MODULATION OF OPIOID AND NMDA RECEPTORS IN PRECLINICAL MODELS OF PARKINSON'S DISEASE AND LEVODOPA-INDUCED DYSKINESIA

by

Andrew J. Flores

A Dissertation Submitted to the Faculty of the

GRADUATE INTERDISCIPLINARY PROGRAM IN PHYSIOLOGICAL SCIENCES

In Partial Fulfillment of the Requirements

For the Degree of

DOCTOR OF PHILOSOPHY

In the Graduate College

THE UNIVERSITY OF ARIZONA

2020

THE UNIVERSITY OF ARIZONA
GRADUATE COLLEGE

As members of the Dissertation Committee, we certify that we have read the dissertation prepared by: Andrew J. Flores, titled: Modulation of Opioid and NMDA Receptors in Preclinical Models of Parkinson's Disease and Levodopa-Induced Dyskinesia

and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy.

Torsten Falk, Ph.D. Date: Oct 12, 2020

Scott J. Sherman, M.D., Ph.D. Date: Oct 13, 2020

Frank Porreca, Ph.D. Date: Oct 9, 2020

Andrew J. Fuglevand, Ph.D. Date: Oct 10, 2020

Michael L. Heien, Ph.D. Date: Oct 12, 2020

Final approval and acceptance of this dissertation is contingent upon the candidate's submission of the final copies of the dissertation to the Graduate College.

I hereby certify that I have read this dissertation prepared under my direction and recommend that it be accepted as fulfilling the dissertation requirement.

Torsten Falk, Ph.D.
Dissertation Committee Co-Chair
Physiological Science GIDP
Date: Oct 12, 2020

Scott J. Sherman, M.D., Ph.D.
Dissertation Committee Co-Chair
Physiological Science GIDP
Date: Oct 13, 2020

ACKNOWLEDGMENTS

I would like to thank the following people for helping me to complete my PhD:

First, I would like to thank my advisor, **Dr. Torsten Falk**, for serving as an excellent mentor, research advisor, and friend. Thank you for all that you have taught me about science and about life, and for believing in me. I am looking forward to accomplishing great things in the future, I will always remember the lessons you have taught me as I move forward with my career.

I also thank the members of my dissertation committee for their support, guidance, and invaluable insight on my research projects. Specifically, I thank my co-mentor **Dr. Scott Sherman** for providing expertise on clinical aspects of Parkinson's disease and drugs used to treat the disease. On several occasions, I had the chance to shadow Dr. Sherman and help in treating actual PD patients in his movement disorders clinic; this experience is something that has motivated me and reminded me of the importance of this research. I thank **Dr. Frank Porreca** for contributing his expertise in the area neuropharmacology and for use of surgical facilities/equiptment and laboratory resources. I thank **Dr. Michael Heien** and members of his lab including **Blake Seaton, James Siegenthaler,** and **Kate Parent** for their expertise related to analytical techniques (FSCAV and HPLC-EC). I would also like to thank **Dr. Andrew Fuglevand** for his guidance and support and for encouraging me to pursue graduate education. I also thank the graduate program in Physiological Sciences, I am fortunate and proud to have had the opportunity to train with the outstanding physiologists in this program.

To **Dr. Mitchell Bartlett**, who was a fellow graduate student in the Falk/Sherman laboratory, I am thankful to have had the opportunity to work with you. I greatly admire your commitment and perseverance. Thank you for everything you taught me about surgery and behavioral analysis. We did a lot of experiments together over the years and spent many hours working together; I'm glad I was

able to work with someone as responsible and dedicated as you. To **Xu Yue**, thank you also for teaching me surgery and behavioral analysis. I thank **Dr. Lisa So** for all her help with behavioral analysis. I want to thank members of the **Porreca lab**, especically **Dr. Jennifer Xie**, for their help over the years. Finally, I am thankful to have had the opportunity to work with a number of amazing and talented undergraduate and high school students over the years. Thank you for all you did to help me.

Finally, I was fortunate enough to have received funding through various sources during my time in this PhD program. Specifically I want to thank **Holly Lopez** and **Dr. Frans Tax** of the **NIH IMSD program** and also the **ARCS Foundation**. Other funding sources are listed in the Acknowledgments section of each chapter.

DEDICATION

This dissertation is dedicated to my family. Thank you for believing in me and supporting me. To my parents, **Karen** and **Tony**, thank you for all you have taught me in life and for all you have done to help me along the way. To my brothers, **Russell** and **Isaac**, thank you for always being there for me. To my fiancée, **Monica**, thank you for being my emotional support and for reminding me to have fun every once in a while. Thank you all, without you, I could not have accomplished this.

Table of Contents

Table of Figures

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Index of Tables

Abstract

Dopamine (DA)-replacement therapy utilizing L-DOPA is the mainstay of symptomatic treatment for Parkinson's disease (PD). A critical complication of this therapy is the development of L-DOPA-induced dyskinesia (LID), which occurs in the majority of patients. Endogenous opioid peptides, including enkephalins and dynorphins, are co-transmitters of dopamine, gamma-aminobutyric acid (GABA), and glutamate neurotransmission in the direct and indirect striatal output pathways, which are disrupted in PD. Alterations in levels of expression of these peptides and their precursors have been implicated both in PD and in the subsequent development and expression of LID. Alterations in *N*-methyl-D-aspartate (NMDA) glutamate transmission have also been implicated in LID; the NMDA receptor antagonist amantadine is the only drug currently approved for the clinical treatment of LID. We utilized pharmacological techniques to investigate the role of altered opioid and NMDA neurotransmission occurring in the direct and indirect striatopallidal output pathways in preclinical models of PD and LID.

In the first study presented (Chapter 2), we show that the antidyskinetic effects of the NMDA receptor antagonist MK-801 are preferential to the indirect pathway. Specifically, we show that MK-801 is capable of suppressing hyperkinetic abnormal involuntary movements (AIMs) induced by a selective D2R agonist, quinipirole but not those induced by a selective D1R agonist, SKF81297. Importantly, MK-801 is capable of suppressing AIMs induced by L-DOPA (levodopa, L-3,4-dihydroxyphenylalanine), which is the most effective clinical treatment for PD, even though this agent likely activates both outflow pathways, and quinpirole, but not those induced by SKF81297. This finding of MK-801 also indicates that the contribution of NMDA receptor-mediated glutamatergic transmission to the expression of LID may be specific to the indirect striatopallidal output pathway.

In Chapter 3, we have investigated the effects of a novel opioid glycopeptide agonist MMP-2200 which has high affinities and activities for mu and delta opioid receptors on AIMs induced separately by L-DOPA, quinpirole, and SKF81297. We also investigated the combined effects of MMP-2200 and MK-801. It was shown that the opioid glycopeptide MMP-2200 reduced AIMs induced by a D2R selective agonist quinpirole, and MMP-2200 modified the effect of MK-801 to result in a potent reduction of L-DOPA-induced AIMs without induction of parkinsonism.

In the studies presented in Chapter 4, we show that the selective kappa opioid receptor antagonist nor-BNI accelerates the rate of development of levodopa-induced AIMs (as opposed to the expression of established AIMs) in a mild striatal 6-OHDA lesion model in a paradigm of chronic gradual dose escalation of L-DOPA to prime LID. Functional restorative effects of nor-BNI on parkinsonian motor deficits were not observed.

Together, the studies described here investigated the roles of alterations in NMDA receptor glutamate transmission and opioid peptide transmission occurring in the direct and indirect striatal output pathways in the context of PD and LID. These findings, using a standard preclinical model of PD and LID may point toward new therapeutic targets and drug candidates for the clinical treatment of LID.

Key words: Parkinson's disease, levodopa-induced dyskinesia, opioid receptors, NMDA receptor antagonist, MMP-2200, nor-BNI, MK-801

Chapter 1: Introduction to Parkinson's Disease and Levodopa-Induced Dyskinesia

1.1 Introduction

Parkinson's disease (PD) is a common age-related neurological disorder that affects approximately 1-2% of the population over the age of 60 (Alves et al., 2008; de Lau & Breteler, 2006; Nussbaum & Ellis, 2003; Tanner & Ben-Shlomo, 1999). It is the second-most common neurodegenerative disease following Alzheimer's disease (Tanner & Aston, 2000). PD is named in honor of James Parkinson (1755–1824), an English surgeon and apothecary who was the first to describe the disease as a neurological disorder (Parkinson, 1817). Clinically, PD is characterized as a hypokinetic movement disorder with cardinal motor features including resting tremor, bradykinesia or akinesia, muscular rigidity, and postural instability (loss of postural reflexes). Motor symptoms are mainly caused by degeneration of dopaminergic nigrostriatal projection neurons in the substantia nigra pars compacta (SNpc) and the resulting depletion of the neuromodulator dopamine in the basal ganglia. Gait disturbance, cognitive impairments and other non-motor and non-dopaminergic features are also present in the majority of patients (Goldman & Postuma, 2014). In addition to nigrostriatal dopamine depletion, another important pathological feature of PD is the presence of intracellular inclusion bodies primarily composed of the neuronal presynaptic protein alpha-synuclein in the brains of PD patients (Forno, 1996; Lewy, 1914).

Current therapeutic approaches to PD include pharmacological restoration of dopaminergic signaling relying primarily on the use of the DA precursor L-DOPA (levodopa) and selective DA receptor agonists and, during advanced stages of the disease, surgical interventions such as deep brain stimulation (DBS) (Jankovic & Aguilar, 2008). It is important to note that these approaches, while effective at alleviating the symptoms of PD, do not alter the course of disease progression (i.e.,

neurodegeneration). Furthermore, L-DOPA therapy is associated with the development of debilitating motor complications including motor fluctuations and abnormal involuntary movements called dyskinesias (Fox et al., 2008). The research presented in this dissertation focuses mainly on the problem of levodopa-induced dyskinesia and details studies that relied on the use of preclinical models of L-DOPA-induced dyskinesia (LID).

1.1.1 Historical background

James Parkinson and paralysis agitans

Parkinson's disease was first described in 1817 by the English surgeon and apothecary James Parkinson in his seminal manuscript *An Essay on the Shaking Palsy,* in which he described a series of six case studies of individuals afflicted with a tremulous, hypokinetic condition he referred to as the shaking palsy or *paralysis agitans* (Parkinson, 1817). In 1872, the disease was renamed in Parkinson's honor by the eminent French neurologist, Jean-Martin Charcot (1825-1893), who further elaborated the clinical description of the disease and is also responsible for realizing the distinction between PD and parkinsonism as a syndrome, which occurs in a number of various PD-plus syndromes (Charcot, 1872; Dauer & Przedborski, 2003; Lees et al., 2009).[1]

1 Charcot differentiated between resting tremor in PD and kinetic tremor associated with other neurological disorders such as multiple systems atrophy, multiple sclerosis, progressive supranuclear palsy, essential tremor, and alcoholism. He was also the first to show that the symptoms of PD could be improved with anticholinergic drugs.

Discovery of the pathological hallmarks of PD

In the early 20th century, two key discoveries were made regarding the neuropathology of PD. First, in 1910, F. H. Lewy described the disease pathology on a cellular and molecular level with his discovery of the presence of cytoplasmic inclusions occurring in the brains of postmortem *paralysis agitans* patients (Lewy, 1914). These inclusions, which are referred to as Lewy bodies and Lewy neurites, are found within neurons and processes in the substantia nigra and in a broad range of other brain regions in individuals with PD, and are recognized today as one of the pathological hallmarks of this disease, although the mechanisms underlying their formation have not yet been resolved (**Figure 1**). Lewy bodies were later found to contain ubiquitin, a highly-conserved molecular signal for the degradation of intracellular substrates (Clague & Urbé, 2010); and in 1998 alpha-synuclein was identified as a main structural component (Spillantini et al., 1997).

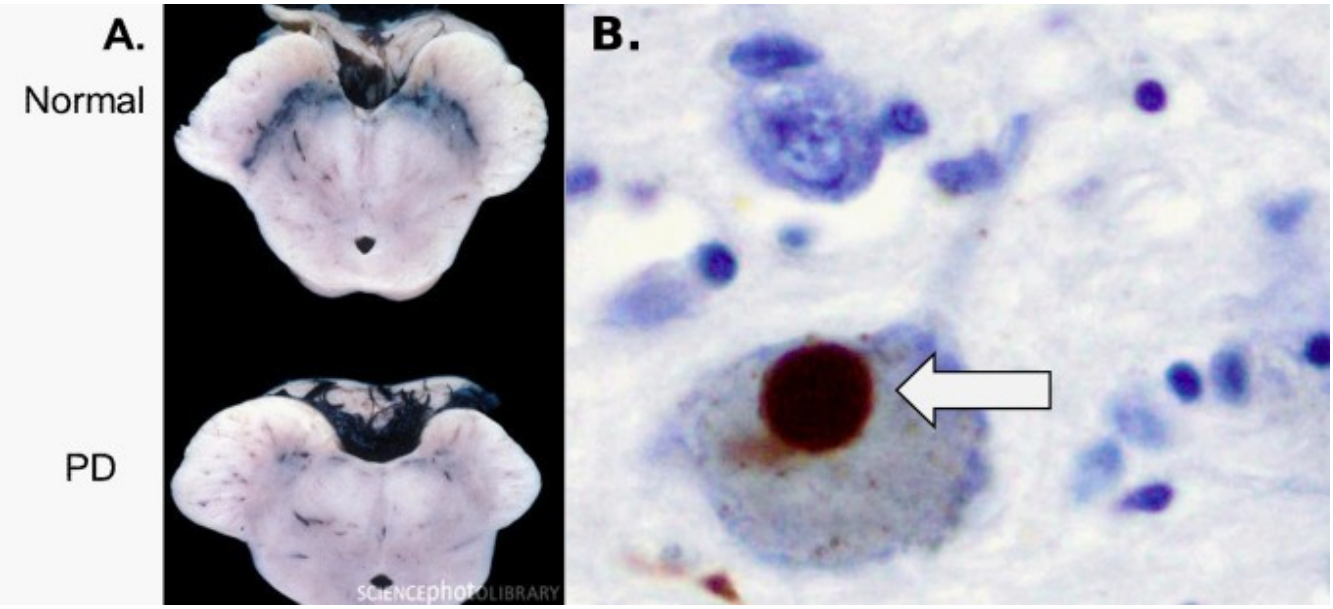

Figure 1. Pathological hallmarks of Parkinson's disease. (A) Loss of pigmented dopaminergic neurons in the substantia nigra pars compacta (SNpc) is readily observable between PD patients and age-matched Normal controls when comparing gross anatomical midbrain dissections. Credit: Science Photo Library https://www.sciencephoto.com/ **(B)** The arrow indicates a cytoplasmic inclusion, called a Lewy body, which is comprised of alpha-synuclein are present in neurons in the brains of PD patients (immunohistochemistry for alpha-synuclein showing positive staining of an intraneural Lewy-body in the Substantia nigra in Parkinson's disease. Credit: Wikipedia https://www.wikipedia.org/

The second important discovery which has contributed to our understanding of PD neuropathology was the description of pallor (loss of pigmentation) of the substantia nigra (SN) in gross midbrain dissections taken from postmortem *paralysis agitans* patients (Trietiakoff, 1919). This phenomenon is now know to be due to loss of dopaminergic neurons in the SN that contain neuromelanin, a pigment synthesized from dopamine that is structurally related to other forms of melanin, which causes them to appear dark in color. This finding proved to be an essential clue that the substantia nigra was the site of lesion in PD (**Figure 1**).

Neuropathological role of dopamine depletion in Parkinson's disease and the advent of levodopa therapy

In the late 1950s, Swedish pharmacologist, Arvid Carlsson performed a series of landmark studies which revealed for the first time that dopamine (DA) functions as a neurotransmitter in the central nervous system (Carlsson et al., 1957). Using a novel, highly sensitive assay developed for measuring chemical concentrations in biological tissue samples, he discovered that DA was concentrated within the basal ganglia nuclei, particularly within the neostriatum, and that these regions were spatially distinct from those in which norepinephrine is concentrated.[2] Carlsson then demonstrated that the catatonic state of animals that had been treated with reserpine was associated with pronounced depletion of DA in these nuclei, and that this deficit could be completely reversed by administration of L-DOPA, the immediate precursor of DA. This finding provided the first direct evidence for a functional role of DA as a neurotransmitter and was an important clue that dopaminergic signaling within the BG played an essential role in movement. Furthermore, it suggested that DA could be the primary neurochemical deficient in the brains of PD patients.

In 1960, soon after Carlsson published his studies on reserpinized animals, Oleh Hornykiewicz verified that dopamine was depleted within the brains of Parkinson's patients and that this deficit was spatially localized to the neostriatum (Ehringer & Hornykiewicz, 1960). This led to the rapid development of L-DOPA therapy as a symptomatic treatment for PD (Birkmayer & Hornykiewicz, 1961). Initially, a cohort of akinetic patients with postencephalitic parkinsonism from the influenza pandemic of 1918 were administered a relatively high-dose (50 to 150 mg) L-DOPA intravenously, which led to their seemingly miraculous awakenings.[3] Then, in 1967, George Cotzias and his

2 Carlsson utilized a spectrofluorophotometer to measure chemical concentrations in tissue samples.

3 Despite this initial success, levodopa therapy remained problematic in several ways; First patients' response to L-DOPA was extremely limited in duration; furthermore, subsequent trials by others failed to reproduce the same rates of

colleagues successfully treated patients with extremely high doses of orally-administered L-DOPA (Cotzias et al., 1967, 1969). Cotzias utilized an approach that relied on gradually increasing doses of L-DOPA over the span of months, thus allowing the drug to gradually attain its full level of efficacy.[4] Finally, in 1969, Yahr conducted a double-blind clinical trial which ultimately established L-DOPA as an effective therapy for treating the motor symptoms of PD (Yahr et al., 1969). Lower doses of L-DOPA could be used with a peripherally acting decarboxylase inhibitor (Papavasiliou et al., 1972). Problems of excessive peripheral DA were resolved through the co-administration of an inhibitor of the aromatic amino acid decarboxylase (AADC) enzyme, using alpha-methyl hydrazine (carbidopa) and later benserazide, which greatly enhances transport[5] of L-DOPA across the blood-brain-barrier, potentiating its effects within the central nervous system while simultaneously preventing accumulation of excess peripheral DA. This allowed for more effective management of PD symptoms through an accelerated induction of optimal L-DOPA efficacy, while using substantially lower doses of L-DOPA.

Soon after levodopa was introduced as a therapy for PD, it was discovered that it is associated with the appearance of adverse motor complications including motor fluctuations and the development of abnormal involuntary movements called levodopa-induced dyskinesias (LIDs). Dyskinesias typically occur during L-DOPA "on" periods, particularly during the peak of each dose, which predominantly consist of choreiform and dystonic abnormal involuntary movements, but may also include athetosis. In contrast, motor fluctuations refer to "off"-states, which are defined by different temporal patterns of returning parkinsonian symptoms, e.g. "wearing-off", "random on-off", and "delayed on" (Fahn et al., 2011). Although, these motor complications had been encountered previously during clinical trials, the antiparkinsonian benefits of L-DOPA therapy were self-evident and

---

success, with some trials even reporting a null effect of L-DOPA therapy altogether.

4 One critical problem was that, in many instances, extremely high doses of L-DOPA were necessary to effectively reverse PD motor symptoms, which caused a number of adverse side effects related to excessive peripheral DA including gastrointestinal effects as well as nausea.

5 L-DOPA is transported across the blood-brain-barrier by the action of large neutral amino acid transporters.

outweighed the risk of side effects, thus development of L-DOPA as a therapy for PD continued and it has remained the 'gold-standard' of symptomatic treatment for PD until the present day.[6]

The presence of motor complications related to chronic use of L-DOPA as a therapy for PD is a significant problem that affects the majority of patients (Fox & Lang, 2008; Obeso, Olanow, et al., 2000). Since the 1970s, various therapeutic strategies have been developed in an attempt to counteract or delay the appearance of adverse side effects of L-DOPA therapy, while attempting to preserve its motor restorative efficacy (Nutt et al., 2000; Olanow et al., 2006; Schapira & Obeso, 2006; Olanow et al., 2013), however, motor complications associated with DA-replacement therapy remain an important treatment-limiting problem that will be encountered by the majority of patients (Smith et al., 2012). Thus there is an ongoing need for the development of novel adjunct therapies that can help to alleviate these debilitating side effects.

Mapping the neural circuits underlying PD pathology and the development of functional surgical approaches to PD therapy

The most important developments following the discovery of the role of DA in movement and the development of levodopa therapy, which have shaped our conceptualization of PD pathology and the clinical treatment of PD, were the mapping of the neural pathways pathologically dysregulated in PD and the development of surgical approaches for treatment of PD. Several key neuroanatomical and pathological studies have helped to shape our current understanding of basal ganglia functional anatomy and systems-level pathophysiology of PD. In the late 1980s and early 1990s, scientists began to map the functional organization of the basal ganglia utilizing data from studies involving rats and

6 The development and success of L-DOPA therapy was a landmark development in the treatment of PD, however, it has also revealed that some symptoms of PD do not respond well to dopaminergic drug therapies. It is thought that these symptoms are more likely associated with dysregulation of other neurotransmitter systems e.g. serotonergic, noradrenergic; treatment of non-motor features remains an important problem in the treatment of PD.

non-human primates. Several studies revealed that unilateral lesions of the ventral midbrain decreased levels of DA in the striatum (Andén et al., 1965, 1964; Goldstein et al., 1966; Poirier & Sourkes, 1965) and that unilateral removal of the striatum decreased the number of catecholaminergic neurons in the SN (Andén et al., 1965). Together, these studies provided evidence that the SN and striatum form part of a common nigrostriatal dopaminergic pathway involved in motor control, and more specifically, that the dorsolateral striatum receives terminal projections from dopaminergic neurons in the SN.

Detailed anatomical and pathological studies of the SN revealed that one particular subregion of the SN, the pars compacta (SNpc), is preferentially affected by the neurodegenerative process (Greenfield & Bosanquet, 1953) and that, within the SNpc, cell loss is further localized to the ventrolateral tier and caudal regions of that nucleus (Fearnley & Lees, 1991). Another study revealed disproportionate degeneration of pigmented DA neurons in the SNpc, relative to non-pigmented DA neurons in this nucleus (Hirsch et al., 1988).[7]

Following characterization of the disease pathology in the SNpc, attention shifted toward studying the molecular basis of DA neurotransmission in striatal neurons. Several key studies (Albin et al., 1989; Brotchie et al., 1993; DeLong, 1990) revealed that the striatum is comprised mostly of GABAergic medium spiny neurons (MSNs), which project either 1) directly to the output nuclei of the basal ganglia (i.e., the substantia nigra pars reticulata (SNr) and the globus pallidus interna) or 2) indirectly, through synapses in the globus pallidus externa (GPe) and subthalamic nucleus (STN). Gerfen et al. (1990) found that striatonigral projecting MSNs predominantly express D1 dopamine receptors and substance P, while striatopallidal projection MSNs express predominantly D2 dopamine receptors and the opioid peptide enkephalin. This established the existence of two anatomically distinct pathways in the basal ganglia, which are segregated according to their expression of dopamine receptor

7 This led some scientists to hypothesisze that neuromelanin could contribute to the selective vulnerability of DA neurons in the SNpc and that neuromelanin or its precursors might be cytotoxic or interfere with cellular physiological processes (Marsden, 1983).

subtype (D1 vs. D2) and corresponding projection targets, thus forming two separate populations of striatal MSNs,[8] which form the so-called *direct* and *indirect* striatal output pathways. This striking anatomical and neurochemical segregation has been further characterized through a series of electrophysiological, genetic, and behavioral experiments, which have provided empirical evidence to support the model (Day et al., 2006; Gong et al., 2003; Kravitz, 2010; Kreitzer & Malenka, 2007).

Classic model of basal ganglia physiology

The development of toxin-based animal models of PD including the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) nonhuman primate (Burns et al., 1983; Di Monte et al., 2000; Langston et al., 1984) and mouse models (Hallman et al., 1984; Heikkila et al., 1984; Sonsalla & Heikkila, 1986), as well as the 6-hydroxydopamine (6-OHDA) rat model (Hokfelt & Ungerstedt, 1973; Ungerstedt & Arbuthnott, 1970; Ungerstedt, 1968) was instrumental to furthering our understanding of the systems level pathophysiology of the disease and led to the development of the classical model of basal ganglia functional circuitry (Albin et al., 1989; DeLong, 1990). The introduction of this model also restored interest in surgical approaches (Bergman et al., 1990; Limousin et al., 1995) eventually leading to the development of deep brain stimulation (DBS).

8 D1 and D2 expressing MSNs together constitute 90% of the neurons in the striatum.

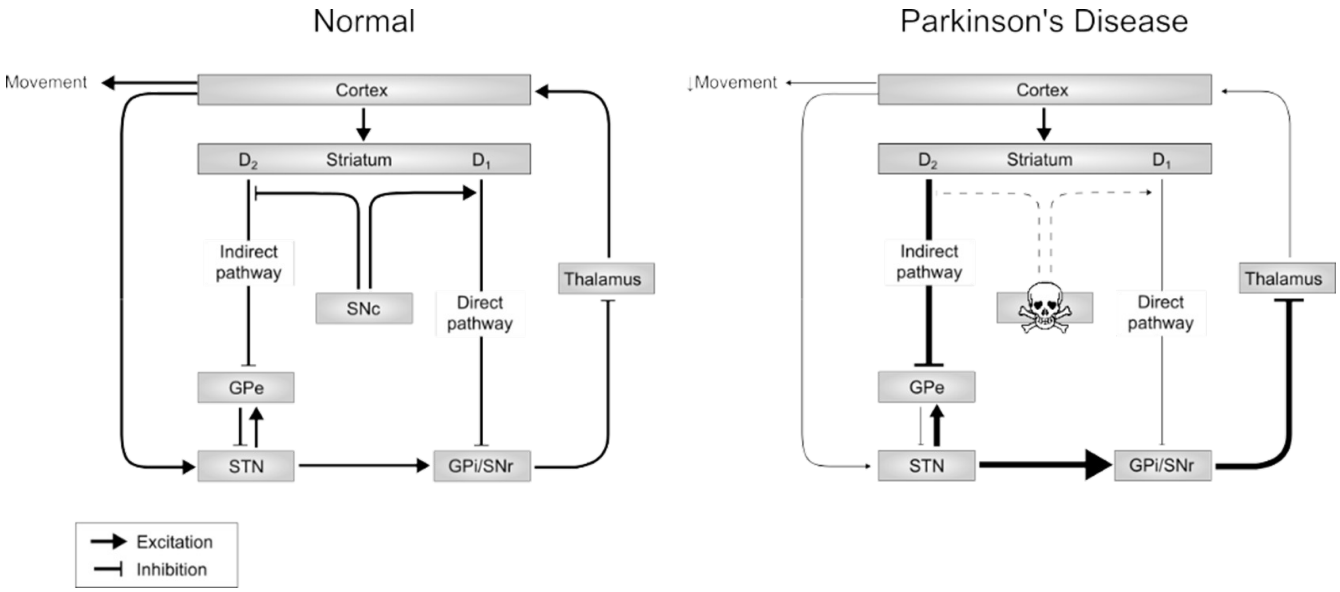

Figure 2. Schematic diagram of the direct and indirect striatal output pathways of the basal ganglia in normal and parkinsonian states. Inhibitory striatal projection neurons, called medium spiny neurons (MSNs) in the striatonigral direct ('Go') pathway express D1 receptors (D1Rs) and utilize dynorphins and substance P as a co-transmitters, while MSNs in the striatopallidal indirect ('No-Go') pathway express D2 receptors (D2Rs) and employ enkephalins as co-transmitters. Dopamine (DA) is excitatory to MSNs in the direct pathway via its activation of D1Rs; it is inhibitory to indirect pathway MSNs via its activation of D2Rs. Arrows represent synaptic connections made by projection neurons between the respective nuclei; the weights of arrows (indicated by their thickness) represent the average linear rate of activation for a given projection. In the normal state (**Left**), the activation rates of MSNs in the direct and indirect pathways are balanced. However, in response to DA depletion in PD (**Right**), activation of MSNs in the direct and indirect pathways are hypo- and hyper-activated respectively. Dashed lines of arrows label the afferent dopaminergic projections from the SNpc to the **striatum** indicate lesion of the nigrostriatal pathway that occurs in PD; the skull-and-crossbones 'toxicity' symbol indicates cell death in the SNpc in PD. Note that this is a simplified diagram of basal ganglia circuitry, some relevant synaptic projections and nuclei, e.g. the pedunculopontine nucleus (PPN), have been omitted for simplicity of visualization. **GPe**, external globus pallidus; **GPi**, internal globus pallidus; **Cortex** includes primary motor cortex, pre-motor cortex, supplementary motor area; **SNc**, substantia nigra pars compacta; **SNr**, substantia nigra pars reticulata; **STN,** subthalamic nucleus; **Thalamus** includes the ventral anterior/ventral lateral nucleui of thalamus. Adapted from Smith, Y., Wichmann, T., Factor, S. A. & DeLong, M. R. Parkinson's disease therapeutics: new developments and challenges since the introduction of levodopa.

The classical model is based on the functional opposition of the direct and indirect striatal output pathways of the basal ganglia (**Figure 2**). The two pathways operate in parallel to one another to modulate motor output (Alexander & Crutcher, 1990). Specifically, activation of the direct striatonigral pathway is excitatory to movement while activation of the indirect striatopallidal pathway is inhibitory to movement (Smith et al., 1998). The principle concept underlying the classic model is that, under normal physiological conditions, the direct and indirect pathways have balanced rates of activation; however, in states of altered DA neurotransmission, as a consequence of oppositional effects of DA on D_1 and D_2 receptors, an imbalance arises in the relative activation rates of the two pathways. In the context of PD-related DA depletion, the indirect striatopallidal pathway is rendered hyperactive while the direct striatonigral pathway is rendered hypoactive; this imbalance is thought underlie the predominantly hypokinetic features of the disease. Thus the net functional effect of striatal DA depletion is enhanced suppression of thalamo-cortical motor output (Obeso, Rodriguez-Oroz, et al., 2000).

In the context of the classic rate model, hyperkinetic abnormal involuntary movements related to DA receptor hyperstimulation (i.e., dyskinesia), in contrast PD symptoms, are thought to result from hyperactivation of the direct pathway, and inhibition of the indirect pathway (Graybiel et al., 2000). This model is referred to as a rate-based model because it explains aspects of basal ganglia physiology and pathology according to linear rates of activation between component nuclei and it is diagrammed such that weights of projections between nuclei (indicated by their thickness) represent linear rate coding of activation. Thus, the classical direct-indirect pathway theoretical framework appears to successfully explain some key aspects of both the hypokinetic motor abnormalities in PD and the hyperkinetic abnormal involuntary movements in LID as a result of the oppositional roles of these pathways in activating and inhibiting motor output (**Figure 2**). In summary, the classical "direct-

indirect" pathway model provides a framework for reasoning about basal ganglia physiology and serves to effectively explain many aspects of disorders that involve dysfunction of these pathways.[9]

Updates to the classical model of basal ganglia physiology

It is now appreciated that the physiology of the BG is more complex than the classical rate-based model developed by Albin and DeLong. In particular, more recent models include operation of the hyperdirect corticosubthalamic pathway in parallel with the direct and indirect striatal output pathways (Nambu, 2004). Furthermore, it is now appreciated that initiation and execution of movement sequences involves coordinated activation of the direct and indirect pathways together (Cui et al., 2013; Tecuapetla et al., 2016). Prior to 2002, models of PD were represented as static diagrams representing linear rate-coding of activation, (i.e., static/non-dynamic average rates of activation) however, it is now appreciated that non-linear dynamical activity plays a critical role in BG physiology and pathophysiology (Obeso et al., 2008). In particular, oscillatory activity and synchronization are now recognized to play an important role in BG physiology (Brown et al., 2001; Hammond et al., 2007, 2008; Plenz & Kital, 1999) and appear to play a fundamental role in information processing and communication by coordinating activity within and across brain regions (Buzsáki & Draguhn, 2004).

The striatum is also spatially and functionally divided into striasomes and matrisomes (or patch-matrix organization) based on their differential expression of histochemical markers (Gerfen et al., 1985, 1987; Gerfen, 1984, 1992; Graybiel & Ragsdale, 1978; Herkenham & Pert, 1981); this represents another level of organizational complexity for which the classical basal ganglia model did not account. Both patch and matrix compartments contain striatal projection neurons belonging to the direct and

9 Other disorders involving dysfunction of the basal ganglia include Huntington's disease, Tourette's syndrome, addiction disorders, chronic pain, depression, and schizophrenia.

indirect output pathways, however, function of motor control has been more closely associated with matrix pathways, whereas cognitive and affective functions have been assigned to the striasome/patch compartments (Prager & Plotkin, 2019). Additionally, there is another functional division of the striatum that is broadly-defined along a dorsolateral-ventromedial axis such that the dorsolateral striatum is generally involved with motor control, while the ventromedial striatum is involved with associative and limbic functions (Voorn et al., 2004).

Surgical approaches & deep brain stimulation

Although interest in surgical approaches for the treatment of PD declined following the advent of L-DOPA therapy, by the mid-1980s, a significant number of PD patients with symptoms refractory to levodopa had accumulated and surgical techniques began to be re-explored (Gardner, 2013). In 1990, Bergman et al. demonstrated reversal of PD motor deficits through ablative lesion of the STN. Another study by Laitinen et al. (1992) showed that stereotactic posteroventral pallidotomy led to remarkable improvement of parkinsonian sysmptoms. However, although such ablative surgical procedures were remarkably effective in treating PD symptoms, their efficacy was ultimately proven inferior to that of deep brain stimulation (DBS) and by 1995, DBS was established as the most effective surgical treatment for PD and it gained FDA approval for that purpose in 2002 (Gardner, 2013).

Deep brain stimulation (DBS) is a procedure that involves chronic high frequency (> 100 Hz)[10] electrical stimulation of specific brain nuclei via surgically implanted electrodes. The procedure involves surgical placement of a generator that transmits a continuous pattern of electrical impulses via multiple contact electrode configurations to target nuclei.[11] As PD neurodegeneration progresses

10 A frequency of 130 Hz is commonly used in DBS.
11 The most common nuclei targeted in PD are the STN and the GPi.

during the course of the disease, many patients develop medication-refractory symptoms and debilitating motor complications including motor fluctuations and LID. Once disease pathology has advanced to this point, pharmacological management of symptoms and disability becomes increasingly intractable. Then, for certain patients, DBS often results in a remarkable degree of symptomatic improvement and enhanced quality of life. In many instances, DBS provides symptomatic alleviation that is superior to levodopa therapy. Motor signs of PD including tremor, bradykinesia, and rigidity, as well as the motor complications associated with chronic DA replacement therapy, are significantly improved by DBS targeting either the STN or GPi.

The discovery of DBS is mostly attributed to Alim-Louis Benabid who was at the Centre Hospitalier Universitaire de Grenoble in France. While using intraoperative electrical stimulation to insure correct localization of lesion placement during ablative surgeries, Benabid discovered that high frequency stimulation could alleviate some of the motor symptoms of PD. In 1991, two independent groups, one led by Benabid and another by Blond and Siegfried, concurrently reported that thalamic DBS improves tremor in patients with advanced PD (Benabid et al., 1991; Blond & Siegfried, 1991). These were the first published reports of modern DBS for the treatment of PD, however, these studies were limited by a lack of effect on other motor symptoms of PD. The aforementioned ablative lesion studies in non-human primates, by Bergman and Laitinen during the early 1990s, suggested that the STN and globus pallidus would be more appropriate targets for DBS in PD. This was subsequently confirmed in studies directly applying DBS to the internal globus pallidus (GPi) and the subthalamic nucleus (STN) (Benazzouz et al., 1993; Limousin et al., 1995; Pollack et al., 1993; Siegfried & Lippitz, 1994). DBS of the STN is currently the preferred target for most patients because it provides improvement for a wide range of PD motor symptoms; however, stimulation of the GPi may be

preferred for patients with L-DOPA refractory symptoms or with LID (Limousin & Martinez-Torres, 2008; Schwalb & Hamani, 2008).

Although DBS has been in use for several decades as a therapy for PD motor symptoms, it apparently does not alter the course of disease progression. Furthermore, its mechanisms of action are not well-understood. In particular, there remains an unexplained paradox related to question of the mechanism through which high-frequency stimulation (HFS) of the STN or GPi can give rise to the same effect on PD motor symptoms as ablative lesions in these nuclei. According to the classic rate-based model, DBS in the STN or GPi should further inhibit movement, yet it reverses PD-related motor inhibition. Therefore it was initially hypothesized that DBS must function as a reversible lesion, however, our current understanding is that it actually increases firing rates in the target and projection nuclei and directly modulates brain activity in a controlled manner (Hammond et al., 2007, 2008). An important pathological feature of PD is the emergence of abnormal hypersynchronous neuronal activity within the beta frequency range (13–35 Hz), particularly within the subthalamic nucleus (Cassidy et al., 2002; Gradinaru et al., 2009; Kühn et al., 2005; Levy et al., 2002; Sharott et al., 2018). Data suggest that DBS acts to alter oscillation frequencies and attenuate aberrant hypersynchronous patterns of neural activity in PD.

Using, *in vivo* electrophysiology to record neural activity in one region of the brain, while simultaneously stimulating another brain region with a DBS electrode, demonstrated that DBS directly modulates pathological oscillatory activity between brain regions. Specifically, DBS suppresses hyper-synchronized oscillations in nuclei of the basal ganglia (Hammond et al., 2007) and reduces the phase-amplitude coupling in the motor cortex (De Hemptinne et al., 2015). This suggests that high-frequency stimulation of the STN or GPi may realize symptomatic benefits through attenuation of PD-related hypersynchronization. Recent advances in DBS include the development of closed-loop DBS, bi-

directional electrodes, which can stimulate as well as record neural activity, and the use of DBS for other neuropsychiatric disorders like obsessive compulsive disorder, Tourette's syndrome, and depression (Rosin et al., 2011).

1.2 Parkinson's disease etiology

Parkinson's disease is a highly complex and heterogeneous disease whose pathogenesis is thought to arise through interactions between multiple genetic and environmental factors.[12] Age is the primary risk factor for the development of PD. The etiology of most cases of PD is unknown; such cases are referred to as *idiopathic* or *sporadic*. This accounts for the majority of PD cases (> 90%) and they occur without any family history of the disease (Bellou et al., 2016), although, there is now considerable evidence that idiopathic PD is linked to mutations in the *LRRK2* (leucine-rich repeat kinase 2) and several other genes without any clear pattern of familial inheritance (Berg et al., 2005; Brice, 2005; Correia Guedes et al., 2010). It is estimated that fewer than 10% of PD cases are linked to genetic mutations occurring in a single gene locus that are inherited as Mendelian dominant or recessive traits. Although monogenic forms of PD are rare, their characterization has helped to elucidate pathological mechanisms underlying idiopathic PD.

