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Modulation of the 5-HT_3 Receptor as a Novel Anti-Dyskinetic Target in Parkinson's Disease

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Résumé

La L-3,4-dihydroxyphénylalanine (L-DOPA) est le traitement le plus efficace de la maladie de Parkinson. Cependant, avec une administration chronique de L-DOPA, les patients développent des complications motrices telles que les dyskinésies. Des études antérieures ont montré que le blocage des récepteurs type 3 de la sérotonine (5-HT_3) réduit les niveaux de dopamine dans les ganglions de la base, suggérant qu'il pourrait atténuer la libération de dopamine qui caractérise l'état dyskinétique. Ici, nous avons étudié les effets de l'ondansétron, un antagoniste hautement sélectif du récepteur 5-HT_3 à diminuer et à prévenir le développement des dyskinésies induites par L-DOPA chez le rat lésé a la 6-hydroxydopamine. Dans la première expérience, les rats sensibilisés avec L-DOPA pour induire des mouvements involontaires anormaux (AIMs), ont reçu L-DOPA en combinaison avec l'ondansétron ou un véhicule. Dans la seconde expérience, les doses efficaces d'ondansétron ont été administrées simultanément avec L-DOPA pendant 22 jours, et la sévérité des dyskinésies a été évaluée. Après 3 jours d'élimination, L-DOPA a été administré en aigu et la sévérité des dyskinésies évaluée. Nous avons trouvé que l'ondansétron 0,0001 mg/kg en combinaison avec L-DOPA, a significativement diminué la sévérité des dyskinésies par rapport à L-DOPA seul. Ondansétron 0,0001 mg/kg, administré en même temps que L-DOPA, a retardé le développement des dyskinésies. L'action anti-dyskinétique de l'ondansétron n'a pas compromis le bénéfice thérapeutique conféré par la L-DOPA. Ces résultats suggèrent que l'antagonisme des récepteurs 5-HT_3 est une stratégie thérapeutique potentiellement nouvelle et efficace pour soulager la sévérité et prévenir le développement des dyskinésies.

Mots-clés : maladie de Parkinson, dyskinésie, sérotonine, récepteur 5-HT_3, L-DOPA, 6-OHDA, rat

Abstract

L-3,4-dihydroxyphenylalanine (L-DOPA) is the most effective treatment for Parkinson's disease However, with chronic administration of L-DOPA, patients develop motor complications such as dyskinesia. Previous studies have shown that 5-HT_3 receptor blockade reduces dopamine levels within the basal ganglia, suggesting that it could mitigate the aberrant dopamine release that characterises the dyskinetic state. Here, we investigated the effects of the highly-selective 5-HT_3 antagonist ondansetron at diminishing the expression of established, and preventing the development of L-DOPA-induced dyskinesia in the 6-hydroxydopamine-lesioned rat. In the first set of experiments, rats were primed with L-DOPA to induce abnormal involuntary movements (AIMs), after which L-DOPA was administered, in combination with ondansetron or vehicle. The effect of ondansetron on L-DOPA anti-parkinsonian action was subsequently determined by the cylinder test. In the second set of experiments, rats were administered effective doses of ondansetron, started concurrently with L-DOPA for 22 days, during which dyskinesia severity was monitored. After a 3-day washout period, an acute challenge of L-DOPA was administered and AIMs severity was assessed. We found that acute challenges of ondansetron 0.0001 mg/kg in combination with L-DOPA, significantly diminished the severity of AIMs compared to L-DOPA alone. Ondansetron 0.0001 mg/kg, when started concurrently with L-DOPA, attenuated the priming process leading to the development of dyskinesia. The anti-dyskinetic action of ondansetron did not compromise the therapeutic benefit conferred by L-DOPA. These results suggest that 5-HT_3 receptor antagonism is a potentially new and effective therapeutic strategy to alleviate the severity, and prevent the development of dyskinesia.

Keywords: Parkinson's disease, dyskinesia, serotonin, 5-HT_3 receptor, L-DOPA, 6-OHDA, rat

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List of abbreviations

α-synuclein	alpha-synuclein
2-DG	2-deoxyglucose
5,7-DHT	5,7-dihydroxytryptamine
5-HT	serotonin
5-HT_{1A}	serotonin 1A receptor
5-HT_{2A}	serotonin 2A receptor
5-HT_3	serotonin 3 receptor
5-HIAA	5-hydroxyindoleacetic acid
5-HTP	5-hydroxytryptophan
6-OHDA	6-hydroxydopamine
8-OHDPAT	8-hydroxy-2-(di-n-propilamino) tetralin
AADC	aromatic acid decarboxylase
AIMs	abnormal involuntary movements
AIMS	Abnormal Involuntary Movement Scale
AL	axial limbs
ALO	axial limbs orolingual
ATP	adenosine triphosphate
ATP13A2	adenosine triphosphatase 13A2
BBB	blood brain barrier
BG	basal ganglia
cAMP	cyclic adenosine monophosphate
C_{max}	peak plasma concentration
CNS	central nervous system
Complex I	NADH:ubiquinone oxidoreductase
COMT	catechol-O-methyltransferase
DA	dopamine
DAT	dopamine transporter
DNA	deoxyribonucleic acid
DOPAC	3,4-dihydroxyphenylacetic acid
EIF4G1	eukaryotic translation initiation factor 4-gamma

ERK	extracellular signal-regulated kinase
EP	entopeduncular nucleus
FBXO7	F-box only protein 7
GABA	γ-amino butyric acid
GBA	glucocerebrosidase
GI	gastrointestinal
GPCR	G protein-coupled receptor
GWAS	genome-wide association studies
GPe	globus pallidus pars externa
GPi	globus pallidus pars interna
HD	Huntington's disease
HVA	homovanillic acid
L-DOPA	L-3,4-dihydroxyphenylalanine
LB	Lewy body
LC-MS/MS	liquid chromatography-tandem mass spectrometry
LN	Lewy neurite
LID	L-DOPA-induced dyskinesia
LRRK2	leucine-rich repeat kinase 2
MAO-B	monoamine oxidase-B
MAPT	microtubule-associated protein tau
mCPBG	1-(m-chlorophenyl)-biguanide
MFB	medial forebrain bundle
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MSN	medium spiny neuron
NMDA	*N*-methyl-D-aspartate
PD	Parkinson's disease
PINK1	PTEN-induced putative kinase 1
PK	pharmacokinetic
PLA2G6	phospholipase A2, group VI
PLTS	persistent low-threshold spiking
RBD	REM sleep behaviour disorder
REM	rapid eye movement

RN	raphe nucleus
RNA	ribonucleic acid
ROS	reactive oxygen species
SEM	standard error of the mean
SERT	serotonin transporter
SNARE	soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor
SNc	substantia nigra pars compacta
SNCA	alpha-synuclein gene
SNr	substantia nigra pars reticulata
STN	subthalamic nucleus
$t_{1/2}$	plasma half-life
t_{max}	time at maximal plasma levels
TH	tyrosine hydroxylase
UDysRS	Unified Dyskinesia Rating Scale
VMAT2	vesicular monoamine transporter type 2
VPS35	vacuolar protein sorting 35
VTA	ventral tegmental area

"You can't ever reach perfection, but you can believe in an asymptote toward which you are ceaselessly striving."

-Paul Kalanithi

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I. Introduction

1. General Introduction

Parkinson's Disease (PD) was initially described in *An Essay on Shaking Palsy* by the British physician James Parkinson in 1817 (1). However, it was only until 1861 that the French neurologist Jean-Martin Charcot, known as "the founder of modern neurology", coined the term "Parkinson's disease" and distinguished bradykinesia as a separate clinical feature of the illness (2). PD is one of the most common neurodegenerative disorders that affects nearly 1% of the population over 65 years of age (3, 4). PD can be defined by four cardinal motor features: tremor at rest, akinesia (or bradykinesia), rigidity and postural instability (5). In addition to these symptoms, many patients are also affected by non-motor symptoms including dementia, autonomic dysfunction, and sleep disorders (6). The onset of PD is gradual and clinical manifestations do not appear until there is a loss of approximately 40-60% of the dopamine (DA) neurons in the substantia nigra (SN) pars compacta (SNc) and about 80% of striatal nerve terminals (7-10). In more severe stages PD, neuronal loss spreads to outside the SNc to regions including the locus coeruleus, raphe nuclei (RN), olfactory bulb and cerebral cortex, and this widespread neurodegeneration may be responsible for the progression of non-motor symptoms of PD (11) (12). The pathological hallmark of the disease is the presence of intracellular proteinaceous inclusions, known as Lewy bodies (LBs), however, the role of LBs in the pathogenesis of PD is still unknown (13). Alpha-synuclein (α-synuclein) is a major component of LBs and recent studies demonstrate that specific α-synuclein conformations are directly toxic to neurons (14, 15) and can propagate via a "prion-like" mechanism of pathogenesis (16).

Currently, the most effective symptomatic drug for PD is the biochemical precursor to DA, L-3,4-dihydroxyphenylalanine (L-DOPA), which helps to relieve motor symptoms by restoring striatal DA levels. However, as L-DOPA is also converted into DA in the peripheral nervous system, chronic L-DOPA therapy results in adverse effects, notably debilitating involuntary movements, termed L-DOPA induced dyskinesia (LID) (17). Moreover, the longer the duration of treatment, the greater the number of PD patients that develop LID and approximately 80 to 90% of patients suffer from LID after 10 years of treatment (18, 19). Patients with advanced PD suffer from these erratic movements that cannot be adequately controlled with existing therapies (20), which underscores the urgent need to develop therapies that attenuate dyskinesia. In recent years, the understanding of neuronal mechanisms that

underlie the pathophysiology of LID has grown and has been associated with events including the pulsatile stimulation of dopaminergic receptors, downstream changes in proteins and genes and abnormalities in non-dopaminergic transmitter systems, which modify the activity of the basal ganglia (BG) circuitry (21).

2. Parkinson's Disease

2.1. Epidemiology of Parkinson's Disease

Epidemiological studies show that PD is an age-related disease with men at higher risk of developing the disease than women (3). In Canada, it is estimated that about 99,000 individuals are living with PD and by 2031, the number of is expected to increase by 65% to 163,700 (22). In addition, Canadians with PD tend to be older individuals with an average age of diagnosis of 66.2 years of age (22).

The global prevalence PD is estimated at between 18 to 300 per 100,000 individuals while the incidence of PD is between 10 and 50 cases per 1000,000 individuals per year, respectively (23, 24). Incidence of PD is heavily age-dependent, and onset is rare before 50 years of age until a sharp increase of incidence is observed after 60 years of age (25). The disease prevalence is estimated at 1% in subjects over 65 years of age and increases to 4.3% in those over the age of 85 (26). Most of the increase is attributed to the general trend of an increasingly ageing population (27). Despite the worldwide distribution of PD, incidence rates may vary among populations. The conflicting results between individuals studies may reflect differences in research methodologies, particularly in case definitions, diagnostic criteria, and the age distribution of the study population (28). A collaborative study in four European countries using similar case-finding methods and diagnostic criteria did not reveal any differences (29). In contrast, a meta-analysis of six studies found a lower prevalence in Africa than in Europe or North America (30), but no significant difference was reported between African Americans and Caucasians living in Mississippi (31). In addition, an autopsy study found that African Americans showed the same prevalence of incidental LB disease when compared with Caucasian populations (32). Similarly, lower prevalence rates have also been reported in some Asian countries (23, 33-35) but some studies have found similar estimates to Western countries (36, 37). Accordingly, differences in environmental exposure or interethnic distribution of

susceptibility genes may also contribute to the ethnic differences in estimates of PD prevalence and incidence (38, 39). Moreover, the variation in PD prevalence reported amongst studies may be related to differences in response rates, survival and case certainty rather than ethnic differences in PD prevalence (31, 38, 40, 41). Thus, the relative contribution of genetic or environmental variations to population differences in PD incidence is still unclear (26).

Some studies report a higher prevalence of PD in men than in women (42-45) with a male-to-female ratio of about 1.5 (46-48) but other studies found no significant differences in PD prevalence between men and women (29, 49, 50). Consistent with prevalence studies, prospective studies have reported a higher incidence of PD in men than in women (29, 42, 51-53). The neuroprotective effects of oestrogens in women and X-linked genetic factors may account for the higher risk of PD in men but their role is still controversial (54).

2.2. Aetiology of Parkinson's Disease

The aetiology of PD is poorly understood but considerable advances in sequencing technology, genetics (55) and clinical studies (20) have contributed to a greater comprehension on the pathogenic processes occurring in PD. The common view today is that PD is a multifactorial disease that arises from the combined effects of exposure to environmental risk factors, genetic susceptibility, and complex genetic-environmental interactions (26, 39). Ageing is the strongest risk factor of developing PD (56) and can be explained by the increasing failure of normal physiological and biochemical processes that lead to the increased vulnerability of DA neurons to toxic insult (57). Growing evidence suggests that impairments in the regulation of protein homeostasis including processes such as protein aggregation, intracellular protein and membrane trafficking and disruptions to the ubiquitin-proteasome and lysosome-autophagy are implicated in the pathogenesis of PD (58). In addition, it has been suggested that the genetics of PD are involved in aberrations in synaptic structure and function (58), which confirmed the importance of mitochondrial dysfunction in toxin models of PD (59).

2.3. Risk factors of Parkinson's disease

2.3.1. Non-genetic risk factors

2.3.1.1. Environmental hypothesis

Evidence linking exposure to agrochemicals, including pesticides and herbicides to an increased risk of PD has been postulated for many years (60, 61). In particular, it has been demonstrated that rotenone (62) and paraquat (63-65) cause nigral dopaminergic cell death in rodents. Furthermore, individuals exposed to pesticides had a 70% higher incidence of developing PD those not exposed (66). Additional environmental factors identified include industrialization, rural environment (67), use of well water (68), intake of various metals, etc. (69-71) but studies show conflicting outcomes (72). Although several studies report a positive association between environmental risk factors and PD, no factor has been consistently implicated as the sole causative agent (73). Similarly, the degree of pesticide exposure that may lead to PD is unknown.

2.3.1.2. Discovery of MPTP-induced Parkinson's disease

The discovery of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced parkinsonism (74) stimulated an interest in exposure to environmental risk factors for PD. MPTP causes the degeneration of nigrostriatal dopaminergic neurons with the loss of striatal dopamine in various species, including primates, cats and mice (75-80). In 1982, several young people developed an acute and severe form parkinsonism, due to MPTP produced during their illegal synthesis process of heroin (74, 81). MPTP is highly lipophilic, and can be metabolized into 1-methyl-4-phenylpyridinium (MPP+), the active toxic molecule, by brain monoamine oxidase B (MAO-B) (75-80, 82). MPP+ is accumulated by high affinity DA transporters (DAT) into the mitochondria of dopaminergic neurons (83). Once inside the mitochondria, MPP+ binds to and inhibits NADH-ubiquinone oxidoreductase I (complex I) of the mitochondrial electron transport chain (84). This results in an impairment of ATP production, elevated intracellular calcium concentration and free radical generation (81). Accumulation of MPP+ in dopaminergic neurons causes neurodegeneration via reactive oxygen species-mediated oxidative stress and results in DA neuron death (85-87). MPTP-induced parkinsonism exhibits similar phenomena to PD, particularly the preferential loss of DA nerve terminals of the putamen and DA neuron

loss of the SNc. Primates exposed to MPTP are responsive to L-DOPA treatment, and develop motor complications after chronic administration (88). Despite the contributions of the MPTP-lesioned animal models to the knowledge of pathways implicated in PD pathogenesis, they do not fully capture all the features of the disease. For example, MPTP-induced parkinsonism is not progressive, an acute rather than chronic increase in α-synuclein occurs and LB formation is absent (57, 64, 89). In addition, the investigation of agents based on MPTP models in clinical trials has not been successful thus far. Thus, the underlying pathways in the MPTP models of PD may not all be shared with those in PD patients (64).

A recent systematic review of meta-analyses identified several environmental factors that are associated with a risk of developing PD (90). Two factors, physical activity and constipation, presented with convincing evidence for a strong association with PD. Several cohort studies support the protective effect of physical activity for PD (91, 92). Also, constipation may be an early premorbid manifestation of PD (93) and laboratory studies and laboratory studies reported an abnormal deposition of α-synuclein within the submucosal and myenteric plexuses of the enteric nervous system (94, 95). Highly significant association for increased PD risk included head injury (96), anxiety or depression (97), while decreased risk is associated with smoking (97) and high uric acid levels (98). Additional non-genetic risk factors significantly associated with development of PD include a decrease in risk with alcohol (99) and coffee consumption (100), whereas pesticides (101), well water (97), and male sex (102, 103) are linked to an increase in risk. Although substantial evidence supports the association of environmental risk factors and PD, the heterogeneity amongst the examined meta-analyses suggests that some associations may reflect reverse causation, residual confounding, information bias and sponsor conflicts. In addition, the variation in cohort studies and case-control studies, differences in exposure assessment (frequency and exposure types) may account for the biased estimates of association. Furthermore, the authors emphasize that mechanisms of several putative risk factors are poorly understood, and additional studies are required to clarify the association between these factors and the risk of developing PD.

2.3.2. Genetic risk factors

Clinical observation of increased prevalence of PD amongst relatives of patients (104, 105) and the discovery of families with genetic forms of parkinsonism (106-109) heightened

interest in the heritability of PD. However, familial aggregation does not necessarily imply genetic causation (110, 111) and a large PD twin study found no increased concordance for PD amongst monozygotic twins (112). However, for subjects with an onset before 50 years of age, a significant concordance rate in identical twins was identified, which suggests young onset PD has a greater genetic component. In contrast, a later twin study using clinical assessment and fluoro-dopa positron emission tomography (PET) to image dopaminergic function reported increased concordance amongst monozygotic twins (113), which supports a role of genetics in PD aetiology.

Significant advances in understanding the pathogenic processes of PD in the past few decades have been made due to the identification of genetic mutations and chromosomal loci associated with parkinsonism (Table I, page 9) (114-116). The majority of PD cases are sporadic (117) but Mendelian loci and the high-risk glucocerebrosidase (GBA) locus collectively account for approximately 10 – 40% of disease risk depending on the population under study (114). Genetic factors that have been identified include mutations in the genes for α-synuclein (SNCA), and leucine-rich repeat kinase 2 (LRRK2*)*, which are responsible for autosomal-dominant PD forms, whereas mutations in the genes PARK2 (Parkin), PARK7 (PTEN-induced putative kinase 1, PINK1), PARK7 (DJ-1), and PARK9 (ATPase 13A2, ATP13A2) account for early-onset autosomal recessive PD forms (117). Recently, two other autosomal dominant PD genes, vacuolar protein sorting 35 (VPS35) and eukaryotic translation initiation factor 4-gamma (EIF4G1), have been identified (118-120). Early candidate gene studies and subsequent meta-analyses provided conclusive evidence demonstrating that polymorphisms in SNCA (121), LRRK2 (122), microtubule-associated protein tau (MAPT) (123) and glucocerebrosidase (GBA) (124) impact PD susceptibility.

High-density arrays of single nucleotide polymorphisms identified genetic susceptibility factors in genome-wide association studies (GWAS), where the frequencies of putative risk alleles are compared in patients and controls (125). Genetic variations may be susceptibility factors or disease modifiers, affecting penetrance, age at onset, severity, and progression (126). The most commonly studied candidate genes include genes involved in DA metabolism, mitochondrial metabolism, detoxification, other neurodegenerative diseases and familial PD (3, 127) and genes associated with putative risk factors for PD including oestrogen receptor gene polymorphisms (128), the tau HI haplotype (129) and the apolipoprotein E epsilon 2 allele (130).

Although the significance of many loci identified with an increase in PD risk is still unknown, they account for a population attributable risk of > 60% (131).

2.3.2.1. Risk Loci

2.3.2.1.1. GBA

The GBA gene encodes a lysosomal enzyme β-glucocerebroside, which is involved in glycolipid metabolism (116). Homozygous GBA mutations cause Gaucher's disease, an autosomal recessive lysosomal storage disease caused by accumulation of glucocerebrosides (132). In contrast, heterozygous mutations in GBA are associated with a higher risk of PD where approximately 5-10% of PD patients have GBA mutations as opposed to an estimated frequency of 1% in healthy controls but this may be underestimated in certain populations (133-137). Carriers of only one mutated allele have a 5-fold increased risk to develop PD compared with non-carriers, which makes GBA one of the strongest genetic risk factors reported to date (134). The high prevalence of PD amongst GBA mutation carriers, which is also age-dependent and rises up to 30% at 80 years of age (138), has led to the suggestion that GBA mutations can act as dominant factors with reduced penetrance rather than simple risk variants (139). The mechanism underlying the association of mutations in GBA with the development of PD and other LB disorders (140) is not known, but may be caused by alterations in lipid metabolism or autophagy and lysosomal function (115). In fact, the mechanism of pathogenicity may be linked to α-synuclein as intracellular glucocerebrosides facilitate the aggregation of α-synuclein into toxic oligomers and fibrils (141), which are the main constituent of LBs (142). Moreover, α-synuclein is primarily degraded through autophagy and GBA mutations interfere with autophagic clearance of α-synuclein fibrils (143, 144). Consequently, fibrils are likely to accumulate in the cell (145, 146), following which they may propagate through cell to cell transmission (147, 148).

Table I: Genes and loci associated with Parkinson's Disease

locus	gene	inheritance	clinical phenotype	pathology
Mendelian Genes				
PARK1 and PARK4	SNCA	AD	parkinsonism with common dementia	LBs
PARK2	Parkin	AR	early-onset, slowly progressing parkinsonism	LBs rarely
PARK3	unknown	AD	late-onset parkinsonism	LBs
PARK5	UCH-L1	AD	late-onset parkinsonism	unknown
PARK6	PINK1	AR	early-onset, slowly progressing parkinsonism	one case with LBs
PARK7	DJ-1	AR	early-onset parkinsonism	unknown
PARK8	LRRK2	AD	late-onset parkinsonism	usually LBs; sometimes tangles or neither
PARK9	ATP13A2	AR	early-onset parkinsonism with Kufor-Rakeb syndrome	unknown
PARK10	unknown	AD	unclear	unknown
PARK11	unknown	AD	late-onset Parkinsonism	unknown
PARK12	unknown	unknown	unclear	unknown
PARK13	HTRA2	unknown	unclear	unknown
PARK 14	PLA2G6	AR	aggressive and complex parkinsonism with pyramidal features	LBs
High-risk locus				
Gaucher's locus	GBA		late-onset parkinsonism	LBs
	SNCA		typical PD	LBs
Low-risk loci				
	LRRK2		typical PD	LBs
FTDP-17	MAPT		dementia, sometimes parkinsonism	neurofibrillary tangles

Adapted from (114-116).

2.3.2.1.2. MAPT

Most of the gene loci discovered through GWAS are present in more than 5% of the population (allele frequencies of > 10%) and carriers of the risk allele have a less than two-fold increase of disease risk over the general population average (114). The majority of these low-risk loci appear to mediate their effect by altering gene expression rather than through translational changes. The MAPT is a protein that can form aggregates similar to α-synuclein and beta-amyloid. Mutations in the MAPT gene cause a range of neurodegenerative phenotypes but some can lead to a typical PD presentation (149). The H1 haplotype at the MAPT locus has been consistently suggested as a risk factor for PD (150, 151) and gene duplications at MAPT cause frontotemporal dementia (152), which suggests that the pathogenic cascades in the tauopathies can provoke severe neurodegeneration leading to parkinsonism (153, 154). Moreover, MAPT promotes the formation of α-synuclein oligomers and fibrils (115) and in transgenic mice that exhibit the LB variant of Alzheimer's disease, cognitive decline is accelerated and associated with amyloid beta, tau and α-synuclein pathologies compared to age-matched control animals (155). Thus, synergistic interactions between α-synuclein and tau may promote their fibrillization and the formation of pathological inclusions characteristic of neurodegenerative diseases.

2.3.2.2. Autosomal dominant forms of Parkinson's disease

2.3.2.2.1. LRRK2

The most common cause of autosomal-dominant Mendelian form of PD is mutations in the LRRK2 at the PARK8 locus, which account for nearly 10% of all familial dominant inherited forms (156). The G2019S kinase domain mutation is the most frequent LRRK2 mutation (157), and responsible for 5-40% of sporadic or dominantly inherited PD, depending on the population studied (158-160). Higher G20119S prevalence rates have been reported in more isolated populations, such as the Ashkenazi Jewish (161) and North African Berber Arab (162) populations, which can be explained by a genetic founder effect (163). Patients with LRRK2 mutations tend to display late-onset PD with symptoms indistinguishable from those of sporadic PD, even though LB pathology is sometimes absent or lacking (164, 165). Thus, the disconnect between clinical manifestations of PD and the presence of LBs (166, 167) supports the theory

that inclusions may not be necessary for neurodegeneration and may instead be a consequence of PD (115). The mechanism underlying the neurodegeneration caused by LRRK2 mutations and its natural substrate are still unknown. However, cell culture studies suggest that neurotoxicity *in vitro* requires intact kinase activity (168, 169), prompting increased interest towards LRRK2 kinase inhibitors (170) as a potential neuroprotective strategy.

2.3.2.2.2. α-synuclein

SNCA mutations are the second most common cause of dominant PD (171) and various studies have reported a link between familial PD and duplications or triplications in the SNCA gene (172). The SNCA genes encodes α-synuclein, which accumulates in LBs predominantly within the brainstem. As LB pathology is also the dominant pathology observed in most cases of LRRK2-related PD, this suggests that SNCA and LRRK2 affect a common pathway that leads to α-synuclein aggregation (139). Moreover, gene triplication leads to earlier onset and faster progression of disease than duplication, which suggests a gene-dose effect between α-synuclein levels and disease severity (173). The link between α-synuclein expression levels and the appearance of PD is well-established across studies, and leads to the hypothesis that a gain-of-function by α-synuclein underlies pathogenesis of PD (115). In addition, recent *in vivo* evidence shows that it binds to and promotes assembly of soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complexes, which are required for the fusion of vesicles to the pre-synaptic membrane (174, 175). Triple knock-out mice lacking α-synuclein also exhibit deficits in SNARE complex assembly and develop accelerated age-associated motor impairments and early-onset mortality, but do not show neurodegeneration (168, 174).

2.3.2.2.3. VPS35

VPS35 gene encodes a major component of the retromer complex involved in endosomal trafficking to the trans Golgi (119, 176). Recent studies have identified a single missense (D620N) mutation in VPS35 as a new cause of autosomal-dominant PD in two independent exome sequencing studies on Swiss (118) and Austrian families (119). Frequency of mutation carriers is low and has been estimated to represent about 0.1% of the PD population (177). Patients with a VPS35 mutation exhibit classical late-onset, L-DOPA responsive parkinsonism similar to that of sporadic PD, with a slightly earlier age at onset (139). Specific deletion

of VPS35 in DA neurons of mice results in PD-like deficits, including loss of DA neurons and accumulation of α-synuclein and early degeneration at 2-3 months of age (178). Consistent with this data, overexpression of human D620N VPS35 variants induce the marked degeneration of SNc DA neurons and axonal pathology (179). In addition, mutations in VPS35 caused extensive mitochondrial fragmentation and cell death as well as functional deficits *in vitro*, in mouse SNc neurons *in vivo*, and in human fibroblasts from PD patient bearing the D620N mutation (180). Defects in macroautophagy, aminomethylphosphonic acid (AMPA) receptor trafficking to dendritic spines or alterations in mitochondrial dynamics and turnover have been proposed as the mechanism underlying VPS35-induced neurodegeneration (181). Although the mode of action by which it causes PD is unclear, modulation of the development of DA neurons via the wingless-related integration site pathway (182, 183) and aberrant brain iron accumulation (184, 185) have been suggested as possible mechanisms. Furthermore, recent studies demonstrate that VPS35 may interact with other PD-linked gene products including LRRK2, SNCA and Parkin (186-190) in a common pathway that leads to the neurodegeneration observed in PD.

2.3.2.3. Autosomal recessive forms of Parkinson's disease

2.3.2.3.1. Parkin, PINK1, DJ-1

Mutations in Parkin are the most common cause of autosomal recessive forms of PD, whereas mutations in PINK1 and DJ-1 are relatively less prevalent (139). Parkin gene mutations account for almost 50% of early-onset recessive familial PD and up to 15% of early onset sporadic cases (191, 192). Pathology underlying Parkin-related PD does not tend to show LBs, unlike the autosomal dominant and idiopathic forms. Clinical manifestation of Parkin mutations is often indistinguishable from that of the sporadic disease except for the earlier age at onset (generally before 45 years of age) (139). Wild-type Parkin, PINK1 and DJ-1 are involved in processes of mitochondrial quality control and regulation such as mitogenesis, mitophagy and mitochondrial homeostasis and transport (139, 163). Studies suggest that the function of these proteins in a mitochondrial quality control pathway is impaired in PD, leading to the accumulation of bioenergetically compromised mitochondria, however, it is unclear how this might give rise to substantial nigral degeneration and PD (193).

2.3.2.3.2. ATP13A2, FBXO7 and PLA2G6

More rarely, recessively inherited forms of atypical parkinsonism are caused by mutations in the ATP13A2, F-box only protein 7 (FBXO7) and phospholipase A2, group VI (PLA2G6) genes (131). Mutations in ATP13A2 were first identified from families with Kufor-Rakeb syndrome, a rare hereditary disease with typical signs of PD that also includes symptoms of more extensive neurodegeneration (194). Loss of function mutations of ATP13A2 underlie an autosomal recessive form of early-onset parkinsonism with pyramidal degeneration and dementia (194) while heterozygous mutations may be a risk factor for PD (195). ATP13A2 mutations likely play a role in lysosome degradation (131) and recent studies demonstrate that ATP13A2 can rescue against α-synuclein toxicity in a yeast, *Caenorhabditis elegans* (*C. elegans*) and neuronal culture model of PD (196). FBXO7 mutations cause early-onset autosomal recessive parkinsonism with pyramidal signs and after an initial favourable response to L-DOPA, patients often develop dyskinesia (197). Most of the reported FBXO7 mutations are loss of function (198) but no neuropathology has been described thus far. Mutations in PLA2G6 cause an early-onset recessive degenerative disorder characterized by spasticity, ataxia and dystonia but adult onset forms can manifest as dystonia-predominant parkinsonism (199) that is responsive to L-DOPA (200). PD associated with PLA2G6 is caused by the homozygous or compound heterozygous inheritance of various missense mutations (201-203).

The clinical and sometimes pathological resemblance of genetic PD to sporadic disease make it a suitable human model to identify at-risk individuals in earlier and possibly prodromal phases of the disease (116). However, monogenic causes of PD represent less than 10% of PD cases in most populations (204) whereas the majority of cases seem to arise from complex interactions among genes and between genes and environmental factors (39). Thus, environmental factors appear to be more important determinants than ethnic and genetic factors in the aetiology of PD. Further efforts are warranted to understand how genetic causes and risk factors of PD play a role in the underlying pathophysiology in hopes of developing targeted therapies that alter disease course (139).

2.4. Pathophysiology of Parkinson's disease

Based on autopsy findings in PD patients, Braak and colleagues postulated that α-synuclein aggregates form in the periphery in early stages of PD before α-synuclein aggregation in the brain (205) and also propose a six-stage system for PD based on the stereotypic pattern of α-synuclein spreading (206). The Braak model is based on the presence of LBs and Lewy neurites (LNs) where the pathogenic process begins in the lower brainstem in the dorsal motor nucleus of the vagus nerve and anterior olfactory structures (206). The disease then spreads rostrally from the dorsal motor nucleus of the vagus nerve through the medulla, pontine tegmentum, midbrain and basal forebrain before ultimately reaching the cerebral cortex. This process follows a specific pattern where susceptible regions are affected in a predictable topographic sequence (207) where severity of the lesions and the clinical manifestations of the disease increase as the pathology ascends from the brainstem (208). Accordingly, *in vitro* (209, 210), *in vivo* (211, 212) and clinical evidence (213, 214) suggest that cell types in the central nervous system (CNS) exhibit a propensity for developing Lewy pathology that shares common features. In spite of the support for Braak's hypothesis, there is criticism around whether it accurately reflects the development of PD in all patients as studies report that Braak staging fails to describe the disease progression in upwards of 50% of α-synuclein immunoreactive cases (215-217). Moreover, the absence of information on the loss of neurons and synaptic connections in the original Braak papers has been the subject of scrutiny (218, 219) as the scientific premises underlying the model remain unclear (220). Thus, the inconsistencies between the Braak model and conflicting reports of the spread of pathology require further study to determine the relationship and likely also require a deeper understanding of the mechanisms underlying the role of α-synuclein in disease progression (207).

