major limitation to its management. Utilizing experimental models of PD, the work presented in this thesis investigates the underlying mechanisms involved in these treatment-related complications.

In **Paper I** we examined changes in autophagy associated with L-Dopa-induced dyskinesia (LID), which consists of dystonic and choreic abnormal involuntary movements tending to occur within a few years from the beginning of L-Dopa treatment. We found that LID is associated with dopamine D1 receptor (D1R)-mediated accumulation of the autophagy-specific substrate p62, a marker of autophagy deficiency. Inhibition of the mammalian target of rapamycin complex 1 with rapamycin counteracted the impairment of autophagy produced by L-Dopa, and reduced dyskinesia, suggesting that autophagy-promoting agents may represent a novel pharmacological approach to the treatment of dyskinesia. In **Paper II** we examined the ability of pre- and post-surgical interventions to reduce the mortality observed in a bilateral 6-hydroxydopamine mouse model reproducing non-motor symptoms of PD. We showed that the survival rate of male and female mice subjected to this lesion differs significantly, with higher mortality among males, and provided a protocol of enhanced care, which nearly eliminates animal loss. The same model was utilized in **Paper III** to recapitulate features of dopamine dysregulation syndrome, a non-motor complication in PD patients associated with pathological overconsumption of dopaminergic medications, far beyond that necessary to correct motor disabilities. We found that L-Dopa acquires rewarding properties in dopamine-depleted mice and this effect was mediated by abnormal D1R transmission in the dorsal striatum. We identified ΔFosB as a potential target to counteract this condition.

Overall, the work presented in this thesis offers a new perspective on underlying, and potentially common, mechanisms involved in motor and non-motor complications induced by dopamine replacement therapy. These studies also reveal the importance of employing appropriate experimental models of PD to identify novel targets for therapeutic interventions.

LIST OF SCIENTIFIC PAPERS

I. Feyder, M., **Plewnia, C. (co-first author),** Lieberman, O. J., Spigolon, G., Piccin, A., Urbina, L., Dehay, B., Li, Q., Nilsson, P., Altun, M., Santini, E., Sulzer, D., Bezard, E., Borgkvist, A., & Fisone, G. Involvement of Autophagy in Levodopa-Induced Dyskinesia. *Movement Disorders*. 2021, 36(5):1137-1146

II. Masini, D., **Plewnia, C.**, Bertho, M., Scalbert, N., Caggiano, V., Fisone, G. A Guide to the Generation of a 6-Hydroxydopamine Mouse Model of Parkinson's Disease for the Study of Non-Motor Symptoms. *Biomedicines*. 2021, 9(6):598

III. **Plewnia, C.**, Masini, D., Fisone, G. Rewarding properties of L-Dopa in experimental parkinsonism are linked to dysregulated dopamine D1 receptor transmission. *Manuscript.*

CONTENTS

LIST OF ABBREVIATIONS

6-OHDA	6-hydroxydopamine
AAV	Adeno-associated virus
AIMs	Abnormal involuntary movements
Atg	Autophagy-related gene or protein
BAC	Bacterial artificial chromosome
cAMP	Cyclic adenosine monophosphate
CNO	Clozapine N-oxide
CPP	Conditioned place preference
D1R	Dopamine D1 receptor
D2R	Dopamine D2 receptor
DARPP-32	Dopamine- and cAMP-regulated phosphoprotein of 32 kDa
DDS	Dopamine dysregulation syndrome
DREADD	Designer Receptors Exclusively Activated by Designer Drugs
DRT	Dopamine replacement therapy
EGFP	Enhanced green fluorescent protein
ERK	Extracellular signal-regulated kinases 1 and 2
GABA	Gamma-aminobutyric acid
GPCR	G protein-coupled receptor
L-Dopa	Levodopa (L-3,4-dihydroxyphenylalanine)
LID	Levodopa-induced dyskinesia
MFB	Medial forebrain bundle
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MSN	Medium spiny neuron
mTORC1	Mammalian target of rapamycin complex 1
NAc	Nucleus accumbens

NHP	Non-human primate
NMS	Non-motor symptoms
PD	Parkinson’s disease
PKA	cAMP-dependent protein kinase A
S6	S6 ribosomal protein
SNc	Substantia nigra pars compacta
TH	Tyrosine hydroxylase
UPS	Ubiquitin–proteasome system
VTA	Ventral tegmental area

1 INTRODUCTION

1.1 Parkinson's disease and symptomatology

Parkinson's disease (PD) is the second most common neurodegenerative disorder after Alzheimer's disease, affecting around 1% of the population over 60 years (de Lau and Breteler, 2006; Lee and Gilbert, 2016). PD was first described in 1817 by James Parkinson in "An Essay on the Shaking Palsy" (Parkinson, 2002) and diagnostic criteria are classically still based on the same motor dysfunctions that were described more than 200 years ago.

The main pathological characteristic of PD is the progressive and irreversible loss of dopamine neurons located in the midbrain within the substantia nigra pars compacta (SNc) (Hassler, 1938; Hornykiewicz, 2006). These neurons project to the dorsal striatum, a major receiving component of the basal ganglia involved in motor function. Clinically, the loss of striatal dopamine innervations in PD patients leads to severe motor impairment including bradykinesia and akinesia, rigidity, postural instability and resting tremor (Samii et al., 2004; Kalia and Lang, 2015; Postuma et al., 2015). These motor symptoms appear when approximately 50% of the dopamine neurons in the SNc are lost and around 80% of the striatal dopamine levels are depleted (Ehringer and Hornykiewicz, 1960; Fearnley and Lees, 1991; Foley and Riederer, 1999). Another pathological hallmark of PD is the occurrence of abnormal protein aggregates, named Lewy bodies. These eosinophilic cytoplasmatic inclusions, with alpha-synuclein as a major constituent, have been considered a marker of neuronal degeneration (Dauer and Przedborski, 2003).

Although PD is classically diagnosed based on motor impairment, it has become evident that PD is also associated with a wide range of non-motor symptoms (NMS). These symptoms range from olfactory dysfunction to neuropsychiatric manifestations such as depression, cognitive impairment, sleep disorders and autonomic dysfunctions (Chaudhuri and Odin, 2010; Kalia and Lang, 2015; Schapira et al., 2017). NMS often appear in the prodromal phase of the disease and may precede the onset of motor symptoms by more than a decade (Rodriguez-Oroz et al., 2009; Postuma et al., 2012). NMS are known to affect almost 90% of patients during the course of the disease and are often underrecognized in clinical practice despite their negative impact on the overall life quality of patients (Martinez-Martin et al., 2011).

Whereas the deficit of dopamine in the basal ganglia is the primary source of motor impairment in PD, the appearance of NMS strongly suggests the involvement of neurotransmitters other than dopamine and brain regions outside the basal ganglia. Indeed, the emergence of these conditions correlates not only with the depletion of dopamine, but also with the concomitant

disruption of noradrenergic, serotonergic and cholinergic transmission (Braak et al., 2003), changing the view of PD from a pure motor to a multisystemic disease.

Additionally, pharmacological treatment of PD is associated with motor and non-motor complications that involve abnormal involuntary movements (dyskinesia) and neuropsychiatric disorders like dopamine dysregulation syndrome (DDS) and impulse control disorders.

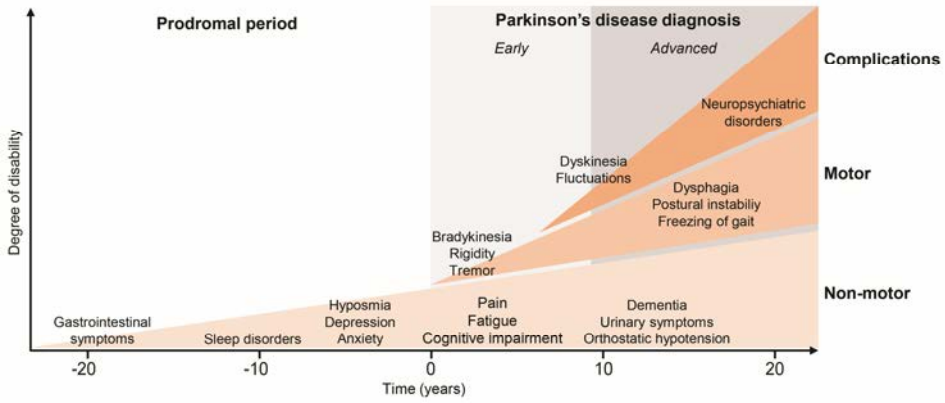

Figure 1. Clinical symptoms and time course of Parkinson's disease. *Adapted from Kalia & Lang, 2015.*

1.2 The midbrain dopaminergic and basal ganglia system

The midbrain dopaminergic system consists primarily of neurons in the SNc and the ventral tegmental area (VTA) with their topographical projections to the striatum and multiple other brain regions (Fig. 2). These neurons play a fundamental role in the execution of voluntary movements and represent a critical link between learning, memory and the expression of these cognitive aspects via movement.

Dopaminergic neurons in the VTA mainly innervate the ventral striatum (nucleus accumbens) and the prefrontal cortex, constituting the mesolimbic and mesocortical pathways. Often referred together as the mesocorticolimbic pathway or the reward circuit, this network plays a key role in emotion-based behavior including reward and motivation (Bjorklund and Dunnett, 2007; Haber, 2008). The nucleus accumbens, generally divided into the two anatomical subregions core and shell, is postulated to function as limbic-motor interface, guiding behavior as a response to rewarding stimuli (Mogenson et al., 1980).

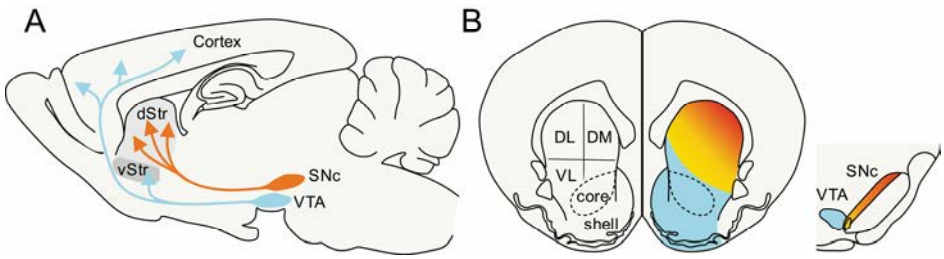

Figure 2. Dopaminergic projections in the rodent brain. (A) Sagittal view of nigrostriatal (orange) and mesocorticolimbic (blue) projections to the dorsal (dStr) and ventral (vStr) striatum (B) Coronal view of dopaminergic projections from SNc and VTA to different striatal subregions (nucleus accumbens core and shell, dorsolateral (DL), dorsomedial (DM) and ventrolateral (VL) striatum. *Adapted from Cenci et al., 2015.*

The dopaminergic neurons in the SNc project via the nigrostriatal tract to the dorsal striatum and are crucial in the control of voluntary movements. The dorsal and ventral regions of the striatum are the main receiving structure of the basal ganglia and, besides the dopaminergic input from the SNc and the VTA, receive glutamatergic inputs from cortex, thalamus, hippocampus and amygdala (Kelley and Domesick, 1982; Kelley et al., 1982; Hunnicutt et al., 2016), as well as a serotonergic input from the dorsal raphe nucleus (Jacobs and Fornal, 1997). Overall, the neurodegenerative process of PD with dopaminergic loss occurring in both nigrostriatal and (even though to a lesser extent) mesolimbic pathways triggers a cascade of basal ganglia dysfunction resulting in motor and non-motor symptoms.