Evidence for environmental causes of PD is related to the incidence of postencephalitic cases of parkinsonism (Casals et al., 1998) and severe acute parkinsonism resulting from self-injection with MPTP-contaminated meperidine (Davis et al., 1979; Langston et al., 1983). Epidemiological studies have also linked the disease to consumption of well water, residence in rural areas, and exposure to pesticides such as rotenone (Betarbet, 2000) and herbicides including paraquat, maneb, and agent

12 The wide heterogeneity in PD clinical subtypes suggests that it may not represent a single disease but rather a spectrum of disorders.

orange (Dauer & Przedborski, 2003; Thiruchelvam et al., 2000, 2003). Parkinsonism with frontotemporal dementia has also been linked to consumption of cycad plant seeds and/or cyanobacteria by indigenous peoples of Guam (Bradley & Mash, 2009; Murch et al., 2004; Spencer et al., 1987). Furthermore, a protective effect of curcumin has been evidenced by epidemiological studies that reported lower prevalence of PD and AD in India (Chainani-Wu, 2003; Donatus et al., 1990; Kelloff et al., 1996). Coffee consumption and cigarette smoking are protective and inversely associated with the incidence of PD (Hernan et al., 2002). There is currently no consensus as to whether or not any racial bias on the incidence of PD exists, however, most prevalence studies suggest that White people are more likely to develop PD than individuals from other racial backgrounds although geographic location is a more reliable predictor than race or ethnicity (Pringsheim et al., 2014; Wright Willis et al., 2010).

1.2.1 Epidemiology

In the United States, approximately one million individuals suffer from PD, and there are 60,000 new cases diagnosed every year (DeMaagd & Philip, 2015; Marras et al., 2018). The disease affects 1-2% of the population over the age of 60 and this rate increases to affect 5% of the population over age 85 (de Lau & Breteler, 2006; Nussbaum & Ellis, 2003). Only 4% of all PD cases are diagnoses before age 50. The disease affects males more often than females with a ratio of 3:2 (Martínez-Rumayor et al., 2009; Miller & Cronin-Golomb, 2010). The most recent estimate of the combined direct and indirect costs of PD in the U.S. amounts to nearly $52 billion per year (Parkinson's Foundation, parkinson.org/research/economic-burden-study). Based on U.S. Census Bureau projections, Marras et al. (2018) estimated that the number of individuals living with PD will be 1.2 million in 2030. It is projected that the number of individuals living with PD worldwide has reached 10 million; the

incidence is higher in industrialized countries. As life expectancy continues to increase worldwide, the prevalence of PD is expected to increase correspondingly. Therefore, research endeavors promoting the development of novel therapies to prevent or treat PD and LID has become an urgent concern.

1.2.2 Advanced age

The primary risk factor for developing PD is advanced age (Calne & Langston, 1983; Tanner & Ben-Shlomo, 1999) and it is also the best predictor of the rate of disease progression (Diederich et al., 2003; Hely et al., 1995; Jankovic & Kapadia, 2001). In particular, there is substantive evidence to suggest that PD and aging share common underlying mechanisms. Mitochondrial dysfunction, oxidative stress, and defective proteostasis play a central role in the cellular and molecular neuropathogenesis of PD (Dauer & Przedborski, 2003). Furthermore, PD and aging are linked through genomic instability (Ambroziak et al., 2015), and mutations and deletions in nuclear (Berg et al., 2005; Brice, 2005; Correia Guedes et al., 2010) and mitochondrial DNA (De Coo et al, 1999; Muller Nedebock et al., 2019).

A number of preclinical primate studies have identified age-related dysfunction of the nigrostriatal dopaminergic system, including depletion of striatal DA, and age-related reduction in motor activity (Collier et al., 2007; Irwin et al., 1994). These findings suggest that age-related changes increase the susceptibility of nigrostriatal dopaminergic neurons to degeneration and that cellular mechanisms involved in normal aging are enhanced and accelerated in PD due to multiple genetic and environmental factors (Sulzer, 2007). Aging is associated with accumulation of damage to cellular macromolecules including membranes, proteins and DNA (Bandy & Davison, 1990; Gems & Partridge, 2013; Kirkwood, 2005; Schumacher et al., 2008; Vijg & Campisi, 2008). Theories

attempting to explain the relationship between aging and neurodegeneration posit that age-related changes increase the vulnerability of neurons to degeneration and that cumulative damage to cellular components such as nuclear DNA (nDNA) and mitochondria, including mitochondrial DNA (mtDNA) is a major factor underlying the process of both aging and neurodegeneration (Collier et al., 2017; Madabhushi et al., 2014; Martin, 2008; Schon & Przedborski, 2011).

1.2.3 Selective vulnerability of SNpc dopaminergic neurons to degeneration

Another important hallmark of aging is altered energy metabolism (Bratic & Trifunovic, 2010; López-Otín et al., 2013; Roberts & Rosenberg, 2006; Swerdlow, 2011; Willis et al., 2002). Similar alterations in mitochondrial respiration have also been linked to PD neuropathology (Mann et al., 1992; Minzuno et al., 1989). Neurons require between 10-20 times more energy than other cell types; roughly 20% and 25% of total oxygen and glucose consumed by the body are dedicated to the brain, although the brain accounts for only 2% of total body mass. Most of this additional energy is used to power synaptic transmission (Harris et al., 2012) as well as in the maintenance and restoration of ion gradients dissipated by signaling processes (Attwell & Laughlin, 2001). It is thought that dopaminergic neurons in the SNpc may possess the highest energy requirements of any neuronal cell type (Pellerin & Magistretti, 1994; Pissadaki & Bolam, 2013; Wellstead & Cloutier, 2011). One aspect of this is that SNpc neurons possess intrinsically generated pacemaking activity, characterized by a 2-4 Hz firing rate, which is reliant on calcium (Chan et al., 2009; Guzman et al., 2009; Puopolo et al., 2007). This reliance on calcium for pacemaking is a major source of energetic stress. Cells maintain a 20,000 fold difference between their intracellular (~100 nM free) and extracellular (mM) concentrations of calcium; excess cytosolic calcium interferes with a wide array of intracellular signaling pathways critical to survival (Clapham, 2007). Removal of calcium from the cytosol is an energy-intensive

process that involves ATP-dependent pumping of cytosolic calcium ions into endoplasmic reticulum stores and extrusion into the extracellular space against an extremely high concentration gradient via the sodium-calcium exchanger (NXC). Finally, another reason for the enormously high energy requirements of SNpc DA neurons is related to their massive and highly complex unmyelinated axonal arborization within the striatum (Matsuda et al., 2009). Neurons with long, sparsely myelinated axons are preferentially vulnerable to PD damage (Braak & Del Tredici, 2004). In conclusion, the unique anatomy and physiology of SNpc dopaminergic neurons is associated with exceptionally high energetic demands, which predisposes dopaminergic neurons in to effects of aging on energy metabolism (Van Laar & Berman, 2009).

1.2.4 Mitochondrial dysfunction

Mitochondrial dysfunction, defined as deficiency in the mechanisms of mitochondrial respiration, is a central component of aging and has been implicated in the pathogenesis of PD. The first evidence linking mitochondrial dysfunction to PD arose in the late 1970s and early 1980s when a series of cases of irreversible parkinsonism clinically indistinguishable from idiopathic PD, including responsiveness to L-DOPA, were documented in individuals who had intravenously self-administered meperidine contaminated with the mitochondrial toxin MPTP (Davis et al., 1979; Langston et al., 1983). MPTP is a nonpolar molecule, which is transported across the blood-brain-barrier, and metabolized to MPP+, a potent inhibitor of complex I of the mitochondrial electron transport chain. This conversion takes place inside of astrocytes and is catalyzed by monoamine oxidase A. MPP+ is then selectively taken up by dopaminergic terminals through the DAT transporter (Dauer & Przedborski, 2003). The finding that complex I inhibition is sufficient to cause a parkinsonian syndrome clinically identical to idiopathic PD suggested that defects in mitochondrial respiration may play a role in the pathogenesis of PD. Indeed, it was confirmed soon thereafter that nigral tissue taken from sporadic PD patients postmortem displayed decreased complex I activity (Mann et al., 1992; Schapira, 1990; Schapira, Cooper, et al., 1990).

However, while the development of PD induced by MPTP is an important clue related to the role of mitochondiral dysfunction in the pathogenesis of PD, the more gradual development of symptoms in sporadic PD argues against a role for acute exposure to environmental toxins. Instead, it is more likely that genetic mutations impairing mitochondrial respiration are a determining factor. Perhaps not surprisingly, mutations in complex I genes were later identified in postmortem sporadic PD brain tissues, particularly within the substantia nigra, including deletions in mitochondrial DNA (Schon & Przedborski, 2011). Additionally, several PD-associated genes, including *SNCA, Parkin, PINK1,*

DJ-1, LRRK2 and *HTR2A*, show a degree of localization to the mitochondria under certain conditions. Paradoxically, however, PD is not maternally inherited except in a very small subset of patients, and no specific mutation in mitochondrial DNA has yet been conclusively linked to PD. Nevertheless, rare but important cases of familial PD establish an important role of impaired mitochondrial function as a factor, as discussed in the next section.

1.2.5 Mitochondrial quality-control deficits

Monogenetic loss of function mutations in PTEN-induced kinase (*PINK1; PARK6*) and *parkin (PARK2)* are two mitochondrial genes that have been conclusively linked to PD (Kitada et al., 1998). Together, the two genes form a quality-control mechanism for removal of damaged mitochondria through the process of mitophagy (Narendra, 2010; Vives-Bauza, 2010). Monogenetic loss of function mutations in *PINK1* and *parkin* have both been linked to early-onset autosomal recessive forms of PD (Valente et al., 2004). While mutations in these genes are rare, a mechanistic understanding of the functions performed by their encoded proteins has revealed important insights related to the potential role of mitochondrial dysfunction in sporadic/idiopathic PD. Since the selective removal of damaged mitochondria by the PINK1/Parkin mitophagy system was described in detail, the role of mitophagy in PD has continued to be an active area of research (Deas et al., 2011).

Selective mitophagy is regulated by *parkin* and *PINK1 (PTEN-induced putative kinase 1)*; mutations in these genes have been linked to autosomal recessive forms of Parkinson's disease (Valente et al., 2004). These proteins have also been shown to mediate the same pathway in response to mitochondrial damage in *Drosophila* and other invertebrates, indicating that the pathway is evolutionarily conserved (Clark et al., 2006; Greene et al., 2003; Park, 2006). *PINK1* encodes PTEN-

induced putative kinase 1 (PINK1), a mitochondrial kinase which has a highly conserved kinase domain with homology to the serine/threonine kinases of the calcium/calmodulin-dependent family of kinases and has a mitochondrial targeting sequence that localizes it to mitochondria *Parkin* encodes an E3 ubiquitin ligase, which ubiquitylates a number of proteins, including proteins on the surface of the outer membrane of mitochondria that are important for targeting autophagic machinery to damaged mitochondria.

1.2.6 Oxidative stress

In addition to mitochondrial dysfunction, there is also substantial evidence that oxidative stress plays an important role in the neuropathogenesis of PD (Schapira & Jenner, 2011). The SNc of postmortem PD patients displays increased levels of oxidized lipids, DNA, proteins and decreased levels of the antioxidant protein glutathione (GSH) (Dias et al., 2013). Mitochondrial respiration normally generates reactive oxygen species (ROS), which in turn causes oxidative damage to cellular macromolecules such as DNA, proteins, and lipids (Boveris et al., 1972). Cells have evolved a number of mechanisms for scavenging free radicals (Ebadi et al., 1996; Jenner et al., 2003; Jenner & Olanow, 2006). Oxidative stress occurs when there is an imbalance between production of ROS and cellular antioxidant activity and/or repair mechanisms (Lotharius & Brundin, 2002). Two important protective reactions include the conversion superoxide radical (O_2^-) to hydrogen peroxide (H_2O_2) by superoxide dismutase, and the reaction of hydrogen peroxide with reduced glutathione to produce H_2O. Nigral dopaminergic neurons also contain iron, which catalyzes the Fenton reaction, through which O_2^- and H_2O_2 can contribute to harmful oxidative stress (Valko et al., 2007). It has been shown that accumulation of iron in the SNc of PD patients is increased relative to control subjects (Brar et al., 2009; Faucheux et al., 2003). Additionally, neuroinflammation can further accelerate ROS production

(Block et al., 2007). Finally, DA metabolism is another important source of free-radicals in the form of DA metabolites (dopaquinones) and auto-oxidation products. Nigral DA neurons are subject to many sources of ROS are therefore thought to be intrinsically more susceptible to oxidative stress induced by excessive ROS generation (Sulzer, 2007).

1.3 Levodopa-induced dyskinesia

DA replacement strategies relying primarily on use of the DA precursor levodopa or DA receptor agonists are the primary symptomatic treatments for PD. Although effective at treating the primary motor symptoms of the disease, over time, patients develop debilitating motor fluctuations and levodopa-induced dyskinesia (LID). In PD and LID, abnormal dopamine receptor stimulation alters the GABAergic striatal efferents of the dopamine D1 receptor-expressing direct striatonigral output pathway and the dopamine D2 receptor-expressing indirect striatopallidal output pathway (**Figure 3**). Abnormal striatal output, in turn, alters the linear firing rate, patterning, and synchronization of neuronal activity in the basal ganglia. These alterations in the basal ganglia subsequently affect the motor cortex, which may manifest as alterations in volitional movement or the appearance of involuntary movements clinically in patients. In rodent models, choreiform and dystonic abnormal involuntary movements (AIMs) are thought to be a correlate of the clinical state of LID (Cenci et al., 2002).

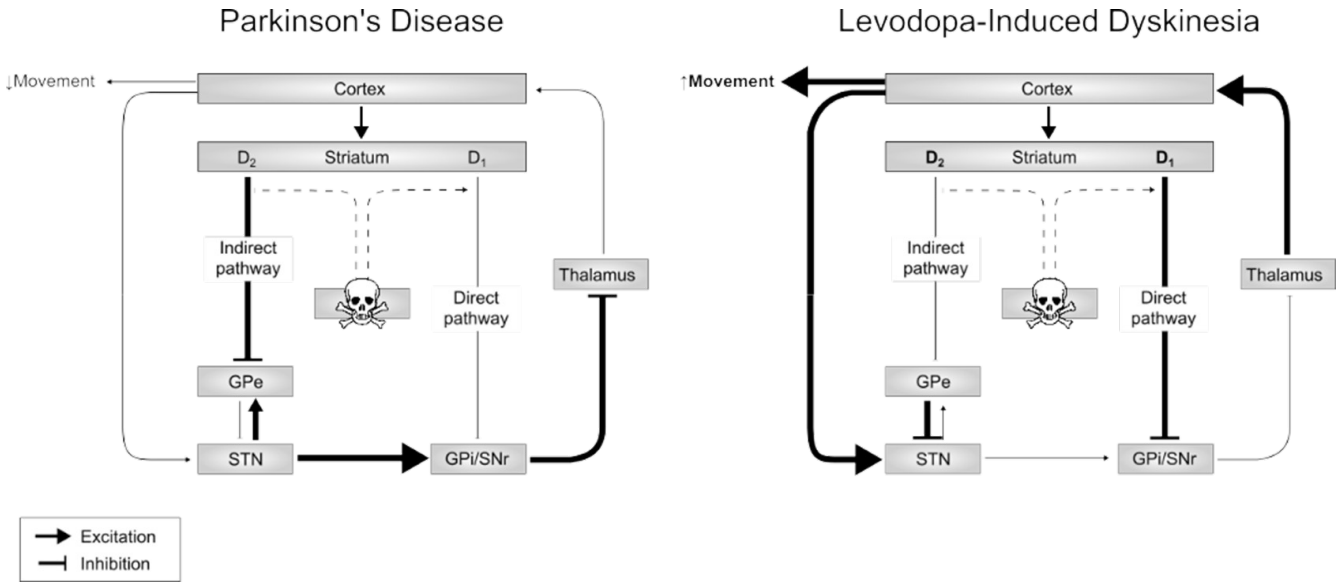

Figure 3. Schematic diagram of the direct and indirect striatal output pathways of the basal ganglia in states of Parkinson's disease and L-DOPA-induced dyskinesia. Alterations in the direct and indirect pathways of the basal ganglia in Parkinson's disease (**Left**) and in levodopa-induced dyskinesia (**Right**). Following administration of levodopa, D1 and D2 receptors on striatal projection neurons are hyperactivated by DA derived from exogenously administred L-DOPA, leading to an imbalance in rates of activation of the direct and indirect output pathways that is opposite to the imbalance realized in the Parkinson's disease dopamine depleted state.

It is thought that the underlying pathophysiology of LID is related to a maladaptive synaptic plasticity that has distinct presynaptic and postsynaptic components linked to the pharmacological replacement of endogenous DA with L-DOPA formulations (Obeso et al., 2000). Nigral dopaminergic neurons have intrinsic pacemaker activity and fire continuously to maintain levels of striatal DA at relatively constant levels so that postsynaptic DA receptors on MSNs receive stable and continuous levels of input via post-synaptic DA receptors (Surmeier et al., 2011). As PD progresses however, the striatum becomes progressively denervated of nigral dopaminergic terminals and, secondarily, striatal

DA levels become increasingly dependent on exogenous administration of L-DOPA. L-DOPA is typically administered orally between 2-4 times daily, which gives rise to progressively more severe fluctuations of dopaminergic tone, as the striatum loses the capacity to homeostatically regualate levels of DA. The resulting presynaptic alterations contribute to the development of LID and motor fluctuations. Postsynaptically, LID is thought to arise from non-physiologic, pulsatile stimulation of DA receptors expressed on striatal projection neurons (SPNs), as well as altered mechanics of DA vesicular release and reuptake pre-synaptically (de la Fuente-Fernandez et al., 2004).

1.3.1 NMDA-receptor antagonists in PD and LID

Preclinical studies in MPTP-lesioned primates and 6-OHDA lesioned rats have demonstrated that selective NMDA receptor antagonists reduce LID but worsen parkinsonism (Gomez-Mancilla & Bédard, 1993; Papa & Chase, 1996a; Paquette et al., 2010; Rupniak et al., 1992). The multifunctional drug with modest NMDA receptor antagonist activity, amantadine, is an established clinical therapy for LID (Goetz et al., 2005; Pahwa et al., 2006). In practice, however, only a proportion (~25%) of patients respond well to amantadine; some patients experience hallucinations, confusion, and other side effects which limit its use. In theory, NMDA receptor antagonists should reduce hyperactivation of the indirect pathway in PD, helping to counteract parkinsonian symptoms (Thomas et al., 2004). In support of this, our data presented in Chapter 2 demonstrate that AIMs induced by levodopa and a selective D2 receptor agonist (quinpirole) can be suppressed by MK-801, while AIMs elicited by the selective D1 receptor agonist SKF81297 were not significantly reduced by MK-801 in the 6-OHDA lesioned preclinical rat model of LID (Flores et al., 2014).

1.3.2 Opioid neurotransmission in PD and LID

The opioid neurotransmission system is prominent in the basal ganglia. Endogenous opioid peptides and their precursors are expressed within the basal ganglia, where they are modulators of GABA, DA, and glutamate neurotransmission. Furthermore, all three opioid receptor subtypes—μ, δ, and κ—are expressed with a high density in the striatum, and are co-localized on GABAergic MSNs and are also expressed in nuclei receiving input from the striatum (Fox & Brotchie, 2011). Of particular importance, striatal GABAergic MSNs are segregated according to their differential expression of opioid peptide co-transmitters. Specifically, the D2 receptor-expressing indirect striatopallidal projection neurons co-express enkephalins (Leu- and Met-) derived from the precursor pre-proenkephalin A (PPE-A), which preferentially bind and activate δ-opioid receptors, whereas the D1 receptor-expressing direct striatonigral projection neurons co-express dynorphin and opioid peptides derived from pre-proenkephalin B (PPE-B); dynorphin, in particular, predominantly activates κ receptors (Breslin et al., 1993; Cuello & Paxinos, 1978; Gerfen et al., 1990; Gerfen & Young, 1988; Seizinger et al., 1984).

PD and LID are both associated with alterations in mRNA and peptide levels of the opioid peptide precursors preproenkephalin-A (PPE-A) and preproenkephalin-B (PPE-B) (Fox & Brotchie, 2011; Huot et al., 2013). Although opioid drugs have not translated into therapies for PD nor LID, further study of the opioid system in PD and LID may reveal novel drug candidates and/or therapeutic targets for these disorders. Here, we investigated the potential of two opioid compounds in preclinical rodent models of PD and LID. First, we investigated the mixed δ/μ-activating glycopeptide MMP-2200 (lactomorphin) in behavioral paradigms related to LID (**Chapter 3**). Then, in another study, we tested the κ-selective opioid receptor antagonist nor-BNI in LID-prevention and PD functional-restoration paradigms (**Chapter 4**).

In the basal ganglia, regulation of opioid peptides is closely associated with regulation of dopamine transmission (Breslin et al., 1993; Cuello & Paxinos, 1978; Gerfen et al., 1990; Seizinger et al., 1984), however, the role played by altered opioid transmission in PD (compensatory vs. pathological) has not been resolved (Fox et al., 2006; Fox & Brotchie, 2011; Sgroi & Tonini, 2018). In response to DA depletion, striatal PPE-A mRNA and enkephalin peptide levels are upregulated (Cenci et al., 1998; Gerfen et al., 1990; Herrero et al., 1995; Nisbet et al., 1995; Sgroi et al., 2016), while striatal PPE-B mRNA and dynorphin are downregulated (Aubert et al., 2007; Cenci et al., 1998; Gerfen et al., 1990; Sgroi et al., 2016; Tel et al., 2002). Furthermore, PPE-A mRNA precedes the appearance of PD motor deficits in MPTP treated nonhuman primates, which also supports a compensatory role (Aubert et al., 2007). In theory, activation of DORs expressed presynaptically in the GPe by enkephalin would atenuate the hyperactivation of the indirect output pathway in PD. In support of this idea, DOR agonists have shown antiparkinsonian actions in other preclinical studies (Hille et al., 2001; Hudzik et al., 2000).

In contrast, following chronic levodopa therapy, levels of PPE-A remain upregulated both in animal models (Westin et al., 2001) and in patients with LID (Calon et al., 2003), which indicates a persistent adaptive role for enkephalin in LID (Tamim et al., 2010). Levels of PPE-B, on the other hand, are reversed and upregulated following chronic levodopa therapy (Sgroi et al., 2016; Tamim et al., 2010; Westin et al., 2001). Furthermore, positron emission tomography (PET) studies have revealed abnormalities in opioid receptors related to enhanced opioid transmission in the striatum of patients with LID (Piccini et al., 1997), suggesting that increased opioid neurotransmission may be associated with the pathophysiology of LID.

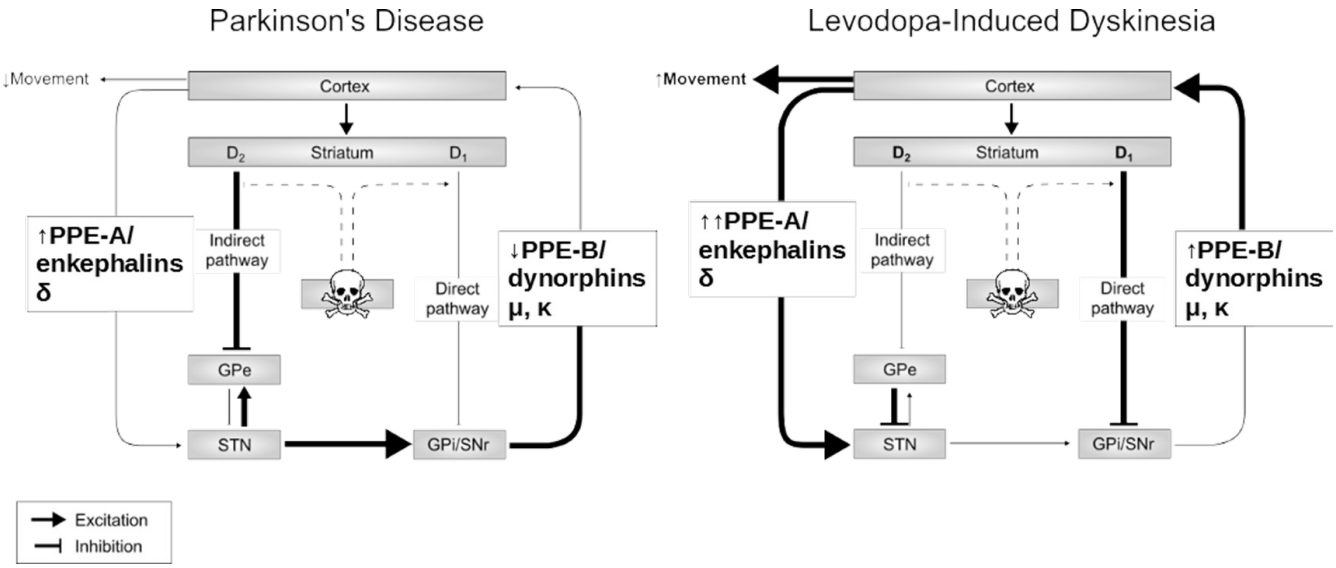

Figure 4. Alterations in the expression of opioid peptides and their precursors are observed in Parkinson's disease and levodopa-induced dyskinesia. In the Parkinson's disease dopamine-depleted state (**left**), PPE-A mRNAs and enkephalin precursors are upregulated, while PPE-B and dynorphin are downregulated. Following chronic levodopa-therapy (**right**), levels of PPE-A are further upregulated, while levels of PPE-B are reversed and upregulated. PPE-A and enkephalins are expressed by striatal neurons and within nuclei of the indirect output pathway, while PPE-B and dynorphin are expressed by striatal neurons and within nuclei in the direct output pathway.

1.3.3 Kappa-opioid neurotransmission in PD and LID

Several lines of evidence suggest that κ-mediated signaling is overactive in LID. In the 6-OHDA rat model of LID, the severity of AIMs correlates strongly with striatal PPE-B mRNA levels (Cenci, Lee, & Björklund, 1998). Another study, showed that striatal PPE-B mRNA levels were increased in both MPTP-lesioned macaques and PD patients with LID (Henry et al., 2003). Other studies have shown that striatal levels of dynorphin are positively correlated with LID (Sgroi, Capper-Loup, Paganetti, & Kaelin-Lang, 2016). Additionally, MALDI-TOF imaging mass spectrometry showed that levels of

dynorphin B and alpha-neoendorphin in the substantia nigra are strongly correlated with the severity of AIMs in the 6-OHDA rat model of LID (Ljungdahl et al., 2011). Furthermore, [^{35}S]GTPγS binding indicated hyperactive κ-mediated signaling in the caudate nucleus and motor cortex in a nonhuman primate model of LID (Chen et al., 2005).

With regard to κ-opioid receptors, Aubert et al. (2007) found that they were unaltered in MPTP-lesioned primates, but that their expression was reduced in the GPe and GPi following chronic L-DOPA treatment. In contrast, KOR expression was decreased in the striatum and substantia nigra, but unaltered in the GP, of 6-OHDA-lesioned rats that expressed AIMs in response to L-DOPA compared with non-dyskinetic animals (Johansson et al., 2001).

Despite this evidence, antagonizing κ receptors with nor-BNI failed to reduce LID in primates (Henry et al., 2001). Furthermore, activation of κ receptors with the selective κ-receptor agonist U50-488 reduced AIMs in the 6-OHDA rat model of LID and dyskinesia in MPTP-lesioned squirrel monkeys (Cox et al., 2007). The current project proposes to evaluate nor-BNI in the 6-OHDA lesion rat model of LID, which to our knowledge, has never been done. Differences in alterations in κ-neurotransmission exist between the 6-OHDA rat and nonhuman primate models of LID; therefore, although nor-BNI failed to reduce LID in nonhuman primates, it may prove to have antidyskinetic efficacy in the 6-OHDA rat model of LID, and the results of this study would fill an important gap in the primary literature.

Kappa-mediated signaling in stress, addiction, and dopamine modulation

KORs play an important role in modulating mood, stress and addiction. It was found that the dysphoric component of stress is encoded by activation of κ-opioid receptors (Land et al., 2008) and that κ-receptor agonists induce dysphoric and aversive states (Xuei et al., 2006) and are potentiators for drug-

seeking behavior (Beardsley et al., 2005; Redila & Chavkin, 2008). It is thought that one component of this dysphoria is a KOR mediated reduction in dopamine levels in the reward circuit, which gives rise to a negative affective state (Chefer et al., 2005; Shippenberg et al., 2006; Spanagel et al., 1994). It is thought that this KOR-dependent reduction in dopamine occurs via the action of dynorphin at presynaptic KORs in the striatum. Because, motor symptoms of PD are caused by depleted levels of striatal DA, we hypothesize that nor-BNI, as a KOR antagonist, could potentially elevate levels of striatal DA, at least transiently, thus restoring motor functionality to 6-OHDA lesioned animals.

KOR agonists were recently evaluated for their therapeutic potential in the treatment of addiction (Hasebe et al., 2004). It is thought that dynorphin, the endogenous ligand for KORs, is a natural addiction control mechanism (Frankel et al., 2008); it has been hypothesized that the dynorphin/ KOR system mediates withdrawal-induced dysphoria and/or stress-induced dysphoria. The KOR activates p38, a mitogen-activated protein kinase (MAPK), through phosphorylation and that activation of p38 is necessary to produce KOR-dependent behaviors (Bruchas et al., 2007).

1.4 The 6-hydroxydopamine rodent model of PD

6-hydroxydopamine (6-OHDA or oxidopamine) was the first neurotoxin used to create an animal model of PD through selective degeneration of nigral dopaminergic neurons projecting to the striatum (Ungerstedt, 1968). It has been used extensively both *in vitro* and *in vivo* to study degeneration of dopaminergic neurons. Models using hemiparkinsonian rodents unilaterally lesioned with 6-OHDA have been instrumental in providing an understanding of the mechanisms of established therapies which reverse PD motor deficits, and continue to be widely used for preclinical studies evaluating the effects novel pharmacological compounds or other experimental therapies.

The 6-OHDA rodent models most commonly rely on the acute induction of unilateral degeneration of nigrostriatal dopaminergic afferents, which in turn give rise to a hemiparkinsonian behavioral syndrome. Pre- and post-synaptic alterations accompanying dopaminergic denervation can then be taken advantage of to induce a distinctive turning behaviors that are quantifiable and highly correlated with the severity of the lesion. Turning is induced using either drugs that induce presynaptic DA release, most commonly amphetamine, or that stimulate DA receptors postsynaptically, such as the non-selective DA receptor agonist apomorphine, or by administration of L-DOPA. The latter approaches rely on the activation of signaling pathways downstream of DA receptors that have developed hypersensitivity of signaling in order to compensate for the loss of dopaminergic input. As a result of the decussation of descending motor control, hemiparkinsonian animals will turn contralaterally to the hemisphere in which DA signaling is stronger; that is, they will turn ipsiversively to the side of the lesion following administration of drugs that stimulate presynaptic DA release and contralaterally following administration of drugs that stimulate DA receptors postsynaptically (e.g., L-DOPA or dopamine agonists) (Hefti et al., 1980; Iancu et al., 2005; Ungerstedt, 1976). Other common behavioral analyses used to measure asymmetric motor impairment include the cylinder test, which is a measure of forelimb preference, or the forelimb-adjusting steps test (FAST), a measure of forelimb akinesia. The unilateral 6-OHDA lesion paradigm has the added advantage of providing an internal control in the unaffected contralateral hemisphere for comparative tissue analysis.

Structurally, 6-OHDA is a dopamine analog whose structure is identical to dopamine with the exception that it possesses an additional aromatic hydroxyl group. Its structural resemblance to DA allows it to be efficiently taken up by neurons expressing either the dopamine reuptake transporters (DAT) or norepinephrine reuptake transporters; therefore, its toxicity is selective for monoaminergic

neurons (Luthman et al., 1989). Selective targeting of dopaminergic neurons is therefore accomplished by co-administration of desipramine, an inhibitor of the norepinephrine reuptake transporter.

The toxicity mechanism of 6-OHDA is thought to be related to oxidative stress, 6-OHDA rapidly reacts with oxygen to form reactive oxygen species (ROS) such as superoxide and hydrogen peroxide. Like DA, 6-OHDA is unable to cross the blood-brain-barrier (BBB); therefore, it must be delivered intracerebrally. This is usually performed stereotaxically using a microinjector to infuse the toxin. To induce selective degeneration of nigrostriatal dopaminergic pathway. requires that that 6-OHDA be delivered directly into nigrostriatal system, targeting either 1) the substantia nigra, 2) the medial forebrain bundle, or into 3) the striatum, where it is taken up into dopaminergic axon terminals. When injected into the striatum, 6-OHDA causes a more progressive degeneration that occurrs over the course of weeks (Przedbroski et al., 1995; Sauer & Oertel, 1994).

Inducing selective degeneration of nigrostriatal dopaminergic pathway requires that 6-OHDA be delivered directly into the nigrostriatal pathway, usually targeting either the medial forebrain bundle (MFB) or the SNpc, or the striatum, where it is taken up into DAergic axons and axon terminals (Dauer & Przedborski, 2003). After administration into the MFB or SN, degeneration occurs rapidly, over the span of hours to days, and leads to a virtually complete degeneration of the nigrostriatal pathway that progresses in an anterograde manner (Jeon et al., 1995). By contrast, when injected into the striatum, 6-OHDA causes partial degeneration that progresses in a retrograde manner over the course of several weeks (Berger et al., 1991; Kirik et al., 1998; Przedbroski et al., 1995; Sauer & Oertel, 1994)but is complete by 4 weeks (Shimohama et al., 2003).

The MFB is a diffuse bundle of axons, consisting of both ascending and descending fibers, that runs between the lateral hypothalamus and the mesencephalon. The dopaminergic neurons in the midbrain of the rat are located in three groups: A8 cells in the retrorubral field, A9 cells in SNpc, and

A10 cells in the VTA (German & Manaye, 1993). In rats, the MFB includes ipsilaterally ascending fibers A10 and A9 dopaminergic neurons (Gerfen, 1992; Marley et al., 1982; Nieuwenhuys et al., 1982; Venero et al., 2002; Yeomans et al., 1988). It has been shown that 6-OHDA toxicity differentially affects A9 and A10 neurons; in particular, A9 neurons in SNpc are much more susceptible to degeneration than A10 neurons following administration of 6-OHDA into either the MFB or the striatum (Grant & Clarke, 2002; Healy-Stoffel et al., 2014; Kirik et al., 1998; Przedbroski et al., 1995). The relative levels of cell loss in the SNpc and VTA depend on a number of parameters including locus of 6-OHDA delivery, number of injection sites, and 6-OHDA dose and infusion volume. For example, intrastriatal 6-OHDA lesions are associated with very minimal cell loss in the VTA, which is non-progressive (Kirik et al., 1998).

1.5 The 6-OHDA model of LID

When administered to rats that have been unilaterally lesioned with 6-OHDA, chronic treatment with L-DOPA causes a gradual development of abnormal involuntary movements (AIMs) that are considered behavioral correlates of clinical LID. AIMs refer to those that affect the forelimb contralateral to the lesion, the trunk (in the form of torsion contralateral to the lesion) and the orofacial musculature (Cenci et al., 1998; Lee et al., 2000; Lundblad et al., 2002; Winkler et al, 2002). AIMs can be quantified on the basis of their topographical distribution, amplitude and duration. Cenci et al. (1998) developed a scoring scale that is commonly used to assess the severity of AIMs in this experimental paradigm that closely reflects the criteria that are used assess LID clinically. L-DOPA-induced AIMs in the 6-OHDA rat model also have the same time course as peak-dose dyskinesia in PD and, like the human disorder, they disrupt physiological motor activities (Lee et al., 2000; Lundblad et al., 2002; Winkler et al., 2002).

The development of L-DOPA-induced AIMs is associated with altered expression of the same molecular correlates of LID in non-human primates. These correlates include striatal upregulation of opioid messenger RNAs (Cenci et al., 1998) and ΔFosB-like transcription factors (Andersson et al., 1999) and changes in opioid-receptor binding within the basal ganglia and related cortical areas (Johansson et al., 2001; Piccini et al., 1997). Drugs with reported antidyskinetic efficacy in Parkinson's disease, such as amantadine also reduce the severity of L-DOPA-induced abnormal involuntary movements in the rat (Lundblad et al., 2002).

1.5.1 Limitations of the 6-OHDA rat model of LID

The appropriateness of utilizing contralateral turning in 6-OHDA lesioned rats as a preclinical correlate of LID has been called into question (Bezard et al., 2001; Blanchet et al., 1998; Chase, 1998; Papa & Chase, 1996). One important criticism of this model is that choreiform and dystonic AIMs of the limb, axial, and orolingual subtypes, which are considered true behavioral correlates of clinically-observed LID, do not correlate with contraversive rotations (i.e., locomotor AIMs). Hypersensitization to turning is thought to be a result of asymmetric DA denervation associated with the unilateral 6-OHDA lesion model, rather than a correlate of clinical LID, although many PD patients do develop the disease asymmetrically. The non-correlation of LAO and locomotor AIMs is further evidenced by the fact that administration of bromocriptine, a selective agonist of D2 receptors, causes pronounced contraversive rotation without induction of LAO AIMs (Lundblad et al., 2002). Furthermore, chronic L-DOPA treatment also induces AIMs in rats with bilateral 6-OHDA lesions that show no propensity for hypersensitization to turning; AIMs develop bilaterally in this paradigm (Lundblad et al., 2002; Winkler et al., 2002).

