Therapeutic potential of LY-404,039 via activation of $mGluR_{2/3}$ in Parkinson's disease

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Abstract

LY-404,039, an orthosteric agonist of metabotropic glutamate receptors 2 and 3 ($mGluR_{2/3}$) may harbour additional agonist effect at dopamine D_2 receptors. LY-404,039 and its pro-drug, LY-2140023, have previously been tested in clinical trials for psychiatric indications and could be repurposed for the treatment of Parkinson's disease (PD). We have recently shown that the $mGluR_{2/3}$ orthosteric agonist LY-354,740 alleviated L-3,4-dihydroxyphenylalanine (L-DOPA)-induced abnormal involuntary movements (AIMs) in the 6-hydroxydopamine (6-OHDA)-lesioned rat, without hampering the anti-parkinsonian action of L-DOPA. LY-354,740 also diminished L-DOPA-induced dyskinesia and psychosis-like behaviours (PLBs) in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned marmoset. Here we seek to take advantage of the possible additional D_2 agonist effect of LY-404,039 and determine its effects on dyskinesia and PLBs, in addition to assessing its potential anti-parkinsonian effect, in the 6-OHDA-lesioned rat and the MPTP-lesioned marmoset models of PD. We have administered LY-404,039 (vehicle, 0.1, 1, 10 mg/kg) to 6-OHDA-lesioned rats, after which the severity of AIMs was assessed. The addition of LY-404,039 10 mg/kg to L-DOPA resulted in a significant reduction of AIMs 60-100 min after administration (54%, $P < 0.05$). LY-404,039 significantly enhanced the anti-parkinsonian effect of L-DOPA, assessed through the cylinder test (76%, $P < 0.01$). Next, we determined the pharmacokinetic (PK) profile of LY-404,039 in the marmoset, to optimise drug administration, following which MPTP-lesioned marmosets were injected L-DOPA with LY-404,039 (vehicle, 0.1, 0.3, 1, 10 mg/kg). The addition of LY-404,039 10 mg/kg resulted in a significant reduction of global dyskinesia (55%, $P < 0.01$) and PLBs (50%, $P < 0.05$), as well as a significant enhancement of the anti-parkinsonian action of L-DOPA (47%, $P < 0.05$). These results further reinforce the paradigm of $mGluR_{2/3}$ orthosteric stimulation at alleviating dyskinesia and PLBs in PD. Moreover, the possible agonist effect of LY-404,039 at D_2 receptors might represent an additional therapeutic asset, in light of the anti-parkinsonian effect obtained here. Because LY-404,039 has already been tested in clinical trials, it could be repurposed for indications related to PD.

Résumé

LY-404,039 est un agoniste orthostérique des récepteurs métabotropiques du glutamate 2 et 3 ($mGluR_{2/3}$) qui peut avoir un effet agoniste supplémentaire sur le récepteur dopaminergique D_2. LY-404,039 et son promédicament, LY-2140023, ont déjà été testés dans des essais cliniques pour des indications psychiatriques et pourraient être réutilisés pour la maladie de Parkinson (MP). Nous avons récemment démontré que l'agoniste orthostérique de $mGluR_{2/3}$, LY-354,740, soulage les mouvements involontaires anormaux (AIMs) induits par la L-3,4-dihydroxyphénylalanine (L-DOPA) chez le rat lésé par la 6-hydroxydopamine (6-OHDA) sans interférer avec l'action anti-parkinsonienne de la L-DOPA. Le LY-354 740 a également réduit la sévérité de la dyskinésie induite par la L-DOPA et les comportements de type psychose (PLBs) chez le ouistiti lésé par le 1-méthyl-4-phényl-1,2,3,6-tétrahydropyridine (MPTP). Ici, nous cherchons à tirer parti de l'effet agoniste D_2 supplémentaire possible du LY-404,039 et à déterminer ses effets sur la dyskinésie et les PLBs, en plus d'évaluer son effet anti-parkinsonien potentiel, chez le rat lésé par la 6-OHDA et le ouistiti lésé par le MPTP. Nous avons administré le LY-404 039 (véhicule, 0,1, 1 et 10 mg/kg) aux rats lésés par la 6-OHDA. Ensuite, la sévérité des AIMs a été évaluée. L'ajout de LY-404,039 10 mg/kg à la L-DOPA a entraîné une réduction significative des AIMs durant la période de 60 à 100 min après l'administration (54 %, $P < 0,05$). LY-404 039 a significativement amélioré l'effet anti-parkinsonien de la L-DOPA, évalué par le test du cylindre (76 %, $P < 0,01$). Ensuite, nous avons déterminé le profil pharmacocinétique (PK) de LY-404,039 chez le ouistiti, afin d'optimiser l'administration du médicament. Ensuite, des ouistitis lésés par le MPTP ont reçu une injection de L-DOPA avec LY-404,039 (véhicule, 0,1, 0,3, 1 et 10 mg/kg). L'ajout de LY-404,039 10 mg/kg a entraîné une réduction significative de la sévérité de la dyskinésie globale (55 %, $P < 0,01$) et des PLBs (50 %, $P < 0,05$), ainsi qu'une amélioration significative de l'action anti-parkinsonienne de la L-DOPA (47 %, $P < 0,05$). Les résultats renforcent davantage le paradigme de la stimulation orthostérique de $mGluR_{2/3}$ pour soulager les dyskinésies et les PLBs dans la MP. Par ailleurs, l'éventuel effet agoniste de LY-404,039 au niveau des récepteurs D_2 pourrait représenter un atout thérapeutique supplémentaire, en vu de l'effet anti-parkinsonien obtenu ici. Étant donné que le LY-404,039 a déjà été testé dans des essais cliniques, il pourrait être repositionné rapidement pour des indications liées à la MP.

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Woojin Kang co-first-authored the manuscript, performed the experiments, analysed the data, drafted the manuscript, and managed the submission and editing process. Imane Frouni co-first-authored the manuscript, performed the experiments, analysed the data, and drafted and reviewed the manuscript. Cynthia Kwan organised the experiments and reviewed the manuscript. Dr. Philippe Huot conceived the project, supervised and organised the experiments, and reviewed the manuscript. Dr. Adjia Hamadjida organised the experiments and reviewed the manuscript. Louis Desbiens performed the experiments and reviewed the manuscript.

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List of Abbreviations

5-HT	serotonin
5-HT_{2A}R	serotonin 2A receptor
6-OHDA	6-hydroxydopamine
7TMDs	seven transmembrane-spanning domains
α-syn	α-synuclein protein
AC	adenylyl cyclase
AIMs	abnormal involuntary movements
ALO	axial, limbs, orolingual
A/R	akinetic/rigid
ATP13A2	ATPase cation transporting 13A2
BBB	blood-brain barrier
BG	basal ganglia
BINA	biphenylindanone A
BST1	bone marrow stromal cell antigen 1
cAMP	cyclic adenosine monophosphate
CNS	central nervous system
COMT	catechol-O-methyltransferase
CRD	Cysteine-Rich Domain
D_2	dopamine 2
DA	dopamine
DAT	dopamine transporter
DJ-1	protein deglycase
GABA	γ-amino butyric acid
GBA	glucosylceramidase beta
GP	globus pallidus
GPCR	G protein-coupled receptor
GPe	globus pallidus pars externa
GPi	globus pallidus pars interna
GDP	guanosine diphosphate
GTP	guanosine 5’-triphosphate

GWAS	genome-wide association study
HD	heptahelical domain
IP_3	inositol 1,4,5-trisphosphate
iPSC	induced pluripotent stem cell
LB	Lewy body
L-DOPA	L-3,4-dihydroxyphenylalanine
LRRK2	leucine-rich repeat kinase 2
MAO-B	monoamine oxidase type B
MAPT	microtubule associated protein tau
MFB	medial forebrain bundle
mGluR	metabotropic glutamate receptor
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
NAc	nucleus accumbens
NAM	negative allosteric modulator
NMDA	*N*-methyl-D-aspartate
PAM	positive allosteric modulator
PARK	Parkinson protein
PD	Parkinson's disease
PEPT1	human peptide transporter 1
PFC	prefrontal cortex
PIGD	postural instability and gait disorder
PINK1	PTEN induced putative kinase 1
PK	pharmacokinetic
PKA	protein kinase A
PLBs	psychosis-like behaviours
PLC	phospholipase C
PNS	peripheral nervous system
PRKN	Parkin
PTEN	phosphatase and tensin homolog
RM	repeated measure
s.c.	subcutaneous

SN	substantia nigra
SNc	substantia nigra pars compacta
SNr	substantia nigra pars reticulata
SNCA	synuclein alpha
STN	subthalamic nucleus
TH	tyrosine hydroxylase
UCH-L1	ubiquitin C-terminal hydrolase L1
VFD	Venus Flytrap Domain
VMAT	vesicular monoamine transporter
VTA	ventral tegmental area

Chapter 1: Introduction

Parkinson's disease (PD) is one of the most common neurodegenerative disorders that affects approximately over 100,000 Canadians and 10 million people worldwide (1). Patients with PD experience both motor and non-motor symptoms, but the disease is primary characterised by its motor symptoms, *i.e*. bradykinesia, rigidity and tremor, the constellation of which is termed parkinsonism (1). Non-motor symptoms include dementia, autonomic dysfunction, depression, constipation, olfactory loss, anxiety, and other sleep and emotional disorders (2). The disease affects both males and females but is statistically more likely to develop in males (3). Age plays a major factor in PD development, as it affects 1% of population above 60 and 3% in population over 80 years, compared to only 0.3% in the general population (4). The exact cause of PD is still largely unknown but viral infection, genetic factors, and environmental toxins likely all contribute to development of the neurodegenerative disease, making PD a multifactorial disease (4). Age causes normal physiological and biochemical processes to degenerate, a phenomenon that is notoriously obvious in the dopamine (DA)-producing neurons of substantia nigra (SN) pars compacta (SNc) in the basal ganglia (BG) in PD (5). Environmental toxins, like pesticides and other agrochemicals, as well as genetic risk factors, like mutations in the phosphatase and tensin homolog (*PTEN*), synuclein alpha (*SNCA* or *PARK1/PARK 4*), Parkin (*PRKN* or *PARK2*), ubiquitin C-terminal hydrolase L1 (*UCH-L1* or *PARK5*), PTEN induced putative kinase 1 (*PINK1* or *PARK 6*), protein deglycase (*DJ-1* or *PARK7*), leucine-rich repeat kinase 2 (*LRRK2* or *PARK 8*), and ATPase cation transporting 13A2 (*ATP13A2* or *PARK9*) genes all contribute to the degeneration of DA neurons of the SNc (6). For instance, mutation in the *SNCA* gene, which codes for the protein α-synuclein (α-syn), causes misfolding and accumulation into clumps known as Lewy bodies (LBs), which leads to early-onset PD (6).

There is no cure for PD, and only symptomatic treatments are available. Administration of DA receptor agonists alleviate motor symptoms of PD (4). However, the nature of their action may cause harmful side effects, rendering them only a temporary solution. L-3,4-dihydroxyphenylalanine (L-DOPA or levodopa) is the most effective therapeutic drug, which alleviates motor symptoms by restoring DA levels in the striatum (7). However, chronic administration of L-DOPA is associated with motor complications, known as L-DOPA-induced dyskinesia and motor fluctuations (7). Eventually, after over 10 years of L-DOPA use, 90-95% of patients suffer from L-DOPA-induced dyskinesia (7-9). Amantadine is the only approved treatment that attenuates the expression of dyskinesia without interfering with the therapeutic

benefits of L-DOPA (10). However, amantadine has limited efficacy as well as adverse side effects such as confusion, hallucinations, leg and feet oedema, etc. (11, 12). Due to these limitations of amantadine, discovering other treatments that alleviate dyskinesia is crucial. Other therapeutic options for the treatment of PD, in addition to L-DOPA and DA receptor agonists, are monoamine oxidase type B (MAO-B) (13) and catechol-O-methyltransferase (COMT) inhibitors (14). In advanced stages of PD, patients may choose surgical interventions such as deep brain stimulation of the subthalamic nucleus (STN) or globus pallidus (GP) pars interna (GPi) (15). However, surgical interventions are limited to patients in advanced stages of the disease due to their invasive nature. Continuous administration of levodopa-carbidopa intestinal gel through an intrajejunal percutaneous tube may also be an option to treat motor fluctuations, as well as dyskinesia to a certain extent (16, 17).

1. Parkinson's disease

1.1. Epidemiology

1.1.1. Incidence, prevalence, and distribution

Based on incidence studies of PD, global PD incidence ranges from < 10 to > 20 new cases in 100,000 annually (18, 19). The incidence increases by 5-to-10-fold from sixth to ninth decade of life span. This wide range of variability in the incidence reports may be due to methodological differences. Estimates of global PD prevalence range from 1 to 2 per 1,000 in unselected populations, but increases to 1% of the population above age 60 (19).

The only unequivocal and consistent risk factor for PD is age. Early onset of PD before age 50 years is rare and a sharp increase of the incidence is observed in higher age groups (20). Based on predictive models, it can be estimated that PD prevalence will double in the next two decades as the global population age increases (18). In addition to age, some reports indicate greater prevalence among men than women by ratio of 1.5 (21), but others report no significance in prevalence between male and female populations (22, 23).

1.1.2. Non-genetic risk factors

Many non-genetic risk factors have been linked to PD. As mentioned previously, the only reliable indicator of PD is age. It must be noted that most of the non-genetic risk factors were examined in retrospective case-control studies or prospective cohort studies, which introduce the

possibility of reverse causality biases (20). Still, it is important to consider such risk factors as they nevertheless have some correlation with progression of the disease.

A large majority of non-genetic risk factors can be categorized into environmental and dietary risk factors. Environmental risk factors include exposure to agrochemicals, pesticides, and herbicides such as rotenone and paraquat, or consumption of heavy metals such as iron, manganese, copper, lead, amalgam, aluminium, or zinc from contaminated water supplies (20, 24, 25). While these risk factors are closely linked to PD, their mechanism is not fully understood. Other environmental factors include cancer, traumatic brain injury, body-mass index, diabetes, blood cholesterol, arterial hypertension, and early life factors such as season of birth, birthweight, parental age, and infections have all been linked to PD with varying degrees of consistency (20, 24, 25). Consumption of coffee and smoking have consistently reduced the risk of PD (20, 24, 25). The biological basis of this inverse correlation is poorly understood. For smoking, the observation may be a result of selective mortality of smokers among people with PD (20). Consumption of alcohol, on the other hand, shows less clear association with risk of PD (20). Dairy and fat have been positively associated with risk of PD while vitamins are negatively associated (20, 24), but these possibilities also require further investigation.

1.1.3. Genetic risk factors

Genetic research efforts in PD have led to identification of 18 distinct chromosomal regions that are convincingly associated with PD, as well as genes within these loci that may or may not contain causative or disease-determining mutations (26). Linkage studies have successfully identified the following genes unequivocally responsible for heritable, monogenic forms of PD: *SNCA* and *LRRK2* in autosomal dominant forms; *PRKN*, *PINK1*, *DJ-1* and *ATP13A2* in autosomal recessive forms (26-28). Monogenic forms of PD, which are caused by a single mutation in one of these inherited autosomal-dominant or autosomal-recessive gene, while rare, are well-established and well-documented due to their early age of onset compared to non-genetic PD (26). However, monogenic forms of PD are rare and highly distinct from the common typical PD, which has far more complex aetiology and involves complex meshing of multiple genetic and environmental factors. As it stands, monogenic forms of PD account for approximately 10% of PD cases (29). For these reasons, genes involved in monogenic forms of PD will not be further discussed in this Thesis. Other genes, such as microtubule associated protein tau (*MAPT*) and glucosylceramidase

beta (*GBA*) genes are known to be associated with increased risk of PD (30). While attempts have been made to elucidate the genes underlying any heritable form of PD by studying Mendelian inheritance patterns, positive family history of PD may be difficult to track due to various components such as reduced penetrance, variable expressivity, affected single heterozygous mutation carriers, and phenocopies (26). More recently, gene mapping, candidate gene studies, and genome-wide association studies (GWAS) have been conducted in attempts to discover genes associated with PD and compile a comprehensive list of all associated genetic risk factors (27, 28, 31, 32). Notably, GWAS in various populations allow for comparison of associated genetic risk factors across populations. For example, GWAS comparison revealed *PARK16, SNCA* and *LRRK2* as common shared risk loci between European and Japanese populations, with bone marrow stromal cell antigen 1 (*BST1*) gene and *MAPT* as loci showing population differences (28). Further investigation among different populations is required to establish a robust database of associated genetic material, which could help with early detection of individuals susceptible to PD progression and contribute to understanding the pathophysiology of idiopathic PD.

1.2. Symptoms

1.2.1. Motor symptoms

The motor symptoms of PD begin mildly on one side of the body, but progressively spread across the entire body, increasing in severity (33). Motor symptoms include 4-6 Hz rest tremor (5), akinetic/rigid (A/R) symptoms marked by bradykinesia and muscular rigidity (34), as well as postural instability and gait disorders (PIGD) symptoms marked by stooped posture, decreased arm swing, and shuffling gait (35). Although bradykinesia, rigidity, tremor, and postural instability are the 4 cardinal motor features of PD, motor symptoms are variably manifested in each patient and clinical diagnosis is not always straightforward. Classically, the marked diversity among the patients is categorized into either tremor-dominant and A/R PD types (34). However, results of clinical studies support other subgroupings, notably bradykinetic PD patients that exhibit rapid deterioration and great cognitive impairment (36).

1.2.2. Non-motor symptoms

In addition to motor impairments, patients with PD may also suffer from non-motor manifestations ranging from gastrointestinal tract symptoms, urinary symptoms, sexual

dysfunction, dementia, olfactory impairment, vision problems, pain, behavioural changes, memory loss, depression/anxiety/anhedonia, psychosis, sleep disturbances, fatigue, hallucinations/delusions, apathy/attention/memory, cardiovascular dysfunction, autonomic dysfunction, and other symptoms that negatively impact their quality of life (37, 38). Although the clinical diagnosis of PD mostly centres on motor symptoms, non-motor symptoms are extremely frequent and often play a dominant role in the management and diagnosis of PD (38, 39). Some individuals with PD tend to exhibit a larger number of different non-motor symptoms and experience them with greater frequency and severity than motor symptoms. Furthermore, as degeneration of the peripheral nervous system (PNS) and key areas that mediate non-motor symptoms such as the olfactory bulb and the olfactory nucleus occur in preclinical stages of PD, some non-motor symptoms such as olfactory deficit, constipation and sleep disturbance precede the development of motor symptoms, sometimes by more than a decade (40, 41). Recent studies have demonstrated that non-motor symptoms may be more disturbing to the patients' quality of life than motor symptoms (42). Non-motor symptoms are often side-lined by both patients and clinicians alike during diagnosis, as these symptoms are still not commonly associated with the classic motor manifestations of PD and patients do not mention them as symptoms (43).

1.3. Pathophysiology

The two hallmarks of PD pathology are degeneration of nigrostriatal DA neurons in the SNc followed by DA depletion in the dorsal striatum (44) and occurrence of LBs and Lewy neurites throughout the central nervous system (CNS) (45). The disruption in DA transmission in the striatum results in imbalance of input and output between the BG nuclei and causes increase in BG output from GPi and SN pars reticulata (SNr) to thalamic neurons, leading to hypoactivation of the cortex (46-48), leading to paucity of movement in patients. In early stages of the disease, the motor symptoms may not be identifiable until there is an estimated 60% reduction of SNc DA neurons and an estimated 80% reduction of striatal DA neurons, as the brain can functionally compensate for the loss up to such levels (49-52). However, such is not the case in advanced stages of PD where damage to DA neurons is widespread and severe (33). In tremor-dominant PD, the medial SN is most affected by DA denervation, whereas A/R dominant PD is characterized by severe denervation in the lateral SN (53, 54). Formation of LBs occurs primarily through aggregation of misfolded α-syn (55). Lewy pathology is not isolated to the CNS, but is also present

in the autonomic nervous system (56). The attempt to categorize the progressive spread of Lewy pathology has resulted in Braak's hypothesis of six stages (57-59). According to Braak's hypothesis, the Lewy pathology originates from the brain stem and olfactory bulb and then spreads through the brain (59). As it progresses, LBs spread to the midbrain, then the lower forebrain/cortex, and eventually throughout the entire cerebral cortex (59).

1.3.1. Dopaminergic pathway

The wide intertwined network of the DA system throughout the CNS implies that DA modulates a wealth of brain function, including but not limited to locomotion, cognition, and movement (60). DA terminals are encountered throughout the entirety of the brain, but DA neurons that originate in the SNc and terminate in the striatum form the nigrostriatal pathway, whereas DA neurons that originate from the ventral tegmental area (VTA) and terminate in the nucleus accumbens (NAc) and cortex form the mesolimbic and mesocortical pathways. The nigrostriatal pathway is the most affected in PD, with the mesolimbic and mesocortical pathways being relatively spared (61, 62). As projecting DA neurons from the SNc play a key role in signal transmission between the SN and the striatum, and to a lesser extent to other BG nuclei such as the GP pars externa (GPe) and GPi, significant denervation along this pathway results in a loss of DA modulatory effects, which leads to the motor symptoms of PD (48, 63). DA is secreted into the extracellular space via presynaptic vesicular release following intra-vesicle packaging by the vesicular monoamine transporter (VMAT) type 2 (64). Extracellular DA that does not bind to DA receptors on the postsynaptic terminals are either metabolized by MAO-B and COMT in the cytosol or absorbed into the presynaptic terminal by the DA transporter (DAT) (65). The synaptic secretion of DA is controlled by presynaptic DA 2 (D_2) autoreceptors (65-67).