The gamma-aminobutyric acid (GABA)ergic medium spiny neurons (MSNs) represent approximately 95% of striatal neurons. Even though morphologically similar, striatal MSNs can be distinguished based on their projection targets and their ability to express different types of dopamine receptors (Kemp and Powell, 1971; Graveland and DiFiglia, 1985; Alexander et al., 1986; Gerfen et al., 1990). The two types are approximately equally distributed throughout the striatum and they are heavily intermingled. Dopamine D1 receptors (D1Rs) are expressed by MSNs directly innervating the output structures of the basal ganglia - substantia nigra pars reticulata and globus pallidus pars interna (entopeduncular nucleus in rodents) - and form the "direct" striatonigral pathway. In contrast, the "indirect" striatopallidal pathway is formed by MSNs that project via the globus pallidus pars externa and the subthalamic nucleus to the output structures and express dopamine D2 receptors (D2Rs). This distinction is the basis of a commonly accepted model of basal ganglia transmission (Fig. 3). The two pathways have opposite effects on thalamo-cortical target neurons, with the direct pathway neurons facilitating motor activity and the indirect pathway neurons suppressing motor activity (Gerfen, 1992). Whereas these opposing effects are assumably driven by long-range projections from MSNs to

their output nuclei, it is important to note that lateral inhibition between MSNs through short-range projections has also been shown to regulate the behavioral output of the basal ganglia (Dobbs et al., 2016). Additionally, recent studies have shown that striatal neurons of both pathways are co-activated during movement initiation and are inactive when the animal is not moving and that the coordinated spatial and temporal activation of specific MSNs, rather than the relative amount of activity, is crucial for the selection and initiation of actions (Cui et al., 2013; Tecuapetla et al., 2016; Klaus et al., 2017).

Alongside MSNs, striatal GABAergic and cholinergic interneurons form dense microcircuits which further modulate basal ganglia transmission (Kawaguchi, 1993).

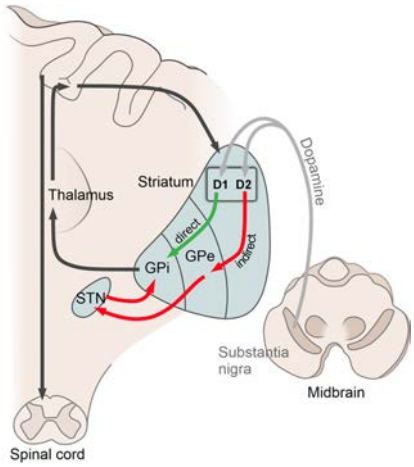

Figure 3. Simplified representation of the basal ganglia circuitry indicating the parallel direct (green) and indirect (red) pathways from the striatum to the basal ganglia output nuclei. The striatum receives excitatory glutamatergic input from the cortex and thalamus and dopaminergic input from the substantia nigra. Two types of dopamine receptors (D1 and D2) are located on different sets of inhibitory GABAergic output neurons (MSNs) in the striatum that give rise to the direct (striatonigral) and indirect (striatopallidal) pathways. D1 MSNs project directly to the globus pallidus pars interna (GPi) and substantia nigra reticulata (SNr), whereas D2 MSNs influence these output nuclei indirectly via intermediate projections to the globus pallidus pars externa (GPe) and subthalamic nucleus (STN). *Adapted from Kandel et al., 2000.*

1.3 Dopamine replacement therapy

In the 1960s, the discovery that parkinsonism results from striatal dopamine depletion (Ehringer and Hornykiewicz, 1960; Hornykiewicz, 1963), and the understanding that L-3,4-dihydroxyphenylalanine (L-Dopa) is the natural precursor of dopamine, opened the way for the use of dopamine-replacing agents to counteract motor symptoms in PD patients.

Since then, the primary symptomatic treatment of PD has been based on the use of dopaminergic drugs, such as L-Dopa and dopamine receptor agonists (Birkmayer and Hornykiewicz, 1961; Mercuri and Bernardi, 2005; Blandini and Armentero, 2014). Administration of L-Dopa compensates for the lack of dopamine and effectively counteracts the cardinal motor symptoms of PD in the early stages of the disease. However, the response to L-Dopa fades during the progression of PD with the need to continuously adjust the drug dosage. Most non-motor symptoms are refractory to the therapeutic effects of L-Dopa and

long-term dopamine replacement therapy (DRT) is accompanied by motor (Obeso et al., 2000) and non-motor complications (O'Sullivan et al., 2009; Voon et al., 2009).

L-Dopa has a relatively short half-life and is therefore administered several times a day to patients and is often combined with a decarboxylase inhibitor (such as carbidopa or benserazide) to prevent peripheral metabolism. This allows the reduction of dosage required for a clinical response by up to 75% and diminishes peripheral side effects (Markham et al., 1974). Unlike dopamine, L-Dopa crosses the blood-brain barrier and in the brain is converted into dopamine by the enzyme aromatic L-amino acid decarboxylase (AADC). AADC is not specific to dopamine neurons and can be found in glia, endothelial cells, and most importantly in serotonergic neurons. The efficiency of these neurons to convert L-Dopa to dopamine is known to cause oxidative stress and deficits in serotonin neurotransmission, and might be linked to the emergence of some of the side effects observed in parkinsonian patients treated with L-Dopa (Carta et al., 2007; Stansley and Yamamoto, 2015).

Dopamine agonists like bromocriptine, pramipexole and ropinirole have also proven their efficacy in clinical practice, either as monotherapy in early PD or adjuvants to L-Dopa in advanced stages of the disease (Goetz et al., 2005; Fox et al., 2011). This approach has been shown to delay the onset of L-Dopa-related motor complications in de novo PD patients (Hubble, 2002). Half-lives of dopamine agonists are much longer than L-Dopa and therefore can produce a more persistent period of dopamine receptor stimulation (Hayes et al., 2010). However, once L-Dopa is introduced, motor complications like dyskinesia will occur regardless of the previous use of dopamine agonists (Hauser et al., 2007; Katzenschlager et al., 2008). Treatment with dopamine agonists has also been associated with the development of neuropsychiatric side effects. The probability of developing these complications, which include impulse control disorders, psychosis and hallucinations, is higher compared to treatment with L-Dopa (Biundo et al., 2017; Voon et al., 2017). Other approaches to prolong the therapeutic effect of L-Dopa include the co-administration of inhibitors of enzymatic dopamine degradation, such as catechol-O-methyltransferase (COMT) or monoamine oxidase (MAO) inhibitors (AlDakheel et al., 2014; Connolly and Lang, 2014). In clinical practice, these compounds have also been shown to worsen or induce motor complications.

Current therapeutic approaches including DRT focus primarily on alleviating the severity of motor symptoms by counteracting the dopaminergic deficits, whereas no neuroprotective and disease-modifying options are available for patients at this time.

1.4 L-Dopa-induced dyskinesia

Motor symptoms in PD patients are commonly treated with the dopamine precursor L-Dopa. Whereas at the beginning of the therapy, L-Dopa is effective (Birkmayer and Hornykiewicz, 1961; Mercuri and Bernardi, 2005), prolonged use of the drug is severely limited by the appearance of dyskinesia, which consists of dystonic and choreic abnormal involuntary movements (Obeso et al., 2000; Fabbrini et al., 2007). Dystonia is a hyperkinetic movement disorder in which the involuntary and repetitive contraction of muscles results in abnormal postures and twisting movements. Choreic movements are brief, irregular and dance-like motions that appear to flow from one muscle to the next.

L-Dopa-induced dyskinesia (LID) usually appears within five years of treatment with L-Dopa (Obeso et al., 2000) and can be even more debilitating than the cardinal symptoms of PD. Dyskinesias have a tremendous impact on the life quality of PD patients, since day-to-day functions including speech, eating, handwriting, walking and balance are severely impaired.

Several risk factors have been proposed to contribute to the development of LID, including early onset of PD, which results in a longer duration of the disease, and L-Dopa therapy (Kumar et al., 2005). In early stages of PD, spared dopaminergic neurons have the ability to release L-Dopa-derived dopamine in a regulated and physiological manner (Jankovic, 2005). But with the progressive degeneration of dopaminergic neurons, those buffering capabilities are lost and administration of L-Dopa leads to supra-physiological, pulsatile stimulation of dopamine receptors. LID is usually most severe during the first two hours following the administration of L-Dopa, which parallels the peak plasma level of the drug in the brain (Tolosa et al., 1975; Fahn et al., 2004). In early stages of the disease, reducing the dose of L-Dopa while maintaining an antiparkinsonian effect minimizes peak-dose LID, but the therapeutic window narrows over time since optimal dose efficiency decreases. Additionally, when striatal dopamine returns to sub-physiological levels, patients suffer from an ‘off” medication state which leads to the re-emergence of parkinsonian symptoms like fixed dystonic postures and necessitates repeated L-Dopa administration. A less common type of LID, which is referred to as diphasic dyskinesia, consists of motor fluctuations during the rising or falling phase of L-Dopa plasma levels (Tedroff et al., 1996; Pavese et al., 2006).

Current pharmacological treatment of LID in PD patients is based on the use of amantadine, a multi-target drug with antagonistic activity at the N-methyl-d-aspartate receptor (NMDA) receptor (Fox et al., 2018). In clinical trials, the co-administration of amantadine with L-Dopa has shown long-term anti-dyskinetic effects, without worsening PD motor symptoms

(Verhagen Metman et al., 1998; Metman et al., 1999; da Silva-Junior et al., 2005; Wolf et al., 2010). However, the use of this drug leads to adverse side effects, including confusion and visual hallucinations (Kong et al., 2017). Advanced therapies, especially at late stages of the disease for patients that suffer from severe motor fluctuations and LID, include continuous subcutaneous infusion of apomorphine (Carbone et al., 2019) or continuous intestinal infusion of L-Dopa (Abbruzzese et al., 2012) or deep brain stimulation (Groiss et al., 2009; Giugni and Okun, 2014).