Several lines of evidence have implicated dysfunctions in the ubiquitin-proteasome system in PD pathogensis (221-223), which have been further supported by the identification of disease-causing mutations in genes encoding proteins involved in protein degradation in PD (224). Impairment of ubiquitination pathways and proteasomal function could result in defects in the clearance of toxic aggregates and result in their accumulation and degeneration of DA neurons (223, 225). Although systemic administration of proteasomal inhibitors modelled a behavioural and pathological phenotype reminiscent of PD (226), this model has been met with

great scrutiny due to the extensive variability in the consequences of *in vivo* proteasomal inhibition (227). Moreover, questions on the molecular connections between these systems and pathogenesis of PD remain, including the divergent fate of misfolded proteins for degradation or inclusion formation, and further studies, that likely exploit advances in genetics and technologies (228), are warranted to clarify this relationship.

2.4.1. Lewy bodies in Parkinson's Disease

Idiopathic or sporadic PD is characterized by the selective loss of neurons and appearance of abnormal cytoplasmic proteinacious aggregates called LBs in the soma or LNs in the processes in DA neurons (173). Immunohistochemistry shows that LBs consist primarily of the protein α-synuclein (229), as well as other proteins such as ubiquitin (230) and parkin co-regulated gene (231). Studies have suggested that misfolded α-synuclein and the deposition of LBs within midbrain neurons could contribute to neuronal damage and cell death (232). In addition to SNc DA neurons, neuronal loss and Lewy pathology also occurs extensively in locus coerulus noradrenergic neurons, RN serotonergic neurons, enteric DA neurons, post-ganglionic sympathetic noradrenergic neurons and olfactory neurons (11, 12).

2.4.2. Alpha-synuclein in Parkinson's disease

α-synuclein is of the main constituents of LBs and LNs, and accumulates widely in central and peripheral neurons of PD patients (233). Given it predominates in pre-synaptic terminals and the nuclear envelope, it plays a role in SNARE-mediated exocytosis and synaptic vesicle transport (234). Moreover, α-synuclein is present in mitochondria in PD brain and may affect mitochondrial function both *in vitro* and *in vivo*, possibly leading to the vulnerability of nigrostriatal DA neurons in PD (235-238). Accordingly, it has been demonstrated that α-synuclein aggregation may be associated with oxidative or nitrosative stress (239-241), which may be important in the pathogenesis in neurodegenerative disorders with LBs, like PD (242). Converging evidence also supports the hypothesis that α-synuclein oligomers (243, 244) and fibrils (245, 246), the pathologic form of α-synuclein, may participate in the propagation of neurodegeneration observed in PD (247). Thus, the misfolded α-synuclein fibrils present in LBs (142) and their self-propagation and spread, reminiscent of a "prion-like" process, suggest that their mode of cell-to-cell transmission is not in disagreement with the Braak staging and a

possible peripheral origin of Lewy pathology (210, 211, 248-250). Furthermore, the prion hypothesis of α-synuclein transmission is supported by evidence from transgenic mouse models of synucleinopathy and viral vector-mediated α-synuclein overexpression in rats (251, 252) and nonhuman primates (253, 254). However, no data from transgenic mice models has reported the spontaneous pathological α-synuclein (255) and conflicting findings surround the specificity of peripheral α-synuclein for PD in humans (255), for instance, some studies have found similar levels of α-synuclein accumulation in the colon of patients with PD compared with healthy controls (256-258).

2.4.3. Oxidative stress in Parkinson's disease

Oxidative stress defines a disequilibrium between the levels of reactive oxygen species (ROS) produced and the ability of a biological system to detoxify the reactive intermediates, ultimately creating a perilous state contributing to cellular damage (259). An increasing body of evidence suggests that in PD, oxidative stress and mitochondrial damage contribute to a sequence of events that lead to the degeneration of DA neurons in the SNc (57, 260-262). In addition to mitochondrial dysfunction, DA metabolism (263), neuroinflammation (259), iron (264), calcium (265) and ageing (266) also contribute to ROS production in the PD brain. Indeed, *post-mortem* brain analyses consistently show increased oxidative damage to lipids (267, 268), proteins (269), deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) (270, 271). Further support for the link between oxidative stress and DA neuronal degeneration has been demonstrated by modelling motor symptoms of PD in toxin-induced animal models that cause oxidative stress such as 6-hydroxydopamine (6-OHDA), MPTP, rotenone and paraquat (272). It has also been suggested that mechanisms that contribute to neurodegeneration act in a feed forward manner where primary insults lead to oxidative stress, which damages key cellular proteins and disrupts lipid membranes that in turn cause more ROS production (259).

2.4.4. Mitochondrial dysfunction in Parkinson's Disease

In addition to the dual role of mitochondria as both a source and target of ROS (273-275), compelling evidence suggests that mitochondrial dysregulation is critical in the pathogenesis of PD (259). Mitochondria are dynamic organelles with important functions in cellular respiration, energy metabolism, calcium homeostasis, stress response and apoptosis

pathways (260). Several groups have reported decreased complex I activity in the SN of PD patients (276-278), and the finding of the downregulation of genes encoding mitochondrial proteins further supports the role of mitochondrial dysfunction in PD (279). In addition, PD-related proteins, including DJ-1, PINK1, Parkin, α-synuclein and LRRK2, are also involved in mitochondria quality control leading to exacerbations of ROS generation and susceptibility to oxidative damage (259).

2.5. Dopaminergic system in Parkinson's disease

DA neurons form four major systems within the mammalian brain: the nigrostriatal, mesolimbic, mesocortical and tuberoinfundibular systems (280) that originate from the A9, A10, and A8 groups of dopamine-containing cells, respectively (281, 282). In the nigrostriatal pathway, projections from dopaminergic neurons with cell bodies in the SN terminate in the striatum (283). The mesolimbic pathway consists of dopaminergic neurons that originate in the ventral tegmental area (VTA) and project to the nucleus accumbens and related limbic regions, whereas VTA neurons that project to the prefrontal cortex establish the mesocortical pathway (284). Last, the tuberohypophyseal pathway consists of dopaminergic projections from the hypothalamus to the pituitary gland, and its secretions regulate prolactin (285). Due to their wide connectivity within distinct pathways, DA neurons exert a variety of functions including locomotion, addiction, reward, learning and memory, cognition, stress and movement (286).

DA is a monoamine neurotransmitter synthesized in a series of enzymatic reactions (287), beginning with the conversion of the amino acid tyrosine into L-DOPA via the rate-limiting enzyme tyrosine hydroxylase (TH) (Figure 1, page 19). L-DOPA is subsequently decarboxylated into DA by the enzyme aromatic acid decarboxylase (AADC). DA is then packaged into pre-synaptic vesicles by the vesicular monoamine transporter type 2 (VMAT2) and released at nerve terminals into the synapse upon stimulation. Released DA bind to DA receptors to elicit a response in the post-synaptic cell and this interaction is important in the modulation of motor function through the BG circuitry. Extracellular DA is either metabolized by MAO-B and catechol-O-methyl transferase (COMT) in the cytosol or transported back into the pre-synaptic terminal via the DAT. Following re-entry of DA into the pre-synaptic neuron, DA can be repackaged into vesicles and recycled or degraded into the metabolite homovanillic acid (HVA).

A deficit in the number of nigrostriatal dopaminergic neurons, characteristic of PD, disrupts the dopaminergic transmission and produces abnormal motor features in affected subjects.

2.5.1. Nigrostriatal dopaminergic pathway

Dopaminergic terminals in the striatum consist of dense innervation of fibres from two specific groups of neurons in the brainstem (282, 288) . The first group are neurons with cell bodies in the VTA that project to the nucleus accumbens and olfactory tubercle. The second group have cell bodies in the SNc and project primarily to the putamen and the caudate nucleus. The tegmental and nigral afferents form the nigrostriatal dopaminergic pathways.

2.5.2. Classification of dopamine receptors and their distribution in the basal ganglia

DA receptors are a family of G protein-coupled receptors (GPCRs) with five subtypes, D1-D5, that are divided into two groups (289). D1-like receptors are comprised of D1 and D5 receptors and mainly couple G proteins (290), which stimulate adenylyl cyclase and cyclic adenosine monophosphate (cAMP) production (291, 292). In contrast, D2-like receptors comprise D2, D3 and D4 receptors; they couple with $G_{\alpha i}$ /$G_{\alpha o}$ and inhibit adenylyl cyclase and negatively regulate cAMP production (293, 294). D1-like receptors have an excitatory effect by stimulating cAMP production, whereas activation of D2-like receptors is inhibitory. D2 receptors are the presynaptic receptors of the dopaminergic system and are responsible for the negative feedback on levels of synaptic DA (295).

Both D1Rs and D2Rs are highly expressed by striatal medium spiny neurons (MSNs) (296, 297) and are present at lower levels in the cortex as compared to the striatum (298). D1 receptors are expressed in striatonigral neurons containing substance P and dynorphin that project to the SN pars reticulata (SNr) and to the globus pallidus (GP) pars interna (GPi), which constitute the direct striatal output pathway (299). In contrast, D2 receptors are predominantly localized in striatofugal neurons expressing encephalin, which project to the GP pars externa (GPe), constituting the indirect pathway (300, 301). D1 receptors are post-synaptic whereas D2 receptors are also localized on pre-synaptic nigrostriatal dopaminergic terminals, on SNc neurons, and on pre-synaptic cortico-striatal terminals where they can inhibit striatal glutamate

release (296, 302, 303). In humans and nonhuman primates, D3 receptors are mostly found in the nucleus accumbens and caudate nucleus-putamen complex but are also localized in the GPi, anterior thalamus, amygdala, hippocampus and cortex (304-306). In the human striatum, there is approximately D3:D2 receptors is approximately 1:2, and D3 receptors can co-localize with both D1 and D2 receptors (305, 307).

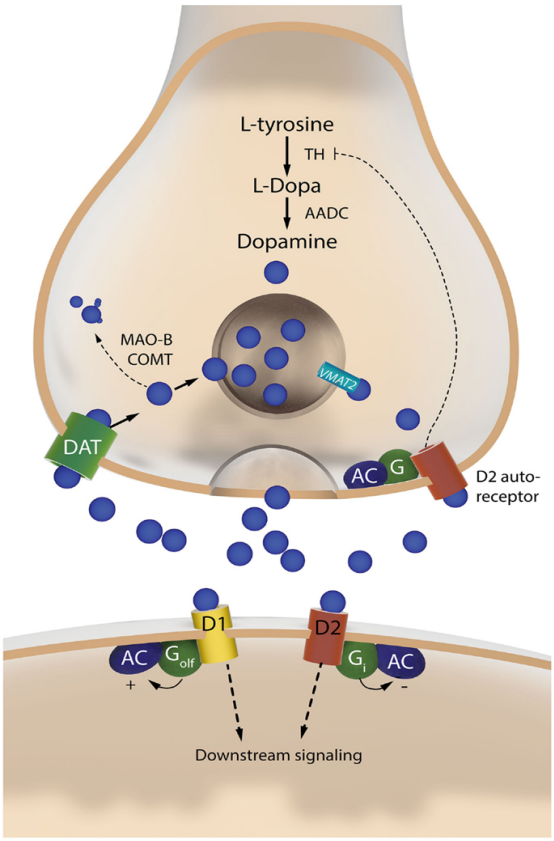


Figure 1: Dopaminergic synapse. After DA is synthesized in the pre-synaptic neuron and released into the nerve terminal, extracellular levels of DA are regulated through several mechanisms. DAT is responsible for the reuptake of DA back into the pre-synaptic neuron, VMAT2 packages DA back into synaptic vesicles, pre-synaptic D2 receptors control DA synthesis and release, and MAO-B and COMT are involved in the extracellular metabolism of DA. Following the release of DA at synaptic terminals, DA can bind to two types of DA receptors on post-synaptic neurons. The D1 receptor is coupled to Golf and activates cAMP-dependent signalling pathways while the D2 receptor is coupled to Gi and inhibits the same pathways. AADC: aromatic L-amino acid decarboxylase; AC: adenylate cyclase; COMT: catechol-o-methyl-transferase; DAT: dopamine transporter; MAO-B: monoamine oxidase B; TH: tyrosine hydroxylase; VMAT2: vesicular monoamine transporter 2. Modified from (308).

2.6. Clinical features of Parkinson's disease

Traditionally described as a motor disorder, numerous brain structures are affected at different time points along the course of the disease manifestation, and both motor and nonmotor symptoms are observed in PD.

2.6.1. Motor symptoms of Parkinson's disease

The four cardinal motor features of PD are the following: bradykinesia, muscular rigidity, resting tremor and impairment of postural balance leading to disturbances in gait and falls. Movement can be normal in early disease (309) due to the redundancy in BG activity and the capacity of the striatum to compensate functionally for lower degrees of DA deficiency (7). However, after the loss of approximately 80% striatal DA and loss 60% SNc DA neurons, motor symptoms begin to appear (7-10). Initially, the symptoms are mild and are usually confined to one side of the body but over the disease course, symptoms are increasingly impairing and involve the contralateral side as well (310). Motor features are heterogeneous and in spite of the lack of consensus on the classification of subtypes, empirical clinical observations suggest the existence of two major subtypes: tremor-dominant PD with a relative absence of other motor symptoms and non-tremor-dominant PD, a phenotype described as akinetic-rigid syndrome and postural instability gait disorder (311). Bradykinesia is defined as difficulty in planning, initiating and executing movements, as well as with performing sequential and simultaneous tasks (312). This often manifests as deficits in fine motor control (312), slower reaction times(313, 314) and slowness in performing daily activities (315-318). Moreover, bradykinesia leads to impairment of the power of voluntary movement (319, 320). Muscle rigidity is described as increased resistance to passive joint movement, and can often lead to a flexed posture (321). The combination of these motor symptoms along with disturbances in gait, leads to greater disability in PD patients. In addition to motor symptoms, PD patients also experience debilitating nonmotor symptoms.

2.6.2. Non-motor symptoms of Parkinson's disease

The spectrum of non-motor features encompasses olfactory dysfunction, sleep disturbances, autonomic dysfunction, gastrointestinal (GI) distress, memory loss and dementia, as well as neuropsychiatric conditions (6, 322-324) (325, 326). Virtually all patients with PD

exhibit at least one nonmotor symptom with an average of 7.8–11.9 nonmotor symptoms per patient (322, 327-330). Moreover, nonmotor symptoms have been reported to affect the quality of life of PD patients to a greater extent than motor features (330-332).

Olfactory dysfunction is of one of the most common nonmotor symptoms in PD that affects over 80% of patients with PD (333), a prevalence with greater sensitivity for PD compared to many other clinical markers. Although robust evidence indicates that olfactory loss precedes PD (334-340), the lead time for olfactory loss is variable, and some patients may develop detectable loss before or after developing parkinsonism (309). In addition, strong evidence supports rapid eye movement (REM) sleep behaviour disorder (RBD) as a predictor of synucleinopathies, most commonly PD with or without dementia LBs (309). RBD is defined as apparent enactment of dreams during REM sleep, associated with a loss of normal REM sleep atonia (341). Five prospective studies have reported that synucleinopathies, *i.e.* PD, dementia with LBs or multiple system atrophy develop in up to 80-90% of patients with RBD (342-347). Moreover, one study showed that α-synuclein deposition was present in brains of 98% of subjects with neurodegenerative disease who also had RBD confirmed by polysomnography (348).

Additional symptoms relating to autonomic dysfunction include constipation, orthostatic hypotension, urinary and sexual dysfunction and somnolence. α-synuclein is abundant in the GI system (256), which led some to speculate that prion-like spreading might occur from the GI tract to the brain (207), and a delay in colon transit time that results in constipation could theoretically facilitate this spreading (309). Although this remains controversial, pathological evidence supports the capacity of α-synuclein to spread (349) and a preliminary report showed that vagotomy may reduce the risk of PD (350). Depression and anxiety are commonly comorbid in PD, however, their potential as a marker is limited by the low relative risks (351-355) and predictive value (309), and highly variable lead times for psychiatric manifestations (353-356). Cognitive impairment is traditionally associated to late stages of PD but mild cognitive changes are also observed in *de novo* PD (357). In general, nonmotor features precede the appearance of motor symptoms in PD, including olfactory dysfunction, RBD, constipation, urinary and sexual dysfunction, which show promise as potential markers of prodromal PD (358).

2.7. Current pharmacotherapy for Parkinson's disease

Currently available therapies are not disease-modifying or neuroprotective and they provide only symptomatic relief for motor features of the disease (359). There are non-pharmacological, pharmacological and surgical treatments that attempt to restore dopaminergic activity using L-DOPA and DA receptor agonists.

2.7.1. L-DOPA

Administration of L-DOPA with a peripheral AADC inhibitor such as carbidopa or benserazide is the most effective treatment for relief of motor symptoms of parkinsonism, particularly for controlling bradykinesia (360). The addition of carbidopa or benserazide enhances the therapeutic benefits of L-DOPA, reduces the dose of L-DOPA required, and minimize peripheral adverse effects (361).

2.7.1.1. Pharmacology of L-DOPA

DA was first synthesized in 1910 (362) and its biochemical precursor was synthesized the following year (363). In a seminal study, Ehringer and Hornykiewicz discovered that DA levels were reduced in the striataum of PD patients (364). Moreover, Hornykiewicz observed a correlation between most of the PD motor symptoms and striatal DA depletion (365). The introduction of DA replacement therapy with L-DOPA in the early 1960s revolutionized symptomatic treatment of PD (366). Unlike DA, L-DOPA crosses the blood brain barrier (BBB), and is effective at alleviating motor features of PD during early stages of treatment (367). Barbeau and colleagues reported an improvement of parkinsonism, mostly with respect to rigidity, after oral administration of L-DOPA to patients with PD (368). Furthermore, Cotzais and colleagues reported that high doses of L-DOPA had marked beneficial effect on the motor symptoms of parkinsonism, mostly bradykinesia and rigidity (369-371).

As AADC is also present outside of the brain, peripheral metabolism of L-DOPA can cause adverse side effects including hypotension, nausea and vomiting (372, 373). Thus, a peripheral acting AADC inhibitor, such as carbidopa or benserazide, that does not cross the BBB is often co-administered, to limit the peripheral decarboxylation of L-DOPA so that more L-DOPA is available to enter the brain, while minimizing the aforementioned peripheral adverse

effects. The addition of peripherally acting AADC inhibitors allowed a reduction in the required dose of L-DOPA up to 60-80% (374, 375), potentiated its efficacy, led to a faster onset of anti-parkinsonian benefit and a reduction of cardiovascular and GI side effects (371, 373, 376).

L-DOPA is absorbed in in the duodenum and proximal jejunum by active transport via the large neutral amino acid system (377, 378). L-DOPA enters the body and brain by active transport (379, 380) and competes with dietary proteins and amino acids (381). Thus, high protein intake can reduce L-DOPA anti-parkinsonian action and indeed, intraduodenal delivery of L-DOPA leads to a decline of motor performance following oral protein intake (382). In the clinic, L-DOPA has a short half-life of 1.5 to 2 hours (383-388). After oral administration, L-DOPA plasma levels reach a maximum about one hour after intake, although this may vary due to the unpredictable absorption (387-389). L-DOPA plasma levels are 10–15-fold higher than L-DOPA levels in the ventricular cerebrospinal fluid (390). COMT inhibitors are often used to extend the duration of L-DOPA anti-parkinsonian action and indeed, tolcapone, entacapone and opicapone increase the area under the curve when administered with L-DOPA (391, 392).

L-DOPA is converted into DA by AADC in DA neurons from the SN and projections (393), serotonin (5-HT) neurons from the raphe complex and their striatal projections (394, 395) and noradrenergic neurons from the locus coeruleus and their projections (396). Due to the characteristic degeneration of the nigrostriatal system in PD, L-DOPA is converted in DA mostly by raphe-striatal 5-HT neurons, and to a lesser extent, by striatal intrinsic AADC-containing interneurons (397-399).

Although L-DOPA is the most effective treatment for PD, chronic administration of L-DOPA is associated with the development of motor complications including motor fluctuations and LIDs (400). LID represent a major limitation of current pharmacotherapy for PD as the majority of patients experience dyskinesia after a few years of treatment (400, 401) and underscores the need to develop effective therapeutic strategies for patients suffering from these involuntary movements.

2.7.2. Dopamine agonists

DA receptor agonists such as ropinirole, pramipexole and rotigotine are commonly employed and their main advantages over L-DOPA are: they do not require enzymatic activation

and have a longer duration of action (402). However, due to their action on DA receptors, their adverse effect profile includes: hallucinations, confusion, nausea, and increased incidence of impulse control disorders including pathological gambling and hypersexuality (403). Apomorphine, a DA agonist, is primarily used as rescue therapy for temporary relief of off-periods of akinesia in patients with fluctuating response to dopaminergic therapy (404), but is not available in Canada.

2.7.3. MAO-B inhibitors

Selective MAO-B inhibitors like selegiline and rasagiline delay the breakdown of DA in the striatum (287). As their efficacy is modest, they can be used as a monotherapy in early PD and in advanced stages of the disease, they can be administered as an adjunct to reduce off time in patients with declining response to L-DOPA (405).

2.7.4. COMT inhibitors

COMT inhibitors block the peripheral degradation of LDOPA, which leads to its increased half-life and enhanced central bioavailability (406). Two COMT inhibitors are available in Canada, tolcapone and entacapone, while opicapone is also available in Europe, although entacapone is preferred because it is not associated with hepatotoxicity (407-409). They are used as adjunctive treatment in patients who develop motor fluctuations to prolong the effect of L-DOPA (410).

2.7.5. Anti-cholinergics and amantadine

Anti-cholinergic agents like trihexyphenidyl and benztropine were historically used for the treatment of PD before the introduction of L-DOPA. Their main therapeutic effect is on tremor and they are only indicated in early PD or as an adjunct to DA replacement therapy. Amantadine modulates dopaminergic and cholinergic transmissions and is also a non-competitive *N*-methyl-D-aspartate (NMDA) receptor antagonist with a modest efficacy and improves parkinsonian symptoms in mildly affected patients with early disease and reduces LIDs in patients with advanced disease.

2.7.6. Treatments for non-motor symptoms

Non-motor symptoms in PD are being increasingly recognized as important issues diminishing the quality of life of patients, although treatment options remain inadequate (6, 326). Thus, medications used to treat related conditions are usually tried in PD, *e.g.* antidepressants for depression, atypical antipsychotics for psychosis, laxatives for constipation, etc. However, several of the currently used interventions lack robust evidence and require further research to discern their role in the management of nonmotor symptoms of PD }(326).

2.8. Surgical interventions for Parkinson's disease

Surgical brain treatments are increasingly attractive options for patients with PD, particularly in advanced stages because they diminish motor fluctuations and decrease dyskinesia severity (411). Deep brain stimulation (DBS) is the most popular surgical intervention and aims to modulate abnormal neuronal activity within a circuit to alleviate symptoms (412). Several clinical trials have demonstrated that stimulation of the subthalamic nucleus (STN) or GPi is effective in moderate to severe cases of PD (413). Inasmuch as its invasiveness, this surgical intervention is considered a symptomatic treatment limited to patients with advanced PD who no longer achieve adequate symptomatic relief with medication (411). As a result, patients who undergo DBS are on average 58.6 years of age with an average disease duration of 12 years (414-417). The precise mechanism(s) underlying the action of DBS is still unclear but the commonly accepted hypothesis is that electrical stimulation of the brain exerts inhibitory effects on structures such as neuron cell bodies close to the current and the output nuclei of the BG (418, 419).

3. L-DOPA induced dyskinesia

3.1. Clinical characteristics of dyskinesia

Dyskinesia, originates from the Greek word (δυσκινησία) meaning "bad movement" and is medically defined as any nonvoluntary movement, and dyskinesia is clinically heterogeneous in presentation and progression (420). Typically, it firsts appear on the more severely parkinsonian side of the body (421) and affects the distal lower limb, followed by an ascending spread but with disease progression, both sides are ultimately affected (422).

LID develops with disease progression and with repeated DA replacement therapy in PD. Although administration of DA agonists can elicit dyskinesia, the prevalence is lower than L-DOPA monotherapy (423, 424). Thus, in attempt to minimize the induction and delay the onset of drug-induced dystonia and chorea, DA agonists may be used in early disease. Alternatively, once fluctuations and dyskinesia appear, DA agonists may allow to reduce the dose of L-DOPA to reduce existing dyskinesia in PD (420). In contrast, a concern with the addition of COMT inhibitors, which delay the breakdown of L-DOPA, is that L-DOPA will have a longer duration of action and may induce dyskinesia (425).

3.2. Timing of dyskinesia

LID expression is based on the timing of appearance in relation to the on-off phenomenon of the patient (420), which is defined as the switch between mobility and immobility in patients treated with L-DOPA (426). On-time refers to periods when the patient is responsive to L-DOPA and experiences improvement in mobility, whereas off-time applies to periods when the patient responds poorly to L-DOPA and impaired motor function including tremor, akinesia or rigidity, which often occurs as an end-of-dose or "wearing off" of the effect of L-DOPA, or because L-DOPA has not been taken (420). In particular, the on period is the most common time during which LID is present, *i.e.* in 70-80% of patients who experience it while being in the on-state (427). During the on period, dopaminergic stimulation in the patient's brain is maximal or increased. Moreover, LID may be categorized into different presentation forms where the most common forms are peak-dose dyskinesia, off-period dyskinesia and diphasic dyskinesia. These forms of LID likely lie somewhere along a clinical continuum and may vary between patients, and even fluctuate between different doses in the same patients. In general, LID appears first in the foot, ipsilaterally to the side of the body more severely affected by parkinsonian symptoms, with inversion of the foot and ankle. A possible explanation may be the early loss of dopaminergic innervation in the dorsolateral striatum, which corresponds somatotopically to the foot area innervated by the SNc (428). Over the course of disease progression, dyskinesia eventually spreads to other body areas, and may follow the pattern of progression of parkinsonian symptoms (429). In addition, the forms of dyskinesia are not mutually exclusive, and a combination of choreic and dystonic movements, and the three types of dyskinesia may be observed in patients throughout the L-DOPA cycle (430).

3.2.1. Peak dose dyskinesia

Peak-dose LID is the most common subtype that occurs at high plasma levels of L-DOPA and coincides with the maximal anti-parkinsonian effect. These are often expressed as spasmodic twitching or jerking in the muscles of the superior extremities and neck. They are predominantly characterized by a choreic phenotype but can also include dystonic movements (431). They tend to be less disabling and less painful than the other forms of dyskinesia.

3.2.2. Off-period dyskinesia

In contrast, off-period dyskinesia occurs when the DA levels are falling and dopaminergic stimulation in the brain is low. Thus, the patient is subsequently in the off state or in the transition from the on to off state. This phenomenon tends occurs more commonly during the night, or prior to the first L-DOPA dose in morning or just after taking the dose (432). Phenotypically, off-period dyskinesia mainly consists of dystonic movements (433) frequently affecting the foot of the more affected side, but can also be segmental or generalized in distribution (431). A characteristic manifestation includes foot inversion and painful flexion of the toes (421). This phenomenon can be combated by taking more dopaminergic medication, particularly longer acting DA agonists and controlled release L-DOPA to avoid a decrease in DA levels over night(434).

3.2.3. Diphasic dyskinesia

Diphasic dyskinesia, also described as dystonia-improvement-dystonia (D-I-D), occurs at two different time points of a single dose cycle, at the beginning and at the end of the treatment effect, separated by an on period of minimal dyskinesia (435). In general, it is assumed at the time points of dyskinesia, L-DOPA levels are rising and falling, respectively. They affect both extremities but tend to affect the lower limbs of the most affected side more (436). It is characterized by repetitive and rapidly alternating dystonia and consists of flexion/extension of the foot or stereotyped movements (437). A notable feature is that while the lower limbs are moving involuntarily, the upper body can exhibit parkinsonian symptoms such as tremor (438).

3.3. Risk factors for the induction of dyskinesia

3.3.1. Priming

Priming is defined as the neurochemical and functional aberrant modifications in the DA-denervated BG that eventually lead to the emergence of dyskinesia in response to the repeated administration of L-DOPA or DA agonists (439). Over time, with repeated treatment, the chance of dopaminergic stimulation eliciting LID is increased and once LID has been established, the severity of dyskinesia increases (440).

Priming is produced by a two-step administration of dopaminergic drugs, including DA agonists and L-DOPA, and, encompasses an induction and expression phase. During the induction phase, the first administration of dopaminergic drugs, followed by the second administration, results in priming, *i.e.* the neurochemical and behavioural sensitization of the animal to subsequent challenge with dopaminergic drugs (441). At the behavioural level, chronic dopaminergic treatment induces dyskinesia that, once established, never stops and progressively increases in severity with further treatment (442). In the expression phase, once LID is established, the brain maintains the primed state such that even in the absence of treatments, a single challenge with L-DOPA or DA agonist will elicit dyskinesia at nearly the same severity (440). In addition, priming is associated with neurochemical maladaptive modifications in the DA-denervated striatum similar to those observed in animal models of dyskinesia induced by chronic DA replacement therapy (443). These include changes in the production of cAMP, phosphorylation of phosphoprotein of 32 kDa, and expression of mRNA encoding immediate early genes, dynorphin and glutamic acid decarboxylase isoform 67, which all regulate the activity of striatal output neurons (443).

The major factors that affect the induction of dyskinetic movements are the extent of nigral dopaminergic cell loss, the type of drug administered, and the method of drug administration (444). Nigral cell degeneration is responsible for plastic changes in BG function that lead to dyskinesia priming (308). The extent of denervation regulates the level and duration of drug exposure required to induce dyskinesia. In general, normal nonhuman and human primates do not develop dyskinesia when treated with chronic pharmacological doses of L-DOPA (445). In contrast, humans or primates exposed to MPTP, which induces nigral cell loss,

and PD patients with severe nigral denervation develop dyskinesia rapidly after starting L-DOPA therapy (446).

The method of drug delivery is regarded as one factor that is important in the development of dyskinesia, in addition to other determinants including nigrostriatal dopamine denervation and dopamine receptor sensitivity. The greater propensity of L-DOPA to induce dyskinesia than DA agonists is attributed to more than differing duration of action and/or plasma half-life and pharmacology of receptors (447). Orally administered L-DOPA has a short half-life of 60-90 minutes (448) and due to its central and peripheral pharmacokinetic effects amongst many other factors, it is associated with a gradually shorter action duration with continued disease progression (449). In contrast, orally administered DA agonists have half-lives of several hours or more, notably cabergoline has a half-life of 72-96 hours (450, 451). Therefore, intermittent oral doses of L-DOPA with a shorter duration of action may be associated with pulsatile stimulation of dopaminergic receptors, whereas administration of longer-acting DA agonists results in the tonic and phasic release of DA and more continuous physiological dopaminergic stimulation, which results in a lower incidence of dyskinesia (452, 453).

The different pharmacological profile of L-DOPA compared to DA agonists may also underlie variations in dyskinesia induction. Orally-administered DA agonists display a higher affinity for D2 DA receptors compared to D1 receptors (454, 455), whereas L-DOPA interacts with all five subtypes of DA receptors present in the basal ganglia (456, 457). Evidence suggests that dyskinesia is associated with specific alterations in D1 receptor function and D2-selective DA agonists may induce less dyskinesia because of this pharmacological difference (458). In addition, several DA agonists also demonstrate affinity for non-dopaminergic receptors, for example, cabergoline also antagonizes 5-HT_{2A} receptors (451), which has been demonstrated to exert an anti-dyskinetic effect (459, 460). Thus, the relatively lower association of dyskinesia with DA agonists than L-DOPA may be due to their lack of selectivity for the D1 receptor and the broader pharmacology of L-DOPA (450).

Moreover, results from clinical trials have been conflicting on the association between continuous delivery of L-DOPA, such as intraduodenal L-DOPA infusion, also known as duodopa, and the risk of dyskinesia development (461). In two small trials, duodopa administration over a 12-h period daily over 6 months but not over a 14-h period daily over 18 months, reduced the severity of dyskinesia (462, 463). In contrast, a recent study reported no

effect of duodopa on dyskinesia severity (464). In addition, this method of continuous drug delivery still encompasses some pulsatility of post-synaptic dopaminergic receptors, and in the event of uninterrupted drug administration, there is the issue of tolerance (461). Therefore, while pulsatile L-DOPA may be implicated in the development of dyskinesia, compared to other factors, the extent of its contribution remains relatively unclear.