1.6 References

Albin, R. L., Young, A. B., & Penney, J. B. (1989). The functional anatomy of basal ganglia disorders. *Trends in Neurosciences*, *12*(10), 366–375. https://doi.org/10.1016/0166-2236(89)90074-X

Alexander, G. E., & Crutcher, M. D. (1990). Functional architecture of basal ganglia circuits: neural substrates of parallel processing. In *Trends in Neurosciences* (Vol. 13, Issue 7, pp. 266–271). Elsevier Current Trends. https://doi.org/10.1016/0166-2236(90)90107-L

Alves, G., Forsaa, E. B., Pedersen, K. F., Dreetz Gjerstad, M., & Larsen, J. P. (2008). Epidemiology of Parkinson's disease. *Journal of Neurology*, *255*(S5), 18–32. https://doi.org/10.1007/s00415-008-5004-3

Ambroziak, W., Koziorowski, D., Duszyc, K., Górka-Skoczylas, P., Potulska-Chromik, A., Sławek, J., & Hoffman-Zacharska, D. (2015). Genomic instability in the PARK2 locus is associated with Parkinson's disease. *Journal of Applied Genetics*, *56*(4), 451–461. https://doi.org/10.1007/s13353-015-0282-9

Andén, N.-E., Dahlström, A., Fuxe, K., & Larsson, K. (1965). Further evidence for the presence of nigro-neostriatal dopamine neurons in the rat. *American Journal of Anatomy*, *116*(1), 329–333. https://doi.org/10.1002/aja.1001160117

Andén, N. E., Carlsson, A., Dahlström, A., Fuxe, K., Hillarp, N. Å., & Larsson, K. (1964). Demonstration and mapping out of nigro-neostriatal dopamine neurons. *Life Sciences*, *3*(6), 523–530. https://doi.org/10.1016/0024-3205(64)90161-4

Andersson, M., Hilbertson, A., & Cenci, M. A. (1999). Striatal fosB expression is causally linked with l-DOPA-induced abnormal involuntary movements and the associated upregulation of striatal prodynorphin mRNA in a rat model of Parkinson's disease. *Neurobiology of Disease*, *6*(6), 461–474. https://doi.org/10.1006/nbdi.1999.0259

Attwell, D., & Laughlin, S. B. (2001). An energy budget for signaling in the grey matter of the brain. *Journal of Cerebral Blood Flow and Metabolism : Official Journal of the International Society of Cerebral Blood Flow and Metabolism*, *21*(10), 1133–1145. https://doi.org/10.1097/00004647-200110000-00001

Aubert, I., Guigoni, C., Li, Q., Dovero, S., Bioulac, B. H., Gross, C. E., Crossman, A. R., Bloch, B., & Bezard, E. (2007). Enhanced Preproenkephalin-B-Derived Opioid Transmission in Striatum and Subthalamic Nucleus Converges Upon Globus Pallidus Internalis in L-3,4-dihydroxyphenylalanine-Induced Dyskinesia. *Biological Psychiatry*, *61*(7), 836–844. https://doi.org/10.1016/j.biopsych.2006.06.038

Bandy, B., & Davison, A. J. (1990). Mitochondrial mutations may increase oxidative stress: Implications for carcinogenesis and aging? *Free Radical Biology and Medicine*, *8*(6), 523–539. https://doi.org/10.1016/0891-5849(90)90152-9

Beardsley, P. M., Howard, J. L., Shelton, K. L., & Carroll, F. I. (2005). Differential effects of the novel kappa opioid receptor antagonist, JDTic, on reinstatement of cocaine-seeking induced by footshock stressors vs cocaine primes and its antidepressant-like effects in rats. *Psychopharmacology*, *183*(1), 118–126. https://doi.org/10.1007/s00213-005-0167-4

Bellou, V., Belbasis, L., Tzoulaki, I., Evangelou, E., & Ioannidis, J. P. A. (2016). Environmental risk factors and Parkinson's disease: An umbrella review of meta-analyses. *Parkinsonism & Related Disorders*, *23*, 1–9. http://linkinghub.elsevier.com/retrieve/pii/S1353802015300614

Benabid, A. L., Pollak, P., Hoffmann, D., Gervason, C., Hommel, M., Perret, J. E., de Rougemont, J., & Gao, D. M. (1991). Long-term suppression of tremor by chronic stimulation of the ventral intermediate thalamic nucleus. *The Lancet*, *337*(8738), 403–406. https://doi.org/10.1016/0140-6736(91)91175-T

Benazzouz, A., Gross, C., Féger, J., Boraud, T., & Bioulac, B. (1993). Reversal of Rigidity and Improvement in Motor Performance by Subthalamic High-frequency Stimulation in MPTP-treated Monkeys. *European Journal of Neuroscience*, *5*(4), 382–389. https://doi.org/10.1111/j.1460-9568.1993.tb00505.x

Berg, D., Schweitzer, K. J., Leitner, P., Zimprich, A., Lichtner, P., Belcredi, P., Brüssel, T., Schulte, C., Maass, S., Nägele, T., Wszolek, Z. K., & Gasser, T. (2005). Type and frequency of mutations in the LRRK2 gene in familial and sporadic Parkinson's disease*. *Brain*, *128*(12), 3000–3011. https://doi.org/10.1093/brain/awh666

Berger, K., Przedborski, S., & Cadet, J. L. (1991). Retrograde degeneration of nigrostriatal neurons induced by intrastriatal 6-hydroxydopamine injection in rats. *Brain Research Bulletin*, *26*(2), 301–307. https://doi.org/10.1016/0361-9230(91)90242-C

Bergman, H., Wichmann, T., & DeLong, M. R. (1990). Reversal of experimental parkinsonism by lesions of the subthalamic nucleus. *Science*, *249*(4975), 1436–1438. https://doi.org/10.1126/science.2402638

Betarbet, R. (2000). Chronic systemic pesticide exposure reproduces features of Parkinson's disease. *Nat. Neurosci.*, *3*.

Bezard, E., Brotchie, J. M., & Gross, C. E. (2001). Pathophysiology of levodopa-induced dyskinesia: potential for new therapies. In *Nature Reviews Neuroscience* (Vol. 2, Issue 8, pp. 577–588). Nature Publishing Group. https://doi.org/10.1038/35086062

Birkmayer, W., & Hornykiewicz, O. (1961). [The L-3,4-dioxyphenylalanine (DOPA)-effect in Parkinson-akinesia]. *Wiener Klinische Wochenschrift*, *73*, 787–788. http://www.ncbi.nlm.nih.gov/pubmed/13869404

Blanchet, P. J., Konitsiotis, S., & Chase, T. N. (1998). Amantadine reduces levodopa-induced dyskinesias in parkinsonian monkeys. *Movement Disorders*, *13*(5), 798–802. https://doi.org/10.1002/mds.870130507

Block, M. L., Zecca, L., & Hong, J. S. (2007). Microglia-mediated neurotoxicity: Uncovering the molecular mechanisms. In *Nature Reviews Neuroscience* (Vol. 8, Issue 1, pp. 57–69). Nat Rev Neurosci. https://doi.org/10.1038/nrn2038

Blond, S., & Siegfried, J. (1991). Thalamic stimulation for the treatment of tremor and other movement disorders. *Acta Neurochirurgica. Supplementum*, *52*, 109–111. https://doi.org/10.1007/978-3-7091-9160-6_30

Braak, H., & Del Tredici, K. (2004). Poor and protracted myelination as a contributory factor to neurodegenerative disorders. In *Neurobiology of Aging* (Vol. 25, Issue 1, pp. 19–23). Elsevier Inc. https://doi.org/10.1016/j.neurobiolaging.2003.04.001

Brar, S., Henderson, D., Schenck, J., & Zimmerman, E. A. (2009). Iron accumulation in the substantia nigra of patients with Alzheimer disease and parkinsonism. *Archives of Neurology*, *66*(3), 371–374. https://doi.org/10.1001/archneurol.2008.586

Bratic, I., & Trifunovic, A. (2010). Mitochondrial energy metabolism and ageing. In *Biochimica et Biophysica Acta - Bioenergetics* (Vol. 1797, Issues 6–7, pp. 961–967). https://doi.org/10.1016/j.bbabio.2010.01.004

Breslin, M. B., Lindberg, I., Benjannet, S., Mathis, J. P., Lazure, C., & Seidah, N. G. (1993). Differential processing of proenkephalin by prohormone convertases 1(3) and 2 and furin. *The Journal of Biological Chemistry*, *268*(36), 27084–27093. http://www.ncbi.nlm.nih.gov/pubmed/8262946

Brice, A. (2005). Genetics of Parkinson's disease: LRRK2 on the rise. *Brain*, *128*(12), 2760–2762. https://doi.org/10.1093/brain/awh676

Brotchie, J., Crossman, A., Mitchell, I., Duty, S., Carroll, C., Cooper, A., Henry, B., Hughes, N., & Maneuf, Y. (1993). Chapter 8 Chemical signalling in the globus pallidus in parkinsonism. *Progress in Brain Research*, *99*, 125–139. https://doi.org/10.1016/S0079-6123(08)61342-4

Brown, P., Oliviero, A., Mazzone, P., Insola, A., Tonali, P., & Di Lazzaro, V. (2001). Dopamine dependency of oscillations between subthalamic nucleus and pallidum in Parkinson's disease. *Journal of Neuroscience*, *21*(3), 1033–1038. https://doi.org/10.1523/jneurosci.21-03-01033.2001

Burns, R. S., Chiueh, C. C., Markey, S. P., Ebert, M. H., Jacobowitz, D. M., & Kopin, I. J. (1983). A primate model of parkinsonism: Selective destruction of dopaminergic neurons in the pars compacta of the substantia nigra by N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Proceedings of the National Academy of Sciences of the United States of America*, *80*(14 I), 4546–4550. https://doi.org/10.1073/pnas.80.14.4546

Buzsáki, G., & Draguhn, A. (2004). Neuronal olscillations in cortical networks. In *Science* (Vol. 304, Issue 5679, pp. 1926–1929). American Association for the Advancement of Science. https://doi.org/10.1126/science.1099745

Calne, D. B., & William Langston, J. (1983). AETIOLOGY OF PARKINSON'S DISEASE. *The Lancet*, *322*(8365–8366), 1457–1459. https://doi.org/10.1016/S0140-6736(83)90802-4

Calon, F., Rajput, A. H., Hornykiewicz, O., Bédard, P. J., & Di Paolo, T. (2003). Levodopa-induced motor complications are associated with alterations of glutamate receptors in Parkinson's disease. *Neurobiology of Disease*, *14*(3), 404–416. https://doi.org/10.1016/j.nbd.2003.07.003

Carlsson, A., Lindqvist, M., & Magnusson, T. (1957). 3,4-Dihydroxyphenylalanine and 5-hydroxytryptophan as reserpine antagonists [16]. In *Nature* (Vol. 180, Issue 4596, p. 1200). Nature Publishing Group. https://doi.org/10.1038/1801200a0

Casals, J., Elizan, T. S., & Yahr, M. D. (1998). Postencephalitic parkinsonism - A review. In *Journal of Neural Transmission* (Vol. 105, Issues 6–7, pp. 645–676). https://doi.org/10.1007/s007020050086

Cassidy, M., Mazzone, P., Oliviero, A., Insola, A., Tonali, P., Lazzaro, V. Di, & Brown, P. (2002). Movement-related changes in synchronization in the human basal ganglia. *Brain*, *125*(6), 1235–1246. https://doi.org/10.1093/brain/awf135

Cenci, M. A., Lee, C. S., & Björklund, A. (1998). L-DOPA-induced dyskinesia in the rat is associated with striatal overexpression of prodynorphin- and glutamic acid decarboxylase mRNA. *European Journal of Neuroscience*, *10*(8), 2694–2706. https://doi.org/10.1046/j.1460-9568.1998.00285.x

Cenci, M. Angela, Whishaw, I. Q., & Schallert, T. (2002). Animal models of neurological deficits: How relevant is the rat? *Nature Reviews Neuroscience*, *3*(7), 574–579. https://doi.org/10.1038/nrn877

Chainani-Wu, N. (2003). Safety and anti-inflammatory activity of curcumin: A component of tumeric (Curcuma longa). *Journal of Alternative and Complementary Medicine*, *9*(1), 161–168. https://doi.org/10.1089/107555303321223035

Chan, C. S., Gertler, T. S., & Surmeier, D. J. (2009). Calcium homeostasis, selective vulnerability and Parkinson's disease. In *Trends in Neurosciences*. https://doi.org/10.1016/j.tins.2009.01.006

Charcot, J. M. (1872). Lecons sur les Maladies du Systeme Nerveux. In *Oeuvres completes de J.M. Charcot* (pp. 155–188). Bureaux du Progres Medical.

Chase, T. N. (1998). Levodopa therapy: Consequences of the nonphysiologic replacement of dopamine. *Neurology*, *50*(5 SUPPL. 5), S17–S25. https://doi.org/10.1212/wnl.50.5_suppl_5.s17

Chefer, V. I., Czyzyk, T., Bolan, E. A., Moron, J., Pintar, J. E., & Shippenberg, T. S. (2005). Endogenous κ-Opioid Receptor Systems Regulate Mesoaccumbal Dopamine Dynamics and Vulnerability to Cocaine. *Journal of Neuroscience*, *25*(20).

Clague, M. J., & Urbé, S. (2010). Ubiquitin: Same molecule, different degradation pathways. In *Cell* (Vol. 143, Issue 5, pp. 682–685). Cell Press. https://doi.org/10.1016/j.cell.2010.11.012

Clapham, D. E. (2007). Calcium Signaling. In *Cell* (Vol. 131, Issue 6, pp. 1047–1058). Cell. https://doi.org/10.1016/j.cell.2007.11.028

Clark, I. E., Dodson, M. W., Jiang, C., Cao, J. H., Huh, J. R., Seol, J. H., Yoo, S. J., Hay, B. A., & Guo, M. (2006). Drosophila pink1 is required for mitochondrial function and interacts genetically with parkin. *Nature*, *441*(7097), 1162–1166. https://doi.org/10.1038/nature04779

Collier, T. J., Kanaan, N. M., & Kordower, J. H. (2017). Aging and Parkinson's disease: Different sides of the same coin? In *Movement Disorders* (Vol. 32, Issue 7, pp. 983–990). John Wiley and Sons Inc. https://doi.org/10.1002/mds.27037

Collier, T. J., Lipton, J., Daley, B. F., Palfi, S., Chu, Y., Sortwell, C., Bakay, R. A. E., Sladek, J. R., & Kordower, J. H. (2007). Aging-related changes in the nigrostriatal dopamine system and the response to MPTP in nonhuman primates: Diminished compensatory mechanisms as a prelude to parkinsonism. *Neurobiology of Disease*, *26*(1), 56–65. https://doi.org/10.1016/j.nbd.2006.11.013

Correia Guedes, L., Ferreira, J. J., Rosa, M. M., Coelho, M., Bonifati, V., & Sampaio, C. (2010). Worldwide frequency of G2019S LRRK2 mutation in Parkinson's disease: A systematic review. In *Parkinsonism and Related Disorders* (Vol. 16, Issue 4, pp. 237–242). Parkinsonism Relat Disord. https://doi.org/10.1016/j.parkreldis.2009.11.004

Cotzias, G. C., Papavasiliou, P. S., & Gellene, R. (1969). Modification of Parkinsonism — Chronic Treatment with L-Dopa. *New England Journal of Medicine*, *280*(7), 337–345. https://doi.org/10.1056/NEJM196902132800701

Cotzias, G. C., Van Woert, M. H., & Schiffer, L. M. (1967). Aromatic Amino Acids and Modification of Parkinsonism. *New England Journal of Medicine*, *276*(7), 374–379. https://doi.org/10.1056/NEJM196702162760703

Cuello, A. C., & Paxinos, G. (1978). Evidence for a long Leu-enkephalin striopallidal pathway in rat brain. *Nature*, *271*(5641), 178–180. http://www.ncbi.nlm.nih.gov/pubmed/23498

Cui, G., Jun, S. B., Jin, X., Pham, M. D., Vogel, S. S., Lovinger, D. M., & Costa, R. M. (2013). Concurrent activation of striatal direct and indirect pathways during action initiation. *Nature*, *494*(7436), 238–242. https://doi.org/10.1038/nature11846

Dauer, W., & Przedborski, S. (2003). Parkinson's Disease: Mechanisms and Models. *Neuron*, *39*(6), 889–909. https://doi.org/10.1016/S0896-6273(03)00568-3

Davis, G. C., Williams, A. C., Markey, S. P., Ebert, M. H., Caine, E. D., Reichert, C. M., & Kopin, I. J. (1979). Chronic parkinsonism secondary to intravenous injection of meperidine analogues. *Psychiatry Research*, *1*(3), 249–254. https://doi.org/10.1016/0165-1781(79)90006-4

Day, M., Wang, Z., Ding, J., An, X., Ingham, C. A., Shering, A. F., Wokosin, D., Ilijic, E., Sun, Z., Sampson, A. R., Mugnaini, E., Deutcch, A. Y., Sesack, S. R., Arbuthnott, G. W., & Surmeier, D. J. (2006). Selective elimination of glutamatergic synapses on striatopallidal neurons in Parkinson disease models. *Nature Neuroscience*, *9*(2), 251–259. https://doi.org/10.1038/nn1632

De Coo, I., Renier, W., Ruitenbeek, W., Ter Laak, H., Bakker, M., Schägger, H., Van Oost, B., & Smeets, H. (1999). A 4-base Pair Deletion in the Mitochondrial Cytochrome B Gene Associated With parkinsonism/MELAS Overlap Syndrome. *Annals of Neurology*, *45*(1). https://doi.org/10.1002/1531-8249(199901)45:1<130::AID-ART21>3.3.CO;2-Q

De Hemptinne, C., Swann, N. C., Ostrem, J. L., Ryapolova-Webb, E. S., San Luciano, M., Galifianakis, N. B., & Starr, P. A. (2015). Therapeutic deep brain stimulation reduces cortical phase-amplitude coupling in Parkinson's disease. *Nature Neuroscience*, *18*(5), 779–786. https://doi.org/10.1038/nn.3997

de la Fuente-Fernandez, R., Schulzer, M., Mak, E., Calne, D. B., & Stoessl, A. J. (2004). Presynaptic mechanisms of motor fluctuations in Parkinson's disease: a probabilistic model. *Brain*, *127*(4), 888–899. https://doi.org/10.1093/brain/awh102

de Lau, L. M., & Breteler, M. M. (2006). Epidemiology of Parkinson's disease. In *Lancet Neurology* (Vol. 5, Issue 6, pp. 525–535). Elsevier. https://doi.org/10.1016/S1474-4422(06)70471-9

Deas, E., Wood, N. W., & Plun-Favreau, H. (2011). Mitophagy and Parkinson's disease: The PINK1-parkin link. In *Biochimica et Biophysica Acta - Molecular Cell Research*. https://doi.org/10.1016/j.bbamcr.2010.08.007

DeLong, M. R. (1990). Primate models of movement disorders of basal ganglia origin. In *Trends in Neurosciences* (Vol. 13, Issue 7, pp. 281–285). Elsevier Current Trends. https://doi.org/10.1016/0166-2236(90)90110-V

DeMaagd, G., & Philip, A. (2015). Parkinson's disease and its management part 1: Disease entity, risk factors, pathophysiology, clinical presentation, and diagnosis. *P and T*, *40*(8), 504–532.

Di Monte, D. A., McCormack, A., Petzinger, G., Janson, A. M., Quik, M., & Langston, W. J. (2000). Relationship among nigrostriatal denervation, parkinsonism, and dyskinesias in the MPTP primate model. *Movement Disorders*, *15*(3), 459–466. https://doi.org/10.1002/1531-8257(200005)15:3<459::AID-MDS1006>3.0.CO;2-3

Diederich, N. J., Moore, C. G., Leurgans, S. E., Chmura, T. A., & Goetz, C. G. (2003). Parkinson disease with old-age onset: A comparative study with subjects with middle-age onset. *Archives of Neurology*, *60*(4), 529–533. https://doi.org/10.1001/archneur.60.4.529

Donatus, I. A., Sardjoko, & Vermeulen, N. P. E. (1990). Cytotoxic and cytoprotective activities of curcumin. Effects on paracetamol-induced cytotoxicity, lipid peroxidation and glutathione depletion in rat hepatocytes. *Biochemical Pharmacology*, *39*(12), 1869–1875. https://doi.org/10.1016/0006-2952(90)90603-I

Ebadi, M., Srinivasan, S. K., & Baxi, M. D. (1996). Oxidative stress and antioxidant therapy in Parkinson's disease. In *Progress in Neurobiology* (Vol. 48, Issue 1, pp. 1–19). Elsevier Ltd. https://doi.org/10.1016/0301-0082(95)00029-1

Ehringer, H., & Hornykiewicz, O. (1960). [Distribution of noradrenaline and dopamine (3-hydroxytyramine) in the human brain and their behavior in diseases of the extrapyramidal system]. *Klinische Wochenschrift*, *38*, 1236–1239. https://doi.org/10.1007/bf01485901

Fahn, S., Jankovic, J., & Hallett, M. (2011). Principles and Practice of Movement Disorders: Second Edition. In *Principles and Practice of Movement Disorders: Second Edition*. Elsevier Inc. https://doi.org/10.1016/C2009-0-44357-5

Faucheux, B. A., Martin, M. E., Beaumont, C., Hauw, J. J., Agid, Y., & Hirsch, E. C. (2003). Neuromelanin associated redox-active iron is increased in the substantia nigra of patients with Parkinson's disease. *Journal of Neurochemistry*, *86*(5), 1142–1148. https://doi.org/10.1046/j.1471-4159.2003.01923.x

Fearnley, J. M., & Lees, A. J. (1991). Ageing and Parkinson's disease: Substantia nigra regional selectivity. *Brain*, *114*(5), 2283–2301. https://doi.org/10.1093/brain/114.5.2283

Financial Burden of Parkinson Disease Is $52 Billion, More Than Double Previous Estimates. (n.d.). Retrieved May 10, 2020, from https://www.ajmc.com/newsroom/the-financial-burden-of-parkinson-disease-is-52-billion-more-than-double-previous-estimates-

Forno, L. S. (1996). Neuropathology of Parkinson's Disease. *Journal of Neuropathology and Experimental Neurology*, *55*(3), 259–272. https://doi.org/10.1097/00005072-199603000-00001

Fox, S. H., & Brotchie, J. M. (2011). Non-Dopaminergic Approaches to the Treatment of Parkinson's Disease. In W. C. Olanow, F. Stocchi, & A. E. Lang (Eds.), *Parkinson's Disease - Non-Motor and Non-Dopaminergic Features* (pp. 443–444). Wiley-Blackwell.

Fox, S. H., Brotchie, J. M., & Lang, A. E. (2008). Non-dopaminergic treatments in development for Parkinson's disease. In *The Lancet Neurology* (Vol. 7, Issue 10, pp. 927–938). https://doi.org/10.1016/S1474-4422(08)70214-X

Fox, S. H., & Lang, A. E. (2008). Levodopa-related motor complications-Phenomenology. *Movement Disorders*, *23*(S3), S509–S514. https://doi.org/10.1002/mds.22021

Fox, S. H., Lang, A. E., & Brotchie, J. M. (2006). Translation of nondopaminergic treatments for levodopa-induced dyskinesia from MPTP-lesioned nonhuman primates to phase IIa clinical studies: Keys to success and roads to failure. *Movement Disorders*, *21*(10), 1578–1594. https://doi.org/10.1002/mds.20936

Frankel, P. S., Alburges, M. E., Bush, L., Hanson, G. R., & Kish, S. J. (2008). Striatal and ventral pallidum dynorphin concentrations are markedly increased in human chronic cocaine users. *Neuropharmacology*, *55*(1), 41–46. https://doi.org/10.1016/j.neuropharm.2008.04.019

Gardner, J. (2013). A history of deep brain stimulation: Technological innovation and the role of clinical assessment tools. *Social Studies of Science*, *43*(5), 707–728. https://doi.org/10.1177/0306312713483678

Gems, D., & Partridge, L. (2013). Genetics of Longevity in Model Organisms: Debates and Paradigm Shifts. *Annual Review of Physiology*, *75*(1), 621–644. https://doi.org/10.1146/annurev-physiol-030212-183712

Gerfen, C. R., Baimbridge, K. G., & Miller, J. J. (1985). The neostriatal mosaic: Compartmental distribution of calcium-binding protein and parvalbumin in the basal ganglia of the rat and monkey. *Proceedings of the National Academy of Sciences of the United States of America*, *82*(24), 8780–8784. https://doi.org/10.1073/pnas.82.24.8780

Gerfen, C R. (1992). The neostriatal mosaic: multiple levels of compartmental organization. *Trends in Neurosciences*, *15*(4), 133–139. http://www.ncbi.nlm.nih.gov/pubmed/1374971

Gerfen, C R, Engber, T. M., Mahan, L. C., Susel, Z., Chase, T. N., Monsma, F. J., & Sibley, D. R. (1990). D1 and D2 dopamine receptor regulated gene expression of striatonigral and striatopallidal neurons. *Science*, *250*(1986), 1429–1432. https://doi.org/10.1126/science.2147780

Gerfen, Charles R. (1984). The neostriatal mosaic: Compartmentalization of corticostriatal input and striatonigral output systems. *Nature*, *311*(5985), 461–464. https://doi.org/10.1038/311461a0

Gerfen, Charles R., & Scott Young, W. (1988). Distribution of striatonigral and striatopallidal peptidergic neurons in both patch and matrix compartments: an in situ hybridization histochemistry and fluorescent retrograde tracing study. *Brain Research*, *460*(1), 161–167. https://doi.org/10.1016/0006-8993(88)91217-6

Gerfen, Charles R, Herkenham, M., & Thibault, J. (1987). The Neostriatal Mosaic: II. Patch-and Matrix-Directed Mesostriatal Dopaminergic and Non-Dopaminergic Systems. In *The Journal of Neuroscience* (Vol. 7, Issue 12).

German, D. C., & Manaye, K. F. (1993). Midbrain dopaminergic neurons (nuclei A8, A9, and A10): Three-dimensional reconstruction in the rat. *Journal of Comparative Neurology*, *331*(3), 297–309. https://doi.org/10.1002/cne.903310302

Goetz, C. G., Poewe, W., Rascol, O., & Sampaio, C. (2005). Evidence-based medical review update: Pharmacological and surgical treatments of Parkinson's disease: 2001 to 2004. In *Movement Disorders* (Vol. 20, Issue 5, pp. 523–539). Mov Disord. https://doi.org/10.1002/mds.20464

Goldman, J. G., & Postuma, R. (2014). Premotor and nonmotor features of Parkinson's disease. *Current Opinion in Neurology*, *27*(4), 434–441. https://doi.org/10.1097/WCO.0000000000000112

Goldstein, M., Anagnoste, B., Owen, W. S., & Battista, A. F. (1966). The effects of ventromedial tegmental lesions on the biosynthesis of catecholamines in the striatum. *Life Sciences*, *5*(23), 2171–2176. https://doi.org/10.1016/0024-3205(66)90078-6

Gomez-Mancilla, B., & Bédard, P. J. (1993). Effect of nondopaminergic drugs on L-dopa-induced dyskinesias in MPTP-treated monkeys. *Clinical Neuropharmacology*, *16*(5), 418–427. http://www.ncbi.nlm.nih.gov/pubmed/8106150

Gong, S., Zheng, C., Doughty, M. L., Losos, K., Didkovsky, N., Schambra, U. B., Nowak, N. J., Joyner, A., Leblanc, G., Hatten, M. E., & Heintz, N. (2003). A gene expression atlas of the central nervous system based on bacterial artificial chromosomes. *Nature*, *425*(6961), 917–925. https://doi.org/10.1038/nature02033

Gradinaru, V., Mogri, M., Thompson, K. R., Henderson, J. M., & Deisseroth, K. (2009). Optical deconstruction of parkinsonian neural circuitry. *Science*, *324*(5925), 354–359. https://doi.org/10.1126/science.1167093

Grant, R. J., & Clarke, P. B. S. (2002). Susceptibility of ascending dopamine projections to 6-hydroxydopamine in rats: Effect of hypothermia. *Neuroscience*, *115*(4), 1281–1294. https://doi.org/10.1016/S0306-4522(02)00385-8

Graybiel, A. M., & Ragsdale, C. W. (1978). Histochemically distinct compartments in the striatum of human, monkey, and cat demonstrated by acetylthiocholinesterase staining. *Proceedings of the National Academy of Sciences of the United States of America*, *75*(11), 5723–5726. https://doi.org/10.1073/pnas.75.11.5723

Graybiel, Ann M., Canales, J. J., & Capper-Loup, C. (2000). Levodopa-induced dyskinesias and dopamine-dependent stereotypies: A new hypothesis. In *Trends in Neurosciences* (Vol. 23, Issue 10 SUPPL., pp. S71–S77). Elsevier Ltd. https://doi.org/10.1016/S1471-1931(00)00027-6

Greene, J. C., Whitworth, A. J., Kuo, I., Andrews, L. A., Feany, M. B., & Pallanck, L. J. (2003). Mitochondrial pathology and apoptotic muscle degeneration in Drosophila parkin mutants. *Proceedings of the National Academy of Sciences of the United States of America*, *100*(7), 4078–4083. https://doi.org/10.1073/pnas.0737556100

Greenfield, J. G., & Bosanquet, F. D. (1953). The brain-stem lesions in Parkinsonism. *Journal of Neurology, Neurosurgery, and Psychiatry*, *16*(4), 213–226. https://doi.org/10.1136/jnnp.16.4.213

Guzman, J. N., Sánchez-Padilla, J., Chan, C. S., & Surmeier, D. J. (2009). Robust pacemaking in substantia nigra dopaminergic neurons. *Journal of Neuroscience*, *29*(35), 11011–11019. https://doi.org/10.1523/JNEUROSCI.2519-09.2009

Hallman, H., Olson, L., & Jonsson, G. (1984). Neurotoxicity of the meperidine analogue N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine on brain catecholamine neurons in the mouse. *European Journal of Pharmacology*, *97*(1–2), 133–136. https://doi.org/10.1016/0014-2999(84)90521-1

Hammond, C., Ammari, R., Bioulac, B., & Garcia, L. (2008). Latest view on the mechanism of action of deep brain stimulation. *Movement Disorders*, *23*(15), 2111–2121. https://doi.org/10.1002/mds.22120

Hammond, C., Bergman, H., & Brown, P. (2007). Pathological synchronization in Parkinson's disease: networks, models and treatments. In *Trends in Neurosciences* (Vol. 30, Issue 7, pp. 357–364). Elsevier. https://doi.org/10.1016/j.tins.2007.05.004

Harris, J. J., Jolivet, R., & Attwell, D. (2012). Synaptic Energy Use and Supply. In *Neuron* (Vol. 75, Issue 5, pp. 762–777). Cell Press. https://doi.org/10.1016/j.neuron.2012.08.019

Healy-Stoffel, M., Ahmad, S. O., Stanford, J. A., & Levant, B. (2014). Differential effects of intrastriatal 6-hydroxydopamine on cell number and morphology in midbrain dopaminergic subregions of the rat. *Brain Research*, *1574*, 113–119. https://doi.org/10.1016/j.brainres.2014.05.045

Hefti, F., Melamed, E., Sahakian, B. J., & Wurtman, R. J. (1980). Circling behavior in rats with partial, unilateral nigro-striatal lesions: Effect of amphetamine, apomorphine, and DOPA. *Pharmacology, Biochemistry and Behavior*, *12*(2), 185–188. https://doi.org/10.1016/0091-3057(80)90353-6

Heikkila, R. E., Hess, A., & Duvoisin, R. C. (1984). Dopaminergic neurotoxicity of 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine in mice. *Science*, *224*(4656), 1451–1453. https://doi.org/10.1126/science.6610213

Hely, M. A., Morris, J. G. L., Reid, W. G. J., O'Sullivan, D. J., Williamson, P. M., Broe, G. A., & Adena, M. A. (1995). Age at onset: the major determinant of outcome in Parkinson's disease. *Acta Neurologica Scandinavica*, *92*(6), 455–463. https://doi.org/10.1111/j.1600-0404.1995.tb00480.x

Herkenham, M., & Pert, C. B. (1981). Mosaic distribution of opiate receptors, parafascicular projections and acetylcholinesterase in rat striatum. *Nature*, *291*(5814), 415–418. https://doi.org/10.1038/291415a0

Hernan, M. A., Takkouche, B., Caamano-Isorna, F., & Gestal-Otero, J. J. (2002). A meta-analysis of coffee drinking, cigarette smoking, and the risk of Parkinson's disease. *Ann. Neurol.*, *52*.

Herrero, M. T., Augood, S. J., Hirsch, E. C., Javoy-Agid, F., Luquin, M. R., Agid, Y., Obeso, J. A., & Emson, P. C. (1995). Effects of l-DOPA on preproenkephalin and preprotachykinin gene expression in the MPTP-treated monkey striatum. *Neuroscience*, *68*(4), 1189–1198. https://doi.org/10.1016/0306-4522(95)00120-8

Hille, C. J., Fox, S. H., Maneuf, Y. P., Crossman, A. R., & Brotchie, J. M. (2001). Antiparkinsonian action of a delta opioid agonist in rodent and primate models of Parkinson's disease. *Experimental Neurology*, *172*(1), 189–198. https://doi.org/10.1006/exnr.2001.7763

Hirsch, E., Graybiel, A. M., & Agid, Y. A. (1988). Melanized dopaminergic neurons are differentially susceptible to degeneration in Parkinson's disease. *Nature*, *334*(6180), 345–348. https://doi.org/10.1038/334345a0

Hokfelt, T., & Ungerstedt, U. (1973). Specificity of 6-hydroxydopamine induced degeneration of central monoamine neurones: an electron and fluorescence microscopic study with special reference to intracerebral injection on the nigro-striatal dopamine system. *Brain Res.*, *60*.

Hudzik, T. J., Howell, A., Payza, K., & Cross, A. J. (2000). Antiparkinson potential of delta-opioid receptor agonists. *European Journal of Pharmacology*, *396*(2–3), 101–107. http://www.ncbi.nlm.nih.gov/pubmed/10822062

Huot, P., Johnston, T. H., Koprich, J. B., Fox, S. H., & Brotchie, J. M. (2013). The Pharmacology of l-DOPA-Induced Dyskinesia in Parkinson's Disease. *Pharmacological Reviews*, *65*(1).

Iancu, R., Mohapel, P., Brundin, P., & Paul, G. (2005). Behavioral characterization of a unilateral 6-OHDA-lesion model of Parkinson's disease in mice. *Behavioural Brain Research*, *162*(1), 1–10. https://doi.org/10.1016/j.bbr.2005.02.023

Irwin, I., DeLanney, L. E., McNeill, T., Chan, P., Forno, L. S., Murphy, G. M., Di Monte, D. A., Sandy, M. S., & Langston, J. W. (1994). Aging and the nigrostriatal dopamine system: a non-human primate study. *Neurodegeneration : A Journal for Neurodegenerative Disorders, Neuroprotection, and Neuroregeneration*, *3*(4), 251–265.

Jankovic, J., & Aguilar, L. G. (2008). Current approaches to the treatment of Parkinson's disease. *Neuropsychiatric Disease and Treatment*, *4*(4), 743–757. http://www.ncbi.nlm.nih.gov/pubmed/19043519

Jankovic, J., & Kapadia, A. S. (2001). Functional decline in Parkinson disease. *Archives of Neurology*, *58*(10), 1611–1615. https://doi.org/10.1001/archneur.58.10.1611

Jenner, P., Hunot, Olanow, Beal, Kordower, Tatton, & Schapira. (2003). Oxidative stress in Parkinson's disease. In *Annals of Neurology* (Vol. 53, Issue SUPPL. 3, pp. S26–S36). https://doi.org/10.1002/ana.10483

Jenner, P., & Olanow, C. W. (2006). The pathogenesis of cell death in Parkinson's disease. In *Neurology* (Vol. 66, Issue 10 SUPPL. 4). Lippincott Williams and Wilkins. https://doi.org/10.1212/wnl.66.10_suppl_4.s24

Jeon, B. S., Jackson-Lewis, V., & Burke, R. E. (1995). 6-Hydroxydopamine Lesion of the Rat Substantia Nigra: Time Course and Morphology of Cell Death. *Neurodegeneration*, *4*(2), 131–137. https://doi.org/10.1006/neur.1995.0016

Johansson, P. A., Andersson, M., Andersson, K. E., & Cenci, M. A. (2001). Alterations in Cortical and Basal Ganglia Levels of Opioid Receptor Binding in a Rat Model of l-DOPA-Induced Dyskinesia. *Neurobiology of Disease*, *8*(2), 220–239. https://doi.org/10.1006/nbdi.2000.0372

Kelloff, G. J., Crowell, J. A., Hawk, E. T., Steele, V. E., Lubet, R. A., Boone, C. W., Covey, J. M., Doody, L. A., Omenn, G. S., Greenwald, P., Hong, W. K., Parkinson, D. R., Bagheri, D., Baxter, G. T., Blunden, M., Doeltz, M. K., Eisenhauer, K. M., Johnson, K., Knapp, G. G., … Sigman, C. C. (1996). Strategy and planning for chemopreventive drug development: Clinical development plans II. *Journal of Cellular Biochemistry*, *63*(S26), 54–71. https://doi.org/10.1002/jcb.240630705

Kirik, D., Rosenblad, C., & Björklund, A. (1998). Characterization of behavioral and neurodegenerative changes following partial lesions of the nigrostriatal dopamine system induced by intrastriatal 6-hydroxydopamine in the rat. *Experimental Neurology*, *152*(2), 259–277. https://doi.org/10.1006/exnr.1998.6848

Kirkwood, T. B. L. (2005). Understanding the odd science of aging. In *Cell* (Vol. 120, Issue 4, pp. 437–447). Cell Press. https://doi.org/10.1016/j.cell.2005.01.027

Kitada, T., Asakawa, S., Hattori, N., Matsumine, H., Yamamura, Y., Minoshima, S., Yokochi, M., Mizuno, Y., & Shimizu, N. (1998). Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature*, *392*(6676), 605–608. https://doi.org/10.1038/33416

Kravitz, A. V. (2010). Regulation of parkinsonian motor behaviours by optogenetic control of basal ganglia circuitry. *Nature*, *466*.

Kreitzer, A. C., & Malenka, R. C. (2007). Endocannabinoid-mediated rescue of striatal LTD and motor deficits in Parkinson's disease models. *Nature*, *445*(7128), 643–647. https://doi.org/10.1038/nature05506

Kühn, A. A., Trottenberg, T., Kivi, A., Kupsch, A., Schneider, G. H., & Brown, P. (2005). The relationship between local field potential and neuronal discharge in the subthalamic nucleus of patients with Parkinson's disease. *Experimental Neurology*, *194*(1), 212–220. https://doi.org/10.1016/j.expneurol.2005.02.010

Laitinen, L. V., Bergenheim, A. T., & Hariz, M. I. (1992). Leksell's posteroventral pallidotomy in the treatment of Parkinson's disease. *Journal of Neurosurgery*, *76*(1), 53–61. https://doi.org/10.3171/jns.1992.76.1.0053

Land, B. B., Bruchas, M. R., Lemos, J. C., Xu, M., Melief, E. J., & Chavkin, C. (2008). The Dysphoric Component of Stress Is Encoded by Activation of the Dynorphin κ-Opioid System. *Journal of Neuroscience*, *28*(2).

Langston, J., Ballard, P., Tetrud, J., & Irwin, I. (1983). Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. *Science*, *219*(4587), 979–980. https://doi.org/10.1126/science.6823561

Lee, C. S., Cenci, M. A., Schulzer, M., & Björklund, A. (2000). Embryonic ventral mesencephalic grafts improve levodopa-induced dyskinesia in a rat model of Parkinson's disease. *Brain : A Journal of Neurology*, *123 (Pt 7*, 1365–1379. http://www.ncbi.nlm.nih.gov/pubmed/10869049

Lees, A. J., Hardy, J., & Revesz, T. (2009). Parkinson's disease. *Lancet (London, England)*, *373*(9680), 2055–2066. https://doi.org/10.1016/S0140-6736(09)60492-X [doi]

Levy, R., Ashby, P., Hutchison, W. D., Lang, A. E., Lozano, A. M., & Dostrovsky, J. O. (2002). Dependence of subthalamic nucleus oscillations on movement and dopamine in Parkinson's disease. *Brain*, *125*(6), 1196–1209. https://doi.org/10.1093/brain/awf128

Lewy, F. H. (1914). Zur pathologischen Anatomie der Paralysis agitans. *Dtsch Ztschr Nervenheilkunde*, *50*, 50–55.

Limousin, P., Pollak, P., Benazzouz, A., Hoffmann, D., Le Bas, J.-F., Perret, J. E., Benabid, A.-L., & Broussolle, E. (1995). Effect on parkinsonian signs and symptoms of bilateral subthalamic nucleus stimulation. *The Lancet*, *345*(8942), 91–95. https://doi.org/10.1016/S0140-6736(95)90062-4

Limousin, Patricia, & Martinez-Torres, I. (2008). Deep Brain Stimulation for Parkinson's Disease. *Neurotherapeutics*, *5*(2), 309–319. https://doi.org/10.1016/j.nurt.2008.01.006

López-Otín, C., Blasco, M. A., Partridge, L., Serrano, M., & Kroemer, G. (2013). The hallmarks of aging. In *Cell* (Vol. 153, Issue 6, p. 1194). Cell Press. https://doi.org/10.1016/j.cell.2013.05.039

Lotharius, J., & Brundin, P. (2002). Pathogenesis of parkinson's disease: Dopamine, vesicles and α-synuclein. *Nature Reviews Neuroscience*, *3*(12), 932–942. https://doi.org/10.1038/nrn983

Lundblad, M., Andersson, M., Winkler, C., Kirik, D., Wierup, N., Cenci Nilsson, M. A., & Cenci, M. A. (2002). Pharmacological validation of behavioural measures of akinesia and dyskinesia in a rat model of Parkinson's disease. *The European Journal of Neuroscience*, *15*(1), 120–132. https://doi.org/10.1046/j.0953-816x.2001.01843.x

Luthman, J., Fredriksson, A., Sundström, E., Jonsson, G., & Archer, T. (1989). Selective lesion of central dopamine or noradrenaline neuron systems in the neonatal rat: motor behavior and monoamine alterations at adult stage. *Behavioural Brain Research*, *33*(3), 267–277. https://doi.org/10.1016/S0166-4328(89)80121-4

Madabhushi, R., Pan, L., & Tsai, L. H. (2014). DNA damage and its links to neurodegeneration. In *Neuron* (Vol. 83, Issue 2, pp. 266–282). Cell Press. https://doi.org/10.1016/j.neuron.2014.06.034

Mann, V. M., Cooper, J. M., Krige, D., Daniel, S. E., Schapira, A. H. V, & Marsden, C. D. (1992). Brain, skeletal muscle and platelet homogenate mitochondrial function in Parkinonson's disease. *Brain*, *115*(2), 333–342. https://doi.org/10.1093/brain/115.2.333

Marley, P. D., Emson, P. C., & Rehfeld, J. F. (1982). Effect of 6-hydroxydopamine lesions of the medial forebrain bundle on the distribution of cholecystokinin in rat forebrain. *Brain Research*, *252*(2), 382–385. https://doi.org/10.1016/0006-8993(82)90407-3

Marras, C., Beck, J. C., Bower, J. H., Roberts, E., Ritz, B., Ross, G. W., Abbott, R. D., Savica, R., Van Den Eeden, S. K., Willis, A. W., & Tanner, C. (2018). Prevalence of Parkinson's disease across North America. *Npj Parkinson's Disease*, *4*(1), 1–7. https://doi.org/10.1038/s41531-018-0058-0

Marsden, C. D. (1983). Neuromelanin and Parkinson's disease. *J. Neural Transm. Suppl.*, *19*.