1.5 Dopamine dysregulation syndrome

Administration of L-Dopa or dopamine agonists attenuates the motor symptoms of PD, but it also acts on dopaminergic systems implicated in addictive and compulsive behaviors. Thus, long-term DRT can lead to a neurobehavioral disorder similar to drug abuse and known as dopamine dysregulation syndrome (DDS). Patients with DDS show a pattern of self-medication, which exceeds the dose required to control motor impairment. These motivation-based disorders are still underdiagnosed in clinical practice and have deleterious social consequences, leading to an increasing demand for more effective therapies (O'Sullivan et al., 2009).

DDS is mainly found in young patients with early onset of the disease. It is thought to have a prevalence of approximately 3-4% among PD patients (Pezzella et al., 2005) and is typically associated with L-Dopa or short-acting dopamine agonists (Gallagher et al., 2007). DDS is characterized by a pathological overconsumption of dopaminergic medications and the use of DRT has been associated with feelings of pleasure and euphoria during on-drug periods in PD patients (Maricle et al., 1995; Castrioto et al., 2013). DDS patients feel under-medicated, ignore advised dose schedules and self-medicate to a state where they only feel “on” when notably dyskinetic. Many patients hoard the drug and seek out multiple providers to increase quantities of medication, not realizing the harm they cause to themselves or their families. Attempts for dose reduction are met with strong resistance and are mostly unsuccessful. Withdrawal can be observed in up to 19% of PD patients and typical symptoms include dysphoria, depression, irritability and anxiety (Lawrence et al., 2003; Rabinak and Nirenberg, 2010). Predisposing factors that may contribute to develop DDS include young-onset of disease, male gender, previous history of substance abuse as well as sensation and novelty-seeking personality traits (Evans et al., 2005; Evans et al., 2009; O'Sullivan et al., 2009; Katzenschlager, 2011).

The progressive neurodegenerative process of PD with dopaminergic loss occurring in both nigrostriatal and mesolimbic pathways combined with DRT is proposed to hyperactivate the mesocorticolimbic pathway leading to the disruption of the reward system. Different mechanisms of psychostimulant addiction including pleasure-seeking, habit models and neuroadaptations in the nucleus accumbens (NAc) and related circuitry are currently being discussed as potentially implicated in DDS (Lawrence et al., 2003; Voon et al., 2011).

In addition to DDS, approximately 15% of PD patients on prolonged dopaminergic treatment develop stereotyped behavior (punding) and impulse control disorders, such as pathological gambling, hypersexuality, as well as compulsive shopping and eating (Weintraub et al., 2010).

These psychiatric conditions represent a major problem for the patients and their relatives and seriously limit the use of drugs commonly employed to combat PD. Whereas the study of the motor complications (i.e. dyskinesia) associated with the use of anti-parkinsonian drugs has been the subject of intense research, much less is known about the mechanisms implicated in the non-motor side effects. Dopaminergic depletion and DRT coincide in all PD patients, suggesting that the repetitive movements of LID and compulsive behaviors share common mechanisms resulting from abnormal DA transmission.

1.6 Dysregulated dopamine transmission in PD and LID

In the striatum, the physiological actions of dopamine are mediated by interaction with distinct dopamine receptors in MSNs. Dopamine receptors belong to the class of G-protein-coupled receptors (GPCR) and are divided into D1-type and D2-type receptors based on their ability to either activate or inhibit the enzyme adenylyl cyclase (AC), which catalyzes the formation of the second messenger cyclic adenosine monophosphate (cAMP). Increased cAMP synthesis in D1R leads to activation of cAMP-dependent protein kinase A (PKA), which phosphorylates target proteins in the cytoplasm and the nucleus. In contrast, inhibition of cAMP synthesis via D2Rs leads to a reduction of the phosphorylation of downstream targets of PKA (Jaber et al., 1996; Missale et al., 1998).

A large body of evidence indicates that the depletion of dopamine occurring in PD leads to sensitization of dopamine receptors. This effect is particularly prominent at the level of D1R located in a large population of MSNs, within the dorsal striatum. Sensitization occurs also, albeit to a lesser extent, at D2R. The enhanced sensitivity of dopamine receptors in combination with pulsatile administration of L-Dopa leads to hyperactivation of multiple signaling cascades,

ultimately resulting in long-term modifications of gene expression and protein synthesis (Aubert et al., 2005; Spigolon and Fisone, 2018) (Fig. 4).

Changes in signal transduction associated with the development and manifestation of LID have been intensively studied during the last decades. In striatal MSNs, D1R-mediated stimulation of PKA leads to the phosphorylation of the dopamine- and cAMP-regulated phosphoprotein of 32 kDa (DARPP-32), which is then converted into a potent inhibitor of protein phosphatase (Hemmings et al., 1984).

Through a mechanism that remains to be understood, the PKA/DARPP-32/protein phosphatase-1 cascade activates the extracellular signal-regulated kinases 1 and 2 (ERK), leading to chromatin remodeling and aberrant gene transcription (Santini et al., 2009b; Spigolon and Fisone, 2018). One important transcription factor that is highly up-regulated in the striatum following chronic L-Dopa treatment and participates in the development of LID, is ΔFosB. Studies in rodent and non-human primate models showed increased levels of ΔFosB (Andersson et al., 1999; Pavon et al., 2006; Westin et al., 2007; Feyder et al., 2016; Beck et al., 2021) a stable, truncated splice variant of the immediate early gene FosB. Importantly, enhanced expression of ΔFosB has also been implicated in the long-term effects produced by substances of abuse (Nestler, 2008).

Dysregulated ERK is also involved in the stimulation of the mammalian target of rapamycin complex 1 (mTORC1), which regulates numerous important physiological functions, including protein synthesis and autophagy. mTORC1 phosphorylates the 4E-binding protein (4E-BP) and the p70 ribosomal protein S6 kinase (S6K), thereby promoting the initiation of mRNA translation (Santini et al., 2010).

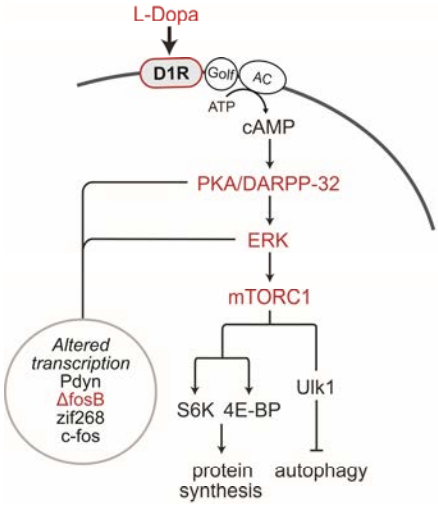

Figure 4. Simplified diagram illustrating some of the major abnormalities related to sensitized D1R-signaling. Abnormal activation of D1R/Gαolf/AC signaling by L-Dopa results in augmented synthesis of cAMP and stimulation of PKA and DARPP-32. Increased PKA/DARPP-32 signaling activates ERK, which controls transcriptional and translational processes. In the nucleus, PKA/DARPP-32 and ERK signaling leads to increased expression of immediate early genes (ΔfosB, zif268, c-fos) and prodynorphin (Pdyn). In addition, activation of ERK promotes mTORC1-dependent signaling, thereby accelerating protein synthesis and inhibiting autophagy through phosphorylation of Unc-51 like kinase 1 (Ulk1). *Adapted from Feyder et. al, 2011.*

Rapamycin is a specific inhibitor of mTORC1-dependent signaling, which acts by binding to the FKBP12-rapamycin binding domain of the mTOR kinase (Li et al., 2014). In dopamine-depleted animals challenged with L-Dopa, the degree of phosphorylation of downstream targets of mTORC1 correlates with the severity of dyskinesia, which can be attenuated by administration of rapamycin (Santini et al., 2009a; Decressac and Bjorklund, 2013).

1.7 mTORC1-mediated inhibition of autophagy

Autophagy is another important biological function regulated by mTORC1. It is an essential process leading to lysosomal degradation of protein aggregates and pathogens, and involved in cellular processes such as phagocytosis, secretion, and exocytosis (Boya et al., 2013).

Activation of mTORC1 inhibits autophagy through phosphorylation of the Unc-51 like kinase 1 (Ulk1), which is regarded as a key step in the negative control exerted on autophagy. mTORC1-mediated phosphorylation prevents Ulk1 from forming a core complex with other autophagy-related proteins (Atg) required for the generation of the autophagosome (Kim et al., 2011). Changes in the autophagic flux can be monitored by measuring the levels of a protein called p62, also known as sequestosome 1. During autophagy, p62 acts as a shuttling protein for ubiquitinated substrates and delivers the cargo to the growing autophagosome for lysosomal degradation (Fig. 5). Accumulation of p62 is therefore regarded as a marker of impaired autophagy (Bjorkoy et al., 2009; Ichimura and Komatsu, 2010). Dysregulated autophagy leads to the accumulation of protein aggregates and damaged organelles, causing pathological disorders including neurodegenerative diseases (Levine and Kroemer, 2008).

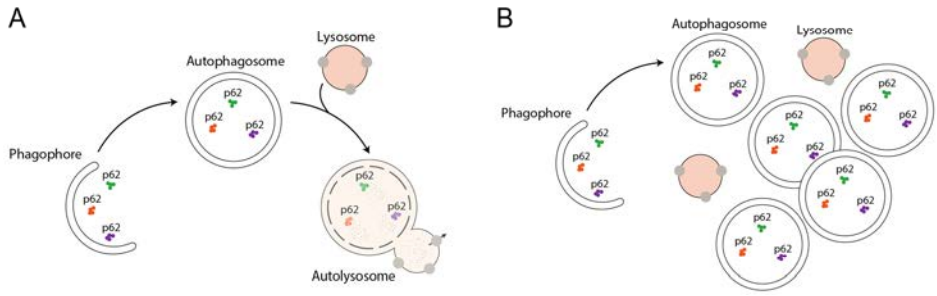

Figure 5. Impaired autophagic flux results in the accumulation of p62. (A) Physiological autophagic flux with lysosomal degradation. (B) Blocked autophagic flux with accumulation of p62 in the autophagosome.

1.8 Intrastriatal signaling in addictive disorders

The primary mechanism of action of psychostimulants is the enhancement of extracellular dopamine, leading to an increased activity of the mesolimbic pathway (Pierce and Kalivas, 1997). It is well established that the rewarding properties of drugs of abuse are mediated by their ability to potentiate dopamine transmission in the ventral striatum (nucleus accumbens) and VTA. However, the transition from goal-directed to compulsive drug-seeking occurs in parallel to a shift of the effects of the drugs from the ventral to the dorsal striatum, which is implicated in habit learning (Belin and Everitt, 2008; Zapata et al., 2010). Therefore, the enhanced responsiveness of the dorsal striatum to dopaminergic drugs after dopamine depletion in PD may promote the acquisition of motor programs involved in addictive and compulsive behaviors.