1.3.2. Lewy bodies and α-synuclein

Aggregation of misfolded pathogenic α-syn-containing LBs is one of the hallmarks of PD pathophysiology (68). The functional role of α-syn in the healthy human body is not fully defined, but it is typically found in small amounts throughout the body, particularly in the liver, muscles, lymphocytes, and red blood cells (69). In the brain, α-syn is highly expressed in presynaptic nerve terminals, particularly in the olfactory bulb, hippocampus, striatum, and thalamus, and is believed to be involved in regulation of synaptic vesicle trafficking and neurotransmitter release with

relevance to DA storage (56, 70, 71). α-Syn is intrinsically unstructured but has significant conformational plasticity. α-Syn monomers form β-sheet rich oligomers that make up a transient population of protofibrils of heterogenous structure (55, 72). These protofibrils likely form more stable amyloid-like fibrils, resulting in aggregation and precipitation to form LBs (72). α-Syn has a prion-like properties, implying that abnormal α-syn spreads from one neuron to another, inducing conformational changes and Lewy pathology in neighbouring neurons, ultimately leading to brain invasion (18, 73). Whereas the initial site of α-syn propagation remains uncertain, observations suggest that the gastrointestinal tract may the origin site (74), whereas others point to the olfactory bulb (75-77). The cause of this initial trigger of α-syn aggregation is unknown, although it is likely that the aggregation is impacted by numerous risk factors of PD, from environmental risk factors, genetic predisposition, and cellular proteostatic mechanism deterioration from age (78, 79). Mutations and triplications of the α-syn gene may be a driving force behind overexpressed α-syn and accelerated formation of LBs in certain cases (80). Missense mutations in the *A30P* and *A53T* display a propensity for excessive self-aggregation to form oligomeric fibrils and LBs (80). Exposure to mitochondrial complex-I inhibitors such as rotenone and paraquat may facilitate aggregation of α-syn (81-83). Oxidative stress may also play a prominent role in α-syn aggregation, particularly in the SNc with aging (84). The stabilization of α-syn with aging likely results in accumulation of α-syn with oxidative damage (84).

According to the Braak staging, once inside the CNS, α-syn invasion follows a 6-stage progression (57-59). In the first stage the Lewy-like aggregates are isolated to the olfactory bulb and dorsal motor nucleus of the vagus nerve, specifically on the medulla oblongata where the nerve fibres that extend to the gut and other visceral organs originate. In the second stage, α-syn spreads along the neural pathways throughout the brain, until the third stage, in which they reach the SN. Throughout the rest of the stages, the α-syn spreads throughout the brain until eventually reaching most of the cerebral hemispheres including the primary cortices, in the sixth stage (59). Of note, the Braak staging remains hypothetical and other models, notably the "brain-first vs body-first model", has recently emerged, showing the complexity of PD pathophysiology (85, 86).

1.4. Treatments

Presently, the standard treatment approach is pharmacotherapy, although severe cases may be amenable to surgical approaches (87). Pharmacotherapy generally involves restoring DA

transmission within the BG, especially the striatum (7, 88, 89). These treatments are effective for symptom relief but are not permanent cures and most elicit negative side effects after chronic administration. The following sections will discuss the most prominent pharmacological treatment avenues for PD symptoms.

1.4.1. L-DOPA

L-DOPA is the most efficacious therapy for the treatment of PD motor symptoms. It is conventionally administered with another molecule, carbidopa or benserazide, two peripheral aromatic L-amino acid decarboxylase inhibitors, to delay the conversion of L-DOPA into DA until it crosses the blood-brain barrier (BBB) and reaches the brain (90). Carbidopa and benserazide also mitigate side effects of L-DOPA and allow to reduce the dosage (87). Chronic administration of L-DOPA almost inevitably leads to development of abnormal involuntary movements (AIMs) commonly known as L-DOPA-induced dyskinesia (87). Patients begin developing dyskinesia after 5 years of treatment (91) and, by 15 years of treatment, 90-95% of patients suffer from dyskinesia (9). Levodopa-carbidopa intestinal gel can also be delivered continuously to the proximal jejunum of advanced PD patients who experience severe motor fluctuation and dyskinesia via a percutaneous gastrojejunostomy tube connected to a portable infusion pump (16, 17).

1.4.2. DA agonists

DA agonists, which directly activate DA receptors, are generally less effective than L-DOPA at treating motor symptoms (92). Particularly, activation of D_2 receptors may play a critical role in mediating the beneficial anti-parkinsonian effect of DA agonists (93). Some have suggested that concurrent D_1 and D_2 receptor activation may confer greater effective anti-parkinsonian action, but the development of drugs with such affinities has been lagging, and most DA agonists preferentially interact with D_2 and D_3 receptors (93). They are administered as a monotherapy to delay the need for L-DOPA therapy, and in turn, the development of dyskinesia, until motor symptoms become too severe and disrupt patients' function and everyday lifestyle (90). They can also be administered in later stages of the disease, as adjunct to L-DOPA (94). DA agonists can be subdivided into ergot derivatives and non-ergot derivatives (93, 95). Ergot-derivatives are first generation of DA agonists that are seldom used nowadays due to the increased risk of valvular heart disease, whereas non-ergot derivatives are second-generation DA agonists, such as now-

conventionally used ropinirole and pramipexole (96). Of note, the use of DA agonists has diminished in the past decade, given their propensity to elicit impulse-control disorders (97, 98).

1.4.3. MAO-B inhibitors

MAO-B inhibitors, as the name implies, inhibit the activity of MAO-B. As MAO-B catalyses the metabolism of DA in the brain, its inhibition increases DA levels in the striatum and reduces PD symptoms (13). Like DA agonists, MAO-B inhibitors may be administered as monotherapy during early stages of PD when symptoms are non-invasive and used to delay the need for L-DOPA therapy (99). Like DA agonists, MAO-B inhibitors can be administered as adjunct to L-DOPA to treat motor fluctuations (90). The most frequently administered MAO-B inhibitors are rasagiline, selegiline, and more recently, safinamide (100-102).

1.4.4. COMT inhibitors

COMT is responsible for peripheral metabolism of L-DOPA (14, 103). Its inhibitors block the enzyme to prolong DA response and mitigate motor fluctuations (104). COMT inhibitors are generally used in combination with L-DOPA as adjunct (103). Entacapone and opicapone are the most frequently used COMT inhibitor (14, 105).

1.4.5. Non-DA drugs

Non-DA drugs primarily interact with neurotransmitter systems other than DA. Non-DA drugs for PD include anticholinergics and amantadine. Anticholinergic drugs antagonise binding of acetylcholine with muscarinic receptors, thereby balancing DA and acetylcholine levels (106). Anticholinergic drugs are not frequently used due to their adverse side effects such as cognitive impairment, dry mouth, constipation, etc. (107). Amantadine is well-known as symptomatic treatment of dyskinesia, but its therapeutic benefit for early PD symptoms is also established (108).

1.4.6. Non-pharmacological therapies

Symptoms of PD are predominantly treated with pharmacological therapies. Nonetheless, certain cases of advanced PD require surgical intervention. Nowadays, deep brain stimulation is the most commonly performed surgical procedure in PD (15, 109, 110). Briefly, deep brain stimulation consists of inserting electrodes inside the brain, usually inside the STN, sometimes the

GPi and, more rarely in PD, the thalamus, in order to regulate the oscillatory activity of the BG (15, 111). These surgical therapies are beyond the scope of this Thesis and will not be discussed in further detail.

1.5. L-DOPA-induced dyskinesia

Dyskinesia develops with chronic use of L-DOPA as treatment for PD symptoms. L-DOPA is the most effective drug for the treatment of PD, and, as mentioned above, a majority of L-DOPA-treated PD patients eventually develop dyskinesia (112-114). In this way, L-DOPA is simultaneously an effective treatment of PD and a dyskinesia inducer. Once L-DOPA-induced dyskinesia has been established, each subsequent exposure to the drug can trigger the re-emergence of the involuntary movements (115). Loss of DA neurons in the SNc facilitates the induction of dyskinesia (116, 117). The extent of nigral DA cell loss seems to determine the level and duration of drug exposure required to induce dyskinesia (7, 118). The exact mechanism(s) of dyskinesia is unclear and has not been elucidated. The most popular proposal involves alterations to the classic model of the direct and indirect output pathways of BG (Figure 1) following striatal DA reduction (115). In early stages of PD, degeneration in the SN and loss of striatal DA results in adaptive changes in striatal organization as well as the outputs from the striatum (119).

Thus, the physiological balance of two major output routes of the striatum, the direct and indirect pathways, is altered in dyskinesia (115). The neurons of the direct pathway express D_1 receptors and project to the GPi and the SNr, which then projects towards the thalamus (120). The neurons of the indirect pathway express D_2 receptors and project to the GPe (120). In the GPe, they synapse with γ-amino butyric acid (GABA) efferent neurons that project to the STN and form synapses with glutamatergic neurons that contact the GPi and SNr (121). In healthy brains, DA inhibits activity along the indirect pathway and promotes activity in the direct pathway (122). Therefore, in PD, the indirect pathway activity is increased with absence of DA control and the direct pathway activity, decreased (123, 124). This results in a cascading response in the BG circuitry that ultimately leads to decreased thalamic neuronal firing, resulting in less cortical activation and, as a corollary, less movement (123, 124). In this state, L-DOPA normalizes PD motor symptoms by restoring DA levels in the striatum (9). However, chronic administration of L-DOPA, which effectively overfloods the striatum with overabundance of DA and overstimulates DA receptors, leads to the opposite phenomenon from the one observed in PD (115). Thus, in the

dyskinetic state, the indirect pathway is underactive, and the direct pathway is overactive, resulting in overfiring of thalamic neurons, leading to excessive cortical activation, which clinically manifest as AIMs (112). Of note, this explanation is an oversimplification of complex processes, but is nevertheless helpful in understanding the underpinnings of dyskinesia in the parkinsonian state (115). Furthermore, the classical view of the BG circuitry, from which the theory is based on, comes with its own controversy. In addition, non-physiological, discontinuous, pulsatile stimulation of striatal DA receptors by intermittent administration of L-DOPA have been associated with development of motor complications (125, 126). To prevent this phenomenon, DA agonists with longer duration of action were administered for more continuous stimulation of striatal DA receptors that results in delayed onset and reduced severity of L-DOPA-induced dyskinesia (127, 128), although this is not as commonly done today. Of note, the exact molecular and physiological mechanisms underlying the effect of pulsatile stimulation on dyskinesia is not clearly understood (128). Thus, further research is required to understand the possible pathways from which dyskinesia truly originates.

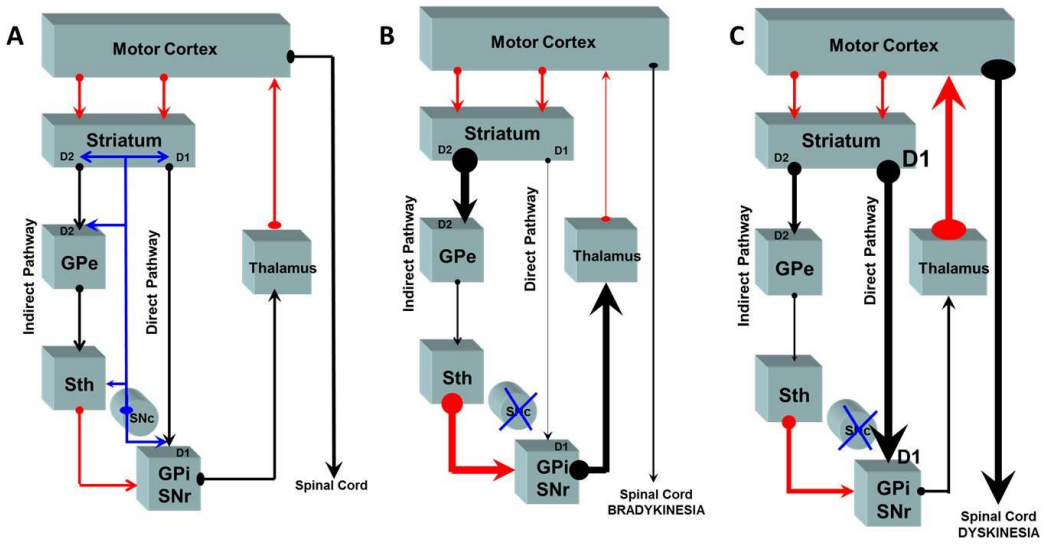

Figure 1. BG motor circuitry in healthy, parkinsonism, and dyskinetic states
In (A), the direct pathway projects to GPi and the indirect pathways projects to GPe. Activity in the BG circuitry is normal. In (B), degeneration of nigrostriatal DA neurons causes underactive direct pathway and overactive indirect pathway, resulting in parkinsonism. In (C), overabundance of DA in the striatum causes underactive indirect pathway and overactive direct pathway, resulting in dyskinesia. GPe, globus pallidus pars externa; Sth, subthalamic nucleus; GPi, globus pallidus pars interna; SNr, substantia nigra pars reticulata; SNc, substantia nigra pars compacta, D_1, DA D_1 receptor and D_2, DA D_2 receptor. Reproduced from Sacnité Albarran et al. 2014 (129) with permission from IntechOpen.

1.5.1. Treatment of L-DOPA-induced dyskinesia

Amantadine, a non-selective *N*-methyl-D-aspartate (NMDA) receptor antagonist that also modulates DA and cholinergic neurotransmission, is prescribed to PD patients to alleviate L-DOPA-induced dyskinesia without concomitantly worsening PD symptoms (10, 130, 131). It is currently the only drug approved by the FDA for the treatment of dyskinesia (10, 94, 132). Despite its efficacy in relieving dyskinesia, neuropsychiatric side effects and possibility of tolerance for amantadine limit its usefulness for the treatment of dyskinesia in PD (10, 133). Clozapine, more commonly known as an anti-psychotic drug, has been shown to alleviate dyskinesia (134) and parkinsonism (135) in randomised controlled trials. However, it too is limited due to the possibility of inducing agranulocytosis (136).

2. Metabotropic Glutamate Receptors

2.1. Overview of mGluRs

Metabotropic glutamate receptors (mGluRs) were first discovered in the mid-1980s, following the observation that glutamate stimulates inositol 1,4,5-trisphosphate (IP_3) production in cultured striatal neurons (137). This was the first discovery to challenge the general belief that all glutamate receptors were ionotropic, ligand-gated ion channel, receptors. The term "metabotropic glutamate receptors" was dubbed in 1987, to distinctly categorize the glutamate receptors that were activated via an indirect metabotropic process (138). Since then, mGluRs have been thoroughly investigated and are part of the group C family of G protein-coupled receptors (GPCRs), a large group of membrane-bound proteins activated by extracellular ligands such as light-sensitive compounds, hormones, peptides, and neurotransmitters (139-141). GPCRs transduce intracellular signals via interactions with G proteins, and consist of a heterotrimeric complex of α, β, and γ subunits (139, 142). GPCRs encompass three main domains: an extracellular N-terminal domain, seven transmembrane-spanning domain (7TMD) α-helices, and an intracellular C-terminal tail. While the GCPR superfamily contains several subgroupings, it is classically divided into 3 main classes (A, B, and C) (142).

2.1.1. Class C GPCRs

The majority of GPCRs belong to class A, the largest class among the 3 classes, consisting of the most diverse GPCRs genes (143). These receptors are often termed the rhodopsin-like

GPCRs and are structurally homologous. In contrast, mGluRs belong to class C GPCRs (143). Class C GPCRs are distinguished from class A GPCRs by a larger extracellular N-terminal domain about 500-600 amino acids long that contains the endogenous ligand-binding site (144, 145). In addition to mGluRs, class C GPCRs is comprised of $GABA_B$ receptors, calcium-sensing receptors, pheromone receptors, chemosensory receptors, taste receptors, etc. (146, 147). Extracellular ligands either bind to the extracellular N-terminal loops or to one of the binding sites within the 7TMDs, which often contain the binding sites for allosteric ligands (148, 149). Binding of ligands activates the associated G-protein subunits by exchanging guanosine 5'-triphosphate (GTP) for guanosine diphosphate (GDP) within the α subunit (150). This allows the α subunit to dissociate from β and γ subunits and the conformation change activates the G-protein (150). The dissociated α subunit modulates the function of various molecules in the intracellular signalling pathways, such as enzymes, ion channels, and transcription factors (150). Inactivation of the G-protein occurs when the bound GTP is hydrolysed to GDP, resulting in reassembly of the subunits (150).

2.1.2. Classification of mGluRs

Eight different types of mGluRs, $mGluR_1$-$mGluR_8$, are subclassified into 3 groups, Group I-III, based on structure, sequence homology, G-protein coupling, ligand-binding profiles, and shared downstream signalling pathways (141). Group I consist of $mGluR_1$ and $mGluR_5$. Group I mGluRs are coupled with $G\alpha_{q/11}$ protein and linked to stimulation of phospholipase C (PLC) signalling, formation of IP_3 hydrolysis, opening of Ca^{2+} ion channels, and increasing cytosolic Ca^{2+} concentrations (151, 152). Group I receptors are also known to modulate other ion channels, such as Na^+ and K^+ channels, thereby having both excitatory and inhibitory effects (153). Group II mGluRs consists of $mGluR_2$ and $mGluR_3$, whereas Group III consists of $mGluR_4$, $mGluR_6$, $mGluR_7$, and $mGluR_8$. Group II and Group III mGluRs are coupled with a $G\alpha_{i/o}$ protein and inhibit the activity of adenylyl cyclase (AC), which in turn prevents the formation of cyclic adenosine monophosphate (cAMP) and mitigates protein kinase A (PKA) activity (152, 154). An overview of mGluRs is provided in Table 1 (141, 151-154).

Table 1. Overview of mGluRs

Group	Receptors/splice variants	CNS expression	Synaptic localization	Signalling pathways of group
Group I	$mGluR_1$ a, b, c, d, e, f Taste $mGluR_1$	• Widespread in neurons • Taste buds	Predominantly postsynaptic	• Phospholipase C stimulation • Stimulation of adenylyl cyclase (some systems) • MAP kinase phosphorylation
	$mGluR_5$ a, b	• Widespread in neurons, astrocytes		
Group II	$mGluR_2$	• Widespread in neurons	Presynaptic and postsynaptic	• Inhibition of adenylyl cylcase • Activation of K^+ channels Inhibition of Ca^{2+} channels
	$mGluR_3$ GRM3Δ2 GRM3Δ4 GRM3Δ2Δ3	• Widespread in neurons, astrocytes		
Group III	$mGluR_4$ Taste $mGluR_4$	• Widespread in neurons, High in cerebellum • Taste buds	Predominantly presynaptic	• Inhibition of adenylyl cylcase • Activation of K^+ channels • Inhibition of Ca^{2+} channels Stimulation of cGMP phosphodiesterase ($mGluR_6$)
	$mGluR_6$ a, b, c	• Retina	Postsynaptic in ON-bipolar retinal cells	
	$mGluR_7$ a, b, c, d, e	• Widespread in neurons	Active zone of presynaptic terminals	
	$mGluR_8$ a, b, c	• Lower and more restricted expression than $mGluR_{4/7}$	Predominantly presynaptic	

mGluR, metabotropic glutamate receptor; MAP, mitogen-activated protein; cGMP, guanosine 3',5'-cyclic monophosphate
Adapted from Niswender and Conn 2010 (141) with permission from Annual Reviews, Inc.

2.2. Properties and physiology of mGluRs

Compared to other GPCRs, mGluRs possess a large extracellular N-terminal domain, called the Venus Flytrap Domain (VFD), made up of 2 globular lobes separated by a hinge (140, 141). The glutamate-binding site lies within the cleft between the 2 lobes. Like other GPCRs, mGluRs form functional dimers through a hydrophobic interaction between lobe I of the VFD in each monomer, stabilized by a disulphide bridge (155). Chimera receptors containing the C-terminal domains of type 1 and type 2 $GABA_B$ receptors have revealed that mGluR dimers are activated by a single molecule of orthosteric agonist, but that binding of 2 agonist molecules, 1 on each monomer, is required for full receptor activation (140, 156, 157). Thus, mGluRs may adopt 3 main conformations, between which the lobes oscillate: open-open (inactive), closed-open (active), and closed-closed (active) (141, 158). Open-open state is when no agonist ligand is bound (158). This inactive state can be stabilized by antagonists. Closed-open and closed-closed states are active states when 1 or 2 agonist molecules are bound to each VFDs and cause different receptor conformations (141). Also present in mGluRs are Cysteine-Rich Domains (CRDs), which propagate the conformational changes in the VFDs from ligand binding (141). This signal is transmitted throughout different domains of the receptor, such as the heptahelical domain (HD)-C terminal tail (159, 160). The signal is transmitted from VFDs to the CRDs through the disulphide bridge formed between Cys-234 of the VFD and Cys-518 of the CRD that cross-links the VTD to the rest of the receptor (160). HDs are 7 transmembrane α-helices present in all GPCRs. The second intracellular loop (i2) of mGluRs may be responsible for G-protein coupling specificity, a process regulated by kinases such as GPCR kinase 2 (141, 161). Most positive allosteric modulators (PAMs) and negative allosteric modulators (NAMs) of mGluRs bind within the HD (162). In contrast to the VFDs, only 1 ligand binding to HD is required for effective G-protein activation (163). The C-terminal of mGluRs is intracellular and modulates G protein coupling (164). It is also where modulatory protein-protein interactions occur, as well as alternative splicing in certain mGluRs (165).