3.4. Risk factors for developing dyskinesia

Processes that underlie the induction of dyskinesia are different from those responsible for the execution of involuntary movements in response to subsequent drug treatment (439). The risk factors for LID include the age of PD onset, duration of L-DOPA treatment and dose, which suggests that the progressive loss of DA neurons and L-DOPA exposure are implicated in the development of LID (465-467). Cotzias and colleagues, who are credited with the first successful use of L-DOPA to treat PD, were also the first to report the incidence of LID (371). Studies subsequently noted the high incidence and variation of LID, and treatment-limiting effect of L-DOPA. Moreover, several studies reported that continuous rather than intermittent exposure to L-DOPA is associated with a decrease in the incidence of LID. Accordingly, continuous infusion of L-DOPA via an intestinal gel increased on time without dyskinesia compared to immediate-release oral formulation of L-DOPA (464).

3.4.1. Duration of L-DOPA therapy

The duration of L-DOPA therapy is considered an important risk factor for the development of LID. After five years of L-DOPA treatment, about 50% of patients are reported to have developed LID (423, 468, 469) and after 15 years, the incidence rises to more than 90% of patients suffering from dyskinesia (19). LID occurs more frequently with longer duration of L-DOPA treatment (470). However, it remains to be determined to what extent each of treatment duration, the effect of neurodegeneration, disease duration and dose contribute to the development and expression of LID (420). Thus, disease duration and severity, are also shown to correlate highly with the duration of therapy as well as the prevalence of dyskinesia.

3.4.2. Impact of L-DOPA dose

In general, L-DOPA does not typically induce dyskinesia in normal individuals (471, 472). In hemiparkinsonian rats, the dosage is critically involved in dyskinesia via loss of synaptic depotentiation (473). That L-DOPA induces dyskinesia and alleviates extrapyramidal symptoms is generally considered as a continuous dose-dependent pharmacological spectrum (474). A landmark paper by Ahlskog and Muenter looked at the epidemiology of LID in studies from the pre-L-DOPA era and from the modern area (468). The frequency of LID between prospective clinical trials and observational studies yielded similar figures and the overall frequency of LID applies to both. However, dyskinesia occurred earlier in the pre-L-DOPA era during L-DOPA treatment than the modern era, which is partially attributed to the longer durations of pre-existing PD. Moreover, the reported difference in LID frequency suggests that greater depletion of dopaminergic striatal terminals may increase the likelihood of developing LID. Similarly, LID occurs later now than in earlier series of studies, which may be explained by the higher dose used in the past, which led to the earlier appearance of dyskinesia (475). A large retrospective study found that the risk of developing LID was a higher initial L-DOPA dose (476), which is in agreement with a later cross-sectional study, adjusted for other risk factors (476). In line with this data, a placebo-controlled clinical trial also reported that patients with LID were taking higher L-DOPA doses at the appearance of dyskinesia compared to patients without dyskinesia (477). However, the cumulative dose of L-DOPA does not differ significantly between the groups, as observed in a retrospective study (478). Furthermore, it is difficult to determine the exact effect of L-DOPA dosage as PD patients may receive different doses at different time points throughout the day as the drug regimen changes throughout the course of disease (420).

Studies suggest that females are more likely to develop dyskinesia than males (479, 480) but it appears that sex cannot fully explain this phenomenon. In fact, given the same dose of L-DOPA with an AADC inhibitor, females are exposed to a higher plasma concentration time curve compared to males, when adjusted for kilogram of body weight (481). It seems that body weight affects the pharmacokinetics of L-DOPA, which in turn, may influence the onset of dyskinesia (480, 482). This is further supported by a subanalysis of a prospective clinical trial, which reported that dose per kilogram body weight is the most significant factor in the

development of LID (483). In contrast, multiple logistic regression analysis found that female gender, absolute dose, body weight and disease duration were insignificant (483). Thus, treatment with high L-DOPA dose relative to patient's body weight seems to be a significant risk factor for LID (484).

3.4.3. Impact of L-DOPA type

L-DOPA is currently available in several formulations including oral standard-release, controlled-release and dispersible tablets. Controlled-release formulations theoretically reduce fluctuations in plasma L-DOPA levels and clinical trials have reported decreased off-time and reduced L-DOPA dosing frequency when compared to immediate-release formulations (485-487). Other studies, however, suggest that controlled-release is not superior to standard release (488) and is associated with increased incidence of dyskinesia (488-492). To date, there is no consensus in selecting one formulation over another in the treatment of dyskinesia. An increasingly popular approach to treat motor complications in advanced PD patients is continuous intrajejunal infusion. Despite its cost and technical demands, this method of delivery improves motor fluctuations in PD and may reduce both duration and severity of dyskinesia (493, 494).

3.4.4. Young age at onset

Several epidemiological studies indicate that a young age at the onset of PD is a significant risk factor for LID (495). In addition, the "DA turnover" to DA synthesis and storage rate are inversely correlated with the onset age of PD (496). Young-onset patients might have more compensatory mechanisms to dopaminergic cell loss in the BG, rendering them more vulnerable to the development of LID (497). Moreover, young-onset patients have more nigral abnormalities while late onset patients have more cortical abnormalities (498). The relationship between the age of onset and the development of LID may be partially explained by genetic influences (499). Some genetically determined forms of PD at young onset have been reported to have a higher risk of developing LID, and a higher prevalence of dyskinesia has been reported in patients with a family history of PD (495, 500-503) than those without (495, 502). Mutations in the genes PARK 2, PARK 6 and PARK 7 are associated with young onset PD, and have been reported to have relatively higher rates of dyskinesia (504-506). Recent studies, however,

disagree with these reports and observe that carriers of PARK2 or PARK8 mutations do not develop LID more frequently than age- and disease duration-matched non-carriers (507, 508).

3.4.5. Genetic risk factors

Genetic factors may also contribute to the variability in incidence, severity and latency from treatment onset, and result in different susceptibility to develop dyskinesia. In addition, the interindividual difference and the high prevalence of LID in young-onset PD patients further supports the possibility of genetic susceptibility for LID (484). Genetic polymorphisms of pre-synaptic and post-synaptic structures could be potential substrates for genetic susceptibility to LID (484) and the occurrence of dyskinesia is associated with specific polymorphisms for the DA receptors or DAT genes (509-511), the COMT gene and the mu-opioid receptor gene.

3.5. Dyskinesia rating scales in Parkinson's disease

Despite the developments in pharmacological and surgical treatments for advanced PD, progress has been limited by the lack of a widely accepted clinical rating scale for dyskinesia (512). The challenge can be attributed to the variability in the anatomical distribution of dyskinesia, intensity of movements, disability or impact on daily living (513). In addition, quantification of dyskinesia using rating scales is subject to inter- and intra-rater reliability and also needs to clearly discriminate from other motor parkinsonian features. Amongst the clinically-available scales used to assess dyskinesia, only a few meet the minimal criteria and it is often at the discretion of the investigators and clinicians to select one that best fits the need of the assessment. Lang-Fahn (514) and PD-DYS-26 (515) are more patient-oriented scales while more objective assessments can be obtained for impairment and disability with the Abnormal Involuntary Movement Scale (AIMS) (516) and the Rush Dyskinesia Rating Scale (517). Although the AIMS displays high inter-rater reality for tardive dyskinesia, data suggests it does not have a specific reference to LID, which limits its use in PD patients (518). In contrast, the Unified Dyskinesia Rating Scale (UDysRS), the newest rating scale developed specifically for the assessment of dyskinesia in PD, encompasses both patient-based and rater-based ratings of disability and impairment, and provides a more comprehensive measurement tool for the burden of dyskinesia (519). Furthermore, the intra-rater and inter-rating reliabilities and reproducibility of the UDysRS have been well established with a strong clinimetric profile (519-521).

3.6. Pharmacological management of dyskinesia

Pharmacological treatment for dyskinesia is based on adjustments to the intervals and doses of dopaminergic treatments, adjunct oral drugs with direct anti-dyskinetic effects and continuous administration of anti-parkinsonian drugs via pumps. To date, only two orally administered agents have been shown to relieve dyskinesia without worsening motor disability, amantadine and clozapine.

Amantadine is a non-selective NMDA receptor antagonist and has been reported to reduce LID in animal models and in PD patients (522). The effectiveness of this agent in LID has provided support for the pathogenic role of changes in striatal NDMA receptors in dyskinesia (522). In addition, a randomized, placebo-controlled trial reported that treatment with amantadine significantly improved on time with dyskinesia and reduced AIMs after a L-DOPA challenge (523). Furthermore, in a placebo-controlled crossover trial, dyskinesia severity was reduced by nearly 50% on amantadine compared with placebo (523). Amantadine is efficacious as an oral anti-dyskinetic drug with a sustained effect that lasts for at least one year (524). However, its potential as a treatment is limited by its ability to worsen neuropsychiatric problems, particularly in elderly patients (525).

Clozapine is an atypical anti-psychotic and although its exact mechanism of action is unclear, it may be attributed to its affinity to 5-HT 2A (5-HT_{2A}) receptors (526). Uncontrolled studies of clozapine for dyskinesia have estimated a reduction by around 50 % with high doses (527). Moreover, a randomized, placebo-controlled 10-week study reported that clozapine is associated with a significant reduction in on-time with dyskinesia compared with placebo, without changes in off-time duration (528). The use of clozapine is limited by potential adverse events, agranulocytosis and myocarditis, although evidence shows that this risk decreases over time (529, 530).

3.7. Surgical options for dyskinesia

Treatment of LID is one of the most common indications for neurosurgery in PD and procedures such as STN-DBS, or pallidotomy or GPi-DBS, which are thought to have a direct effect on dyskinesia, can provide relief of motor symptoms of PD and help control LID (531). Since the introduction of “chronic” high-frequency DBS by the French neurosurgeon Alim

Benabid (532), DBS procedures have been pivotal to improve motor symptoms (533), whereas ablative surgery is considered an alternative and only used when DBS is not feasible (415, 534, 535). However, controversial issues including the timing of therapeutic intervention, the selection of stimulation target and adverse effects (536) as well as the restricted selection criteria and invasiveness of these procedures present considerable risks to patients with PD compared to pharmacological interventions. Thus, the present Thesis will focus more on the relevance of pharmacological management of LID but advances in DBS and pallidotomy are reviewed in (412, 444).

3.8. Basal ganglia circuitry in dyskinesia

The interaction between DA and post-synaptic receptors is crucial in the modulation of motor function and classically described by the BG circuitry. BG are a group of interconnected nuclei located bilaterally in the diencephalon and midbrain and contain the striatum, GPe, GPi, SNc, SNr and STN (537). The BG form a neural network that relays information from the motor cortex to the thalamus, forming closed "cortico-striato-thalamo-cortical" loops (538). In addition to an involvement in the planning, initiation, and execution of voluntary movement (539, 540), these structures have broader roles in motor learning, executive functions, behaviours and emotions (541, 542). Thus, these loops functionally convey information for both motor and non-motor processes (543).

Clinical-pathological observations during the 20th century found that lesions to the putamen, GP and STN were associated with movements disorders (544, 545), which heavily influenced our understanding of BG function (546). There are two opposing views on the anatomical substrate of information processing at the BG, the "parallel processing" and "information funneling" hypotheses (547). The "parallel processing" hypothesis infers that the processing of different types of cortical information is largely independent via parallel and segregated circuits (547). Five parallel circuits have been identified thus far: motor, oculomotor, orbitofrontal, dorsolateral prefrontal, lateral orbitofrontal and anterior cingulate loops (543). Each circuit appears to receive inputs from separate cortical areas, travel portions of the BG and thalamus and project back upon cortical input areas, forming a partially "closed" loop (543, 548-550). In contrast, the "information funneling" hypothesis proposes the convergence of cortical information along the cortico-striato-pallido/nigro-thalmo-cortical system, and emphasizes the

contrast between the three-dimensional geometry of the axonal and dendritic arborizations in the striato-pallido-nigral circuit (547).

3.8.1. Physiological state

The BG and related nuclei can be categorized into input, output and intrinsic nuclei (551). Input nuclei receive incoming information from differences sources, primarily from cortical, thalamic and nigral areas. The primary input structure of the BG is the striatum, which receives excitatory input from the cortex. In contrast, output nuclei send basal ganglia information to the thalamus and consist of the GPi and the SNr. Finally, intrinsic nuclei such as the GPe the STN and the SNc are the intermediary between the input and output nuclei in the relay of information.

3.8.1.1. Structures

3.8.1.1.1. Striatum

The striatum serves as the major input structure of the BG and the origin of its name refers to the striated appearance of white corticofugal fibres (552). The caudate nucleus and putamen are referred to as the striatum (553). In primates, the fibres of the internal capsule separate the caudate nucleus medially and the putamen laterally (554), whereas in rodents, the structures are fused together (555). There is also a ventral component of the striatum defined as the nucleus accumbens, which is functionally and anatomically connected to limbic brain structures (556). Functionally, the caudate-putamen complex is associated with movement regulation, whereas the ventral striatum is involved in mediating neurological functions relating to motivation, reward and emotion (557, 558). The striatum receives different afferent projections, including dopaminergic fibres from the midbrain (559), serotonergic fibres from the dorsal and medial raphe nucleus (560) and noradrenergic fibres from the locus coeruleus (561). Glutamatergic fibres originating from the cerebral cortex project to the striatum in a somatotopically-organized manner (562-564). The targets of cortical input are gamma-amino butyric acid (GABA)-containing inhibitory MSNs (565, 566) that represent of the majority of striatal neurons. These GABAergic output cells are homogenously distributed such that the striatum lacks a distinct cytoarchitectural organization, in contrast with the laminar organization of the cortex (555). MSNs are divided into two subtypes, which form the direct and indirect

pathways (567). The remaining striatal neurons are larger in size and serve as local interneurons composed of three subtypes: 2% contain either parvalbumin or calretinin and 1% contain the peptides somatostatin and acetylcholine (568, 569). Striatal interneurons are distributed in axons and the majority synapse onto spiny projection neurons (569).

3.8.1.1.2. Substania nigra

The SN is a midbrain structure that comprises two distinct components, the SNr and the SNc. The name “substantia nigra”, also referred to as “locus niger”, or black substance, refers to the high concentration of neuromelanin, a dark pigment derived from L-tyrosine, found in dopaminergic neurons (570). In contrast, the SNr, consists of dendritic arborizations, and is located ventrally to the SNc (571). Functionally, the SNr is a major output structure of the BG circuitry involved in sensorimotor integration and organization of behaviour (572). Dopaminergic neurons of the SNc project to the caudate nucleus and putamen, where they synapse with MSNs and release dopamine (557, 573).

3.8.1.1.3. Subthalamic nucleus

The STN is the uniquely placed in the BG circuitry as the sole structure emitting glutamatergic fibres (299). It receives excitatory glutamatergic afferents from the frontal lobes with especially large inputs from the motor cortex (574, 575). The STN also receives inhibitory GABAergic input from the GPe (576). In contrast, the STN sends excitatory glutamatergic output to the GPi, GPe and SNr (577-580).

3.8.1.1.4. Output nuclei

The output nuclei, the GPi/SNr complex, act as a single functional unit through inhibitory projections to the thalamus, which in turn, influences frontal lobe cortical regions (299). The GPi and SNr have similar cyto- and chemoarchitectural characteristics and to some extent, similar types of afferent and efferent systems (546). They receive afferent projections including glutamatergic afferents from the STN and inhibitory GABAegic afferents from the striatum and GPe (299). In both structures, there is a relative segregation of limbic and sensorimotor inputs (581). The GPi and SNr consist of inhibitory GABAergic neurons with a

firing rate of discharge that tonically inhibits their targets (546). Their output predominantly projects to the ventral anterior, ventral lateral and mediodorsal thalamus (543, 582, 583).

3.8.1.2. Direct versus indirect pathways

To mediate voluntary movement, equilibrium is maintained between two opposing pathways originating in the striatum and regulated by the SNc DA projections that synapse with striatal GABAergic neurons (299).

The first population of MSNs express D1 receptors and preproenkephalin-B, an opioid peptide cleaved to produce co-transmitters including substance P and dynorphin (584). This population forms the direct pathway, and provide direct inputs to the output neurons of the GPi and to the SNr (Figure 20A, page 39). These structures then project to the ventral lateral and centro-median parts of the motor thalamus, which in turn, project towards the motor cortex. Striatofugal neurons subsequently send GABAergic projections inhibiting the tonic activity of GPi/SNr, which leads to a dis-inhibition of thalamic glutamatergic neurons. The thalamus then sends an excitatory glutamatergic projection that activates the motor cortex. The behavioural result is a facilitation of voluntary movement (585).

In contrast, the second population of MSNs express enkpephalin and D2 receptors (584). These neurons project towards relay structures prior to arriving on the GPi/SNr and form the indirect pathway (Figure 2A, page 39). D2 neurons of the striatum project to the GPe, which in turn, sends GABAergic efferent fibres to the STN. The STN then provides excitatory projections to the output neurons of the BG. Striatopallidal MSNs subsequently emit an inhibitory GABAergic projection to the GPe, and further to the glutamatergic neurons of the STN. In turn, the STN activates the GPi/SNr. Both nuclei send an inhibitory GABAergic projection to the motor thalamus (ventral lateral and centro-medial nuclei) that leads to a decrease in excitatory thalamic output to the motor cortex. The net result is suppression of voluntary movement (585).

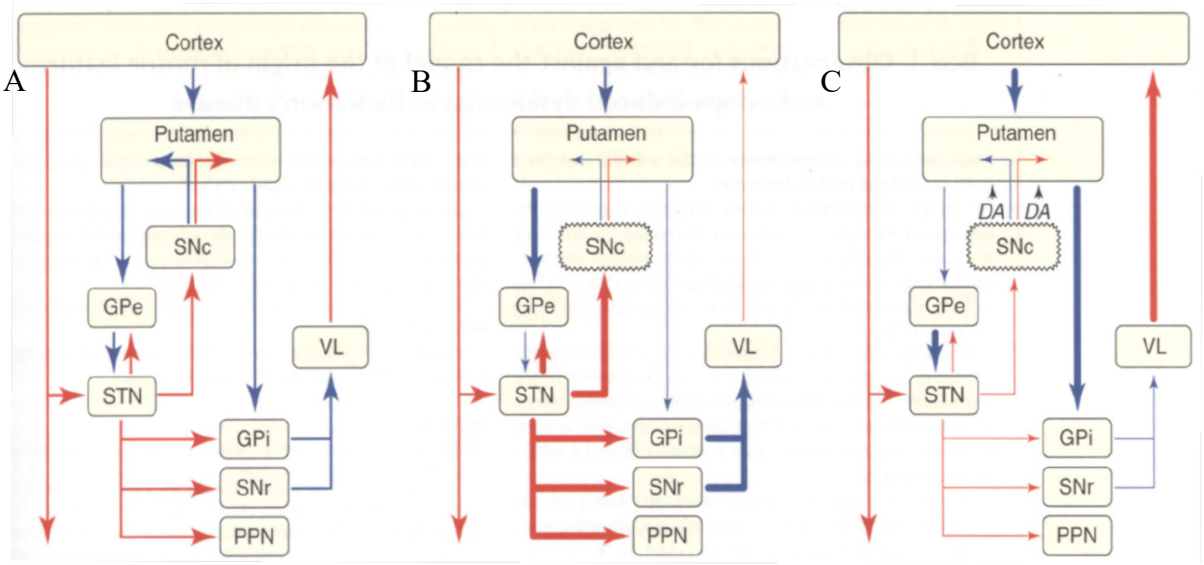


Figure 2: Schematic diagram of the classical BG circuitry describing different states. A. The model under physiological conditions shows an equilibrium between the direct and indirect pathways. B. The loss of nigrostriatal DA neurons in PD leads to the hyperactivity of the indirect pathway and the underactivity of the direct pathway. C. Chronic administration of L-DOPA and the eventual development of LID may be due to the hyperactivity of the direct pathway and underactivity of the indirect pathway. Reproduced from Obeso (586).

3.8.2. Pathophysiological state: parkinsonism and dyskinesia

3.8.2.1. Basal ganglia circuitry in Parkinson's Disease

In PD, the extensive degeneration of SNc dopaminergic neurons disrupts the equilibrium between the direct and indirect pathways and favours the hypokinetic state (7). Activity of the striatofugal neurons of the direct pathway diminishes, whereas the MSNs of the indirect pathway become overactive, motor symptoms arise as a result of this imbalance (Figure 2B, page 38). With striatal dopamine loss, both pathways lead to the inhibition of GPe and subsequent dis-inhibition of the glutamatergic fibres of the STN (572). The consequent hyperactivity of the GPi/SNr causes the inhibition of motor thalamic nuclei and decreased thalamic output. The result is the underactivity of motor cortical areas, which is reported to occur in the primary sensory motor cortex and supplementary motor area (587) in the parkinsonian state. In a groundbreaking series of experiments by Mitchell and colleagues, the neuronal metabolic marker 2-deoxyglucose (2-DG) was used to reveal the activity states of BG subnuclei in the MPTP-lesioned nonhuman primates (588-590). It was reported that the STN

was hyperactivated, while the GPe and thalamic nuclei were hyperinhibited (588-590). These findings suggested that output structures of the BG are hyperactivated in PD (590), and were later confirmed through electrophysiology and mRNA expression studies (591-593). However, assessment of levels of neural activity in BG based on metabolic markers instead of electrophysiological methods is difficult to interpret because the increase in metabolism can reflect excitatory or inhibitory processes and the balance between pre- and post-synaptic metabolic activity is unclear (594).

3.8.2.2. Basal ganglia circuitry in dyskinesia

Early attempts to describe the pathophysiology of LID proposed a disequilibrium between the direct and indirect pathways that is opposite with respect to PD (595-597). A greater emphasis was placed on the indirect pathway in the pathogenesis of the dyskinetic state and it was proposed that MSNs of the indirect pathway become underactive, leading to disinhibition of the GPe (Figure 2C, page 38) (538). Subsequently, this causes the over-inhibition of the STN and leads to the underactivity of the GPi/SNr. This imbalance dis-inhibits the motor thalamus and motor cortex, giving rise to the overactivation of motor cortical areas and consequent excessive abnormal movements, which characterize the dyskinetic PD patients (598, 599). In MPTP-lesioned nonhuman primates, Mitchell and co-workers demonstrated that at the peak dose of DA agonist-induced dyskinesia, there was an increased uptake of 2-DG in the STN and GPi, indicating that these structures were hyperinhibited (600). In addition, there was a decrease in the uptake of 2-DG in the motor thalamus, which reflected its hyperactivated state in dyskinesia (600). In contrast, the proposed underactivity of the indirect pathway in LID is generally inconsistent with experimental findings, which presents limitations of the classic model of BG circuitry (538). For example, the underactivation of the indirect pathway due to an overactive GPe is not consistently seen in dyskinetic MPTP-lesioned nonhuman primates (601). Furthermore, MSNs of the indirect pathway are not necessarily underactive, as levels of striatal PPE-A mRNA are actually upregulated in dyskinetic compared to non-dyskinetic PD patients (602, 603).

Bezard and colleagues later emphasized the role of the direct pathway in the pathogenesis of LID (604). The authors suggest that underactive/abnormal firing of the BG output nuclei in dyskinesia (591, 605-610) is primarily caused by overactivation of MSNs of the

direct pathway. Indeed, functional overactivity of the direct pathway in LID has been demonstrated at the cellular level by upregulated striatal mRNA expression of PPE-B and prodynorphin (602, 611-614), and the supersensitization of striatal D1 receptors (614). Moreover, treatment with the selective dopamine D1 receptor agonist ABT-431 in PD patients elicits dyskinesia to a similar extent to that of L-DOPA (615), which further supports the hypothesis of the hyperactivation of the direct pathway in dyskinesia. Overall, these findings are consistent with the mechanism suggested in the classic functional model, where the hyperactive direct pathway mediates over-inhibition of the BG output, resulting in the underactivation of these nuclei.

Recently, Nadjar and colleagues showed that both the phenotype and the targets of striatofugal neurons, are preserved after dopamine denervation in the parkinsonian state and even after chronic L-DOPA treatment in non-dyskinetic and dyskinetic subjects (616). Although these results suggest that the phenotypical plasticity of the striatofugal system is not affected by the experimental condition, it does not exclude the possibility of plastic changes in the striatum (617). In fact, the size of the dendritic tree and density of dendritic spines of MSNs is reduced in the striatum of PD patients compared with controls (618), consistent with the pruning reported to occur in rodents (619) and in MPTP-primates (620, 621). Taken together, these plastic changes contribute to the development of L-DOPA related adverse events by altering the flow of information through the striatum and the rest of the BG (617).

3.8.3. Present: changes to the classic model

In the 1980s, based on the anatomy, neurochemistry, and electrophysiology studies available at the time, the classic model of the functional organization of the BG circuitry was first proposed (617). As discussed above, the classic model is based on the segregation of the direct and indirect pathways where neural processing occurs in a feed-forward manner to achieve behavioural outcome (538). Although the classic functional model has advanced our understanding of functional mechanisms in normal and disease states, the model is too simplistic and limited to describe the pathophysiology underlying PD and LID (538). For example, underactivity of the BG output nuclei in dyskinesia (605, 608, 609) cannot fully account for disease pathogenesis (607). Similarly, lesioning the GPi does not result in dyskinesia (539, 622) and in fact, pallidotomy of the GPi effectively alleviates LID in MPTP-lesioned primates (623)

and PD patients (624, 625), which is contrary to the outcome proposed by the classic functional model.

Experimental reports demonstrate a greater complexity in the neural organization and information processing within the BG (617). This has prompted deviation from the classical model originally described by Alexander and Crutcher (543) to a new functional model, which considers the dynamic neural network in the BG circuitry (597, 626). As demonstrated by major experimental findings in PD and LID, the BG are not simply a "go through" structure, where the connectivity and functional interactions occur along a unilateral fashion along the cortico-basal ganglia-thalamo-cortical circuits (627, 628). The organization of the BG circuitry appears to be interconnected rather than segregated as striatofugal axons consistently collateralize to both the GPe and GPi (629). In addition, major changes to the classic indirect pathway include the GPe as a key structure for inhibitory modulation of the striatum and output nuclei (628, 630, 631) and the STN as another major input station that receives and sends glutamatergic projections (551, 574, 632). Thus, the model now incorporates internal feedback loops (597, 626), and reciprocal connections are found between many nuclei of the circuitry (633-635). Furthermore, the reorganization includes functional dual di-synaptic control of the GPe and GPi (597, 628) via parallel cortical projections to the striatum and STN (538). The corticostriatal projection uses the striatum to exert inhibition of the pallidal segments (538) while the cortico-STN projection uses the STN to mediate fast excitatory input to these structures (636). Indeed, parallel cortico-basal ganglia loops have been confirmed in humans by fMRI and PET studies (637).

3.8.4. Serotonergic system and basal ganglia: implication in Parkinson's Disease and dyskinesia

The basal ganglia are enriched with a variety of neurotransmitters such as DA, glutamate, acetylcholine and 5-HT (638). In the last decade, there has been growing interest in the role of the serotonergic system in PD and LID, which will be further discussed in detail (section: 3.10. Serotonergic system in dyskinesia, page 47). Briefly, serotonergic neurons in the raphe nucleus project axonal fibres to multiple brain areas including the basal ganglia (639). In the DA-denervated brain in PD, striatal serotonergic terminals contribute increasingly to the conversion of L-DOPA into DA (640, 641). However, the lack of auto-regulatory feedback mechanisms to

control DA release results in the aberrant release of DA into the extracellular space (639). Consequently, fluctuations in DA levels lead to the supersensitivity of postsynaptic dopaminergic receptors and the expression of dyskinesia (642).

The 5-HT receptors are divided in 7 classes (5-HT_{1-7}) with at least 14 subtypes and are all members the GPCR family, except for the 5-HT_3 receptor (see section: 5-HT_3 receptors, page 52). These GPCRs activate an intracellular second messenger to mediate excitatory or inhibitory neurotransmission (643). 5-HT neurons express three subtypes of autoreceptors, amongst which the 5-HT_{1A} and 5-HT_{1B} are the most abundant (644). 5-HT_{1A} receptors are found in the soma and on dendrites (645, 646), whereas 5-HT_{1B} receptors are more abundant in terminals (647) and together, these autoreceptors fine-tune the synaptic release of 5-HT to maintain synaptic levels within a physiological range (645). Agonists of these receptors reduce neurotransmitter release from 5-HT neurons (645, 648-650), and since DA and 5-HT are localized in the same synaptic vesicles after exogenous L-DOPA administration, it is conceivable that they also decrease the release of L-DOPA derived DA from 5-HT terminals (394, 640, 651).

The BG nuclei receive serotonergic afferents that mainly originate from the dorsal raphe nuclei (see review in (652)). Furthermore, the BG contain 5-HT, its metabolite 5-hydroxyindoleacetic acid (5-HIAA) (560, 653, 654), the 5-HT transporter (SERT) and 5-HT receptors 5-HT_1 to 5-HT_7. The distribution of 5-HT receptors in these structures is heterogeneous and varies between species (655). 5-HT modulates the activity of BG nuclei by acting on 5-HT receptors and helps maintain the balance between the direct and indirect pathways (656).

Parent and colleagues conducted an immunohistochemistry study to visualize the 5-HT system innervation of BG in human and nonhuman primates (657). The SN is the most densely innervated BG subnucleus and nigral 5-HT innervation originates from axons and arborizes immediately upon entering the SN (657). In addition, 5-HT mainly exerts an inhibitory effect on the activity of SNc DA neurons projecting to the striatum and SNr GABAergic neurons projecting to the thalamus and brainstem in humans and primates (657). Conversely, 5-HT depletion decreases firing rate and increases burst activity of SNr neurons (658). In spite of the apparent inhibitory effect of 5-HT input on the SNc (659, 660), lesioning the dorsal RN does

not alter SNc activity (661), so it is still unclear how 5-HT transmission modulates the activity of dopamainergic SNc neurons (655).

In addition, the striatum receives dense serotonergic afferents from the dorsal RN, where local administration of 5-HT inhibits the majority of the striatal cells (662-664). Furthermore, many 5-HT variscosities in the striatum could be visualized in close apposition with the pigmented cell bodies of the SNc (657). Stimulation of pre-synaptic 5-HT_{1A} and 5-HT_{1B} receptors inhibits striatal 5-HT release (648, 665) and activation of the 5-HT_{1A} receptor also decreases glutamate release from corticostriatal projections (666-668). In contrast, the 5-HT_2 receptor exerts an inhibitory effect on striatal neuron activity, mainly by targeting MSNs (669, 670).

In the STN, 5-HT can act at the pre- and post-synaptical levels and, given the functional diversity of 5-HT receptors, exert multiple effects (671-673). The activity of STN neurons is modulated by 5-HT afferents and in primates, 5-HT axonal varicosities are apposed almost only upon dendritic spines or branches and many terminals do not form synaptic contacts (657). Pharmacologic lesion of the DRN and 5-HT depletion increases STN firing frequency and bursting activity *in vivo* (674, 675).

Electrophysiology studies in rats indicate that 5-HT controls its effect by both pre- and post-synaptic mechanisms at the pallidal level (676). The decrease in 5-HT concentrations can lead to changes in pallidal activity and contribute to abnormal synchronous oscillations in BG components (657). Moreover, in the GPe, 5-HT depletion decreases the firing frequency and increases the proportion of bursty and irregular neurons (677), these results have been confirmed by a patch-clamp recording study where 5-HT perfusion increased the firing rate of GPe neurons (678). In addition, 5-HT can decrease the pre-synaptic release of glutamate and GABA from subthalamopallidal and striatopallidal terminals, respectively, by acting on 5-HT_{1B} receptors (679).

In PD and subsequent L-DOPA replacement therapy, the 5-HT system adapts to DA depletion by adopting anatomical and functional transformations (655). However, the changes occurring after dopaminergic lesion in animal models of PD differ across research groups, which may reflect methodological differences including the parkinsonian state, the age of the animals, injection site, toxin concentration, and the time between surgery and performing the studies (655). Similarly, striatal 5-HT levels have been reported to be increased (680, 681), unchanged

(641, 682) or decreased (683) in parkinsonian animals. On the other hand, the dorsal RN also undergoes adaptive changes after the dopaminergic degeneration such as the increased 5-HT_{1A} expression in MPTP-lesioned primates (684). Overall, the effects of 5-HT in the BG depend on the specific nucleus and its receptor population (655). For example, 5-HT exerts an inhibitory action on striatal MSNs via direct or indirect activation of 5-HT receptors, as well as in the STN and SNr *in vivo*, whereas the overall effect of 5-HT is excitatory in the GPe (655).

3.9. Dopaminergic system in dyskinesia

Mechanisms involved in the pathophysiology of LID are complex and have been investigated in studies using animal models and parkinsonian patients.