In line with the hyperactivation of multiple signaling cascades produced by administration of L-Dopa in PD models, various drugs of abuse also increase ERK phosphorylation in the NAc and striatum, which mediates behavioral effects and promotes long-term synaptic plasticity in brain areas involved in reward processing (Valjent et al., 2004). Furthermore, the role of mTORC1 has been suggested for reward-related behaviors (Dayas et al., 2012; Morisot et al., 2018). Importantly, accumulation of ΔFosB is known as a critical factor in the development of all forms of behavioral and drug addictions. For example, acute exposure to cocaine induces FosB, whereas chronic exposure induces accumulation of its stable splice variant ΔFosB in D1R MSNs (Nestler, 2008). Similar findings are observed with many other drugs of abuse as well as with natural rewards such as food, sex, and wheel running (Wallace et al., 2008).

2 RESEARCH AIMS

The general aim of this thesis was to explore the mechanisms at the basis of motor and neuropsychiatric complications associated with the administration of L-Dopa in experimental models of PD and identify possible targets for counteracting interventions.

The specific aims were:

1) To study the involvement of autophagy in the development of L-Dopa-induced dyskinesia

2) To identify complications in the generation of a bilateral 6-OHDA mouse model of PD and provide an improved protocol with low mortality

3) To examine the mechanisms involved in neuropsychiatric complications produced by dopamine replacement therapy

3 METHODOLOGICAL CONSIDERATIONS

3.1 Experimental animal models of PD

Experimental models are essential to advance the understanding of the etiology, pathology and molecular mechanisms of diseases. The use of animal models in preclinical research has played a key role in the evaluation of potential therapeutic strategies against PD.

Animal models are expected to closely match human pathology. An ideal model of PD should recapitulate pathological and clinical features involving motor and non-motor symptoms, preferably with quantifiable behavioral, histological and biochemical aspects in order to study the effects of therapeutic interventions. Additionally, the progressive neurodegenerative nature of the disease and age-dependent onset should be reflected. None of the currently available models recapitulates all features of PD but many of them reproduce specific subsets. Therefore, the use of PD models requires an understanding of their intrinsic limitations and their incomplete representations of the human pathology. Thus far, despite the progressive development of genetic models of PD, neurotoxins have remained the most efficient tool to produce selective neuronal death and associated behavioral deficiencies. The four most popular neurotoxins used in experimental parkinsonism are 6-hydroxydopamine, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), rotenone, and paraquat (Bove et al., 2005).

3.1.1 The 6-OHDA mouse model

6-hydroxydopamine (6-OHDA) is the first dopamine neurotoxin discovered (Ungerstedt, 1968) and the most widely used to induce an experimental lesion of the nigrostriatal pathway (Buhidma et al., 2020). 6-OHDA is a hydroxylated analog of dopamine and noradrenaline which is taken up by catecholamine transporters, leading to neuronal death through a combination of oxidative stress-induced cytotoxicity and impaired mitochondrial function (Glinka and Youdim, 1995; Blum et al., 2001; Tieu, 2011).

Since 6-OHDA does not cross the blood-brain barrier, the model requires an intracranial injection in the region of interest (Thoenen and Tranzer, 1973). The resulting dopaminergic depletion remains stable for several months, thereby simplifying experimental evaluation. The magnitude of the lesion depends on the amount and concentration of 6-OHDA injected, the site of injection and the species used, allowing to model different stages of the human pathology (Deumens et al., 2002). Injections of 6-OHDA into the SNc or the medial forebrain bundle (MFB) (**paper I**), rapidly produce a lesion, which results in a nearly complete loss of

dopaminergic cell bodies in the SNc in the first 12 h, followed by degeneration of striatal terminals and depletion of striatal dopamine in the following 7-10 days (Jeon et al., 1995; Sarre et al., 2004). Injections of 6-OHDA in the dorsal striatum (**papers II** and **III**) allow for a better control of the extent of the lesion due to the larger size of this structure compared to the SNc or MFB. Intrastriatal injections produce degeneration of dopaminergic nerve terminals during the first 24 h, followed by a slow and progressive retrograde loss of nigrostriatal neurons over a period of up to 3 weeks (Sauer and Oertel, 1994; Przedborski et al., 1995; Stott and Barker, 2014).

The limitation of the 6-OHDA model lies mainly in the acute and rapid effect of the toxin which does not replicate the progressive nature of PD. Additionally, the neurodegenerative response to 6-OHDA is not accompanied by the appearance of Lewy body inclusions, which is a pathological marker of PD. Furthermore, due to the invasive character of the procedure, which requires stereotactic injection of the toxin into the brain, and the concurrent neurodegenerative processes, 6-OHDA models are characterized by high mortality rates and require intense post-surgical care (**paper II**). Low survival rates after 6-OHDA lesion have previously been reported (Rodriguez et al., 2001; Deumens et al., 2002; Lundblad et al., 2004) and are thought to be caused by reduced feeding motivation (i.e. aphagia and adipsia), followed by weight loss and dehydration (Ungerstedt, 1971; Longo, 1973; Kaakkola and Teravainen, 1990; Casas et al., 2000).

Despite those limitations, the relative selectivity of 6-OHDA has been the basis of its popularity and has been an essential tool to model symptoms of PD, as well as complications associated with antiparkinsonian treatments. The unilateral injection of 6-OHDA (**paper I**) has been extensively used to study motor symptoms of PD by assessing spontaneous or drug-induced rotational behavior and other asymmetrical movements produced in response to anti-parkinsonian drugs, such as L-Dopa-induced dyskinesia (Ungerstedt and Arbuthnott, 1970; Cenci and Lundblad, 2007). In contrast, bilateral injections of 6-OHDA (**papers II** and **III**) are a more appropriate choice for the study of NMS, to avoid asymmetric movements interfering with behavioral analyses. Partial lesions induced by injecting 6-OHDA into the dorsal striatum have been associated with mild motor symptoms, thereby reproducing the early stages of PD. NMS observed in this model of PD range from cognitive and affective deficits to olfactory impairment and disruption of circadian rhythm and sleep (De Leonibus et al., 2007; Bonito-Oliva et al., 2014a; Bonito-Oliva et al., 2014b; Masini et al., 2017; Masini et al., 2018).

In addition to its effect on the dopamine system, injection of 6-OHDA results in the degeneration of the noradrenaline system, and reduced serotonin levels (Breese and Traylor,

1971; Commins et al., 1989; Santiago et al., 2010; Bonito-Oliva et al., 2014a). This indicates that this model is not limited to the classical degeneration of the dopaminergic system and can also be successfully employed to study NMS of PD, which are often refractory to standard DRT.

3.1.2 The MPTP non-human primate model

PD modeling in non-human primates (NHP) has been essential in advancing our understanding of the pathophysiology and neural mechanisms underlying the disease, owing to their anatomic and genetic similarity to humans. MPTP, the most common neurotoxin used to induce PD in NHP such as macaques (*macaca mulatta*) (**paper I**), leads to behavioral, biochemical and pathological deficits that closely resemble human parkinsonism (Langston and Ballard, 1983) with excellent response to dopaminergic drugs and development of long-term motor complications. After systemic administration, MPTP crosses the blood-brain barrier and is converted to 1-methyl-4-phenylpyridium ion (MPP+), predominantly in non-DA neurons like serotonergic neurons and glia (Chiba et al., 1984; Westlund et al., 1985). Once it is released in the extracellular space, MPP+ is selectively transported into dopaminergic neurons through the dopamine transporter and cell death occurs through inhibition of mitochondrial complex I function and increased oxidative stress (Langston et al., 1983; Javitch et al., 1985). The ability of MPTP to produce nigral and striatal dopamine cell loss similar of human PD, with higher sensitivity of dopaminergic neurons in the SNc than VTA, mimics another fundamental resemblance to the PD pathology in humans (Moratalla et al., 1992; Varastet et al., 1994; Dauer and Przedborski, 2003; Blesa et al., 2010).

An often raised weakness of the model is the absence of Lewy body inclusions, but researchers have reported intraneural eosin-positive inclusions which are reminiscent of Lewy bodies (Forno et al., 1986; Forno et al., 1993) and overexpression of alpha-synuclein in NHP treated chronically with MPTP (Kowall et al., 2000; Emborg, 2007). Ethical and logistic restrictions are the main limitations of the NHP model in the use of PD research.

3.2 Transgenic mice and viruses

The availability and variety of transgenic mouse models are a major advantage in the study of neurological and psychiatric diseases. By manipulating the genome of mice, models that mimic specific aspects of diseases can be created to identify underlying mechanisms and test potential

treatments, providing a valuable tool for investigating the genetic, molecular, and behavioral aspects of these conditions.

The Cre-Lox Recombination system, a site-specific recombination technology, is one of the most widely used approaches nowadays to carry out DNA modifications like deletions, insertions, translocations and inversions at specific sites. The cyclization recombination enzyme (Cre) originally derived from bacteriophage P1, recombines a pair of short target sequences called Lox sequences (Hoess and Abremski, 1985; Sauer, 1998). Based on the orientation of these LoxP (locus of crossing-over [X] of P1) sites with respect to one another, the gene of interest will be excised/integrated or inverted by Cre. If the LoxP sites are in the same orientation (direct repeats), the DNA is excised, effectively deleting the region between the two LoxP sites. Conversely, if the LoxP sites are in the opposite orientation (inverted repeats), the DNA between the two sites is inverted, and it can be reinserted in the opposite direction, allowing to introduce point mutations into the protein of interest (Zhang and Lutz, 2002).

The creation of transgenic mice using bacterial artificial chromosomes (BAC) is another important genetic engineering technique based on large-insert DNA clones derived from bacterial plasmids that can be used as vehicles to transport and manipulate genes of interest. In this case, the natural specificity of neurons in expressing certain proteins can be utilized as a tool for targeted expression of Cre or fluorescent reporters linked to the endogenous promoter of such proteins within a particular cellular group. In this thesis, BAC transgenic lines like Drd1a-Cre (EY262) and Drd1a- or Drd2-EGFP mice are based on the expression of the Cre recombinase or enhanced green fluorescent protein (EGFP) reporter gene under the control of the Drd1a or Drd2 promoter, respectively (Gong et al., 2003; Gong et al., 2007). The Drd1a- and Drd2-EGFP transgenic mice allowed us to examine the involvement of the two different populations of MSNs in the activation of signaling cascades in L-Dopa-induced dyskinesia (**paper I**) and psychiatric complications induced by L-Dopa (**paper III**). By combining Drd1a-Cre BAC transgenic mice with the Cre-Lox technology, conditional knockout mice of Atg7 in D1R-expressing MSNs ($Atg7^{F/F}$;Drd1a-$Cre^{+/-}$ mice) were generated (Komatsu et al., 2005). These mice lack the gene coding for Atg7, a core Atg involved in autophagosome formation, and allowed us to study if D1R-mediated hyperactivation of mTORC1 involved in dyskinesia is accompanied by reduced autophagy (**paper I**).