2.3. $MGluR_{2/3}$

$MGluR_2$ is encoded by *GRM2* on chromosome 3 and $mGluR_3$ is encoded by *GRM3* on chromosome 7 (166). $MGluR_2$ and $mGluR_3$ share ~ 70% sequence homology, which is highly conserved for the ligand binding site (167, 168). Both receptors are expressed on neurons and

astrocytes in the CNS (169). Both receptors possess all key features of GPCRs, including extracellular N-terminal domain, 7TMD, and intracellular C-terminal domain. MGluRs form a heteromeric complex with other GPCRs (155). For example, $mGluR_2$ has been shown to be colocalized in several brain regions with $mGluR_4$, notably in the striatum, where $mGluR_2$ and $mGluR_4$ form a heterocomplex, displaying unique pharmacological profile in response to allosteric modulators (155). $MGluR_2$ also displays protein-protein interactions with the serotonin (5-HT) type 2A receptor ($5\text{-}HT_{2A}R$) (170).

Modulation of $mGluR_{2/3}$ regulates glutamate release and maintains homeostasis in the synaptic cleft (8, 171). In recent years, $mGluR_{2/3}$ as modulators of glutamatergic transmission have been studied as a novel target for the treatment of diseases such as schizophrenia and PD (172-175). Receptors coupled with $G\alpha_{i/o}$ proteins are classically associated with inhibition of AC and cAMP formation (176). As such, $mGluR_{2/3}$ regulate ion channels and downstream signalling pathways via dissociation of α from $\beta\gamma$ subunits. Furthermore, both Group II and III mGluRs are also associated with activation of mitogen-activated protein kinase and phosphatidyl inositol kinase pathways (177). It is via these complex network of signalling pathways that these mGluRs modulate synaptic transmission (140, 141, 177).

2.3.1. Localization of $mGluR_{2/3}$ in the brain

$MGluR_{2/3}$ are predominantly localized on presynaptic terminals (141), though not entirely absent in the postsynaptic terminal, where they function as inhibitors of glutamatergic transmission (178, 179). While $mGluR_2$ is localized mostly to neurons at the presynaptic terminal, $mGluR_3$ is expressed at both the presynaptic and postsynaptic terminals and in glial cells (178, 180).

Quantitative receptor autoradiography studies of the rat brain using [^{3}H]-LY-354,740, an orthosteric agonist of Group II receptors, revealed high density of $mGluR_{2/3}$ binding in the accessory olfactory bulb, limbic cortex, striatum, cerebellar granular layer, outer molecular layer of the dentate gyrus, molecular layer and stratum lucidum of the hippocampus, the antero-ventral thalamic nuclei, as well as the neocortex (181).

Immunohistochemistry revealed an intense reactivity in the external plexiform and granule cell layers of the accessory olfactory bulb (181). In the cerebral neocortex, layers I, III, and IV displayed highest levels of $mGluR_2$ and $mGluR_3$ immunolabelling. In the striatum, the caudate

putamen and NAc were strongly labelled. In the thalamus, the anteroventral thalamic nuclei and the reticular thalamic nucleus showed the highest levels of $mGluR_{2/3}$ immunoreactivity.

In situ hybridisation histochemistry revealed that layer IV displayed the most $mGluR_2$ and $mGluR_3$ transcripts, but $mGluR_3$ mRNA were distributed throughout the cortical layer, even in the corpus callosum. In the striatum, only $mGluR_3$ transcripts were detected. In the thalamus, $mGluR_2$ transcripts were found in both the anteroventral and reticular nuclei, while $mGluR_3$ transcripts were found only in the reticular nucleus (181).

2.3.2. Dimerization with 5-HT_{2A}R

As mentioned in Section 2.3, recent studies have indicated that $mGluR_2$ may form functional heterodimers with 5-HT_{2A}R in the isocortex (182-184). These two receptors are co-expressed in the same population of cortical neurons, and both have been implicated as the mechanism of action of anti-psychotic drugs (177, 185-188). It is suggested that dimerization between $mGluR_2$ and 5-HT_{2A}R results in similar downstream effects, where $mGluR_2$ activation and 5-HT_{2A}R antagonism both mediate the anti-psychotic effects (189). Once formed, the 5-HT_{2A}R-$mGluR_2$ complex likely promotes allosteric cross-talk between the two receptors as well (182). In mice, the heterodimerization with $mGluR_2$ increased the affinity of $5HT_{2A}$R for hallucinogenic drugs and activation of $mGluR_2$ suppressed those responses (182). Furthermore, $mGluR_2$ knockout mice did not display behavioural effects to hallucinogenic drugs, suggesting that 5-HT_{2A}R-$mGluR_2$ complex, not 5-HT_{2A}R receptor alone, is necessary for neuropsychological responses to hallucinogens (190). Finally, post-mortem analysis of untreated schizophrenic brains showed significantly altered receptor densities in cortical membranes, increased 5-HT_{2A}R levels and decreased $mGluR_{2/3}$ levels (182). It should be noted that there is no evidence that $mGluR_3$ subtype forms functional dimers with $5HT_{2A}$R.

2.4. Pharmacological modulation of $mGluR_{2/3}$

As the two Group II receptors share ~ 70% sequence homology, most of the orthosteric agonists identified so far are dual $mGluR_{2/3}$ activators (168). Ligands that selectively bind to $mGluR_2$ and/or $mGluR_3$ can be generalized into two categories: selective orthosteric agonists/antagonists and selective allosteric modulators (191). The first non-selective Group II mGluR orthosteric agonists to be discovered was (1*S*,3*R*)-ACPD (192, 193), and subsequent

investigation led to identification of agonists with greater selectivity. Once the conformationally constrained bicyclo[3.1.0]alkane-based glutamate $mGluR_{2/3}$ orthosteric agonist LY-354,740 was identified, various dual $mGluR_{2/3}$ agonists with nanomolar potency were delineated from the unique scaffold (168). Examples of such selective agonists are oxygen containing LY-379,268, sulphur containing LY-389,795, LY-404,039, and LY-459,477, as well as $mGluR_2$ agonist/$mGluR_3$ antagonist LY-541,850 (194-198). Peptidyl prodrugs such as LY-544,344 and LY-2140023 were also developed to improve oral absorption and bioavailability (199-201). LY-2812223 and its L-alanine prodrug LY-2979165 have been developed as well and have recently entered clinical testing (202, 203).

Selective allosteric modulators, on the other hand, consist of a group of large chemical diversity. Allosteric modulators, both PAMs and NAMs, are an emerging class of orally available molecules with greater selectivity and better modulatory control at $mGluR_2$ over $mGluR_3$ or $mGluR_3$ over $mGluR_2$, depending on their primary target, in contrast to orthosteric ligands, which tend to be equipotent at both $mGluR_2$ and $mGluR_3$ (168). Allosteric modulators do not bind to $mGluR_{2/3}$ directly but instead bind to an allosteric site in the HD to mediate the response to glutamate (141). The majority of $mGluR_{2/3}$ PAMs are related to either prototypical $mGluR_2$ PAMs LY-487,379 or biphenylindanone A (BINA), which both reverse PCP-induced hyperlocomotion in rodents (141, 204).

Many $mGluR_2$ activators have been investigated for their therapeutical potential in treating various neuropsychiatric disorders, and several have successfully reached clinical development phases for the treatment of neurological disorders such as schizophrenia and anxiety (168). LY-354,740 was one of the first $mGluR_{2/3}$ agonists to have demonstrated tolerability and safety profiles in clinical trials with patients with panic disorder (205). While unable to reach clinical significance compared to placebo, LY-354,740 has also been investigated for possible anxiolytic effects (206, 207). Other $mGluR_2$ activators such as AZD8529 (schizophrenia; Phase II) (208), LY-2979165 (bipolar disorder; Phase I) (209), ADX71149/JNJ40411813 (schizophrenia; Phase II, epilepsy; Phase II) (210, 211), LY-544,344 (anxiety disorders; Phase II) (212), and LY-2300559 (migraine; Phase II) (213) have all successfully reached clinical trials (168). $mGluR_2$ activators have been investigated for working memory processes and cognitive symptoms of schizophrenia as well (168, 214).

2.5. Effect of $mGluR_{2/3}$ activation on L-DOPA-induced dyskinesia

$MGluR_{2/3}$ activation has recently been studied as a treatment of L-DOPA-induced dyskinesia, because of their expression at the cortico-striatal synapse and their role in regulating glutamatergic transmission (172, 215-217). Indeed, it has been proposed that L-DOPA-induced dyskinesia is the result of incoming overactive striatal glutamatergic transmission that triggers excessive excitatory effects, notably through hyperactivation of post-synaptic glutamate receptors such as NMDA and $mGluR_5$ (218). Amantadine is thought to reduce the dyskinetic effect by blocking NMDA, thereby diminishing glutamatergic transmission (219). Despite having no interactions with NMDA receptors, the paradigm of $mGluR_{2/3}$ activation possesses potential as an alternative way to reduce over-active striatal glutamatergic transmission upon activation and the dyskinetic manifestation that follows (8, 171).

Recent studies with the $mGluR_{2/3}$ orthosteric agonist LY-354,740, which has 6-fold potency difference for $mGluR_2$ over $mGluR_3$ (220), in the 6-hydroxydopamine (6-OHDA)-lesioned rat and the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned marmoset models of PD, have successfully demonstrated that activation of $mGluR_{2/3}$ not only significantly diminishes already established L-DOPA-induced dyskinesia in both species, but also reduces severity of psychosis-like behaviours (PLBs) and enhances the anti-parkinsonian action of L-DOPA in the marmoset (172). These results are comparable to that of amantadine in terms of alleviating established dyskinesia in both the 6-OHDA-lesioned rat (221, 222) and the MPTP-lesioned marmoset (223-225). Administration of LY-354,740 also attenuated the development of dyskinesia when commenced concurrently with the first dose of L-DOPA in the rat (172).

Similar studies with the $mGluR_2$ PAM LY-487,379 in 6-OHDA-lesioned rats (226) and MPTP-lesioned marmosets (216, 227), as well as with the $mGluR_2$ PAM CBiPES in the MPTP-lesioned marmoset (173) have also shown efficacy in attenuating dyskinesia and PLBs, while providing additional anti-parkinsonian action when administered with L-DOPA. As $mGluR_2$ positive allosteric modulation is the putative mechanism of action of these PAMs (228), these studies provide mechanistic insight into $mGluR_2$ activation as the source of anti-dyskinetic, anti-psychotic, and anti-parkinsonian effects when $mGluR_{2/3}$ agonists are administered. On the other hand, it also reveals that $mGluR_3$ stimulation likely does not confer much anti-dyskinetic action upon dual $mGluR_{2/3}$ orthosteric activation. Rather, $mGluR_3$ stimulation may counter or dampen any anti-dyskinetic effects by amplifying $mGluR_5$ signalling (229), whose negative allosteric

modulation has recently been investigated as an anti-dyskinetic approach in PD (230, 231). Reversal of these anti-dyskinetic and anti-psychotic effects of LY-354,740 and LY-487,379 via administration of $mGluR_{2/3}$ orthosteric antagonist LY-341,495 provided incremental evidence that the $mGluR_2$ signalling pathway is the mechanism of action of those therapeutic effects (232).

2.6. LY-404,039

LY-404,039 is a nanomolar potent orthosteric agonist of Group II mGluRs (196, 233). LY-404,039 is highly selective for $mGluR_{2/3}$, displaying > 100-fold selectivity for the receptors compared to other receptors that are conventionally targeted by anti-psychotic medications (196, 233). LY-404,039 displays no affinity for other mGluRs (234), nor does it demonstrate affinity for receptors involved in the mechanism of clinically effective anti-psychotic and anxiolytic medications (233). Functionally, the drug suppresses electrically evoked excitatory activity in the striatum and 5-HT-induced L-glutamate release in the prefrontal cortex (PFC) by inhibiting cAMP formation in cells expressing $mGluR_{2/3}$ (206, 233, 235). Its modulatory role in glutamatergic activity in brain regions associated with psychiatric disorders suggests valuable potential for treatment of neuropsychiatric disorders (235, 236). In addition, unlike other $mGluR_{2/3}$ agonists, LY-404,039 was found to inhibit the binding of the D_2 antagonist [^{3}H]-domperidone, to the human cloned D_2 receptor with dissociation constants of 8.2-12.6 nM at high-affinity state D_2 receptors, indicating an additional affinity at D_2 receptors (237). This is lower than the dissociation constants of 92-149 nM at human $mGluR_{2/3}$ (233), demonstrating that D_2 receptors would be considerably occupied at clinically-relevant doses (237). Furthermore, LY-,404,039 bears structural similarities with the DA chemical model and comfortably fits the tetrahedral model of the D_2 receptor, lending support for a LY-404,039-D_2 interaction and DA neurotransmission modulation (238). However, this distinct affinity of LY-404,039 remains controversial and requires further investigation (233).

The methionine prodrug of LY-404,039, pomaglumetad methionil (LY-2140023), has been developed to enhance the oral bioavailability of LY-404,039, the active moiety, in humans and has undergone clinical trials as treatment for schizophrenia (203, 239-243). Based on human data, the estimated bioavailability of the prodrug is 68% (201). The prodrug is actively absorbed in the gastrointestinal tract by the human peptide transporter 1 (PEPT1) and undergoes complete or partial hydrolysis to release the active moiety during transit through the intestinal epithelium by the action of esterase enzymes (201). LY-2140023 has reached Phase III clinical stage for

treatment of schizophrenia (240, 242). During earlier clinical trials, LY-2140023 given at 80 mg per day was effective, well-tolerated, and deemed safe as a potential monotherapy for positive, negative, and other symptoms in patients with schizophrenia (200, 239, 242). In contrast, rodents exhibit high plasma esterase activity and most, if not all, of the prodrug is converted to active moiety and is not detected in the circulation (201).

3. Animal Models of Parkinson's disease

Animal models are utilized in PD studies for the comprehension of the molecular mechanisms underlying the disease and discovery of novel and effective treatments (244). They reproduce some core elements of clinical phenotype of the disease, although they are generally limited in mimicking the progressive degenerative nature of the disease (60). PD models can be generated through neurotoxin-based, genetic-based, or α-syn-based approaches in a wide range of animals. Neurotoxin-based models recapitulate the degeneration of DA neurons in the SNc and reduced DA concentrations in the striatum (245, 246). These models involve administration of toxins such as 6-OHDA, MPTP, paraquat, and rotenone (246). Genetic-based animal models involve manipulation of disease-causing genes to generate transgenic models (244). Such targets include transgenic overexpression of *SNCA* or *LRRK2* in mice models as well as knockout and knockdown of *DJ-1*, *PINK1* or *PRKN* in mice (247-251). α-Syn-based models include transgenic mice overexpressing α-syn, injection of pre-formed fibrils in the brain or gut to produce LB-like pathology in the brain of animals, viral mediated overexpression with either recombinant adeno associated virus or lentivirus vectors expressing α-syn (58, 247, 252-262). There are also recent models that combine both neurotoxin administration and genetic manipulation. One model utilizes in vitro midbrain organoids from the induced pluripotent stem cell (iPSC) of PD patients and genetically modified iPSCs of healthy controls for studying human PD progression (244, 263-266). Another model induces neuroinflammation in the midbrain that may lead to DA neuron degeneration to explore the role of immune system and a potential novel avenue for PD pathogenesis (267-270).

While these models are engineered for PD studies, individually, these models do not wholly mimic the complexities of human PD and are limited in recapitulating the clinical features of the disease (271, 272). It must be emphasized that neurotoxin-based models lack LB formation and model an advanced stage of PD (60, 271). Other models, such as the haloperidol-induced catalepsy

or the reserpine-treated rat, do not even trigger a degenerative process (273). As for genetic models, some do not manifest parkinsonism; they are primarily used to understand specific aspects of the pathogenesis of PD (244, 274). Therefore, it is imperative to select a model that is the most likely to answer the questions specific to the study.

The two animal models of PD that we employ in the experiments detailed later in this Thesis are the 6-OHDA-lesioned rat and the MPTP-lesioned marmoset, which are both highly characterized to study the effects of experimental drugs on dyskinesia and parkinsonism and, in the case of the marmoset, dyskinesia, parkinsonism, and PLBs (60, 275). The following sections will discuss both animal models of PD in depth and provide reasons why they are suitable models for the experimental work conducted in this Thesis.

3.1. 6-OHDA

6-OHDA is a hydroxylated analogue of DA, with similar molecular structure to DA and noradrenaline (276). It was first isolated in 1959 (277, 278) and investigated for its effects on catecholaminergic neurons in 1963, before becoming one of the most popular neurotoxins in PD models (278). Once administered, 6-OHDA is selectively taken up by catecholaminergic neurons via DAT and noradrenaline transporters, where the neurotoxin accumulates in the cytosol or mitochondria (279, 280). Accumulated 6-OHDA inhibits mitochondrial complex I and forms free radical with molecular oxygen, leading to oxidative stress, mitochondrial damage and, eventually, apoptosis (275, 281). However, as 6-OHDA accumulates in catecholaminergic neurons, both DA and noradrenergic neurons are destroyed by the neurotoxin (271, 276). Therefore, animals are often pre-treated with a noradrenaline transporter blocker, desipramine, prior to the injection of 6-OHDA for selective lesioning of DA neurons and preservation of noradrenergic neurons (282). Studies have shown that 6-OHDA-induced degeneration of catecholamine neurons is comparable to the degeneration caused by injection of iron, suggesting a role for iron in the toxicity of 6-OHDA (275).

3.1.1. The 6-OHDA-lesioned rat

6-OHDA does not cross the BBB when administered systemically (283, 284). Thus, it must be injected in situ by stereotaxic surgery (283, 284). Currently, the three common injection sites are SN, medial forebrain bundle (MFB), and striatum, although early studies have utilized

intracerebroventricular injection to achieve bilateral degeneration of DA neurons (285-289). Injection of 6-OHDA at each site induces varying extent of DA denervation, but the neurotoxin can be injected virtually anywhere along the nigrostriatal tract. Injections into the SN or the MFB are followed by degeneration of DA neurons within 12 h and striatal DA levels begin diminishing 2-3 days later (275). While the magnitude of the DA neuron lesion also varies according to the amount of 6-OHDA injected and the species used, it remains highly effective and causes near complete (> 97%) DA neuron lesions if injected in the MFB, the most appropriate to model advanced PD (290, 291). Injections in the striatum cause a partial, more progressive and retrogradely induced neuron death than injection in the other sites (275). Upon repeated administration of L-DOPA, 6-OHDA-lesioned rats exhibit AIMs, a rat analogue of human dyskinesia (282, 292, 293).

3.1.2. Bilateral and unilateral 6-OHDA lesions

In the first 6-OHDA rat models, the animals were bilaterally lesioned with injections in lateral ventricles (294). Bilaterally 6-OHDA-lesioned rats displayed severe motor symptoms, such as catalepsy, akinesia, adipsia and aphagia (294, 295). These debilitating phenotypes eventually recovered due to compensatory mechanisms in which the remaining functional DA neurons in the nigrostriatal pathway took up increased function, DA turnover and receptor sensitivity (296). However, the animals remained severely difficult to motivate and train and required intensive nursing care (297). Thus, the bilateral lesion model is no longer widely used and has largely been replaced by the unilateral lesion model (295).

The unilateral 6-OHDA-lesioned rat is also known as the hemi-parkinsonian rat model, and the intact hemisphere may serve as internal control structure when post-mortem experiments are carried (298, 299). Unlike bilateral injections, unilateral 6-OHDA-injection generates asymmetric and observable parkinsonian behaviours. Furthermore, upon administration of L-DOPA or other DA receptor agonists (300), unilaterally lesioned rats rotate preferentially away from the side of the lesion (295). In contrast, in the presence of amphetamine, lesioned rats display rotations preferentially towards the side of the lesion, likely due to the imbalance in DA release between the two striata (301). Compensatory hypersensitivity in the DA system of the unaffected striatum may also contribute to this rotational behaviour (302).

3.1.3. Behavioural tests in the 6-OHDA-lesioned rat

Rats do not exhibit observable parkinsonian features following unilateral 6-OHDA lesion of the nigrostriatal pathway (301). Absence of idiopathic PD features such as bradykinesia and tremor in rats presents difficulty for assessing the extent of lesion (295). Therefore, the degree of DA denervation must be assessed via behavioural tests to demonstrate sufficient motor deficits for parkinsonian studies (292, 293, 301). These tests are also performed to estimate the anti-parkinsonian efficacy of treatment and the severity of treatment-related motor complications (295). In context of the experiments conducted for this Thesis, these behavioural evaluations were crucial in distinguishing whether any anti-dyskinetic effect of the treatment interfered with the anti-parkinsonian action of L-DOPA. The motor test performed for this study to assess the degree of parkinsonism and the effect of the experimental molecule on the parkinsonism was the cylinder test. In addition to motor tests that assess parkinsonism in unilateral 6-OHDA-lesioned rats, AIMs scale exists for assessment of AIMs (292, 293, 301, 303, 304).