Martin, L. J. (2008). DNA damage and repair: Relevance to mechanisms of neurodegeneration. In *Journal of Neuropathology and Experimental Neurology* (Vol. 67, Issue 5, pp. 377–387). https://doi.org/10.1097/NEN.0b013e31816ff780

Martínez-Rumayor, A., Arrieta, O., Sotelo, J., & García, E. (2009). Female gender but not cigarette smoking delays the onset of Parkinson's disease. *Clinical Neurology and Neurosurgery*, *111*(9), 738–741. https://doi.org/10.1016/j.clineuro.2009.07.012

Matsuda, W., Furuta, T., Nakamura, K. C., Hioki, H., Fujiyama, F., Arai, R., & Kaneko, T. (2009). Single nigrostriatal dopaminergic neurons form widely spread and highly dense axonal arborizations in the neostriatum. *Journal of Neuroscience*, *29*(2), 444–453. https://doi.org/10.1523/JNEUROSCI.4029-08.2009

Miller, I. N., & Cronin-Golomb, A. (2010). Gender differences in Parkinson's disease: Clinical characteristics and cognition. In *Movement Disorders* (Vol. 25, Issue 16, pp. 2695–2703). https://doi.org/10.1002/mds.23388

Müller-Nedebock, A. C., Brennan, R. R., Venter, M., Pienaar, I. S., van der Westhuizen, F. H., Elson, J. L., Ross, O. A., & Bardien, S. (2019). The unresolved role of mitochondrial DNA in Parkinson's disease: An overview of published studies, their limitations, and future prospects. In *Neurochemistry International* (Vol. 129). Elsevier Ltd. https://doi.org/10.1016/j.neuint.2019.104495

Nambu, A. (2004). A new dynamic model of the cortico-basal ganglia loop. *Progress in Brain Research*, *143*, 461–466. https://doi.org/10.1016/S0079-6123(03)43043-4

Narendra, D. P. (2010). PINK1 is selectively stabilized on impaired mitochondria to activate Parkin. *PLoS Biol.*, *8*.

Nieuwenhuys, R., Geeraedts, L. M. G., & Veening, J. G. (1982). The medial forebrain bundle of the rat. I. General introduction. *Journal of Comparative Neurology*, *206*(1), 49–81. https://doi.org/10.1002/cne.902060106

Nisbet, A. P., Foster, O. J., Kingsbury, A., Eve, D. J., Daniel, S. E., Marsden, C. D., & Lees, A. J. (1995). Preproenkephalin and preprotachykinin messenger RNA expression in normal human basal ganglia and in Parkinson's disease. *Neuroscience*, *66*(2), 361–376. http://www.ncbi.nlm.nih.gov/pubmed/7477878

Nussbaum, R. L., & Ellis, C. E. (2003). Alzheimer's Disease and Parkinson's Disease. *New England Journal of Medicine*, *348*(14), 1356–1364. https://doi.org/10.1056/NEJM2003ra020003

Nutt, J. G., Obeso, J. A., & Stocchi, F. (2000). Continuous dopamine-receptor stimulation in advanced Parkinson's disease. In *Trends in Neurosciences* (Vol. 23, Issue 10 SUPPL., pp. S109–S115). Elsevier Ltd. https://doi.org/10.1016/S1471-1931(00)00029-X

Obeso, J A, Rodriguez-Oroz, M. C., Rodriguez, M., DeLong, M. R., & Olanow, C. W. (2000). Pathophysiology of levodopa-induced dyskinesias in Parkinson's disease: problems with the current model. *Annals of Neurology*, *47*(4 Suppl 1), S22-32; discussion S32-4. http://www.ncbi.nlm.nih.gov/pubmed/10762129

Obeso, José A., Olanow, C. W., & Nutt, J. G. (2000). Levodopa motor complications in Parkinson's disease. *Trends in Neurosciences*, *23*, S2–S7. https://doi.org/10.1016/S1471-1931(00)00031-8

Obeso, Jose A., Rodríguez-Oroz, M. C., Benitez-Temino, B., Blesa, F. J., Guridi, J., Marin, C., & Rodriguez, M. (2008). Functional organization of the basal ganglia: Therapeutic implications for Parkinson's disease. *Movement Disorders*, *23*(SUPPL. 3). https://doi.org/10.1002/mds.22062

Obeso, José A., Rodriguez-Oroz, M. C., Rodriguez, M., Lanciego, J. L., Artieda, J., Gonzalo, N., & Olanow, C. W. (2000). Pathophysiology of the basal ganglia in Parkinson's disease. *Trends in Neurosciences*, *23*, S8–S19. https://doi.org/10.1016/S1471-1931(00)00028-8

Olanow, C. W., Obeso, J. A., & Stocchi, F. (2006). Continuous dopamine-receptor treatment of Parkinson's disease: scientific rationale and clinical implications. *The Lancet Neurology*, *5*(8), 677–687. https://doi.org/10.1016/S1474-4422(06)70521-X

Pahwa, R., Factor, S. A., Lyons, K. E., Ondo, W. G., Gronseth, G., Bronte-Stewart, H., Hallett, M., Miyasaki, J., Stevens, J., Weiner, W. J., & Quality Standards Subcommittee of the American Academy of Neurology. (2006). Practice Parameter: Treatment of Parkinson disease with motor fluctuations and dyskinesia (an evidence-based review): [RETIRED]. *Neurology*, *66*(7), 983–995. https://doi.org/10.1212/01.wnl.0000215250.82576.87

Papa, S. M., & Chase, T. N. (1996). Levodopa-induced dyskinesias improved by a glutamate antagonist in Parkinsonian monkeys. *Annals of Neurology*, *39*(5), 574–578. https://doi.org/10.1002/ana.410390505

Papavasiliou, P. S., Cotzias, G. C., Düby, S. E., Steck, A. J., Fehling, C., & Bell, M. A. (1972). Levodopa in Parkinsonism: Potentiation of Central Effects with a Peripheral Inhibitor. *New England Journal of Medicine*, *286*(1), 8–14. https://doi.org/10.1056/NEJM197201062860102

Paquette, M. A., Anderson, A. M., Lewis, J. R., Meshul, C. K., Johnson, S. W., & Paul Berger, S. (2010). MK-801 inhibits l-DOPA-induced abnormal involuntary movements only at doses that worsen parkinsonism. *Neuropharmacology*, *58*(7), 1002–1008. https://doi.org/10.1016/j.neuropharm.2010.01.003

Park, J. (2006). Mitochondrial dysfunction in Drosophila PINK1 mutants is complemented by parkin. *Nature*, *441*.

Parkinson, J. (1817). *An Essay on the Shaking Palsy*. Wittingham and Rowland.

Pellerin, L., & Magistretti, P. J. (1994). Glutamate uptake into astrocytes stimulates aerobic glycolysis: A mechanism coupling neuronal activity to glucose utilization. *Proceedings of the National Academy of Sciences of the United States of America*, *91*(22), 10625–10629. https://doi.org/10.1073/pnas.91.22.10625

Piccini, P., Weeks, R. A., & Brooks, D. J. (1997a). Alterations in opioid receptor binding in Parkinson's disease patients with levodopa-induced dyskinesias. *Annals of Neurology*, *42*(5), 720–726. https://doi.org/10.1002/ana.410420508

Piccini, P., Weeks, R. A., & Brooks, D. J. (1997b). Alterations in opioid receptor binding in Parkinson's disease patients with levodopa-induced dyskinesias. *Annals of Neurology*, *42*(5), 720–726. https://doi.org/10.1002/ana.410420508

Pissadaki, E., & Bolam, J. P. (2013). The energy cost of action potential propagation in dopamine neurons: clues to susceptibility in Parkinson's disease . In *Frontiers in Computational Neuroscience* (Vol. 7, p. 13). https://www.frontiersin.org/article/10.3389/fncom.2013.00013

Plenz, D., & Kital, S. T. (1999). A basal ganglia pacemaker formed by the subthalamic nucleus and external globus pallidus. *Nature*, *400*(6745), 677–682. https://doi.org/10.1038/23281

Poirier, L. J., & Sourkes, T. L. (1965). Influence of the substantia nigra on the catecholamine content of the striatum. *Brain*, *88*(1), 181–192. https://doi.org/10.1093/brain/88.1.181

Pollack, P., Benabid, A. L., Gross, C., Gao, D. M., Laurent, A., Benazzoiz, A., Hoffmann, D., Gentil, M., & Perret, J. (1993). Effects of the stimulation of the subthalamic nucleus in Parkinson disease. *Rev Neurol (Paris)*, *149*(3), 175–176.

Prager, E. M., & Plotkin, J. L. (2019). Compartmental function and modulation of the striatum. In *Journal of Neuroscience Research* (Vol. 97, Issue 12, pp. 1503–1514). John Wiley and Sons Inc. https://doi.org/10.1002/jnr.24522

Pringsheim, T., Jette, N., Frolkis, A., & Steeves, T. D. L. (2014). The prevalence of Parkinson's disease: A systematic review and meta-analysis. In *Movement Disorders* (Vol. 29, Issue 13, pp. 1583–1590). John Wiley and Sons Inc. https://doi.org/10.1002/mds.25945

Przedbroski, S., Leviver, M., Jiang, H., Ferreira, M., Jackson-Lewis, V., Donaldson, D., & Togasaki, D. M. (1995). Dose-dependent lesions of the dopaminergic nigrostriatal pathway induced by instrastriatal injection of 6-hydroxydopamine. *Neuroscience*, *67*(3), 631–647. https://doi.org/10.1016/0306-4522(95)00066-R

Puopolo, M., Raviola, E., & Bean, B. P. (2007). Roles of subthreshold calcium current and sodium current in spontaneous firing of mouse midbrain dopamine neurons. *Journal of Neuroscience*, *27*(3), 645–656. https://doi.org/10.1523/JNEUROSCI.4341-06.2007

Redila, V. A., & Chavkin, C. (2008). Stress-induced reinstatement of cocaine seeking is mediated by the kappa opioid system. *Psychopharmacology*, *200*(1), 59–70. https://doi.org/10.1007/s00213-008-1122-y

Roberts, S. B., & Rosenberg, I. (2006). Nutrition and aging: Changes in the regulation of energy metabolism with aging. In *Physiological Reviews* (Vol. 86, Issue 2, pp. 651–667). Physiol Rev. https://doi.org/10.1152/physrev.00019.2005

Rosin, B., Slovik, M., Mitelman, R., Rivlin-Etzion, M., Haber, S. N., Israel, Z., Vaadia, E., & Bergman, H. (2011). Closed-loop deep brain stimulation is superior in ameliorating parkinsonism. *Neuron*, *72*(2), 370–384. https://doi.org/10.1016/j.neuron.2011.08.023

Rupniak, N. M. J., Boyce, S., Steventon, M. J., Iversen, S. D., & Marsden, C. D. (1992). Dystonia induced by combined treatment with L-dopa and MK-801 in parkinsonian monkeys. *Annals of Neurology*, *32*(1), 103–105. https://doi.org/10.1002/ana.410320118

Sauer, H., & Oertel, W. H. (1994). Progressive degeneration of nigrostriatal dopamine neurons following intrastriatal terminal lesions with 6-hydroxydopamine: A combined retrograde tracing and immunocytochemical study in the rat. *Neuroscience*, *59*(2), 401–415. https://doi.org/10.1016/0306-4522(94)90605-X

Schapira, A. H. V., & Obeso, J. (2006). Timing of treatment initiation in Parkinson's disease: A need for reappraisal? In *Annals of Neurology* (Vol. 59, Issue 3, pp. 559–562). https://doi.org/10.1002/ana.20789

Schapira, A H. (1990). Anatomic and disease specificity of NADH CoQ1 reductase (complex I) deficiency in Parkinson's disease. *J. Neurochem.*, *55*.

Schapira, Anthony H., & Jenner, P. (2011). Etiology and pathogenesis of Parkinson's disease. In *Movement Disorders* (Vol. 26, Issue 6, pp. 1049–1055). Mov Disord. https://doi.org/10.1002/mds.23732

Schapira, A. H. V., Cooper, J. M., Dexter, D., Clark, J. B., Jenner, P., & Marsden, C. D. (1990). Mitochondrial Complex I Deficiency in Parkinson's Disease. *Journal of Neurochemistry*, *54*(3), 823–827. https://doi.org/10.1111/j.1471-4159.1990.tb02325.x

Schon, E A, & Przedborski, S. (2011). Mitochondria: the next (neurode)generation. *Neuron*, *70*.

Schumacher, B., Garinis, G. A., & Hoeijmakers, J. H. J. (2008). Age to survive: DNA damage and aging. In *Trends in Genetics* (Vol. 24, Issue 2, pp. 77–85). https://doi.org/10.1016/j.tig.2007.11.004

Schwalb, J. M., & Hamani, C. (2008). The History and Future of Deep Brain Stimulation. *Neurotherapeutics*, *5*(1), 3–13. https://doi.org/10.1016/j.nurt.2007.11.003

Seizinger, B. R., Grimm, C., Höllt, V., & Herz, A. (1984). Evidence for a selective processing of proenkephalin B into different opioid peptide forms in particular regions of rat brain and pituitary. *Journal of Neurochemistry*, *42*(2), 447–457. http://www.ncbi.nlm.nih.gov/pubmed/6141221

Sgroi, S., Capper-Loup, C., Paganetti, P., & Kaelin-Lang, A. (2016). Enkephalin and dynorphin neuropeptides are differently correlated with locomotor hypersensitivity and levodopa-induced dyskinesia in parkinsonian rats. *Experimental Neurology*, *280*, 80–88. https://doi.org/10.1016/j.expneurol.2016.03.024

Sgroi, S., & Tonini, R. (2018). Opioidergic Modulation of Striatal Circuits, Implications in Parkinson's Disease and Levodopa Induced Dyskinesia. *Frontiers in Neurology*, *9*, 524. https://doi.org/10.3389/fneur.2018.00524

Sharott, A., Gulberti, A., Hamel, W., Köppen, J. A., Münchau, A., Buhmann, C., Pötter-Nerger, M., Westphal, M., Gerloff, C., Moll, C. K. E., & Engel, A. K. (2018). Spatio-temporal dynamics of cortical drive to human subthalamic nucleus neurons in Parkinson's disease. *Neurobiology of Disease*, *112*, 49–62. https://doi.org/10.1016/j.nbd.2018.01.001

Shimohama, S., Sawada, H., Kitamura, Y., & Taniguchi, T. (2003). Disease model: Parkinson's disease. In *Trends in Molecular Medicine* (Vol. 9, Issue 8, pp. 360–365). Elsevier Ltd. https://doi.org/10.1016/S1471-4914(03)00117-5

Shippenberg, T. S., Chefer, V. I., Zapata, A., & Heidbreder, C. A. (2006). Modulation of the Behavioral and Neurochemical Effects of Psychostimulants by κ-Opioid Receptor Systems. *Annals of the New York Academy of Sciences*, *937*(1), 50–73. https://doi.org/10.1111/j.1749-6632.2001.tb03558.x

Siegfried, J., & Lippitz, B. (1994). Bilateral chronic electrostimulation of ventroposterolateral pallidum: A new therapeutic approach for alleviating all parkinsonian symptoms. *Neurosurgery*, *35*(6), 1126–1130. https://doi.org/10.1227/00006123-199412000-00016

Smith, Y., Bevan, M. D., Shink, E., & Bolam, J. P. (1998). Microcircuitry of the direct and indirect pathways of the basal ganglia. In *Neuroscience* (Vol. 86, Issue 2, pp. 353–387). Elsevier Ltd. https://doi.org/10.1016/S0306-4522(98)00004-9

Smith, Y., Wichmann, T., Factor, S. A., & DeLong, M. R. (2012). Parkinson's disease therapeutics: new developments and challenges since the introduction of levodopa. *Neuropsychopharmacology*, *37*.

Sonsalla, P. K., & Heikkila, R. E. (1986). The influence of dose and dosing interval on MPTP-induced dopaminergic neurotoxicity in mice. *European Journal of Pharmacology*, *129*(3), 339–345. https://doi.org/10.1016/0014-2999(86)90444-9

Spanagel, R., Almeida, O. F. X., Bartl, C., & Shippenberg, T. S. (1994). Endogenous κ-opioid systems in opiate withdrawal: role in aversion and accompanying changes in mesolimbic dopamine release. *Psychopharmacology*, *115*(1–2), 121–127. https://doi.org/10.1007/BF02244761

Spillantini, M. G., Schmidt, M. L., Lee, V. M. Y., Trojanowski, J. Q., Jakes, R., & Goedert, M. (1997). α-synuclein in Lewy bodies [8]. In *Nature* (Vol. 388, Issue 6645, pp. 839–840). Nature Publishing Group. https://doi.org/10.1038/42166

Sulzer, D. (2007). Multiple hit hypotheses for dopamine neuron loss in Parkinson's disease. In *Trends in Neurosciences* (Vol. 30, Issue 5, pp. 244–250). https://doi.org/10.1016/j.tins.2007.03.009

Surmeier, D. J., Guzman, J. N., Sanchez-Padilla, J., & Schumacker, P. T. (2011). The role of calcium and mitochondrial oxidant stress in the loss of substantia nigra pars compacta dopaminergic neurons in Parkinson's disease. In *Neuroscience* (Vol. 198, pp. 221–231). https://doi.org/10.1016/j.neuroscience.2011.08.045

Swerdlow, R. H. (2011). Brain aging, Alzheimer's disease, and mitochondria. In *Biochimica et Biophysica Acta - Molecular Basis of Disease* (Vol. 1812, Issue 12, pp. 1630–1639). Biochim Biophys Acta. https://doi.org/10.1016/j.bbadis.2011.08.012

Tamim, M. K., Samadi, P., Morissette, M., Grégoire, L., Ouattara, B., Lévesque, D., Rouillard, C., & Di Paolo, T. (2010). Effect of non-dopaminergic drug treatment on Levodopa induced dyskinesias in MPTP monkeys: Common implication of striatal neuropeptides. *Neuropharmacology*, *58*(1), 286–296. https://doi.org/10.1016/j.neuropharm.2009.06.030

Tanner, C. M., & Aston, D. A. (2000). Epidemiology of Parkinson's disease and akinetic syndromes [In Process Citation]. *Current Opinion in Neurology*, *13*(4), 427–430. https://journals.lww.com/co-neurology/Abstract/2000/08000/Epidemiology_of_Parkinson_s_disease_and_akinetic.10.aspx

Tanner, C. M., & Ben-Shlomo, Y. (1999). Epidemiology of Parkinson's disease. *Advances in Neurology*, *80*, 153–159. http://www.ncbi.nlm.nih.gov/pubmed/10410715

Tecuapetla, F., Jin, X., Lima, S. Q., & Costa, R. M. (2016). Complementary Contributions of Striatal Projection Pathways to Action Initiation and Execution. *Cell*, *166*(3), 703–715. https://doi.org/10.1016/j.cell.2016.06.032

Tel, B. C., Zeng, B. Y., Cannizzaro, C., Pearce, R. K. B., Rose, S., & Jenner, P. (2002). Alterations in striatal neuropeptide mRNA produced by repeated administration of L-DOPA, ropinirole or bromocriptine correlate with dyskinesia induction in MPTP-treated common marmosets. *Neuroscience*, *115*(4), 1047–1058. https://doi.org/10.1016/S0306-4522(02)00535-3

Thiruchelvam, M., Brockel, B. J., Richfield, E. K., Baggs, R. B., & Cory-Slechta, D. A. (2000). Potentiated and preferential effects of combined paraquat and maneb on nigrostriatal dopamine systems: Environmental risk factors for Parkinson's disease? *Brain Research*, *873*(2), 225–234. https://doi.org/10.1016/S0006-8993(00)02496-3

Thiruchelvam, Mona, McCormack, A., Richfield, E. K., Baggs, R. B., Tank, A. W., Di Monte, D. A., & Cory-Slechta, D. A. (2003). Age-related irreversible progressive nigrostriatal dopaminergic neurotoxicity in the paraquat and maneb model of the Parkinson's disease phenotype. *European Journal of Neuroscience*, *18*(3), 589–600. https://doi.org/10.1046/j.1460-9568.2003.02781.x

Thomas, A., Iacono, D., Luciano, A. L., Armellino, K., Di Iorio, A., & Onofrj, M. (2004). Duration of amantadine benefit on dyskinesia of severe Parkinson's disease. *Journal of Neurology, Neurosurgery and Psychiatry*, *75*(1), 141–143. www.jnnp.com

Trietiakoff, C. (1919). *Contribution a L'étude de L'anatomie Pathologique de Locus Niger de Soemmerling*. Universite de Paris.

Ungerstedt, U, & Arbuthnott, G. (1970). Quantitative recording of rotational behaviour in rats after 6-hydroxydopamine lesions of the nigrostriatal dopamine system. *Brain Res.*, *24*.

Ungerstedt, Urban. (1968). 6-hydroxy-dopamine induced degeneration of central monoamine neurons. *European Journal of Pharmacology*, *5*(1), 107–110. https://doi.org/10.1016/0014-2999(68)90164-7

Ungerstedt, Urban. (1976). 6-Hydroxydopamine-induced degeneration of the nigrostriatal dopamine pathway: The turning syndrome. *Pharmacology And Therapeutics: Part B: General And Systematic*, *2*(1), 37–40. https://doi.org/10.1016/0306-039X(76)90016-7

Valente, E. M., Abou-Sleiman, P. M., Caputo, V., Muqit, M. M. K., Harvey, K., Gispert, S., Ali, Z., Del Turco, D., Bentivoglio, A. R., Healy, D. G., Albanese, A., Nussbaum, R., González-Maldonado, R., Deller, T., Salvi, S., Cortelli, P., Gilks, W. P., Latchman, D. S., Harvey, R. J., … Wood, N. W. (2004). Hereditary early-onset Parkinson's disease caused by mutations in PINK1. *Science*, *304*(5674), 1158–1160. https://doi.org/10.1126/science.1096284

Valko, M., Leibfritz, D., Moncol, J., Cronin, M. T. D., Mazur, M., & Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. In *International Journal of*

Biochemistry and Cell Biology (Vol. 39, Issue 1, pp. 44–84). Int J Biochem Cell Biol. https://doi.org/10.1016/j.biocel.2006.07.001

Van Laar, V. S., & Berman, S. B. (2009). Mitochondrial dynamics in Parkinson's disease. In *Experimental Neurology*. https://doi.org/10.1016/j.expneurol.2009.03.019

Venero, J. L., Revuelta, M., Cano, J., & Machado, A. (2002). Time Course Changes in the Dopaminergic Nigrostriatal System Following Transection of the Medial Forebrain Bundle: Detection of Oxidatively Modified Proteins in Substantia Nigra. *Journal of Neurochemistry*, *68*(6), 2458–2468. https://doi.org/10.1046/j.1471-4159.1997.68062458.x

Vijg, J., & Campisi, J. (2008). Puzzles, promises and a cure for ageing. In *Nature* (Vol. 454, Issue 7208, pp. 1065–1071). Nature Publishing Group. https://doi.org/10.1038/nature07216

Vives-Bauza, C. (2010). PINK1-dependent recruitment of Parkin to mitochondria in mitophagy. *Proc. Natl Acad. Sci. USA*, *107*.

Voorn, P., Vanderschuren, L. J. M. J., Groenewegen, H. J., Robbins, T. W., & Pennartz, C. M. A. (2004). Putting a spin on the dorsal-ventral divide of the striatum. In *Trends in Neurosciences* (Vol. 27, Issue 8, pp. 468–474). Elsevier Current Trends. https://doi.org/10.1016/j.tins.2004.06.006

Warren Olanow, C., Kieburtz, K., Rascol, O., Poewe, W., Schapira, A. H., Emre, M., Nissinen, H., Leinonen, M., & Stocchi, F. (2013). Factors predictive of the development of Levodopa-induced dyskinesia and wearing-off in Parkinson's disease. *Movement Disorders*, *28*(8), 1064–1071. https://doi.org/10.1002/mds.25364

Wellstead, P., & Cloutier, M. (2011). An energy systems approach to Parkinson's disease. *Wiley Interdisciplinary Reviews: Systems Biology and Medicine*, *3*(1), 1–6. https://doi.org/10.1002/wsbm.107

Westin, J. E., Andersson, M., Lundblad, M., & Cenci, M. A. (2001). Persistent changes in striatal gene expression induced by long-term l -DOPA treatment in a rat model of Parkinson's disease. *European Journal of Neuroscience*, *14*(7), 1171–1176. https://doi.org/10.1046/j.0953-816x.2001.01743.x

William Langston, J., Forno, L. S., Rebert, C. S., & Irwin, I. (1984). Selective nigral toxicity after systemic administration of 1-methyl-4-phenyl-1,2,5,6-tetrahydropyrine (MPTP) in the squirrel monkey. *Brain Research*, *292*(2), 390–394. https://doi.org/10.1016/0006-8993(84)90777-7

Willis, M. W., Ketter, T. A., Kimbrell, T. A., George, M. S., Herscovitch, P., Danielson, A. L., Benson, B. E., & Post, R. M. (2002). Age, sex and laterality effects on cerebral glucose metabolism in healthy adults. *Psychiatry Research - Neuroimaging*, *114*(1), 23–37. https://doi.org/10.1016/S0925-4927(01)00126-3

Winkler, C., Kirik, D., Björklund, A., & Cenci, M. A. (2002a). L-DOPA-induced dyskinesia in the intrastriatal 6-hydroxydopamine model of parkinson's disease: relation to motor and cellular parameters of nigrostriatal function. *Neurobiology of Disease*, *10*(2), 165–186. http://www.ncbi.nlm.nih.gov/pubmed/12127155

Wright Willis, A., Evanoff, B. A., Lian, M., Criswell, S. R., & Racette, B. A. (2010). Geographic and ethnic variation in Parkinson disease: A population-based study of us medicare beneficiaries. *Neuroepidemiology*, *34*(3), 143–151. https://doi.org/10.1159/000275491

Xuei, X., Dick, D., Flury-Wetherill, L., Tian, H.-J., Agrawal, A., Bierut, L., Goate, A., Bucholz, K., Schuckit, M., Nurnberger, J., Tischfield, J., Kuperman, S., Porjesz, B., Begleiter, H., Foroud, T., & Edenberg, H. J. (2006). Association of the κ-opioid system with alcohol dependence. *Molecular Psychiatry*, *11*(11), 1016–1024. https://doi.org/10.1038/sj.mp.4001882

Yahr, M. D., Duvoisin, R. C., Schear, M. J., Barrett, R. E., & Hoehn, M. M. (1969). Treatment of Parkinsonism With Levodopa. *Archives of Neurology*, *21*(4), 343–354. https://doi.org/10.1001/archneur.1969.00480160015001

Yeomans, J. S., Maidment, N. T., & Bunney, B. S. (1988). Excitability properties of medial forebrain bundle axons of A9 and A10 dopamine cells. *Brain Research*, *450*(1–2), 86–93. https://doi.org/10.1016/0006-8993(88)91547-8

Chapter 2: Differential effects of the NMDA receptor antagonist MK-801 on dopamine receptor D1- and D2-induced abnormal involuntary movements in a preclinical model

Andrew J. Flores[a,b], Mitchell J. Bartlett[a], Lisa Y. So[a], Nicholas D. Laude[c], Kate L. Parent[c], Michael L. Heien[c], Scott J. Sherman[a], Torsten Falk[a,b]

a Department of Neurology, University of Arizona, Tucson, b Graduate Interdisciplinary Program in Physiological Sciences, c Department of Chemistry and Biochemistry, University of Arizona, Tucson, AZ 85721, USA

Published in *Neuroscience Letters*
564 (2014), 48-52
https://doi.org/10.1016/j.neulet.2014.02.004

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4825183/

Contributions

Conception and design: Torsten Falk, Andrew J. Flores

Data collection: Andrew J. Flores, Mitchell J. Bartlett, Lisa Y. So, Nicholas D. Laude, Kate L. Parent

Data analysis and interpretation: Andrew J. Flores, Torsten Falk

Drafting the article: Torsten Falk, Andrew J. Flores, Scott J. Sherman

Critical revision of article: Torsten Falk, Scott J. Sherman, Michael L. Heien, Andrew J. Flores, Mitchell J. Bartlett

Highlights

- MK-801 worsens D1R-induced abnormal involuntary movements (limb/axial/orolingual).
- MK-801 does not affect D1R-induced abnormal involuntary locomotor movements.
- MK-801 blocks D2R-induced abnormal involuntary movements (limb/axial/orolingual).
- MK-801 induces abnormal ipsilateral rotations in D2R-treated animals

Abstract

Dopamine-replacement therapy with L-DOPA is still the gold standard treatment for Parkinson's disease (PD). One drawback is the common development of L-DOPA-induced dyskinesia (LID) in patients, which can be as disabling as the disease itself. There is no satisfactory adjunct therapy available. Glutamatergic transmission in the basal ganglia circuitry has been shown to be an important player in the development of LID. The *N*-methyl-d-aspartate (NMDA) receptor antagonist MK-801 has previously been shown to reduce L-DOPA-induced abnormal involuntary movements (AIMs) in a rat preclinical model but only at concentrations that worsen parkinsonism. We investigated the contribution of the direct and indirect striatofugal pathways to these effects. In the direct pathway, dopamine D1 receptors (D1R) are expressed, whereas in the indirect pathway, dopamine D2 receptors (D2R) are expressed. We used the 6-hydroxydopamine-lesioned hemi-parkinsonian rat model initially primed with L-DOPA to induce dyskinesia. When the rats were then primed and probed with the D1R agonist SKF81297, co-injection of MK-801 worsened the D1R-induced limb, axial, and orolingual (LAO) AIMs by 18% (predominantly dystonic axial AIMs) but did not aggravate parkinsonian hypokinesia as reflected by a surrogate measure of ipsiversive rotations in this model. In contrast, when the rats were then primed and probed with the D2R agonist quinpirole, co-injection of MK-801 reduced D2R-induced LAO AIMs by 89% while inducing ipsiversive rotations. The data show that only inhibition of the indirect striatopallidal pathway is sufficient for the full anti-dyskinetic/pro-parkinsonian effects of the NMDA receptor antagonist MK-801, and that MK-801 modestly worsens dyskinesias that are due to activation of the direct striatonigral pathway alone. This differential activation of the glutamatergic systems in D1R- and D2R-mediated responses is relevant to current therapy for PD which generally includes a mixture of dopamine agonists and L-DOPA.

2.1 Introduction

Parkinson's disease (PD) is a prevalent neurodegenerative disease characterized by a hypokinetic movement disorder with symptoms of tremor, rigidity, and bradykinesia (Olanow et al., 2009). These motor symptoms correspond to the loss of dopaminergic neurons with cell bodies located in the substantia nigra and axonal projections to the striatum. The most common treatment for PD consists of dopamine (DA) replacement therapy utilizing either the DA precursor L-DOPA or DA receptor agonists. These therapies become unsatisfactory as the disease progresses due to a variety of short-term and long-term side effects that occur with dose escalation, including LID. Therefore, there is an urgent need to develop non-dopaminergic therapies (Fox et al., 2008) or adjunctive therapies that can directly block dyskinesia while not worsening the cardinal Parkinson motor symptoms.

There is evidence that the glutamatergic system contributes to the development of LID, although the underlying mechanisms are not completely understood. Glutamate activates two different types of receptors widely expressed in the brain, including the basal ganglia; (1) the metabotropic glutamate receptors (mGluRs) involved in slow synaptic transmission and (2) the ionotropic glutamate receptors (iGluRs) involved in fast synaptic transmission (Greengard, 2001). Imaging data marking activated *N*-methyl-d-aspartate (NMDA) receptors, suggest that L-DOPA administration leads to elevated NMDA receptor activity in the caudate, putamen, and precentral cortex in PD patients with LID, but not in those without LID (Ahmed et al., 2011). Binding studies in human postmortem tissue demonstrated that levels of the NR1/NR2B NMDA receptor are increased in the putamen of PD patients with LID compared to those without LID (Calon et al., 2002). The non-competitive NMDA receptor antagonist MK-801 has been shown to reduce L-DOPA-induced abnormal involuntary movements (AIMs) in a rat model of LID but only at concentrations that worsen parkinsonism (Paquette et al., 2010); however, the separate contributions of the two major striatofugal pathways to

this effect have not been investigated. In the direct striatonigral pathway, D1Rs are expressed, whereas in the indirect striatopallidal pathway, D2Rs are expressed (Gerfen & Surmeier, 2011). DA agonists with activity at D1R and D2R have been reported to produce dyskinesias with similar incidence and severity to L-DOPA (Taylor et al., 2006), but those depend solely on postsynaptic mechanisms since these drugs do not induce any DA release.

In the current study we utilized a preclinical rat LID model first established by Cenci's group (Dekundy et al., 2007), modified by Paquette et al., 2010, and we investigated the effect of MK-801 on dyskinesias either induced by a direct pathway-specific D1R agonist or an indirect pathway-specific D2R agonist.

2.2 Material and methods

2.2.1 Animals

Male Sprague-Dawley rats (250 g; Charles River, Wilmington, MA), were used and housed in a temperature and humidity controlled room with 12 h reversed light/dark cycles with food and water available *ad libitum*. All animals were treated as approved by the Institutional Animal Care and Use Committee at the University of Arizona and in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals. Both the number of animals used and their suffering were minimized.

2.2.2 The unilateral 6-hydroxydopamine (6-OHDA)-lesion rat PD model

Animals were injected with 20 µg of freshly prepared 6-OHDA hydrochloride (5.0 µg/µl in 0.9% sterile saline with 0.02% ascorbic acid; Sigma-Aldrich, St. Louis, MO) into 2 locations (10 µg per site) in the

medial forebrain bundle (MFB) at coordinates: AP −2.8, ML −1.8, DV −8.0 and AP −4.7, ML −1.5, DV −7.9 (Paxinos and Watson, 1997). The rate of injection was 0.5 µl/min using a Stoelting microinjector (Stoelting Co., Wood Dale, IL). The Hamilton syringe was left in place for 5 additional minutes to prevent backflow of solution. Rats were pretreated (30 min prior to 6-OHDA) with 12.5 mg/kg desipramine (Sigma-Aldrich) given intraperitoneally (*i.p.*) to prevent damage to noradrenergic neurons.

2.2.3 Induction of LID in unilateral lesioned rats

1) Two weeks after surgery, the unilateral 6-OHDA-lesioned rats were injected with d-amphetamine (5.0 mg/kg, *i.p.*; Sigma-Aldrich) to induce asymmetrical DA release. The number of ipsiversive rotations during 1 min intervals every 5 min was counted for a total of 60 min. 2) Rats with ≥4 rotations/min were selected and treated for 3 weeks with 7 mg/kg L-DOPA (with 14 mg/kg benserazide, both *i.p.*; Sigma–Aldrich).

2.2.4 Behavioral analysis in the LID rat model

L-DOPA-, D1R- and D2R-induced AIMs were scored by an experimentally blinded investigator (Dekundy et al., 2007) with a minimum of 3 days between testing sessions. Drugs were tested once in a within-subjects crossover design of drugs and own vehicle for each condition (only SKF81297 + MK-801 condition was repeated to confirm the modest effect). In order to quantify the severity of the AIMs, rats were observed individually during their night, in their standard cages every 20th min at 20–180 min after an injection of L-DOPA. As described in the literature (Paquette et al., 2010), AIMs were classified into four subtypes: (1) limb; (2) axial; (3) orolingual; (4) locomotor, and each was scored on a severity scale from 0 to 4. The sums of AIMs scores per testing session were used

for statistical analyses. Drugs: SKF81297; quinpirole; SCH23390; eticlopride (Tocris Bioscience/R&D Systems, Inc., Minneapolis, MN); MK-801 hydrogen maleate (Sigma–Aldrich); all dissolved in 0.9% sterile saline (irrigation-USP; VWR, Brisbane, CA). MK-801 was injected *i.p.* 20 min prior to other drugs.

2.2.5 Measurement of striatal dopamine and serotonin content

Rat brains were washed in chilled Tris buffer (pH 7.4, 15 mM Tris, 125 mM NaCl, 2.5 mM KCl, 2 mM $CaCl_2$) for 30 s and placed in a chilled brain block. Coronal brain slices were collected and a 2 mm steel biopsy punch was used to sample tissue from the striatum. Samples from left and right hemispheres were collected and immediately flash frozen on an aluminum pan at −70 °C. Samples massed at 2.5 ± 0.5 mg, were placed in 1.5 mL homogenization vials with 100 µL of 0.1 N $HClO_4$ (aq), manually homogenized (15 strokes) using a disposable pestle and stored at −80 °C for up to 2 weeks prior to analysis. High performance liquid chromatography with electrochemical detection (HPLC-EC) was used to separate and quantify DA, 3,4-dihydroxyphenylacetic acid, serotonin, and 5-hydroxyindoleacetic acid (Mefford et al., 1980).

2.2.6 Western analysis of striatal tyrosine hydroxylase (TH) content

After the tissue punch, described above, the remaining striata from left and right hemispheres were immediately flash frozen and stored at −80 °C. Total protein was prepared by homogenizing (BBX24-CE Bullet Blender homogenizer, Next Advance, Inc., Averill Park, NY) striatal tissue in lysis buffer.

Semi-quantitative western analysis was conducted as described (Yue et al., 2014). 10 μg of protein was loaded for each sample to measure TH and β-Actin as internal control.

2.2.7 Data analysis

Statistical analysis was performed using GraphPad Prism 5.1 software (GraphPad Software, Inc., La Jolla, CA), in Origin 9.0 and Microsoft Excel 2013. Differences between each drug treatment and its own vehicle in the behavioral analysis were assessed using two-tailed paired Student's *t*-tests. The null hypothesis was rejected when $p < 0.05$.