Another methodological approach used in this thesis is based on the use of transgenic mice in combination with viral strategies to express designer receptors exclusively activated by designer drugs (DREADD) in a subset of neurons within an anatomically restricted area of the

striatum. For this approach, Drd1a-Cre mice were injected with a fluorescent-tagged Cre-inducible-adeno-associated virus (AAV) carrying the gene for the inhibitory Gi-DREADD (pAAV5-hSyn-DIO-hM4D(Gi))-mCherry). DREADDs are artificially engineered protein receptors that represent a powerful chemogenetic tool to manipulate the activity of selected neurons and investigate their role in behavior and disease (Roth, 2016; Atasoy and Sternson, 2018). DREADD are modified GPCR insensitive to endogenous ligands but selectively activated by designer drugs like clozapine-N-oxide (CNO), the inactive metabolite of the antipsychotic drug clozapine (Armbruster et al., 2007; Zhu and Roth, 2014). Whereas clozapine binds with different affinities to multiple neurotransmitter receptors (including acetylcholine, dopamine and serotonin receptors), CNO has very low potency at these receptors. The dose of CNO must be kept as low as possible and appropriate controls are necessary, since clozapine-like side effects have been described due to potential back-metabolism (Jann et al., 1994; Gomez et al., 2017). The effects of the inhibitory DREADD, hM4Di, on physiology and behavior are caused by (a) CNO-induced hyperpolarization and reduction of neuronal firing due to G-protein-mediated activation of inwardly rectifying potassium channels (GIRKs) and (b) inhibition of synaptic release by suppression of calcium channel activity (Armbruster et al., 2007; Stachniak et al., 2014). In MSNs, which are characterized by a relatively hyperpolarized resting membrane potential, the synaptic silencing function of hM4Di seems to be more relevant for inhibiting the neuronal output than the suppression of electrical activity.

Stereotaxic viral-mediated expression of DREADD can vary between subjects and requires subsequent biochemical evaluation of viral transduction. Nevertheless, DREADD offer a relatively non-invasive, reversible and selective manipulation of neuronal activity.

3.3 Methods of behavioral analysis

Motor and non-motor behavioral experiments on different models of PD were performed according to the experimental questions. All animals were habituated to the experimental environment and protocols were balanced regarding groups, treatments and time of the day. All behavioral procedures are described in detail in the individual papers.

3.3.1 Rating L-Dopa-induced dyskinesia

Chronic administration of L-Dopa in parkinsonian models induces dyskinesia, which is classically characterized by abnormal involuntary movements.

The mouse model we used to reproduce LID is based on the unilateral injection of 6-OHDA in the MFB (**paper I**), which leads to a near total loss of the nigrostriatal pathway in the hemisphere ipsilateral to the lesion and asymmetric movements. After chronic treatment (10 days) with L-Dopa, dyskinetic behavior was assessed by scoring rotational behavior and abnormal involuntary movements (AIMs) according to a severity scale. AIMs can be quantified based on their amplitude and duration, and represent repetitive, purposeless movements that are not within the range of any normal rodent behavior, affecting the side of the body contralateral to the lesion (Cenci et al., 1998; Cenci and Lundblad, 2007). They can be divided into limb (repetitive jerks or dystonic twirls), axial (flexion of the neck or torsion of the upper trunk) and orolingual (jaw movements and tongue protrusion) AIMs. In the unilateral 6-OHDA model, development of sensitization to rotational effects induced by DRT has been regarded as a marker of dyskinesia. However, this initial view has been challenged by studies showing that rotational sensitization can be induced by drugs with low dyskinesiogenic potential and does not allow to distinguish between dyskinetic and anti-akinetic effects of drugs (Lundblad et al., 2002). Additionally, this type of sensitization is associated with increased immediate early gene expression in the dorsomedial striatum, which is involved in associative function. In contrast, the development of axial, forelimb and orolingual involuntary movements, is associated with increased immediate early genes in the dorsolateral striatum which is involved in motor control. For this reason, AIMs are generally considered a more reliable marker of LID (Sebastianutto et al., 2016; Peng et al., 2019).

Chronic treatment (4-5 months) with L-Dopa in the MPTP NHP model results in dyskinesia similar to human LID presenting choreic–athetoid (characterized by constant writhing and jerking motions), dystonic, and sometimes ballistic movements (large-amplitude flinging, flailing movements). Both NHP and mouse models present peak-dose dyskinesia that disrupts physiological motor activities.

3.3.2 Measuring rewarding effects of drugs

Modeling addiction-like behaviors in rodent models comes with the challenge of replicating a complex behavioral pathology with relatively simple behavioral methods (Kuhn et al., 2019). Non-contingent (experimenter-administered) models in which animals are passively exposed to rewarding substances are commonly used to study the rewarding or aversive effects of drugs and were used within the frame of this thesis (**paper III**).

The behavioral sensitization model is based on the augmented motor-stimulant response that occurs with repeated, intermittent exposure to a constant drug dose and is commonly assessed

by monitoring motor activity. Within addictive disorders, it is proposed to model the increased drug craving observed in human psychostimulant abusers. The repeated administration of psychostimulants such as cocaine, amphetamine and ethanol induces locomotor hyperactivity (Segal and Mandell, 1974; Everitt and Wolf, 2002) that can even be observed after long drug-free periods (Robinson and Berridge, 1993). Moderate drug doses in repeated intermittent administration schedules proved more effective at inducing sensitization than continuous exposure to high or escalating drug doses (Robinson and Berridge, 1993; Vezina and Leyton, 2009). Interestingly, sensitization to psychostimulants can be induced after a few exposures or even a single injection of the drug (Robinson et al., 1982; Jackson and Nutt, 1993; Vanderschuren et al., 2001; Valjent et al., 2010) and the expression of sensitized locomotor response is significantly enhanced in context-dependent environments (Badiani and Robinson, 2004). Behavioral sensitization requires D1R activation in the VTA and is generally considered to be mediated by an increased release of dopamine in the ventral striatum (Henry and White, 1991; Vezina, 1996; Camarini et al., 2011). However, behavioral sensitization to drugs of abuse has also been shown to involve noradrenergic and serotonergic systems (Lanteri et al., 2008; Tassin, 2008). Interestingly, cross-sensitization between drugs has been described in several cases (Vezina et al., 1989; Itzhak and Martin, 1999; Valjent et al., 2010), suggesting that despite their individual binding sites in the brain, common mechanisms underlie the development of behavioral sensitization.

The conditioned place preference paradigm is a form of pavlovian conditioning and has become the most widely used non-contingent assay to determine rewarding or aversive effects of drugs (Bardo and Bevins, 2000; Tzschentke, 2007). In this test, an animal is conditioned to associate a particular environment (context) with a drug (stimulus) and a different environment with the absence of the drug. After conditioning, a choice test between both contexts in the absence of the stimulus determines possible rewarding properties of the drug, indicated by an increased time spent in the drug-paired environment compared to the initial preference. Numerous studies revealed that various substances of abuse, such as cocaine, amphetamine, morphine, nicotine, alcohol, and cannabis induce CPP (Bardo and Bevins, 2000; Garcia Pardo et al., 2017) and established the involvement of the reward circuitry with dopaminergic, glutamatergic, GABAergic, cholinergic, noradrenergic and serotonergic systems in the induction and maintenance of CPP (Tzschentke, 2007).

The design of the apparatus can differ in the number of compartments, the context may vary in visual (size, shape, color, pattern), tactile (flooring) or olfactory cues and conditioning protocols are based on individual durations and number of drug exposures (Prus et al., 2009;

Kuhn et al., 2019). Experiments in this thesis (**paper III**) were carried out in a two-compartment apparatus, limiting the choice of the animal to one of the two chambers. In contrast, an also commonly used three-compartment apparatus offers the animal a central passage area between the experimental chambers, which is referred to as a neutral zone. The choice of the apparatus is important, as it presents the animal with a decision between a "forced" and an "unforced" choice (Prus et al., 2009). The additional compartment is considered a neutral starting point, but a potential bias for the compartment the animal was placed in during the test session can be avoided with appropriate balancing of experimental groups.

Another important consideration concerning the CPP paradigm is the choice between a "biased" versus an "unbiased" experimental design (Prus et al., 2009). In an unbiased CPP setup, the assignment to a drug-paired compartment is decided by the researcher, regardless of the initial preferences animals displayed in the pre-conditioning test. This design can be reliably used when animals do not show a strong preference for one compartment prior to conditioning. The choice for a setup with tactile cues (**paper III**) was accompanied by an initial preference for one of the compartments, indicative of a biased CPP design. Therefore, the preference of each individual mouse for a particular environment was assessed prior to conditioning and drug-pairings were assigned to the least-preferred compartment. In this case, overcoming the initial aversion for that environment and developing a preference for the initially non-preferred compartment is understood as the establishment of a CPP.

One major advantage of the CPP paradigm lies in its ability to evaluate the conditioned reward effects in a drug-free state, thereby avoiding potential drug-mediated impairment of the animals (Cunningham et al., 2006). The test is also sensitive to the effects of low drug doses, but it is important to consider the specific time course of the drug response and adjust the number of conditioning sessions depending on the animal model and type of drug. In the case of drugs with strong rewarding characteristics, establishing a CPP necessitates fewer conditioning sessions, whereas drugs with less pronounced rewarding properties might demand a greater number of conditioning sessions.

3.4 Ethical considerations

This thesis is mainly based on the use of mouse models of Parkinson's disease. To reproduce the salient feature of this disorder, animals are surgically operated to inject a neurotoxin,

resulting in relatively selective brain damage. This, together with pharmacological, genetic and behavioral approaches previously described, raises questions regarding animal welfare.

The use of these models is necessary to address the questions at the center of the studies described in this thesis, which deal with the assessment of complex behaviors and require transgenic interventions. Furthermore, the information on rodent models of PD available in the literature is much more extensive in comparison to that obtained from models generated in other species, making it simpler to design and control our studies and to reduce to a minimum the number of animals necessary to reach reliable conclusions. Thus, the mouse model represents an acceptable compromise between approaches in vitro, or in lower vertebrates, and approaches in NHP, which also pose more challenging ethical questions.

Behavioral studies employing pharmacological agents are generally characterized by high variability. Therefore, to obtain reliable results, a relatively large number of animals per experimental group is necessary. We avoided long test batteries as each experimental procedure may affect the performance of the animals in the subsequent behavioral paradigm, which contributes to an increased number of animals. Given these considerations, experiments can only be carried out if there is sufficient evidence that the tests applied will be useful to model clinically relevant symptoms described in PD patients. Keeping this in mind, we always strived to use the minimal number of animals necessary to reach consistent results for each behavioral domain. Furthermore, the invasive character of the 6-OHDA model requires intense post-surgical care and raises questions concerning the animals' well-being. Close monitoring of individual animals throughout the last years has resulted in the improvement of a standard surgical protocol (**paper II**), leading to a nearly complete elimination of lesion-related animal mortality and ultimately to a reduction of the number of animals needed to conduct the studies.