In general, DA cell loss in the nigrostriatal pathway and chronic administration of L-DOPA or DA agonists, are viewed as necessary conditions for the appearance of LID (685).

3.9.1. Dopamine receptor supersensitivity and dopamine sensitization

Denervation-induced supersensitivity of DA receptors has been recognized as a plausible mechanism of LID. However, it is likely more complex than simply an increase in the density of striatal DA receptors (686-689); thus, according to this theory, then LID might appear with first dose of L-DOPA. However, LID does not usually emerge at the first exposure to L-DOPA but gradually develops over years of L-DOPA therapy, as discussed above. The development of LID appears to be related to an increase in the activity of D1, D2, D3 receptor subtypes while additional studies are required to discern the contribution of D4 and D5 receptors (685).

3.9.2. D1-like family of dopamine receptors

In early studies in primates, it was shown that D1 agonists were as effective as D2 agonists to improve parkinsonian symptoms, while inducing less dyskinesia (690-692). Later studies, however, implicated a more important role of D1 receptors in dyskinesiogenesis. A study in drug-naïve MPTP-lesioned primates found that chronic administration of a D1 receptor agonist led to the development of dyskinesia (693). Consistent with this data, recent studies in 6-OHDA-lesioned rats reported that D1 agonists induce dyskinesia, and that pharmacological blockade of D1 receptors was more effective than D2 receptor antagonism at alleviating dyskinesia (457, 694, 695). Furthermore, genetic knockout of D1 receptors completely

suppressed LID in parkinsonian mice, whereas D2 receptor knockout mice developed LID similar to wild-type mice (696).

Autoradiographic studies on D1 receptors *in vivo* and on *post-mortem* tissues of animal models and in PD patients have been conducted with no general consensus (697), which may be due to the differences in experimental assays and the subregion of the striatum measured (685). Although the association between the expression of D1 receptors and dyskinesia is unclear, the sensitivity of D1 receptors, measured by GTPγS binding, was reported to be linearly related to the severity of LID (686). L-DOPA induced a decrease in the sensitivity of D1 receptors in non-dyskinetic MPTP-lesioned primates, whereas its sensitivity was increased in dyskinetic animals (686). Moreover, D1 receptors are internalized in the cytoplasm in 6-OHDA-lesioned rats compared to normal rats (698), which is also observed in PD patients, where D1 receptors are preferentially localized to the cytoplasm compared to healthy controls (699). However, it is unclear if the change in subcellular distribution of D1 receptors is a consequence of distribution in development, priming process or expression of LID (685).

D1 receptors interact with a variety of receptors and trigger signalling pathways that have an influence on dyskinesia development. D1 receptors interact with ionotropic glutamate NMDA receptors at the post-synaptic striatal level, and may also form hetero-oligomeric complexes (700). This interaction affects the trafficking, signalling and desensitization of both receptors (701, 702) and importantly, these complexes are lost in dyskinetic 6-OHDA-lesioned rats (703). Furthermore, extracellular signal-regulated kinase (ERK) is part of the intracellular pathways of both NMDA and D1 receptors (704). ERK intracellular signalling is associated with LID priming process (705) and the expression of LID is reduced with pharmacological inhibition of ERK intracellular signalling (706, 707). On the other hand, dopaminomimetic agents induce the expression of transcription factors such as c-jun, c-fos, ΔFosB, FosB in striatal neurons in normal (708, 709) and hemiparkinsonian animals (350) and require the activation of D1 but not D2 receptors (709). No study has reported the involvement of the D5 receptor in PD and LID (685).

3.9.3. D2-like family of dopamine receptors

Once primed to express LID, D2 agonists will trigger abnormal involuntary movements (AIMs) in 6-OHDA-lesioned rats (710, 711) and LID in MPTP-lesioned primates (712, 713) and PD patients (714), producing greater dyskinesia than D1 agonists (690). Results from autoradiographic studies were more consistent than with D1 receptors, but again, the inconsistency observed could be due to variation in experimental assays and the striatal region studied (685). Expression of D2 receptors in the striatum remained unchanged (715, 716) or increased (717, 718) with MPTP lesion in primates and in untreated PD patients (719, 720). D2 receptor agonists reduced MPTP-induced upregulation of D2 receptors (721, 722) not as efficiently as L-DOPA whereas D1 receptor agonists had no effect or produced an increase of D2 receptors (686). A PET study in *de novo* PD patients reported similar observations (723, 724). *In situ* hybridization studies in primates demonstrated that MPTP induced an upregulation of striatal D2 receptor mRNA that was completely reversed by L-DOPA treatment (725) or unaffected (686, 726), whereas D2 receptor agonists decreased or reversed this expression (727). The influence of D2 receptor trafficking in PD and LID is not yet established (685).

3.10. Serotonergic system in dyskinesia

The chemical structure of 5-HT was identified in 1953 (728) and a few years later, its function as a neurotransmitter in the CNS was proposed (729). 5-HT plays major roles in the regulation of mood and emotion, cognition, feeding and satiety, circadian and sleep-wake cycle regulation, pain, and motor control through a variety of receptor subtypes (730, 731). 5-HT is synthesized from L-tryptophan by the following reactions: the tryptophan hydroxylase enzyme generates 5-hydroxytryptophan (5-HTP), which is then converted to 5-HT by the AADC, the same enzyme that catalyzes the conversion of L-DOPA into DA. In the brain, 5-HT neurons are clustered within the midbrain raphe nuclei (areas B1-B9) (732) and include the dorsal, median, magnus, obscuris, and pontis RN (733). Anatomical studies further subdivided the midbrain RN into two main groups based on position within the brainstem and axonal projections. The rostral group, which contains the dorsal RN comprises the B7 and B8 cell clusters whereas the median RN consists of the B5, B8 and B9 cell clusters. 5-HT plays a key role in the CNS as 5-HT neuron soma from the midbrain send projections throughout the entire CNS (734). In fact, one 5-HT cell body can be responsible for up to 500,000 cortical varicosities (735), which underscores the

widely distributed innervations derived from these nuclei, including the majority of 5-HT innervation to the forebrain regions (736, 737). Moreover, within the dorsal RN, 5-HT neurons are topographically organized and studies also demonstrate that 5-HT neurons within each sub-region differ morphologically, electrophysiologically, and molecularly with respect to receptor expression (738).

There is a growing appreciation for the multifaceted effect of 5-HT in PD and recent studies suggest that the 5-HT system is heavily implicated in the pathophysiology of LID (641). In animal models of PD, it has been suggested that the 5-HT system is an important source of striatal L-DOPA derived DA release (641, 739-741). In PD patients undergoing L-DOPA therapy with severe degeneration of the nigrostriatal DA system and compromised function in the remaining DA neurons (742-744), the 5-HT neuron derived "false transmitter" can help improve motor disability (641). With chronic administration of L-DOPA, and particularly in advanced stages of the disease, the lack of a regulatory mechanism to control synaptic neurotransmitter levels can provoke dyskinesia (745).

3.10.1. Pre-clinical evidence for the involvement of the serotonergic system in dyskinesia

Pharmacological studies also demonstrate the involvement of 5-HT neurons in the appearance of LID in animal models, as discussed above. It is generally assumed that in early stages of disease, L-DOPA is taken up into spared nigrostriatal DA neurons of PD animal models and patients, converted into DA, stored into vesicles and released in a physiologically regulated manner (746). The resultant DA release from these dopaminergic terminals within the striatum accounts for the therapeutic action of L-DOPA and is finely regulated by D2 autoreceptors and the DAT (746). With disease progression, however, fewer DA terminals can convert exogenous L-DOPA into DA. Other cellular compartments can compensate for the loss of DA neurons in mediating the conversion of L-DOPA to DA and neurotransmitter release. 5-HT neurons possess the same enzymatic machinery as dopaminergic terminals as they express AADC and VMAT2 (394, 395, 747) and thus, are able to convert L-DOPA to DA and to mediate its storage into synaptic vesicles (746). Various studies have demonstrated that 5-HT neurons can store and release DA *in vivo* and *in vitro* (644). The first report implicating 5-HT neurons as a source of DA release was provided by Tanaka and colleagues (743). In the study, removal

of 5-HT innervation by 5,7-dihydroxytryptamine (5,7-DHT) administration reduced L-DOPA derived extracellular DA levels by about 80% in the striatum of 6-OHDA-lesioned rats (743). Importantly, this also led to a near-complete suppression of LID in L-DOPA primed parkinsonian rats. Furthermore, the same group also showed a similar reduction in extracellular DA levels after co-administration of the 5-HT_{1A} agonist 8-hydroxy-2-(di-n-propilamino)tetralin (8-OHDPAT) with L-DOPA (744). Another group used a similar approach to demonstrate that lesion to the 5-HT system suppresses L-DOPA induced rotational behaviour and striatal c-Fos expression in 6-OHDA-lesioned rats (748). Taken together, these studies suggest that the action of L-DOPA in PD depends, at least in part, on its conversion to DA in 5-HT neurons (308).

Risk factors that underlie the development of dyskinesia suggests that it is the progression of dopaminergic degeneration rather than the duration of L-DOPA treatment that is responsible for the emergence of LID over time (749). Indeed, parkinsonian animals only develop severe dyskinesia with extensive DA denervation whereas partially lesioned animals show no or only mild dyskinesia (745). Using a viral vector delivery of short hairpin RNA for TH, Ulusoy and colleagues induced significant DA deficiency, and reported that DA-depleted rats were resistant to the induction of dyskinesia following administration of high dose of L-DOPA as opposed to control animals with similar striatal DA depletion (750). These findings may be explained by the relatively preserved striatal DA terminals after inhibition of DA synthesis, which act as a buffering system for exogenous L-DOPA (745). Rat transplantation studies also confirmed the ability of pre-synaptic DA compartment to prevent excessive DA receptor stimulation, as L-DOPA primed dyskinetic rats tended to normalize response to L-DOPA after receiving ventral mesencephalic dopaminergic neuronal grafts into the lesioned striatum (739, 751). Similarly, in the clinic, as neurodegeneration progresses in PD patients, susceptibility to dyskinesia also increases over time (752). DA surges reflect uptake and conversion of exogenous L-DOPA by cells other than nigrostriatal DA neurons (443). Accordingly, several studies have demonstrated that 5-HT neurons become the main source of DA release in severely DA-denervated animals and that 5-HT neuron derived DA release is important in LID (308). In 6-OHDA lesioned rats, Carta and colleagues demonstrated that DA release from the 5-HT system is responsible for the appearance of LID (641). In fact, animals

subject to toxic lesion of the 5-HT system by the neurotoxin 5,7-DHT or pharmacological blockade of these neurons by 5-HT_{1A} and 5-HT_{1B} agonists leads to the silencing of dyskinesia upon treatment with L-DOPA (641). Moreover, studies consistently found that 5-HT_{1A} and 5-HT_{1B} agonists exert anti-dyskinetic effects in animal models of LID (641, 753-755) .

In early stages of disease, the therapeutic efficacy of L-DOPA and the physiological release of DA by 5-HT neurons is beneficial because the remaining DA terminals can buffer the 5-HT neuron-derived DA and avoid excessive post-synaptic DA receptor stimulation (642). DA D2 autoreceptors are located on the pre-synaptic membrane and activate a feedback control mechanism to fine-tune neurotransmitter release and allow the maintenance of physiological-like synaptic DA levels (745). However, with disease progression, the therapeutic efficacy of L-DOPA is partly compromised and, 5-HT neurons contribute increasingly to the conversion of exogenously administered L-DOPA to DA, eventually producing excessive DA receptor activation (745). Moreover, unlike DA neurons, 5-HT neurons lack autoregulatory feedback to control DA release (465, 756). As a result, the non-physiological release of DA leads to large fluctuations in synaptic DA levels, causing pulsatile stimulation of striatal DA receptors and aberrant downstream signalling cascade (757).

3.10.1.1. 5-HT_1 agonists in the treatment of dyskinesia

Munoz and colleagues observed that a combination of 5-HT_{1A} and 5-HT_{1B} receptor agonists, using low doses of 8-OH-DPAT and CP-94253, synergistically suppressed L-DOPA induced AIMs in 6-OHDA-lesioned rats (758). These results were also obtained in dyskinetic MPTP-lesioned macaques (758). Accordingly, a rat microdialysis study reported a reduction of extracellular DA levels that account for the potent anti-dyskinetic effect of 5-HT_{1A} and 5-HT_{1B} receptor agonists (759). In a rat PET study, Nahimi and co-workers showed that administration of 8-OH-DPAT reverses L-DOPA induced decrease of [^{11}C]-raclopride binding and increases extracellular DA in 6-OHDA lesioned rats (760).

Agonists for the 5-HT_{1A} receptor have shown acute and chronic efficacy in animal models and clinical studies for LID but at the expense of the therapeutic efficacy of L-DOPA (641, 740, 741, 761-763). The 5-HT_{1A} receptor agonist sarizotan demonstrated efficacy in reducing dyskinesia in rodent and primate models of PD, as well as in idiopathic PD patients in early open-label studies (762). However, the anti-dyskinetic effect was not significantly

different compared to placebo in two Phase III clinical trials (764). Similarly, the partial non-selective 5-HT_{1A} receptor agonist buspirone reduced LID in patients (765) but two other studies found that this effect compromised the therapeutic efficacy of L-DOPA (766, 767). 5-HT_{1B} receptor agonists can produce anti-dyskinetic effects in animal models of PD but no clinical trials have been performed with these yet (768).

3.10.1.2. 5-HT_{2A} antagonists in the treatment of dyskinesia

5-HT_{2A} receptors are localized post-synaptically and in general, they exert an excitatory effect (768). Rahi and colleauges demonstrated an increase in 5-HT_{2A} receptors in the striatum of dyskinetic primates when compared with non-dyskinetic animals (769). Preclinical and clinical studies have shown the efficacy of drugs acting on 5-HT_{2A} receptors in controlling L-DOPA-induced motor complications (770, 771) but the results are contradictory. In MPTP-lesioned primates, the selective 5-HT_{2A} inverse agonist pimavanserin reduced LID without worsening motor scores (459), whereas another antagonist, ritanserin, alleviated LID but worsened L-DOPA anti-parkinsonian action (772). In addition, the 5-HT_{2A} antagonist volinanserin did not reduce LID in hemiparkinsonian rats (773). Thus, further work is required to establish whether 5-HT_{2A} antagonists can be beneficial in dyskinetic patients (768).

3.10.1.3. Clinical evidence for the involvement of the serotonergic system in dyskinesia

Clinical evidence on the effectiveness of 5-HT modulation in LID is still scarce (745). An open-label double-blind study on the efficacy of sarizotan, a partial 5-HT_{1A} receptor agonist in dyskinetic patients was terminated for lack of efficacy (764, 774). This may be attributed to its antagonistic activity at the D2 receptors (775), as well as its action on only the 5-HT_{1A} receptor, whereas experimental evidence has demonstrated that simultaneous targeting of 5-$HT_{1A/1B}$ auto-receptors exerts a synergistic effect to attenuate LID (741, 758). In fact, a Phase II study with the mixed 5-$HT_{1A/1B}$ agonist eltoprazine has shown promising results (776).

Consistent with microdialysis experiments in rats, a PET study provided support for the association between dyskinesia and dysregulated DA release (752). Dyskinetic patients showed higher synaptic DA levels one hour after L-DOPA administration compared to non-dyskinetic subjects, which led the authors to propose that dyskinetic patients have difficulty maintaining

DA levels within certain limits, likely caused by progressive degeneration of DA neurons and consequent reduced ability to mediate controlled DA release. In line with this view, a PET study using the radioligand [^{11}C]-3-amino-4-(2-dimethylaminomethylphenylsulfanyl)-benzonitrile to evaluate 5-HT terminal function and the radioligand [^{11}C]-raclopride to evaluate striatal DA release found that dyskinetic PD patients showed relative preservation of serotonergic terminals and no difference compared to non-dyskinetic PD patients (777). Furthermore, in dyskinetic PD patients, the same L-DOPA dose induced higher striatal synaptic DA concentration in PD patients with LIDs compared to non-dyskinetic PD patients, which is in agreement with previous studies (752, 778). Administration of the partial 5-HT$_{1A}$ agonist buspirone, prior to L-DOPA treatment, reduced L-DOPA evoked rise in striatal synaptic DA and attenuated LID (777). Further dividing the LID group by severity into milder and severe forms, it was found that buspirone modulated DA levels to a greater extent in PD patients with mild LIDs compared to those with severe LIDs (777). The authors concluded that striatal serotonergic terminals contribute to LID in human PD via aberrant processing of exogenous L-DOPA and release of DA as false neurotransmitter. Finally, the SERT-to-DAT binding ratio increases in PD patients that experience LIDs, when compared with non-dyskinetic PD patients, which further supports the notion that when dopaminergic innervation in the striatum is low, the 5-HT system is critical to the development of LIDs (779).

Collectively, these experimental findings provide strong evidence supporting the pivotal role of 5-HT neurons in the induction and expression of LID. An important challenge for future clinical studies will be to preserve the therapeutic effect of L-DOPA following pharmacological dampening of 5-HT neuron activity (745).

4. 5-HT$_3$ receptor

Although many serotonergic drugs tested have demonstrated efficacy in reducing dyskinesia, most have been at the expense of impairing L-DOPA anti-parkinsonian action (639). In MPTP-lesioned non-human primate models of PD, for example, selective 5-HT$_{1A}$ and 5-HT$_{1B}$ receptor agonists reduced dyskinesia but induced suppression of locomotor activity and increased motor disability (780, 781). The 5-HT$_3$ receptor is an interesting target to study in the context of dyskinesia because several drugs are clinically available to modulate its function and

the 5-HT_3 receptor has been shown to modulate striatal DA release, as discussed in detail (section: 5-HT_3 receptors in Parkinson's Disease and L-DOPA-induced dyskinesia, page 57).

The 5-HT_3 receptor is the sole ligand-gated ion channel amongst the 5-HT receptor family, while the other 5-HT receptors are all metabotropic GPCRs that modulate an intracellular second messenger system (782). The 5-HT_3 receptor was first identified in the guinea pig ileum, and then more widely distributed in the peripheral nervous system (PNS) (783). The presence of 5-HT_3 receptors in the brain was initially a subject of controversy (784) until adequate ligands were developed to conduct membrane binding and autoradiography studies (785-788), which suggested the presence of 5-HT_3 receptors in the CNS. In the PNS, the activation of 5-HT_3 receptors regulates autonomic, parasympathetic and sensory functions (789). 5-HT_3 receptors located on vagal sensory afferents exert pronounced effects on the cardiovascular system (790, 791) and also control motility and peristalsis throughout the gastrointestinal tract (792). In addition, 5-HT_3 receptors regulate nociceptive processing (793-795), which is consistent with their expression in the dorsal root ganglion and neurons in dorsal horn of the spinal cord (795-798). In the CNS, 5-HT_3 receptors in the hippocampus and nucleus accumbens are implicated in anxiety (799). Moreover, 5-HT_3 receptors are implicated in drug addiction and alcohol consumptions in rats (800) and in humans (801) and they are also important for cognitive function in elderly patients (802).

4.1. Localization of 5-HT_3 receptors

The distribution of 5-HT_3 receptors has been studied through autoradiographic, immunohistochemistry and *in situ* hybridization techniques with variation across different species (803), likely reflecting differences in the methodology and choice of ligands. In the PNS, 5-HT_3 receptors have been detected on pre- and post-ganglionic autonomic neurons and on neurons of the sensory and enteric nervous system (804-806).

In the CNS, when compared to other 5-HT receptors, the 5-HT_3 receptor displays a relatively lower density (805, 807). The highest density of 5-HT_3 receptors are found within the dorsal vagal complex in the brainstem (807, 808), which comprises the nucleus tractus solitarius, area postrema and dorsal motor nucleus of the vagus nerve (784). Outside the brainstem, the highest levels of 5-HT_3 receptors are expressed in regions such as the hippocampus (788), amygdala and superficial layers of the cerebral cortex (807, 808). However, the distribution

within the forebrain displays species variations, and in humans, for example, there are relatively high levels of 5-HT_3 receptors within the caudate nucleus and putamen (809) whereas low levels are detected within cortical regions (809-811). In contrast, autoradiographic and homogenate binding studies in the rat brain have demonstrated high levels of 5-HT_3 receptors in the neocortex (786, 812-816), hippocampus (786, 812-815) , amygdala (786, 812-814) and dorsal vagal complex (813, 814), whereas a low density of 5-HT_3 receptors is detected in the striatum (786, 815), RN (813), SN (813) and nearly absent in the cerebellum (786, 815). An autoradiographic binding study conducted in the mouse, ferret and rabbit brains also showed a similar distribution of 5-HT_3 receptors within the brain (808). Moreover, an immunohistochemistry study in the Syrian hamster brain also reported similar results with high levels of the 5-HT 3A (5-HT_{3A}) subunit within the neocortex and amygdala, and intermediate levels in the striatum, SN, GP and DRN (817). In general, 5-HT_3 receptors are concentrated in regions involved in the initiation and coordination of the vomiting reflex, which may explain the relevance of 5-HT_3 receptor antagonists in chemotherapy-induced emesis (782) as well as pain processing and control of anxiety (818).

Consistent with the mapping of 5-HT_3 receptors in autoradiographic studies, *in situ* hybridization studies indicate that in the rodent brain, 5-HT_{3A} receptor mRNA transcripts are similarly distributed to radiolabelled 5-HT_3 receptor binding sites (819). 5-HT_3 mRNA is present in interneurons in the hippocampus and prefrontal cortex (820, 821) and this distribution indicates that 5-HT_3 receptors may mediate the indirect inhibition of excitatory pyramidal neurons via activation of GABAergic interneurons. Furthermore, 5-HT_3 receptor-like immunoreactivity is primarily associated with GABA-containing neurons in the cerebral cortex and hippocampus that often co-localize with the peptide hormone cholecystokinin (804, 822, 823) or the calcium-binding protein calbindin (804).

4.2. 5-HT_3 receptor subtypes and properties

5-HT_3 receptors share electrophysiological and structural patterns with the nicotinic acetylcholine and GABA type A receptors, other members of the Cys-loop superfamily (824). A functional channel consists of five symmetrically-arranged subunits that surround a central ion-conducting pore (825). In rodents, two subunits have been cloned thus far: 5-HT_{3A} (826) and 5-HT_{3B} (827) receptor subunits, whereas three additional subunits have been identified in

humans: 5-HT_{3C}, 5-HT_{3D} and 5-HT_{3E} subunits (828-830). The subunits can be arranged to form a homomeric (5-HT_{3A} only) receptor or heteromeric (5-HT_{3A} and 5-HT_{3B} subunits) receptor (827). Although the 5-HT_{3C}, 5-HT_{3D} and 5-HT_{3E} subunits are likely to form only heteromeric receptors with 5-HT_3 receptor subunits, their function is still being debated (828, 831).

In contrast to the 5-HT_{3A} subunits, the 5-HT_{3B} subunit does not form functional homopentameric channels because of its retention in the endoplasmic reticulum (832). 5-HT_3 receptors in the CNS and PNS may be constructed of different subunits and, although it is known that all receptors contain the 5-HT_{3A} subunit, the distribution of the 5-HT_{3B} subunit is still unclear (833). Immunohistochemical studies suggested that the expression of the 5-HT_{3B} subunit is restricted to the PNS (798, 834) but *in situ* hybridization studies showed that the 5-HT_{3B} subunit mRNA is present in the human brain (827). Furthermore, immunocytochemical studies report that 5-HT_{3B} subunits are found in rat hippocampal neurons (835). Thus, it has been proposed that the 5-HT_{3B} subunit is either present in low levels in the CNS or in very discrete localized cell populations (833). The function of 5-HT_3 receptors depends on receptor composition (836, 837), and expression of the 5-HT_{3B} subunits leads to an increase in single channel conductance and lower permeability to Ca^{2+} (827, 838). Furthermore, heteromeric receptors show faster activation and deactivation kinetics than homomeric receptors (839). The differences observed between *in vitro* and *in vivo* studies (803) may be explained by the fact that *in vitro* studies tend to be performed in cultured cells expressing only homomeric 5-HT_3 receptors (840). Whether the 5-HT_{3B} subunit is a major determinant of 5-HT_3 receptor function in the CNS is still being debated (836, 841, 842) and may depend on species-specific expression patterns (843).

4.3. Physiology and pharmacology of 5-HT_3 receptors

The 5-HT_3 receptor is permeable to Na^+, K^+ and Ca^{2+} (844, 845) and its function depends on whether it localizes to nerve terminals or post-synaptic cells (803). Differences in the cellular localization of pre- and/or post-synaptic 5-HT_3 receptors within different cerebral regions appear to depend on the nature of the neuron that bears these receptors (846, 847). The preferential localization on nerve endings is consistent with a physiological role of the 5-HT_3 receptor in the control of neurotransmitter release (848). Activation of pre-synaptic 5-HT_3 receptors is followed by rapid membrane depolarization, which causes a rapid rise in cytosolic

Ca^{2+} concentration by inducing Ca^{2+} influx and mobilizing intracellular Ca^{2+} stores, and modulates the release of neurotransmitters and neuropeptides including DA, cholecystokinin, acetylcholine, GABA, substance P or 5-HT itself (849, 850). In contrast to 5-HT_3 receptors found predominantly in pre-synaptic regions associated with axons and terminals, in the hippocampus, they are mostly located on post-synaptic receptors in somatodendritic regions (846). Here, activation of post-synaptic 5-HT_3 receptors leads to depolarization by Na^+ influx and K^+ efflux (851) where it mediates fast synaptic transmission (852, 853). Furthermore, pre- and post-synaptic 5-HT_3 receptors exhibit distinct electrophysiological profiles with differences in single channel conductance, kinetics and re-sensitization time-course (827, 854, 855). For example, the permeation properties differ such that pre-synaptic 5-HT_3 receptors are highly permeable to Ca^{2+} (851, 856-858), whereas post-synaptic receptors are less permeable to Ca^{2+} compared to Na^+ and K^+ (844, 859).

Activation of the 5-HT_3 receptor by its physiological ligand 5-HT, leads to the influx of cations through the open ion channel, which causes depolarization of the cell (860). In addition to 5-HT, DA may be another endogenous ligand for 5-HT_3 receptors, as it displays low-affinity agonism of the 5-HT_3 receptor (861). Frequently used 5-HT_3 receptor agonists are 1-(*m*-chlorophenyl)-biguanide (mCPBG), 2-methyl-5-HT and phenylbiguanide. However, they do not readily penetrate the BBB (862, 863), which limits their usefulness in *in vivo* studies. In recent years, SR57227A has been proposed as a high affinity agonist of the 5-HT_3 receptor. SR57227A may be a useful tool to study the function of 5-HT_3 receptor in both *in vitro* and *in vivo* studies (864) given its ability to cross the BBB and its affinity to central 5-HT_3 receptors (863, 865, 866). However, due to the emetogenic and anxiogenic effects of 5-HT_3 agonists, they have no therapeutic potential (839).

In contrast to 5-HT_3 receptor agonists, a variety of highly specific and potent antagonists have been developed in the last three decades and they are currently the gold standard to treat chemotherapy-induced emesis (867). 5-HT_3 receptor antagonists can be identified by the suffix setron, and competitively bind to the orthosteric ligand binding site of 5-HT_3 receptors (837). However, the nature of receptor antagonism varies, which may account for differences in their pharmacokinetic profile (868). The affinities of common 5-HT_3 receptor antagonists are in the low nanomolar concentration range (7.73 to 10.45 nM) and include dolasetron, ondansetron,

granisetron, tropisetron and palonosetron (869). 5-HT_3 antagonists are only non-selective at concentrations 100-fold or greater in excess of those required to antagonize the 5-HT_3 receptor (870, 871) and their non-selective effects include agonism of 5-HT receptors (870, 872), antagonism of non-5-HT_3 receptors (873, 874), and local blockade of ionotropic receptors (875).

In spite of the actions cited above, pharmacological blockade of 5-HT_3 receptors does not modify normal animal behaviour or physiological function in healthy volunteers except for intestinal transit time (818). However, 5-HT_3 receptor antagonists demonstrated clinical efficacy in various forms of emesis like chemotherapy-induced, radiotherapy-induced, and post-operative emesis (867, 876) and extends to other indications such as irritable bowel syndrome (877, 878), anxiety (879), chronic fatigue syndrome (880), alcohol abuse (881), fibromyalgia (882) and migraine (883). Although the use of 5-HT_{3A} knockout mice has not contributed much to the role of 5-HT_3 receptors (805), studies that investigate the effects of specific genetic alterations of 5-HT_3 receptors (884) may further illuminate the function of these receptors.

4.4. 5-HT_3 receptors in Parkinson's Disease and L-DOPA-induced dyskinesia

Pharmacological modulation of the 5-HT system, particularly the 5-HT_{1A} and 5-HT_{1B} receptors, as discussed above, has demonstrated efficacy in preclinical and clinical studies of dyskinesia. Although the 5-HT_3 receptor has been understudied in the context of PD and its role is unknown in LID, its distribution in BG draws attention to the potential of the 5-HT_3 receptor as a novel therapeutic target for dyskinesia. Administration of pharmacological compounds including clozapine (528, 885, 886), mirtazapine (887), quetiapine (770), AQW051(888) and AZD0328 (889) have reduced the severity of dyskinesia in animal models of PD and/or in clinical settings. However, given the non-selective effects of these therapeutic agents, namely as antagonists of the 5-HT_3 receptor (837, 890-896), it is conceivable that pharmacological blockade of 5-HT_3 receptors may have contributed to the anti-dyskinetic effect of these compounds.

The 5-HT_3 receptor is poorly characterized in the BG compared to other members of the 5-HT receptor family (652) and only a few radioligand binding experiments have used highly-specific drugs to study its distribution in these nuclei. There is some controversy amongst the

literature concerning the variation in the binding sensitivity of the selected ligands (897). For instance, radioligand binding of the 5-HT_3 receptor within the rodent striatum differed depending on the molecule used, with low levels with the antagonist radioligands [3H]-GR 65630 (786) while strong levels of binding were observed with the agonist radioligand [3H]-mCPBG (814) and the antagonist radioligand [125I]-iodozacopride (898). Some authours have reported the presence of 5-HT_3 receptors in the striatum of different mammals and intriguingly, they observed relatively higher receptor densities of 5-HT_3 receptors in human striatum compared to rat striatum (788, 809, 812, 813, 899-903), although the functional significance of this species difference is poorly understood. Of note, homogenate binding studies from patients with Huntington's disease (HD) and PD suggest that the 5-HT_3 receptor is localized to GABAergic output neurons of the caudate putamen and not predominantly located on DA neurons (904). In fact, the density of 5-HT_3 receptors was not affected by the neurodegeneration associated with PD, whereas a significant proportion of HD cases showed decreased 5-HT_3 receptor binding in the striatum. HD is neuropathologically characterized by the degeneration of neurons with cell bodies within the caudate putamen, which include MSNs. Thus, these studies suggest that at least a proportion of 5-HT_3 receptors is localized on neurons which degenerate in HD but not on DA terminals which degenerate in PD (904). Furthermore, the use of rat striatal synaptosomes showed the presence of functional pre-synaptic 5-HT_3 receptors as well as its known post-synaptic localization (851, 856, 857). In contrast, membrane binding assays and immunolabelling of the rat and human brains only detected low levels of the 5-HT_3 receptor in the SN (813, 898, 900, 905).

The dysregulated release of DA in the striatum is a potential pre-synaptic mechanism responsible for the progression of dyskinesia in parkinsonian animals and patients with PD. In addition to the distribution of the 5-HT_3 receptor in the striatum and SN, studies also demonstrate that the 5-HT_3 receptor modulates central dopaminergic activity. Indeed, 5-HT agonists stimulate the striatal release of DA *in vitro* (865, 906-910) and *in vivo* (911, 912), which is reversed by 5-HT_3 antagonists (913). In further support of these findings, behavioural studies also report that the 5-HT_3 receptor modifies nigrostriatal DA transmission-mediated motor responses such as stereotypy (914), orofacial dyskinesia (915) or rotations (863). Ondansetron is a potent and highly-selective prototypical 5-HT_3 receptor antagonist used as an anti-emetic in

patients receiving cancer chemotherapy (916). It has been demonstrated that ondansetron reduces basal concentration of DA in the nucleus accumbens (917) and modifies mesolimbic DA activity in the rat and marmoset brains (918, 919), such as the inhibition of amphetamine induced hyperactivity. Moreover, in clinical studies, administration of ondansetron led to improvements in tardive dyskinesia and psychotic symptoms (920) and attenuated psychosis in advanced PD patients (921, 922).