2.3 Results

2.3.1 Establishing the levodopa-induced dyskinesia rat model

We injected 6-OHDA unilaterally into the MFB of 15 rats and measured the development of a lesion with amphetamine-induced rotational tests 2 weeks post-surgery. For the LID model, we chose 9 rats that showed an average of a minimum of 4 ipsiversive rotations/min. These rats showed significant ipsiversive rotations (5.7 ± 0.3 ipsiversive rotations/min, mean ± SEM; $n = 9$), at a rate that has been shown previously to correspond with >90% destruction of dopaminergic neurons (Dekundy et al., 2007). The lesioned rats were treated with L-DOPA (7 mg/kg) daily for 3 weeks; all 9 developed significant L-DOPA-induced AIMs and were chosen for the experiment. At the end of the experiments, the severity of the dopaminergic denervation was measured. Compared to the non-lesioned side there was a 90% depletion of striatal DA content evident in the lesioned side whereas the levels of serotonin remained unchanged, as determined by HPLC-EC analysis (data not shown). The striatal TH content,

determined by semi-quantitative western analysis, was also reduced by 90% on the lesioned side, indicating a similar loss of dopaminergic terminals (data not shown). The L-DOPA-induced AIMs in 3 separate testing sessions (3–4 days apart) after the 21 days of priming are shown in Fig. 1 A,B. All 4 subtypes of AIMs were present in the animals.

2.3.2 MK-801 worsens D1-receptor-induced LAO AIMs while not affecting rotational behavior

Next the rats were primed 3 times (3–4 days apart) with the D1R agonist SKF81297 (0.8 mg/kg, *s.c.*) as shown in Fig. 2. Both LAO AIMs (Fig. 2A) and locomotor AIMs (Fig. 2B) were elicited by SKF81297. These could be completely blocked by the D1R antagonist SCH23390 at 1 mg/kg, *i.p.* (Fig. 2C,D). Co-injection of MK-801 (0.3 mg/kg, *i.p.*) led to a significant increase of the D1R-induced LAO AIMs by 18% (Fig. 2E), while not inducing any ipsiversive rotations (Fig. 2F). The time course shows that the effect of MK-801 lasted throughout the time SKF81297 elicited AIMs (data not shown). Specifically, the 9.5% increase in choreiform limb AIMs and the 33% increase in the orolingual AIMs after MK-801 did not reach significant levels (Fig. 2G), while MK-801 significantly increased dystonic axial AIMs by 33% (Fig. 2H).

2.3.3 MK-801 blocks D2-receptor-induced LAO and locomotor AIMs

Next, the rats were primed 3 times (3–4 days apart) with the D2R agonist quinpirole (0.2 mg/kg, *i.p.*) as shown in Fig. 3. Both LAO AIMs (Fig. 3A) and locomotor AIMs (Fig. 3B) were elicited by quinpirole. These could be completely blocked by the D2R antagonist eticlopride at 0.1 mg/kg, *i.p.* (Fig. 3C,D). Co-injection of MK-801 (0.3 mg/kg, *i.p.*) led to a significant reduction of D2R-induced LAO AIMs by

89% (Fig. 3E), while also inducing ipsiversive rotations (Fig. 3F), similar to what has been shown previously for MK-801 in rats primed and probed with L-DOPA alone (Paquette et al., 2010). The time course did show that the effect of MK-801 lasted throughout the 3 h recording period for both measures (data not shown). Specifically, MK-801 significantly reduced choreiform limb, orolingual and dystonic axial AIMs by 84%, 97.5% and 72% respectively (Fig. 3G and H).

2.4 Discussion

In this study we investigated the contribution of the direct and indirect striatofugal pathways to the anti-dyskinetic and pro-parkinsonian effects of the NDMA receptor antagonist MK-801. MK-801 has previously been shown to effectively block LIDs in a rat preclinical model but only at a dose that also worsens the parkinsonian behavior (Paquette et al., 2010). Here, we show that MK-801 affects AIMs in unilaterally-lesioned dyskinetic rats differentially when separately activating the two basal ganglia pathways. AIMs elicited by a D2R-specific agonist, inhibiting the indirect striatopallidal pathway, were effectively blocked by MK-801, whereas the AIMs due to a D1R-specific agonist, activating the direct striatonigral pathway, were moderately worsened. The worsening effect size is small, but it is important to point out that with the paradigm used the rats displayed very severe dyskinesias already (>50 total LAO AIMs), indicating a potential ceiling effect.

The anti-dyskinetic activity of the current clinically used compound amantadine is partially dependent on its weak NMDA receptor antagonism (Paquette et al., 2012), and it is known to reduce L-DOPA-induced AIMs in rodents (Kobylecki et al., 2011; Paquette et al., 2012). Amantadine has recently been shown to mildly reduce D2R-induced AIMs by 40% while having no effect at all on D1R-induced AIMs (Kobylecki et al., 2011) in the rat LID model. Here we show that the strong non-

competitive NMDA receptor antagonist MK-801 not only fails to reduce D1R-induced AIMs, as amantadine does, but actually significantly increases these AIMs by 18%. In contrast, we found that MK-801 still potently inhibits the D2R-induced AIMs by 89%, which is much more pronounced than the effect of amantadine in this paradigm. These data are in agreement with MK-801 being a stronger NMDA receptor antagonist and show that blocking NMDA receptors modestly worsened dyskinesias that are due to D1R activation. In line with our results are data showing that intrastriatal infusion of MK-801 failed to inhibit D1R-mediated induction of motor activity in DA-depleted animals (Campbell et al., 2006). The differential effects of this strong NMDA receptor antagonist are in contrast to the effects on the basal ganglia pathways of a serotonin 1A receptor antagonist that reduces AIMs induced by L-DOPA, D1R, or D2R agonists (Dupre et al., 2007). Interestingly, in this case L-DOPA-induced rotations were reduced, D1R-induced rotations were increased, and D2R-induced rotations remained unchanged. A serotonin 2A receptor antagonist, on the other hand, does not reduce L-DOPA-induced AIMs but reduces D1R-induced rotations (Taylor et al., 2006),

Unilateral 6-OHDA-lesions of the MFB, irrespective of the priming drug (L-DOPA or SKF81297), led to a reduction of basal levels of striatal glutamate when compared to sham-lesioned primed rats (Dupre et al., 2011), while high dose L-DOPA administration (25 and 100 mg/kg) in hemi-parkinsonian rats has been shown to enhance striatal glutamate levels (Jonkers et al., 2002; Robelet et al., 2004). Our data complement a study that showed L-DOPA treatment produces a moderate augmentation of glutamate levels in the DA-depleted striatum, whereas the D1R agonist SKF81297 did not, also suggesting that enhancement of extracellular striatal glutamate may be important for the expression of LID and not necessarily D1R-mediated dyskinesia (Dupre et al., 2011). Similar to that study, we detected similar severities of LAO AIMs produced by L-DOPA, SKF81297, and quinpirole, with differences in pharmacokinetics and with both L-DOPA's and especially quinpirole's effects

lasting longer than SKF81297. The differences in the elicited total locomotor AIMs between L-DOPA (5–7 locomotor AIMs), and the agonists (12–15 locomotor AIMs) can be attributed to different pharmacokinetics of the agonists vs. L-DOPA. SKF81297 has a similar time course as L-DOPA but induces more locomotor AIMs per min, whereas quinpirole induces less locomotor AIMs per min but for an extended time period, being still fully effective at 180 min, when L-DOPA and SKF81297 are not very effective any more.

In a non-human primate model of LID a different competitive NMDA antagonist (LY235959) has been shown at a high dose to reduce chorea but exacerbate dystonia (Papa & Chase, 1996). The data from a rodent model presented here indicate that the worsening of the dystonic dyskinesia (axial AIMs) by a NMDA antagonist might be driven by effects on the direct pathway, while NMDA antagonism reduces choreiform limb, orolingual and dystonic dyskinesias induced solely by inhibiting the indirect pathway.

One caveat of this study is that the rats have been sequentially tested with D1R and D2R agonists, antagonists and MK-801. It is known that the effects of some drugs can affect cellular and molecular responses for weeks, and repeated administration of MK-801 can lead to behavioral sensitization, which could be contributing factors to the effects described. The fact that there were more than 3 weeks between the MK-801 treatments in the D1R and D2R experiments and the double-dissociation of the MK-801 effects on D1R vs. D2R activation argue against a major impact on the acute effect.

MK-801 has been shown to alter the effects of priming on L-DOPA or D1R-induced changes in neuropeptide mRNA levels in the striatal output neurons. The increased behavioral responsiveness to a D1R agonist after priming with L-DOPA is primarily related to the up-regulation of dynorphin mRNA in the DA-depleted striatum (Van De Witte et al., 2002). Pretreatment with MK-801 has been shown to

reduce D2R-induced contralateral rotations and ipsilateral striatal Fos expression in 6-OHDA-lesioned rats (Pollack & Haisley, 2001). Changes in indirect pathway neurons might be an important feature of the dyskinetic striatum, since the loss of spines and their corticostriatal connections occurs specifically in the indirect pathway neurons following DA depletion (Day et al., 2006), and increase in excitability seems to be caused by the loss of inhibitory signaling at D2R (Shen et al., 2008). Direct pathway neurons retain a form of long-term depression (LTD) comparable to that seen in untreated parkinsonian rats, while long-term potentiation (LTP) at indirect pathway neurons in dyskinetic animals is characterized as unstable (Belujon et al., 2010). These data would be in line with excessive NMDA receptor activity in the dyskinetic state playing a more important role in the indirect pathway than in the direct pathway.

In conclusion, we show that the strong non-competitive NMDA receptor antagonist MK-801 leads to modest worsening of D1R-induced dyskinesias, especially dystonic dyskinesias, while effectively reducing D2R-induced dyskinesias. The degree of reduction is of the same magnitude as the established reduction of L-DOPA-induced dyskinesias. These results point to differences in glutamatergic regulation of the striatonigral and striatopallidal systems in L-DOPA-induced dyskinesias.

2.5 Acknowledgements

We would like to thank Dr. F. Porreca, Department of Pharmacology, University of Arizona, for insightful discussions, use of his equipment and laboratory. Support: American Parkinson's Disease (APDA) Research Grant (T.F., S.J.S.), APDA medical summer fellowship (M.J.B.), and the Jerry T. and Glenda G. Jackson Fellowship in Parkinson's Research to the University of Arizona.

2.6 Figures

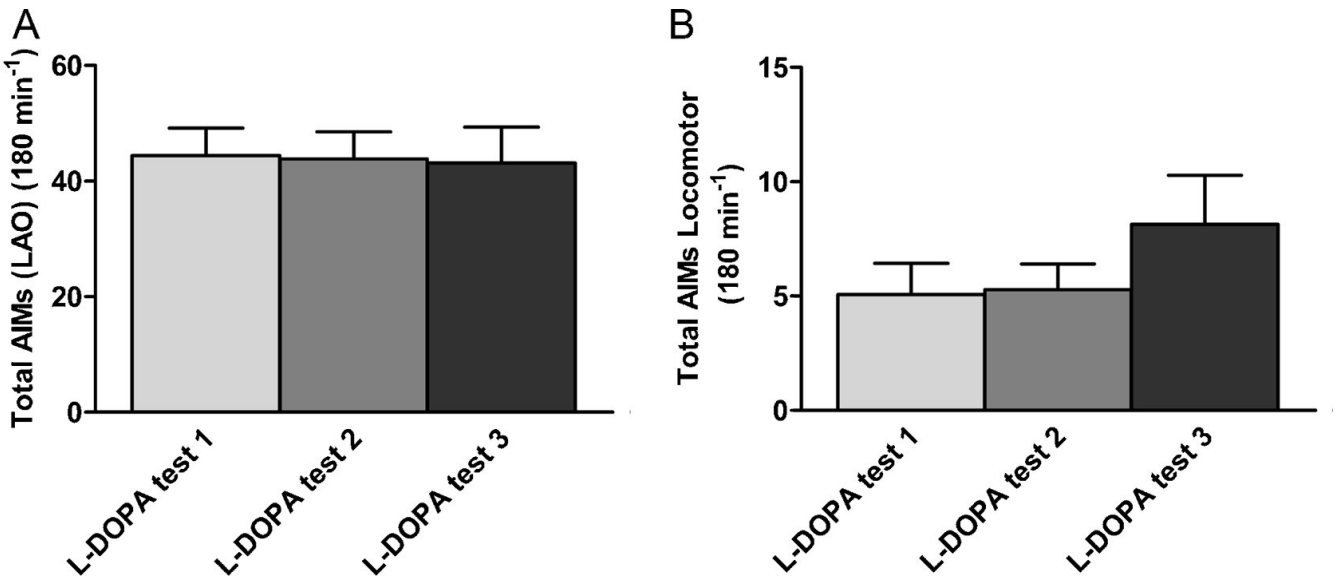

Figure 1. Establishing the L-DOPA-induced AIMs model. After 21 days of L-DOPA-treatment L-DOPA-induced AIMs were established. (A) The graph shows the mean LAO AIMs (±SEM) at 3 testing sessions in weeks 3 and 4 post-L-DOPA onset (n = 9). (B) The graph depicts the mean locomotor AIMs (±SEM) elicited by L-DOPA (n = 7).

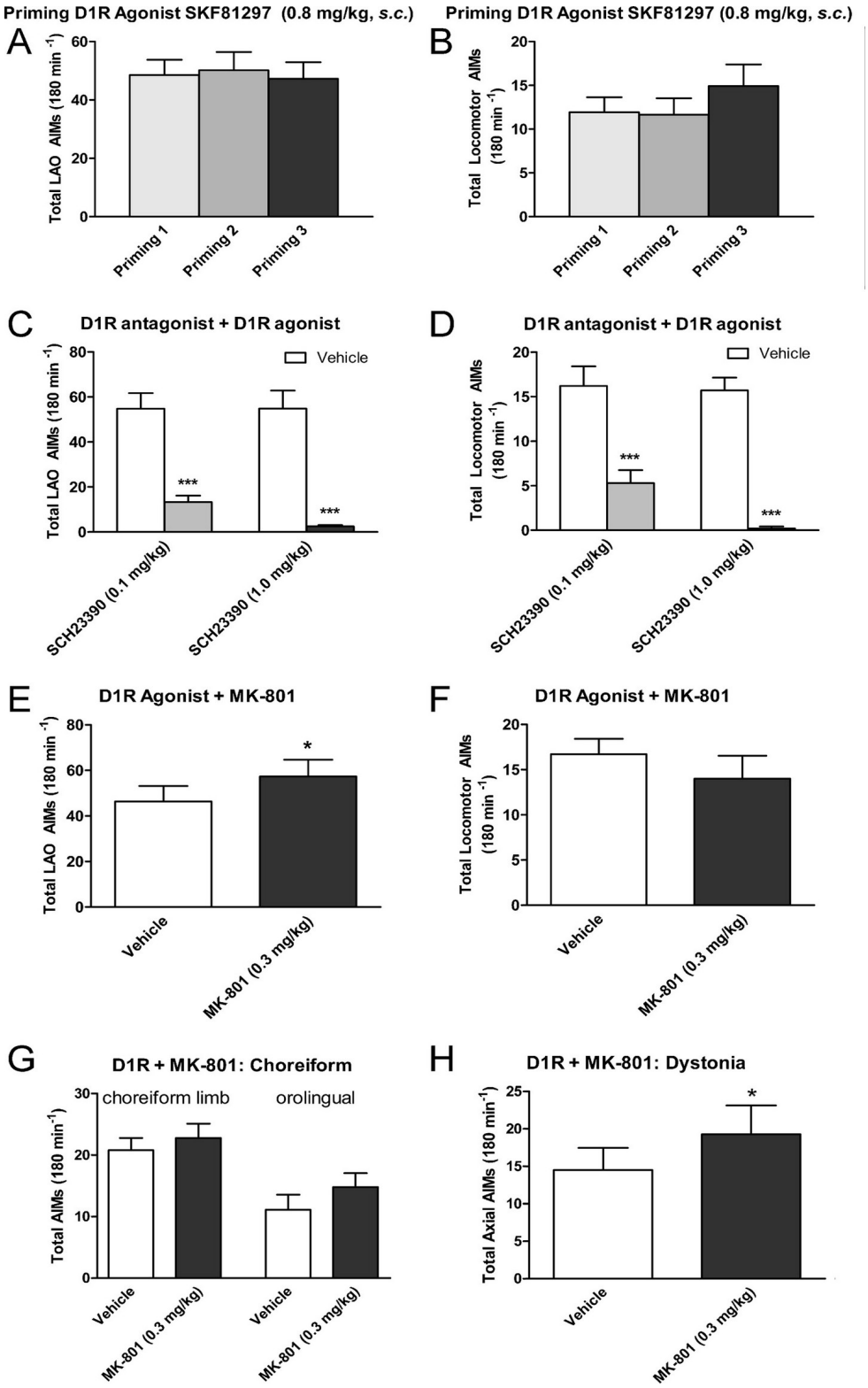
Priming D1R Agonist SKF81297 (0.8 mg/kg, s.c.)
A
Total LAO AIMs (180 min -1)
Priming 1
Priming 2
Priming 3
Priming D1R Agonist SKF81297 (0.8 mg/kg, s.c.)
B
Total Locomotor AIMs (180 min -1)
Priming 1
Priming 2
Priming 3
C
D1R antagonist + D1R agonist
Vehicle
Total LAO AIMs (180 min -1)
***
***
SCH23390 (0.1 mg/kg)
SCH23390 (1.0 mg/kg)
D
D1R antagonist + D1R agonist
Vehicle
Total Locomotor AIMs (180 min -1)
***
***
SCH23390 (0.1 mg/kg)
SCH23390 (1.0 mg/kg)
E
D1R Agonist + MK-801
Total LAO AIMs (180 min -1)
*
Vehicle
MK-801 (0.3 mg/kg)
F
D1R Agonist + MK-801
Total Locomotor AIMs (180 min -1)
Vehicle
MK-801 (0.3 mg/kg)
G
D1R + MK-801: Choreiform
choreiform limb
orolingual
Total AIMs (180 min -1)
Vehicle
MK-801 (0.3 mg/kg)
Vehicle
MK-801 (0.3 mg/kg)
H
D1R + MK-801: Dystonia
Total Axial AIMs (180 min -1)
*
Vehicle
MK-801 (0.3 mg/kg)

Figure 2. Establishing the D1R agonist-induced AIMs model. 3 priming sessions with the D1R agonist SKF81297 (0.8 mg/kg, *s.c.*) induce AIMs in rats with established L-DOPA-induced-AIMs. (A) Mean LAO AIMs at the 3 priming sessions using SKF81297. (B) Mean locomotor AIMs elicited by SKF81297. (C) The D1R agonist-induced LAO AIMs were blocked by the D1R antagonist SCH23390. (D) The D1R agonist-induced locomotor AIMs were also blocked by the D1R antagonist SCH23390. MK-801 (0.3 mg/kg) worsens D1R agonist-induced LAO AIMs. In (E) mean LAO AIMs are shown. (F) The graph depicts mean locomotor AIMs, and shows no effect of MK-801 to either block locomotor AIMS or induce ipsiversive AIMs. In (G and H) mean choreiform limb, orolingual and dystonic axial AIMs are depicted separately, showing the effect of MK-801 is predominantly on dystonic AIMs, and to lesser extent on orolingual AIMs. All graphs show the mean data ± SEM acquired after injection of drugs. Significant differences from vehicle control experiments in the same testing sessions are depicted by asterisks, ***$p < 0.001$, *$p < 0.05$ (n = 7–9; two-tailed t-tests).

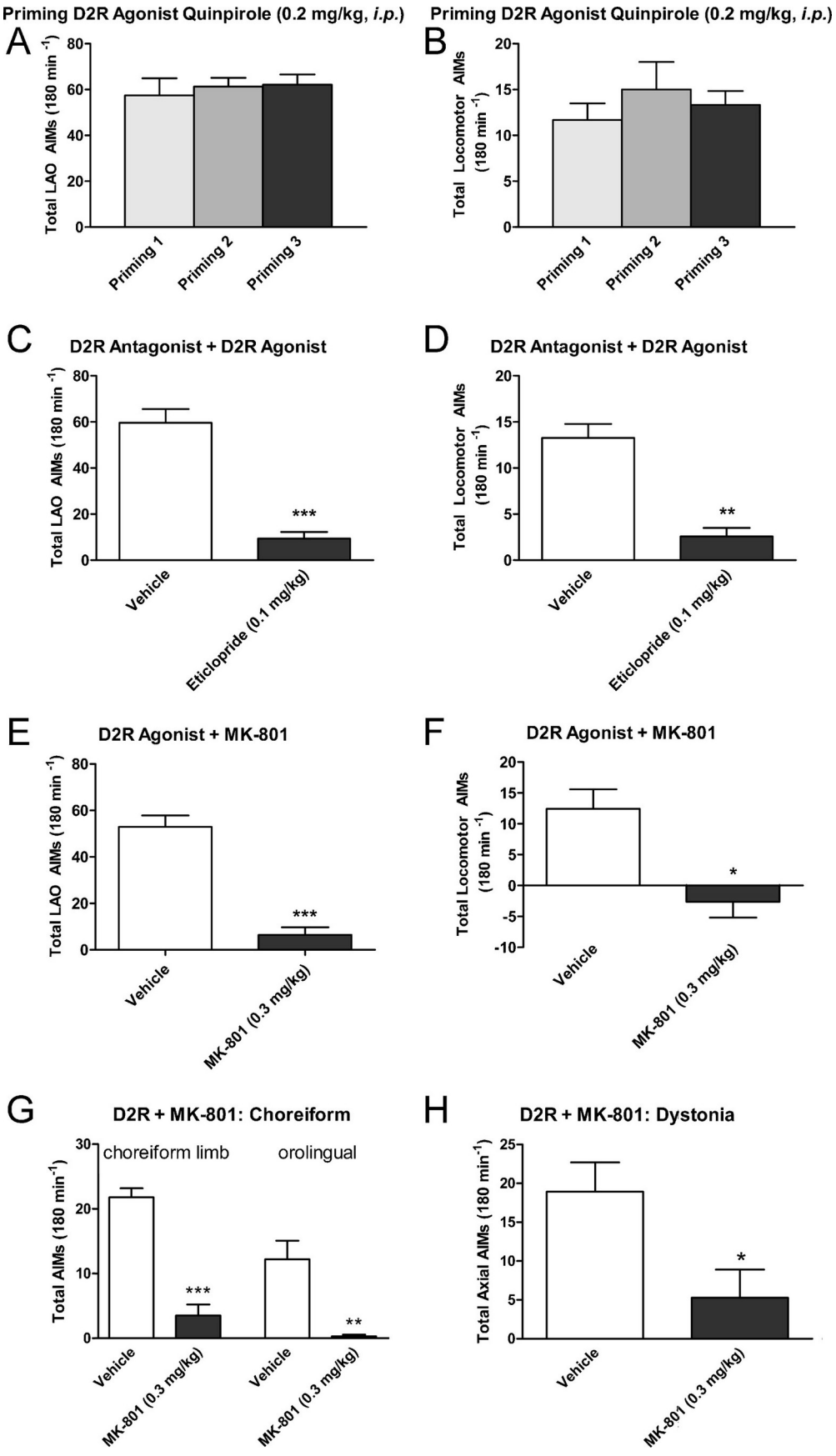
A
Priming D2R Agonist Quinpirole (0.2 mg/kg, i.p.)
Total LAO AIMs (180 min -1)
Priming 1
Priming 2
Priming 3
B
Priming D2R Agonist Quinpirole (0.2 mg/kg, i.p.)
Total Locomotor AIMs (180 min -1)
Priming 1
Priming 2
Priming 3
C
D2R Antagonist + D2R Agonist
Total LAO AIMs (180 min -1)
***
Vehicle
Eticlopride (0.1 mg/kg)
D
D2R Antagonist + D2R Agonist
Total Locomotor AIMs (180 min -1)
**
Vehicle
Eticlopride (0.1 mg/kg)
E
D2R Agonist + MK-801
Total LAO AIMs (180 min -1)
***
Vehicle
MK-801 (0.3 mg/kg)
F
D2R Agonist + MK-801
Total Locomotor AIMs (180 min -1)
*
Vehicle
MK-801 (0.3 mg/kg)
G
D2R + MK-801: Choreiform
Total AIMs (180 min -1)
choreiform limb
orolingual
***
**
Vehicle
MK-801 (0.3 mg/kg)
Vehicle
MK-801 (0.3 mg/kg)
H
D2R + MK-801: Dystonia
Total Axial AIMs (180 min -1)
*
Vehicle
MK-801 (0.3 mg/kg)

Figure 3. Establishing the D2R agonist-induced AIMs model. 3 priming sessions with the D2R agonist quinpirole (0.2 mg/kg, *i.p.)* induce AIMs in rats with established L-DOPA-induced-AIMs. (A) LAO AIMs at the 3 priming sessions using. (B) Mean locomotor AIMs elicited by quinpirole. (C) The D2R agonist-induced LAO AIMs were blocked by the D2R antagonist eticlopride. (D) The D2R agonist-induced locomotor AIMs were also blocked by the D2R antagonist eticlopride. MK-801 (0.3 mg/kg) blocks D2R agonist-induced AIMs (quinpirole, 0.2 mg/kg, *i.p.*). In (E) mean LAO AIMs are shown. (F) The graph depicts mean locomotor AIMs after quinpirole injection, and shows that MK-801 induced ipsiversive locomotor AIMs. Mean choreiform limb, orolingual (G) and dystonic axial (H) AIMs are depicted separately, showing that all are effectively reduced by MK-801. All graphs show the mean data ± SEM (n = 7–9) after injection of drugs. Significant differences from vehicle control experiments in the same testing sessions are depicted by asterisks, ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$ (n = 7–9; two-tailed t-tests).

2.7 References

Ahmed, I., Bose, S. K., Pavese, N., Ramlackhansingh, A., Turkheimer, F., Hotton, G., Hammers, A., & Brooks, D. J. (2011). Glutamate NMDA receptor dysregulation in Parkinson's disease with dyskinesias. *Brain*, *134*(4), 979–986. https://doi.org/10.1093/brain/awr028

Belujon, P., Lodge, D. J., & Grace, A. A. (2010). Aberrant striatal plasticity is specifically associated with dyskinesia following levodopa treatment. *Movement Disorders*, *25*(11), 1568–1576. https://doi.org/10.1002/mds.23245

Calon, F., Birdi, S., Rajput, A. H., Hornykiewicz, O., Bédard, P. J., & Di Paolo, T. (2002). Increase of preproenkephalin mRNA levels in the putamen of Parkinson disease patients with levodopa-induced dyskinesias. *Journal of Neuropathology and Experimental Neurology*, *61*(2), 186–196. http://www.ncbi.nlm.nih.gov/pubmed/11853020

Campbell, B. M., Kreipke, C. W., & Walker, P. D. (2006). Failure of MK-801 to suppress D1 receptor-mediated induction of locomotor activity and striatal preprotachykinin mRNA expression in the dopamine-depleted rat. *Neuroscience*, *137*(2), 505–517. https://doi.org/10.1016/j.neuroscience.2005.09.024

Day, M., Wang, Z., Ding, J., An, X., Ingham, C. A., Shering, A. F., Wokosin, D., Ilijic, E., Sun, Z., Sampson, A. R., Mugnaini, E., Deutcch, A. Y., Sesack, S. R., Arbuthnott, G. W., & Surmeier, D. J. (2006). Selective elimination of glutamatergic synapses on striatopallidal neurons in Parkinson disease models. *Nature Neuroscience*, *9*(2), 251–259. https://doi.org/10.1038/nn1632

Dekundy, A., Lundblad, M., Danysz, W., & Cenci, M. A. (2007). Modulation of l-DOPA-induced abnormal involuntary movements by clinically tested compounds: Further validation of the rat dyskinesia model. *Behavioural Brain Research*, *179*(1), 76–89. https://doi.org/10.1016/j.bbr.2007.01.013

Dupre, K. B., Eskow, K. L., Negron, G., & Bishop, C. (2007). *The differential effects of 5-HT 1A receptor stimulation on dopamine receptor-mediated abnormal involuntary movements and rotations in the primed hemiparkinsonian rat*. https://doi.org/10.1016/j.brainres.2007.05.005

Dupre, K. B., Ostock, C. Y., Jaunarajs, K. L. E., Button, T., Savage, L. M., Wolf, W., & Bishop, C. (2011). *Local modulation of striatal glutamate efflux by serotonin 1A receptor stimulation in dyskinetic, hemiparkinsonian rats*. https://doi.org/10.1016/j.expneurol.2011.02.012

Fox, S. H., Brotchie, J. M., & Lang, A. E. (2008). Non-dopaminergic treatments in development for Parkinson's disease. In *The Lancet Neurology* (Vol. 7, Issue 10, pp. 927–938). https://doi.org/10.1016/S1474-4422(08)70214-X

Gerfen, C. R., & Surmeier, D. J. (2011). Modulation of Striatal Projection Systems by Dopamine. *Annual Review of Neuroscience*, *34*(1), 441–466. https://doi.org/10.1146/annurev-neuro-061010-113641

Greengard, P. (2001). The neurobiology of slow synaptic transmission. In *Science* (Vol. 294, Issue 5544, pp. 1024–1030). https://doi.org/10.1126/science.294.5544.1024

Jonkers, N., Sarre, S., Ebinger, G., & Michotte, Y. (2002). MK801 suppresses the L-DOPA-induced increase of glutamate in striatum of hemi-Parkinson rats. In *Brain Research* (Vol. 926). www.elsevier.com/locate/bres

Kobylecki, C., Hill, M. P., Crossman, A. R., & Ravenscroft, P. (2011). Synergistic antidyskinetic effects of topiramate and amantadine in animal models of Parkinson's disease. *Movement Disorders*, *26*(13), 2354–2363. https://doi.org/10.1002/mds.23867

Lundblad, M., Andersson, M., Winkler, C., Kirik, D., Wierup, N., Cenci Nilsson, M. A., & Cenci, M. A. (2002). Pharmacological validation of behavioural measures of akinesia and dyskinesia in a rat model of Parkinson's disease. *The European Journal of Neuroscience*, *15*(1), 120–132. https://doi.org/10.1046/j.0953-816x.2001.01843.x

Mefford, I. N., Gilberg, M., & Barchas, J. D. (1980). Simultaneous determination of catecholamines and unconjugated 3,4-dihydroxyphenylacetic acid in brain tissue by ion-pairing reverse-phase high-performance liquid chromatography with electrochemical detection. *Analytical Biochemistry*, *104*(2), 469–472. https://doi.org/10.1016/0003-2697(80)90101-3

Papa, S. M., & Chase, T. N. (1996). Levodopa-induced dyskinesias improved by a glutamate antagonist in Parkinsonian monkeys. *Annals of Neurology*, *39*(5), 574–578. https://doi.org/10.1002/ana.410390505

Paquette, M. A., Anderson, A. M., Lewis, J. R., Meshul, C. K., Johnson, S. W., & Paul Berger, S. (2010). MK-801 inhibits l-DOPA-induced abnormal involuntary movements only at doses that worsen parkinsonism. *Neuropharmacology*, *58*(7), 1002–1008. https://doi.org/10.1016/j.neuropharm.2010.01.003

Paquette, M. A., Martinez, A. A., Macheda, T., Meshul, C. K., Johnson, S. W., Berger, S. P., & Giuffrida, A. (2012a). Anti-dyskinetic mechanisms of amantadine and dextromethorphan in the 6-OHDA rat model of Parkinson's disease: Role of NMDA vs. 5-HT1A receptors. *European Journal of Neuroscience*, *36*(9), 3224–3234. https://doi.org/10.1111/j.1460-9568.2012.08243.x

Pollack, A. E., & Haisley, E. C. (2001). (No Title). In *Brain Research* (Vol. 897). www.elsevier.com/locate/bres

Robelet, S., Melon, C., Guillet, B., Salin, P., & Kerkerian-Le Goff, L. (2004). Chronic L-DOPA treatment increases extracellular glutamate levels and GLT1 expression in the basal ganglia in a

rat model of Parkinson's disease. *European Journal of Neuroscience*, *20*(5), 1255–1266. https://doi.org/10.1111/j.1460-9568.2004.03591.x

Shen, W., Flajolet, M., Greengard, P., & Surmeier, D. J. (2008). Dichotomous dopaminergic control of striatal synaptic plasticity. *Science*, *321*(5890), 848–851. https://doi.org/10.1126/science.1160575

Taylor, J. L., Bishop, C., Ullrich, T., Rice, K. C., & Walker, P. D. (2006). Serotonin 2A receptor antagonist treatment reduces dopamine D1 receptor-mediated rotational behavior but not l-DOPA-induced abnormal involuntary movements in the unilateral dopamine-depleted rat. *Neuropharmacology*, *50*(6), 761–768. https://doi.org/10.1016/j.neuropharm.2005.12.004

Van De Witte, S. V., Groenewegen, H. J., & Voorn, P. (2002). MK-801 alters the effects of priming with L-DOPA on dopamine D1 receptor-induced changes in neuropeptide mRNA levels in the rat striatal output neurons. *Synapse*, *43*(1), 1–11. https://doi.org/10.1002/syn.1113

Winkler, C., Kirik, D., Björklund, A., & Cenci, M. A. (2002). L-DOPA-induced dyskinesia in the intrastriatal 6-hydroxydopamine model of parkinson's disease: relation to motor and cellular parameters of nigrostriatal function. *Neurobiology of Disease*, *10*(2), 165–186. http://www.ncbi.nlm.nih.gov/pubmed/12127155

Yue, X., Hariri, D. J., Caballero, B., Zhang, S., Bartlett, M. J., Kaut, O., Mount, D. W., Wüllner, U., Sherman, S. J., & Falk, T. (2014). Comparative study of the neurotrophic effects elicited by VEGF-B and GDNF in preclinical in vivo models of Parkinson's disease. *Neuroscience*, *258*, 385–400. https://doi.org/10.1016/j.neuroscience.2013.11.038

Chapter 3: The combination of the opioid glycopeptide MMP-2200 and a NMDA receptor antagonist reduced L-DOPA-induced dyskinesia and MMP-2200 by itself reduced dopamine receptor 2-like agonist-induced dyskinesia

Andrew J.Flores[ab], Mitchell J.Bartlett[ae], Brandon K.Root[a], Kate L.Parent[c], Michael L. Heien[c], Frank Porreca[d], Robin Polt[c], Scott J.Sherman[a], Torsten Falk[abd]

a Department of Neurology, b Graduate Interdisciplinary Program in Physiological Sciences, c Department of Chemistry & Biochemistry and BIO5 Institute, d Department of Pharmacology, The University of Arizona, Tucson, AZ, 85724, e Graduate Program in Medical Pharmacology, The University of Arizona, Tucson, AZ, 85724, USA

Published in *Neuropharmacology*

141 (2018), 260-271

https://doi.org/10.1016/j.neuropharm.2018.09.005

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6309213/

Contributions

Conception and design: Torsten Falk, Robin Polt, Andrew J. Flores
Data collection: Andrew J. Flores (surgery, drug administration, behavioral analysis, IHC), Mitchell J. Bartlett (drug administration, behavioral analysis), Kate L. Parent (HPLC analysis), Brandon K. Root (surgery)
Data analysis and interpretation: Andrew J. Flores, Torsten Falk
Drafting the article: Andrew J. Flores, Torsten Falk, Scott J. Sherman
Critical revision of article: Torsten Falk, Scott J. Sherman, Robin Polt, Frank Porreca, Michael L. Heien, Andrew J. Flores, Mitchell J. Bartlett

Highlights

- The opioid glycopetide MMP-2200 has modest antiparkinsonian activity.
- MMP-2200 reduces dyskinesia induced by activation of D2R-like dopamine receptors.
- The NMDA receptor antagonist MK-801 is anti-dyskinetic, but also pro-parkinsonian.

• MMP-2200 + MK-801 reduces L-DOPA-induced dyskinesia.
• MMP-2200 does suppress the pro-parkinsonian activity of MK-801.

Abstract

Dopamine (DA)-replacement therapy utilizing levodopa-DOPA is the gold standard symptomatic treatment for Parkinson's disease (PD). A critical complication of this therapy is the development of levodopa-DOPA-induced dyskinesia (LID). The endogenous opioid peptides, including enkephalins and dynorphin, are co-transmitters of dopaminergic, GABAergic, and glutamatergic transmission in the direct and indirect striatal output pathways disrupted in PD, and alterations in expression levels of these peptides and their precursors have been implicated in LID genesis and expression. We have previously shown that the opioid glycopeptide drug MMP-2200 (a.k.a. Lactomorphin), a glycosylated derivative of Leu-enkephalin mediates potent behavioral effects in two rodent models of striatal DA depletion. In this study, the mixed mu-delta agonist MMP-2200 was investigated in standard preclinical rodent models of PD and of LID to evaluate its effects on abnormal involuntary movements (AIMs). MMP-2200 showed antiparkinsonian activity, while increasing L-DOPA-induced limb, axial, and oral (LAO) AIMs by ~10%, and had no effect on dopamine receptor 1 (D1R)-induced LAO AIMs. In contrast, it markedly reduced dopamine receptor 2 (D2R)-like-induced LAO AIMs. The locomotor AIMs were reduced by MMP-2200 in all three conditions. The *N*-methyl-*d*-aspartate receptor (NMDAR) antagonist MK-801 has previously been shown to be anti-dyskinetic, but only at doses that induce parkinsonism. When MMP-2200 was co-administered with MK-801, MK-801-induced pro-parkinsonian activity was suppressed, while a robust anti-dyskinetic effect remained. In summary, the opioid glycopeptide MMP-2200 reduced AIMs induced by a D2R-like agonist, and

MMP-2200 modified the effect of MK-801 to result in a potent reduction of L-DOPA-induced AIMs without induction of parkinsonism.

3.1 Introduction

Parkinson's disease (PD) is a common neurodegenerative disease characterized by a hypokinetic movement disorder with motor symptoms of resting tremor, bradykinesia, muscular rigidity and postural instability (Olanow, 2009). The underlying pathology of PD is associated with chronic, progressive degeneration of dopaminergic neurons with cell bodies located in the substantia nigra pars compacta (SNpc) and axon terminals projecting to the striatum. With the degeneration of nigral dopaminergic neurons, levels of striatal dopamine (DA) become progressively depleted, thus causing an imbalance in the so-called direct and indirect striatal output pathways of the basal ganglia and the corresponding development of hypokinetic motor features. DA replacement strategies relying primarily on use of the DA precursor levodopa (L-DOPA), which act to restore striatal levels of DA, continue to be the mainstay of treatment for the symptoms of PD. These therapies become unsatisfactory over time, however, due to the need for dose escalation and the development of debilitating motor complications including on-off effects and levodopa-induced dyskinesia (LID). Therefore, there is an urgent need to develop non-dopaminergic adjunct therapies (Fox et al., 2008; Hille et al., 2001) that can modify the course of development of LID and/or directly ameliorate established LID. Although opioid drugs have not currently found utility in the treatment of PD, they are known to have a profound influence on motor function and reward behavior by acting as metabotropic modulators of DA, GABA, and glutamate neurotransmission. Furthermore, the striatum expresses a high density of both μ- and δ-opioid receptors (Fox et al., 2006). Striatal medium spiny

neurons (MSNs) utilize the opioid peptides including dynorphin and enkephalins as co-transmitters, and alterations in levels of these peptides have been consistently observed in PD and LID. Striatopallidal projection neurons of the indirect pathway express enkephalins derived from the precursor preproenkephalin A (PPE-A, Penk), while the striatonigral neurons of the direct pathway express opioid peptides derived from preproenkephalin B (PPE-B, Pdyn) (Cuello & Paxinos, 1978; Gerfen, 1990). Enkephalins are endogenous ligands for δ-opioid receptors, while opioid peptides derived from PPE-B are predominantly endogenous ligands for μ, and κ opioid receptor subtypes (Breslin et al., 1993; Seizinger et al., 1984). In PD following dopamine depletion, striatal levels of PPE-A mRNA and enkephalin are upregulated, whereas levels of PPE-B mRNA and dynorphin are downregulated (Cenci et al., 1998; Nisbet et al., 1995; Sgroi et al., 2016).