3.1.3.1. The cylinder test

The cylinder test, also known as the limb-use asymmetry test, evaluates the preferential use of each forepaw to determine the extent of unilateral 6-OHDA lesion of the nigrostriatal pathway (295). The cylinder test is performed with the rat placed in a transparent cylinder (30 cm high and 20 cm in diameter) without prior habituation period (301). The shape of the cylinder enables rats to explore the enclosure by periodic rearing (305, 306). During each rear, the rats use their forelimbs to hold themselves against the cylindrical wall (306). Each weight bearing contact on the wall is scored, separated by forepaw ipsilateral and contralateral to the side of the lesion, as well as contacts by both forepaws simultaneously. At the end of the scoring session, percentage of contact for each paw in total wall contact can be calculated to determine the degree of unilateral DA loss. In normal rats, the percentages of right and left forepaw use are comparable to each other. In unilaterally 6-OHDA-lesioned rats, a preferential use of the ipsilateral forepaw in $\geq$ 70% of all wall contacts is indicative of $\geq$ 88% nigrostriatal DA-lesion, which is widely used as a threshold for animal inclusion in studies (306). Therefore, the cylinder test is a sensitive measure of the extent of nigrostriatal denervation following unilateral 6-OHDA lesion. Unlike the rotation paradigms described in Section 3.1.3.2., the cylinder test does not require administration of DA drugs for evaluation of motor function, which maintains the animals in a drug-naïve state, if

required (301, 306). In addition, the cylinder test is sensitive to pharmacological reversal in forepaw asymmetry by administration of L-DOPA and DA agonists, as the anti-parkinsonian action of these compounds is reflected in the rearing scores (301, 303). The disadvantage of the cylinder test is that it cannot be performed too frequently, as the rats' interest in exploring the novel environment gradually decreases with multiple sessions, along with the repeated wall contacts (295).

3.1.3.2. Abnormal Involuntary Movements

The AIMs rating scale evaluates the severity of dyskinesia following administration of L-DOPA to rodents (282). Before the development of the AIMs rating scale (292), most experimental studies of L-DOPA-induced dyskinesia were performed in MPTP-lesioned non-human primates (282). While primate models do offer the advantage of demonstrating movement patterns comparable to humans, they are accordingly more expensive, time-consuming, and not readily available for many laboratories (282, 307).

In 1970s, Ungerstedt developed a "rotometer" system to quantify behavioural asymmetry in 6-OHDA-lesioned rats, in which rotational behaviour contralateral to the side of lesion was measured following administration of L-DOPA or DA agonists (308, 309). Ungerstedt's rotometry model was used for a while as the gold standard in rodent research, but its interpretation was not straightforward (303). Thus, the behavioural component(s) underlying rotations, either dyskinesia, an anti-parkinsonian effect, motor fluctuations, or a combination thereof, was difficult to precisely ascertain, which limited its translational potential (303). The rotometry model, while still in use today, has been mostly replaced by the development of the AIMs rating scale in combination with a test to assess motor disability, such as the cylinder test (303).

Cenci and colleagues developed the AIMs rating scale for the 6-OHDA-lesioned rat treated with L-DOPA/benserazide that accounts for all abnormal movements and postures exhibited by dyskinetic rats (282). These abnormal movements are divided into 4 subcategories of axial, limbs, orolingual (ALO), and locomotive, which parallels L-DOPA-induced dyskinesia in PD patients. "Axial" AIMs is a category that comprises of lateral flexion of the neck and/or torsional movements of the upper trunk towards the contralateral side of the lesion (282). "Limbs" AIMs category comprises hyperkinetic, jerky stepping movements of the forelimb contralateral to the lesion, and/or small circular movements of the forelimb to and from the snout (282). Severe limbs

AIMs phenotypes include slow and forceful positioning, as well as fast and irregular movements. “Orolingual” AIMs category scores facial, tongue, and masticatory movements (282). These include bursts of empty masticatory movements, variable degree of jaw opening, lateral translocations of the jaw, twitching of facial muscles, and protrusion of the tongue towards the contralateral side of the lesion. “Locomotive” AIMs, less used because of the difficulty of interpreting rotational behaviour, include locomotion towards the contralateral side of the lesion (282). During the locomotion process, rats must have at least three paws on the floor for it to be scored. These 4 AIMs categories are rated for both the percentage of observation time a dyskinetic movement is expressed and the amplitude of the dyskinetic movements. Having 2 different AIMs rating scales increases the dynamic range of the total dyskinesia score per observation session (282).

In these behavioural tests, there must be a discrimination between normal and abnormal movements, the same way that natural behaviours are distinguished from dyskinetic movements (282, 301). Generally, abnormal movements are restricted to the side of the body contralateral to the lesion and can be distinguished from normal movements with little familiarity with normal rodent behaviours (282). Particularly, orolingual AIMs is scored in a conservative manner due to the fact that isolated orolingual movements are difficult to distinguish between abnormal movements induced by L-DOPA or normal movements induced by falling asleep, waking up, or stress (282). However, even abnormal orolingual movements can be identified by the asymmetrical involuntary nature of their exhibition (282). In some cases, AIMs may almost mimic normal rodent physiological responses to stimuli, which makes them difficult to score consistently. That notwithstanding, the rating scale provides suggestions for discriminating and scoring dyskinesias for various cases. Ultimately, Cenci and colleagues state that activity in any given category should be interpreted in context of the global pattern of activation at each given time period (282).

For 6-OHDA-lesioned rodents to develop AIMs, an induction phase in which they are injected daily with L-DOPA/benserazide for 10-14 days is required (282). This is followed by 2-4 injections per week for maintaining a stable dyskinetic phenotype over the long-term. Approximately 50-80% of L-DOPA-treated 6-OHDA-lesioned rats develop AIMs by the end of the treatment period with a daily dose of 6-10 mg/kg/day L-DOPA and 15 mg/kg/day benserazide; it remains unclear why some rats do not develop AIMs, despite being severely lesioned and exposed to L-DOPA (282, 301). The latency for the first appearance of dyskinetic movements may

vary between 1-13 days among individual rats, which can be shortened using higher doses of L-DOPA (301, 310). To rate AIMs severity, animals are placed individually in transparent cylinders separated by ~ 15cm on a table, so the animals can be observed from all angles (282). The rats are observed for 2 minutes every 20 minutes for 3 hours following the injection of L-DOPA. While the AIMs score rating was designed for both rats and mice, there are some differences between the two species and ratings are more difficult to perform in mice due to their smaller size and higher movement speed (282). Rating scales for duration and amplitude are provided in Tables S1 and S2 (see Appendix).

3.2. MPTP

MPTP-induced parkinsonism was discovered accidentally in 1983, when four substance abusers developed a parkinsonian syndrome after self-administered intravenous injection of a potent analogue of the drug meperidine, also known as synthetic heroin (311). It was apparent that MPTP was synthesized as a biproduct during underground laboratory preparations of the drug. While the first reported case to officially identify MPTP as the cause of lesion in the nervous system was in 1983 (312, 313), it is likely that the first case of MPTP-induced parkinsonism was reported previously in 1979 but MPTP was not identified as the responsible contaminant at the time (314). Contaminant MPTP likely caused sporadic outbreaks of parkinsonism among substance abusers across North America for 8 years prior to the initial report (315). Following the discovery of MPTP, its propensity to cause parkinsonism was first investigated in non-human primates in 1984, when it was administered to rhesus macaques (*Macaca mulatta*) (316). Following this discovery, MPTP-induced primate models of PD were engineered in the squirrel monkey (*Saimiri sciureus*) (317), common marmoset (*Callithrix jacchus*) (318), cynomolgus macaque (*Macaca fascicularis*) (319), African green monkey (*Chlorocebus sabaeus*) (320), pig-tailed macaque (*Macaca nemestrina*) (321), vervet monkey (*Cercopithecus aethiops*) (322), bonnet monkey (*Macaca radiata*) (323), Japanese monkey (*Macaca fuscata*) (324), capuchin monkey (*Sapajus apella*) (325), and the baboon (*Papio papio*) (326).

3.2.1. MPTP neurotoxicity

Unlike 6-OHDA, MPTP crosses the BBB after systemic administration due to its lipophilic nature (327). Once inside the brain, MPTP is converted into 1-methyl-4-phenyl-2,3-

dihydropyridium (MPDP) by MAO-B and then spontaneously oxidized into the potent DA neurotoxin 1-methyl-4-phenylpyridinium ion (MPP^+) in non-DA cells of the SN such as astrocytes (327). MPP^+, the metabolite responsible for the neurotoxic effects, is then released into the extracellular space and is taken up by DA neurons through the DAT due to its structural similarity to DA (328). Inside DA neurons, MPP^+ accumulates in the mitochondria and inhibits complex I in mitochondrial respiration, resulting in production of reactive oxygen species (ROS) (275). Increased ROS concentration leads to oxidative stress, inflammation, and apoptosis of DA neurons (275). On the other hand, MPP^+ in neurons can also be sequestrated into synaptic vesicles via the VMAT as a protective measure against the neurotoxin (329). MPTP-induced parkinsonism in human is highly comparable to idiopathic PD, as all cardinal symptoms such as bradykinesia, rigidity, resting tremor, flexed posture, and loss of postural reflexes are present (273, 295). However, it does not replicate the neuropathology of PD, as pathological study of 3 human subjects with MPTP-induced parkinsonism revealed no LBs (330, 331).

3.2.2. MPTP-induced animal models of Parkinson's disease

MPTP can be administered to various species such as cats, dogs, guinea pigs, sheep, rats and frogs, but most common models are with mice and non-human primates (271, 328, 332). Generally, rodents are less sensitive to MPTP toxicity than humans and primates (333). The C57BL/6 mouse strain is sensitive to MPTP and significantly more vulnerable than other mouse strains in terms of mesencephalic DA neuronal loss (275, 334). Rats, on the other hand, do not develop parkinsonism from MPTP as seen in mice and primates, and require significantly higher doses of MPTP to achieve DA depletion that is comparable to that achieved in mice (328). Accordingly, the MPTP-lesioned rat is rarely used to model PD. Conversely, the MPTP-lesioned mouse currently represents one of the most practical models to study neuropathological and neurochemical model of PD, as it also provides some economic advantages (334). For these reasons, mice are currently one of the most used species in MPTP studies. Disadvantage of the MPTP-lesioned mouse model is that mice lesioned with MPTP do not develop persistent and progressive motor symptoms and require significantly higher doses of MPTP on a mg/kg basis than primates to induce a loss of DA neurons (328, 334). Various regimens of MPTP administration have been described in the primate, e.g. subcutaneous (s.c.), intravenous, intra-arterial, etc. (275, 295). These regimens for MPTP administration vary according to species and

desired phenotypes (328). Furthermore, MPTP-lesioned primates treated with chronic injection of L-DOPA display involuntary movements, such as dyskinesia, comparable to those observed in PD patients, making it a suitable model for this study (275, 295, 328).

3.2.3. The MPTP-lesioned marmoset

In the common marmoset, MPTP is often administered s.c. at the dose of 1-2 mg/kg once daily for 5 days (335-338). This method of administration results in > 95% DA reduction in the striatum. Other methods, such as 6 mg/kg s.c. over 9 days, resulting in > 95% DA reduction within the striatum (339), or 1.25–2.5 mg/kg s.c. twice weekly over 5-10 months, leading to > 95% DA reduction within the striatum (340), were also reported. More recently, studies have administered a lower dose of MPTP such as 0.05 mg/kg intravenously 2-3 times per week for longer periods of time in an attempt to mimic the progressive neurodegenerative process of PD that other administration paradigms fail to capture (328, 341-343). Some marmosets were shown to recover over time, at least partly, from parkinsonian phenotypes induced by an earlier systemic administration of MPTP (344). These marmosets rarely regain the motor deficits, despite further administration of high dose MPTP or more severe reduction in DA neurons in the SNc, which suggests functional compensation by non-DA neurotransmitters (344). The topographic pattern of DA cell loss in the striatum of MPTP-lesioned marmoset closely resembles that in human PD (273, 275), though it lacks the formation of LBs (345).

3.2.3.1. Behavioural assessment in the MPTP-lesioned marmoset

The rating scales for assessment of parkinsonism and dyskinesia in MPTP-lesioned marmoset model are presented in detail in Tables S3 and S4 (see Appendix). The severity rating scales are based upon manifestations observed in the clinic in patients with PD, which the MPTP-lesioned marmoset reproduces closely (337, 346-349). Briefly, the parkinsonism rating scale includes range of movement, bradykinesia, posture, attention/alertness. The dyskinesia rating scale includes chorea and dystonia. The rating scale for assessment of PLBs is presented in full detail in Table S5 (see Appendix). The rating scale for PLBs has also been developed and validated from clinical rating scales (225, 350-352). PLBs rating scale encompasses hyperkinesia, hallucinatory-like response to apparent non-stimuli, obsessive grooming, and stereotypies.

3.2.3.2. Clinical predictability value

Based on unbiased reviews of the literature, the MPTP-lesioned marmoset demonstrates high translational predictive effectiveness of forecasting clinical efficacy as it relates to the effect of anti-dyskinetic, anti-parkinsonian, and anti-psychotic drugs (353, 354). More specifically, the MPTP-lesioned marmoset demonstrates a positive predictive value of 76.9% and a false-positive rate of 15.6% for experimental drugs on dyskinesia, positive predictive value of 86.9% and a false-positive rate of 41.7% for experimental drugs of parkinsonism, and positive predictive value of 100% for experimental drugs on psychosis (353, 354). Compared to the other commonly used MPTP-lesioned primate models of PD, the marmoset model boasts the highest predictive value as it relates to clinical effectiveness of experimental drugs on parkinsonism and psychosis and high predictive value for experimental drugs on dyskinesia (353). Thus, MPTP-lesioned marmoset is the ideal MPTP-lesioned primate model for assessment of our drug of interest, following the evaluation of its efficacy in the 6-OHDA-lesioned rat. Use of the MPTP-lesioned marmoset will enhance the validity of our results and maximize the chances of the drug success in eventual clinical trials.

Hypothesis and Aims

As presented in Section 2.5, there is growing evidence that modulating glutamatergic transmission via $mGluR_{2/3}$ activation reduces dyskinesia. However, none of the agents tested also exhibited putative agonistic effects at D_2 receptors. The present study aims to investigate whether LY-404,039, which activates $mGluR_{2/3}$ and has an additional agonistic effect at D_2 receptor, could elicit a more robust anti-parkinsonian action than other $mGluR_{2/3}$ activators tested so far, in addition to alleviate dyskinesia and PLBs.

We hypothesized that:

1. Administration of LY-404,039 alleviates L-DOPA-induced AIMs, in the 6-OHDA-lesioned rat;
2. Administration of LY-404,039 enhances the anti-parkinsonian action of L-DOPA, in the 6-OHDA-lesioned rat;
3. Administration of LY-404,039 alleviates L-DOPA-induced dyskinesia, in the MPTP-lesioned marmoset;
4. Administration of LY-404,039 alleviates L-DOPA-induced PLBs, in the MPTP-lesioned marmoset;
5. Administration of LY-404,039 enhances the anti-parkinsonian benefit of L-DOPA, in the MPTP-lesioned marmoset.

We aimed to:

1. Assess the effect of LY-404,039 on L-DOPA-induced dyskinesia, in the 6-OHDA-lesioned rat;
2. Determine whether LY-404,039 confers additional anti-parkinsonian benefit, when administered with L-DOPA, in the 6-OHDA-lesioned rat;
3. Investigate the effect LY-404,039 on L-DOPA-induced dyskinesia, in the MPTP-lesioned marmoset;
4. Measure the effect of LY-404,039 on L-DOPA-induced PLBs, in the MPTP-lesioned marmoset;
5. Assess the effect of LY-404,039 on the anti-parkinsonian action of L-DOPA, in the MPTP-lesioned marmoset.

Positive results here will provide incremental evidence that orthosteric stimulation of $mGluR_{2/3}$ may alleviate both L-DOPA-induced dyskinesia and PD psychosis. Furthermore, and specific to LY-404,039, the additional agonistic effect at DA D_2 receptors might prove to be advantageous in the treatment of parkinsonian disability, conferring it potential to address simultaneously dyskinesia, psychosis and parkinsonism.

Chapter 2: LY-404,039 in the 6-OHDA-lesioned rat

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Running head: LY-404,039 and dyskinesia in the 6-OHDA rat

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Abstract

LY-404,039 is an orthosteric agonist at metabotropic glutamate 2 and 3 ($mGlu_{2/3}$) receptors with possible additional agonist effect at dopamine D_2 receptors. LY-404,039 and its pro-drug, LY-2140023, have previously been tested in clinical trials for psychiatric indications and could therefore be repurposed if they were shown to be efficacious in other conditions. We have recently demonstrated that the $mGlu_{2/3}$ orthosteric agonist LY-354,740 alleviated L-3,4-dihydroxyphenylalanine (L-DOPA)-induced abnormal involuntary movements (AIMs) in the 6-hydroxydopamine (6-OHDA)-lesioned rat, without hampering the anti-parkinsonian action of L-DOPA. Here, we seek to take advantage of a possible additional D_2-agonist effect of LY-404,039 and see if anti-parkinsonian benefit might be achieved in addition to the anti-dyskinetic effect of $mGlu_{2/3}$ activation. To this end, we have administered LY-404,039 (vehicle, 0.1, 1 and 10 mg/kg) to 6-OHDA-lesioned rats, after which the severity of axial, limbs and oro-lingual (ALO) AIMs was assessed. The addition of LY-404,039 10 mg/kg to L-DOPA resulted in a significant reduction of ALO AIMs over 60-100 min (54%, $P < 0.05$). In addition, LY-404,039 significantly enhanced the anti-parkinsonian effect of L-DOPA, assessed through the cylinder test (76%, $P < 0.01$). These results provide further evidence that $mGlu_{2/3}$ orthosteric stimulation may alleviate dyskinesia in PD and, in the specific case of LY-404,039, a possible D_2-agonist effect might also make it attractive to address motor fluctuations. Because LY-404,039 and its pro-drug have been administered to humans, they could possibly be advanced to Phase IIa trials rapidly for the treatment of motor complications in PD.

Key words: $mGlu_2$, $mGlu_3$, D_2, Parkinson's disease, 6-OHDA-lesioned rat, dyskinesia, LY-404,039

1. Introduction

L-3,4-dyhydroxyphenylalanine (L-DOPA) is the most effective therapeutic drug for alleviation of motor symptoms of Parkinson's disease (PD), which is achieved by restoring dopamine levels in the striatum (Brotchie, 2005; Huot et al., 2013). However, chronic administration of L-DOPA ultimately leads to motor complications, including L-DOPA induced dyskinesia (Dawson and Dawson, 2003). Eventually, 90-95% of patients suffer from dyskinesia after 15 years of dopaminergic therapy (Hely et al., 2005). Amantadine, a N-methyl-D-aspartate (NMDA) glutamate receptor antagonist, is the only FDA-approved treatment for L-DOPA-induced dyskinesia. However, amantadine may lead to adverse effects such as hallucinations (Riederer et al., 1992), suggesting that amantadine may not be a suitable option for some PD patients, emphasising the need to discover new anti-dyskinetic therapeutics.

We have recently identified activation of metabotropic glutamate 2/3 ($mGlu_{2/3}$) receptors as a novel therapeutic approach in PD, either via orthosteric stimulation (Frouni et al., 2019) or positive allosteric modulation (Hamadjida et al., 2020; Frouni et al., 2021) to reduce dyskinesia in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned marmoset. $mGlu_{2/3}$ receptors appear as target of interest based on their regulatory role in glutamatergic neurotransmission. Thus, $mGlu_{2/3}$ receptors are densely expressed at pre-synaptic terminals in the striatum, where they decrease excitatory synaptic transmission at the cortico-striatal synapse, which may underlie their beneficial effects in experimental parkinsonism (Picconi et al., 2002).

Building on the discoveries mentioned above, we elected to perform further experiments in the 6-OHDA-lesioned rat to perform whether the $mGlu_{2/3}$ orthosteric agonist (OA) LY-404,039 would effectively alleviate dyskinesia. Although it remains controversial, LY-404,039 may elicit an additional agonist effect at dopamine D_2 receptors (Seeman, 2013), a property not shared by other $mGlu_{2/3}$ activators. This uniquely positions this molecule, as it may elicit a robust anti-parkinsonian effect when administered, as a therapeutic adjunct to L-DOPA and suggests that, in addition to reducing dyskinesia, it might alleviate parkinsonism. LY-404,039 and its pro-drug LY-2140023 have undergone clinical trials for schizophrenia and therefore have known pharmacokinetic (PK) profile and documented safety and tolerability profiles in human (Adams et al., 2013; Liu et al., 2012; Mehta et al., 2018; Patil et al., 2007; Zhang et al., 2015). As LY-404,039 and its pro-drug have already been assessed in clinical settings, they could potentially be

repurposed for indications related to PD, following demonstration of efficacy in pre-clinical experiments.