5. Animal models of Parkinson's disease

Animal models of PD, categorized into toxin or genetic, have led to the discovery of novel symptomatic treatments and uncovered mechanisms underlying some features of the disease (59, 923-925). Pharmacological interventions can be used to mimic the motor deficits of parkinsonism including neuroleptic-induced catalepsy (926) or reserpine-induced akinesia (927, 928). The most characterized neurotoxin-induced animals models are the complex I inhibitor of the respiratory chain MPTP in primates (925, 929) and mice (283, 930) and the 6-OHDA rodent model of PD (931). On the other hand, genetic models in mice have more recently been developed (924) including the transgenic overexpression of mutant genes (α-synuclein and LRRK2) or the knockdown or knockout of autosomal recessive genes (PINK1, PARKN, DJ-1) (932), and in transgenic mice, preformed fibrils seed Lewy pathology and decrease survival time (933). Moreover, viral vector-mediated overexpression of α-synuclein in rats (251, 252, 934) and primates (253, 254, 935) have reproduced hallmarks of PD including Lewy-like synucleinopathy, progressive dopaminergic cell loss, and even a parkinsonian behavioural phenotype in rodents (936). Although genetic models have provided greater insight into the molecular mechanisms underlying PD, the recapitulation of genetic alterations discussed above (see section: genetic risk factors of PD, page 5) tends to elicit modest loss of DA neurons (937-940) and does not fully capture the neuropathology of PD (924). Moreover, in spite of the reports of alterations in the motor function and behaviour of animals (939, 941, 942), the behavioural phenotypes are often distinct from the human condition (924). As the experimental work presented in this Thesis was conducted in the 6-OHDA-lesioned rat, the next sections will discuss in depth this animal model of PD.

5.1. The 6-OHDA-lesioned rat

The 6-OHDA-lesioned rat model is the classical and most widely used toxin-based animal model used for both *in vitro* and *in vivo* investigations, and its popularity may be attributed to its cost-effectiveness and minimal labour requirements (943). The catecholamine neurotoxin 6-OHDA is transported into cell bodies and fibres of both dopaminergic and noradrenergic neurons and destroys these neurons on the ipsilateral side relative to its administration (944). Its neurotoxic effect is based on the inhibition of mitochondrial respiratory enzymes (945), which causes oxidative stress and mitochondrial damage (946). Subsequently, these neurons can no longer exert their normal physiological functions and ultimately, die (947). As 6-OHDA has poor penetration across the BBB, it is injected intra-cerebrally into the site of interest (948). Furthermore, the neurotoxin is only relatively selective since it has the ability to destroy dopaminergic and noradrenergic fibres, a concern when injecting into the medial forebrain bundle (MFB), which includes ascending fibre systems from the raphe pontine nucleus and locus coeruleus, respectively (946). Therefore, to achieve the selective destruction of dopaminergic neurons and spare noradrenergic fibres, subjects are pre-treated with despiramine, a noradrenaline transporter blocker, prior to the 6-OHDA lesion (946).

The neurotoxin 6-OHDA can be injected virtually anywhere along the nigrostriatal tract and a seminal study found that 6-OHDA could be injected at the origin of the ascending nigrostriatal DA pathway to produce a nearly complete depletion of DA in the ipsilateral striatum (949). Injection of 6-OHDA into the MFB leads to a close to complete nigrostriatal lesion, with up to 100% loss of dopaminergic terminals in the striatum (950). Generally, 6-OHDA is injected into one of three target sites: SNc, MFB or the striatum (951), where it induces varying degrees of DA denervation (946), depending on what the experimental end-point is. To model PD, the animal model should recapitulate both dopaminergic cell loss and behavioural deficits associated with idiopathic PD (944). For instance, injections of the toxin at the origin of nigrostriatal DA bundle produce large (>97%) DA depleting lesions, which model an advanced stage of PD, while injections in the terminal field of the nigrostriatal pathway produce a partial and slower progressing lesion (952, 953) that would be of greater interest for neuroprotective interventions.

In the present study, it is of interest to study the effects of a therapeutic intervention that alleviate AIMs in the 6-OHDA-lesioned rat model, which resembles more advanced stages of PD. Thus, the injection of 6-OHDA into the MFB, which results in severe DA denervation, was more appropriate for the following experiments.

5.1.1. Injection of 6-OHDA into the MFB

The nigrostriatal dopaminergic pathway consists of the A9 cell group, located in the SNc (944). Axons of these neurons run along the MFB and terminate in the dorsal striatum (954). In PD, the dopaminergic neurons of the A9 cell group undergo extensive loss and cause a dramatic decline in striatal DA, leading to motor impairments (955). Unilateral injection of the MFB causes total destruction of A9 and A10 cell groups (956), resulting in near total depletion of DA in the ipsilateral striatum, denervation supersensitivity of post-synaptic DA receptors in ipsilateral striatum and the characteristic rotational behaviour in response to both D-amphetamine and apomorphine (944).

Although bilateral 6-OHDA lesions of the MFB more closely resemble the bilateral pathology observed in idiopathic PD (944, 957), the survival and problems with daily living such as swallowing and adipsia of the animals limits the use of the bilateral model (958).

Unilateral lesioning the MFB causes can asymmetry in the motor behaviour of rats. Following lesion, rats initially tend to turn preferentially towards the side of the lesion (949, 959), a postural motor asymmetry of behaviour that may recover only slightly if depletion is near total (944). When challenged with drugs acting on the DA system, rat displays active rotational behaviour due to the imbalance in DA activity between two striata, which causes rotational asymmetry such that the animal rotates away from the side of greater activity (931).

5.1.2. Compensation

An important consideration is that by inducing a DA-depleting lesion in rats, animals transition from a normal state to a state of severe parkinsonian symptoms (944). It is possible that compensatory mechanisms come into action to antagonize these neurobiological deficits, for instance, in the rat, PD symptoms may recover to some extent over time (960-963), whereas human idiopathic PD is a progressive disease with PD symptoms worsening with time (944). In

addition, unilateral 6-OHDA-lesioned rats shows compensatory serotonergic hyperinnervation of striatum, an effect that is not observed in human PD (964). This animal model of PD should be interpreted with caution as it may not be predictive of changes in 5-HT receptors in PD.

6. Behavioural testing

6.1. Cylinder test

It is crucial to assess the extent of DA-denervating lesions in animal models and tests of physiological motor behaviour can be performed to estimate lesion severity. Although many interventions have demonstrated anti-dyskinetic efficacy in animal models, their action may at times be due to a motor depressant effect, which is of no benefit in the context of PD as it would exacerbate parkinsonian symptoms (965). Therefore, it is important to assess that the anti-parkinsonian efficacy of a treatment does not interfere with physiological motor behaviour. With the cylinder test, a measure of forelimb use during spontaneous exploration (966), it is possible to determine whether the efficacy of the anti-dyskinetic treatment compromises the therapeutic efficacy of L-DOPA.

The cylinder test, originally described by Schallert and Tillerson, assesses the independent use of each forelimb during explorative activity (966). This test takes advantage of the rats' innate drive to explore a novel environment by standing on their hind limbs, known as rearing, and using their forelimbs to contact the wall (965). Forelimb asymmetry is scored as independent weight bearing contacts on the cylinder wall of the ipsilateral and contralateral forepaws, as well as movements by both forepaws (944). Subsequently, investigators compute a limb use asymmetry score that expresses the performance of each limb as a percentage of total wall contacts. Normal rats use the right and left forepaw indifferently in this test, whereas unilaterally 6-OHDA-lesioned rats use the forepaw contralateral to lesion in about 10-30% of total supporting wall contacts (885). Use of the paw ipsilateral to lesion in ≥70% of all wall contacts is indicative of 88% nigrostriatal dopaminergic denervation and is used as a cut-off threshold for animal inclusion in studies (967).

The cylinder test offers several advantages as a measure of physiological motor performance (965). On a conceptual level, the test is a true measure of spontaneous forelimb use as the movements exhibited by the rat in the testing cylinder are identical to those performed

in its home cage. In addition, it is simple, objective and rapidly executed and does not require pre-training of animals or extensive manipulation. Moreover, the inter-rater reliability is very high ($r > 0.95$), even with relatively inexperienced raters (966). Some investigators have argued against drug-induced rotational behaviour as a reliable indicator of nigrostriatal DA depletion (948, 968). Accordingly, the cylinder test is a drug-free test sensitive to the disrupting effects of DA denervating lesions and allows the animals to remain in a drug-naïve state, which is critical to the success of certain experimental paradigms. In addition, 6-OHDA-lesioned rats show robust forepaw asymmetry and the cylinder test is sensitive to the motor improvement produced by anti-parkinsonian compounds (see section: administration of ondansetron does not impair the therapeutic efficacy of L-DOPA in the cylinder test, page 96). Treatment with L-DOPA improves performance in the cylinder test, *i.e.* greater use of the contralateral parkinsonian forepaw, and is disrupted by the appearance of L-DOPA induced AIMs. The main drawback of the cylinder test is its relatively narrow dynamic range (from 20-50% of the contralateral forepaw) and with many repetitions of the test, the animal loses interest in exploring the novel environment and the total number of wall contacts gradually declines (965). Therefore, it is important to limit the frequency of testing sessions to a twice-weekly or weekly basis to avoid compromising test sensitivity.

6.2. ALO AIMs

Traditionally, experimental studies of LID were exclusively performed in nonhuman primates. Existing literature on LID in the parkinsonian rodent assumed that the responsiveness to L-DOPA could only be measured with tests of contralateral rotation and many investigators expressed scepticism about modelling PD symptoms and treatment related dyskinesia in rodents (885). In fact, it was believed that only primates could show the repertoire of movement disorders displayed by patients (604), which could not be evaluated in rats. For decades, the Ungerstedt model was the gold standard model of rodent research (931) and used as a screen for potential anti-parkinsonian agents where, following administration of drugs that stimulate dopamine receptors, the animal turns away the site of lesion, *i.e.* displays contralateral turning (969).

In the late 1990s, Cenci and collaborators were the first to develop the AIMs rating scale in the L-DOPA-treated 6-OHDA-lesioned rat (602). In addition to the sensitized rotational

behaviour displayed by dyskinetic rats, they also exhibit abnormal movements and postures affecting the trunk, limb and orofacial muscles contralateral to lesion. The quantification of AIMs in rats gradually replaced the test of contralateral rotation (970, 971), which does not always correlate with the development of dyskinesia (885). L-DOPA-induced AIMs in rats present functional and phenomenological analogies to LID in PD patients (965). Phenotypically, the movements are complex and involve different muscle groups that include tonic torsion of the upper trunk and neck and are associated with repetitive head movements and rapid flexion of the forelimb, similar to the choreiform on-dyskinesia exhibited by patients (965). Functionally, these movements are involuntary and disabling as are LID in PD patients (965).

The standard L-DOPA treatment to induce dyskinesia consists of a priming phase of single daily intraperitoneal injections of L-DOPA for two to three weeks, depending on the dose of L-DOPA administered, followed by a maintenance of priming by two to four injections per week to maintain stable dyskinesia over long-term (965). Once established, the brain maintains its primed state, and even after stopping the initial therapy, a single acute L-DOPA or DA agonist administration can elicit LID at nearly the same severity (440). With a daily dose of 6-10 mg/kg L-DOPA (combined with 15 mg/kg benserazide), approximately 50 to 80% of the treated rats develop AIMs by the end of the treatment period (965). The latency for the initial appearance of dyskinesia varies amongst individual rats (602) and the incidence of dyskinesia can be boosted and its latency shortened with higher L-DOPA doses (972).

To quantify drug-induced AIMs, rats are individually placed in a transparent cylinder and observed for two minutes every twenty minutes for three hours following the injection of L-DOPA (885, 973). Rat AIMs are classified into four subtypes based on their topographic distribution: axial AIMs, which are dystonic postures or choreiform twisting of the neck and body towards the side contralateral to lesion; limb AIMs, which are abnormal purposeless movements of the forelimb and digits contralateral to lesion; orolingual AIMs are empty jaw movements and contralateral tongue protrusions; and locomotive AIMs which are increased locomotion with contralateral side bias (946). Although locomotive AIMs, contralateral rotation, are part of the dyskinetic expression, it is not a specific predictor of dyskinesia as it may result from increased locomotor activity in rats that display sensorimotor asymmetry, as discussed above. The relative presentation of different AIM subtypes may differ amongst the

animals but is very consistent in the same animal upon repeated testing (965). To increase the sensitivity of the test, the authours included an additional scale based on the amplitude of dyskinetic movements (Table IV, page II). The amplitude scale is scored simultaneously with the duration scale as described above (based on the duration and frequency). Lastly, only movements that are phenomenologically distinguishable from stereotypic behaviour for the rat are classified as dyskinetic, whereas enhanced manifestations of normal motor activities (grooming, gnawing, rearing and sniffing) are not included in the rating (974).

In seminal studies, Lundblad and colleagues compared the effects of drugs with varying dyskinesiogenic potential on motor performance in the 6-OHDA-lesioned rat model (885). They reported an attenuation of AIMs by non-dopaminergic compounds with proven anti-dyskinetic efficacy in patients and/or primates. In contrast, AIMs were not induced by anti-parkinsonian treatments with low dyskinetic potential in primates (975). These studies were the first demonstrations that clinically-relevant measures of parkinsonian akinesia and dyskinesia could be obtained in rats (885). Furthermore, it demonstrated that rat AIMs share similar pharmacological properties to primate models of LID, particularly with respect to modulation of neurotransmitter systems to control the expression of dyskinesia (885, 973, 976-978).

Another study compared the effects of various non-dopaminergic compounds on both L-DOPA-induced AIMs and L-DOPA-induced motor improvement in 6-OHDA lesioned rats (973). Again, the AIMs model demonstrated a high degree of predictive validity as interventions with proven anti-dyskinetic action in primate models and in PD patients also modulated rodent axial, limb and orolingual (ALO) AIMs. However, treatments that specifically alleviated the severity of trunk, limb and orofacial dyskinesia did not necessarily reduce locomotive AIM scores. Treatments that specifically produced a decrease in ALO AIMs scores neither interfered with normal rat behaviour (*e.g.* locomotion, exploration, grooming) nor affected the locomotive AIM scores or rotarod activity.

7. Objectives and hypotheses

As presented above, growing evidence supports a pivotal role of the 5-HT system in the pathogenesis of LID. However, no studies have evaluated the effect of 5-HT_3 receptors on dyskinesia expression. Thus, the present study seeks to determine and validate the efficacy of

5-HT_3 receptor antagonism as a new therapeutic strategy to alleviate L-DOPA-induced AIMs in the 6-OHDA-lesioned rat model of PD. More specifically, we hypothesize that:

1. 5-HT_3 receptor blockade reduces the severity of established L-DOPA-induced AIMs;
2. 5-HT_3 receptor blockade attenuates the priming process that leads to the development of L-DOPA induced AIMs;
3. 5-HT_3 receptor blockade does not impair the therapeutic efficacy of L-DOPA on parkinsonian features.

To validate these hypotheses, we will meet the following aims:

1. To determine the effect of acute challenges of the highly-selective 5-HT_3 antagonist ondansetron at alleviating established L-DOPA-induced AIMs;
2. To determine the effect of selective 5-HT_3 receptor blockade with ondansetron on the development of L-DOPA-induced AIMs, in the context of a *de novo* study;
3. To assess whether the anti-dyskinetic benefit of 5-HT_3 antagonism with ondansetron is achieved without compromising the anti-parkinsonian action of L-DOPA.

Positive outcomes of the proposed experiments would provide support for 5-HT_3 receptor antagonism as a new and effective therapeutic approach to alleviate L-DOPA induced dyskinesia in the 6-OHDa lesioned rat model of PD. Moreover, positive results would demonstrate the potential of a new target to achieve an anti-dyskinetic effect without impairing L-DOPA anti-parkinsonian action. Given that ondansetron and other 5-HT_3 receptor antagonists are clinically available and well tolerated, positive outcomes could rapidly lead to Phase IIa clinical trials, and enhance the quality of life of D patients.

II. Material and methods

Animals

Adult female Sprague-Dawley rats (225 – 250 g, Charles River, Saint-Constant, Canada) were group-housed in a temperature, humidity- and light-controlled environment (under 12-h light/dark cycle, on 07:00) with free access to food and water. Experimental protocols were approved by Centre de Recherche du Centre Hospitalier de l'Université de Montréal Animal Care Committee in agreement to guidelines established by the Canadian Council on Animal Care. Upon arrival, rats were left undisturbed to acclimatize to the housing conditions for at least 5 days before experiments.

Dose-finding pharmacokinetic study

Based on doses of ondansetron used in the literature (979), a preliminary dose-finding pharmacokinetic (PK) study was conducted to determine clinically-relevant plasma levels of ondansetron in the rat. Blood was collected from animals ($N = 2$) by jugular vein puncture using a sparse sampling technique, as previously described (980), serial blood samples of 150 µL were collected prior to, and at the following time points: 2 min, 5 min, 15 min, 30 min, 1 h, 2 h, 3 h, and 4 h after subcutaneous administration of ondansetron 0.01 mg/kg. Samples were gently inverted and placed on ice pending centrifugation (1500g for 10 minutes at 4°C). Following centrifugation, aliquots of approximately 80 µL of plasma were stored at -80°C until analysis. The analytical method to quantify ondansetron in the plasma consisted of protein precipitation followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS), which was done in collaboration with Dr Francis Beaudry and Ms Fleur Gaudette from Faculté de Médecine Vétérinaire de l'Université de Montréal and Centre de Recherche du Centre Hospitalier de l'Université de Montréal.

Unilateral 6-OHDA lesion

Animals were rendered hemi-parkinsonian by unilateral injection of 6-OHDA into the right MFB as previously described (967). Animals were pre-treated with pargyline (5 mg/kg) and desipramine (10 mg/kg) 30 min prior to surgery. Under general anaesthesia (3% isoflurane in 95% O_2, 5% CO_2), rats were positioned onto a stereotaxic frame (Kopf Instruments, Tujunga, USA). 6-OHDA (7 mg/mL) was dissolved in 0.02% ascorbic saline. The 6-OHDA solution (2.5

µL) was injected into the right MFB using a 10 µL Hamilton microsyringe with a 30–gauge needle (at a flow rate of 0.5 µL/min) at the following coordinates: anteroposterior -2.8 mm, lateromedial -2.0 mm and dorsoventral -9.0 mm from Bregma and skull surface (981), with the incisor bar set at 3.3 mm below ear bars. The injection was done over 5 min and the needle was left in place for an additional 5 min before slowly retracting the needle to avoid reflux. At the end of surgery and for two additional days post-op, animals received carprofen (5 mg/kg) as analgesic treatment.

Cylinder test

Following a 14-day post-lesion recovery period, animals underwent the cylinder test to assess the extent of dopaminergic degeneration (966). Rats were placed in a transparent cylinder (14 cm diameter × 18 cm height) and recorded for 10 min. A mirror was placed behind the cylinder to enable the evaluator to count forelimb movements when the animal was turned away from the camera. Several behaviours were scored to determine the extent of forelimb-use asymmetry displayed by the animal during the 10-min period and analysed *post hoc*. The first limb to contact the wall during a rear or weight-shifting movement was scored as an independent wall placement for that limb. A subsequent placement of the other limb on the wall while maintaining the initial movement was scored as a "both" movement. A simultaneous placement of both forepaws on the walls was also considered a "both" movement. Another wall movement score was attributed only if both paws were removed from the vertical surface. Only animals exhibiting preferential use of the un-lesioned forelimb in ≥ 70% of the rears were selected to undergo further behavioural pharmacological testing. As mentioned above, this rearing asymmetry score indicative of ≥ 88% striatal dopamine depletion (967).

Drug treatments

Despiramine hydrochloride, pargyline hydrochloride, 6-OHDA hydrobromide, L-DOPA methyl ester, benserazide hydrochloride and ondansetron hydrochloride were purchased from Sigma-Aldrich (St Louis, USA). All drugs were dissolved in saline (sodium chloride 0.9% w/v) except for 6-OHDA and ondansetron hydrochloride, which were dissolved in ascorbate-saline and dimethyl sulfoxide at 100 mg/mL, respectively, and the latter was diluted to the appropriate concentrations in saline. All solutions were subcutaneously injected in a volume of 1.0 mL/kg

body weight. In the acute challenge study, treatments were randomized according to a Latin square design and behavioural testing sessions were separated by at least 48 h of drug washout.

Experimental design

The experimental design for the acute challenges and *de novo* studies is described in Figure 3, page 70.

Acute challenges of ondansetron study

6-OHDA lesion surgery was performed on 35 Sprague-Dawley rats. Following assessment of parkinsonism, 18 lesioned animals (~ 51%) exhibiting severe rearing asymmetry were selected and were primed daily with L-DOPA/benserazide (10/15 mg/kg, from now on referred to as L-DOPA) for 14 days to elicit stable and reproducible AIMs. Once AIMs were expressed, on days on behavioural testing, rats were administered L-DOPA (6/15 mg/kg) in combination with ondansetron (0.0001, 0.001, 0.01 0.1 and 1mg/kg) or vehicle, and AIMs were assessed, as described below (Section: ratings of AIMs, page 71), by a blinded rater.

De novo ondansetron study

In another set of experiments, rats were rendered hemi-parkinsonian by 6-OHDA injection in the MFB as described above. Following recovery and after assessment of the extent of lesion, rats were administered a once daily treatment of L-DOPA (6/15 mg/kg) in combination with ondansetron (0.0001 mg/kg, group 1, or 0.001 mg/kg, group 2, both $n = 9$) or vehicle (group 3, $n = 7$) for 22 days. ALO AIMs were assessed on days 1, 8, 15 and 22 by an experimenter blinded to treatment conditions. After a 3-day washout period, animals were administered an acute 6/15 mg/kg L-DOPA challenge and AIMs severity was assessed.

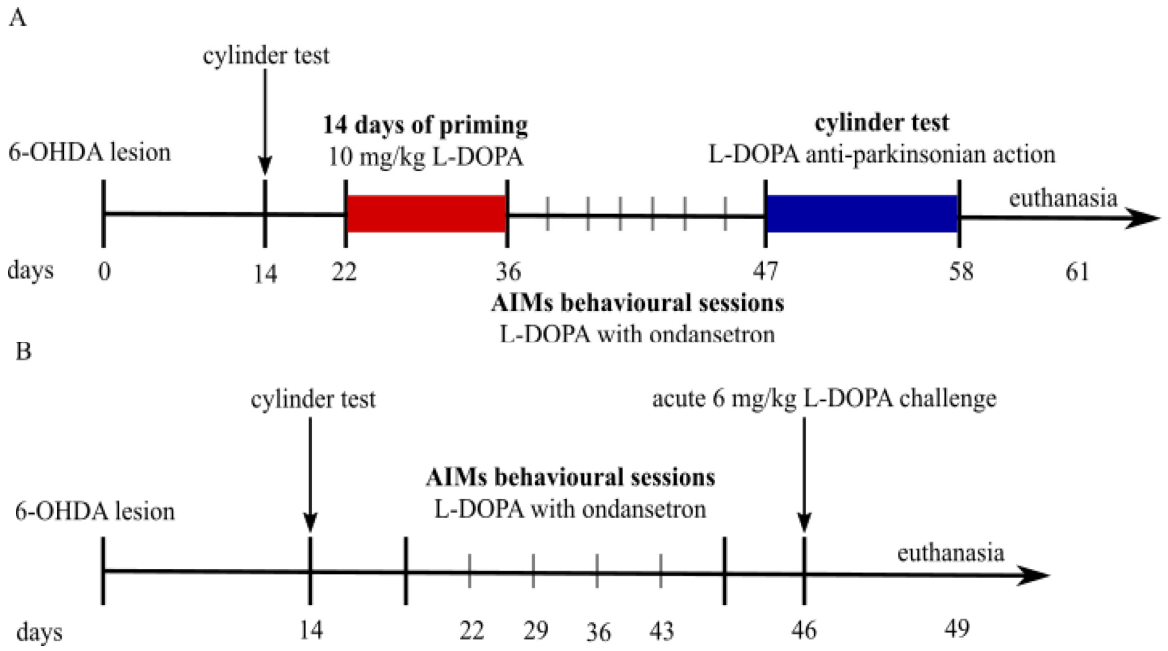


Figure 3: Schematic representation of the experimental design. A. Timeline of the acute challenge experiments. 6-OHDA lesioned animals underwent a L-DOPA priming phase to induce dyskinesia and the effect of acute ondansetron on the severity of ALO AIMs was evaluated. **B.** Timeline of the de novo experiments. Animals received treatment concurrently as their first L-DOPA dose, and this treatment regimen was maintained daily with weekly assessments of the progression of ALO AIMs, followed by an acute L-DOPA challenge after a washout period.

Ratings of AIMs

On days of behavioural scoring, after injection of L-DOPA, rats were put in individual glass cylinders and observed for 2 minutes every 20 minutes over a 180 min testing session, as previously described (967). The severity of dyskinesia was evaluated using a validated rat AIMs scale, where ALO AIMs were each scored (885). Each AIMs subtype was rated on a duration severity scale from 0 to 4 (Table III, page I) in each monitoring interval where: 0 = no dyskinesia; 1 = occasional signs of dyskinesia; 2 = frequent signs of dyskinesia; 3 = continuous dyskinesia but interrupted by external stimuli and 4 = continuous dyskinesia not interrupted by external stimuli. The amplitude of AIMs was rated from 0 to 4 (Table IV, page II). Axial AIMs are the twisting of the neck and upper body toward the contralateral side to the lesion and amplitude are rated according to the following scale: 1= sustained deviation of the head and neck at ~30° angle; 2 = sustained deviation of the head and neck at an angle of 60° or more; 3

= sustained twisting of the head, neck and upper trunk at an angle greater than 60° but up to 90° and 4 = sustained twisting of the head, neck and trunk at an angle greater than 90°, causing the rat to lose balance from a bipedal position. Limbs AIMs consist of jerky or dystonic movements of the contralateral limb and are rated as follows: 1 = tiny movements of the paw around a fixed position; 2 = movements leading to visible displacement of the limb; 3 = large displacement of the limb with contraction of shoulder muscles and 4 = vigorous limb displacement of maximal amplitude, with concomitant contraction of shoulder and extensor muscles. Orolingual AIMs consist of movement of jaw muscles and tongue protrusions and amplitude are rated as: 1 = twitching of facial muscles accompanied by small masticatory movements without jaw opening; 2 = twitching of facial muscles accompanied by masticatory movements that occasionally result in jaw opening; 3 = movements with broad involvement of facial muscles and masticatory muscles, with frequent jaw opening and occasional tongue protrusion and 4 = involvement of all of the above muscles to the maximal possible degree. The ALO AIMs score represents the sum of axial, limbs and orolingual AIMs scores during the behavioural session, and this expression of AIMs scores is sensitive to the anti-dyskinetic effects of drugs used in the clinic (885, 973). The axial limbs (AL) AIMs score represents the sum of axial and limbs AIMs scores on all monitoring periods.

Assessment of L-DOPA anti-parkinsonian action

To assess whether the anti-dyskinetic effect of ondansetron affects the therapeutic efficacy of L-DOPA, preference for the un-lesioned forelimb in the cylinder test was evaluated as described above. Rats used in the acute challenge study underwent a 3-day washout period, after which they were administered a low dose of L-DOPA (3/15 mg/kg), to avoid triggering AIMs, in combination with vehicle or ondansetron (0.0001 0.001, 0.01, 0.1, 1 mg/kg). 45 min later, at peak anti-parkinsonian action, animals underwent the cylinder test, in which the number of rears of each paw was counted, *post hoc*, by a treatment-blinded experimenter.

Perfusions

Perfusions were performed at least 48 h post-administration of L-DOPA. Under general anaesthesia (4% isoflurane in 95% O_2, 5% CO_2), rats were euthanised by exsanguination by

transcardial perfusion with saline (982). After the toe pinch-response was used to determine depth of anaesthesia, animals were secured in a supine position on the work surface. Using sharp scissors, incisions were made along the thoracic midline and lateral to the ventral ribcage to expose the thoracic field. The rib cage was then cut through to open up the thoracic cavity and, using blunt scissors, the diaphragm was separated from the chest wall and the tissue connecting the sternum to the heart was cleared. The heart was subsequently secured with blunt forceps before an 18-gauge perfusion needle was inserted into the left ventricle and the needle position was secured by clamping a hemostat near the point of entry. An incision to right atrium was then made with scissors and animals were infused with a steady flow of their corresponding body weights of 0.9% saline (until the fluid exiting the right atrium was clear). Brains were rapidly removed, flash-frozen at – 55ºC in isopentane and stored at -80ºC until collection of striatal tissue.

LC-MS/MS analysis for dopamine and its metabolites

The extent of nigrostriatal lesion was determined LC-MS/MS (983). A 30-µm diameter tissue punch of the left and right striata was obtained and deposited into separate 1.5 mL sterile microcentrifuge tubes and stored at –80ºC until LC-MS/MS analysis (984). A piece of comparable size of cerebellum was also dissected from each brain as a control (985). Quantification of the biogenic amine DA and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and HVA in the striatal punches is currently being conducted in collaboration with Dr Lehka Sleno from Université de Québec à Montréal and the results will be presented in a peer-review article.

Statistical Analysis

Pharmacokinetic study

Calculations of the peak plasma concentration (C_{max}), time of C_{max} (t_{max}) and half-life ($t_{1/2}$) were done with Microsoft Office Excel (Microsoft Corporation, Redmond, USA) (986).

Cylinder test

Data from the cylinder test were graphed as the mean ± standard error of the mean (SEM) and were analysed using one-way analysis of variance (ANOVA) with the Greenhouse-Geisser correction; *post hoc* comparisons with the lesion control and L-DOPA/vehicle control groups were performed using Tukey's *post hoc* test.

Taking into consideration the variation in standard deviations across treatment conditions in Figures 10B and 10C, a measure of effect size was provided using Glass' delta (see Tables V and VI in Appendices III and IV). This estimate is recommended for unequal variances and under the assumption that any measure on control is untainted by the effect, the standard deviation of the control group is used to standardize the differences between means to minimize bias (987) as calculated by the formula for Glass' delta (Figure 11 in Appendix V).

Acute challenges of ondansetron study

AIMs scores were expressed as the median with interquartile interval. In the acute study, comparisons of AIM scores used the cumulative score over the entire testing session or the peak of L-DOPA action, the interval from 40-120 min post-drug administration. Results were analysed using nonparametric Friedman test, followed by Dunn's *post hoc* test.

De novo ondansetron study

The *de novo* AIMs timecourse analysis was performed using two-way ANOVA, followed by Tukey's *post hoc* test. Data from the *de novo* challenge AIMs scores underwent a squareroot transformation (988), and were subsequently analysed by one-way ANOVA, followed by Tukey's *post hoc* test.

The threshold for statistical significance was assigned at $P < 0.05$. Statistical analyses were performed with GraphPad Prism 7.03 (GraphPad Software Inc., La Jolla, California, USA).

III. Results

Pharmacokinetic profile of ondansetron

As illustrated in Figure 4 (page 76), the plasma levels of subcutaneous administration ondansetron are mapped over a time course. The ondansetron PK parameters assessed in the preliminary dose-finding ondansetron PK are summarised in Table II (page 76). C_{max} was 2.31 ng/mL while t_{max} and $t_{1/2}$ were 15 and 39 min, respectively.

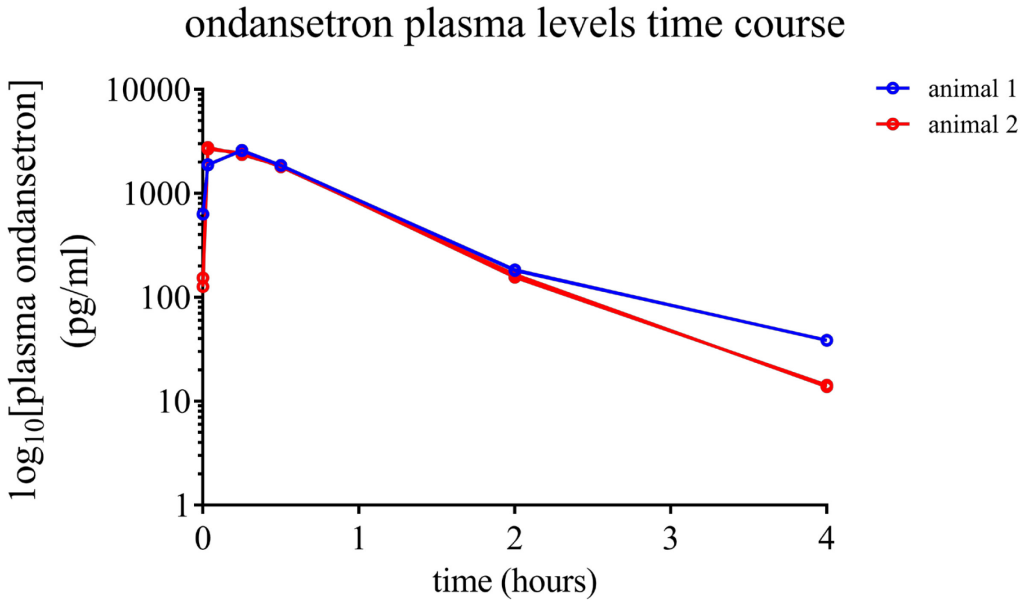


Figure 4: Plasma levels of ondansetron in a preliminary pharmacokinetic study. Logarithmic (log10) time course of ondansetron plasma levels following subcutaneous administration of 0.01 mg/kg ondansetron (n = 2). Data are presented as the mean.