Following long-term L-DOPA therapy leading to the development of dyskinesia, levels of the opioid peptides enkephalin and dynorphin, and mRNAs encoding their precursors are elevated in animal models of PD (Cenci et al., 1998; Duty and Brotchie, 1997; Engber et al., 1991; Henry et al., 1999; Samadi et al., 2006). Postmortem studies of PD patients with LID, revealed increased expression of striatal PPE-A (Calon et al., 2002; Nisbet et al., 1995) and PPE-B expression (Henry et al., 2003). These alterations suggest that the increased enkephalin transmission could be a mechanism that compensates for the downstream consequences for DA depletion in PD.

Given this background, novel opioid compounds could provide an important non-dopaminergic treatment for PD and other movement disorders. From our perspective, opioid glycopeptides offer several advantages over alkaloid-based small molecule compounds, as the likelihood of toxicity and side effects due to the production of active metabolites is greatly reduced with glycopeptide-based drugs versus alkaloids, as opioid glycopeptides are metabolized merely to di- and tri-peptides, amino acids and sugars (Bilsky et al., 2000; Gengo & Chang, 2003; Portoghese et al., 1987), while morphine and similar alkaloid-like drugs produce a cascade of toxic metabolites, which vary from individual to

individual. On the other hand, the need for active metabolism can also be a problem, for example, 10% of patients cannot demethylate codeine, and thus obtain no benefit from the drug (Crews et al., 2014). MMP-2200 is a glycosylated derivative of the opioid peptide Leu-enkephalin that has mixed-δ/μ opioid receptor agonist activity. The relative activity of MMP-2200 on μ vs. δ opioid receptors is 1:2 (Lowery et al., 2011). It has excellent blood-brain barrier penetration following systemic (*i.p.*) administration as determined by microdialysis (Mabrouk et al., 2012), reaches the dorsolateral striatum at high levels, and has been shown to produce potent behavioral effects on movements related to dopaminergic hyper-stimulation following striatal dopamine depletion (Yue et al., 2011). In mouse models of pain MMP-2200 has reduced liability for addiction-associated behavior compared to morphine (Bilsky et al., 2000; Elmagbari et al., 2004; Lowery et al., 2011). In this study, we set out to test if MMP-2200 also has anti-dyskinetic properties, using a well-established rodent model of LID that employs quantification of limb-axial-oral involuntary movements and dopamine-induced rotational movements. We also tested the combination of MMP-2200 + the *N*-methyl-d-aspartate receptor (NMDAR) antagonist MK-801, based on the hypothesis that activation of the opioid system in the basal ganglia by MMP-2200, as demonstrated in prior work (Yue et al., 2011), will lead to downstream activation of the dopaminergic system and might reduce the known pro-parkinsonian activity of the anti-dyskinetic drug MK-801 (Paquette et al., 2010; Flores et al., 2014).

3.2 Materials and methods

3.2.1. Stereotaxic surgery

Male Sprague-Dawley rats (250 g; Charles River, Wilmington, MA), were used and housed in a temperature and humidity-controlled room with 12 hour light/dark cycles with food and water available

ad libitum. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Arizona and conformed to the guidelines of the National Institutes of Health, and also comply with the ARRIVE guidelines. The number of animals used and their suffering was minimized. Animals were acclimated in the animal facility for 1 week prior to surgery. 1) For the severe unilateral 6-OHDA lesion, a total volume of 4.0 µL freshly prepared 6-hydroxydopamine hydrobromide solution (Sigma-Aldrich, St. Louis, MO; 2.5 µg per 1.0 µL in 0.9% sterile saline with 0.02% ascorbic acid) was injected into two locations in the medial forebrain bundle with the following coordinates (AP -2.8 and -4.7, ML -1.8 and -1.5, D -8.0 and -7.9) according to the atlas of Paxinos & Watson (2017). Injections were carried out at a rate of 1.0 µL per min using a 10 µL syringe and needle (33 gauge, 22 mm, 45° tip; Hamilton Company, Reno, NV) with a Stoelting microinjector (Stoelting Co., Wood Dale, IL); the needle was left in place post-injection for a further 3 min to prevent any backflow of 6-OHDA solution upon withdrawal. Rats were pretreated 30 min prior to the 6-OHDA infusion with 12.5 mg/kg (*i.p.*) desipramine to prevent uptake of 6-OHDA by noradrenergic neurons. Following a 2-week period, animals were screened for unilateral dopaminergic denervation using the amphetamine-induced rotations test.

3.2.2 Amphetamine-induced rotations

To evaluate the severity/extent of DAergic lesions, unilateral 6-OHDA-lesioned rats were injected with amphetamine (5.0 mg/kg, *i.p.*) to induce asymmetrical dopamine release from the SNpc projections to the striatum. For each animal, total ipsiversive rotations were counted for a total of 60 min after the injection and the average number rotations per minute were calculated. Two weeks post-surgery, rats showing an average of ≥ 4 full rotations per min ipsilateral to the side of the 6-OHDA lesion were

selected for further experimentation in the dyskinesia model. This number of rotations has previously been shown to correspond to levels of striatal dopamine depletion > 90% (Dekundy et al., 2007).

3.2.3 Forelimb adjusting steps (FAS) test

Motor performance and anti-PD activity of L-DOPA was measured using a procedure previously described (Chang et al., 1999; Eskow et al., 2007). A 0.9-meter distance is marked off on a smooth table. The rat is held by the experimenter with one hand supporting, but obstructing movement of the hindlimbs. The hind limbs are then slightly raised off the surface of the table. The experimenter's second hand is used to support and obstruct the movement of the forelimb not being tested. The forelimb being tested is allowed to touch the table and then moved sideways over the set distance at a rate of 0.1 m/s. The experimenter moves the forelimb first in the backhand direction, followed by the forehand. Each forelimb was tested three times per session. The number of adjusting steps made in each direction and trial was counted to give an average score. All tests were performed by a blinded observer.

3.2.4. L-DOPA-induced AIMs

Severely lesioned rats were primed on L-DOPA (7 mg/kg; with 14 mg/kg benserazide) via once-daily injections (*i.p.*) for 21 consecutive days. The animals were then injected twice per week thereafter to maintain the dyskinetic state. AIMs were assessed by an observer blind to treatment condition, using an adapted version of the Abnormal Involuntary Movement Scale described by Cenci et al. (1998), previously described by Paquette et al. (2010). Each animal was rated on a scale of 0 (absent) to 4 (most severe) on each of four subscales (limb dyskinesia, axial dystonia, oral dyskinesia, and contraversive rotation). Limb, axial, oral, and locomotor type AIMs were based on 1 min observation

intervals conducted every 20 min time point for 180 minutes after administration of drugs. Specifically, for limb dyskinesia, rating of 1 indicated 1 discrete period of abnormal movement, 2 indicated 3 or more discrete periods of abnormal movement, and 3-4 indicated continuous abnormal movement, with 4 indicating that AIMs could not be interrupted by a loud tap on the test cage. For axial dystonia, a rating of 1 indicated a contralateral bias in head orientation, 2 indicated a contralateral bias in head and upper body orientation, and 3-4 indicated a severe contralateral bias in head and upper body orientation (i.e., head above the tail in a four-paw stance), with 4 indicating loss of balance (i.e., falling). For oral dyskinesia, a rating of 1 indicated 2-3 bouts of mastication, 2 indicated more than 3 bouts of mastication, and 3-4 indicated continuous mastication, with 4 indicating the presence tongue protrusions. For L-DOPA-induced contraversive rotation, a rating of 1 indicated 2-3 contraversive turns, 2 indicated more than 3 contraversive turns, and 3-4 indicated continuous contraversive rotation, with a 4 indicating that these could not be interrupted by a loud tap on the cage. When ipsiversive rotation was observed, this was rated identically to contraversive rotation, except a negative score was provided to indicate ipsiversive directionality. Limb, axial, and oral subscale scores were summed to create a composite LAO AIMs score, while Locomotor AIMs scores were considered separately from LAO scores. Total scores for AIMs, as well as for each subscale, were summed over the entire duration of the experiment (180 minutes).

3.2.5 Pharmacological treatments and procedure

A within-subjects crossover design was utilized to control for variability between experiments. In this design, each experiment consisted of two days of testing, 3-4 days apart. On the first testing day, half of the animals received the experimental treatment while the other half received the vehicle control; on

the second testing day, the conditions were reversed. The assignment of animals to vehicle vs. experimental conditions on the first day was randomized. This experimental paradigm was designed to control for within-subject variability and to ensure that no progressive changes in the animals' baseline level of AIMs occurs. An important aspect of our experimental design is that a separate vehicle control test is performed for each drug and/or drug combination tested. All drugs were dissolved in 0.9% USP grade sterile saline and delivered via intraperitoneal (*i.p.*) injection at a volume of 1 ml/kg, except for SKF81297, which was dissolved in a vehicle mixture of 20% DMSO: 80% saline and was delivered subcutaneously (*s.c.*). The procedure for the establishment of AIMs induced by the selective dopamine receptor agonists was performed in the same manner as described in (Flores et al., 2014). The novel opioid glycopeptide MMP-2200 (*N*-Tyr-D-Thr-Gly-Phe-Leu-Ser-(*O*-β-D-lactose)-CONH; *a.k.a.* Lactomorphin; as 1xAcOH salt) is a GMP-compliant material synthesized by UCB Bioproducts, Belgium; the structure of MMP-2200 is described in detail in Do Carmo et al. (2008). L-DOPA (levodopa; L-3,4-dihydroxyphenylalanine methyl ester hydrochloride), benserazide hydrochloride (DL-serine 2-(2,3,4-trihydroxybenzyl) hydrazide hydrochloride) and (+)-MK-801 ((5*S*,10*R*)-(+)-5-Methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine) hydrogen maleate were purchased from Sigma-Aldrich, St. Louis, MO; the highly selective non-peptide δ-opioid receptor (DOR) antagonist naltrindole (17-(Cyclopropylmethyl)-6,7-dehydro-4,5α-epoxy-3,14-dihydroxy-6,7-2',3'-indolomorphinan hydrochloride), the selective D_1R agonist SKF81297 (0.8 mg/kg, *s.c.*) and the selective D_2R-D_3R agonist quinpirole (0.2 mg/kg, *i.p.*) were purchased from Tocris Bioscience/R&D Systems, Inc., Minneapolis, MN. Naltrindole (3 mg/kg, *i.p.*) was administered 10 min prior to testing.MMP-2200, SKF81297 (0.8 mg/kg, *s.c.*) and quinpirole (0.2 mg/kg, *i.p.*) were administered at the beginning (t = 0 min) of testing. The naltrindole dose was chosen based on our prior work showing that this dose blocks effects of MMP-2200 in 6-OHDA-lesioned rats (Yue et al., 2011). MK-801 was given 25 min prior to testing.

The total study *N* is 39. In a first pilot *cohort 1* $n = 12$ animals received unilateral 6-OHDA lesions and $n = 10$ developed a sufficiently severe PD lesion. The average amphetamine-induced rotation counts per min was 5.05 (SEM = 0.05). These animals were then primed with L-DOPA for 3 weeks and $n = 8$ developed LID. These LID animals were tested for effects of MMP-2200 on L-DOPA-induced AIMs. In *cohort 2,* $n = 15$ animals received unilateral 6-OHDA lesions, and $n = 10$ developed a sufficiently severe PD lesion. The average amphetamine-induced rotation counts per min was 5.89 (SEM = 0.38). These animals were then primed with L-DOPA for 3 weeks and $n = 10$ developed LID. These were sequentially tested with L-DOPA, MMP-2200, naltrindole, and MK-801, followed by the SKF81297 and quinpirole experiments. In *cohort 2,* three animals died from unrelated causes during these long-term studies over several months, therefore there is only $n = 9$-10 for the MK-801 experiments, and $n = 7$-8 for the SKF81297 and quinpirole experiments. For the final analysis of the effects of MMP-2200 on L-DOPA AIMs, the data from *cohort 1* and *cohort 2* were combined to increase power ($n = 17$-18). The three animals in *cohorts* 1 and 2 that were showing L-DOPA-induced LAO AIMs, but did not exhibit any L-DOPA-induced locomotor AIMs to begin with, excluded from the analysis of the effects of MMP-2200 on L-DOPA-induced contralateral locomotor AIMs. In the *cohort 3,* $n = 12$ animals received unilateral 6-OHDA-lesions, and $n=10$ developed a sufficiently severe PD lesion. The average amphetamine-induced rotation counts per min was 7.3 (SEM = 1.1). In these 10 PD animals, MMP-2200 was tested with the FAS test to evaluate anti-parkinsonian activity. These animals were then primed with L-DOPA and $n = 7$ developed dyskinesia after 3 weeks. In these 7 LID animals the effect of L-DOPA + MMP-2200 + MK-801 on the non-drug induced FAS test was evaluated.

3.2.6 Euthanasia and brain tissue harvest

Rats were sacrificed 1 day after the last dose of drug with Euthasol (0.35 mg/kg, *i.p.*; Virbac, Fort Worth, TX, United States). For a quantitative measure of the extent of the PD-lesion with our protocol, for $n = 7$ animals, the whole brains were extracted and prepped for semi-quantitative western analysis of tyrosine hydroxylase (TH) and quantitative DA measurements, as described below in section 2.7. In the preparation for the DA measurement, one sample was not recovered, leading to an $n = 6$ for that analysis. For the remaining animals, the whole brains were extracted after transcardial perfusion-fixation with phosphate buffered saline (PBS) followed by cold 4% paraformaldehyde in PBS, and further fixed overnight in 4% paraformaldehyde. Immunohistochemical analysis (IHC) was done for visual verification of lesion, as described below in section 2.8.

3.2.7 DA, serotonin and TH measurements

Rat brains were washed in chilled Tris buffer (pH = 7.4, 15 mM Tris, 125 mM NaCl, 2.5 mM KCl, 2 mM $CaCl_2$) for 30 s and placed in a chilled brain block. Coronal brain slices were collected and a 2 mm steel biopsy punch was used to sample tissue from the striatum. Samples from left and right hemispheres were collected and immediately flash frozen on an aluminum pan at -70 °C. Samples massed at 2.5 ± 0.5 mg, were placed in 1.5 mL homogenization vials with 100 μL of 0.1 N $HClO_4$ (aq), manually homogenized (15 strokes) using a disposable pestle and stored at -80 °C for up to 2 weeks prior to analysis. High performance liquid chromatography with electrochemical detection (HPLC-EC) was used to separate and quantify DA, 3,4-dihydroxyphenylacetic acid (DOPAC), serotonin, and 5-hydroxyindoleacetic acid (5-HIAA), per established protocol (Mefford et al., 1980). After the tissue punch, described above, the remaining striata from left and right hemispheres were immediately flash frozen and stored at −80 °C. Total protein was prepared by homogenizing and semi-quantitative

western analysis of striatal tyrosine hydroxylase (TH) content was conducted as described to quantify the extent of the 6-OHDA-lesion (Flores et al., 2014). 10 μg of protein was loaded for each sample to measure TH and β-Actin as internal control.

3.2.8 Immunohistochemical staining

The IHC analysis was performed as described in detail in prior work (Yue et al., 2011). 40 μm sections yielding the substantia nigra and the striatum were obtained using a vibratome (Pelco 101 Series-1000, Pelco, Clovis, CA) and mounted on standard glass slides (Fisher Scientific). Next, the sections were stained with a rabbit anti-TH primary antibody (1:10,000 for 24 hours at 4°C; Millipore) and a biotinylated donkey anti-rabbit immunoglobulin G secondary antibody (1:1,000 for 2 hours at RT; Millipore). To eliminate nonspecific binding prior to immunostaining endogenous peroxidase activity was blocked using 0.3% H_2O_2 for 30 min at room temperature (RT), the slides were then washed 3 times for 5 min with Tris buffer (pH = 7.6) and submerged in blocking solution (1% normal donkey serum, 0.1% Triton-X-100, Tris-HCl buffer, pH 7.6) for 2 hours at RT. The signal from the secondary antibody was amplified using the ABC reagent (Vectastain Elite ABC Kit, PK-6100) according to the manufacturer's protocol. We used DAB substrate (Vector Laboratories, Burlingame, CA) as the chromogen for final visualization of TH immunoreactivity.

3.2.9 Statistical analyses

All statistical analyses were performed with Prism 7 software (Graphpad, La Jolla CA). Nonparametric Kruskal-Wallis tests with Dunn's multiple comparisons *post hoc* tests were performed to compare total LAO and locomotor AIMs scores. AIMs scores at individual time points during each experiment were tested against vehicle control with two-tailed Wilcoxon matched pairs signed rank sum tests with

Holm-Bonferroni *post hoc* tests to correct for multiple comparisons (Holm, 1979). One-way ANOVA (with Tukey *post hoc* tests) was performed for the FAS test analysis. Two-way ANOVA (with Bonferroni *post hoc* tests) was used for the analysis of striatal dopamine and serotonin content in lesioned and non-lesioned hemispheres. For all statistical analyses, the null hypothesis was rejected when $p < 0.05$.

3.3 Results

3.3.1 *Post hoc* analyses to verify the 6-OHDA lesion

As has been shown in prior work (Bartlett et al., 2016; Flores et al., 2014; Yue et al., 2011), the medial forebrain bundle (MFB) lesion protocol in our hands leads to > 90 % depletion of striatal DA content on the lesioned side of the brain, while leaving levels of serotonin unaltered. The mean number of amphetamine-induced ipsiversive rotations for PD animals (n = 30) included in this study was 6.08 (SEM = 0.66), indicating a > 90 % PD-lesion (i.e. > 90 % dopamine denervation of nigrostriatal projections from the SNpc). In *post hoc* analyses, we observed a reduction of striatal and nigral TH immunoreactivity (**Figure 1A and B**). This was quantified in a subset of animals with semi-quantitative western analysis of TH and showed greater than a 90% reduction of TH in the lesioned hemisphere compared to the intact hemisphere (mean relative intensity TH/ ✔ -actin: intact side = 2.73; lesioned side = 0.30; $p = 0.016$; $n = 7$; two tailed t-test). There is also a 95% loss of striatal DA on the lesioned side, while serotonin and metabolite levels remained unchanged ($F_{[1,22]} = 290.47$, $p < 0.0001$, two-way ANOVA, Bonferroni *post hoc* tests) as shown in **Figure 1C**.

3.3.2 MMP-2200 does not interfere with L-DOPA's antiparkinsonian activity and has antiparkinsonian activity on its own

After L-DOPA-induced dyskinesia was established (i.e., after 21 days of L-DOPA priming), we validated that MMP-2200 does not interfere with the antiparkinsonian action of L-DOPA by using the FAS (forelimb adjusting steps) test paradigm, a measure of forelimb akinesia. The PD lesion-induced reduction in the use of the contralateral front paw was reversed by L-DOPA treatment, and this rescue was not altered by co-injection of MMP-2200 with L-DOPA (one-way ANOVA, $F_{[3,30]} = 281.5$; $p < 0.0001$, Tukey multiple comparisons *post hoc* tests; comparison of each group to post-lesion yielded $p < 0.0001$, all other *post hoc* comparisons were not statistically significant) as shown in **Figure 2A**. In a separate cohort, we tested the effects of MMP-2200 by itself in PD animals on akinesia using the FAS test (**Figure 2B**). In these unilaterally 6-OHDA-lesioned animals, a clear reduction in the contralateral forelimb steps is evident, as expected when compared to baseline. Treatment with MMP-2200 (20 mg/kg; *i.p.*) leads to a significant increase in the percentage of the contralateral steps indicating a modest antiparkinsonian activity (paired sample *t*-test, t = 2.961, two-tailed $p = 0.0159$, $n = 10$).

3.3.3 MMP-2200 differentially affects LAO and locomotor AIMs induced by L-DOPA; these effects are blocked by naltrindole

MMP-2200 at both doses (10 and 20 mg/kg) significantly increased LAO AIMs relative to their respective vehicle control experiments, as shown in **Figures 3A,B**, which plots the time course data for the entire experiment. Two-tailed Wilcoxon matched-pairs signed rank tests with Holm-Bonferroni corrections for multiple comparisons were performed at each individual time point ($n = 17$-18). This is also shown in **Figure 4A** for the total AIMs scores, Wilcoxon matched-pairs signed rank tests with

Holm-Bonferroni corrections for multiple comparisons vs. vehicle control on total AIMs scores; MMP-2200, 10 mg/kg: $W = 137$, $p = 0.002$, $n = 17$; MMP-2200, 20 mg/kg: $W = 137$, two-tailed $p = 0.0024$, $n = 18$). MMP-2200 significantly decreased locomotor AIMs induced by L-DOPA as shown in the time course in **Figures 3C, D** (two-tailed Wilcoxon matched-pairs signed rank tests with Holm-Bonferroni corrections for multiple comparisons, $n = 14$). In **Figure 4B** for the total AIMs scores are shown (Wilcoxon matched-pairs signed rank tests with Holm-Bonferroni corrections for multiple comparisons vs. vehicle control MMP-2200, 10 mg/kg: $W = -81$, two-tailed $p = 0.011$, $n = 14$); MMP-2200, 20 mg/kg: $W = -89$, two-tailed $p = 0.0116$, $n = 14$). The effect of MMP-2200 to enhance LAO AIMs (at both 10 and 20 mg/kg) was blocked by systemic administration (*i.p.*) of the selective DOR antagonist naltrindole at 3 mg/kg (Wilcoxon matched-pairs signed rank tests with Holm-Bonferroni corrections for multiple comparisons vs. vehicle control on total AIMs scores; MMP-2200, 10 mg/kg: $W = -28$, two-tailed $p = 0.504$, $n = 10$; MMP-2200, 20 mg/kg: $W = 16$, two-tailed $p = 0.75$, $n = 10$), as shown in **Figure 4A**. Naltrindole alone did not alter the expression of LAO AIMs when compared to its vehicle control in the crossover design (Wilcoxon matched-pairs signed rank tests with Holm-Bonferroni corrections for multiple comparisons, $W = 2$, two-tailed $p = 0.9336$, $n = 10$). This indicates that the effect of MMP-2200 on L-DOPA-induced AIMs is mediated by a pathway utilizing the DOR system. For locomotor AIMs, naltrindole blocked the effects of MMP-2200 at the 10 mg/kg dose (Wilcoxon matched-pairs signed rank tests with Holm-Bonferroni corrections for multiple comparisons vs. vehicle control were performed on total AIMs scores; MMP-2200, 10 mg/kg: $W = -8$, two-tailed $p > 0.9999$, $n = 10$; , $W = -25$, two-tailed $p = 0.6738$, $n = 10$), as shown in **Figure 4B.** Comparison of the different LAO vehicle control experiments using a Kruskal-Wallis test did not reveal any significant differences; this provides evidence that the level of expression of LAO AIMs in the vehicle control experiments remained stable across time ($H = 4.647$, $p \approx 0.2246$, $n = 5$). The same was true for locomotor AIMs vehicle control experiments ($H = 1.175$, $p \approx 0.8821$, $n = 5$).

3.3.4 Effects of MMP-2200 on D_1R-induced AIMs

We then tested the individual contributions of the direct and indirect striatal output pathways on the expression of DA receptor-mediated AIMs. A D_1R-induced dyskinesia model was established as previously described in Flores et al. (2014). Briefly, animals initially primed with L-DOPA were treated with the selective D_1R agonist SKF18297 (0.8 mg/kg, *s.c.*) three times to ensure stable expression of AIMs in response to the D_1R agonist. Co-administration of MMP-2200 (20 mg/kg) led to significant reduction in SKF81297-induced locomotor AIMs, as shown in **Figure 5B**, which plots the time course data for the entire experiment (Wilcoxon matched-pairs signed rank tests with Holm-Bonferroni corrections for multiple comparisons were performed at each individual time point, $n = 7$). In **Figure 5D** the total scores are shown (Wilcoxon matched-pairs signed rank test vs. vehicle control on total AIMs scores; $W = -24$, $p = 0.0469$, $n = 7$). However, MMP-2200 had no effect on LAO AIMs in this paradigm, as shown in **Figure 5A** for the whole time course (Wilcoxon matched-pairs signed rank tests Holm-Bonferroni corrections for multiple comparisons were performed at each individual time point, $n = 8$). In **Figure 5C** the total scores are depicted (Wilcoxon matched-pairs signed rank test vs. vehicle control was performed on total AIMs scores; $W = 6$, $p = 0.7422$, $n = 8$).

3.3.5 MMP-2200 reduces D_2R-induced AIMs

Next, the rats were primed with the selective D_2R agonist quinpirole (0.2 mg/kg, *i.p.*) three times to ensure stable expression of AIMs in response to the D_2R agonist. Both LAO and locomotor AIMs were elicited by quinpirole, as previously shown by Flores et al. (2014). Co-administration of MMP-2200 (20 mg/kg) with quinpirole led to a marked reduction (40%) in LAO, as shown in **Figure 6A,** which

plots the time course data for the entire experiment (two-tailed Wilcoxon matched-pairs signed rank tests Holm-Bonferroni corrections for multiple comparisons were, $n = 7$). This is further shown in **Figure 6C,** which shows the total LAO scores (Wilcoxon matched-pairs signed rank test vs. vehicle control on total AIMs scores; $W = -28$, two-tailed $p = 0.0156$, $n = 7$). Locomotor AIMs were reduced by ~80% overall as illustrated by the time course data in **Figure 6B** (Wilcoxon matched-pairs signed rank tests Holm-Bonferroni corrections for multiple comparisons, $n = 7$) and in **Figure 6D** for the total scores (Wilcoxon matched-pairs signed rank test vs. vehicle control on total AIMs scores; $W = -21$, two-tailed $p = 0.0313$, $n = 7$). The data on the effects of MMP-2200 for all three treatments are summarized in **Table 1**.

Table 1. Summary of the effects of MMP-2200 on LAO and locomotor AIMs induced by L-DOPA, the selective D1 receptor agonist, SKF81297, and the selective D2 receptor agonist, quinpirole.

AIMs Inducer	Striatal Output Pathway	Change in LAO AIMs		Change in Locomotor AIMs	
		%	*p*-value	%	*p*-value
L-DOPA	Direct and Indirect	~10 ↑	0.0024	~50 ↓	0.0116
SKF81297	Direct	~0	*n.s.*	~40 ↓	0.0469
Quinpirole	Indirect	~40 ↓	0.0156	~80 ↓	0.0313

Two-tailed Wilcoxon matched-pairs rank sums tests vs. corresponding vehicle control

MMP-2200 (20 mg/kg) administration led to a small increase in L-DOPA-induced LAO AIMs, had no effect on SKF81297-induced LAO AIMs. MMP-2200 significantly decreased locomotor AIMs to differing degrees for the AIMs inducers. *n.s.* = not significant; ↑ = increase; ↓ = decrease.

3.3.6 Effects of co-administration of MMP-2200 and MK-801

In the present study, we have also confirmed that MK-801 (0.3 mg/kg, *i.p.*) produces a pro-parkinsonian effect, reliably inducing rotations ipsiversive to the side of the 6-OHDA lesion, which is a correlate of pro-parkinsonian activity in this model, and producing akinesia in the FAS test. The time course plots of these experiments show that the effect of MK-801 lasted throughout the entire duration of the 3-hour testing period for both LAO and locomotor AIMs measures, as shown in **Figure 7 A, D** (two-tailed Wilcoxon matched-pairs signed rank tests Holm-Bonferroni corrections for multiple comparisons were performed at each individual time point, $n = 10$). In **Figure 8A** the total LAO AIMs are shown (Kruskal-Wallis test with Dunn's multiple comparisons *post hoc* tests ($H = 4.647$, $p < 0.0001$, $n = 6$), and in **Figure 8B** the total locomotor AIMs (Kruskal-Wallis test with Dunn's multiple comparisons *post hoc* test ($H = 4.647$, $p < 0.0001$, $n = 6$).

Importantly, co-injection of MMP-2200 at both 10 and 20 mg/kg completely abolished ipsiversive rotations induced by MK-801, while simultaneously preventing the induction of contraversive rotations induced by L-DOPA, as shown in the time course data in **Figure 7E, F** (two-tailed Wilcoxon matched-pairs signed rank tests Holm-Bonferroni corrections for multiple comparisons were performed at each individual time point, $n = 9$-10). This indicates that the pro-parkinsonian effect was blocked by MMP-2200, while a robust anti-dyskinetic effect on LAO AIMs remains, as shown in the time course data in **Figure 7B, C** (two-tailed Wilcoxon matched-pairs signed rank tests Holm-Bonferroni corrections for multiple comparisons were performed at each individual time point, $n = 9$-10). This is further depicted in **Figure 8A** for the total LAO AIMs (Kruskal-Wallis test, $H = 44.32$, $p < 0.0001$, $n = 6$, with Dunn's multiple comparisons *post hoc* tests; MMP-220, 10 mg/kg: mean rank diff. = -8.55, two-tailed $p > 0.9999$, $n = 10$; MMP-2200, 20 mg/kg: mean rank diff. = -8.472, two-tailed $p >$

0.9999, $n = 9$), and in **Figure 8B** for total locomotor AIMs (Kruskal-Wallis test, $H = 35.32$, $p < 0.0001$, $n = 6$, with Dunn's multiple comparisons *post hoc* tests; MMP-2200, 10 mg/kg: MMP-220, 10 mg/kg: mean rank diff. = -16.85, $p = 0.0592$, $n = 10$; MMP-2200, 20 mg/kg: mean rank diff. = -18.08, two-tailed $p = 0.0404$, $n = 9$).

Naltrindole does not interfere to an appreciable extent with the effect of MMP-2200 on ipsiversive rotations elicited by MK-801; MK-801 combined with MMP-2200 (20 mg/kg) still leads to a significant reduction in locomotor AIMs following naltrindole administration. **Figure 8B** shows the total locomotor scores (Kruskal-Wallis test, $H = 35.32$, $p < 0.0001$, $n = 6$, with Dunn's multiple comparisons *post hoc* tests; MMP-2200, 10 mg/kg: mean rank diff. = -10.18, $p > 0.9999$, $n = 10$; MMP-2200, 20 mg/kg: mean rank diff. = -10.78, two-tailed $p = 0.9490$, $n = 9$). In the case of LAO AIMs, the administration of naltrindole combined with 20 mg/kg MMP-2200 does significantly interfere with the anti-dyskinetic action of MK-801 (Kruskal-Wallis test, $H = 35.32$, $p < 0.0001$, $n = 6$, with Dunn's multiple comparisons *post hoc* tests; MMP-2200, 10 mg/kg: mean rank diff. = -17.64, $p > 0.3096$, $n = 10$; MMP-2200, 20 mg/kg: mean rank diff. = -28.44, two-tailed $p = 0.0091$, $n = 9$), as shown in **Figure 8A**.

Finally, it's important to note that comparison of the different LAO vehicle control experiments using a Kruskal Wallis test did not reveal any significant differences; this provides evidence that the level of expression of LAO AIMs in the vehicle control experiments remained stable across time ($H = 5.517$, $p \approx 0.3561$, $n = 6$). The same was true for locomotor vehicle control experiments ($H = 4.557$, $p \approx 0.4723$, $n = 6$).

In a separate cohort of LID animals with baseline LAO AIMs of 24.5 ± 5.0 (mean ± SEM) and locomotor AIMs of 4.6 ± 1.6 (mean ± SEM), we further evaluated if the antiparkinsonian activity of MMP-2200 can counter the MK-801-induced pro-parkinsonian activity using a non-drug-induced behavior. FAS test data from these LID animals showed that indeed MMP-2200 successfully

counteracted the pro-parkinsonian activity of MK-801 in this paradigm measuring forelimb akinesia (**Figure 9**). One-way ANOVA (with Tukey *post hoc* tests) were performed on contralateral steps ($F_{[6,27]} = 32.13$; $p = 0.0001$) and ipsilateral ($F_{[6,27]} = 21.2$; $p < 0.0001$) in the FAS test paradigm (**Figure 9A**). As anticipated, Tukey multiple comparisons *post hoc* tests showed that the proportion of contralateral forelimb steps in both the post-lesion and MK-801 conditions was significantly decreased compared to baseline, owing to the unilateral lesion—both yielding *p*-values < 0.0001 (mean differences of -44.57 and -47.14 respectively). Administration of the noncompetitive NMDA-receptor antagonist MK-801 by itself markedly suppresses the expression of L-DOPA-induced AIMs; therefore, the proportion of contralateral forelimb steps in this condition remained low and were not significantly different from their post-lesion state ($p = 0.4950$). In the L-DOPA + MMP-2200 (10 mg/kg) + MK-801 condition, a significant reduction in the proportion of contralateral steps relative to baseline remained (mean difference = -21.86 , $p = 0.0385$). However, the proportion of contralateral steps in this condition was significantly higher than that realized in the MK-801 alone condition (mean difference = 25.29, $p = 0.0430$), indicating that 10 mg/kg MMP-2200 reduces the pro-parkinsonian effect of MK-801. Contralateral steps in the L-DOPA + MMP-2200 (20 mg/kg) + MK-801 condition were significantly increased relative to both post-lesion and MK-801 alone conditions (mean difference = 33.57, $p = 0.0008$ and mean difference = 36.14, $p = 0.0003$ respectively), further demonstrating that the pro-parkinsonian activity of MK-801 is suppressed by this high dose of MMP-2200. In fact, addition of MMP-2200 at this higher 20 mg/kg dose restored the proportion contralateral forelimb steps to a level that was no longer significantly different from the prelesion baseline (mean difference = -11, $p = 0.3539$). Therefore, MMP-2200 can successfully counteract the pro-parkinsonian effects of MK-801without compromising the antiparkinsonian action of L-DOPA itself.

With regard to ipsilateral forelimb steps (**Figure 9B**), which are controlled by the intact side of the brain and are not attributed to L-DOPA hypersensitivity, the mean and variance of forelimb steps in

the baseline and post-lesion conditions are nearly identical, as expected. Tukey multiple comparisons *post hoc* tests showed that following administration of L-DOPA + MK-801, a vast reduction in the proportion of ipsiversive steps was observed relative to these first two conditions (mean difference = -39.57, $p = 0.008$ and mean difference = -41.86, $p = 0.0032$ respectively). This motor-suppressive effect is probably due to antagonism of NMDAR glutamate receptors by MK-801. From this akinetic state, coadministration of MMP-2200 at both 10 and 20 mg/kg with L-DOPA and MK-801 led to a significant increase and rescue of ipsiversive forelimb steps (mean difference = 30.57, $p = 0.0011$ and mean difference = 26.29, $p = 0.0152$ respectively). The movement-restorative effects of MMP-2200 in both instances were complete enough so that neither MMP-2200 in either the 10 or 20 mg/kg condition was statistically different from baseline or post-lesion data.

3.4 Discussion

MMP-2200, also known as Lactomorphin, is a synthetic opioid glycopeptide, structurally-based on Leu-enkephalin, which has mixed-δ/μ opioid receptor agonist activity and a favorable pharmacological profile compared to alkaloid-based opioid compounds (Li et al., 2012). We have previously shown that MMP-2200 has potent behavioral effects in rodent models of PD, where it blocks amphetamine-induced rotations in unilaterally 6-OHDA-lesioned animals and blocks dopaminergic hypersensitivity in animals with reserpine-induced dopamine depletion (Xu Yue et al., 2011). Since amphetamine releases dopamine non-selectively from nigrostriatal terminals, we could not determine to what extent MMP-2200 was acting on the direct vs. the indirect pathway. We now report a more detailed pharmacological study of MMP-2200, in which we separately activate the direct and indirect striatal outflow pathways of the basal ganglia using selective dopamine receptor agonists. We first could demonstrate that MMP-2200 not only does not interfere with the antiparkinsonian activity of L-DOPA,

but has a modest antiparkinsonian activity by itself as well, which is also consistent with its ability to reduce amphetamine-induced rotations. We also demonstrate that MMP-2200 attenuates four separate modalities of rotational behavior: (1) contraversive rotations induced by L-DOPA, (2 & 3) contraversive rotations induced by selective stimulation of D_1- and D_2-like receptors and (4) ipsiversive rotations induced by the selective NMDA receptor antagonist MK-801. In the unilateral 6-OHDA-lesion model, the contraversive rotations are thought to reflect dopamine hypersensitivity and are related to the clinical phenomenon of LID, while ipsiversive rotations reflect either worsening of the parkinsonian state (i.e MK-801, *vide infra*), or activation of the intact hemisphere (i.e. amphetamine). We also employed quantified measures of limb, axial and orolingual (LAO) AIMs, which represent a further experimental refinement in modeling the clinical phenomenon of LID.

3.4.1 Effects of MMP-2200 on dopamine agonists and selective modulation of the indirect striatal outflow pathway of the basal ganglia

In the present study, we used selective pharmacological stimulation of D_1 and D_2-like receptors in order to examine the differential effects of MMP-2200 in the direct and indirect pathway. We show that MMP-2200 selectively blocks dyskinesia produced by D_2R-like agonist activity. Hemi-parkinsonian animals were initially primed with L-DOPA and then probed sequentially with L-DOPA, the selective D_1R agonist SKF81297, and finally the selective D_2R-like agonist quinpirole (see **Table 1** in the Results section). We show that MMP-2200 selectively reduces D_2R-like agonist-induced LAO AIMs while having no comparable effect against LAO AIMs induced by the selective D_1R agonist. A similar result was obtained with regard to locomotor (contralateral rotational) behavior: MMP-2200 completely blocked the effects of the D_2R-like agonist, while only producing a partial reduction in

rotational movements driven by the D_1R agonist. Taken together these results are consistent with an effect of MMP-2200 that is predominantly selective for the indirect pathway. The ability of MMP-2200 to offset the dyskinesia produced by the D_2R-like agonists is a significant finding given that drugs with D_2R-like agonism are used extensively for the treatment of PD, e.g. pramipexole, ropinirole, and rotigitine. When these clinically-approved drugs are used in early stages of Parkinson's disease as monotherapy, they effectively reduce the motor symptoms of PD but do not cause dyskinesia (Holloway et al., 2004; Rascol et al., 2000). In fact, dopamine agonists play an important role in L-DOPA sparing strategies used in the therapeutic management of PD (Connolly & Lang, 2014). When dopamine agonists are used in combination with L-DOPA to treat more advanced stages of PD, however, they contribute to the overall severity and duration of dyskinesia (Hauser et al., 2007; Holloway et al., 2009; Katzenschlager et al., 2008). With further development, MMP-2200 could be positioned to treat dyskinesia in these more advanced patients with LID that are being treated with combinations of L-DOPA and dopamine agonists.

The lack of effect of MMP-2200 on D_1R-induced dyskinesia could, in theory, limit the therapeutic efficacy of MMP-2200, to the extent that a considerable number of currently prescribed antiparkinsonian drugs exhibit D_1R agonism. Nevertheless, pramipexole and ropinirole, the most commonly prescribed oral dopamine agonists, have no appreciable D_1R activity (Millan et al., 2002). Other agonists with predominant D_2R-like activity include the transdermal drug rotigotine and the injectable agonist apomorphine; while these agonist do exhibit a low level of D_1R agonism, they are more highly selective for D_2Rs (Chen et al., 2009; Millan et al., 2002; Scheller et al., 2009). L-DOPA, the mainstay of treatment for PD, and also the major pharmacological contributor to LID, activates all subtypes of DA receptors belonging to both the D_1 and D_2 families of DA receptors, as discussed specifically below. While D_1R agonism is thought important for complete control of parkinsonian motor symptoms, few therapeutic advances have been made with selective D_1R agonists (Jenner, 2005)

due to issues with multiple side effects. Non-pharmacological evidence also supports the clinical relevance of the indirect pathway: the highly efficacious surgical therapy for PD, deep brain stimulation, has been proven to be a highly effective when targeting the subthalamic nucleus (Krack et al., 1997; P. Limousin et al., 1995), which preferentially modulates the indirect pathway. Therefore, selective modulation of the indirect striatal output pathway continues to be an important therapeutic target for PD and the further mechanistic study of MMP-2200 and similar opioid drugs with the goal of clinical development for patients with uncontrolled dyskinesia appears warranted.