2. Methods

2.1 Animals

Adult female Sprague-Dawley rats (250-275 g, Charles River Laboratories, Saint-Constant, QC, Canada) were group-housed under conditions of controlled temperature (21 ± 1 °C), humidity (55%) and light (under 12 h light/dark cycle, on 07:00), with free access to food and water. Upon arrival, rats were left undisturbed for one week to acclimatise. All procedures were approved by the Montreal Neurological Institute-Hospital (The Neuro) Animal Care Committee in accordance with the regulations defined by the Canadian Council on Animal Care.

2.2 LY-404,039 and dose selection

LY-404,039 was purchased from MilliporeSigma (Oakville, ON, Canada). The doses of LY-404,039 employed here, 0.1, 1 and 10 mg/kg, were selected based upon its previously disclosed PK profile in the rat (Rorick-Kehn et al., 2007b), as well as on our own data (Kang et al., 2022), to reach maximal plasma concentrations < 1,000 ng/mL, which were shown to be well tolerated in clinical trials (Annes et al., 2015; Mehta et al., 2018; Patil et al., 2007).

2.3 Induction of hemi-parkinsonism

Rats were pre-treated with pargyline (5 mg/kg, MilliporeSigma) and desipramine (10 mg/kg, MilliporeSigma) sub-cutaneously (s.c.), 30 min prior to surgery to prevent damage to noradrenergic neurons (Ungerstedt, 1968). Rats were anaesthetised using isoflurane (2-4%) in 100% oxygen (1 L/min) and placed into a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA). Then, rats were injected with 2.5 µL of 6-OHDA hydrobromide (7 µg/µL in 0.9% NaCl containing 0.02% ascorbic acid; MilliporeSigma) in the right medial forebrain bundle (Huot et al., 2015; Kwan et al., 2020) at the following coordinates, according to the Paxinos and Watson's atlas of the rat brain (Paxinos and Watson, 2013): antero-posterior: − 2.8 mm, medio-lateral: − 2.0 mm, dorso-ventral: − 9.0 mm from Bregma and skull surface. 6-OHDA was injected over 5 min at a flow rate of 0.5 µL/min and the syringe was left in place for 5 min after injection prior to slowly retracting the needle to avoid reflux.

2.4 Assessment of parkinsonism and induction of dyskinesia

Following a three-week post-lesion recovery period, the degree of parkinsonism was assessed using the cylinder test (Schallert et al., 2000). The cylinder test was performed in a transparent cylinder (14 cm diameter × 28 cm height) for 10 min. Animals were recorded for post hoc behavioural analysis by a blinded experienced rater. A mirror was placed behind each cylinder to enable the evaluator to count forepaw movements when the animal was turned away from the camera. The first paw to contact the wall during a rear was scored as an independent wall placement for that paw. If a subsequent placement of the other paw on the wall occurred while the initial placement was maintained, it was attributed a score of "bilateral". A simultaneous placement of both forepaws on the walls was also scored as a "bilateral" movement. Use of the forepaw ipsilateral to the lesion in ≥ 70% of the rears is indicative of ≥ 88% striatal dopamine depletion (Schallert et al., 2000). We have used this as an inclusion threshold here, and only animals exhibiting preferential use of the un-lesioned forepaw in ≥ 70% of the rears were selected to undergo dyskinesia induction. Following the cylinder test, severely lesioned hemi-parkinsonian rats were administered for 14 days with once daily injections of L-DOPA/benserazide (10/15 mg/kg, both from MilliporeSigma) to induce stable axial, limbs and oro-lingual (ALO) abnormal involuntary movements (AIMs), the rodent equivalent of dyskinesia (Cenci and Lundblad, 2007). The dose of 10/15 mg/kg L-DOPA/benserazide was used solely during the dyskinesia induction phase.

2.5 Assessment of ALO AIMs

On days of behavioural testing, rats were administered L-DOPA/benserazide (6/15 mg/kg, from this point forward referred to as L-DOPA) in combination with LY-404,039 (0.1, 1 and 10 mg/kg) or vehicle (0.9% NaCl). The dose of 6/15 mg/kg was used in all of the experiments in which ALO AIMs were rated. Then, rats were placed in individual transparent glass cylinders where ALO AIMs were scored. Treatments were administered s.c. and were randomised according to a Latin square design. Behavioural testing sessions were separated by at least 48 h of drug washout. AIMs were scored for 2 minutes every 20 min over a 180 min testing session (Kwan et al., 2020, 2021). ALO AIMs were rated by an observer blinded to treatment, according to a protocol described by Cenci and Lundblad (Cenci and Lundblad, 2007), which includes both time-

based, i.e., “standard”, and severity-based, i.e., “amplitude”, ratings. ALO AIMs standard was rated according to the following scale: 0 = no dyskinesia; 1 = occasional signs of dyskinesia, present for less than 50% of the observation period; 2 = frequent signs of dyskinesia, present for more than 50% of the observation period; 3 = continuous dyskinesia, interrupted by external stimuli and 4 = continuous dyskinesia not interrupted by external stimuli. Axial AIMs amplitude was 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 between 30° and 60°; 3 = sustained twisting of the head, neck and upper trunk at an angle between 60° and 90° and 4 = sustained twisting of the head, neck and trunk at an angle ≥ 90°, causing the rat to lose balance from a bipedal position. Limbs AIMs amplitude was scored as such: 1 = tiny movements of the paw around a fixed position; 2 = movements leading to a visible displacement of the whole limb; 3 = large displacement of the whole limb with visible contraction of shoulder muscles and 4 = vigorous limb displacement of maximal amplitude, with concomitant contraction of shoulder and extensor muscles. Oro-lingual AIMs amplitude was rated as such: 1 = twitching of facial muscles accompanied by small masticatory movements without jaw opening; 2 = twitching of facial muscles accompanied by masticatory movements that result in jaw opening; 3 = movements with broad involvement of facial and masticatory muscles, with frequent jaw opening and occasional tongue protrusions and 4 = involvement of all the above muscles to the maximal possible degree. Cumulative ALO AIMs scores indicate the sum of ALO AIMs standard or of ALO AIMs amplitude over different consecutive measurement time points. Integrated ALO AIMs scores indicate the result of ALO AIMs amplitude × ALO AIMs standard (Ohlin et al., 2011).

2.6 Evaluation of L-DOPA anti-parkinsonian action

The effect of LY-404,039 on the anti-parkinsonian action of L-DOPA was assessed through the cylinder test, as described above. Following completion of the experiments in which ALO AIMs were rated, rats entered a 3-day washout period, after which they were administered a low dose of L-DOPA (3/15 mg/kg s.c.), in combination with vehicle or LY-404,039 (0.1, 1 and 10 mg/kg s.c.), in a randomised within-subject design. This low dose L-DOPA is sufficiently high to produce an anti-parkinsonian effect without triggering AIMs (Frouni et al., 2019; Kwan et al., 2020) and was used only for the cylinder test experiments. After 45 min, at peak anti-parkinsonian

action, animals underwent the cylinder test. The number of rears of each forepaw was counted, post hoc, by a treatment-blinded experimenter.

2.7 Immunohistochemistry

After completion of the behavioural experiments, rats were perfused trans-cardially with 0.9% NaCl and brains were extracted and snap-frozen in isopentane at -56°C. Brains were sectioned, unfixed, into 12 μm sections, on a cryostat at -20°C. Immunohistochemical staining was performed on brain sections containing the striatum. Slides were post-fixed by immersion in pre-cooled (-20°C) acetone for 10 min and air dried for 20 min. Sections were rinsed for 3 × 5 min in Tris buffered saline (TBS; 100 mM Tris-Cl, pH 7.40, containing 240 mM NaCl). Once rinsed, sections were quenched in 0.5% H_2O_2 for 10 min, followed by rinse for 3 × 5 min in TBS. Then, sections were incubated for 1 h in 10% normal goat serum (NGS) and 5% bovine serum albumin (BSA) in TBS containing 0.3% Triton X-100, followed by rinse for 3 × 5 min in TBS. Sections were then incubated with a mouse monoclonal antibody raised against tyrosine hydroxylase (TH, 1:1000, MilliporeSigma, #MAB318) in 5% NGS and 2% BSA in TBS containing 0.1% Triton X-100 (TBS-T) overnight at 4°C. The following day, sections were rinsed for 3 × 5 min in TBS. The next incubation was in the presence of a goat anti-mouse biotinylated secondary antibody (1:200, Invitrogen, Waltham, MA, USA, #31800) in 5% NGS and 2% BSA in TBS-T for 1 h, followed by rinse for 3 × 5 min in TBS. The final incubation was in avidin-biotin complex detection kit (ABC; Vector Laboratories, Burlingame, CA, USA, #PK-6100) for 2 h, followed by rinse for 3 × 5 min in TBS. Antibodies were visualised in TBS-T containing 1.25 mg/mL nickel ammonium sulphate hexahydrate (MilliporeSigma, #574988), 0.25 mg/mL 3,3'-diaminobenzidine (MilliporeSigma, #D5637) and 0.015% H_2O_2. Sections were dried after another TBS wash, rehydrated in water, and dehydrated in 50%, 70%, and 100% alcohol solutions. Once dehydrated, sections were cleared with xylene and coverslipped using Permount mounting medium (Fisher Scientific, Pittsburgh, PA, USA, #SP15-100). TH-immunoreactivity was quantified by densitometric analysis on sections containing the striatum (Bregma ~ +1.20 mm). Images were captured by a Nikon Eclipse E800 microscope (Nikon Corporation, Minato City, Japan, available at The Neuro Microscope Core Facility) using Stereo Investigator software (MBF Bioscience, Williston, VT, USA, version 11). Mean relative optical density (OD) was measured in the ipsilateral and contralateral striata of four adjacent brain sections in ImageJ software (NIH, Bethesda, MD, USA, version 1.53c).

2.8 Statistical analysis

Cylinder test data for assessment of parkinsonism are graphed as the mean ± standard error of the mean (SEM) and were analysed by one-way repeated measure (RM) analysis of variance (ANOVA) followed by Tukey's post-test. Cumulative AIMs scores are presented as the median with semi-interquartile range and were analysed using Friedman test followed by Dunn's post-test. The effect of LY-404,039 on L-DOPA anti-parkinsonian action is presented as the mean ± SEM and was analysed by one-way RM ANOVA followed by Tukey's post-test. Mean relative striatal TH OD values were analysed by Student's t-test by comparing the lesioned and the un-lesioned striata. Statistical significance was set to $P < 0.05$. Statistical analyses were computed using GraphPad Prism 7.0d (GraphPad Software Inc, San Diego, CA, USA).

3. Results

3.1 Extent of parkinsonism and dopaminergic denervation

As shown in Figure 1, 6-OHDA-lesioned rats displayed marked forepaw asymmetry while rearing [$F_{(1.001,13.02)} = 141.2$, $P < 0.0001$; one-way RM ANOVA], with preferential use of the right (un-lesioned) forepaw in 78.05% of wall contacts when compared to 0.19% with the left (lesioned) forepaw and 21.76% with both forepaws, respectively (both $P < 0.0001$, Tukey's post-test), assessed by the cylinder test. Accordingly, there was a significant decrease in TH immunoreactivity when comparing the left and right striata (Figure 2A), with a reduction of ≈ 86% on the lesioned side, compared to the un-lesioned side ($t_{(13)} = 7.457$, $P < 0.0001$, Figure 2B).

3.2 Effect of LY-404,039 on global L-DOPA-induced ALO AIMs

Over the total experimental period, administration of LY-404,039 significantly reduced the severity of global ALO AIMs standard (Friedman Statistic [FS] = 8.023, $P < 0.05$, Figure 3A). There was no significant reduction between 10 mg/kg and vehicle, but significant reduction by ≈ 14% ($P < 0.05$, Dunn's post-test) was observed when comparing 10 and 1 mg/kg.

Administration of LY-404,039 significantly alleviated the severity of global ALO AIMs amplitude (FS = 21.46, $P < 0.0001$, Figure 3B). The addition of LY-404,039 10 mg/kg reduced the severity of ALO AIMs amplitude by ≈ 35%, when compared to vehicle ($P < 0.01$, Dunn's post-test). When comparing 10 mg/kg to other doses, there was ≈ 31% ALO AIMs amplitude reduction

from 0.1 mg/kg ($P < 0.001$, Dunn's post-test) and ≈ 33% reduction from 1 mg/kg ($P < 0.001$, Dunn's post-test). LY-404,039 0.1 and 1 mg/kg did not significantly alter the severity of ALO AIMs amplitude when compared to vehicle.

Acute challenges of LY-404,039 also significantly decreased the severity of integrated ALO AIMs (FS = 21.51, $P < 0.0001$, Figure 3C). Adding LY-404,039 10 mg/kg to L-DOPA reduced the severity of integrated ALO AIMs by ≈ 39%, when compared to vehicle ($P < 0.01$, Dunn's post-test). When comparing 10 mg/kg to other doses, there was ≈ 34% integrated ALO AIMs reduction from 0.1 mg/kg ($P < 0.01$, Dunn's post-test) and ≈ 39% reduction from 1 mg/kg ($P < 0.001$, Dunn's post-test). LY-404,039 0.1 and 1 mg/kg did not significantly alter the severity of integrated ALO AIMs when compared to vehicle.

3.3 Effect of LY-404,039 on L-DOPA-induced ALO AIMs from 60-100 min

Over the 60-100 minutes period (time course showed in Supplementary Figure 1A), LY-404,039 did not produce any effect on ALO AIMs standard (FS = 6.409, $P = 0.0933$, Figure 4A).

In contrast to its effect on ALO AIMs standard, LY-404,039 significantly alleviated the severity of ALO AIMs amplitude over 60-100 min (FS = 21.39, $P < 0.0001$, Figure 4B and Supplementary Figure 1B). Thus, adding LY-404,039 10 mg/kg to L-DOPA reduced the severity of ALO AIMs amplitude over 60-100 min by ≈ 41%, when compared to vehicle ($P < 0.01$, Dunn's post-test). When comparing 10 mg/kg to other doses, there was ≈ 44% ALO AIMs amplitude reduction from 0.1 mg/kg ($P < 0.001$, Dunn's post-test) and ≈ 43% reduction from 1 mg/kg ($P < 0.05$, Dunn's post-test). LY-404,039 0.1 and 1 mg/kg did not significantly alter the severity of ALO AIMs amplitude over 60-100 min when compared to vehicle.

Acute challenges of LY-404,039 also significantly reduced the severity of integrated ALO AIMs over 60-100 min (FS = 13.93, $P < 0.01$, Figure 4C and Supplementary Figure 1C). The addition of LY-404,039 10 mg/kg to L-DOPA reduced the severity of integrated ALO AIMs over 60-100 min by ≈ 54%, when compared to vehicle ($P < 0.05$, Dunn's post-test). When comparing 10 mg/kg to other doses, there was a ≈ 47% ALO AIMs amplitude reduction from 0.1 mg/kg ($P < 0.05$, Dunn's post-test) and ≈ 51% reduction from 1 mg/kg ($P < 0.05$, Dunn's post hoc test). Lower doses of LY-404,039 did not significantly alter the severity of integrated ALO AIMs over 60-100 min when compared to vehicle.

3.5 Effects of LY-404,039 on L-DOPA anti-parkinsonian action

In Figure 5, we show that administration of L-DOPA with LY-404,039 attenuated the severity of parkinsonism [$F_{(2.446, 31.8)} = 19.16$, $P < 0.0001$; one-way RM ANOVA]. The addition of LY-404,039 10 mg/kg resulted in a significant decrease in percentage of right forepaw use compared to L-DOPA alone, by ≈ 76% ($P < 0.01$, Tukey's post-test). In contrast, adding LY-404,039 0.1 and 1 mg/kg did not hinder, nor enhance, the anti-parkinsonian action of L-DOPA, as right forepaw use was similar across all treatments when compared to vehicle and remained lower than when animals were not administered L-DOPA.

4. Discussion

Our results demonstrate that administration of LY-404,039 in combination with L-DOPA significantly attenuates the severity of dyskinesia, while enhancing the anti-parkinsonian action of L-DOPA. Our results provide incremental evidence that acute orthosteric activation of $mGlu_{2/3}$ receptors represents an effective therapeutic strategy to reduce dyskinesia in PD. In our study, LY-404,039 10 mg/kg consistently elicited the greatest ALO AIMs reduction. Whether higher doses of LY-404,039 would have led to greater AIMs reduction remains unknown. Here, we elected not to administer such higher doses, as they would have led to plasma exposure greater than these known to be well tolerated in humans (Annes et al., 2015; Mehta et al., 2018; Patil et al., 2007; Rorick-Kehn et al., 2007b). Of note, no PK experiments were conducted in patients with PD, so it is possible that subtle changes to dosing might have to be carried in the parkinsonian state, depending on the safety and tolerability of LY-404,039 in the condition. Indeed, our previous work in the 6-OHDA-lesioned rat with both LY-354,740 and LY-487,379 has revealed U-shaped dose-response curves to the anti-dyskinetic effect of $mGlu_2$ and $mGlu_{2/3}$ activators. It is noteworthy that this U-shaped dose-response curve was not encountered in our work with $mGlu_2$ and $mGlu_{2/3}$ activators in the MPTP-lesioned marmoset (Frouni et al., 2019; Frouni et al., 2021; Sid-Otmane et al., 2020).

Excess of glutamatergic transmission in the striatum is a defining characteristic of L-DOPA-induced dyskinesia (Cenci and Konradi, 2010). Accordingly, amantadine is thought to diminish dyskinesia through reduction of overactive glutamatergic neurotransmission (Sharma et al., 2018). Although mechanistically different from amantadine, the anti-dyskinetic effect of LY-

404,039 in this study may also be due to restoration of glutamatergic balance following activation of $mGlu_{2/3}$ receptors (Fabbrini et al., 2007; Muguruza et al., 2016).

LY-404,039 harbours affinity at both $mGlu_2$ and $mGlu_3$ receptors (Rorick-Kehn et al., 2007a). Which of these 2 receptors, if not both, is primarily responsible for the anti-dyskinetic effect remains speculative. That being said, we propose that LY-404,039 acted primarily through $mGlu_2$ activation, rather than agonism at $mGlu_3$ receptors. Indeed, $mGlu_3$ receptor activation amplifies signalling at $mGlu_5$ receptors (Di Menna et al., 2018). Currently, negative allosteric modulation of $mGlu_5$ receptors is being investigated as a potential anti-dyskinetic paradigm, with dipraglurant being assessed for this indication in two clinical trials (NCT04857359, NCT05116813). Speculatively, cross-activation of $mGlu_5$ receptors following $mGlu_3$ stimulation might counter potential anti-dyskinetic benefits. For this reason, we suggest that the agonistic action at $mGlu_2$ receptors is the primary mechanism whereby LY-404,039 reduced dyskinesia.

In addition, LY-404,039 enhanced the anti-parkinsonian action of L-DOPA. It is possible that this anti-parkinsonian effect may be mediated by its purported interactions with the dopamine D_2 receptor. Whereas head-to-head comparisons were not made, in the previous work we conducted in the 6-OHDA-lesioned rat with the $mGlu_{2/3}$ OA LY-354,740 (Frouni et al., 2019) and the $mGlu_2$ positive allosteric modulator LY-487,379 (Hamadjida et al., 2020), which reportedly do not interact with D_2 receptors (Johnson et al., 2003; Monn et al., 1997), we did not find that LY-354,740 and LY-487,379 enhance the anti-parkinsonian action of L-DOPA to such an extent. This possible D_2-agonist effect of LY-404,039 might explain why it increased the therapeutic effect of L-DOPA on parkinsonism to a seemingly greater extent than LY-354,740 and LY-487,379. Further experiments are warranted to characterise this possible agonist action at D_2 dopamine receptors in experimental parkinsonism.

An important point to consider if $mGlu_{2/3}$ agonists were to undergo further development for PD is that some $mGlu_{2/3}$ antagonists are recently being developed as anti-depressants, which raises the possibility that $mGlu_{2/3}$ activators might lead to depressive symptoms (Chaki, 2017). As anxiety and depression are common features in PD (Hely et al., 2005), patients who would eventually be administered $mGlu_{2/3}$ agonists would have to be monitored carefully for the occurrence of mood disorders. In addition, chronic studies will eventually have to be conducted to assess the drug efficacy over longer period of time. Interestingly, it should be noted that, while we did not assess the neuroprotective or TH fibre resprouting ability of LY-404,039 in the current

experiments, there has been a study with a different $mGlu_{2/3}$ OA, LY-379,268, that assessed the neuroprotective effect of a 7-day administration of $mGlu_{2/3}$ orthosteric stimulation in the 6-OHDA-lesioned rat, by measuring TH-immunoreactivity in the striatum and SN as an indication of neuroprotection (Murray et al., 2002). The authors found significant protection in the striatum as well as some protection in the SN which failed to reach significance (Murray et al., 2002). Whether such findings would also be encountered with other $mGlu_{2/3}$ OAs such as LY-404,039 remains to be determined, but induction of dopaminergic fibre sprouting in the striatum might result in broader potential uses of these agents in PD.