All experimental approaches described in this thesis have been approved by the national ethical committees for animal research (ethical permits N114/15; 12148-17; 14673-2022). Our laboratory has a very good animal welfare record and operates in a state-of-the-art facility, under strict veterinary supervision, in compliance with the 3Rs policy. Humane endpoints have been defined for all experiments and are implemented as a standard laboratory practice.

4 RESULTS AND DISCUSSION

4.1 Sensitization of the mTORC1 signaling cascade and its association with impaired autophagy in LID

The mTORC1 signaling cascade is involved in the regulation of protein synthesis (Wang and Proud, 2006; Costa-Mattioli et al., 2009; Ma and Blenis, 2009), but is also known to inhibit autophagy (Boya et al., 2013; Zhu et al., 2019). Therefore, in **paper I**, we wanted to examine whether the hyperactivation of mTORC1, which has been previously involved in dyskinesia (Santini et al., 2009a), is accompanied by reduced autophagy. To establish a correlation between impaired autophagy and dyskinesia, we used the unilateral 6-OHDA mouse and MPTP NHP model of PD to examine changes in autophagy associated with chronic L-Dopa administration.

We found that in the striatum of dopamine-depleted mice, repeated daily administration of L-Dopa, which induces severe AIMs, increased the levels of p62, a marker of autophagy impairment (Bjorkoy et al., 2009; Ichimura and Komatsu, 2010) (Fig. 6A). In line with these results, p62 was also significantly increased in the MPTP non-human primate model of LID.

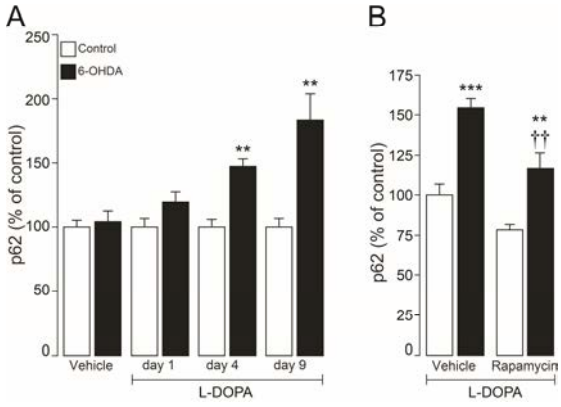

Figure 6. Chronic administration of L-Dopa reduces autophagy in the striata of 6-OHDA mice and is counteracted by the administration of rapamycin. Western blot analysis showing (A) accumulation of p62 in the striatum following chronic L-Dopa treatment and (B) effect of rapamycin on p62 levels. *Adapted from Feyder et al., 2021.*

The administration of rapamycin, a selective inhibitor of mTORC1, has previously been shown to reduce dyskinetic behavior in rodent models of PD (Santini et al., 2009a; Subramaniam et al., 2011; Decressac and Bjorklund, 2013; Calabrese et al., 2020). This effect has been related to its action on mTORC1 downstream targets involved in the control of protein synthesis (Klann, 2009; Santini et al., 2009a), but the mechanisms at the basis of the anti-dyskinetic properties of this drug remain to be determined.

In line with this question, we found that the increase of p62 observed in the mouse model of PD was reduced by rapamycin (Fig. 6B) and accumulation of p62 was limited to D1R-expressing MSNs located in the dopamine-depleted striatum (Fig. 7). Chronic administration of L-Dopa was accompanied by mTORC1-dependent phosphorylation of Ulk1 in the dopamine-depleted striatum and this effect, similarly to the enhancement of p62, was prevented by administration of the D1R antagonist SCH23390. In contrast, raclopride, a D2R antagonist, did not modify the effect of L-Dopa. These results indicate that the impairment of autophagy induced by L-Dopa is caused by D1R-mediated activation of mTORC1 in the MSNs of the direct pathway. It is important to note that the development of LID depends on combined dysregulated transmission in D1R and D2R MSNs. Notably, not only the blockade of D1R but also the inhibition of D2R reduces LID in mouse models of PD (Sebastianutto et al., 2016; Alcacer et al., 2017). Considering these results, the inability of raclopride to reduce the accumulation of p62 associated with LID suggests that the anti-dyskinetic action of this drug occurs through a parallel mechanism, which circumvents the effects of reduced autophagy in D1R MSNs.

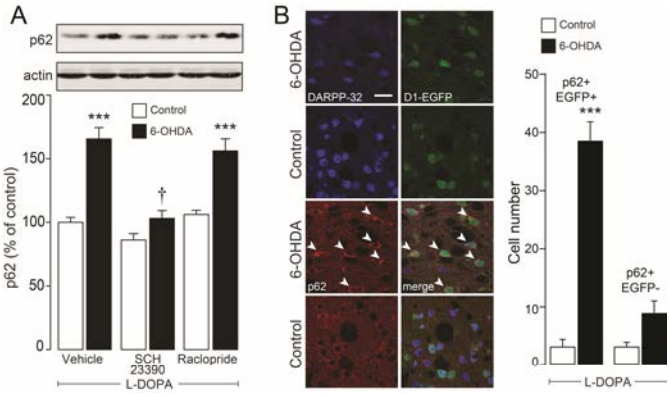

Figure 7. The impairment of autophagy induced by L-Dopa is mediated by D1R. (A) Levels of p62 following chronic L-Dopa treatment in combination with SCH23390 or raclopride. (B) Immunofluorescence analysis of p62 in D1-EGFP mice. *Adapted from Feyder et al., 2021.*

To examine the possibility that the effect of rapamycin depends on its ability to promote autophagy via inhibition of mTORC1, we tested the effect of rapamycin in mice lacking the gene coding for Atg7, a core Atg involved in autophagosome formation. In those animals, loss of Atg7 specifically in D1R MSNs resulted in the constitutive impairment of autophagy, indicated by a large accumulation of p62 (Fig. 8A). We found that, in contrast to control mice ($Atg7^{F/F}$), rapamycin did not normalize p62 levels in Atg7 knockout mice ($Atg7^{F/F}$;Drd1a-$Cre^{+/-}$), indicating that in these mice rapamycin loses its ability to promote autophagy. Importantly, the anti-dyskinetic effect of rapamycin was also abolished, indicating that a

significant proportion of the anti-dyskinetic effect exerted by this drug depends on its ability to promote autophagy (Fig. 8B).

Phenotypic consequences due to the loss of autophagy in D1R MSNs include a reduced body weight, as well as behavioral deficits and disruptions in striatal function compared to control mice (Lieberman et al., 2020). Motor learning in the accelerating rotarod task was impaired in Atg7 knockout mice, but this test has been shown to be highly sensitive to body weight (McFadyen et al., 2003). Importantly, locomotor activity in an open field test was not affected by the genotype. In line with these results, locomotive (rotational) AIMs, which are regarded as an index of motor impairment, were similar in Atg7 knockout mice when compared to control.

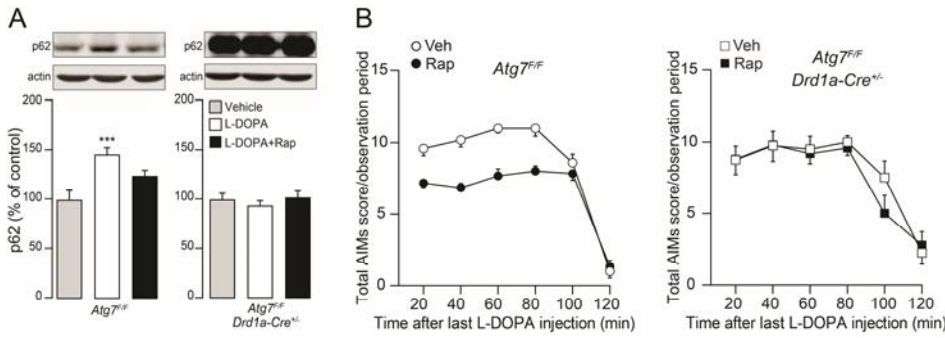

Figure 8. Rapamycin fails to promote autophagy and reduce dyskinesia in mice lacking Atg7 in D1R MSNs as indicated by (A) the lack of effect on p62 accumulation and (B) the dyskinetic response produced by administration of L-Dopa in control ($Atg7^{F/E}$) and knockout ($Atg7^{F/F}$;Drd1a-$Cre^{+/-}$) mice. *Adapted from Feyder et al., 2021.*

Liebermann et. al showed that deletion of Atg7 in D1R MSNs reduced dendritic spine density. This effect is reminiscent of the increase in spine pruning observed in the same neuron type following dopamine depletion and potentially contributes to worsening the effects of 6-OHDA. Interestingly, the constitutive impairment of autophagy caused by inactivation of Atg7 did not enhance the dyskinetic response to L-Dopa. A possible explanation is that the severity of LID in Atg7 deficient mice has reached a peak level which prevents the exacerbation of AIMs.

Besides autophagy, the other crucial degradation pathway in eukaryotic cells is the ubiquitin-proteasome system (UPS), in which proteins tagged by certain types of polyubiquitin chains are selectively recognized and removed by the proteasome (Schwartz and Ciechanover, 2009). Although the UPS and autophagy mechanisms were primarily thought to be largely distinct catabolic pathways, recent studies have revealed common mechanisms such as reciprocal

cross-talks between UPS and autophagy involving the protein p62, as well as cell signaling pathways and transcription factors (Lilienbaum, 2013; Liu et al., 2016; Kocaturk and Gozuacik, 2018). Importantly, LID has also been associated with D1R-mediated impairment of the ubiquitin-proteasome system (Scholz et al., 2008; Berthet et al., 2012; Barroso-Chinea et al., 2015), suggesting that LID is accompanied by compromised activity of the two major catabolic systems in MSNs of the direct pathway. Interestingly, in hippocampal neurons, autophagy has been shown to preserve long-term depression by reducing the degradation of glutamate receptors (Shehata et al., 2012). This finding is in line with the idea that autophagy-promoting agents may act by counteracting the loss of synaptic downscaling observed in LID (Picconi et al., 2003; Calabresi et al., 2016).

Overall, these results indicated that augmented responsiveness at D1R leads to impaired autophagy and that this effect is linked to the emergence of LID. This suggests that autophagy-promoting drugs, which are currently used to treat metabolic disorders (e.g. diabetes) and cancer, may be repositioned as a therapeutic strategy against dyskinesia. Further supporting this possibility, the anti-diabetic drug metformin, which promotes autophagy via activated protein kinase-mediated regulation of Ulk1 and mTORC1 (Li and Chen, 2019; Lu et al., 2021), has been shown to reduce LID in a rodent model of PD (Ryu et al., 2018).