Table II: Ondansetron pharmacokinetic parameters in the 6-OHDA-lesioned rat

	ondansetron
C_{max} (ng/mL)	2.31
t_{max} (min)	15
$t_{1/2}$ (min)	39

C_{max}: maximal plasma concentration; t_{max}: time at maximal plasma levels $t_{1/2}$: plasma half-life. Ondansetron 0.01 mg/kg was administered subcutaneously to the animals (n = 2). Data are presented as the mean.

Extent of dopaminergic denervation assessed in the cylinder test

Following 6-OHDA lesion, only animals that displayed marked forelimb asymmetry, i.e. used the right (un-lesioned) forepaw to initiate ≥ than 70% of wall contacts during the cylinder test, a score that is indicative of ≥ 88% striatal DA depletion (967), were selected for the behavioural studies. As shown in Figure 5A (page 77), animals that underwent the acute challenges of ondansetron study displayed forelimb asymmetry ($F(2, 39) = 296.6$, $P < 0.0001$, one-way ANOVA) with marked preferential use of the right forepaw in 83% of wall contacts when compared to 0.4% with the left forepaw and 15% with both forepaws, respectively (both $P < 0.0001$, Tukey's *post hoc* test). As illustrated in Figure 5B (page 77), in the *de novo* ondansetron study, animals also displayed marked forelimb asymmetry ($F(2, 78) = 1017$, $P < 0.0001$, one-way ANOVA), and preferred the use of the right forepaw in 85% rears when compared with 0.8% and 15% of rears using the left forepaw and both forepaws, respectively (both $P < 0.0001$, Tukey's *post hoc* test).

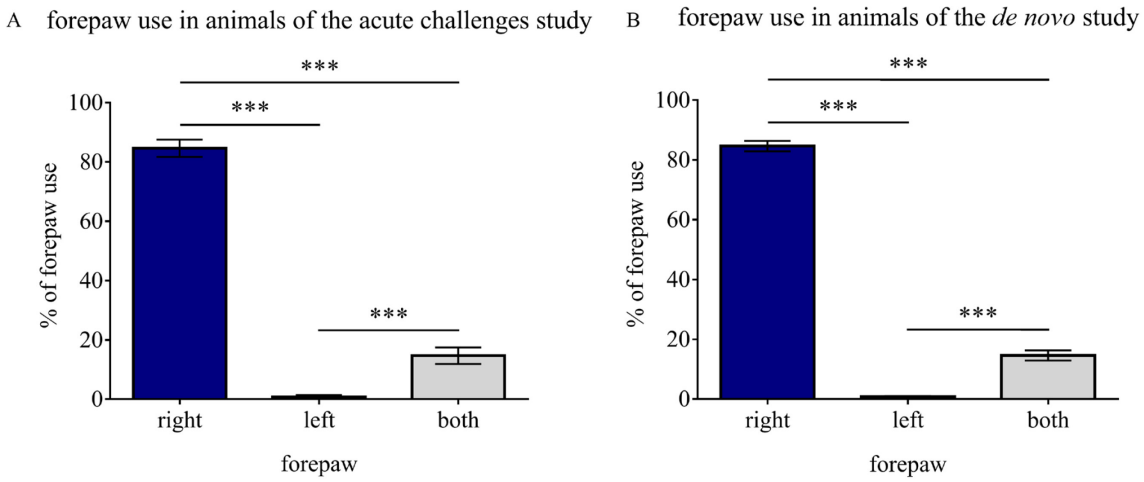


Figure 5: Performance in the cylinder test in drug-naïve lesioned animals. A. Animals (n = 18) selected to undergo acute challenges of ondansetron show a marked preference for the un-lesioned (right) forepaw in 83% of rears compared with 0.4% and 17% of rears using the lesioned (left) forepaw and both forepaws, respectively. **B.** In the *de novo* ondansetron study, animals (n = 24) prefer the right forepaw in 85% of rears while the left forepaw and both forepaws, only accounted for 0.8% and 15% of rears, respectively. Data are presented as the mean ± SEM. **: $P < 0.01$, ****: $P < 0.0001$.

Acute challenges of ondansetron at 0.0001 mg/kg significantly alleviated the severity of established AIMs

In the acute challenges of ondansetron experiments, daily administration of L-DOPA 10 mg/kg for two weeks to 6-OHDA-lesioned rats induced stable and reproducible dyskinetic behaviour. Animals subsequently received acute challenges of ondansetron or vehicle in combination with L-DOPA and AIMs severity was assessed.

Duration of axial AIMs

As shown in Figure 6A and 6B (page 80), administration of ondansetron in combination with L-DOPA reduced the duration of cumulative and peak axial AIMs (Friedman Statistic [FS] = 32.16, $P < 0.001$ and FS = 21.14, $P < 0.01$, respectively). Thus, when ondansetron 0.0001 mg/kg and 0.001 mg/kg was added to L-DOPA, the duration of cumulative axial AIMs was reduced by 55% and 52%, respectively, when compared to L-DOPA/vehicle ($P < 0.001$ and $P < 0.01$, Dunn's *post hoc* test, Figure 6A, page 80). The addition of ondansetron 0.0001 mg/kg also reduced peak axial AIMs duration when compared with vehicle by 49%, compared to L-DOPA alone ($P < 0.01$, Dunn's *post hoc* test, Figure 6B, page 80). Peak axial AIMs duration was also reduced with treatment of ondansetron 0.001, 0.01 and 1 mg/kg compared to vehicle but did not reach statistical significance.

Duration of limbs AIMs

As illustrated in Figure 6C (page 80), adding ondansetron to L-DOPA resulted in a significant reduction in the duration of cumulative limbs AIMs (FS = 20.76, $P < 0.001$). Thus, administration of ondansetron 0.0001 and 0.001 mg/kg in combination with L-DOPA reduced the duration of cumulative limbs AIMs was reduced by 49% and 41%, respectively, when compared with L-DOPA/vehicle ($P < 0.01$ and $P < 0.05$, Dunn's *post hoc* test). Administration of ondansetron did not significantly diminish the duration of peak limbs AIMs (FS = 10.71, $P > 0.05$, Figure 6D, page 80). The duration of limbs AIMs scores was also reduced with treatment of ondansetron 0.001, 0.01 0.1 and 1 mg/kg compared to vehicle but did not reach statistical significance.

Duration of orolingual AIMs

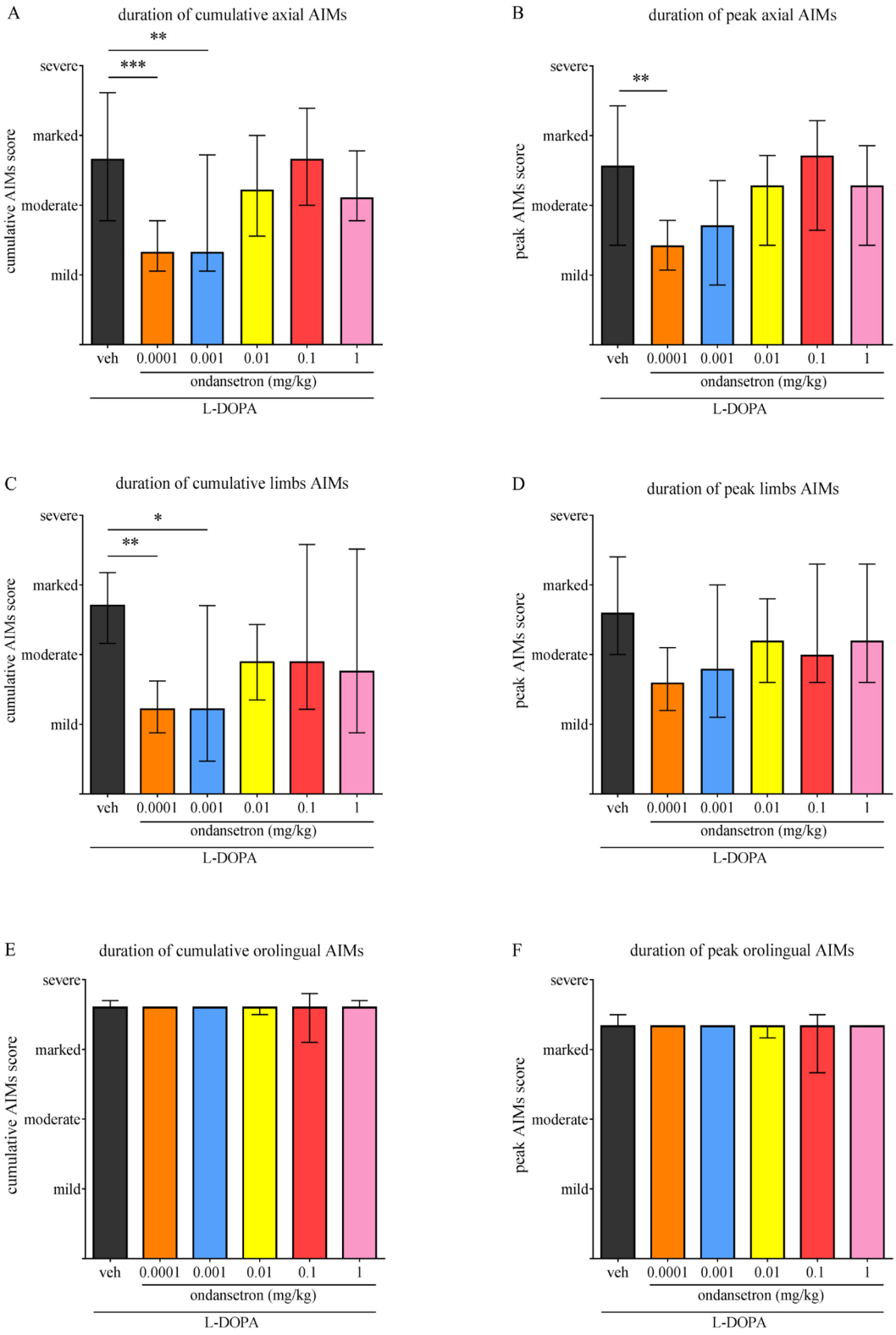
As illustrated in Figures 6E and 6F (page 80), the severity and duration of cumulative and peak orolingual AIMs was comparable between the doses of ondansetron (0.0001, 0.001, 0.01, 0.1 and 1 mg/kg) and vehicle (FS = 5.826, $P > 0.05$ and FS = 4.833, $P > 0.05$, respectively).

Duration of AL AIMs

As shown in Figures 6G and 6H (page 81), the addition of ondansetron to L-DOPA resulted in a significant decrease in the duration of cumulative and peak AL AIMs (FS = 29.01, $P < 0.0001$ and FS = 19.43, $P < 0.01$, respectively). Thus, treatment with ondansetron 0.0001 and 0.001 mg/kg led to a marked decrease in the duration of cumulative AL AIMs by 57% and 47%, respectively, when compared with L-DOPA/vehicle ($P < 0.001$ and $P < 0.01$, Dunn's *post hoc* test, Figure 6G, page 81). Animals that received ondansetron 0.0001 mg/kg also exhibited a 54% reduction in the duration of peak AL AIMs, when compared with vehicle ($P < 0.001$, Dunn's *post hoc* test, Figure 6H, page 81). The duration of cumulative and peak AL AIMs scores was also diminished with administration of ondansetron 0.001, 0.01, 0.1 and 1 mg/kg compared to vehicle but this was not statistically significant.

Duration of ALO AIMs

As shown in Figures 6I and 6J (page 81), administration of ondansetron in combination with L-DOPA led to a significant reduction in the duration of cumulative and peak ALO AIMs, respectively (FS = 23.93, $P < 0.0001$ and FS = 17, $P < 0.001$). Thus, administration of ondansetron 0.0001 and 0.001 mg/kg decreased the duration of cumulative ALO AIMs by 53% and 43%, respectively, when compared with vehicle ($P < 0.01$ and $P < 0.05$, Dunn's *post hoc* test, Figure 6I, page 81). The duration of peak ALO AIMs was reduced with ondansetron 0.0001 mg/kg compared to vehicle by 51% ($P < 0.01$, Dunn's *post hoc* test, Figure 6J, page 81). In addition, cumulative and peak ALO AIMs scores were also diminished with treatment of ondansetron 0.001, 0.01, 0.1 and 1 mg/kg compared to vehicle but did not reach statistical significance.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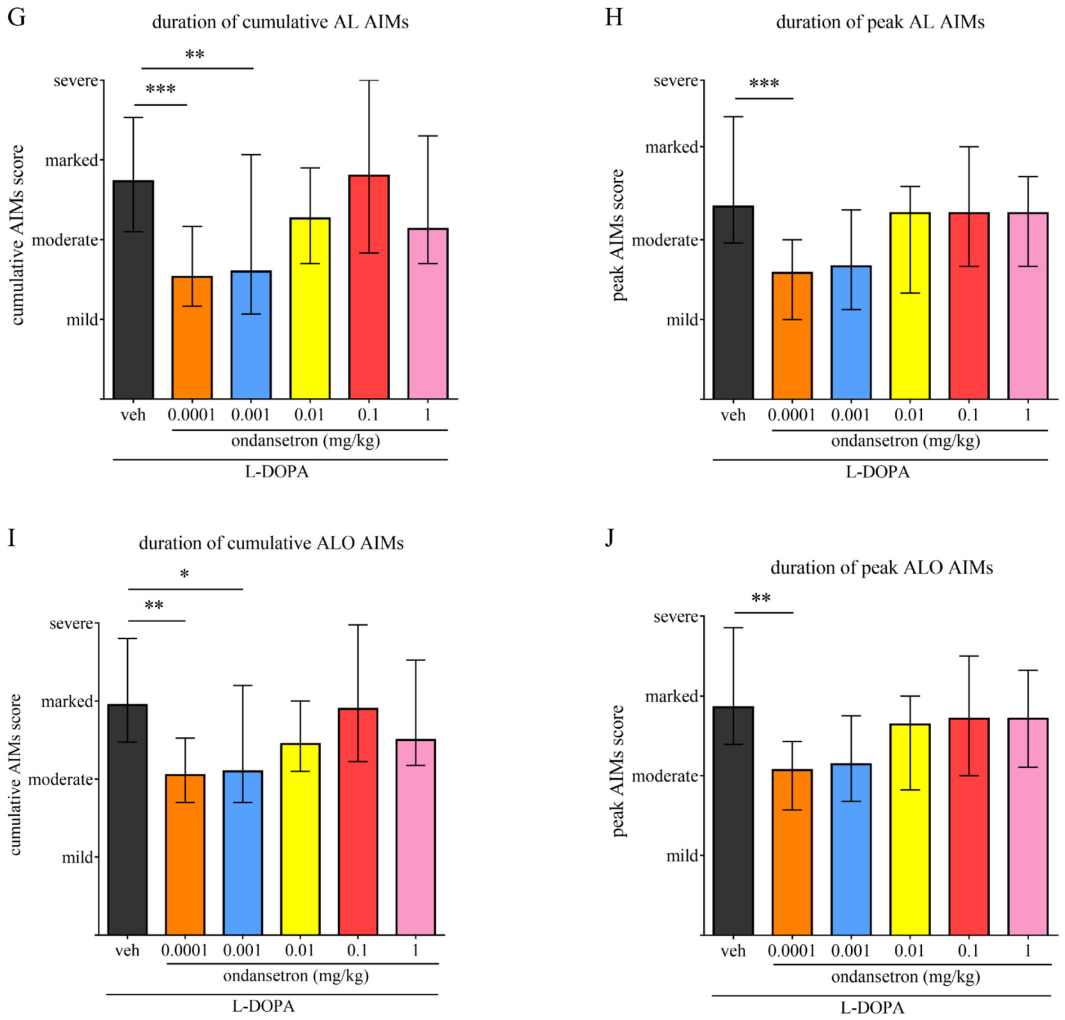


Figure 6: Effect of acute challenges of ondansetron on the duration of established L-DOPA induced AIMs. A. Administration of 0.0001 mg/kg ondansetron in combination with L-DOPA diminished the duration of cumulative and **B.** peak axial AIMs when compared with vehicle. **C.** Similarly, L-DOPA/ondansetron 0.0001 mg/kg resulted in a reduction in the duration of cumulative limbs AIMs, when compared with vehicle but had **D.** no effect on peak limbs AIMs when compared with L-DOPA/vehicle. **E. F.** Adding ondansetron to L-DOPA had no effect on orolingual AIMs when compared to L-DOPA/vehicle. **G.** With respect to the duration of AL AIMs, ondansetron 0.0001 mg/kg resulted in less severe cumulative AL AIMs, **H.** as well as a decrease in the duration of peak AL AIMs. **I.** The combination of ondansetron 0.0001 mg/kg and L-DOPA led to a significant reduction in the severity of cumulative ALO AIMs **J.** and a marked decrease in the duration of peak AIMs scores. Cumulative and peak duration AIMs scores are expressed as median with interquartile interval. $n = 18$ for all treatment conditions. *: $P < 0.05$; **: $P < 0.01$, ***: $P < 0.001$ and ****: $P < 0.0001$.

Amplitude of axial AIMs

As illustrated in Figures 7A and 7B (page 84), during the assessment of AIMs, ondansetron significantly decreased the amplitude of cumulative and peak axial AIMs was reduced with ondansetron (FS = 54.34, $P < 0.0001$ and FS = 46.61, $P < 0.01$, respectively). Thus, after administration of ondansetron 0.0001 and 0.001 mg/kg, the amplitude of axial AIMs diminished by 67% and 66%, respectively, when compared with L-DOPA/vehicle (both $P < 0.0001$, Dunn's *post hoc* test, Figure 7A, page 84). In addition, peak axial AIMs also diminished with 0.001 and 0.001 mg/kg ondansetron by 60% and 61%, respectively, when compared with vehicle (both $P < 0.0001$, Dunn's *post hoc* test, Figure 7B, page 84). Higher doses of ondansetron (0.01, 0.1 and 1 mg/kg) also reduced cumulative and AIMs amplitude scores but did not reach statistical significance.

Amplitude of limbs AIMs

As shown in Figures 7C and 7D (page 84), the addition of ondansetron to L-DOPA resulted in a decrease in the amplitude of cumulative and peak limbs AIMs, respectively, when compared with L-DOPA/vehicle (FS = 28.79, $P < 0.0001$ and FS = 20.48, $P < 0.01$, respectively). Thus, ondansetron 0.0001 and 0.001 mg/kg diminished the amplitude of cumulative limbs AIMs by 48% and 55%, respectively, compared to vehicle ($P < 0.01$ and $P < 0.001$, Dunn's *post hoc* test, Figure 7C, page 84). In addition, animals treated with ondansetron 0.0001 and 0.001 mg/kg exhibited a decrease in peak axial AIMs amplitude scores, compared to vehicle, by 44% and 45%, respectively (both $P < 0.05$, Dunn's *post hoc* test, Figure 7D, page 84). The amplitude of AIMs scores was also reduced with higher doses of ondansetron compared to vehicle but did not reach statistical significance.

Amplitude of orolingual AIMs

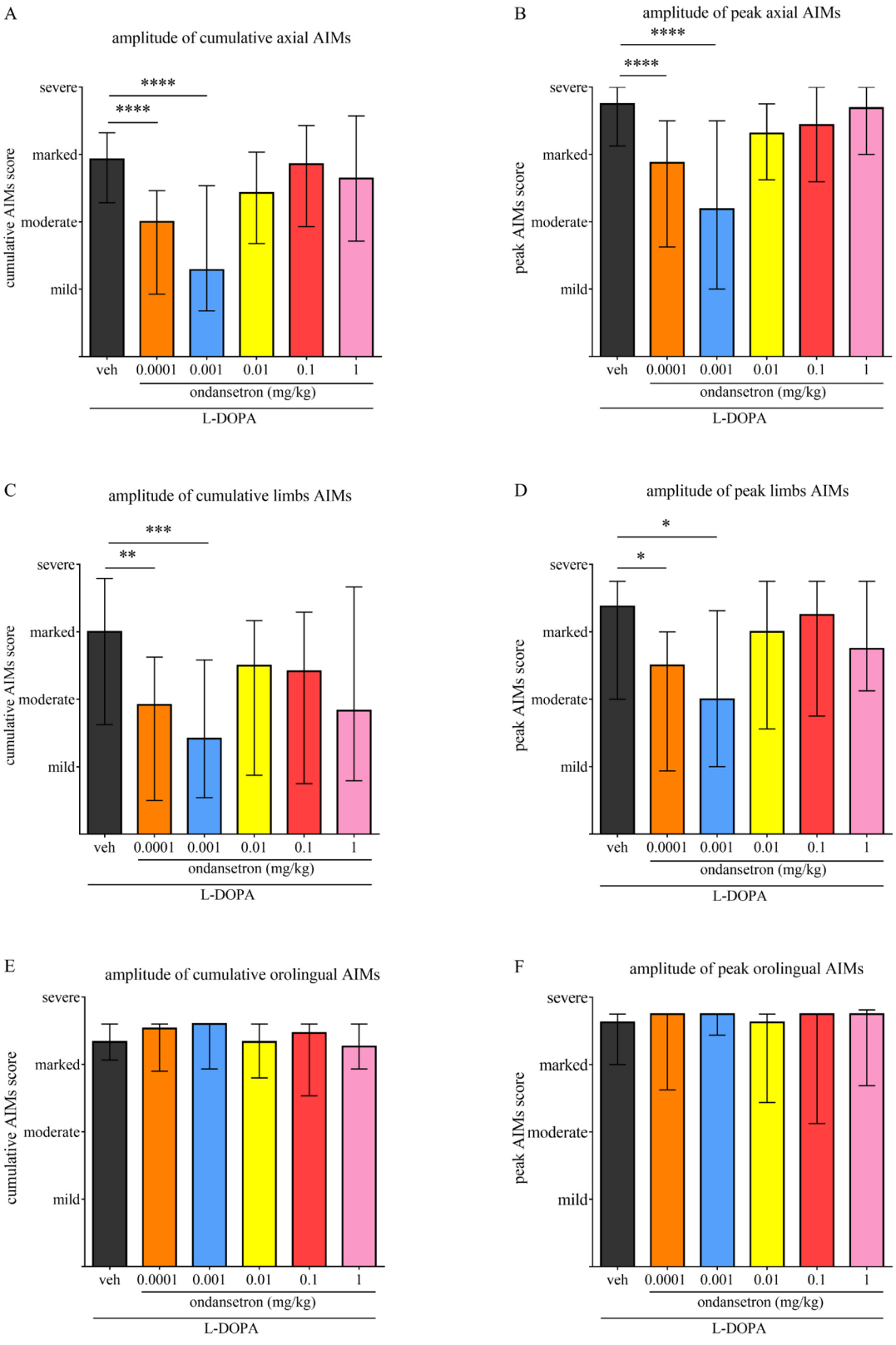
As shown in Figures 7E and 7F (page 84), treatment with ondansetron (0.0001, 0.001, 0.01 0.1 and 1 mg/kg) resulted in similar amplitude severity levels of cumulative and peak orolingual AIMs when compared with vehicle (FS = 1.744 and FS = 3.46, both $P > 0.05$).

Amplitude of AL AIMs

As illustrated in Figures 7G and 7H (page 85), when administered with L-DOPA, ondansetron reduced the amplitude of cumulative and peak AL AIMs (FS = 47.25 and FS = 40.49, both $P < 0.0001$). Thus, treatment with ondansetron 0.0001 mg/kg and 0.001 mg/kg resulted in a 61% and 64% decrease in the amplitude of cumulative amplitude AL AIMs scores, respectively, when compared with vehicle ($P < 0.0001$ and $P < 0.001$, Dunn's *post hoc* test, Figure 7G, page 85). Furthermore, peak AL AIMs amplitude was diminished with ondansetron 0.0001 and 0.001 mg/kg by 60% and 58%, respectively, compared to vehicle (both $P < 0.0001$, Dunn's *post hoc* test, Figure 7H, page 85).

Amplitude of ALO AIMs

As shown in Figures 7I and 7J (page 85), adding ondansetron in combination with L-DOPA led to a significant reduction in the amplitude of cumulative and peak ALO AIMs, respectively (FS = 30.07, $P < 0.0001$ and FS = 22.3, $P < 0.001$). Thus, adding ondansetron 0.0001 and 0.001 mg/kg decreased the amplitude of cumulative ALO AIMs by 51% and 54%, respectively, when compared with vehicle ($P < 0.01$ and $P < 0.001$, Dunn's *post hoc* test, Figure 7I, page 85). In addition, the amplitude of peak ALO AIMs was also reduced with ondansetron 0.001 and 0.001 mg/kg, when compared to vehicle, by 51% and 44%, respectively ($P < 0.01$ and $P < 0.05$, Dunn's *post hoc* test, Figure 7J, page 85).


A
amplitude of cumulative axial AIMs
cumulative AIMs score
severe
marked
moderate
mild
****
****
veh
0.0001
0.001
0.01
0.1
1
ondansetron (mg/kg)
L-DOPA
B
amplitude of peak axial AIMs
peak AIMs score
****
****
C
amplitude of cumulative limbs AIMs
***
**
D
amplitude of peak limbs AIMs
*
*
E
amplitude of cumulative orolingual AIMs
F
amplitude of peak orolingual AIMs

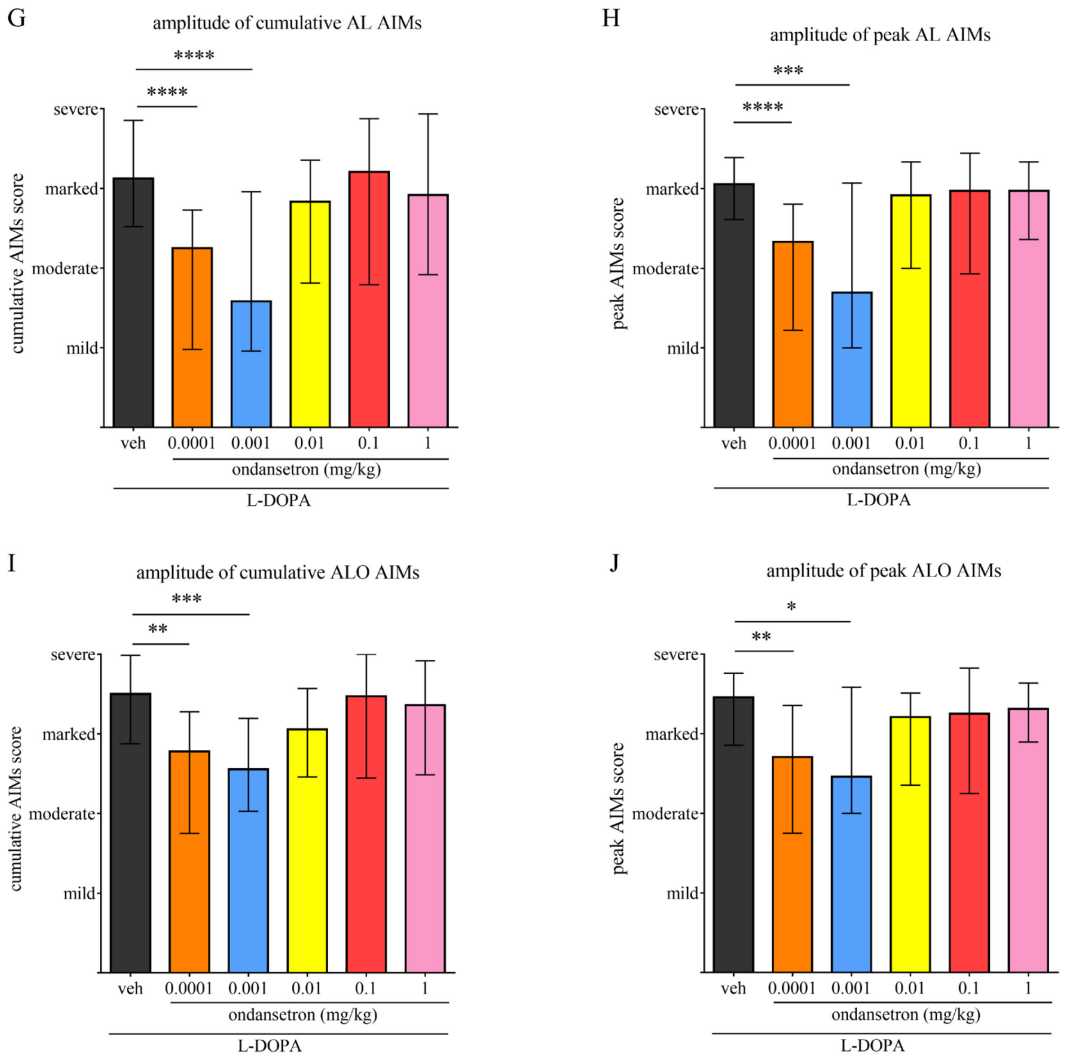


Figure 7: Effect of acute ondansetron treatment on the amplitude of established L-DOPA induced AIMs. A. When administered with L-DOPA, 0.0001 mg/kg ondansetron alleviated the severity of cumulative and **B.** peak axial AIMs when compared with vehicle. **C.** Similarly, ondansetron 0.0001 mg/kg/L-DOPA resulted in a reduction in the amplitude of cumulative limbs AIMs, when compared with vehicle; **D.** this effect was also observed in the lower amplitude of peak limbs AIMs when compared with vehicle/L-DOPA. **E.** The addition of ondansetron led to comparable amplitude in cumulative orolingual AIMs severity, **F.** as well as peak amplitude orolingual AIMs, when compared with vehicle. **G.** With respect to the amplitude of AL AIMs, ondansetron 0.0001 mg/kg resulted in less severe cumulative AL AIMs, and **H.** a decrease in the amplitude of peak AL AIMs. The combination of ondansetron 0.0001 mg/kg and L-DOPA resulted in a significant reduction in **I.** cumulative ALO AIMs severity and **J.** peak ALO AIMs scores. Cumulative and peak duration AIMs scores are expressed as median with interquartile interval. n = 18 for all treatment conditions. *: $P < 0.05$; **: $P < 0.01$, ***: $P < 0.001$ and ****: $P < 0.0001$.

De novo study

De novo treatment with ondansetron attenuates the development of L-DOPA-induced AIMs

Axial AIMs

As illustrated in Figure 8A (page 89), the addition of ondansetron to L-DOPA resulted in a decrease in the duration of axial AIMs during the 22-day priming phase (F_{time} (3,88) = 2.285, $P > 0.05$; $F_{treatment}$ (2,88) = 8.111; $P < 0.001$; and $F_{interaction}$ (6,88) = 0.354, $P > 0.05$, two-way ANOVA). On day 8, animals treated with 0.0001 mg/kg ondansetron/L-DOPA exhibited a 53% reduction in the duration of axial AIMs when compared with the vehicle/L-DOPA group ($P < 0.05$, Tukey's *post hoc* test). The amplitude of axial AIMs was significantly different across the three treatment groups (F_{time} (3,88) = 1.29, $P > 0.05$; $F_{treatment}$ (2,88) = 3.62; $P < 0.05$; and $F_{interaction}$ (6,88) = 0.23, $P > 0.05$, two-way ANOVA, Figure 8B, page 89). Ondansetron 0.001 mg/kg shows a slight reduction in the duration of axial AIMs whereas, the amplitude axial AIMs was increased with respect to the vehicle-treated animals.

Limbs AIMs

As shown in Figure 8C (page 89), administration of ondansetron in combination with L-DOPA did not lead to significant reduction in the duration of limbs AIMs (F_{time} (3,88) = 2.496; $F_{treatment}$ (2,88) = 2.87; and $F_{interaction}$ (6,88) = 0.6427, each $P > 0.05$, two-way ANOVA). Similarly, ondansetron did not significantly diminish the amplitude of limbs AIMs (F_{time} (3,88) = 1.455; $F_{treatment}$ (2,88) = 1.181; and $F_{interaction}$ (6,88) = 0.2405, each $P > 0.05$, two-way ANOVA, Figure 8D, page 89).