3.4.2 Effects of MMP-2200 on L-DOPA-induced AIMs

The effects of MMP-2200 in the LID model are complicated when L-DOPA itself is used as the probe. Since L-DOPA itself can be thought of as driving both the direct and indirect pathways, e.g. having both D_1R and D_2R agonist effects, we expected that MMP-2200 would partially block the effect of L-DOPA in the setting of dopamine hypersensitivity. Indeed, the effects of MMP-2200 on rotational behavior are consistent with this line of reasoning, where a significant reduction in locomotor AIMs was found as expected. However, MMP-2200 was not effective in ameliorating LAO AIMs in our model. Instead, there was a small increase in LAO AIMs, which was blocked by pretreatment with the selective DOR antagonist naltrindole. This suggests that the δ-agonist activity of MMP-2200's dual action at μ- and δ- opioid receptors is responsible for the worsening of LAO-AIMs. This finding is in agreement with the results of a study done by (Billet et al., 2012) in which the selective DOR agonist [d-Pen^2, d-Pen^5]-enkephalin (DPDPE), which like MMP-2200 is a structural analog of Leu-enkephalin, significantly increased LAO AIMs in the 6-OHDA lesion rat model of LID. We do not have a simple explanation for why MMP-2200 is effective against D_2R-like-induced dyskinesia but not even partially

effective against L-DOPA LAO-AIMs (see section 4.4). These results are consistent, though, with the complex array of effects of L-DOPA compared to selective dopamine agonists; L-DOPA acts at all of the five dopamine receptors sub-types which are found in multiple anatomic regions, including extra-striatal regions (Obeso et al., 2000). Thus, it is not surprising that L-DOPA results are not fully predicted by the combined results of the selective D_1R and D_2R-like agonists. In this respect it is of interest that dopamine D3 receptors (D_3R) are found in both the direct and indirect pathways, though differentially after L-DOPA priming (Solís et al., 2017). Currently, a number of studies have implicated alterations in D_3R expression and signaling in LID. Chronic L-DOPA treatment has previously been shown to increase D_3R expression in the dorsal striatum in rats unilaterally lesioned with 6-OHDA (Bordet et al., 1997; Guillin et al., 2001). This increase in D_3R expression correlates with hypersensitization to L-DOPA (Guigoni et al., 2005). Data from a recent study by (Solís et al., 2017) provides evidence that D_3R-mediated signaling plays an important role specifically in the development of dyskinesia and revealed that chronic L-DOPA therapy leads to increase D_3R expression predominantly in D_1R-expressing striatal MSNs of the direct pathway, and to much lesser degree in D_2R expressing striatal MSNs of the indirect pathway. Furthermore, they showed that genetic deletion of D_3Rs attenuates LID. Recent data also support that a functional, synergistic interaction between D_1R and D_3R exists to both behaviorally and biochemically to drive dyskinesia in hemi-parkinsonian rats (Lanza et al., 2018). Considering that the up-regulation of D_3R in LID principally happens in the direct pathway and the main anti-dyskinetic effect of MMP-2200 instead is likely to be located in the indirect pathway, we would argue that changes in D_3R activity do not play a major role in explaining our findings. In order to investigate this directly in the future, experiments with more selective D_3R antagonists would be required.

3.4.3 Combined effects of MMP-2200 and MK-801

In prior work, we have demonstrated that the potent non-competitive NMDAR antagonist MK-801 can also selectively block LID via a mechanism that involves the indirect pathway (Flores et al., 2014) Given that MMP-2200 strongly modulates the indirect pathway, we tested the effects of MMP-2200 in conjunction with MK-801. We report here, for the first time, that co-administration of an opioid receptor agonist (MMP-2200) and MK-801 produces a combined effect that is characterized by a robust suppression of L-DOPA-induced locomotor and LAO AIMs, with reduced induction of parkinsonism. This is an important finding, since MK-801 is extremely effective at blocking dyskinesia in the pre-clinical model, but only at doses that worsen the parkinsonian symptoms (Paquette et al., 2010), indicated by inducing ipsiversive rotations in this model and by preventing L-DOPA's therapeutic effects to reduce impaired sensorimotor function tested with the Vibrissae-Stimulated Forelimb Placement test (Flores et al., 2014, Pacquette et al., 2010). The pro-parkinsonian effect of MK-801 is a major liability for future drug development of novel anti-dyskinesia agents with highly specific NMDAR blocking properties. The clinically used drug amantadine is a multifunctional drug, with its weak NMDAR-antagonism as one contributor to its clinical efficacy in PD (Paquette et al., 2012) among other not identified mechanisms. When compared to MK-801, amantadine shows even at high doses weaker anti-dyskinetic activity in rodents (50% reduction), compared to MK-801 (>90% reduction), but importantly, amantadine does not have the liability to induce any pro-parkinsonian locomotor activity as MK-801 does. In this regard it is of interest that low sub-anesthetic doses of ketamine, another multifunctional drug with weak non-competitive NMDAR-antagonism amongst its activities, do lead to reduction of LID as well, while also not inducing pro-parkinsonian locomotor activity (Bartlett et al., 2016), but rather showing antiparkinsonian activity by itself . Since ketamine is known to bind opioid receptors and having agonist properties (Fink and Ngai, 1982; Gupta

et al., 2011), this would also suggest opioid agonism together with highly specific NMDAR-antagonism might allow a strong anti-dyskinetic activity to occur without worsening of the PD symptoms at the same time. Together with that information our data suggests, that a potent highly specific NMDAR blocker could still find clinical utility if used in combination with MMP-2200 or a similar DOR agonist.

Our results with experiments utilizing MK-801 show that the balance of δ and μ opioid receptor activity of MMP-2200 is an important determinant of its effects on AIMs. If we block the δ activity with naltrindole, the remaining μ activity interferes with the anti-dyskinetic activity of MK-801 (**Figure 8**). This finding is consistent with prior studies of the μ opioid system in LID, which would predict that μ-agonists would worsen LID. Accordingly, there is evidence that MOR antagonists have anti-dyskinetic action in non-human primate (NHP) models of LID (Henry et al., 2001; Koprich et al., 2011). It should be noted though, that the literature is not entirely consistent when comparing results from different animal models and clinical studies (Elmagbari et al., 2004; S. Fox et al., 2004; Henry et al., 2001; Klintenberg et al., 2002; Manson et al., 2001; Rascol et al., 1994). Nevertheless, there is sufficient evidence to consider that δ- and μ-opioid systems may work in balanced opposition.

3.4.4 Relation of experimental findings to the classical model of basal ganglia

We may explain the results of our experiments, in part, by using the framework of the classical model of basal ganglia function. According to the classical theory and supported by computational modeling (Moustafa et al., 2008) the parkinsonian "off" state is explained by an imbalance between the direct and indirect pathways: the indirect pathway becomes hyperactive while the direct pathway becomes underactive. The segregated expression of PPE-A and PPE-B mRNAs as well as differential expression of μ- and δ-opioid receptors by MSNs in the two respective pathways (Lindskog et al.,

1999; Noble & Cox, 2002) suggest that enkephalins modulate neurotransmission in the indirect pathway while dynorphins modulate neurotransmission in the direct pathway. The exact role of the neuropeptides in controlling the activity of the basal ganglia circuitry is less clear, though it is evident that there are important alterations in the peptidergic systems that occur in the setting of PD. The precursor mRNA for PPE-A has been shown to be upregulated in states of striatal dopamine depletion (Gerfen et al., 1990; Nisbet et al., 1995; Westin et al., 2001). In fact, NHP studies have demonstrated that alterations in levels of expression of PPE-A precede the appearance of motor deficits related to DA depletion (Bezard, Ravenscroft, et al., 2001). Furthermore, long-term, chronic L-DOPA therapy leading to LID increases levels of expression of PPE-A mRNAs beyond what is seen in states of DA depletion alone (Calon et al., 2002; Henry et al., 1999). Though these alterations are well-established, we do not know whether the upregulation of enkephalinergic transmission in the indirect pathway contributes to the pathology of PD or whether it is a compensatory mechanism. The results of our experiments with MMP-2200 are most consistent with the viewpoint that in the condition of LID, enkephalin upregulation in the indirect pathway is a compensatory reaction that partially restores homeostasis in the basal ganglia circuitry. The action of MMP-2200, via enhancing the endogenous effect of enkephalin, is able to more fully restore the proper balance between the direct and indirect pathway and thus provides a potent anti-dyskinetic effect in our model of LID.

The same line of reasoning might suggest that our test article, MMP-2200 might worsen the parkinsonian “off” state (dopamine depletion without pharmacological replacement) by exacerbating the pathologically increased enkephalin-ergic transmission in the indirect pathway. Importantly though, we did not observe this effect: MMP-2200 failed to display any pro-parkinsonian effects whether used alone or in combination with other drugs. Rather, MMP-2200 actually reversed the pro-parkinsonian effect of the NMDAR antagonist MK-801. We postulate that this beneficial property

arises from the balanced and dual activity of MMP-2200 at both μ and δ receptors, and by extension a dual effect to increase activity of both the direct and indirect striatal outflow pathways. These balanced effects mean that MMP-2200 alone has little or no effect on normal locomotion, but "clamps" down the striatal outflow and thus resists any perturbation away from the normal balance. This explains the results of the experiments with MK-801 where MMP-2200 has both anti-dyskinetic effects and anti-parkinsonian effects. If further studies validate this concept, then MMP-2200 or similar agents could have a special utility in the treatment of advanced PD, where rapid fluctuations between the "on", "on with dyskinesia" and "off" states produce substantial morbidity.

3.4.5 Potential limitations of the experimental design

The design of this study presents some limitations with respect to the sequential order in which the experiments were performed using each of the three AIMs-inducing drugs: L-DOPA, SKF81297, and quinpirole. The experimental modeling of LID typically involves the initial priming of the animals with L-DOPA prior to a secondary priming and testing with DA receptor agonists. In order to minimize the use of animals, we performed experiments on L-DOPA induced AIMs first, and subsequently tested the selective dopamine receptor agonists. We acknowledge the potential for lasting changes in plasticity that may have carried over from previous phases of the study to affect later phases of the study. Since the experiments testing the effects of MMP-2200 on D_2R agonist-induced AIMs were performed last in the study we cannot rule out that repeated exposure to MMP-2200, or prior exposure to D_1R agonists may have confounded the results. The differential effects of MMP-2200 on locomotor and LAO AIMs, could simply be related to fundamental mechanistic differences between locomotor and LAO AIMs. The predictive validity of L-DOPA-induced contralateral turning behavior (i.e. locomotor AIMs) as a correlate of human LID is highly debated. Rather, it has been suggested

(Lundblad et al., 2002) that while contralateral rotations reflect the extent of unilateral DA-denervation/ depletion in the 6-OHDA rat model, LAO AIMs are a better behavioral correlate of human LID. For example, locomotor AIMs can be induced by drugs that have low potential for inducing dyskinesia clinically and are not attenuated by drugs that have demonstrated anti-dyskinetic efficacy in patients (Dekundy et al., 2007; Lundblad et al., 2002, 2005). Thus, LAO AIMs have greater predictive validity as a surrogate measure of LID occurring in PD patients, and therefore, in this context, it is perhaps not surprising that the effects of MMP-2200 on L-DOPA induced LAO and locomotor AIMs in the present study do not correlate with one another.

3.4.6 Conclusion

In conclusion, systemic administration of the mixed δ/μ opioid glycopeptide MMP-2200 reduced limb, axial, orolingual, and locomotor abnormal involuntary movements (AIMs) induced by a selective D_2R agonist, possibly strengthening the homeostatic upregulation of enkepahlins in response to development of dyskinesia. In addition, MMP-2200 modified the effect of the NMDAR antagonist MK-801 to result in a potent reduction of L-DOPA-induced limb, axial and orolingual AIMs with reduced induction of parkinsonism, mitigating the pro-parkinsonian activity that MK-801 has by itself. Since DA receptor agonists are used extensively in current clinical practice our data suggest that it could be worthwhile to further explore the therapeutic potential of drugs structurally related to endogenous opioid peptides as adjuncts to established therapies. In the case of MMP-2200, μ agonism might be a liability for drug development, given potential for abuse, tolerance, and addiction associated with traditional opioids active at the μ receptor. Our studies indicate that structural changes to enhance the δ-selectivity would preserve the anti-dyskinetic properties of the molecule and perhaps improve the suitability as a therapeutic agent. The μ-activity is important however for the balanced effect and may

prove to be an acceptable pharmacodynamics property when fully evaluated. Indeed, it should be remembered that neuropathic pain has only been recently acknowledged as a major comorbidity of PD and pain related to motor fluctuations remains a poorly addressed non-motor symptom. In this regard, development of new therapies based on the opioid peptide transmitters is all the more urgent.

3.5 Acknowledgments

This work was supported by the American Parkinson's Disease Association, the Jackson Fellowship to the University of Arizona Department of Neurology (T.F. & S.J.S.), the Office of Naval Research Grants 14-02-01-0471, 14-05-1-0807 (R.P.), the National Science Foundation (CHE-607917), the National Institutes of Health (NINDS-NS-052727; R.P. & NIH #T35HL007479 Grant for Short-Term Training: Students in Health Professional Schools; M.J.B. & B.K.R.), and the ARCS Foundation (A.F.).

3.6 Figures

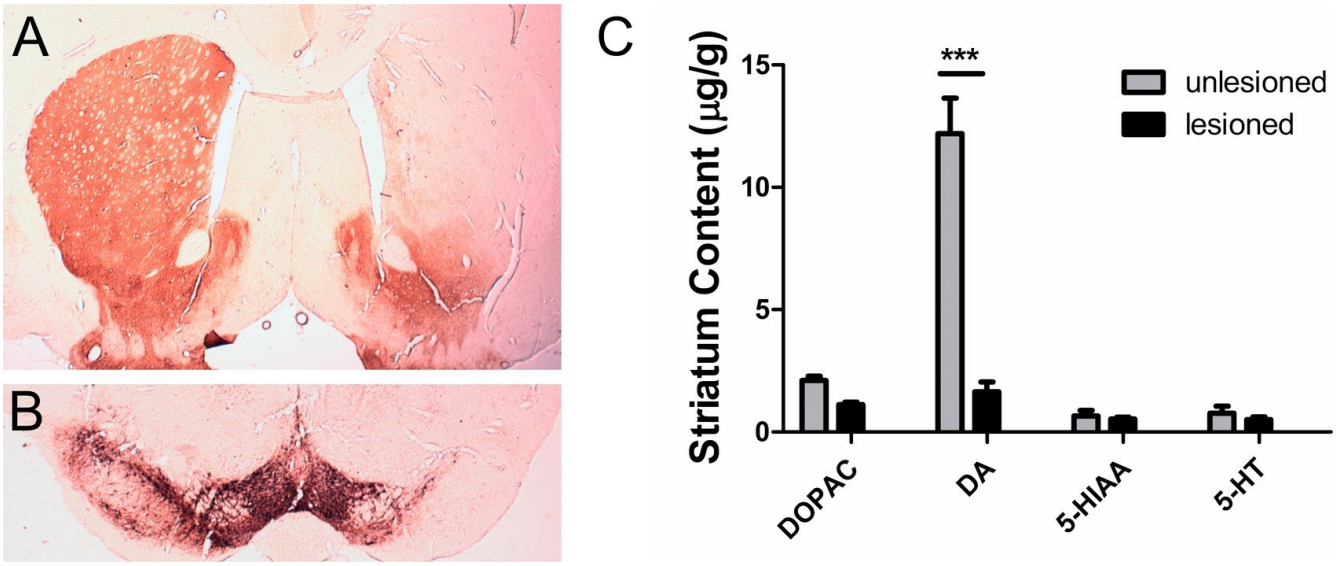

Figure 1. Verification of 6-OHDA lesion. Unilateral injections of 20 µg 6-OHDA into the MFB created a severe lesion in the substantia nigra. The presence of the unilateral lesion was verified using tyrosine hydroxylase (TH) immunoreactivity as a marker of dopaminergic neurons. The loss of dopaminergic cell bodies in the substantia nigra (**A**) and the loss of dopaminergic terminals in the striatum (**B**) are visible in the example photomicrographs after immunohistochemical staining for TH. (**C**) As expected, the striatal dopamine (DA) level measured with HPLC-EC was reduced by over 95% in the lesioned hemisphere, while the level of serotonin (5-HT) and the respective metabolites remained unchanged. Mean values ± SEM are plotted, n = 6; *** $p < 0.0001$, two-way ANOVA, Bonferroni *post hoc* tests.

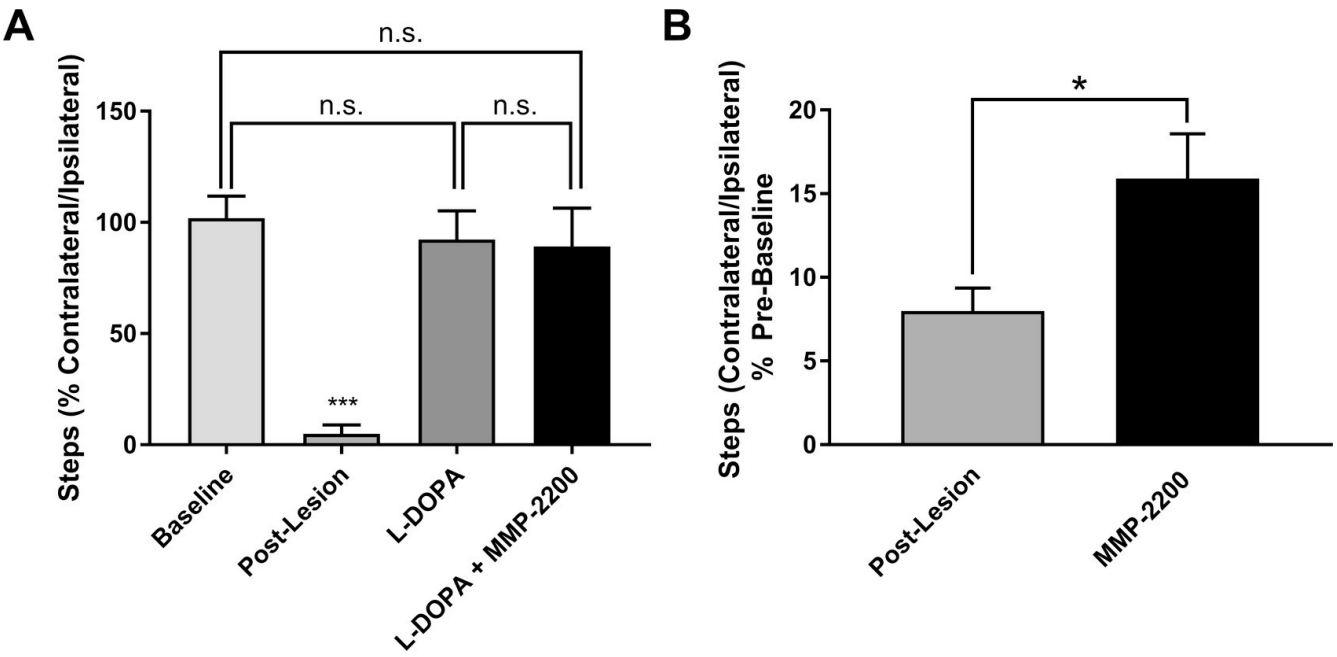

Figure 2. MMP-2200 does not interfere with the therapeutic effects of L-DOPA and acts antiparkinsonian by itself. **(A)** In the Forelimb Adjusting Steps Test (FAST), MMP-2200 does not interfere with the anti-parkinsonian effect of L-DOPA. Mean percentage of contralateral/ipsilateral steps ± SEM using the FAST paradigm are plotted for the pre-lesion baseline and post-lesion tests and for the tests of L-DOPA (7 mg/kg, *i.p.*) and L-DOPA (7 mg/kg, *i.p.*) + MMP-2200 (20 mg/kg, *i.p.*); n = 9. *n.s.* = not significant, *** $p < 0.0001$. **(B)** MMP-2200 (20 mg/kg, *i.p.*) by itself displays modest antiparkinsonian activity in the FAST paradigm as well as in this separate cohort of animals. The bar graph depicts the mean percentage of contralateral/ipsilateral steps ± SEM using the FAST paradigm and is plotted for the post-lesion baseline and then following administration of MMP-2200 at 20 mg/kg, n = 10, * $p < 0.05$. Statistics were done prior to data normalization.

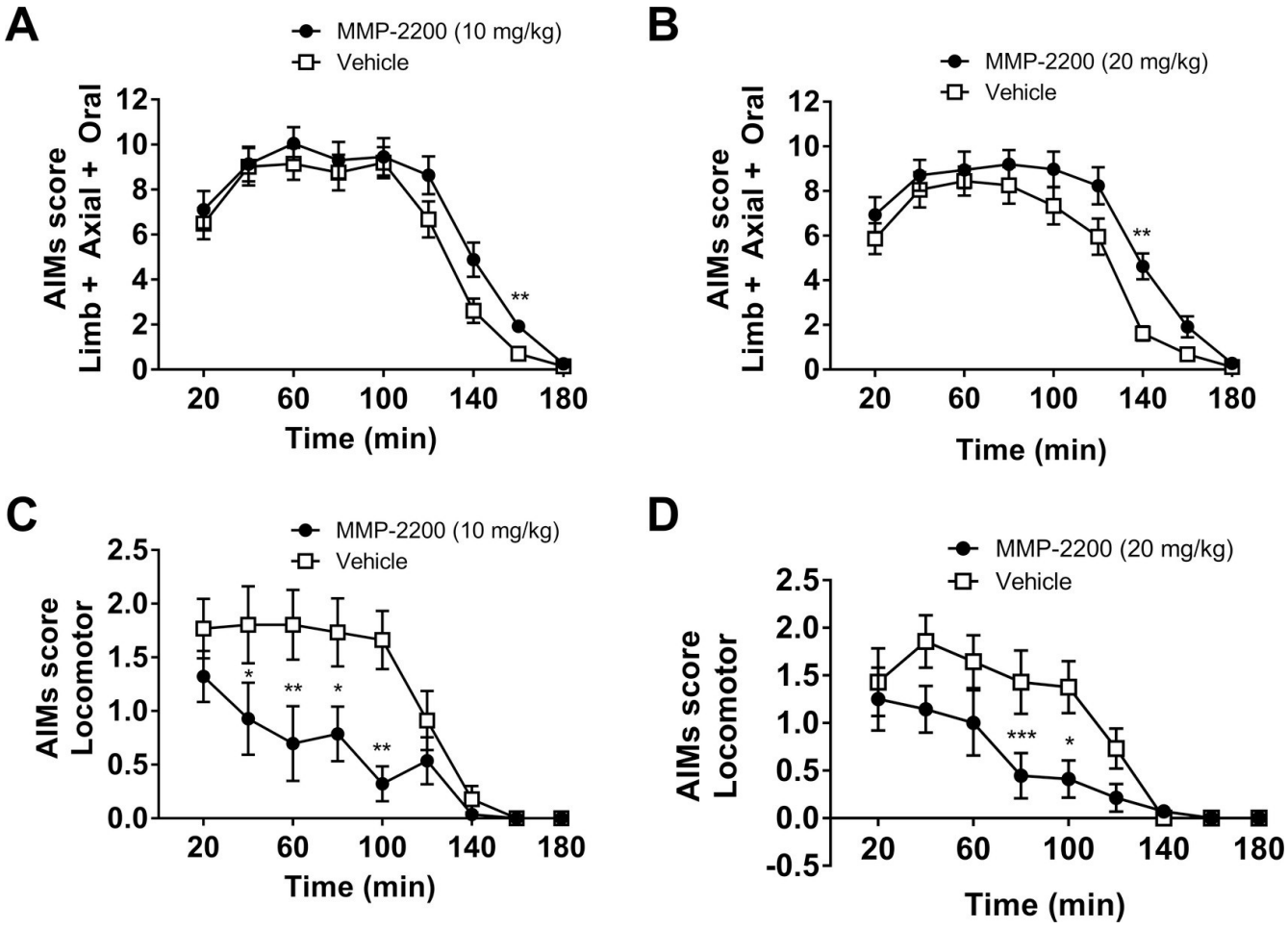

Figure 3. The effect of MMP-2200 on L-DOPA-induced AIMs. In the top panels, LAO AIMs scores are plotted and in the lower panels the L-DOPA-induced locomotor scores (contraversive rotations) are shown (7 mg/kg L-DOPA). Co-injection of **(A)** 10 mg/kg and **(B)** 20 mg/kg MMP-2200 increases L-DOPA-induced LAO AIMs scores by ~10% (mean AIMs score ± SEM; n = 17-18; ** $p < 0.01$). Conversely, co-injection of **(C)** 10 mg/kg and (**D**) 20 mg/kg MMP-2200 led to a marked 50% decrease in L-DOPA-induced contraversive rotations compared to the L-DOPA-only vehicle control (mean AIMs score ± SEM; n = 14; *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$).

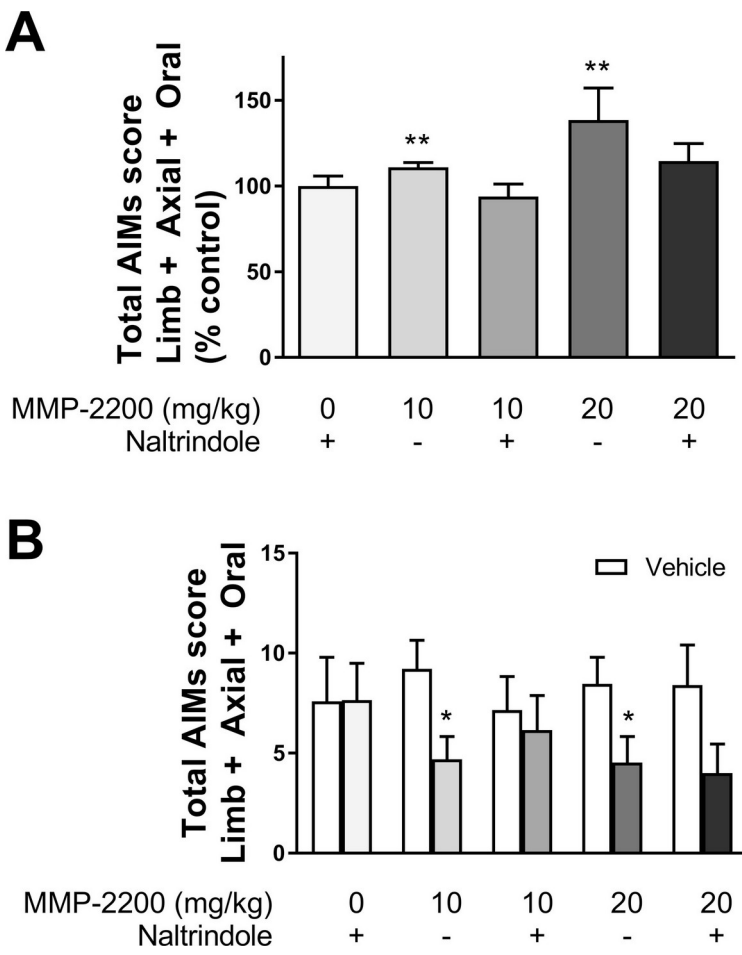

Figure 4. The effects of MMP-2200 on L-DOPA-induced LAO and locomotor AIMs scores are reversed by the selective delta-opioid receptor antagonist naltrindole. (**A**) LAO AIMs: Both 10 mg/kg and 20 mg/kg MMP-2200 doses lead to a modest but statistically significant increase in LAO AIMs compared to vehicle control. Naltrindole (3 mg/kg) blocked the MMP-2200-induced increase in LAO AIMs at both concentrations of MMP-2200 tested. The data are plotted as % of L-DOPA-only vehicle control (mean total AIMs score ± SEM; n = 10-18; ** $p < 0.01$, statistics done on raw AIMs data before normalization to % control). (**B**) Locomotor AIMs: Both 10 and 20 mg/kg MMP-2200 significantly reduce L-DOPA-induced locomotor AIMs (contraversive rotations) by ~50%. This reduction was blocked by the δ-specific antagonist naltrindole in the 10 mg/kg MMP-2200 experiment. The effect of 20 mg/kg MMP-2200 was not significantly reduced by naltrindole (mean total AIMs score ± SEM; n = 10-14; * $p < 0.05$). LAO and Locomotor AIMs scores obtained during the vehicle control testing sessions were stable and not significantly different between experiments.

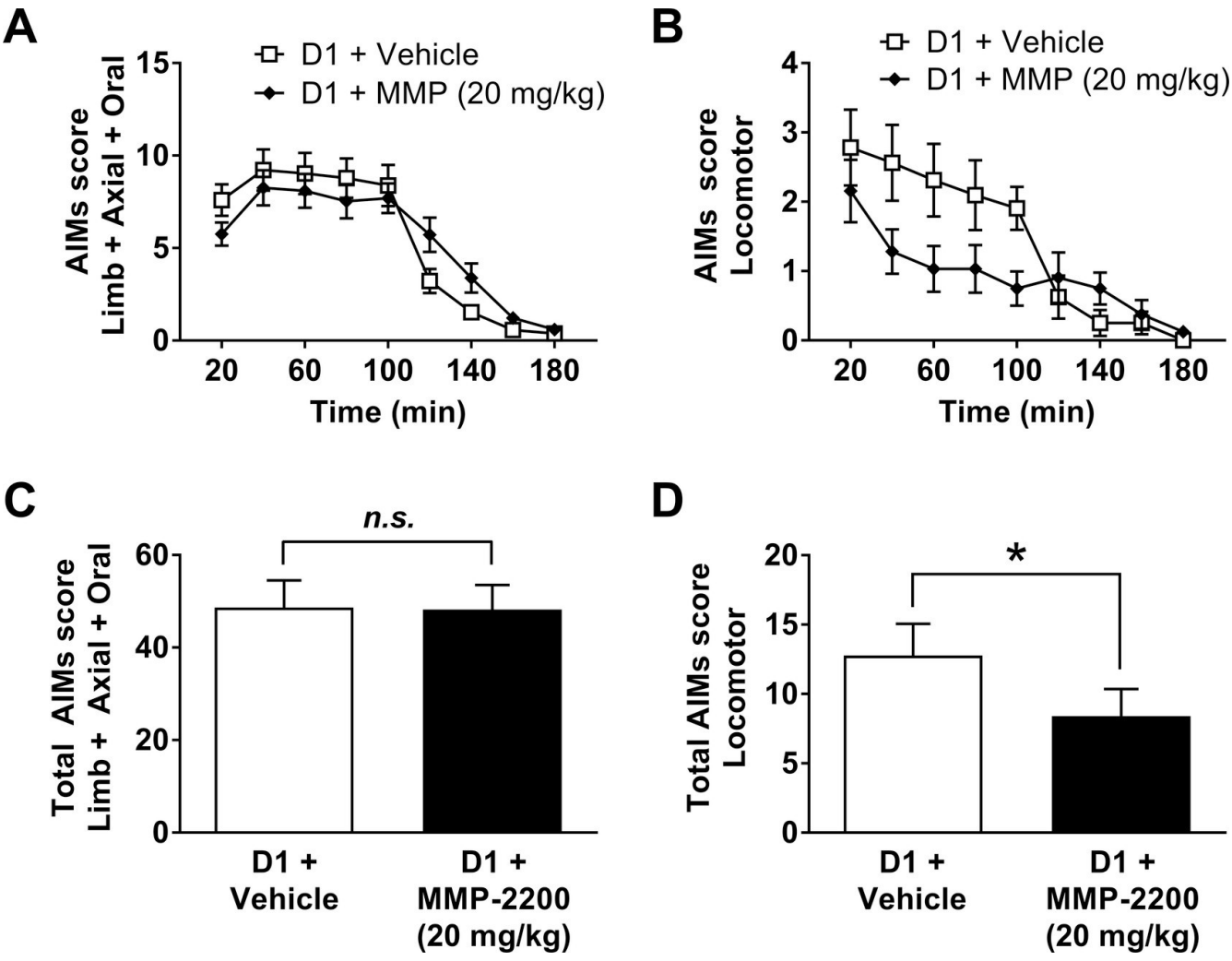

Figure 5. D_1R agonist-induced AIMs: MMP-2200 has no effect on LAO AIMs, but significantly reduces locomotor AIMs. (**A**) Mean total LAO AIMs scores (± SEM) are plotted. MMP-2200 (20 mg/kg, *i.p.*) had no effect on the SKF81297 (0.8 mg/kg, *s.c.*)-induced LAO AIMs. (**B**) Mean total locomotor AIMs scores (± SEM) are graphed; a reduction of SKF81297-induced locomotor AIMs by MMP-2200 is evident. (**C**) The graph shows the time-course of action of MMP-2200 on D_1R agonist-induced LAO-AIMs. (**D**) The graph depicts the time-course of the action of MMP-2200 on D_1R agonist-induced locomotor AIMs. In all graphs, AIMs scores were acquired over a 180 min interval following administration of drugs, using a within-subjects crossover design (* $p < 0.05$; $n = 8$).

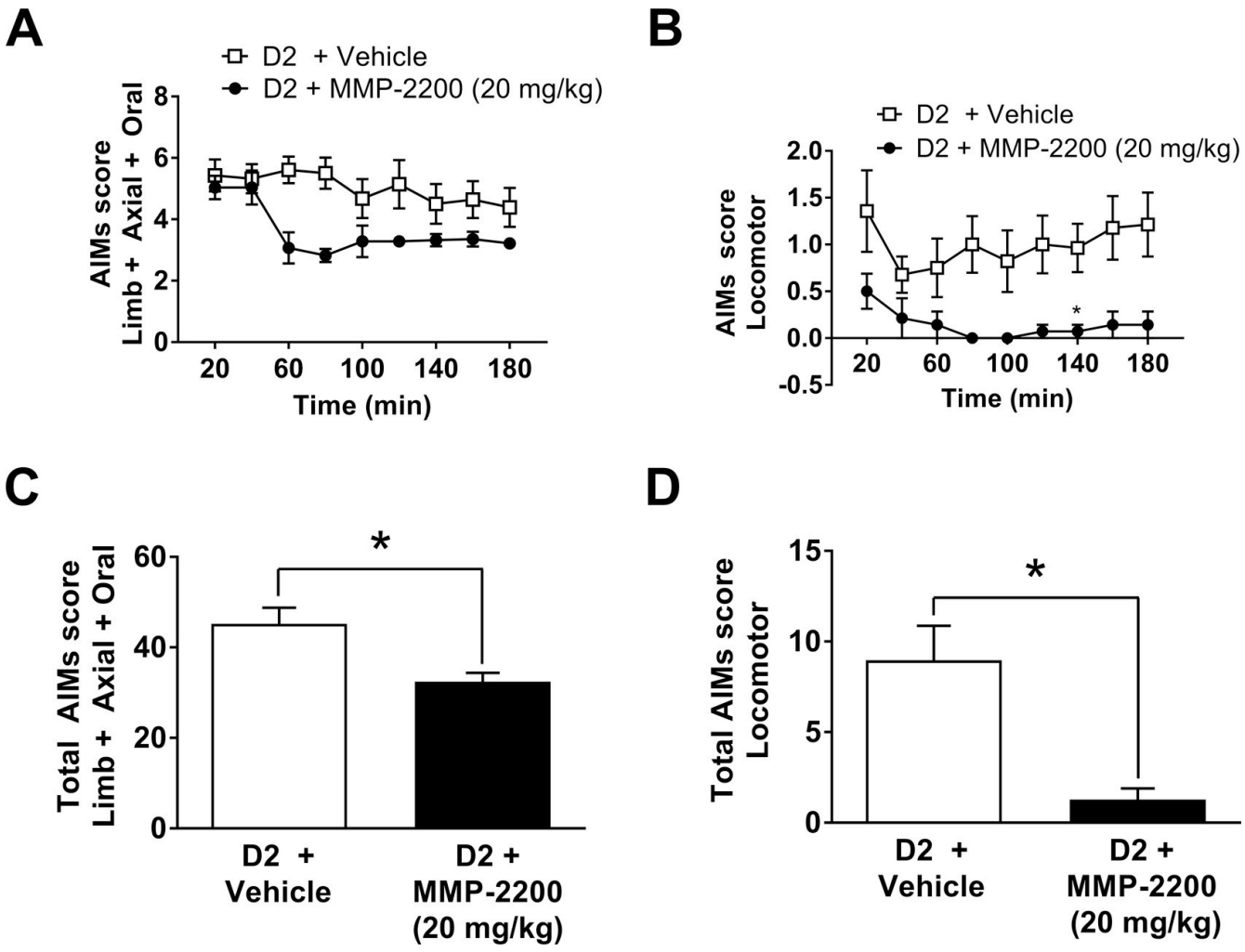

Figure 6. MMP-2200 reduces both D_2R agonist-induced LAO and locomotor AIMs. (**A**) The graph shows mean total LAO AIMs scores (± SEM). MMP-2200 (20 mg/kg, *i.p.*) significantly reduces quinpirole (0.2 mg/kg, *i.p.*)-induced LAO AIMs by ~40%. (**B**) The graph depicts mean total locomotor AIMs scores (± SEM) following quinpirole injection; MMP-2200 (20 mg/kg, *i.p.*) co-administration efficiently blocks locomotor AIMs. (**C**) The graph shows the time-course of the action of MMP-2200 on D_2R agonist-induced LAO-AIMs. (**D**) The graph depicts the time-course of action of MMP-2200 on D_2R agonist-induced locomotor AIMs. In all graphs data were acquired over a 180 min period following administration of drugs, using a within-subjects crossover design (* $p < 0.05$; $n = 7$).

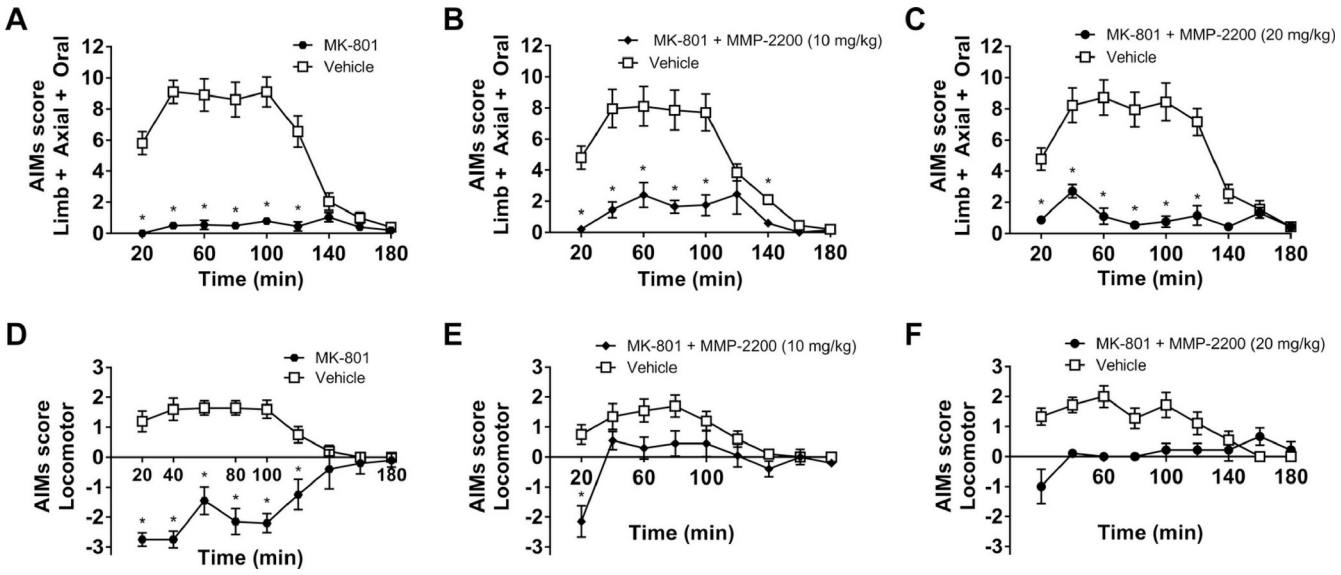

Figure 7. **The effect of MK-801 and MMP-2200 on L-DOPA-induced AIMs.** The non-competitive NMDA receptor antagonist MK-801 has previously been shown to have potent anti-dyskinetic activity only at a dose that also induces parkinsonism. Time-course plots are shown: (**A**) MK-801 (0.3 mg/kg) reduces LAO AIMs by > 90%. (**D**) MK-801 (0.3 mg/kg) induces pro-parkinsonian ipsiversive rotations, indicative of an induced parkinsonian state. Co-administration of 10 mg/kg MMP-2200 (**B** and **E**) and 20 mg/kg MMP-2200 (**C** and **F**) with MK-801 have no impact on the anti-dyskinetic efficacy of MK-801 on LAO AIMs (**B** and **C**), while both L-DOPA-induced contraversive rotations and MK-801-induced ipsiversive rotations are completely abolished **(E** and **F**); mean AIMs scores ± SEM; n = 9-10; * $p < 0.05$.