4.2 Dopaminergic depletion in the bilateral partial lesion model

The unilateral 6-OHDA model has been extensively used to study motor symptoms of PD and motor complications in response to anti-parkinsonian drugs (**paper I**). However, the bilateral partial 6-OHDA model (**papers II** and **III**) is the more appropriate choice for the study of NMS. Whereas unilateral lesions are associated with asymmetric posture and movements, the study of NMS requires an animal model allowing unbiased measurements of the motor repertoire in behavioral tests. Injection of 6-OHDA in a large brain area like the striatum allows for adequate control of the extent of dopamine depletion, particularly when using mice. Therefore, striatal 6-OHDA injections can be designed to produce a partial loss of dopamine, associated with the mild motor symptoms observed during the initial stages of PD.

The bilateral partial 6-OHDA mouse model utilized in this thesis was initially developed in our research group to study non-motor symptoms in PD (Bonito-Oliva et al., 2014b). It is generated by a bilateral injection of 6-OHDA into the dorsolateral striatum, resulting in the partial degeneration of the midbrain dopaminergic system. The lesion does not affect spontaneous motor activity or exploratory behavior, as measured in the novel home cage and open field test.

Furthermore, animals do not show deficits on fixed speed tests in the rotarod or the grip strength test, but reduced vertical activity and subtle gait impairments that include step disruptions affecting the hind limbs, which do not interfere with behavioral evaluation in the range of this thesis. This model has been shown to reproduce a variety of NMS, including memory deficits, depression- and anxiety-like behavior, impaired olfactory discrimination and disrupted circadian rhythm (Bonito-Oliva et al., 2014a; Bonito-Oliva et al., 2014b; Masini et al., 2017).

The relatively small impairment in motor function observed in this lesion is accompanied by reductions in dopamine neurons in the SNc (~60%) and striatal dopamine innervation (~55%) similar to those occurring in PD patients at the onset of motor symptoms (Fig. 9). The injection of 6-OHDA results in a restricted lesion mostly limited to the dorsolateral striatum (~75%) and its dopaminergic afferents from the SNc. The impairment of the nigrostriatal system is accompanied by more limited damage in the VTA and ventral striatum (10-15%), which is in line with the relative sparing of these structures observed in PD patients, where dopaminergic projections are not equally affected (Hirsch et al., 1988). The most striking cell loss can be found in the ventrolateral tier of the SNc which project to the dorsolateral striatum (Kish et al., 1988; Damier et al., 1999). It should be kept in mind, that despite their more moderate character, the neuronal loss in the VTA and the associated impairment of the mesocorticolimbic dopamine system may contribute to cognitive and affective comorbidities.

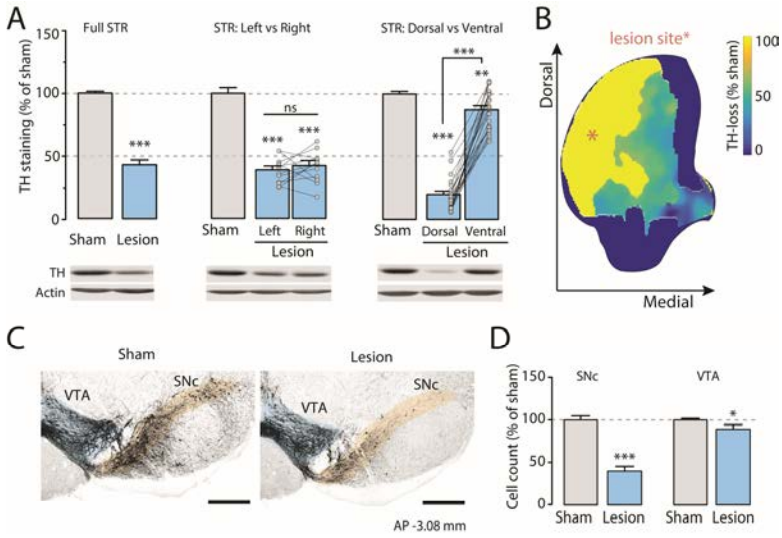

Figure 9. Effect of the striatal bilateral 6-OHDA lesion on TH immunoreactivity. (A) Percentage of TH-loss in striatal tissue of 6-OHDA mice compared with sham mice quantified by western blot. (B) Coronal view of the lesion site, showing the percentage of TH-fiber loss in the striatum. Total depletion is shown in yellow. (C) Representative images and (D) quantification of TH-positive cells within SNc (orange shade) and VTA (blue shade) of sham and lesion mice. *Adapted from Masini et al., 2021.*

Additionally, without pretreatment of selective norepinephrine reuptake inhibitors like desipramine, the injection of 6-OHDA also affects non-dopaminergic systems like noradrenergic neurons in the locus coeruleus (Bonito-Oliva et al., 2014a). This reproduces the parallel degeneration of DA and NE neurons described in PD patients (Braak et al., 2003).

4.3 Implementation of an improved protocol to reduce mortality in partial bilateral lesion mice

Unfortunately, due to its invasive stereotactic administration and rapid neurodegenerative action, the use of 6-OHDA is frequently associated with significant post-surgical mortality. The high mortality rate in this model has been reported previously (Rodriguez et al., 2001; Deumens et al., 2002; Lundblad et al., 2004) but is most likely underrepresented in literature due to its conflict of interest. Low survival rates are thought to be caused by reduced feeding motivation (i.e. aphagia and adipsia), followed by weight loss and dehydration. To reduce this problem, mice with a unilateral 6-OHDA lesion in the MFB have been treated with post-surgical interventions, including supplemental nutrition, rehydration, and external temperature control. Various combinations of these procedures decreased mortality to 20–10% (Thiele et al., 2011; Glajch et al., 2012; Boix et al., 2015) and in some cases even eliminated it (Francardo et al., 2011; Sebastianutto et al., 2016). Implementing a protocol using a lower dose of 6-OHDA combined with extensive post-operative care also has been shown to successfully increase weight recovery and survival rates in mice with a unilateral MFB lesion (Guillaumin et al., 2022).

Animals with a bilateral lesion are particularly sensitive to 6-OHDA, mainly due to the severe character and broader effect of 6-OHDA, requiring extensive pre- and post-surgical support and recovery time. In **paper II** we found that the survival rates of male and female mice subjected to the bilateral 6-OHDA lesion differ significantly, with a much higher mortality among males (96.1% survival rate in females and 79.2% in males). Our results indicate that the first week after 6-OHDA injection is critical for the recovery of male mice and that mice operated at 2.5–3 months of age are the experimental group at higher risk of mortality.

The higher mortality is mainly associated with the persistent blocking of the urethra and penis prolapse affecting males during the weeks following surgery, which may lead to chronic obstructive uropathy. To address this issue, we developed an enhanced care protocol with improved pre- and post-surgical interventions combined with more frequent daily checks to prevent urologic complications. Weight gain as a result of pre-operative food supplementation

appears to be a critical factor for effective post-surgical recovery. The enhanced care protocol increased the male survival rate to 92.3% (Fig. 10).

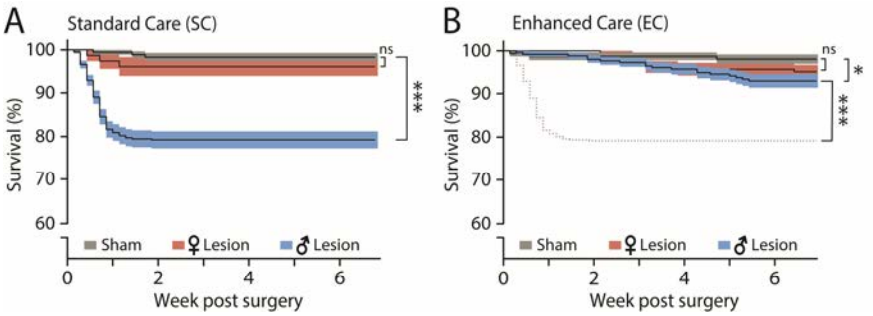

Figure 10. Enhanced perioperative care improves survival following bilateral injection of 6-OHDA. Survival curves of sham and lesion (male and female) mice subjected to (A) standard or (B) enhanced care protocol. *Adapted from Masini et al., 2021.*

The dramatic gender difference with female mice displaying a survival rate similar to control mice, suggests that the use of this gender is preferable in combination with a simpler care protocol. Nevertheless, it is important to consider that gender is associated with the prevalence and severity of particular non-motor symptoms. For example, affective disorders and pain are more commonly diagnosed in women, while men exhibit a greater susceptibility to cognitive disorders and disturbances in sleep (Martinez-Martin et al., 2012; Solla et al., 2012; Szewczyk-Krolikowski et al., 2014).

Reduction in animal loss facilitates the use of this model to study non-motor comorbidities, which represent an urgent clinical problem in the management of PD. The step-by-step surgical protocol and implementations to increase survival with enhanced pre- and post-surgical care are also applicable to other types of 6-OHDA models.

4.4 Dopamine dysregulation syndrome in a mouse model of PD

In **paper III** we focused on neuropsychiatric non-motor complications induced by DRT and the examination of underlying mechanism in the bilateral lesion model of PD. In this previously described model (cf. **paper II**) we were able to mimic addictive-like behavior frequently observed in response to the gold-standard anti-parkinsonian medication L-Dopa.

The rewarding and motor-stimulant properties of L-Dopa, reminiscent of behavioral sensitization, were examined in the CPP paradigm. We found that PD mice developed a place

preference, as shown by the enhanced time spent in the compartment associated with the administration of L-Dopa. In contrast, control mice did not show any place preference when subjected to the same conditioning protocol (Fig. 11A). This effect was accompanied by an enhanced motor-stimulant response to L-Dopa in 6-OHDA lesion mice, observed already after the first administration of the drug (Fig. 11B). These results suggest that L-Dopa induces behavioral sensitization and acquires rewarding properties as a result of dopamine depletion. The effects of L-Dopa are congruent with clinical reports of PD patients experiencing feelings of pleasure and euphoria during on-drug periods (Maricle et al., 1995; Castrioto et al., 2013) in contrast to low-mood elevation caused by L-Dopa in healthy humans (Liggins et al., 2012).