Orolingual AIMs

As illustrated in Figure 8E (page 89), adding ondansetron to L-DOPA did not significantly affect the duration of orolingual AIMs (F_{time} (3,88) = 1.052; $F_{treatment}$(2,88) = 0.9368; and $F_{interaction}$ (6,88) = 0.5472, each $P > 0.05$; two-way ANOVA). In contrast, administration of ondansetron resulted in a significant decrease in the amplitude of orolingual AIMs (F_{time} (3,88) = 0.8776, $P > 0.05$; $F_{treatment}$ (2,88) = 38.12; $P < 0.0001$; and $F_{interaction}$ (6,88) = 1.105, $P > 0.05$, two-way ANOVA, Figure 8F, page 89). Thus, ondansetron 0.0001 mg/kg

significantly reduced the amplitude of orolingual AIMs on days 1, 8, 5 and 22 by 21%, 32%, 37% and 24%, respectively, when compared with vehicle ($P < 0.05$, $P < 0.001$, $P < 0.0001$ and $P < 0.05$, Tukey's *post hoc* test). The dose of 0.001 mg/kg ondansetron showed a similar trend in the development of orolingual AIMs as the vehicle group.

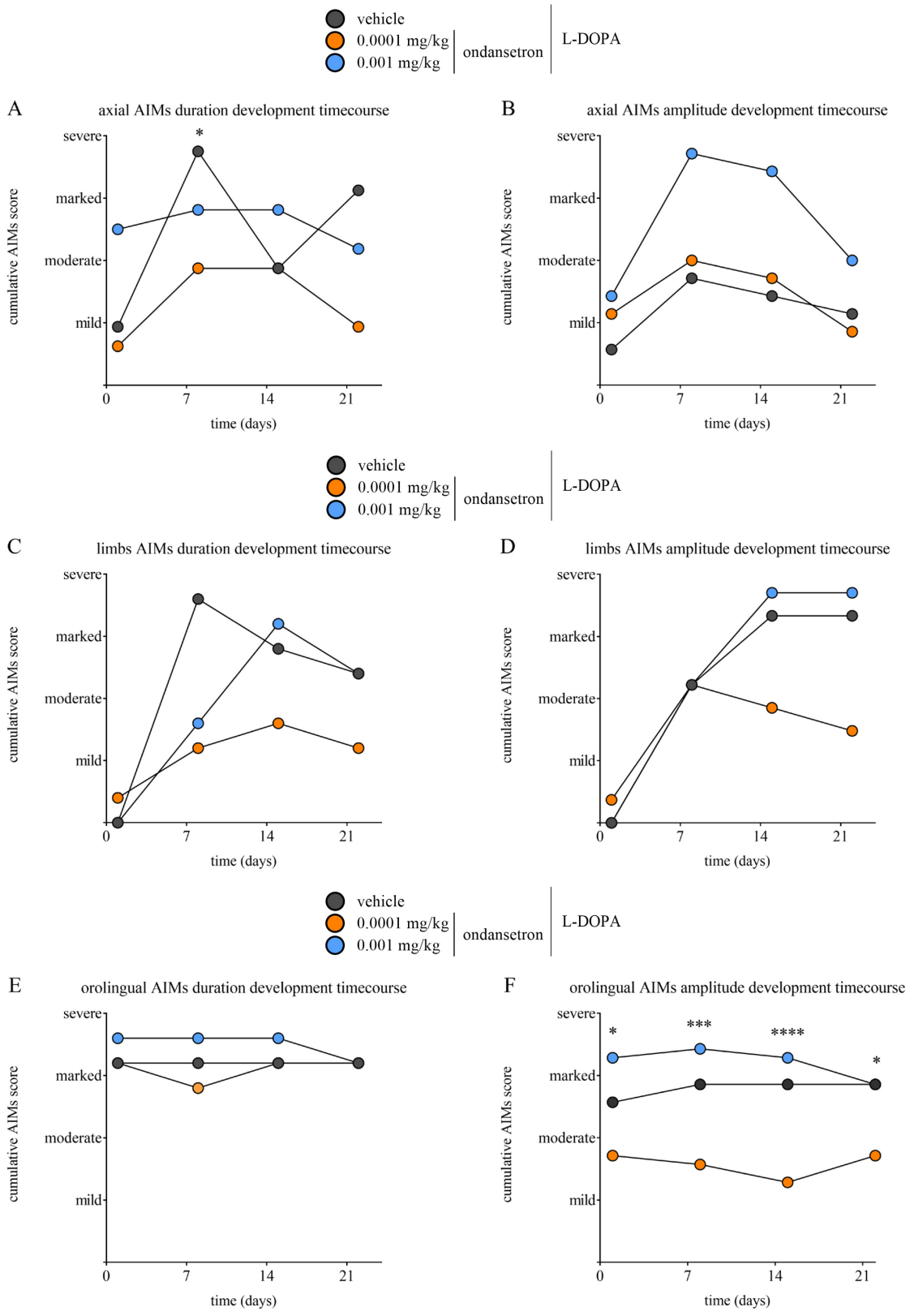
AL AIMs

As shown in Figure 8G (page 90), the addition of ondansetron significantly reduced the duration of AL AIMs (F_{time} (3,88) = 3.064; $P < 0.05$; $F_{treatment}$ (2,88) = 7.953; $P < 0.001$; and $F_{interaction}$ (6,88) = 0.4133, $P > 0.05$, two-way ANOVA). Thus, on day 8, animals that received ondansetron 0.0001 mg/kg with L-DOPA exhibited a 53% shorter duration of AL AIMs, when compared with those that received vehicle/L-DOPA ($P < 0.05$, Tukey's *post hoc* test). In contrast, ondansetron did not lead to a significant reduction in the amplitude of AL AIMs (F_{time} (3,88) = 1.623; $F_{treatment}$ (2,88) = 2.959; and $F_{interaction}$ (6,88) = 0.1974, each $P > 0.05$, two-way ANOVA) but it appears that animals administered 0.0001 mg/kg ondansetron exhibited a slight decrease in the amplitude AL AIMs, when compared with the control group (Figure 8H, page 90). Treatment with 0.001 mg/kg ondansetron produced a modest decrease in the duration of AL AIMs with respect to vehicle-treated animals while the severity of amplitude was unaffected.

ALO AIMs

As illustrated in Figure 8I (page 90), adding ondansetron resulted in a significant reduction in the duration of ALO AIMs (F_{time} (3,88) = 2.969; $P < 0.05$; $F_{treatment}$ (2,88) = 8.797; $P < 0.001$; and $F_{interaction}$ (6,88) = 0.6006; $P > 0.05$, two-way ANOVA). Thus, on day 15, administration of ondansetron 0.0001 mg/kg in combination with L-DOPA led to a 33% decrease in the duration of ALO AIMs, when compared with vehicle ($P < 0.01$, Tukey's *post hoc* test). Similarly, ondansetron treatment also diminished the amplitude of ALO AIMs (F_{time} (3,88) = 0.3526; $P > 0.05$; $F_{treatment}$ (2,88) = 13.87; $P < 0.0001$; and $F_{interaction}$ (6,88) = 0.9623; $P > 0.05$, two-way ANOVA). On day 8, 0.0001 mg/kg ondansetron led to a 31% reduction in the amplitude of ALO AIMs, when compared with vehicle ($P < 0.05$, Tukey's *post hoc* test, Figure 8J, page 90). On day 15, ondansetron 0.001 mg/kg showed an 49% increase in the amplitude of ALO AIMs, when compared with the 0.0001 mg/kg dose ($P < 0.01$, Tukey's *post hoc* test).

Timecourse graphs that present the data as median with interquartile range are shown in Figure 12 (Appendices VI and VII).


vehicle
0.0001 mg/kg
0.001 mg/kg
ondansetron
L-DOPA
A
axial AIMs duration development timecourse
B
axial AIMs amplitude development timecourse
C
limbs AIMs duration development timecourse
D
limbs AIMs amplitude development timecourse
E
orolingual AIMs duration development timecourse
F
orolingual AIMs amplitude development timecourse
cumulative AIMs score
severe
marked
moderate
mild
0
7
14
21
time (days)
*
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****

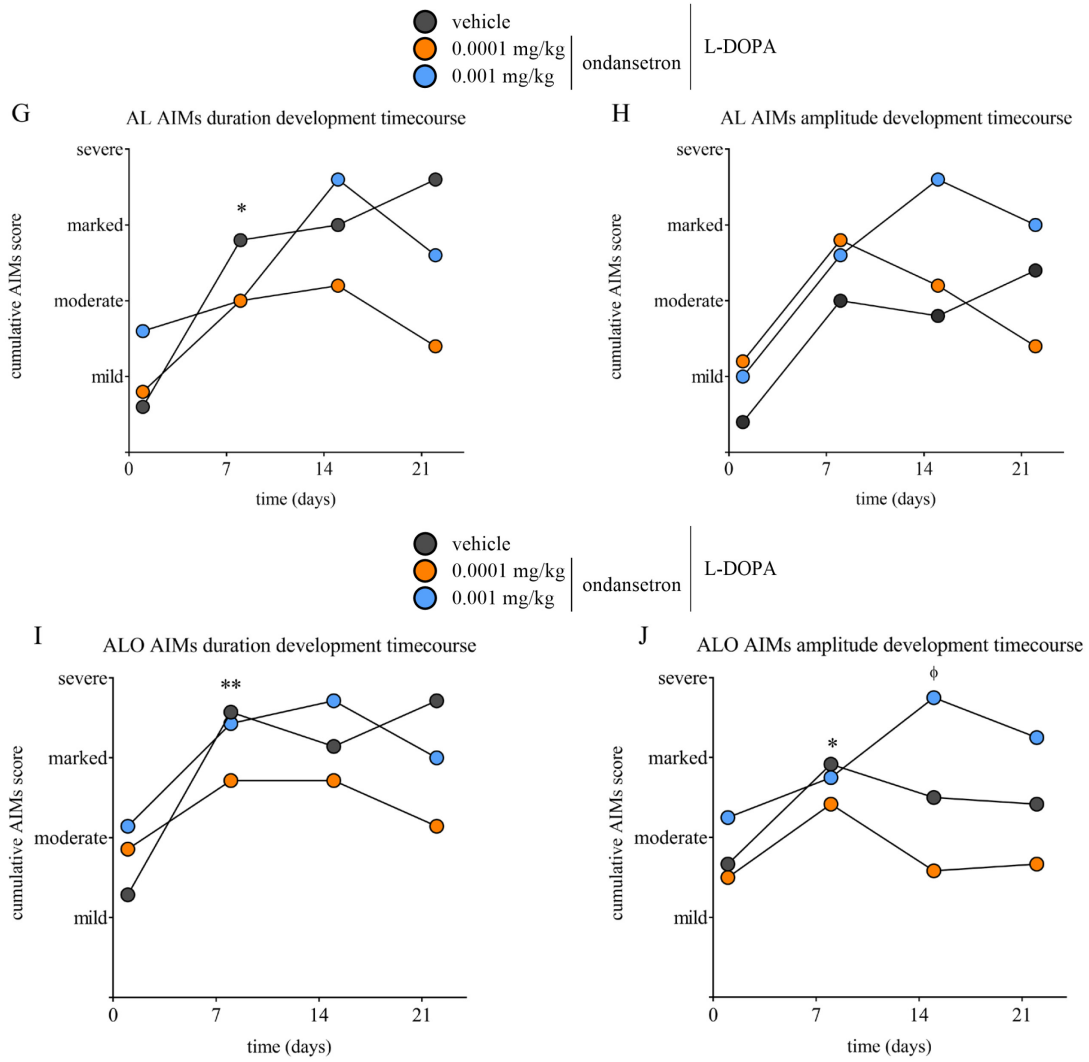

Figure 8: Time course of the development of AIMs during the 22-day priming phase of the *de novo* ondansetron study. On day 8 of priming, **A.** the duration of axial AIMs was significantly reduced in animals previously treated with L-DOPA/0.0001 mg/kg ondansetron (n = 9), by 53%, when compared with L-DOPA/vehicle (n = 7) ($P < 0.05$, Tukey's *post hoc* test). **B.** In contrast, the amplitude of axial AIMS was not significantly reduced in animals that were previously treated with L-DOPA/ondansetron 0.0001 mg/kg (n = 9) compared to animals that received L-DOPA/vehicle. **C.** The duration and **D.** amplitude of limbs AIMs was comparable between animals administered ondansetron 0.0001 mg/kg and vehicle. **E.** The duration of orolingual AIMs was not affected by ondansetron treatment. **F.** Administration of L-DOPA/ondansetron 0.0001 mg/kg resulted in a significant decrease in the amplitude of orolingual AIMs on days 1, 8, 5 and 22 by 21%, 32%, 37% and 24%, respectively, when compared with vehicle ($P < 0.05$, $P < 0.001$, $P < 0.0001$ and $P < 0.05$, Tukey's *post hoc* test). **G.** On day 8, when administered with L-DOPA, 0.0001 mg/kg ondansetron significantly reduced the duration of AL AIMs, by 53%, when compared with L-DOPA/vehicle ($P < 0.05$,

Tukey's *post hoc* test). **H.** The amplitude of AL AIMs was comparable between L-DOPA/0.0001 mg/kg ondansetron and L-DOPA/vehicle. **I.** Administration of ondansetron 0.0001 mg/kg resulted in a significant decrease in the duration of ALO AIMs on day 15, by 33%, when compared with L-DOPA/vehicle ($P < 0.01$, Tukey's *post hoc* test). **J.** The amplitude of ALO AIMs was reduced in animals that were treated with L-DOPA/ondansetron 0.0001 mg/kg, by 31%, compared to animals that received L-DOPA/vehicle ($P < 0.05$, Tukey's *post hoc* test). Data are graphed as the median. *: $P < 0.05$; **: $P < 0.01$, ***: $P < 0.001$, ****: $P < 0.0001$ vehicle versus ondansetron 0.0001 mg/kg; ϕ: $P < 0.01$ ondansetron 0.0001 mg/kg versus ondansetron 0.001 mg/kg.

De novo treatment with ondansetron attenuates the development of ALO AIMs

To determine if any reduction of AIMs observed during the priming phase was due to a symptomatic effect by ondansetron or resulted from an interference with AIMs development, following the 22-day priming phase, rats entered a 3-day washout period, after which they were administered an acute L-DOPA challenge (Figure 9, page 93).

As shown in Figures 9A and 9B (page 93), rats previously administered L-DOPA/ ondansetron demonstrated a reduction in the duration of cumulative and peak ALO AIMs ($F(2,22) = 4.814$ and $F(2,22) = 4.389$, both $P < 0.05$, one-way ANOVA). Treatment with L-DOPA/ondansetron 0.0001 mg/kg and 0.001 mg/kg did not statistically diminish the duration of cumulative and peak ALO AIMS when compared with L-DOPA/vehicle (both $P > 0.05$, Tukey's *post hoc* test). In contrast, the dose of 0.0001 mg/kg ondansetron led to a 32% and 47% decrease in the duration of cumulative and peak ALO AIMs, respectively, when compared with the dose of 0.001 mg/kg (both $P < 0.05$, Tukey's *post hoc* test).

As illustrated in Figure 9C (page 93), the addition of ondansetron led to a significant reduction in the amplitude of cumulative ALO AIMs ($F(2,22) = 5.996$, $P < 0.01$, one-way ANOVA). Thus, administration of L-DOPA/ondansetron 0.0001mg/kg resulted in a decrease in the amplitude of cumulative ALO AIMs by 33% and 34%, respectively, when compared with L-DOPA/vehicle and L-DOPA/ondansetron 0.001 mg/kg, respectively (both $P < 0.05$, Tukey's *post hoc* test). As shown in Figure 9D (page 93), ondansetron diminished the amplitude of peak ALO AIMs ($F(2,22) = 3.513$, $P < 0.05$, one-way ANOVA). Thus, ondansetron 0.0001 mg/kg led to a significant reduction in the amplitude of peak ALO AIMs by 47%, when compared with the dose of 0.001 mg/kg ($P < 0.05$, Tukey's *post hoc* test).

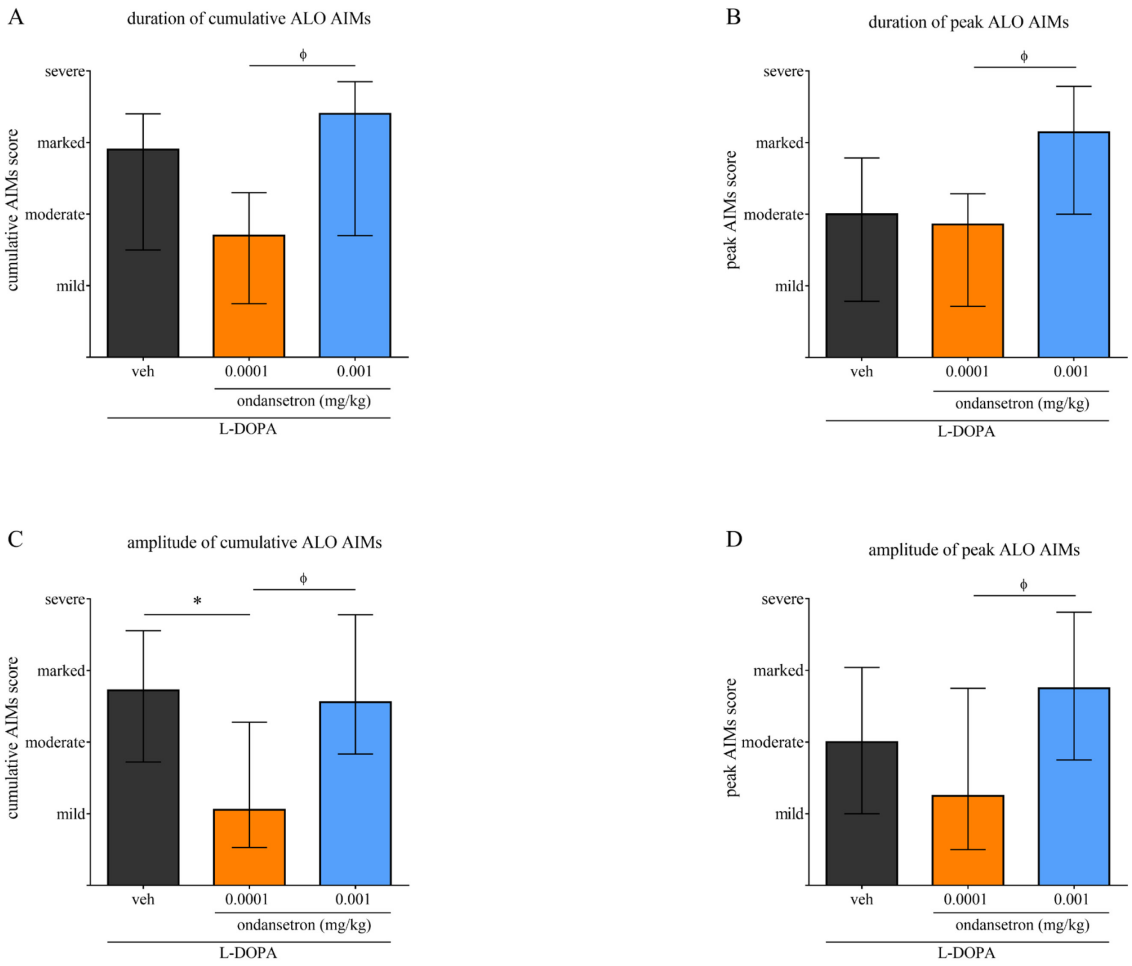

Figure 9: Effect of ondansetron on the duration and amplitude of cumulative and peak AIMs severity during an acute 6 mg/kg L-DOPA challenge. During an acute L-DOPA challenge, rats that were previously administered L-DOPA/ondansetron 0.0001 mg/kg (n = 9) during the priming period exhibited similar **A.** cumulative and **B.** peak duration ALO AIMs, when compared with L-DOPA/vehicle (n = 6). In combination with L-DOPA, 0.0001 mg/kg ondansetron led to a 32% and 47% decrease in the duration of cumulative and peak ALO AIMs, respectively, when compared with the dose of 0.001 mg/kg (n = 9), (both $P < 0.05$, Tukey's *post hoc* test). **C.** In contrast, previous addition of ondansetron 0.0001 mg/kg ondansetron with L-DOPA resulted in a significant reduction in the amplitude of cumulative ALO AIMs, by 33% and 34%, when compared with L-DOPA/vehicle and L-DOPA/0.001 mg/kg ondansetron, respectively (both $P < 0.05$, Tukey's *post hoc* test). **D.** Ondansetron 0.0001 mg/kg led to a significant decrease in the amplitude of peak ALO AIMs by 47%, when compared with the dose of 0.001 mg/kg ($P < 0.05$, Tukey's *post hoc* test). Cumulative and peak AIM scores are expressed as median with interquartile interval. *: $P < 0.05$ vehicle versus ondansetron 0.0001 mg/kg; ϕ: $P < 0.05$ ondansetron 0.0001 mg/kg versus ondansetron 0.001 mg/kg.

Administration of ondansetron does not impair the therapeutic efficacy of L-DOPA in the cylinder test

Following the acute challenges of ondansetron, 6-OHDA-lesioned rats were then subject to a 3 mg/kg L-DOPA challenge to assess whether ondansetron treatment impairs L-DOPA anti-parkinsonian action, as measured by the cylinder test (Figure 10, page 96). As illustrated in Figure 10A (page 96), treatment conditions significantly improved use of the left (lesioned) forepaw in making wall contacts (FS = 28.54, $P < 0.0001$). Although administration of L-DOPA did not alter performance ($P > 0.05$, Dunn's *post hoc* test), ondansetron 0.0001 mg/kg and 1 mg/kg resulted in a significant increase the number of rears using the impaired forepaw when compared to post-surgery performance (both $P < 0.001$, Dunn's *post hoc* test).

As shown in Figure 10B (page 96), administration of L-DOPA alone or in combination with ondansetron led to a decrease in the use of the right (un-lesioned) forepaw (FS = 34.89, $P < 0.0001$). When 6-OHDA-lesioned rats were administered L-DOPA, there was a significant decrease in the number of rears using the un-lesioned side by 34% ($P < 0.01$, Dunn's *post hoc* test). This decrease in rears with the un-lesioned forepaw remained present when ondansetron 0.0001, 0.001, 0.01, 0.1 or 1 mg/kg was combined with L-DOPA by 49%, 38%, 41%, 61% and 41%, respectively (each, $P < 0.001$, $P < 0.01$, $P < 0.001$, $P < 0.0001$ and $P < 0.0001$, Dunn's *post hoc* test). There was no difference between the number of rears using the un-lesioned side between L-DOPA/vehicle and L-DOPA/ondansetron, regardless of the dose of ondansetron (each $P > 0.05$, Dunn's *post hoc* test).

As illustrated in Figure 10C (page 96), 6-OHDA-lesioned animals that received L-DOPA or L-DOPA/ondansetron, demonstrated a significant increase in rears using both forepaws (FS = 21.52, $P < 0.0001$). Following administration of L-DOPA, there was a significant increase in rears using both forepaws, by 156%, compared to drug-naïve animals ($P < 0.05$, Dunn's *post hoc* test). In combination with L-DOPA, ondansetron 0.0001, 0.01 and 0.1 mg/kg increased the use of both forepaws by 114%, 114% and 163%, respectively ($P < 0.05$, $P < 0.05$ and $P < 0.001$, respectively, Dunn's *post hoc* test). There was no difference between the number of rears using both forepaws to rear between L-DOPA/vehicle and L-DOPA ondansetron, regardless of the dose of ondansetron (each $P > 0.05$, Dunn's *post hoc* test).

Results of the effect size calculated by Glass' delta, which accounts for the variation in standard deviations across treatment conditions in Figures 10B and 10C, are presented in Tables V and VI (Appendices III and IV).

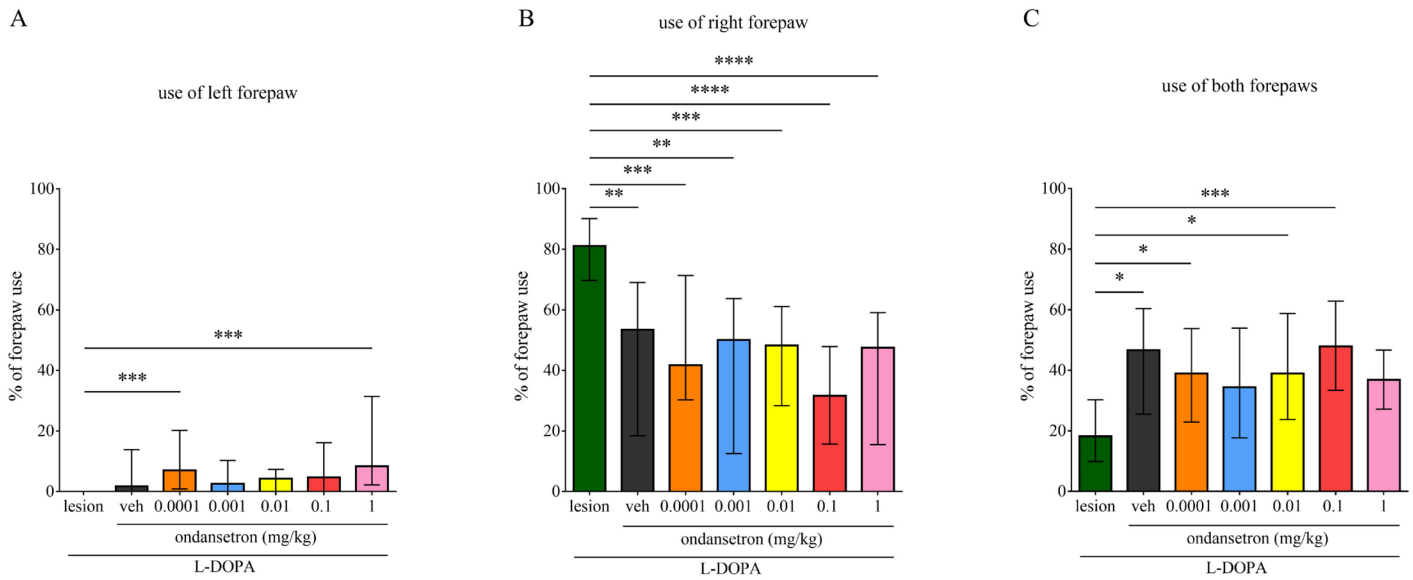

Figure 10: Use of forepaws across treatment conditions. A. Left forepaw use across treatments. Drug-naïve 6-OHDA-lesioned rats (n = 18) did not use the left (lesioned) forepaw during rearing. Following administration of L-DOPA (3/15 mg/kg), there was no significant change in the number of rears with the lesioned forepaw. The addition of ondansetron 0.0001 and 1 mg/kg to L-DOPA significantly improved use of the lesioned forepaw, when compared with post-surgery performance. **B.** Right forepaw use across treatments. 6-OHDA-lesioned rats (n = 18) used the right (un-lesioned) forepaw in 83% of rears. When 6-OHDA-lesioned rats were administered L-DOPA (3/15 mg/kg), there was a significant decrease in the number of rears using the un-lesioned side by 40%. This decrease in rears with the un-lesioned forepaw remained present when ondansetron 0.0001, 0.001, 0.01, 0.1 or 1 mg/kg was combined with L-DOPA by 48%, 39%, 46%, 57% and 51%, respectively. **C.** Use of both forepaws across treatments. 6-OHDA-lesioned rats (n = 18) used both forepaws during 17% of rears. Administration of L-DOPA (3/15 mg/kg) led to a significant increase in rears using both forepaws by 118%. In combination with L-DOPA, ondansetron 0.0001, 0.01 and 0.1 mg/kg increased the use of both forepaws by 113%, 121% and 159%, respectively. Data are graphed as median with interquartile range. *: $P < 0.05$; **: $P < 0.01$, ***: $P < 0.001$, ****: $P < 0.0001$.

IV. Discussion

These results demonstrate that ondansetron significantly diminishes the severity of established L-DOPA-induced AIMs compared to L-DOPA alone, without compromising the anti-parkinsonian action of L-DOPA. Furthermore, administration of ondansetron, when begun concurrently with L-DOPA, attenuates the development of AIMs compared to L-DOPA alone. Taken together, these results suggest that 5-HT_3 receptor antagonism represents a novel and effective therapeutic strategy to reduce LID in PD. The following discussion will focus on how 5-HT_3 receptor blockade may be an effective approach to reduce the severity of LID.

Limitations and future directions

In light of the findings reported in the present thesis, it is also important to highlight the limitations of the experiments. First, the pharmacokinetic study characterized peak ondansetron levels, previously unknown, in the rat with the doses used. However, determining brain levels of ondansetron in human is hardly feasible for ethical reasons, so it is not possible to compare or correlate plasma levels of ondansetron with brain levels. Further studies are thus needed to determine the plasma and brain concentration profile of ondansetron and brain ondansetron levels in the brain associated with maximum anti-dyskinetic activity. Second, the primary endpoint of this Master's thesis was to assess the effect of ondansetron on the severity of dyskinesia in a PD animal model. This preclinical study modelled the administration of ondansetron in the clinic as an adjunct to L-DOPA and demonstrated that it achieved its effect on dyskinesia without impairing the therapeutic efficacy of L-DOPA. It would be interesting to study the effect of ondansetron on basal PD disability in a subsequent set of experiments. Third, in the 6-OHDA-lesioned rat, an increase in SERT levels is reported in the striatum of dyskinetic animals and, furthermore, levels of SERT correlate with AIMs severity (989, 990). In addition, following 2-3 weeks of either low or high doses of L-DOPA, SERT levels also increase in the rat striatum (990). Interestingly, the increase in striatal SERT levels appears to be a dose-dependent effect of L-DOPA-induced axonal sprouting (990). Similarly, in the MPTP-lesioned macaque model, there is an increase in the number of 5-HT axon varicosities in the striatum, which is particularly pronounced where DA denervation is most severe (991). Although these studies may be in accordance with the agreed upon role of serotonergic terminals in the pathophysiology of dyskinesia (736), further anatomical characterization in the 6-OHDA-lesioned rat model is needed before inferring their action on raphe-striatal pathways. Fourth, the

acute challenges of ondansetron study demonstrated the acute suppression of L-DOPA induced AIMs in the 6-OHDA lesioned rat model. Whether this effect would be maintained over the long-term remains unknown, and a chronic ondansetron study, where ondansetron would be administered over several days, with regular assessments of AIMs severity, could determine whether the anti-dyskinetic efficacy of ondansetron is maintained or if tolerance develops. Fifth, most of the thesis was a behavioural pharmacology study and is not informative on the mechanism whereby 5-HT_3 receptor blockade reduces the severity of established and prevents the development of dyskinesia. Thus, we are actively seeking answers on elucidating the mechanism that underlies the action of 5-HT_3 blockade, notably by conducting studies that will shed light on the brain areas involved in mediating the anti-dyskinetic effect of antagonising 5-HT_3 receptors. Sixth, several highly-selective 5-HT_3 receptor antagonists are clinically available with differing affinity for the receptor and duration of action, and it would be of further interest to determine whether the pharmacodynamics or pharmacokinetic profiles of a drug influence its anti-dyskinetic efficacy.

Pharmacokinetic study of clinically relevant doses of ondansetron

The preliminary dose-finding PK study, which aimed at assessing whether there was a ceiling effect to anti-dyskinetic efficacy of higher doses of ondansetron, found that ondansetron 0.01 mg/kg led to a C_{max} of 2.31 ng/mL. Clinical studies have described oral administration of ondansetron in healthy volunteers and a single oral dose of 8 mg measured a C_{max} of 19.9 – 31.2 ng/mL (992, 993). The dose of 0.0001 mg/kg ondansetron that conferred the therapeutic benefit in the behavioural studies is likely well below plasma levels in humans and thus, well tolerated in humans. On the other hand, the higher doses of 0.1 and 1 mg/kg ondansetron likely led to 10-fold to 100-fold higher than well tolerated plasma levels in humans, which limits the translational potential of administering higher doses of ondansetron in the clinic, especially if the dosing regimen requires exceeding maximum tolerated doses of ondansetron. As the PK of subcutaneous ondansetron administration has not been assessed in humans, it is not possible to make a valid comparison to assess whether the dose of 0.01 mg/kg ondansetron is clinically relevant (993). It is thus warranted to conduct PK studies of subcutaneous oral administration in primates to confirm anti-dyskinetic doses of ondansetron are clinically relevant.