In summary, we have demonstrated, in the 6-OHDA-lesioned rat model of PD, that orthosteric stimulation of $mGlu_{2/3}$ receptors with LY-404,039 10 mg/kg significantly reduces the expression of dyskinesia. In the case of LY-404,039, a further interest specifically for PD comes from its possible agonist effect at D_2 receptors, which could have mediated its adjunct anti-parkinsonian effect here. These results suggest that LY-404,039, a clinic-ready compound, might alleviate both dyskinesia and motor fluctuations in patients with idiopathic PD.

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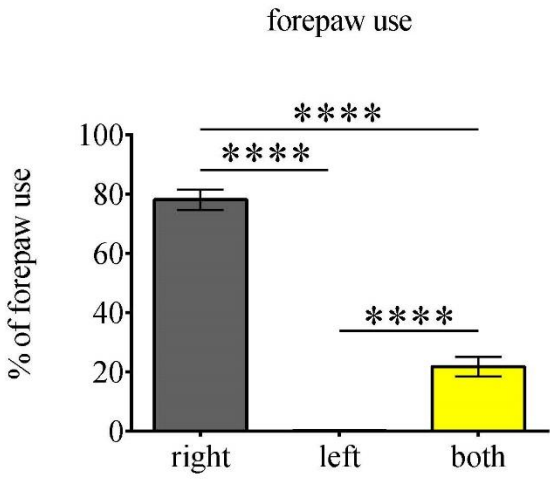
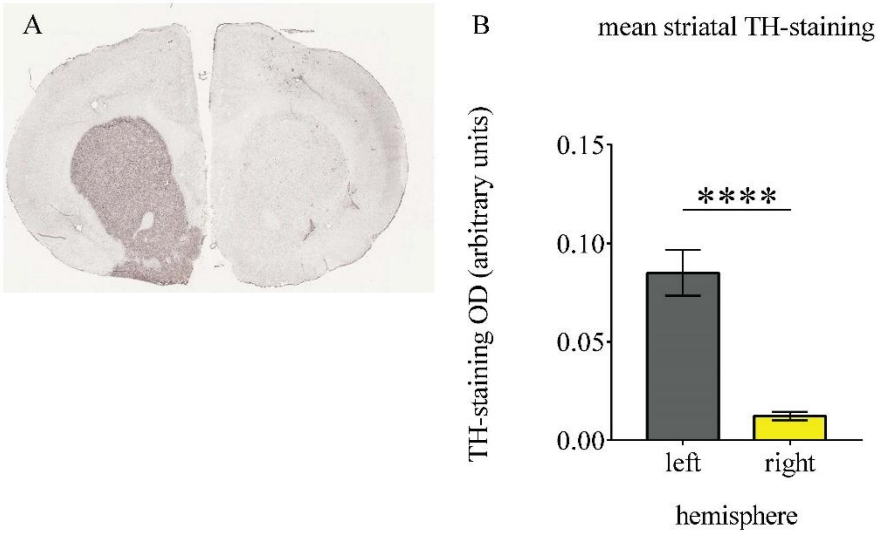
Figures

Figure 1



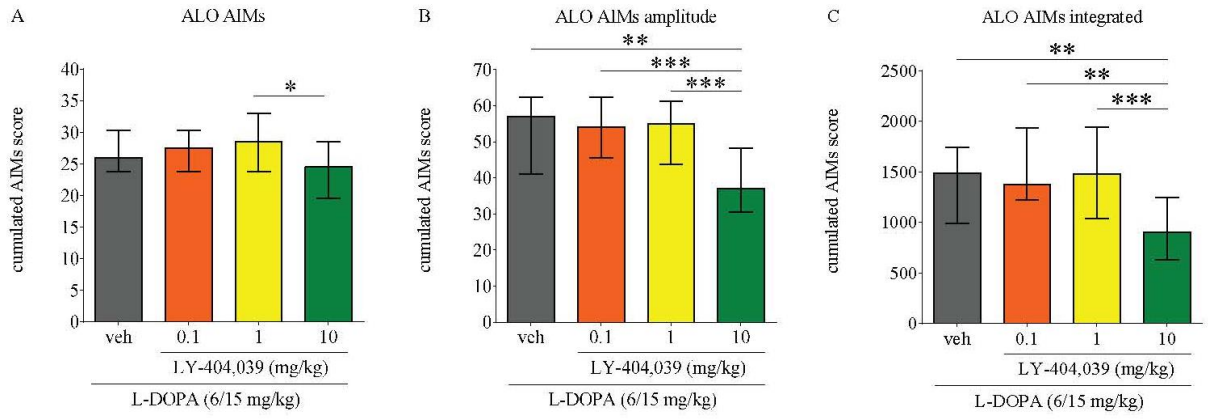
Rears using the right, left, and both forepaws were assessed through the cylinder test. N = 14 rats. Data are presented as the mean ± SEM.

Figure 2



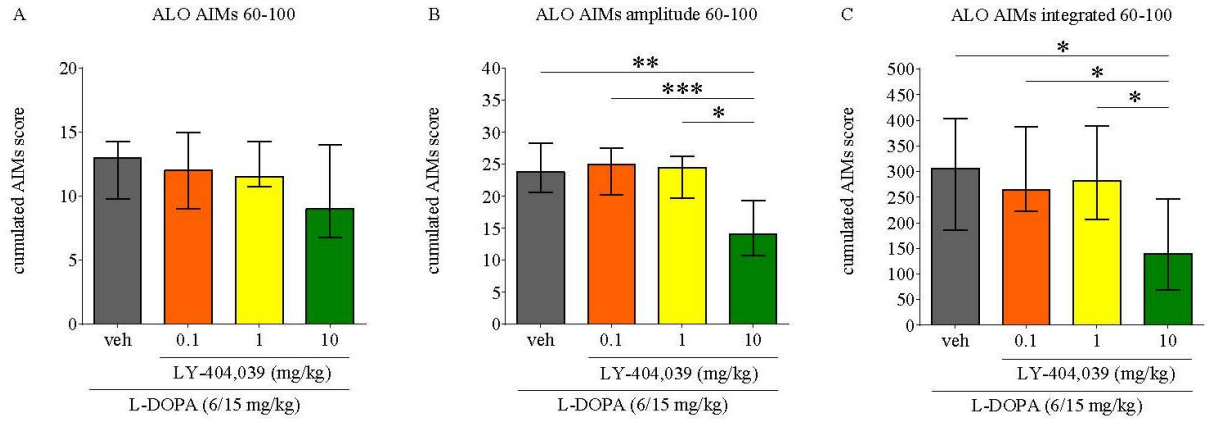
(A) Examples of TH staining from the striatum [left striatum, dark; right striatum light]. (B) Quantification of the optical density of TH-staining in the striatal sections. There was significant reduction of TH striatal immunoreactivity by ≈ 86% when comparing the left and right TH-staining optical density (OD). Data are presented as the mean ± SEM of arbitrary OD values. ****: $P < 0.0001$.

Figure 3



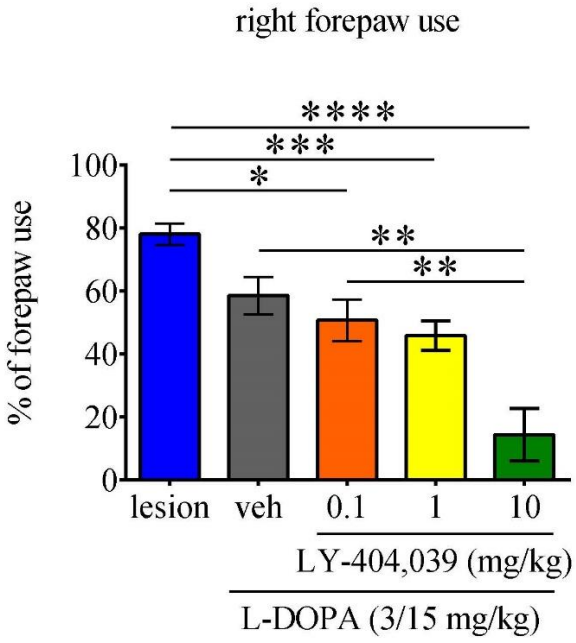
Effect of LY-404,039 on established L-DOPA-induced ALO AIMs. ALO AIMs scores (cumulative score over the entire 180 min experimental period) in 6-OHDA-lesioned rats (N = 14) treated with L-DOPA in combination with LY-404,039 (0.1, 1 and 10 mg/kg) or vehicle. (A) LY-404,039 significantly decreased ALO AIMs standard by ≈ 14% between 1 mg/kg and 10 mg/kg. (B) LY-404,039 10 mg/kg significantly reduced ALO AIMs amplitude, by ≈ 35%, when compared to L-DOPA/vehicle treatment. LY-404,039 10 mg/kg significantly diminished ALO AIMs amplitude, by ≈ 31% when compared to 0.1 mg/kg and by ≈ 33% when compared to 1 mg/kg. (C) LY-404,039 10 mg/kg significantly reduced integrated ALO AIMs, by ≈ 39%, when compared to L-DOPA/vehicle treatment. LY-404,039 10 mg/kg significantly reduced integrated ALO AIMs, by ≈ 34% when compared to 0.1 mg/kg, and by ≈ 39% when compared to 1 mg/kg. ALO AIMs scores are graphed as the median with semi-interquartile range. *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

Figure 4



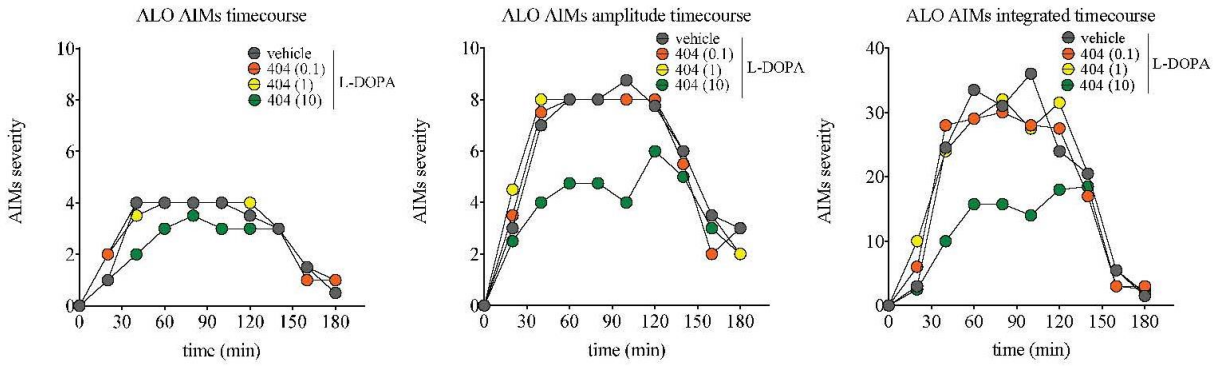
Effect of LY-404,039 on L-DOPA-induced ALO AIMs 60-100 min after treatment administration (Supplementary Figure 1). ALO AIMs scores from 60-100 min after treatment, in 6-OHDA-lesioned rats (N = 14) treated with L-DOPA in combination with LY-404,039 (0.1, 1 and 10 mg/kg) or vehicle. (A) LY-404,039 did not significantly reduce the severity of ALO AIMs standard. (B) LY-404,039 10 mg/kg significantly reduced ALO AIMs amplitude, by ≈ 41%, when compared to L-DOPA/vehicle. LY-404,039 10 mg/kg significantly decreased ALO AIMs amplitude, by ≈ 44%, when compared to 0.1 mg/kg, and by ≈ 43% when compared to 1 mg/kg. (C) LY-404,039 10 mg/kg significantly diminished integrated ALO AIMs, by ≈ 54% when compared to L-DOPA/vehicle. LY-404,039 10 mg/kg significantly reduced integrated ALO AIMs, by ≈ 47% when compared to 0.1 mg/kg and by ≈ 51% when compared to 1 mg/kg. ALO AIMs scores are graphed as the median with semi-interquartile range. *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

Figure 5



Effect of LY-404,039 on L-DOPA anti-parkinsonian action. 6-OHDA-lesioned rats (N = 14) displayed pronounced rearing asymmetry in the cylinder test, favouring the use of the right (un-lesioned) forepaw in ≈ 78% of wall contacts. Following administration of L-DOPA with different doses of LY-404,039, the preferential use of the un-lesioned forepaw was significantly decreased by ≈ 35% with 0.1 mg/kg, ≈ 41% with 1 mg/kg, and ≈ 82% with 10 mg/kg. There was significant decrease between vehicle and 10 mg/kg by ≈ 76%, as well as between 0.1 and 10 mg/kg by ≈ 72%. Data presented as the mean ± SEM. *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$. ****: $P < 0.0001$.

Supplementary Figure 1



Time course of L-DOPA induced axial, limbs and oro-lingual (ALO) abnormal involuntary movements (AIMs) in 6-OHDA-lesioned rats treated with L-DOPA in combination with vehicle or LY-404,039 0.1, 1 and 10 mg/kg. ALO AIMs (A), amplitude (B) and integrated (C) are presented. Data presented as the median.

Bridge: Investigating the anti-dyskinetic and anti-psychotic effects of LY-404,039 in animal models of PD

In Chapter 2, we have demonstrated that orthosteric activation of $mGluR_{2/3}$ with LY-404,039 alleviates the severity of dyskinesia and enhances the anti-parkinsonian action of a low dose of L-DOPA in the 6-OHDA-lesioned rat model of PD. In Chapter 3, we sought to validate the results by investigating whether LY-404,039 would elicit similar anti-dyskinetic and anti-parkinsonian efficacy in the MPTP-lesioned marmoset. Furthermore, we sought to investigate whether LY-404,039 would have anti-psychotic effects as well, as it is possible to study the effects of experimental molecules on PLBs in the parkinsonian marmoset, although this was not a primary endpoint of this Thesis, whose focus is on the effect of LY-404,039 on dyskinesia and parkinsonism. As mentioned previously (Section 3.2.3.2), the MPTP-lesioned marmoset model has high predictive validity in successfully predicting the success of clinical trial for treatment of dyskinesia, psychosis, and parkinsonism (353, 354), and positive results would therefore provide compelling pre-clinical data that would justify envisioning assessing the therapeutic potential of LY-404,039 in PD in the context of clinical trials.

As presented in Section 2.6, LY-404,039 and its prodrug LY-2140023 underwent clinical testing for the treatment of schizophrenia, including a Phase III trial (240). These studies have garnered a wealth of data pertaining to the safety, tolerability, and PK profile in human. In the experiments detailed in the next Chapter, we first sought to determine doses that would lead to plasma exposure comparable to that achieved in the clinic, following which we proceeded to an efficacy study.

Chapter 3: LY-404,039 in the MPTP-lesioned marmoset

In press in Archives of Pharmacology.

Title: The $mGluR_{2/3}$ orthosteric agonist LY-404,039 reduces dyskinesia, psychosis-like behaviours, and parkinsonism in the MPTP-lesioned marmoset

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Authors' roles:

1) Research project: A. Conception, B. Organisation, C. Execution.

2) Manuscript: A. Writing of the first draft, B. Review and Critique.

WK: 1C, 2A; SGN: 1C; DB: 1C; IF: 1C, 2A, 2B; CK: 1B, 2B; AH: 1B, 2B; JCG: 1B, 2B; FG: 1B, 1C, 2B; FB: 1B, 2B PH: 1A, 1B, 2B

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Council of Canada, Healthy Brains for Healthy Lives and Biogen Inc. FB is the holder of the Canada Research Chair in metrology of bioactive molecule and target discovery.

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Abstract

Purpose: LY-404,039, an orthosteric agonist of metabotropic glutamate 2 and 3 receptors ($mGluR_{2/3}$) that may harbour additional agonist effect at dopamine D_2 receptor. LY-404,039 and its pro-drug, LY-2140023, have previously entered clinical trials as treatment option for schizophrenia. They could therefore be repurposed, if proven efficacious, for other conditions, notably Parkinson's Disease (PD). We have previously shown that the $mGluR_{2/3}$ orthosteric agonist LY-354,740 alleviated L-3,4-dihydroxyphenylalanine (L-DOPA)-induced dyskinesia and psychosis-like behaviours (PLBs) in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned marmoset. Unlike LY-404,039, LY-354,740 does not stimulate dopamine D_2 receptors, suggesting that LY-404,039 may elicit broader therapeutic effects in PD. Here we sought to investigate the effect of this possible additional dopamine D_2-agonist action of LY-404,039 by assessing its efficacy on dyskinesia, PLBs and parkinsonism in the MPTP-lesioned marmoset.

Methods: We first determined the pharmacokinetic profile of LY-404,039 in the marmoset, in order to select doses resulting in plasma concentrations known to be well tolerated in the clinic. Marmosets were then injected L-DOPA with either vehicle or LY-404,039 (0.1, 0.3, 1, and 10 mg/kg).

Results: The addition of LY-404,039 10 mg/kg to L-DOPA resulted in a significant reduction of global dyskinesia (by 55%, $P < 0.01$) and PLBs (by 50%, $P < 0.05$), as well as reduction of global parkinsonism (by 47%, $P < 0.05$).

Conclusion: Our results provide additional support of the efficacy of $mGluR_{2/3}$ orthosteric stimulation at alleviating dyskinesia, PLBs and parkinsonism. Because LY-404,039 has already been tested in clinical trials, it could be repurposed for indications related to PD.

Key words: $mGluR_2$, $mGluR_3$, dopamine D_2, Parkinson's disease, MPTP-lesioned marmoset, dyskinesia, LY-404,039, psychosis-like behaviours, parkinsonism

Introduction

Chronic administration of the dopamine precursor L-3,4-dyhydroxyphenylalanine (L-DOPA), the most effective treatment of Parkinson's Disease (PD), ultimately leads to motor complications such as L-DOPA induced dyskinesia (Dawson and Dawson 2003). Ninety-five percent of patients eventually suffer from dyskinesia after 15 years of L-DOPA therapy (Hely et al. 2005). On top of the motor complications, 50% of patients with advanced PD also suffer symptoms of psychosis (Hely et al. 2005). Amantadine, which is thought to act primarily through antagonism of N-methyl-D-aspartate (NMDA) glutamate receptors, is the only United States FDA-approved treatment for dyskinesia (Rascol et al. 2021). However, development of tolerance (Thomas et al. 2004) and psychiatric side effects such as confusion, hallucinations, etc. (Postma and Van Tilburg 1975), limit the use of amantadine. Due to these limitations of amantadine, discovering novel treatments to alleviate dyskinesia is crucial.

Metabotropic glutamate 2 receptors ($mGluR_2$) are densely expressed at the pre-synaptic terminals in the striatum, where they modulate glutamatergic transmission (Picconi et al. 2002). Dyskinetic symptoms from chronic L-DOPA administration are thought to result from hyperactive glutamatergic transmission in the synaptic cleft of neurons in the striatum (Cenci 2014; Huot et al. 2013). Enhanced glutamatergic transmission triggers excessive activation of the ionotropic glutamate receptors, notably through hyperactivation of post-synaptic glutamate receptors such as NMDA and $mGluR_5$ (Reiner and Levitz 2018). $mGluR_{2/3}$ create a negative feedback mechanism to regulate excessive glutamate release and maintain homeostasis in the synaptic cleft (Gregory and Goudet 2021). Dyskinesia expression could therefore be diminished by reducing glutamate levels, notably via $mGluR_{2/3}$ activation. $mGluR_{2/3}$ are also connected to other neuropsychiatric disorders such as anxiety disorders, depression, mood disorders, addiction, and psychotic disorders (Conn and Jones 2009).

We have previously shown that $mGluR_2$ activation, via both orthosteric stimulation (Frouni et al. 2019) and positive allosteric modulation (Frouni et al. 2021; Nuara et al. 2020; Sid-Otmane et al. 2020) reduces dyskinesia in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned marmoset, a non-human primate with high predictive value of the success of drugs in clinical settings (Beaudry and Huot 2020; Veyres et al. 2018). Here, we have expanded our study of mGluR2 receptor activation with the $mGluR_{2/3}$ orthosteric agonist (OA) LY-404,039 (pomaglumetad) on the severity of dyskinesia. We concurrently assessed the effects of LY-

404,039 on psychosis-like behaviours (PLBs), and parkinsonian disability. LY-404,039 is a potent $mGluR_{2/3}$ orthosteric agonist (Rorick-Kehn et al. 2007). Because of poor oral bioavailability (Annes et al. 2015), a pro-drug (LY-2140023 [pomaglumetad methionyl]) was developed and tested in the clinic (Downing et al. 2014; Patil et al. 2007) and has well established safety, tolerability and pharmacokinetic (PK) profiles in human, suggesting that it could be repurposed for the treatment of PD. An additional interest of LY-404,039 in the specific context of PD stems from a possible interaction with dopamine D_2 receptors. It has been shown that LY-404,039 displays significant affinity for the dopamine D_2 receptor, with a dissociation constant at D_2^{High} of 8.2-12.6 nM (Seeman 2013; Seeman and Guan 2009). This is lower than the dissociation constants of 92 nM–149 nM for human $mGluR_{2/3}$, which suggests that LY-404,039 will bind to D_2 at clinical doses. This potential agonist action at dopamine D_2 receptors suggest that it may have an anti-parkinsonian effect, although the D_2 agonist effect remains unclear and controversial.