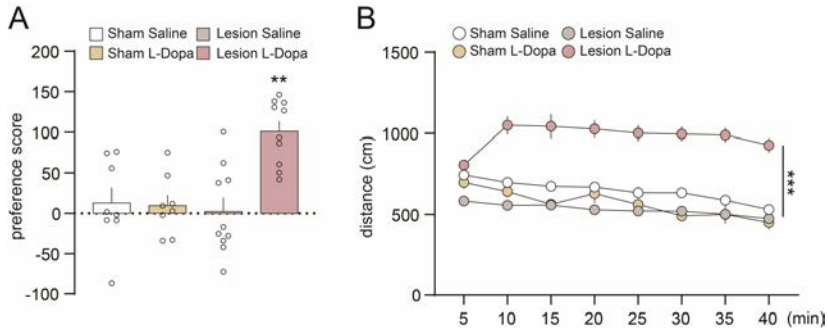

Figure 11. L-Dopa-induced conditioned place preference and motor-stimulation in 6-OHDA lesion mice. (A) Preference score calculated as post- minus pre-conditioning time spent in the drug-paired compartment. (B) Locomotor activity during the conditioning phase in the drug-paired compartment. *Adapted from Plewnia et al., unpublished manuscript.*

Similar results to those described in this study were obtained in an α-synuclein rat model of PD, in which L-Dopa displayed psychostimulant-like properties in a CPP paradigm and decreased the palatability of a nondrug reward (Engeln et al., 2013b). In other 6-OHDA rat models with nigrostriatal or mesolimbic degeneration, L-Dopa failed to show motivational properties (Zengin-Toktas et al., 2013; Carvalho et al., 2017). These discrepancies can be explained by species-specific differences, variations in route of administration (oral vs. i.p.) and dosage of L-Dopa, and the CPP protocol employed in the studies. In our experiments, conditioning was performed with a larger number of sessions and extended duration of drug exposure compared to the studies in rats. These adaptations were implemented to avoid the potential interference of reduced long-term recognition memory observed in the same mouse model (Bonito-Oliva et al., 2014b). In a recent study, administration of L-Dopa in a 6-OHDA rat model resulted in increased 50-kHz ultrasonic vocalizations, which are considered a marker of positive emotional states in rats (Simola et al., 2021). These effects were observed

immediately after drug administration as well as in response to environmental stimuli that were previously paired with the drug.

Dopamine D2/D3 receptor agonists clinically used to alleviate the motor dysfunction of PD, also displayed rewarding effects in several rat models of PD (Riddle et al., 2012; Engeln et al., 2013a; Zengin-Toktas et al., 2013). However, unlike L-Dopa, the properties of pramipexole were found to be intrinsically rewarding since they were also observed in naive rats. Lesion of the nigrostriatal system potentiated the rewarding properties of pramipexole, since a higher dose was needed to induce CPP in naive rats (Riddle et al., 2012). In contrast, in a rat self-administration procedure, a combined nigrostriatal and mesostriatal lesion did not alter the reinforcing properties of pramipexole (Engeln et al., 2013a).

4.5 Sensitized D1R transmission in a mouse model of DDS

The rewarding effect exerted by L-Dopa in the PD mouse model was accompanied by abnormal signal transduction in D1R MSNs within the dorsal striatum. These changes included hyperactivation of ERK and mTORC1 signaling, accompanied by a large accumulation of the transcription factor ΔFosB (Fig. 12A). ΔFosB is known to be upregulated in response to the administration of addictive drugs and other chronic stimuli which may be equivalent to the non-physiologic, pulsatile administration of L-Dopa in PD.

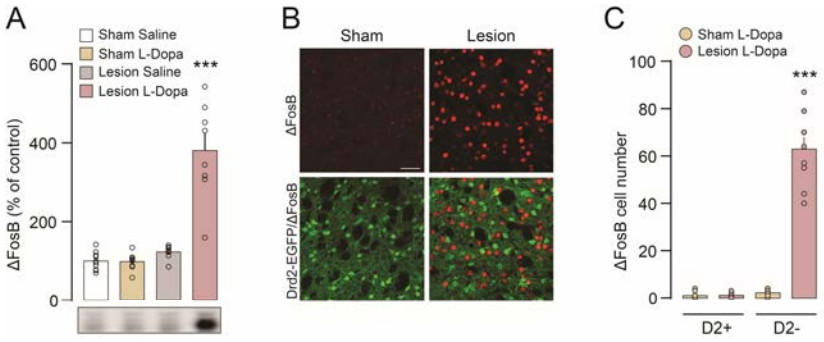

Figure 12. L-Dopa-induced hyperactivation of ΔFosB occurrs selectively in striatal D1R MSNs of 6-OHDA mice. (A) Western blot quantification of ΔFosB in the striata of sham and lesion mice treated with L-Dopa or saline. (B) Representative immunostaining and (C) quantification of ΔFosB (red) in the dorsal striatum of sham or 6-OHDA lesion Drd2-EGFP (green) mice treated with L-Dopa. *Adapted from Plewnia et al., unpublished manuscript.*

Injection of 6-OHDA in BAC transgenic mice expressing enhanced green fluorescent protein (EGFP) under the control of the promoter for the D2R confirmed that the effect of L-Dopa is

cell-type specific and limited to EGFP-negative neurons, which correspond to the D1R-expressing neurons of the direct nigrostriatal pathway (Fig. 12B). In agreement with this finding, pharmacological inactivation of D1R with the antagonist SCH23390 abolished the hyperactivation of the ERK and mTORC1 signaling cascades and the accumulation of ΔFosB. Importantly, SCH23390 also prevented the development of place preference in the CPP test, thereby counteracting the psychostimulant effect of L-Dopa. In line with these results, selective chemogenetic inhibition of hM4Di-expressing D1R MSNs also counteracted the rewarding properties of L-Dopa in the CPP test.

The involvement of D1R activation in the rewarding properties of dopamine-replacing agents, has been challenged by a study showing that administration of the D1R agonist, SKF81297, induces place preference in naive rats, but exerts instead aversive effects in rats with a combined 6-OHDA lesion of the SNc and VTA (Zengin-Toktas et al., 2013). This opposite response may depend on the abnormal stimulation produced by SKF81297 on sensitized D1R of 6-OHDA lesion rats. Indeed, the rewarding effect of SKF81297 measured in the CPP test is biphasic, with large doses failing to produce a significant place preference (Graham et al., 2007). Along these lines of thought, the rewarding properties displayed by L-Dopa in 6-OHDA lesion mice are likely dependent on its ability to activate D1R within a range compatible with the induction of place preference.

Rewarding properties of pramipexole in a 6-OHDA rat model of PD were accompanied by enhanced expression of ΔFosB involving different subregions of the striatum, including both D1 and D2 receptor-expressing neurons (Engeln et al., 2013a). This differs from the accumulation of ΔFosB observed in our model and suggests that the ability of L-Dopa to gain psychostimulant-like properties differs from that of pramipexole by being more strictly dependent on the loss of dopamine in the dorsal striatum, and the development of D1R sensitization.

The D1R-dependent increase of striatal ΔFosB expression produced by L-Dopa in the PD mouse model is accompanied by activation of the ERK and mTORC1 signaling pathways. Pharmacological inhibition of these intracellular cascades has been shown to counteract L-Dopa-induced dyskinesia (Santini et al., 2007; Santini et al., 2009a), but did not prevent the ability of L-Dopa to induce place preference. In addition, combined administration of ERK or mTORC1 inhibitors with L-Dopa failed to produce a full normalization of ΔFosB levels. Considering the well-established role of ΔFosB in drug addiction it is possible that the rewarding properties displayed by L-Dopa in 6-OHDA lesion mice are linked to persisting abnormal expression of this transcription factor.

We hypothesize that the sensitization of dopamine receptors produced by the loss of dopamine in the dorsal striatum in PD is involved in the development of psychiatric complications like DDS. While it is well established that the ventral striatum plays a key role in mediating the reinforcing effects of drugs of abuse through its dopaminergic innervation (Ungless et al., 2001; Saal et al., 2003; Wise, 2004), transition from initial voluntary, to habitual and ultimately compulsive drug use occurs in parallel to a shift of the effects of the drugs from the ventral to the dorsal striatum (Everitt and Robbins, 2013). According to this process, environmental stimuli gain motivational power through their predictive association with drugs, a phenomenon often referred to as context-dependent sensitization. These mechanisms are facilitated during repeated administration of psychostimulants and involve changes in dopamine systems and signaling pathways which are similar to those seen in learning (Berke and Hyman, 2000).

Based on this, we propose that, in PD, the sensitization of dopamine receptors produced by the loss of neurotransmitter in the dorsal striatum would strengthen this shift by enhancing the responsiveness to L-Dopa, ultimately facilitating the acquisition of motor programs involved in addictive-like behaviors such as those observed in DDS.

5 CONCLUSION AND FUTURE PERSPECTIVES

Advancing new therapeutic strategies for managing the motor and non-motor complications induced by dopamine replacement therapy in PD relies on the understanding of underlying physiological mechanisms. The experimental models to answer this critical question are only partially reproducing the pathological mechanisms and symptoms observed in humans and their choice depends on the specific aspects of the disease under investigation.

The work presented in this thesis relied on the use of different models of PD based on neurotoxin-mediated degeneration of distinct dopaminergic structures to investigate treatment-related complications. Our studies have identified abnormalities in dopamine receptor transmission, which are involved in the development of motor and non-motor complications following administration of L-Dopa, which is still the most effective treatment for PD.

We found that in the unilateral 6-OHDA mouse and MPTP NHP model of PD, dyskinesia, a serious motor disorder caused by administration of L-Dopa, is associated with molecular changes linked to impaired autophagy. We also showed that a considerable proportion of the anti-dyskinetic action of rapamycin depends on its ability to promote autophagy via inhibition of mTORC1. These results suggest the use of autophagy-promoting agents in future research to investigate their potential impact on the management of dysfunctional dopamine transmission in dyskinesia. A number of these compounds are currently used for the treatment of cancer and metabolic disorders, thereby facilitating their repositioning as anti-dyskinetic drugs.

The bilateral partial 6-OHDA mouse model has been previously employed to reproduce multiple non-motor symptoms commonly associated with PD. The use of this model is complicated by high mortality, particularly among male mice. We established a protocol with several pre- and post-surgical interventions, which increased the survival rate and reduced the recovery time of the animals. Reduced mortality will facilitate the use of this model to study non-motor comorbidities, which represent an urgent clinical problem in the management of PD.

In the same model, we were able to replicate features of dopamine dysregulation syndrome, a neuropsychiatric complication induced by dopamine replacement therapy. We found that L-Dopa acquired addictive-like properties in dopamine-depleted mice and that this effect was mediated by abnormal dopamine transmission in the dorsal striatum. Interestingly, this

abnormality was associated with accumulation of the transcription factor ΔFosB in a specific population of striatal projection neurons. Future studies may establish a causal role for this specific signaling component in the etiology of DDS. Other studies may further characterize the rewarding effect conferred by the lesion on L-Dopa using additional behavioral paradigms such as operant self-administration. Finally, it will be important to investigate the impact of vulnerability factors conferring individual predisposition to develop L-Dopa-induced neuropsychiatric disorders.

Overall, these studies will help to clarify some of the most urgent issues related to the management of PD and will contribute to the advancement of more effective therapeutic approaches.

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