5-HT_3 blockade with ondansetron alleviates previously established AIMs without impairing the anti-parkinsonian efficacy of L-DOPA

In the present study, during the course of treatment, ondansetron at 0.0001 mg/kg consistently produced the lowest AIMs severity whereas the vehicle produced the most severe AIMs treatment, except for the orolingual AIMs. Accordingly, the duration and amplitude ranking time courses consistently show that the cumulative and peak AIMs with ondansetron 0.0001 mg/kg are significantly reduced, when compared to vehicle. Importantly, ondansetron reduced the severity of cumulative AIMs as well as the peak severity of AIMs, which coincides with the peak L-DOPA concentration, suggesting that ondansetron may alleviate dyskinesia throughout the time period they are expressed, regardless of their intensity, which has important therapeutic implications.

The dose of 0.0001 mg/kg ondansetron was the most effective at alleviating established AIMs, particularly the axial and limbs components, compared to the more cumulative measures of AL and ALO AIMs. Orolingual AIMs are often more difficult to score compared to the other dyskinetic parameters and, as they are more subtle, they may be overestimated or overlooked (965). In the investigators' experience, milder severity levels present with subtle differences while at higher severity, more prominent AIMs of the neck and upper body as well as the forelimb can mask the appearance of orolingual AIMs. Indeed, treatment with ondansetron did not produce a significant reduction on the duration or the amplitude of orolingual AIMs. Thus, while ALO AIMs evaluate the anti-dyskinetic effect of a treatment on the sum of the three components, it is also of interest to express the data as AL AIMs, which are arguably relatively more disabling than orolingual AIMs. Interestingly, despite the lack of effect on the severity and temporal profile of orolingual AIMs, ondansetron appeared to have a more pronounced effect on the severity of ALO than AL AIMs, suggesting that the efficacy of the anti-dyskinetic effect is maintained on cumulative dyskinetic parameters.

A criticism that could be raised on the scale used here is that while it evaluates the severity of ALO AIMs across both duration and amplitude, it does not differentiate between dystonic and hyperkinetic dyskinesia, which could be correlates of dystonia and chorea,

respectively. Steece-Collier and collaborators have introduced a modification to the scale that scores dystonic and hyperkinetic axial and limbs AIMs separately (994), and might allow the detection of differential effects of treatments on these subtypes of dyskinesia (965), and could possibly have enabled us to make predictions as to whether 5-HT_3 blockade might be more effective, in clinical settings, to reduce dystonia, chorea, or both.

The bell-shaped dose-response curve is often ascribed to 5-HT_3 receptor antagonists for indications in preclinical and clinical studies including anxiety (995), depression (996, 997), drug addiction (998) and migraines (883). In general, the maximum response is already observed in the microgram dose range, while higher doses are ineffective (848). However, the mechanism underlying the dose-response curve still lacks satisfactory explanation and it remains to be determined whether this is due to the pharmacology of individual compounds or a group-specific characteristic (818). Thus, in our experiments, lower doses of ondansetron produced the greatest relief of dyskinesia. The most favoured mechanism proposes that, at high concentrations of 5-HT_3 antagonists, there is mutual steric hindrance at the receptor, which refers to an inappropriate interaction of a ligand to its receptor that results in a conformational change in the receptor that prevents the binding of ligands to the receptor" (999-1001) or, more speculatively, additional effects due to low-affinity binding to other receptors. Although ondansetron binds with low affinity to the 5-HT_{1A}, 5-HT_{1B}, α-adrenergic and opioid receptors, its binding to high-affinity 5-HT_3 receptor sites is about 250- and 500-fold higher that of the other receptors (786, 870, 1002). Alternatively, as the density of 5-HT_3 receptors varies between different brain regions with one density type being completely inhibited at low concentrations and the other type only at high concentrations of 5-HT receptor antagonists, which could explain contrary effects (818), *e.g.* a therapeutic effect, triggered by blockade of 5-HT_3 receptors within one brain area, could be off-set when 5-HT_3 receptors from another brain region are completely antagonised (882), which could explain the lack of efficacy of higher doses of ondansetron.

According to the classic model of BG circuitry, dyskinesia may arise as the result of overactivity of the direct pathway and/or underactivity of the indirect pathway (299, 572, 580). Some autoradiographic studies report the expression of 5-HT_3 receptors on GABAergic MSNs of the direct and indirect pathways (638). In view of the anti-dyskinetic results described above, it is possible that, at lower doses of ondansetron, the compound antagonizes inhibitory GABAergic projections neurons of the striatum, and may preferentially block pre-synaptic 5-

HT_3 receptors on MSNs of the direct pathway over MSNs of the indirect pathway. Here, ondansetron would act on the pre-synaptic nerve terminal to prevent the entry of cations into the neurons and their subsequent depolarization followed by release of neurotransmitters (849, 850). As a result, the propagation of inhibitory GABAergic signal to the EP/SNr is reduced, which would diminish hyperactivity of the direct pathway in LID, and may lead to the dampening of AIMs observed with lower doses of the compound.

5-HT_3 receptors are also found on striatal GABAergic interneurons, notably the stomatostatin-/nitric oxide synthase- and calretinin-expressing interneuron subtypes. These two major interneuron populations exhibit high input resistance and persistent low-threshold spiking (PLTS) in response to intracellular depolarization or excitatory synaptic stimulation (1003). Although the output of individual PLTS interneurons is relatively weak and sparse, they may form inhibitory synapses onto distal dendrites of MSNs (1004), which is consistent with SOM+ GABAergic terminals that have been observed on the dendrites of MSNs (1005). Moreover, it is possible that the low connectivity of PLTS interneurons is strengthened under disease states (1006), where they release neuromodulators such as neuropeptide Y, SOM and NO, which more speculatively, may modulate the activity of striatal GABAergic MSNs of the direct pathway (1007-1010).

In addition to their action on MSNs from the direct pathway, antagonism of 5-HT_3 receptors on striatal GABAergic interneurons may exert an inhibitory effect on the activity of the direct pathway that partially restores inhibition of motor cortical areas to physiological levels. Although the presence of AADC expression in striatal interneurons may be controversial (1011, 1012), they could represent another source of L-DOPA derived DA (1013, 1014). Here, ondansetron may prevent extra synaptic DA release by blocking the 5-HT_3 receptor on striatal interneurons, thereby diminishing fluctuations in DA levels associated with the appearance of LIDs (745). In contrast, at higher doses of ondansetron, the compound may also antagonize 5-HT_3 receptors on MSNs of the indirect pathway. This could theoretically result in further disturbance to the equilibrium between the direct and indirect pathways and lead to a greater disinhibition of the motor thalamus and motor cortex, exacerbating existing L-DOPA-induced AIMs.

It should be noted though, that such mechanisms have yet to be demonstrated experimentally, which is further emphasised by the fact that there is currently no consensus in

the literature on the distribution of 5-HT_3 receptors in the rodent BG. In the rodent striatum, studies have reported a variety of expression patterns ranging from weak to moderate expression of the 5-HT_3 receptors. However, a recent immunostaining study in the mouse brain has attempted to address the limitations of these binding studies by using 5-HT_{3A} receptor-green fluorescent protein transgenic mice to map the 5-HT_{3A} receptor subunit in the mouse brain (897). The authours reported moderately high expression of the 5-HT_{3A} receptor subunit in the striatum, slight expression in the thalamus but no expression in the GP and SN. Thus, based upon our results, assuming that the striatum is the structure that mediates, at least in part, the anti-dyskinetic effect of 5-HT_3 blockade, these weak-moderate expression levels of the5-HT_{3A} receptor are probably sufficient to mediate a behavioural effect. Another possibility is that 5-HT_3 expression is altered in the dyskinetic state, but studies on the expression of 5-HT_3 in the dyskinetic state have yet to be performed.

Nevertheless, 5-HT_3 receptors appear to be well situated to modulate the release of neurotransmitters within the BG, which may be responsible for their anti-dyskinetic effect. Thus, anatomical, electrophysiological and behavioural studies have suggested an important functional crosstalk between 5-HT and DA pathways in the BG. In particular, it has been demonstrated that the 5-HT_3 receptor mediates changes in striatal dopamine release *in vitro* (1015) and *in vivo* (911, 912, 1016). In fact, a microdialysis study reported that intrastriatal injection of the 5-HT_3 antagonists 3-tropanyl-indole-3-carboxylate, MDL-72222 or ondansetron attenuated 5-HT or morphine-induced striatal DA release, which suggests that 5-HT acts at 5-HT_3 receptors to facilitate DA release in the striatum (1017, 1018). Consistent with these results, systemic administration of the 5-HT_3 antagonists ondansetron and MDL 72222 did not affect basal DA efflux in the striatum (1018, 1019). Taken together, these results suggest that the 5-HT_3 receptor regulates evoked nigrostriatal DA release (1020), which is dependent on depolarization and the concomitant elevation of both DA and 5-HT tones (1021). Furthermore, in rat striatal slices, application of the selective 5-HT_3 agonists 2-methyl-5-HT and phenylbiguanide increased endogenous release of DA (1022), and DA release induced by 5-HT and the 5-HT_3 agonist 2-methyl-5-HT was attenuated by the 5-HT_3 antagonist ICS 205-930 (1023). In addition, in the dyskinetic state, where 5-HT fibres mediate DA release; administration of ondansetron may reduce the excitatory 5-HT innervation to the striatum, and this would lead to a reduction of DA release, which would translate behaviourally by a reduction

of dyskinesia. Lastly, it has been suggested that 5-HT_3 receptor may reduce the number of electrically-active and firing neurons within the SN and VTA and produce reductions in evoked DA release within the striatum and nucleus accumbens (1024); here again, this reduction of DA release might translate, at the behavioural level, by a reduction of dyskinesia. Furthermore, behavioural experiments have also suggested that pharmacological modulation of the 5-HT_3 receptor modifies nigrostriatal DA-induced motor responses, for instance, intrastriatal injection of the 5-HT_3 agonists SR-57227A or 2-methyl-5-HT in mice led to contralateral rotations that were abolished with systemic administration of the 5-HT_3 antagonist ondansetron (863). Taken together, these studies implicate the involvement of the 5-HT_3 receptor in DA release in the striatum, and it can be inferred that administration of ondansetron may have attenuated this excessive release of DA and led to the reduction in the severity of ALO AIMs.

L-DOPA derived DA release also occurs outside the striatum and other brain regions that receive 5-HT innervation display increased extrasynaptic DA release that may contribute to the development of LID (1025). Volume transmission of DA and its overflow past its release site can lead to the interaction of DA with multiple synapses (1026), and this volume increases even further with the degeneration of DA axons and loss of DAT activity in PD (1027). Given that extrasynaptic DA receptors are activated further from release sites (1027), pharmacological modulation of DA release in the striatum may exert effects on other BG nuclei including the STN, the entopeduncular nucleus (EP), the rodent homologue to the primate GPi, the SNr as well as the thalamus. The STN is considered the major driving force in the BG circuitry (554, 1028); thus, alterations in its activity by the 5-HT_3 receptor could represent an important site of action to reduce dyskinesia. Intrasubthalamic injection of the 5-HT_3 agonist mCPBG in rats induced contralateral turning behaviour, which was suppressed by lesion of the SNr (1029), which suggests that contralateral rotations could result from decreased excitatory input from the STN to the SNr, which in turn enhanced the activity of the ipsilateral motor thalamus (1029). In spite of the lack of autoradiographic studies on the distribution of the 5-HT_3 receptor in the STN, these findings suggest the presence of functional 5-HT_3 receptors in the STN. In line with the localization of 5-HT_3 receptors to GABAergic neurons, including in the striatum (814, 897) and neocortex (804, 822, 823), blockade of 5-HT_3 receptor may exert an inhibitory effect on subthalamic neurons. The consequent increase in glutamatergic output to the EP/SNr causes the inhibition of the motor thalamus and motor cortex, which might alleviate the severity of

dyskinesia. However, additional autoradiographic and immunohistochemical studies need to be conducted to confirm the expression of 5-HT_3 receptors in the STN.

Effect of ondansetron and ALO AIMs on L-DOPA anti-parkinsonian action

In agreement with previous studies (885, 973, 1030), L-DOPA improved 6-OHDA lesion-induced forelimb use asymmetry at the cylinder test. Importantly, this effect of L-DOPA was maintained after ondansetron was administered in our experiments, which indicates that ondansetron did not impair L-DOPA anti-parkinsonian effect.

A limitation of the cylinder test is that it can be impaired by AIMs. To circumvent this limitation, we used L-DOPA 3 mg/kg when performing the test, a dose that should theoretically not trigger AIMs. However, studies report that even such small doses of L-DOPA can trigger AIMs after chronic dopaminergic therapy (1031, 1032), and an interference of AIMs in the scoring occurred in our study with higher doses of ondansetron, with which AIMs were not reduced, which rendered difficult accurate rearing assessment. Retrospectively, a lower dose of L-DOPA, might have been sufficient to assess the anti-dyskinetic action of L-DOPA without eliciting AIMs in the animals. Alternatively, other tests of physiological motor behaviour such as the rotarod test (1033), open field test (1034) or stepping test (1035) should be administered in conjunction with the cylinder test as they may show greater sensitivity to detect motor activity, although severe AIMs could theoretically alter the performance at these tests as well.

Effect of ondansetron on the development of ALO AIMs

In the present study, chronic L-DOPA administration for 22 days in 6-OHDA-lesioned rats induced the expression of AIMs and the severity increased over time before reaching a plateau. In contrast, in animals treated with both ondansetron 0.0001 mg/kg and L-DOPA the development of AIMs was significantly attenuated. Animals treated with ondansetron 0.001 mg/kg and L-DOPA showed a similar progression in the development of AIMS as the control-DOPA/vehicle group, as opposed to the modest anti-dyskinetic effect observed in the acute challenge study. Following the 22-day priming phase, animals were subject to an acute L-DOPA challenge to assess whether ondansetron treatment interfered with the priming process that led

to the development of dyskinesia, or if the apparent dyskinesia reduction during the priming phase was due to a symptomatic effect of the compound. If ondansetron indeed attenuates the development of dyskinesia, we would expect the L-DOPA/vehicle group of animals to display more severe AIMs when compared to the L-DOPA/ondansetron groups upon administration of L-DOPA alone after the washout period that followed the priming phase. We found that, upon acute administration of L-DOPA alone after the priming phase, the cumulative ALO AIMs severity was significantly diminished in animals that were primed with L-DOPA/ondansetron 0.0001 mg/kg.

Collectively, our results suggest that 5-HT_3 blockade acutely diminishes AIMs severity and interferes with the development of dyskinesia. Quite interestingly is the fact that, by looking at the different time course figures illustrating the development of AIMs during the *de novo* study, ondansetron appeared to have little symptomatic effect, which is in contradiction with the less severe AIMs when animals were administered L-DOPA alone after the priming phase. A possible explanation may be the development of tachyphylaxis to ondansetron. Further studies are needed to explore this possible tolerance to the therapeutic effect of the drug, as any administration in clinical settings would entail chronic intake of the drug; as such, tachyphylaxis might reduce the translational potential of ondansetron as a treatment for dyskinesia.

It is important to note that during the 22-day study, animals in the vehicle group had relatively moderate AIMs scores and consequently, any effect of treatment appears to be rather subtle. Furthermore, even during the L-DOPA challenge, several vehicle-treated animals had no to minimal dyskinesia, while amongst those that displayed dyskinesia, it was only to a moderate level, despite the fact that they all had significant rearing asymmetry at the cylinder test, indicative of severe nigrostriatal lesion. This finding was not unexpected and is in agreement with the literature (473, 965, 1036), as mentioned before, as several 6-OHDA-lesioned rats do not develop AIMs when exposed to L-DOPA, regardless of the severity of their nigrostriatal DA denervation. However, no or mild dyskinesia in the control group renders it more difficult to detect an effect in the active group, which is a limitation of conducting *de novo* studies in the 6-OHDA-lesioned rat. Here however, this consideration did not prevent us from finding significant results.

Although we have used the term “priming” on several instances in this Thesis, it should be pointed out that the existence of such a process has been debated. Indeed, the definition of

priming as receptor sensitivity (604, 699) or behavioural manifestation (441), depends on the research group. Moreover, several paradigms are used to study priming and some consider that priming occurs after a single administration of a dopaminergic drug whereas others indicate that at least two injections are required (441, 1037). Thus, it was proposed that dyskinesia development could be related to plastic changes induced by dopaminergic denervation in the striatum or accelerate aberrant changes induced by the dopaminergic lesion (440). Furthermore, the authors argue that DA replacement therapy may affect the propensity of treatment to elicit dyskinesia by increasing the likelihood of dyskinesia development, decreasing the threshold dose of drug needed to induce dyskinesia and shifting the dyskinesia dose-response curve to the left (474). Thus, the first intake and subsequent administration of L-DOPA or DA agonists may sensitize to the actual mechanisms that underlie LID but not induce them.

Chronic L-DOPA administration in the 6-OHDA-lesioned rat induces the expression of ΔFosB protein in the DA-denervated striatum of animals that develop LID (1038). Increased ERK1/2 phosphorylation correlates with increased ΔFosB and dyskinesia in the DA-depleted striatum of 6-OHDA-lesioned rats (974, 1039). Therefore, the use of biological molecular markers such as ΔFosB and ERK1/2 phosphorylation may provide a cellular measure of dyskinesia that is complementary to the behavioural assessment of dyskinesia. Further studies are needed to determine if *de novo* administration of 5-HT_3 antagonists interfere with these molecular changes that are associated with the dyskinetic state.

V. Conclusion

There remains many gaps in our understanding of the pathophysiology of LID, and this unmet medical need continues to undermine the quality of life of PD patients. Here, we have used the 5-HT_3 receptor antagonist ondansetron, because it is highly-selective and clinically available. Inasmuch as its high selectivity, the therapeutic benefit conferred by ondansetron is likely to be mediated exclusively through 5-HT_3 receptor blockade. Our results therefore indicate that 5-HT_3 receptor antagonism is a new and promising therapeutic strategy to alleviate established, and prevent the development of, LID in PD. Importantly, the anti-dyskinetic action was achieved without compromising L-DOPA anti-parkinsonian action, a problem that has hindered the development of several potential anti-dyskinetic approaches in the past few years (741, 1040-1042).

Further studies are needed to characterise the potential of this exciting new therapeutic paradigm to alleviate LID. Given the effect of acute challenges of ondansetron 0.0001 mg/kg on the severity of established ALO AIMs, it would be of further interest to investigate whether chronic administration of treatment can maintain this anti-dyskinetic effect. Hence, following the two-week induction priming phase, animals would be administered L-DOPA/ondansetron on a daily basis for three weeks and ALO AIMs severity would be assessed at regular intervals. This would differ from the *de novo* experiments that seek to assess the effect of treatment on the development of AIMs as here, L-DOPA is administered to the animals so that they already exhibit AIMs before L-DOPA/experimental drug is administered. In addition, experiments in the gold standard model of PD, the MPTP-lesioned nonhuman primate model, could validate the efficacy of 5-HT_3 receptor antagonism on dyskinesia as well as another complication of L-DOPA therapy, psychosis. Given the clinical availability of 5-HT_3 receptors, positive outcomes in the MPTP-lesioned primate model could facilitate the testing of these compounds in Phase IIa clinical trials in the context of dyskinesia.

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VII. Appendix

Table III: Duration rating scale of ALO AIMs in the 6-OHDA-lesioned rat

Parameter	Score
axial	0: no dyskinesia 1: occasional signs of dyskinesia, present < 50% of observation time 2: frequent signs of dyskinesia, present > 50% of the observation time 3: dyskinesia present during the entire observation period, but suppressible by external stimuli 4: continuous dyskinesia not suppressible by external stimuli
limbs	0: no dyskinesia 1: occasional signs of dyskinesia, present < 50% of observation time 2: frequent signs of dyskinesia, present > 50% of the observation time 3: dyskinesia present during the entire observation period, but suppressible by external stimuli 4: continuous dyskinesia not suppressible by external stimuli
orolingual	0: no dyskinesia 1: occasional signs of dyskinesia, present < 50% of observation time 2: frequent signs of dyskinesia, present > 50% of the observation time 3: dyskinesia present during the entire observation period, but suppressible by external stimuli 4: continuous dyskinesia not suppressible by external stimuli

Table IV: Amplitude rating scale of ALO AIMs in the 6-OHDA-lesioned rat

Parameter	Score
axial	0: no dyskinesia 1: sustained deviation of the head and neck at about a 30º angle 2: sustained deviation of the head and neck between an angle of 30º and 60º 3: sustained twisting of the head, neck and upper trunk, at an angle between 60º and 90º 4: sustained twisting of the head, neck and trunk at maximal amplitude, causing the rat to lose balance from a bipedal position
limbs	0: no dyskinesia 1: tiny movements of the paw around a fixed position 2: displacement of the whole limb (horizonal or up-and-down) 3: large displacement of the limb with visible contraction of shoulder muscles 4: vigorous limb displacement of maximal amplitude, with contraction of both shoulder groups and extensor muscles
orolingual	0: no dyskinesia 1: twitching of facial muscles accompanied by small masticatory movements without jaw opening 2: twitching of facial muscles accompanied by masticatory movements, occasional jaw opening 3: movements involving facial muscles and masticatory muscles, frequent jaw opening and occasional tongue protrusion 4: involvement of all of the above muscles to the maximal possible degree

Table V: Glass' delta of right forepaw use across ondansetron treatments

dose of ondansetron (mg/mL)	lesion	veh	0.0001	0.001	0.01	0.1	1
mean	82.31	50.79	52.07	43.17	46.03	34	43.98
standard deviation	11.57	27.67	24.09	28.38	24.64	25.76	21.15
Glass' delta	-	2.72	2.61	3.38	3.14	4.18	3.31

Table VI: Glass' delta of both forepaw use across ondansetron treatments

dose of ondansetron (mg/mL)	lesion	veh	0.0001	0.001	0.01	0.1	1
mean	17.09	41.03	34.83	35.28	34.88	45.95	34.56
standard deviation	11.26	22.49	19.93	26.59	21.54	24.85	15.15
Glass' delta	-	2.13	1.58	1.62	1.58	2.56	1.55

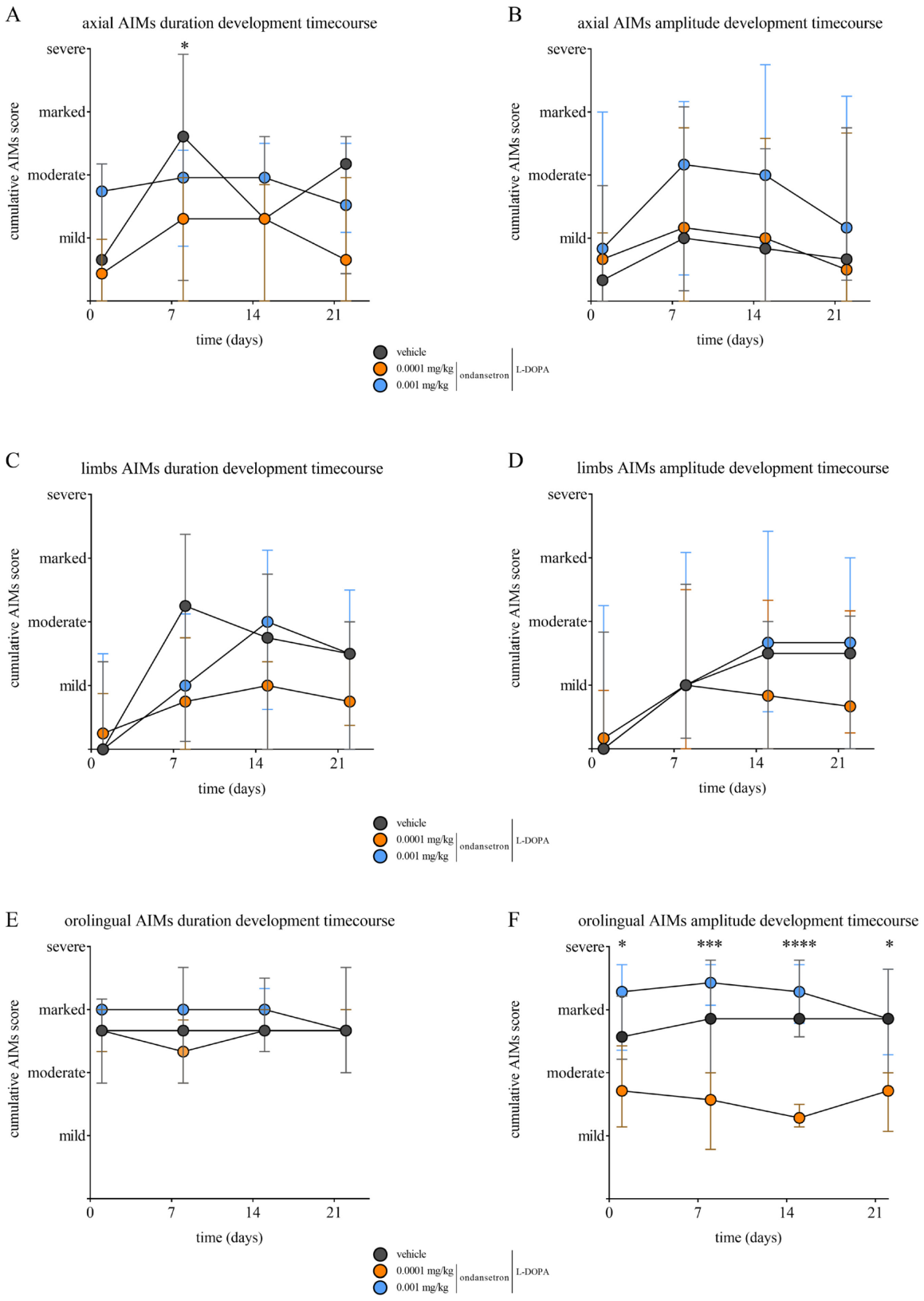
Figure 11: Equation to calculate Glass' delta

$$\text{Glass' delta} = \frac{|\overline{X_2} - \overline{X_1}|}{\sqrt{s^2}_{\text{control}}}$$

$\overline{X_1}$ = mean of control group

$\overline{X_2}$ = mean of experimental group

s_{control} = standard deviation of control group


A
axial AIMs duration development timecourse
B
axial AIMs amplitude development timecourse
C
limbs AIMs duration development timecourse
D
limbs AIMs amplitude development timecourse
E
orolingual AIMs duration development timecourse
F
orolingual AIMs amplitude development timecourse
cumulative AIMs score
severe
marked
moderate
mild
0
7
14
21
time (days)
*
***
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vehicle
0.0001 mg/kg
0.001 mg/kg
ondansetron
L-DOPA

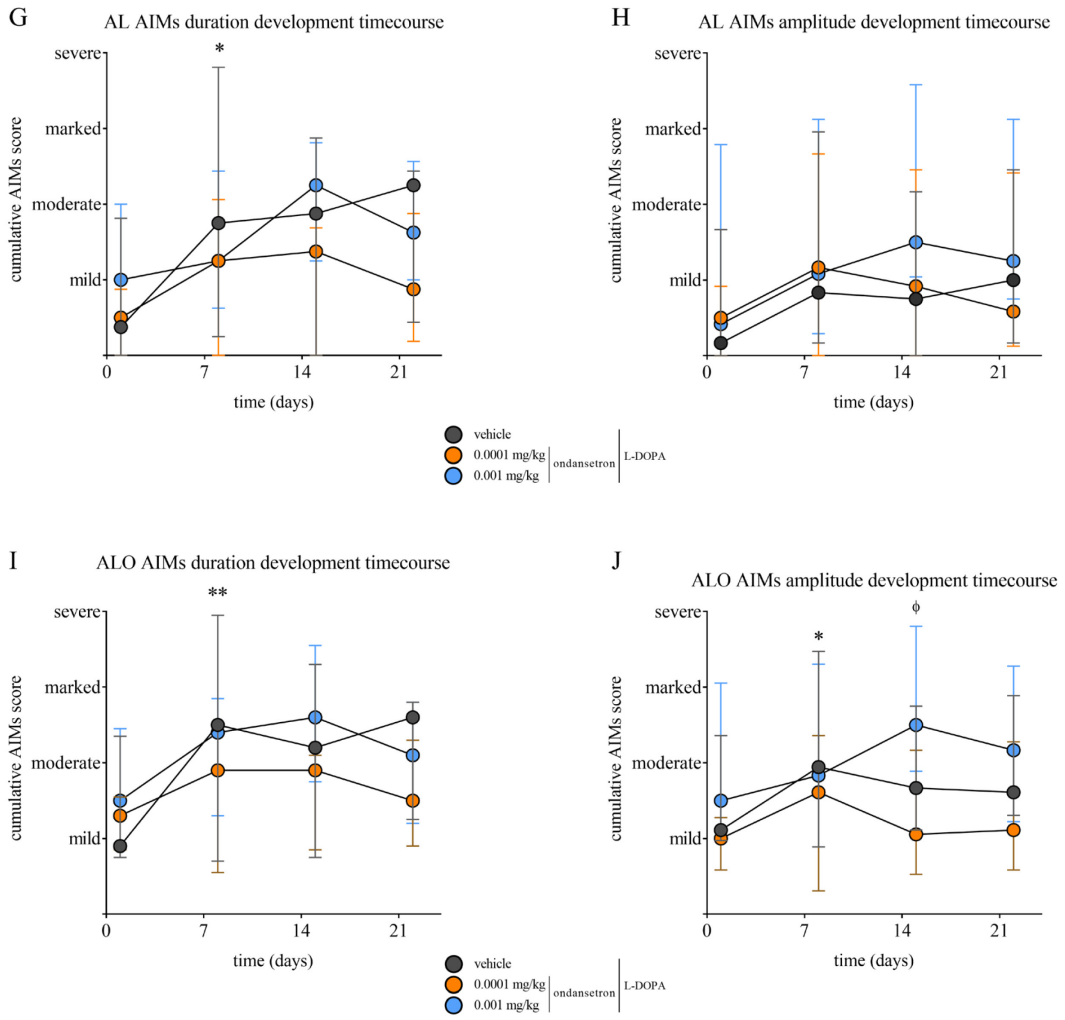

Figure 12: Time course of the development of AIMs during the 22-day priming phase of the *de novo* ondansetron study. On day 8 of priming, **A.** the duration of axial AIMs was significantly reduced in animals previously treated with L-DOPA/0.0001 mg/kg ondansetron (n = 9), by 53%, when compared with L-DOPA/vehicle (n = 7) ($P < 0.05$, Tukey's *post hoc* test). **B.** In contrast, the amplitude of axial AIMS was not significantly reduced in animals that were previously treated with L-DOPA/ondansetron 0.0001 mg/kg (n = 9) compared to animals that received L-DOPA/vehicle. **C.** The duration and **D.** amplitude of limbs AIMs was comparable between animals administered ondansetron 0.0001 mg/kg and vehicle. **E.** The duration of orolingual AIMs was not affected by ondansetron treatment. **F.** Administration of L-DOPA/ondansetron 0.0001 mg/kg resulted in a significant decrease in the amplitude of orolingual AIMs on days 1, 8, 5 and 22 by 21%, 32%, 37% and 24%, respectively, when

compared with vehicle ($P < 0.05$, $P < 0.001$, $P < 0.0001$ and $P < 0.05$, Tukey's *post hoc* test). **G.** On day 8, when administered with L-DOPA, 0.0001 mg/kg ondansetron significantly reduced the duration of AL AIMs, by 53%, when compared with L-DOPA/vehicle ($P < 0.05$, Tukey's *post hoc* test). **H.** The amplitude of AL AIMs was comparable between L-DOPA/0.0001 mg/kg ondansetron and L-DOPA/vehicle. **I.** Administration of ondansetron 0.0001 mg/kg resulted in a significant decrease in the duration of ALO AIMs on day 15, by 33%, when compared with L-DOPA/vehicle ($P < 0.01$, Tukey's *post hoc* test). **J.** The amplitude of ALO AIMs was reduced in animals that were treated with L-DOPA/ondansetron 0.0001 mg/kg, by 31%, compared to animals that received L-DOPA/vehicle ($P < 0.05$, Tukey's *post hoc* test). Data are graphed as the median with interquartile range. *: $P < 0.05$; **: $P < 0.01$, ***: $P < 0.001$, ****: $P < 0.0001$ vehicle versus ondansetron 0.0001 mg/kg; ϕ: $P < 0.01$ ondansetron 0.0001 mg/kg versus ondansetron 0.001 mg/kg.