Methods

Animals

Twelve common marmosets (*Callithrix jacchus*; 300-450g; McGill University breeding colony) were used in the experiments detailed below, 6 for the PK experiments and 6 for the behavioural studies, with an equal number of female and male animals in all settings. Animals were aged between 2 and 6 years old at the time of experiments, were housed in groups of 2 under conditions of controlled temperature (24 ± 1 °C), humidity (50 ± 5%) and light (12 h light/dark cycle, on 07:15 a.m.). They had unlimited access to water, with food (Mazuri® marmoset jelly, boiled eggs, boiled pasta, nuts, legumes) and fresh fruits served twice daily. Cages were enriched with primate toys and perches. Animals were acclimatised to handling, sub-cutaneous (s.c.) injections, as well as transfers to observation cages prior to the experiments. Marmosets were cared for in accordance with a protocol approved by McGill University and the Montreal Neurological Institute-Hospital (The Neuro) Animal Care Committees, both in accordance with the regulations of the Canadian Council on Animal Care.

The marmosets were previously used in other studies. Marmosets utilised in the PK studies were given a 30-day washout period before the start of the current experiments, during which they were not administered any drug or chemical substance. MPTP-lesioned marmosets had also been employed in previous studies. Prior to the experiments reported here, they were allowed a 30-day

washout period, to ensure complete washout of previous treatments. During this washout, they were only administered L-DOPA/benserazide on a daily basis to maintain the dyskinesia and PLB phenotypes stable. No animals were excluded for any behavioural reasons.

Pharmacokinetic profile of LY-404,039

As we have previously reported (Gaudette et al. 2017; Gaudette et al. 2018; Kwan et al. 2021), we used a sparse sampling technique to collect a minimal volume of blood from marmosets (Tse and Nedelman 1996). Following administration of LY-404,039 (0.3 mg/kg s.c.; MilliporeSigma, Oakville, ON, Canada), blood samples were collected at 10 time points, baseline, 5 min, 10 min, 15 min, 30 min, 1 h, 2 h, 4 h, 6 h, and 8 h. Additional samples were collected at 30 min, 1 h, and 2 h from marmosets after s.c. administration LY-404,039 0.1, 0.3, and 1 mg/kg. Plasma was isolated by centrifugation and stored at -80° until analysis. Levels of LY-404,039 were determined by high-performance liquid chromatography and tandem mass spectrometry (Kang et al. 2022). Plasma PK parameters were determined from the mean concentration value at each time point by a non-compartmental analysis method using PKSolver (Rowland M and TN. 1995; Zhang et al. 2010). Area under the curve (AUC) was calculated using the linear trapezoidal rule. $AUC_{0\text{-}t}$, $AUC_{0\text{-}\infty}$, maximal plasma concentration (C_{max}), time to C_{max} (T_{max}), elimination rate constant (λ_z) terminal half-life ($T_{1/2}$), relative clearance (CL/F), relative volume of distribution (V_z/F) and mean residence time (MRT) were all calculated.

Induction of Parkinsonism, dyskinesia and psychosis-like behaviours

Six marmosets were rendered parkinsonian by daily injections of MPTP hydrochloride (2 mg/kg, s.c., MilliporeSigma) over 5 days. Animals were given a month recovery period for development and stabilisation of parkinsonian symptoms. Animals were then orally administered L-DOPA/benserazide (15/3.75 mg/kg, MilliporeSigma) once daily for a minimum of 30 days, a treatment schedule that was shown to elicit stable dyskinetic and psychotic phenotypes (Hamadjida et al. 2018a; Hamadjida et al. 2018b; Hamadjida et al. 2018c, d; Hamadjida et al. 2017) and lead to clinically relevant plasma levels of L-DOPA (Huot et al. 2012b).

Assessment of parkinsonism, dyskinesia and PLBs

On days of assessment, marmosets were administered LY-404,039 (0.1, 0.3, 1, 10 mg/kg s.c.) or vehicle (0.9% NaCl) in combination with L-DOPA (15/3.75 mg/kg s.c., MilliporeSigma). Drug administration followed a randomised schedule according to a within-subjects design that ensured all animals received all treatments. Following administration of treatment, each marmoset was placed in individual observation cages (36 × 33 × 22 in) that contained water, food, and a wooden perch, and left undisturbed for 6 h. Treatments were separated by minimum of 72 h for complete drug clearance. Behaviours were recorded via webcam. Dyskinesia, PLBs and parkinsonism were all scored post hoc using previously validated scales (Fox et al. 2010; Huot et al. 2012a; Huot et al. 2011, 2014; Visanji et al. 2006) by a single experienced rater blinded to the treatment, to minimise inter-individual variability in the scoring of each animal. Over the course of a 6 h observation period, behaviours were examined for 5 min every 10 min. Parkinsonian disability was assessed for range of movement, bradykinesia, posture, and attention/alertness. Range of movement was scored on a scale from 0 to 9, where 0 = running, jumping and use of limbs for different activities and 9 = no movement. Bradykinesia was scored from 0 to 3, where 0 = normal initiation and speed of movement and 3 = prolonged freezing, akinesia and immobile. Postural abnormality was scored 0 or 1, where 0 = normal balance with upright body posture and head is held up and 1 = impaired balance, prone body posture with head down. Attention/alertness was scored 0 or 1, where 0 = normal head checking and movement of neck is smooth in different directions and in small movements and 1 = less or no head checking, and head is in one position for more than 50% of the time. Global parkinsonian disability score was calculated as a combination of the behaviours mentioned above for each observation period using this formula: (range of movement × 1) + (bradykinesia × 3) + (posture × 9) + (alertness × 9), with 36 as the highest possible parkinsonian disability score per 5-min period. Dyskinesia rating evaluated chorea and dystonia, which were both scored on a scale from 0 to 4, where 0 = absent, 1 = mild, present less than 70% of the observation period and animal can eat and perform normal activity, 2 = moderate, 3 = marked and 4 = severe, present more than 70% of the observation period and animal is unable to perform normal activity. The PLBs rating scale measured each of hyperkinesia (0-4), hallucinatory-like behaviour (0-4), repetitive grooming (0-4) and stereotypies (0-4); the PLBs score attributed during any observation period was the most severe of these 4 behaviours.

Statistical analysis

Time courses of parkinsonian disability, dyskinesia and PLB scores are presented as the median. The AUC of the time courses are graphed as the mean ± SEM (referred to as "global" scores hereafter) and was analysed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Parkinsonian disability, dyskinesia and PLB scores at peak dose (90-150 min after administration) are graphed as the median with individual values and were analysed using Friedman followed by Dunn's post hoc test. Statistical analyses were performed with GraphPad Prism 9.4.1 (GraphPad Software Inc., USA).

Results

LY-404,039 was well tolerated by marmosets, regardless of the dose administered. We did not notice any adverse effect. The original data files are available as supplementary materials.

Pharmacokinetic profile of LY-404,039

Plasma PK parameters of LY-404,039 in the marmoset are shown in Table 1. After s.c. administration of LY-404,039 at 0.3 mg/kg, C_{max} was 731.7 ng/mL, T_{max} was observed at 1 h and a $T_{1/2}$ was 32 min. Additionally, C_{max} of 211.6 ng/mL and 2625.4 ng/mL was observed following s.c. administration at doses of 0.1 and 1 mg/kg, respectively, suggesting concentration–time profiles appear linear.

Effect of LY-404,039 on dyskinesia

As presented in Figure 1A and 1B, LY-404,039 significantly diminished global dyskinesia severity ($F_{(4,25)} = 4.223$, $P < 0.01$, one-way ANOVA). Specifically, the addition of LY-404,039 1 and 10 mg/kg to L-DOPA significantly alleviated global dyskinesia when compared to vehicle, by ≈ 44% ($P < 0.05$, Tukey's post hoc test) and ≈ 55% ($P < 0.01$, Tukey's post hoc test), respectively.

As shown in Figure 1C, LY-404,039 also diminished the severity of peak dose dyskinesia (Friedman Statistic (FS) = 17.41, $P < 0.01$), LY-404,039 1 and 10 mg/kg significantly reduced peak dyskinesia severity, by ≈ 45% ($P < 0.05$, Dunn's post hoc test) and ≈ 55% ($P < 0.01$, Dunn's post hoc test) respectively, when compared to vehicle treatment.

Effect of LY-404,039 on PLBs

Figure 2A shows the time course over the 6h observation period and Figure 2B shows the AUC of the time course. We found that LY-404,039 significantly diminished global PLBs severity ($F_{(4,25)} = 3.273$, $P < 0.05$, one-way ANOVA). Specifically, when added to L-DOPA, LY-404,039 10 mg/kg alleviated global PLBs when compared to vehicle, by ≈ 50% ($P < 0.05$, Tukey's post hoc test).

LY-404,039 also reduced the severity of peak dose PLBs (FS = 16.73, $P < 0.01$, Figure 2C). Thus, LY-404,039 1 and 10 mg/kg significantly alleviated peak PLBs severity by ≈ 38% ($P < 0.05$, Dunn's post hoc test) and ≈ 53% ($P < 0.01$, Dunn's post hoc test), respectively, when compared to vehicle treatment.

Effect of LY-404,039 on parkinsonism

In Figure 3A and 3B, we show that LY-404,039 had a significant effect on global parkinsonism severity ($F_{(4,25)} = 3.274$, $P < 0.05$, one-way ANOVA). Thus, combining LY-404,039 10 mg/kg with L-DOPA diminished global parkinsonian disability when compared to vehicle, by ≈ 47% ($P < 0.05$, Tukey's post hoc test, Figure 3B).

Discussion

In the experiments reported here, we have demonstrated the effects of LY-404,039 as an adjunct to L-DOPA. LY-404,039 significantly attenuated the severity of dyskinesia and PLBs, while enhancing the anti-parkinsonian benefits of L-DOPA. The result of this study provides further support for acute $mGluR_{2/3}$ orthosteric activation as an effective therapeutic strategy to reduce motor and non-motor complications in PD. LY-404,039 10 mg/kg consistently elicited the most significant reduction in dyskinesia, PLBs, and parkinsonism, while the dose of 1 mg/kg also displayed anti-dyskinetic efficacy. Whereas it cannot be ruled out that greater effects on each of dyskinesia, PLBs and parkinsonism might have been obtained had we administered higher doses of LY-404,039, we ultimately elected not to do so, as such doses would have led to plasma exposure greater than that documented to be well tolerated in human (Annes et al. 2015; Patil et al. 2007; Rorick-Kehn et al. 2007; Mehta et al. 2018). It should be noted that U-shaped anti-dyskinetic dose-response curves were observed with $mGluR_2$ activators in the 6-hydroxydopamine (6-OHDA)-lesioned rat (Frouni et al. 2019; Hamadjida et al. 2020), but not in the MPTP-lesioned marmoset (Frouni et al. 2019; Frouni et al. 2021; Sid-Otmane et al. 2020), including here.

Although the specific mechanism(s) and cellular populations underlying the therapeutic effect of $mGluR_{2/3}$ activation remain poorly characterised, reduction of glutamatergic neurotransmission and restoration of glutamatergic balance in the striatum may play an important role (Fabbrini 2007; Muguruza et al. 2016). This is due to the fact that overactive glutamatergic transmission due to increased glutamatergic levels in the striatum is a defining characteristic of dyskinesia (Cenci and Konradi 2010). The brain regions at which $mGluR_{2/3}$ are expressed also support this possibility, as they are strategically located pre-synaptically at the cortico-striatal pathway in the basal ganglia. $mGluR_{2/3}$ agonists decrease pre-synaptic glutamate release and blunt the hyper-glutamatergic condition that is associated with the dyskinetic state. Specifically, it is believed that $mGluR_{2/3}$ activation activates $G_{\alpha i/o}$ protein, which in turn inhibits adenylate cyclase (AC) that converts ATP to cAMP (Li et al. 2015). The resulting lower cAMP level limits the activity of various ion channels and members of serine/threonine-specific protein kinase A (PKA) family (Anwyl, 1999), which in turn diminishes pre-synaptic glutamate release. While mechanistically different, amantadine achieves a similar effect by antagonising glutamate binding to NMDA receptors to mitigate the over-activity of the direct striatal output pathway in dyskinesia (Sharma et al. 2018).

As LY-404,039 interacts in a virtually equipotent manner with both $mGluR_2$ and $mGluR_3$, the individual roles of each receptor in the anti-dyskinetic mechanism of $mGluR_{2/3}$ agonists remain undetermined. However, we would suggest that the compound acted primarily through activation of $mGluR_2$ rather than $mGluR_3$. Indeed, there is a cross-talk between $mGluR_3$ and $mGluR_5$, resulting in an increase of $mGluR_5$ downstream signalling following $mGluR_3$ activation (Di Menna et al. 2018). As $mGluR_5$ negative allosteric modulation is considered a potential anti-dyskinetic strategy (Bezard et al. 2014; Tison et al. 2016), any activating action at $mGluR_3$ might result in a worsening of dyskinesia severity, thereby countering the anti-dyskinetic benefits conferred by $mGluR_2$ activation.

Regarding the anti-psychotic effects, we would propose that $mGluR_2$ activation within the infero-temporal cortex may be the primary mechanism through which LY-404,039 diminished L-DOPA-induced PLBs. Thus, antagonism of serotonin (5-HT) type 2A receptors ($5\text{-}HT_{2A}R$) with pimavanserin (Cummings et al. 2014) alleviated PD psychosis in a randomised controlled clinical trial and pimavanserin is now used in the United States for the treatment of PD psychosis. $5\text{-}HT_{2A}R$ and $mGluR_2$ receptors form functional heterodimers, in which $5\text{-}HT_{2A}R$ antagonism and $mGluR_2$

stimulation produce similar downstream signalling effects (Fribourg et al. 2011), which could theoretically underlie the anti-psychotic action of LY-404,039.

In addition, we found that LY-404,039 significantly augmented the anti-parkinsonian action of L-DOPA. Whereas no head-to-head comparison was performed in the current series of experiments, this enhancement appears to be of greater magnitude when compared to our previous work in the same animal model with other $mGluR_{2/3}$ activators such as OA LY-354,740 (Frouni et al. 2019) and the positive allosteric modulator (PAM) LY-487,379 (Sid-Otmane et al. 2020). For instance, LY-354,740 enhanced the anti-parkinsonian action of L-DOPA by 17% (Frouni et al. 2019), while the additional anti-parkinsonian benefit obtained with LY-487,379 was 15% (Sid-Otmane et al. 2020). It is noteworthy that the degree to which LY-404,039 enhanced the anti-parkinsonian action of L-DOPA is almost threefold greater than that of LY-354,740 and LY-487,379 at 47%. We propose two possible explanations for these differences. First, it is possible that the once described possible dopamine D_2-agonist effect of LY-404,039 may have played a role in this extra anti-parkinsonian effect (Seeman 2013; Seeman and Guan 2009). Second, we previously tested the $mGluR_2$ PAM CBiPES in the MPTP-lesioned marmoset (Frouni et al. 2021) and discovered a 43% enhancement of L-DOPA anti-parkinsonian action. CBiPES is structurally derived from LY-487,379 but has improved PK properties and increased brain exposure (Johnson et al. 2005). Although we have not determined brain concentrations of LY-404,039 in the marmoset, it is possible that better PK/pharmacodynamic properties of the compound may explain its seemingly greater anti-parkinsonian effect, when compared to LY-354,740 and LY-487,379. On a similar note, it would be interesting to eventually assess the anti-parkinsonian action of LY-404,039 as monotherapy, especially considering its purported interaction with dopamine D_2 receptors.

LY-404,039 and its prodrug LY-2140023, which enhances the oral bioavailability of LY-404,039, have already undergone clinical trials for the treatment of schizophrenia (Adams et al., 2013; Liu et al., 2012; Mehta et al., 2018; Patil et al., 2007; Zhang et al., 2015). In one such trial, LY-2140023 (or olanzapine as an active control) was administered to patients with considerable psychopathology of schizophrenia (Patil et al., 2007). They reported that the treatment was not only safe and well-tolerated, but the patients showed statistically significant improvements in both positive and negative symptoms of schizophrenia compared to placebo, demonstrating its anti-psychotic properties. In subsequent trials, patients treated with LY-2140023 did not significantly

improve compared to placebo (Adams et al. 2013). In addition, LY-2140023 was tested in a magnetic resonance imaging study (Zhang et al., 2015), a magnetic resonance imaging study (Mehta et al. 2018), as well as a pharmacogenetic study (Liu et al., 2012). LY-404,039 and LY-2140023 have therefore entered several clinical trials, during which they were safe and well tolerated.

In summary, our results provide additional evidence that orthosteric stimulation of $mGluR_{2/3}$ receptors may be an effective approach for simultaneously reducing dyskinesia and psychosis, while providing additional anti-parkinsonian effect, when combined with L-DOPA. In the case of LY-404,039, a possible agonistic effect at dopamine D_2 receptors might make this molecule especially suited as an adjunct therapeutic in PD. Moreover, as mentioned above, LY-404,039 is essentially ready for clinical testing in PD patients, as it has previously undergone studies in the clinic for psychiatric conditions.

Declarations

Funding: PH has research support from Parkinson Canada, Fonds de Recherche Québec – Santé, the Weston Brain Institute, the Michael J Fox Foundation for Parkinson's Research, the Natural Sciences and Engineering Research Council of Canada and Healthy Brains for Healthy Lives. FB is the holder of the Canada Research Chair in metrology of bioactive molecule and target discovery.

Conflicts of interest: none

Authors contribution

PH conceived research. CK, AH, JCG, FG, FB, PH organised experiments. WK, SGN, DB, IF, FG conducted experiments. WK, IF wrote the manuscript. All authors read and approved the manuscript.

The Authors declare that all data were generated in-house and that no paper mill was used.

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Ethics approval

Experiments were approved by McGill University and the Montreal Neurological Institute Animal Care Committees, which are in accordance with the regulations defined by the Canadian Council on Animal Care (Animal Use Protocol 2017-7922).

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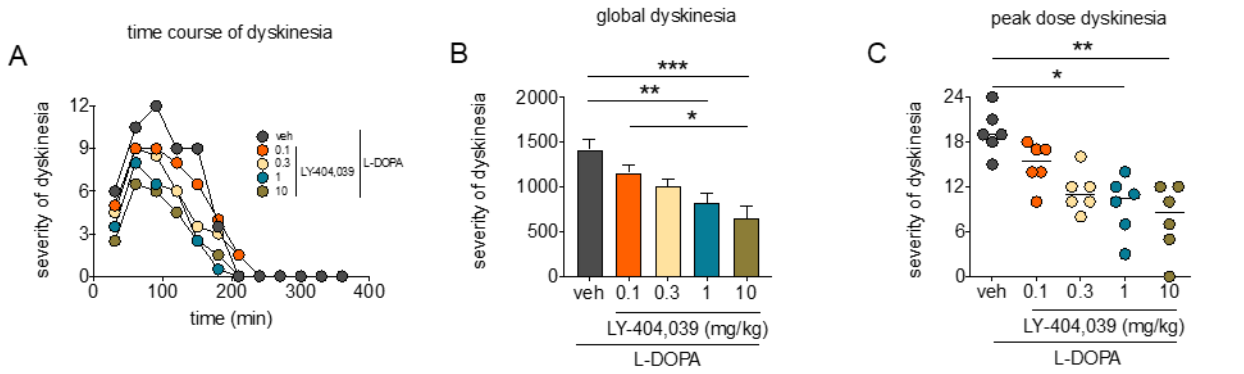
Figures

Figure 1. Effect of LY-404,039 on L-DOPA induced dyskinesia in the MPTP-lesioned marmoset



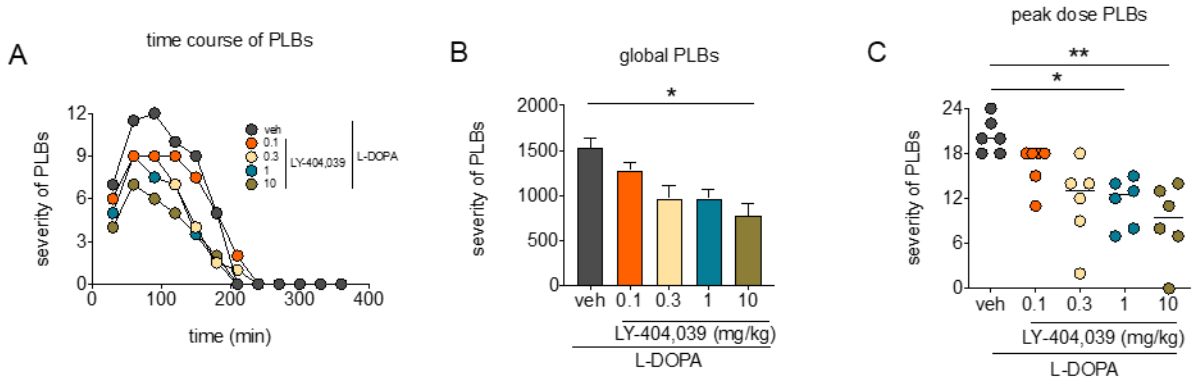
(A) Time course of dyskinesia over the 6 h observation period. Each time point represents the median cumulated dyskinesia scores for every 5 min observation period during the preceding 30 min. The maximal dyskinesia score at any time point is 12.

(B) Area under the curve of dyskinesia time course. LY-404,039 1 and 10 mg/kg significantly reduced the global dyskinesia, by ≈ 44% and ≈ 55%, respectively.

(C) Dyskinesia severity at peak dose (90-150 min after treatment administration). LY-404,039 1 and 10 mg/kg reduced the severity of peak dose dyskinesia by ≈ 45% and ≈ 55%, respectively. The maximal dyskinesia score at any time point is 24.

Data are presented as the median (A), the mean ± SEM (B), and the median with individual values (C). *: $P < 0.05$; **: $P < 0.01$.

Figure 2. Effect of LY-404,039 on L-DOPA induced PLBs in the MPTP-lesioned marmoset



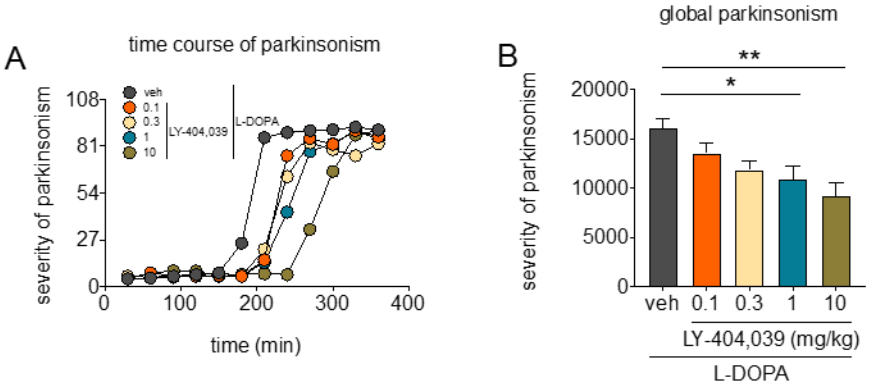
(A) Time course of PLBs over the 6 h observation period. Each time point represents the median cumulated PLB scores for every 5 min observation period during the preceding 30 min. The maximal PLB score at any time point is 12.

(B) Area under the curve of PLBs time course. LY-404,039 10 mg/kg significantly reduced the global PLBs ≈ 50%.

(C) PLBs severity at peak dose (90-150 min after treatment administration). LY-404,039 1 and 10 mg/kg reduced the severity of peak dose PLBs, by ≈ 38% and ≈ 53%, respectively. The maximal PLB score at any time point is 24.

Data are presented as the median (A), the mean ± SEM (B), and the median with individual values (C). *: $P < 0.05$; **: $P < 0.01$.

Figure 3. Effect of LY-404,039 on parkinsonian disability in the MPTP lesioned marmoset



(A) Time course of parkinsonism over the 6 h observation period. Each time point represents the median cumulated parkinsonism scores for every 5 min observation period during the preceding 30 min. The maximal parkinsonism score at any time point is 108.

(B) Area under the curve of parkinsonism time course. LY-404,039 10 mg/kg significantly enhanced the anti-parkinsonian action of L-DOPA. Administration of LY-404,039 10 mg/kg parkinsonism reduced global parkinsonian disability by ≈ 47% when compared to L-DOPA alone. Data are presented as the median (A) and the mean ± SEM (B). *: $P < 0.05$

Table

Table 1. Derived PK parameters in the plasma following s.c. administration of LY-404,039

Dose	0.1 mg/kg	0.3 mg/kg	1.0 mg/kg
PK parameters		mean	
AUC_{0-t} (ng h/mL)		1062.4	
$AUC_{0-\infty}$ (ng h/mL)		1063.0	
C_{max} (ng/mL)	211.6	731.7	2625.4
T_{max} (h)	0.5	1.0	0.5
λ_z (1/h)		1.30	
$T_{1/2}$ (h)		0.53	
CL / F (L/h/kg)		0.28	
V_z / F (L/kg)		0.22	
MRT (h)		1.26	

AUC: area under the curve; CL/F: relative clearance; C_{max}: maximal plasma concentration; F: bioavailability; MRT: mean residence time; PK: pharmacokinetic; $T_{1/2}$: terminal half-life; T_{max}: time to maximal plasma concentration; V_z: relative volume of distribution.

Data are presented as the mean.

Chapter 4: Discussion

In the experiments reported in this Thesis, we have first demonstrated the effects of the $mGluR_{2/3}$ agonist LY-404,039 as an adjunct to L-DOPA in the 6-OHDA-lesioned rat. LY-404,039 significantly attenuated the severity of AIMs in 6-OHDA-lesioned rats and enhanced the anti-parkinsonian benefits of a low dose of L-DOPA. Subsequently, we confirmed its anti-dyskinetic efficacy in the MPTP-lesioned marmoset and found that it also enhanced the anti-parkinsonian action of an optimal dose of L-DOPA. We propose that the enhancement of the anti-parkinsonian effects is due to the drug apparent agonist effect at D_2 receptors. Furthermore, LY-404,039 elicited significant anti-psychotic benefit in addition to its therapeutic effects on motor symptoms. The collective results of this study provide additional evidence for the potential of $mGluR_{2/3}$ orthosteric activation as an effective therapeutic strategy to reduce motor and non-motor complications in PD.

$MGluR_{2/3}$ activation alleviates AIMs while simultaneously augmenting anti-parkinsonian action of L-DOPA in the 6-OHDA-lesioned rat

In our study in the 6-OHDA-lesioned rat, orthosteric stimulation of $mGluR_{2/3}$ with LY-404,039 10 mg/kg significantly reduced the severity of global and peak dose AIMs. This significant reduction was not solely when compared to the vehicle treatment. Thus, the anti-dyskinetic effect achieved with the 10 mg/kg was greater than the one conferred by lower doses of LY-404,039. Of note, the AIMs reduction was not reflected in the global ALO AIMs duration or peak dose ALO AIMs duration, meaning LY-404,039 had no significant effect on the length of observable AIMs. This is not the case with other $mGluR_{2/3}$ activators. The results of ALO AIMs test in rats administered $mGluR_{2/3}$ agonist LY-354,740 or the $mGluR_2$ PAM LY-487,379 demonstrated significant reduction in AIMs duration as well as AIMs amplitude, although, in rats administered LY-354,740, reduction in AIMs duration was not as effective as in AIMs amplitude (172, 226). Thus, it does not seem to be the case that the lack of significant AIMs duration reduction in this study is a trend amongst other $mGluR_{2/3}$ activators. Regardless, as demonstrated by global integrated ALO AIMs and integrated peak dose ALO AIMs, which combined the value of both duration and amplitude, the reduction of AIMs conferred by LY-404,039 remains statistically significant. In addition, LY-404,039 enhanced the anti-parkinsonian action of L-DOPA, whereby LY-404,039 10 mg/kg resulted in a significant decrease in percentage of right forepaw use compared to L-DOPA alone during the cylinder test. In contrast, lower doses of LY-404,039 had no effect on the anti-parkinsonian action of L-DOPA, as right forepaw use was similar

across all treatments when compared to vehicle. Throughout the study, we have suggested that an interaction with D_2 receptor may be a mechanism of action for the augmented anti-parkinsonian benefit of LY-404,039. In previous work from the lab with the orthosteric agonist LY-354,740 (172) and the PAM LY-487,379 (226), which do not interact with D_2 receptors (228, 355), none of the two molecules enhanced the anti-parkinsonian action of L-DOPA, suggesting that the distinct pharmacological profile of LY-404,039 may account for its additional therapeutic benefit.

MGluR$_{2/3}$ activation attenuates dyskinesia, PLBs, and parkinsonism in the MPTP-lesioned marmoset

In our study in the MPTP-lesioned marmoset, similar reduction in motor symptoms was obtained by adding LY-404,039 to L-DOPA. As in the 6-OHDA-lesioned rat, the MPTP-lesioned marmoset displayed most significant reduction in both global and peak dose dyskinesia following administration of LY-404,039 10 mg/kg, the highest dosage tested in the study. The 10 mg/kg dosage also exhibited the greatest efficacy at reversing global parkinsonism, consistent with our findings in the 6-OHDA-lesioned rat. In addition, in the MPTP-lesioned marmoset, the dose of 1 mg/kg also elicited significant reductions in both global and peak dose dyskinesia, while also alleviating parkinsonism. We also found that LY-404,039 10 mg/kg significantly reduced global and peak dose PLBs. Like dyskinesia and parkinsonism, peak dose PLBs were attenuated significantly by 1 mg/kg as well. We proposed in the manuscript that mGluR$_2$ activation in the infero-temporal cortex may be a mechanism through which LY-404,039 reduces PLBs. To this possibility, we would like to add that mGluR$_{2/3}$ activation in the PFC may be another mechanism from which the anti-psychotic benefit is derived, as studies have shown that LY-404,039 suppressed 5-HT-induced L-glutamate release in the PFC by inhibiting cAMP formation in neurons expressing mGluR$_{2/3}$ (206, 233, 235). In addition, co-expression of mGluR$_{2/3}$ and 5-HT$_{2A}$R in the same population of PFC neurons has been reported and may be involved in psychosis associated with schizophrenia (182, 356). Post-mortem analysis of schizophrenic brains displayed up-regulated 5-HT$_{2A}$R and down-regulated mGluR$_2$ in the cortex, a pattern susceptible to psychosis (182). While LY-404,039 does not directly interact with 5-HT$_{2A}$R, 5-HT$_{2A}$R and mGluR$_2$ form functional heterodimers that may underlie its anti-psychotic action (182-184, 213). 5-HT$_{2A}$R antagonism and mGluR$_{2/3}$ stimulation in the PFC result in similar downstream signalling that contribute to the anti-psychotic actions of LY-404,039 (182, 183, 189).

Dose-response curve

Previous work with the $mGluR_{2/3}$ agonist LY-354,740 and the $mGluR_2$ PAM LY-487,379 in the 6-OHDA-lesioned rat has revealed U-shaped dose-response curves to their anti-dyskinetic effect (172, 226). Contrary to these findings, a U-shaped dose-response curve was not observed here with LY-404,039 in the 6-OHDA-lesioned rat. In contrast to the rat experiments, previous studies with $mGluR_{2/3}$ activators in the MPTP-lesioned marmoset (172, 173, 216) did not observe a U-shaped dose-response curve, in agreement with the results we obtained here with LY-404,039 in the marmoset. While there is presently no satisfactory explanation for the lack of the U-shaped dosage response curve in the rat experiments we performed with LY-404,039, it could possibly be attributed to the doses tested. Indeed, we selected the doses based on the PK profile of LY-404,039 profiles from rodent and human literature, as well as from our own experiments (196, 201, 203, 242, 357), to test the effect of doses that would lead to plasma exposure levels known to be well tolerated in the clinic. Thus, we elected to administer doses no higher than 10 mg/kg, despite the fact that LY-404,039 10 mg/kg consistently elicited the most significant therapeutic benefits in both rat and marmoset studies. Whereas higher doses might have elicited a greater symptomatic relief, results obtained with higher doses of LY-404,039 would have led to plasma levels greater than those documented to be safe and well tolerated in human subjects, which would have diminished the translational relevance of our work. Of note, although the PK profile of LY-404,039 in the clinic has been disclosed, no PK experiments were conducted in human subjects with PD, therefore it is possible that subtle changes to dosing might have to be carried in the parkinsonian state, depending on the safety and tolerability of LY-404,039 in the PD population.

In the experiments reported in this Thesis, we elected to inject LY-404,039 s.c. rather than administering its prodrug LY-2140023 orally, as the conversion of LY-2140023 to LY-404,039 in the marmoset has not been studied and it is unclear that we would have achieved the same bioavailability obtained in humans. We also decided against administering LY-404,039 orally, because of its poor oral bioavailability (201, 242), although it is important to mention that its absolute oral bioavailability in the marmoset has not been studied. In clinical trials, LY-2140023 would likely have been utilised to maximise oral absorption, but in the studies reported in this Thesis, we wanted to directly assess the efficacy of LY-404,039, not LY-2140023. S.c. administration removes oral bioavailability and gastrointestinal absorption as extraneous variables.

Limitations and future studies

LY-404,039 is only the second $mGluR_{2/3}$ orthosteric agonist to be studied for alleviating dyskinesia and psychosis in experimental parkinsonism, the other being LY-354,740 (172). While both are $mGluR_{2/3}$ orthosteric agonists, LY-404,039 possesses a possible D_2 agonistic effect that distinguishes it from LY-354,740. The fact that there is a paucity of literature that explored $mGluR_{2/3}$ agonists for alleviating dyskinesia and psychosis emphasises the importance of our study. It must also be noted that testing the LY-404,039 efficacy in the MPTP-lesioned marmoset, which has high validity at predicting the success of clinical trials for dyskinesia, psychosis, and parkinsonism, essentially makes LY-404,039 ready for clinical testing in PD. Furthermore, given the additional D_2 agonistic action of LY-404,039, investigating LY-404,039 as monotherapy, not just as an adjunct with L-DOPA, to alleviate motor symptoms may prove fruitful.

Although the results of our experiments provide compelling evidence supporting $mGluR_{2/3}$ activation for the treatment of PD, our understanding of the mechanism(s) underlying the effects of this therapeutic target remains limited and the explanations that we tentatively provided above remain speculative. Further studies that would try to uncover the mechanism(s) whereby $mGluR_{2/3}$ activation alleviate dyskinesia, PLBs and parkinsonism are warranted. Furthermore, the experiments reported here consisted of acute challenge experiments; chronic studies are required to determine whether tolerance to the benefits of LY-404,039 would occur over time. In addition, it would be interesting to determine if, as LY-354,740 (172) and LY-487,379 (226) were shown to do, LY-404,039 also diminishes the development of dyskinesia when started concurrently with the introduction of L-DOPA in the context of a de novo study.

Chapter 5: Conclusion & Summary

Our results have demonstrated the efficacy of the $mGluR_{2/3}$-D_2 agonist LY-404,039 at alleviating dyskinesia and enhancing the anti-parkinsonian action of L-DOPA, in both 6-OHDA-lesioned rat and MPTP-lesioned marmoset models of PD. In addition, LY-404,039 diminished PLBs in the MPTP-lesioned marmoset. Coupled with previous work performed by our lab, the studies described in this Thesis provide further support for the use of $mGluR_{2/3}$ activators in PD. Because LY-404,039 and its prodrug have previously been assessed in the clinic, they could be repurposed quickly for the treatment of PD. It is our hope that the results we obtained will be useful in the development of novel therapeutics for the treatment of PD, to alleviate the suffering of the individuals suffering from this condition.

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Appendix

Table S1. 6-OHDA-lesioned rat ALO AIMs duration rating scale

Parameter	Score
Axial	0 = no dyskinesia 1 = occasional signs of dyskinesia, which are present during < 50% of the observation time 2 = frequent signs of dyskinesia that are present during > 50% of the observation time 3 = dyskinesia is present during the entire observation time, but it is suppressible by external stimuli 4 = continuous dyskinesia that is not suppressible by external stimuli
Limbs	0 = no dyskinesia 1 = occasional signs of dyskinesia, which are present during < 50% of the observation time 2 = frequent signs of dyskinesia that are present during > 50% of the observation time 3 = dyskinesia is present during the entire observation time, but it is suppressible by external stimuli 4 = continuous dyskinesia that is not suppressible by external stimuli
Orolingual	0 = no dyskinesia 1 = occasional signs of dyskinesia, which are present during < 50% of the observation time 2 = frequent signs of dyskinesia that are present during > 50% of the observation time 3 = dyskinesia is present during the entire observation time, but it is suppressible by external stimuli 4 = continuous dyskinesia that is not suppressible by external stimuli

Adapted from Cenci and Lundblad 2007 (282) with permission from John Wiley and Sons.

Table S2. 6-OHDA-lesioned rat ALO AIMs amplitude rating scale

Parameter	Score
Axial	0 = No dyskinesia 1 = Sustained deviation of the head and neck, at ~ 30° angle 2 = Sustained deviation of the head and neck, angle ≤ 60° 3 = Sustained twisting of the head, neck, and upper trunk at an angle > 60° but ≤ 90° 4 = Sustained twisting of the head, neck, and trunk at maximal amplitude (angle > 90°), 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 = Movements resulting in a visible displacement of the whole limb either sideways or up-and-down 3 = Large displacement of the whole limb with visible contraction of shoulder muscles 4 = Vigorous limb displacement of maximal possible amplitude, with conspicuous contraction of both shoulder muscle 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 noticeable masticatory movements, occasionally leading to jaw opening 3 = Movements with broad involvement of facial muscles and masticatory muscles. Jaw opening is frequent, tongue protrusion occasional 4 = All the above muscle categories are involved to the maximal possible degree

Adapted from Cenci and Lundblad 2007 (282) with permission from John Wiley and Sons.

Table S3. Marmoset parkinsonian disability rating scale

Parameter	Score
Range of movement	0 = running, jumping and use of limbs for different activities 1 = climbing up and down the walls of the cage or along perch 2 = climbing onto wall of cage or perch 3 = hopping on floor of cage 4 = walking around floor 5 = on ceiling, wall of cage or perch, movement of limbs, but no locomotion 6 = on ceiling, wall of cage or perch, movement of head or trunk 7 = on the floor of the cage, movement of limb, but no locomotion 8 = on the floor of the cage, movement of head 9 = no movement
Bradykinesia	0 = normal initiation and speed of movement 1 = slight slowing of movement 2 = moderate slowing of movement, marked freezing, difficulty initiating and maintaining movement 3 = prolonged freezing, akinesia, inability to move
Posture	0 = normal balance, upright posture, head held up 1 = impaired balance, crouched posture, head down
Attention/alertness	0 = normal head checking movements, movement of neck in variable directions, smooth, small movements 1 = reduced or absent head checking, head in one position for > 50% of observation period

For each of the behavioural parameters tested, the score assigned is the most representative of each category over a 5-min period. Global parkinsonian disability score = [(range of movement × 1) + (bradykinesia × 3) + (posture × 9) + (alertness × 9)]; 36 as the highest possible parkinsonian disability score per 5-min period, 6 as the normal score (337, 346, 347, 349).
Adapted from Silverdale et al. 2004 (349) with permission from Elsevier.

Table S4. Marmoset dyskinesia disability rating scale

Parameter	Score
Chorea	0 = activity absent 1 = mild, fleeting, rare, present < 30% of the observation period 2 = moderate, present > 30% of the observation period, but not interfering with normal activity 3 = marked, at times interfering with normal activity, present < 70% of the observation period 4 = severe, continuous, replacing normal activity, present > 70% of the observation period
Dystonia	0 = activity absent 1 = mild, fleeting, rare, present < 30% of the observation period 2 = moderate, present > 30% of the observation period, but not interfering with normal activity 3 = marked, at times interfering with normal activity, present < 70% of the observation period 4 = severe, continuous, replacing normal activity, present > 70% of the observation period

For each of the behavioural parameters tested, the score assigned is the most representative of dyskinesia over a 5-min period. The dyskinesia score attributed was the most disabling of any of the two behavioural parameters observed during the 5-min period (337, 346, 347).
Adapted from Gomez-Ramirez et al. 2006 (348) with permission from John Wiley and Sons.

Table S5. Marmoset PLBs rating scale

Parameter	Score
Hyperkinesia	Locomotor activity: running jumping or climbing, that is faster than normal and/or inability of animal to remain in one position for > 5 seconds without exhibiting locomotion 0 = activity absent 1 = present for < 30% of assessment time and not disabling (animal can walk, run, and eat) 2 = present for > 30% of assessment time and not disabling 3 = present for < 30% and disabling (interference with walking, running, eating – takes over normal activity) 4 = present for > 30% and disabling
Hallucinatory-like response to apparent non-stimuli	Tracking: head movement following non-apparent stimuli (> 10 seconds/min) and/or Staring: head still, looking in one direction at non-apparent stimulus for extended period (> 10 seconds/min) 0 = activity absent 1 = present for < 30% of assessment time and not disabling (animal can walk, run, and eat) 2 = present for > 30% of assessment time and not disabling 3 = present for < 30% and disabling (interference with walking, running, eating – takes over normal activity) 4 = present for > 30% and disabling
Obsessive grooming	Grooming or scratching repetitively (> 5 times/min) 0 = activity absent 1 = present for < 30% of assessment time and not disabling (animal can walk, run, and eat) 2 = present for > 30% of assessment time and not disabling 3 = present for < 30% and disabling (interference with walking, running, eating – takes over normal activity) 4 = present for > 30% and disabling
Stereotypies	a) Side-to-side repetitive whole body jumping movements on floor of cage (>2 times/min) b) Head checking movements that are repetitive, quick, side-to-side, exaggerated large amplitude, often with associated body movements (> 3 times/min)

	c) Circling behaviour – whole body turning in circles (or 360° rotations) on floor, cage wall, perch, or ceiling (> 2 times/min) d) Fiddling with and/or repetitively grasping at cage bars with forearms (> 2 times/min) 0 = activity absent 1 = present for < 30% of assessment time and not disabling (animal can walk, run, and eat) 2 = present for > 30% of assessment time and not disabling 3 = present for < 30% and disabling (interference with walking, running, eating – takes over normal activity) 4 = present for > 30% and disabling

For each of the behavioural parameters tested, the score assigned is the most representative of psychosis-like behaviour over a 5-min period. The psychosis-like behaviour score attributed was the most disabling of any of the four behavioural parameters observed during the 5-min period (225, 350, 351).
Adapted from Fox et al. 2014 (350) and Kwan et al. 2021 (352) with permission from Cambridge University Press and Springer Nature respectively.