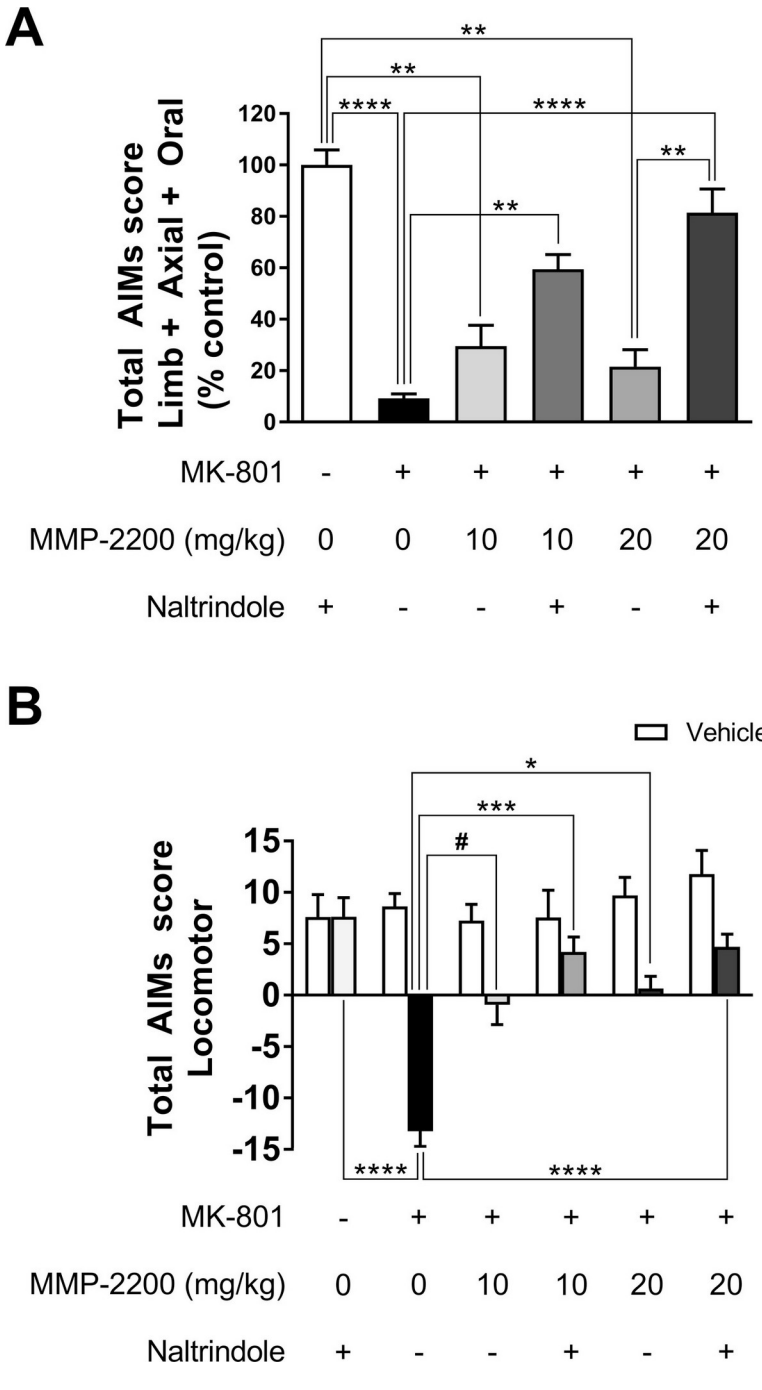

Figure 8. **The modulatory activity of MMP-2200 on MK-801 effects is altered by the selective delta-opioid receptor antagonist naltrindole.**(**A**) MK-801 (0.3 mg/kg) administration leads to a robust suppression of LAO AIMs in this model. LAO AIMs: Co-injection of 10 mg/kg and 20 mg/kg MMP-2200 did not have a significant effect on the anti-dyskinetic effect of MK-801 on LAO AIMs. Naltrindole (3 mg/kg) administration attenuates the anti-dyskinetic effect of MK-801 + MMP-2200. The data are plotted as % of L-DOPA-only vehicle control (mean±SEM; n = 9-10; **** $p < 0.0001$, ** $p < 0.01$, * $p < 0.05$, statistics done on raw AIMs data before normalization to % control). All groups are significantly decreased from vehicle control. (**B**) MK-801 (0.3 mg/kg, *i.p.*) produces a pro-parkinsonian effect, inducing rotations ipsiversive to the side of the 6-OHDA lesion, which is a correlate of parkinsonian activity in this model. Locomotor AIMs: Both 10 and 20 mg/kg MMP-2200 doses abolished MK-801-induced locomotor AIMs. This was significantly changed by the δ-opioid specific antagonist, leading to contraversive rotations instead (mean AIMs ±SEM; n = 9-10; ****$p <$

0.0001, ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$, $^{\#}p = 0.058$, statistics on raw AIMs data). Vehicle LAO and locomotor AIMs were not significantly different between different experimental conditions.

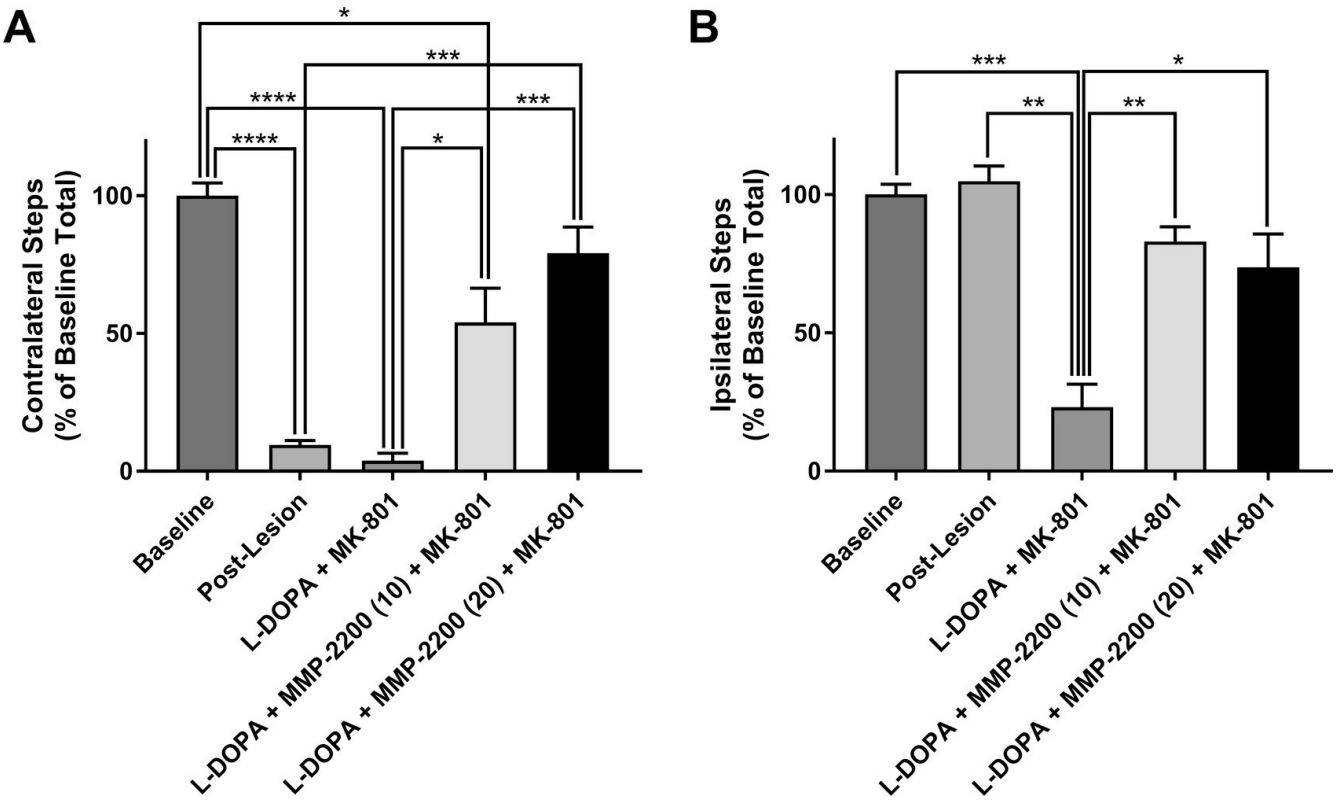

Figure 9. MMP-2200 suppresses the pro-parkinsonian activity of MK-801. Using the FAS test we could show that MK-801 (0.3 mg/kg; *i.p.*) completely blocks the activity of L-DOPA (7 mg/kg; *i.p.*) to counter the akinesia in the contralateral limb and MK-801 also significantly decreases the proportion of ipsiversive steps relative to baseline and postlesion, as has been shown by others prior. In the graph depicted in (**A**) the mean contralateral steps (±SEM) are plotted, and in (**B**) the mean ipsilateral steps (±SEM) are depicted. A reversal of the pro-parkinsonian MK-801 effect is evident after co-injection (*i.p.*) of 10 mg/kg MMP-2200 + MK-801, and at 20 mg/kg of MMP-2200 + MK-801 there is no significance difference from prelesion baseline ±SEM; $n = 7$; statistics were performed on data before normalization. **(A)** ****$p < 0.0001$, ***$p < 0.001$, *$p < 0.05$; **(B)** ***$\mathbf{p < 0.001}$, **$p < 0.01$, *$\mathbf{p < 0.05}$.

3.7 References

Bartlett, M. J., Joseph, R. M., LePoidevin, L. M., Parent, K. L., Laude, N. D., Lazarus, L. B., Heien, M. L., Estevez, M., Sherman, S. J., & Falk, T. (2016). Long-term effect of sub-anesthetic ketamine in reducing l-DOPA-induced dyskinesias in a preclinical model. *Neuroscience Letters*, *612*, 121–125. https://doi.org/10.1016/j.neulet.2015.11.047

Bezard, E., Ravenscroft, P., Gross, C. E., Crossman, A. R., & Brotchie, J. M. (2001). Upregulation of Striatal Preproenkephalin Gene Expression Occurs before the Appearance of Parkinsonian Signs in 1-Methyl-4-phenyl- 1,2,3,6-tetrahydropyridine Monkeys. *Neurobiology of Disease*, *8*(2), 343–350. https://doi.org/10.1006/nbdi.2000.0375

Billet, F., Costentin, J., & Dourmap, N. (2012). Influence of corticostriatal δ-opioid receptors on abnormal involuntary movements induced by L-DOPA in hemiparkinsonian rats. *Experimental Neurology*, *236*(2), 339–350. https://doi.org/10.1016/j.expneurol.2012.04.017

Bilsky, E. J., Egleton, R. D., Mitchell, S. A., Palian, M. M., Davis, P., Huber, J. D., Jones, H., Yamamura, H. I., Janders, J., Davis, T. P., Porreca, F., Hruby, V. J., & Polt, R. (2000). Enkephalin glycopeptide analogues produce analgesia with reduced dependence liability. *Journal of Medicinal Chemistry*, *43*(13), 2586–2590. http://www.ncbi.nlm.nih.gov/pubmed/10891118

Bordet, R., Ridray, S., Carboni, S., Diaz, J., Sokoloff, P., & Schwartz, J. C. (1997). Induction of dopamine D3 receptor expression as a mechanism of behavioral sensitization to levodopa. *Proceedings of the National Academy of Sciences of the United States of America*, *94*(7), 3363–3367. https://doi.org/10.1073/PNAS.94.7.3363

Breslin, M. B., Lindberg, I., Benjannet, S., Mathis, J. P., Lazure, C., & Seidah, N. G. (1993). Differential processing of proenkephalin by prohormone convertases 1(3) and 2 and furin. *The Journal of Biological Chemistry*, *268*(36), 27084–27093. http://www.ncbi.nlm.nih.gov/pubmed/8262946

Calon, F., Birdi, S., Rajput, A. H., Hornykiewicz, O., Bédard, P. J., & Di Paolo, T. (2002). Increase of preproenkephalin mRNA levels in the putamen of Parkinson disease patients with levodopa-induced dyskinesias. *Journal of Neuropathology and Experimental Neurology*, *61*(2), 186–196. http://www.ncbi.nlm.nih.gov/pubmed/11853020

Cenci, M. A., Lee, C. S., & Björklund, A. (1998). L-DOPA-induced dyskinesia in the rat is associated with striatal overexpression of prodynorphin- and glutamic acid decarboxylase mRNA. *European Journal of Neuroscience*, *10*(8), 2694–2706. https://doi.org/10.1046/j.1460-9568.1998.00285.x

Chang, J.-W., Wachtel, S. ., Young, D., & Kang, U.-J. (1999). Biochemical and anatomical characterization of forepaw adjusting steps in rat models of Parkinson's disease: studies on medial

forebrain bundle and striatal lesions. *Neuroscience*, *88*(2), 617–628. https://doi.org/10.1016/S0306-4522(98)00217-6

Chen, H., & Chan, D. C. (2009). Mitochondrial dynamics-fusion, fission, movement, and mitophagy-in neurodegenerative diseases. *Human Molecular Genetics*. https://doi.org/10.1093/hmg/ddp326

Connolly, B. S., & Lang, A. E. (2014). Pharmacological Treatment of Parkinson Disease. *JAMA*, *311*(16), 1670. https://doi.org/10.1001/jama.2014.3654

Crews, K. R., Gaedigk, A., Dunnenberger, H. M., Leeder, J. S., Klein, T. E., Caudle, K. E., Haidar, C. E., Shen, D. D., Callaghan, J. T., Sadhasivam, S., Prows, C. A., Kharasch, E. D., & Skaar, T. C. (2014). Clinical Pharmacogenetics Implementation Consortium Guidelines for Cytochrome P450 2D6 Genotype and Codeine Therapy: 2014 Update. *Clinical Pharmacology & Therapeutics*, *95*(4), 376–382. https://doi.org/10.1038/clpt.2013.254

Cuello, A. C., & Paxinos, G. (1978). Evidence for a long Leu-enkephalin striopallidal pathway in rat brain. *Nature*, *271*(5641), 178–180. http://www.ncbi.nlm.nih.gov/pubmed/23498

Dekundy, A., Lundblad, M., Danysz, W., & Cenci, M. A. (2007). Modulation of l-DOPA-induced abnormal involuntary movements by clinically tested compounds: Further validation of the rat dyskinesia model. *Behavioural Brain Research*, *179*(1), 76–89. https://doi.org/10.1016/j.bbr.2007.01.013

Do Carmo, G. P., Polt, R., Bilsky, E. J., Rice, K. C., & Negus, S. S. (2008). Behavioral pharmacology of the mu/delta opioid glycopeptide MMP2200 in rhesus monkeys. *The Journal of Pharmacology and Experimental Therapeutics*, *326*(3), 939–948. https://doi.org/10.1124/jpet.108.138180

Elmagbari, N. O., Egleton, R. D., Palian, M. M., Lowery, J. J., Schmid, W. R., Davis, P., Navratilova, E., Dhanasekaran, M., Keyari, C. M., Yamamura, H. I., Porreca, F., Hruby, V. J., Polt, R., & Bilsky, E. J. (2004). Antinociceptive structure-activity studies with enkephalin-based opioid glycopeptides. *The Journal of Pharmacology and Experimental Therapeutics*, *311*(1), 290–297. https://doi.org/10.1124/jpet.104.069393

Eskow, K. L., Gupta, V., Alam, S., Park, J. Y., & Bishop, C. (2007). The partial 5-HT1A agonist buspirone reduces the expression and development of l-DOPA-induced dyskinesia in rats and improves l-DOPA efficacy. *Pharmacology Biochemistry and Behavior*, *87*(3), 306–314. https://doi.org/10.1016/J.PBB.2007.05.002

Finck A. Donald, M. D., & Ngai S. H., M. D. (1982). Opiate Receptor Mediation of Ketamine Analgesia. *Anesthesiology*, *56*(4), 291–297. http://dx.doi.org/

Flores, A. J., Bartlett, M. J., So, L. Y., Laude, N. D., Parent, K. L., Heien, M. L., Sherman, S. J., & Falk, T. (2014). Differential effects of the NMDA receptor antagonist MK-801 on dopamine receptor D1- and D2-induced abnormal involuntary movements in a preclinical model. *Neuroscience Letters*, *564*. https://doi.org/10.1016/j.neulet.2014.02.004

Fox, S. H., & Lang, A. E. (2008). Levodopa-related motor complications-Phenomenology. *Movement Disorders*, *23*(S3), S509–S514. https://doi.org/10.1002/mds.22021

Fox, S. H., Lang, A. E., & Brotchie, J. M. (2006). Translation of nondopaminergic treatments for levodopa-induced dyskinesia from MPTP-lesioned nonhuman primates to phase IIa clinical studies: Keys to success and roads to failure. *Movement Disorders*, *21*(10), 1578–1594. https://doi.org/10.1002/mds.20936

Fox, S., Silverdale, M., Kellett, M., Davies, R., Steiger, M., Fletcher, N., Crossman, A., & Brotchie, J. (2004). Non-subtype-selective opioid receptor antagonism in treatment of levodopa-induced motor complications in Parkinson's disease. *Movement Disorders : Official Journal of the Movement Disorder Society*, *19*(5), 554–560. https://doi.org/10.1002/mds.10693

Gengo, P. J., & Chang, K.-J. (2003). Mixed Opioid Receptor Agonists as a New Class of Agents for the Treatment of Moderate to Severe Pain. In *The Delta Receptor* (pp. 231–244). CRC Press. https://doi.org/10.1201/9780203025765.ch14

Gerfen, C. R. (1990). D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. *Science*, *250*.

Guigoni, C., Aubert, I., Li, Q., Gurevich, V. V., Benovic, J. L., Ferry, S., Mach, U., Stark, H., Leriche, L., Håkansson, K., Bioulac, B. H., Gross, C. E., Sokoloff, P., Fisone, G., Gurevich, E. V., Bloch, B., & Bezard, E. (2005). Pathogenesis of levodopa-induced dyskinesia: focus on D1 and D3 dopamine receptors. *Parkinsonism & Related Disorders*, *11*, S25–S29. https://doi.org/10.1016/J.PARKRELDIS.2004.11.005

Guillin, O., Diaz, J., Carroll, P., Griffon, N., Schwartz, J.-C., & Sokoloff, P. (2001). BDNF controls dopamine D3 receptor expression and triggers behavioural sensitization. *Nature*, *411*(6833), 86–89. https://doi.org/10.1038/35075076

Gupta, A., Devi, L. A., & Gomes, I. (2011). Potentiation of μ-opioid receptor-mediated signaling by ketamine. *Journal of Neurochemistry*, *119*(2), 294–302. https://doi.org/10.1111/j.1471-4159.2011.07361.x

Hauser, R. A., Rascol, O., Korczyn, A. D., Jon Stoessl, A., Watts, R. L., Poewe, W., De Deyn, P. P., & Lang, A. E. (2007). Ten-year follow-up of Parkinson's disease patients randomized to initial therapy with ropinirole or levodopa. *Movement Disorders*, *22*(16), 2409–2417. https://doi.org/10.1002/mds.21743

Henry, B, Crossman, A. R., & Brotchie, J. M. (1999). Effect of repeated L-DOPA, bromocriptine, or lisuride administration on preproenkephalin-A and preproenkephalin-B mRNA levels in the striatum of the 6-hydroxydopamine-lesioned rat. *Experimental Neurology*, *155*(2), 204–220. https://doi.org/10.1006/exnr.1998.6996

Henry, Brian, Duty, S., Fox, S. H., Crossman, A. R., & Brotchie, J. M. (2003). Increased striatal pre-proenkephalin B expression is associated with dyskinesia in Parkinson's disease. *Experimental Neurology*, *183*(2), 458–468. https://doi.org/10.1016/S0014-4886(03)00064-5

Henry, Brian, Fox, S. H., Crossman, A. R., & Brotchie, J. M. (2001). μ- and δ-Opioid Receptor Antagonists Reduce Levodopa-Induced Dyskinesia in the MPTP-Lesioned Primate Model of Parkinson's Disease. *Experimental Neurology*, *171*(1), 139–146. https://doi.org/10.1006/EXNR.2001.7727

Hille, C. J., Fox, S. H., Maneuf, Y. P., Crossman, A. R., & Brotchie, J. M. (2001). Antiparkinsonian action of a delta opioid agonist in rodent and primate models of Parkinson's disease. *Experimental Neurology*, *172*(1), 189–198. https://doi.org/10.1006/exnr.2001.7763

Holloway, R. G., Shoulson, I., Fahn, S., Kieburtz, K., Lang, A., Marek, K., McDermott, M., Seibyl, J., Weiner, W., Musch, B., Kamp, C., Welsh, M., Shinaman, A., Pahwa, R., Barclay, L., Hubble, J., LeWitt, P., Miyasaki, J., Suchowersky, O., … Parkinson Study Group. (2004). Pramipexole vs Levodopa as Initial Treatment for Parkinson Disease. *Archives of Neurology*, *61*(7), 1044–1053. https://doi.org/10.1001/archneur.61.7.1044

Holloway, R., Marek, K., Biglan, K., Dick, A., Fahn, S., Julian-Baros, E., Kamp, C., Kieburtz, K., Lang, A., McDermott, M., Seibyl, J., Shinaman, A., Shoulson, I., Weiner, W., Pahwa, R., Grimes, D., Miyasaki, J., Johnston, L., Panisset, M., … Hunter, C. W. (2009). Long-term Effect of Initiating Pramipexole vs Levodopa in Early Parkinson Disease. *Archives of Neurology*, *66*(5), 563–570. https://doi.org/10.1001/archneur.66.1.nct90001

Holm, S. (1979). A Simple Sequentially Rejective Multiple Test Procedure. In *Scandinavian Journal of Statistics* (Vol. 6, pp. 65–70). WileyBoard of the Foundation of the Scandinavian Journal of Statistics. https://doi.org/10.2307/4615733

Jenner, P. (2005). *Principles of Treatment in Parkinson's Disease* (A. H. V. Schapira & C. W. Olanow (eds.)).

Katzenschlager, R., Head, J., Schrag, A., Ben-Shlomo, Y., Evans, A., Lees, A. J., & Parkinson's Disease Research Group of the United Kingdom. (2008). Fourteen-year final report of the randomized PDRG-UK trial comparing three initial treatments in PD. *Neurology*, *71*(7), 474–480. https://doi.org/10.1212/01.wnl.0000310812.43352.66

Klintenberg, R., Svenningsson, P., Gunne, L., & Andrén, P. E. (2002). Naloxone reduces levodopa-induced dyskinesias and apomorphine-induced rotations in primate models of parkinsonism. *Journal of Neural Transmission (Vienna, Austria : 1996)*, *109*(10), 1295–1307. https://doi.org/10.1007/s00702-002-0715-6

Koprich, J. B., Fox, S. H., Johnston, T. H., Goodman, A., Le Bourdonnec, B., Dolle, R. E., Dehaven, R. N., Dehaven-Hudkins, D. L., Little, P. J., & Brotchie, J. M. (2011). The selective mu-opioid

receptor antagonist adl5510 reduces levodopa-induced dyskinesia without affecting antiparkinsonian action in mptp-lesioned macaque model of Parkinson's disease. *Movement Disorders*, *26*(7), 1225–1233. https://doi.org/10.1002/mds.23631

Krack, P., Pollak, P., Limousin, P., Benazzouz, A., & Benabid, A. (1997). Stimulation of subthalamic nucleus alleviates tremor in Parkinson's disease. *The Lancet*, *350*(9092), 1675. https://doi.org/10.1016/S0140-6736(97)24049-3

Lanza, K., Meadows, S. M., Chambers, N. E., Nuss, E., Deak, M. M., Ferré, S., & Bishop, C. (2018). Behavioral and cellular dopamine D1 and D3 receptor-mediated synergy: Implications for L-DOPA-induced dyskinesia. *Neuropharmacology*, *138*, 304–314. https://doi.org/10.1016/J.NEUROPHARM.2018.06.024

Li, Y., Lefever, M. R., Muthu, D., Bidlack, J. M., Bilsky, E. J., & Polt, R. (2012). Opioid glycopeptide analgesics derived from endogenous enkephalins and endorphins. *Future Medicinal Chemistry*, *4*(2), 205–226. https://doi.org/10.4155/fmc.11.195

Limousin, P., Pollak, P., Benazzouz, A., Hoffmann, D., Le Bas, J.-F., Perret, J. E., Benabid, A.-L., & Broussolle, E. (1995). Effect on parkinsonian signs and symptoms of bilateral subthalamic nucleus stimulation. *The Lancet*, *345*(8942), 91–95. https://doi.org/10.1016/S0140-6736(95)90062-4

Lindskog, M., Svenningsson, P., Fredholm, B. B., Greengard, P., & Fisone, G. (1999). Activation of dopamine D2 receptors decreases DARPP-32 phosphorylation in striatonigral and striatopallidal projection neurons via different mechanisms. *Neuroscience*, *88*(4), 1005–1008. https://doi.org/10.1016/S0306-4522(98)00411-4

Lowery, J. J., Raymond, T. J., Giuvelis, D., Bidlack, J. M., Polt, R., & Bilsky, E. J. (2011). In vivo characterization of MMP-2200, a mixed δ/μ opioid agonist, in mice. *The Journal of Pharmacology and Experimental Therapeutics*, *336*(3), 767–778. https://doi.org/10.1124/jpet.110.172866

Lundblad, M., Andersson, M., Winkler, C., Kirik, D., Wierup, N., Cenci Nilsson, M. A., & Cenci, M. A. (2002). Pharmacological validation of behavioural measures of akinesia and dyskinesia in a rat model of Parkinson's disease. *The European Journal of Neuroscience*, *15*(1), 120–132. https://doi.org/10.1046/j.0953-816x.2001.01843.x

Lundblad, M., Usiello, A., Carta, M., Håkansson, K., Fisone, G., & Cenci, M. A. (2005). Pharmacological validation of a mouse model of l-DOPA-induced dyskinesia. *Experimental Neurology*, *194*(1), 66–75. https://doi.org/10.1016/j.expneurol.2005.02.002

Mabrouk, O. S., Falk, T., Sherman, S. J., Kennedy, R. T., & Polt, R. (2012). CNS penetration of the opioid glycopeptide MMP-2200: a microdialysis study. *Neuroscience Letters*, *531*(2), 99–103. https://doi.org/10.1016/j.neulet.2012.10.029

Manson, A. J., Katzenschlager, R., Hobart, J., & Lees, A. J. (2001). High dose naltrexone for dyskinesias induced by levodopa. *Journal of Neurology, Neurosurgery, and Psychiatry*, *70*(4), 554–556. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1737317&tool=pmcentrez&rendertype=abstract

Mefford, I. N., Gilberg, M., & Barchas, J. D. (1980). Simultaneous determination of catecholamines and unconjugated 3,4-dihydroxyphenylacetic acid in brain tissue by ion-pairing reverse-phase high-performance liquid chromatography with electrochemical detection. *Analytical Biochemistry*, *104*(2), 469–472. https://doi.org/10.1016/0003-2697(80)90101-3

Millan, M. J., Maiofiss, L., Cussac, D., Audinot, V., Boutin, J.-A., Newman-Tancredi, A., Lejeune, F., Rivet, J., Brocco, M., Duqueyroix, D., Nicolas, J., Boutin, J., & Newman-Tancredi, A. (2002). Differential actions of antiparkinson agents at multiple classes of monoaminergic receptor. I. A multivariate analysis of the binding profiles of 14 drugs at 21 native and cloned human receptor subtypes. *The Journal of Pharmacology and Experimental Therapeutics*, *303*(2), 791–804. https://doi.org/10.1124/jpet.102.039867

Moustafa, A. A., Sherman, S. J., & Frank, M. J. (2008). A dopaminergic basis for working memory, learning and attentional shifting in Parkinsonism. *Neuropsychologia*, *46*(13), 3144–3156. https://doi.org/10.1016/J.NEUROPSYCHOLOGIA.2008.07.011

Nisbet, A. P., Foster, O. J., Kingsbury, A., Eve, D. J., Daniel, S. E., Marsden, C. D., & Lees, A. J. (1995). Preproenkephalin and preprotachykinin messenger RNA expression in normal human basal ganglia and in Parkinson's disease. *Neuroscience*, *66*(2), 361–376. http://www.ncbi.nlm.nih.gov/pubmed/7477878

Noble, F., & Cox, B. M. (2002). Differential Regulation of D1 Dopamine Receptor- and of A2a Adenosine Receptor-Stimulated Adenylyl Cyclase by μ-, δ1-, and δ2-Opioid Agonists in Rat Caudate Putamen. *Journal of Neurochemistry*, *65*(1), 125–133. https://doi.org/10.1046/j.1471-4159.1995.65010125.x

Obeso, J. A., Rodriguez-Oroz, M. C., Rodriguez, M., DeLong, M. R., & Olanow, C. W. (2000). Pathophysiology of levodopa-induced dyskinesias in Parkinson's disease: problems with the current model. *Annals of Neurology*, *47*(4 Suppl 1), S22-32; discussion S32-4. http://www.ncbi.nlm.nih.gov/pubmed/10762129

Olanow, C. W. (2009). A double-blind, delayed-start trial of rasagiline in Parkinson's disease. *N. Engl. J. Med.*, *361*.

Paquette, M. A., Anderson, A. M., Lewis, J. R., Meshul, C. K., Johnson, S. W., & Paul Berger, S. (2010). MK-801 inhibits l-DOPA-induced abnormal involuntary movements only at doses that worsen parkinsonism. *Neuropharmacology*, *58*(7), 1002–1008. https://doi.org/10.1016/j.neuropharm.2010.01.003

Paquette, M. A., Martinez, A. A., Macheda, T., Meshul, C. K., Johnson, S. W., Berger, S. P., & Giuffrida, A. (2012). Anti-dyskinetic mechanisms of amantadine and dextromethorphan in the 6-OHDA rat model of Parkinson's disease: role of NMDA vs. 5-HT1A receptors. *European Journal of Neuroscience*, *36*(9), 3224–3234. https://doi.org/10.1111/j.1460-9568.2012.08243.x

Paxinos, G., & Watson, C. (2017). *The Rat Brain in Stereotaxic Coordinates* (7th ed.). Academic Press.

Portoghese, P. S., Lipkowski, A. W., & Takemori, A. E. (1987). Binaltorphimine and nor-binaltorphimine, potent and selective k-opioid receptor antagonists. *Life Sciences*, *40*(13), 1287–1292. https://doi.org/10.1016/0024-3205(87)90585-6

Rascol, O, Fabre, N., Blin, O., Poulik, J., Sabatini, U., Senard, J. M., Ané, M., Montastruc, J. L., & Rascol, A. (1994). Naltrexone, an opiate antagonist, fails to modify motor symptoms in patients with Parkinson's disease. *Movement Disorders : Official Journal of the Movement Disorder Society*, *9*(4), 437–440. https://doi.org/10.1002/mds.870090410

Rascol, Olivier, Brooks, D. J., Korczyn, A. D., De Deyn, P. P., Clarke, C. E., & Lang, A. E. (2000). A Five-Year Study of the Incidence of Dyskinesia in Patients with Early Parkinson's Disease Who Were Treated with Ropinirole or Levodopa. *New England Journal of Medicine*, *342*(20), 1484–1491. https://doi.org/10.1056/NEJM200005183422004

Scheller, D., Ullmer, C., Berkels, R., Gwarek, M., & Lübbert, H. (2009). The in vitro receptor profile of rotigotine: a new agent for the treatment of Parkinson's disease. *Naunyn-Schmiedeberg's Archives of Pharmacology*, *379*(1), 73–86. https://doi.org/10.1007/s00210-008-0341-4

Seizinger, B. R., Grimm, C., Höllt, V., & Herz, A. (1984). Evidence for a selective processing of proenkephalin B into different opioid peptide forms in particular regions of rat brain and pituitary. *Journal of Neurochemistry*, *42*(2), 447–457. http://www.ncbi.nlm.nih.gov/pubmed/6141221

Sgroi, S., Capper-Loup, C., Paganetti, P., & Kaelin-Lang, A. (2016). Enkephalin and dynorphin neuropeptides are differently correlated with locomotor hypersensitivity and levodopa-induced dyskinesia in parkinsonian rats. *Experimental Neurology*, *280*, 80–88. https://doi.org/10.1016/j.expneurol.2016.03.024

Solís, O., Garcia-Montes, J. R., González-Granillo, A., Xu, M., & Moratalla, R. (2017). Dopamine D3 Receptor Modulates l-DOPA-Induced Dyskinesia by Targeting D1 Receptor-Mediated Striatal Signaling. *Cerebral Cortex*, *27*(1), bhv231. https://doi.org/10.1093/cercor/bhv231

Westin, J. E., Andersson, M., Lundblad, M., & Cenci, M. A. (2001). Persistent changes in striatal gene expression induced by long-term l -DOPA treatment in a rat model of Parkinson's disease. *European Journal of Neuroscience*, *14*(7), 1171–1176. https://doi.org/10.1046/j.0953-816x.2001.01743.x

Yue, X., Falk, T., Zuniga, L. A., Szabò, L., Porreca, F., Polt, R., & Sherman, S. J. (2011). Effects of the novel glycopeptide opioid agonist MMP-2200 in preclinical models of Parkinson's disease. *Brain Research*, *1413*, 72–83. https://doi.org/10.1016/j.brainres.2011.07.038

Chapter 4: The kappa opioid receptor antagonist nor-BNI accelerates development of L-DOPA-induced dyskinesia in a preclinical model of mild dopamine depletion

Andrew J. Flores[a,b], Mitchell J. Bartlett[a,d], Blake T. Seaton[c], Grace Samtani[a], Morgan R. Sexauer[a], James R. Siegenthaler[c], Nathan C. Weintraub[d], Dong Lu[d], Michael L. Heien[c], Frank Porreca[d], Scott J. Sherman[a], Torsten Falk[a,b,d,*]

[a] Department of Neurology, [b] Graduate Interdisciplinary Program in Physiological Sciences, [c] Department of Chemistry & Biochemistry and BIO5 Institute, [d] Department of Pharmacology, The University of Arizona, Tucson, AZ 85724, USA

* Corresponding author: Torsten Falk, Ph.D.
Dept. of Neurology
P.O. Box 245023
University of Arizona
1501 N. Campbell Ave.
Tucson, AZ 85724
USA.
Phone: + 520 626 3927
E-mail address: tfalk@u.arizona.edu

Keywords: Levodopa-induced dyskinesia; Parkinson's disease; 6-hydroxydopamine; striatal lesion; κ-opioid receptor (KOR); norbinaltorphimine (nor-BNI)

Contributions

Conception and design: Torsten Falk, Andrew J. Flores, Mitchell J. Bartlett

Data collection: Andrew J. Flores (surgery, drug administration, behavioral analysis, sectioning, IHC), Mitchell Bartlett (drug administration, behavioral analysis), Blake T. Seaton (FSCAV analysis), Grace Samtani (behavioral analysis, sectioning), Morgan R. Sexauer (TH-ir analysis), James R. Siegenthaler (HPLC analysis), Nathan C. Weintraub (FSCAV analysis), Dong Lu (FSCAV analysis)

Data analysis and interpretation: Andrew Flores, Blake T. Seaton, Torsten Falk

Drafting the article: Andrew J. Flores, Torsten Falk, Blake T. Seaton

Critical revision of article: Torsten Falk, Scott J. Sherman, Robin Polt, Frank Porreca, Michael L. Heien, Andrew J. Flores, Mitchell J. Bartlett

Highlights

- The selective kappa-opioid receptor antagonist nor-BNI accelerated the development AIMs in a paradigm of prolonged levodopa-induced dyskinkesia development.
- nor-BNI did not show motor-restorative effects in model of mild dopamine-depletion.
- nor-BNI did not alter the level of expression of levodopa-induced AIMs once they were fully established.
- Systemic nor-BNI administration did not alter tonic DA levels in the dorsolateral striatum.

Abstract

Levels of the opioid peptide dynorphin, an endogenous ligand selective for kappa-opioid receptors (KORs), and its mRNA and pro-peptide precursors are differentially dysregulated in Parkinson's disease (PD) and following the development of L-DOPA-induced dyskinesia (LID). It remains unclear, however, whether these alterations contribute to the pathophysiological mechanisms underlying PD motor impairment and the subsequent development of LID, or whether they are part of compensatory mechanisms that attempt counteract pathological changes. Here, we sought to investigate the effects of the KOR antagonist nor-BNI 1) in the dopamine (DA)-depleted PD state and 2) during the development phase of LID, i.e., during the critical priming period. While nor-BNI administration (3 mg/kg; *s.c.*) did not lead to any functional restoration in the DA-depleted state, a change in the dose-dependent development of abnormal voluntary movements (AIMs) in response to escalating doses of L-DOPA in a rat PD model with a mild striatal lesion was seen. We tested 5 escalating dose of L-DOPA (6, 12, 24, 48, 72 mg/kg; *i.p.*), and nor-BNI accelerated the development of LAO AIMs at the lower doses of 12 and 24 mg/kg L-DOPA. However, after dosing with 72 mg/kg L-DOPA, AIMs were not significantly different between control and nor-BNI groups. In summary, while nor-BNI did not have

any functional restorative effect in the hemi-parkinsonian rats, nor-BNI did significantly accelerated the rate of development of LID induced by chronic, escalating doses of L-DOPA in a mild striatal-lesion rat PD model, but did not affect the overall severity once LID was fully established.

4.1 Introduction

Alterations in the expression of endogenous opioid peptides and their precursors, and in the expression of opioid receptors, have been observed in Parkinson's disease (PD) and levodopa (L-DOPA)-induced dyskinesia (LID), however the exact nature of the role played by these alterations in the pathophysiology of these disorders is unknown. Following dopamine (DA) depletion, striatal levels of proenkephalin (PPE-A, Penk) mRNA and enkephalin are upregulated, whereas levels of prodynorphin (PPE-B, Pdyn) mRNA and dynorphin in the striatum are downregulated (Calon et al., 2002; Henry et al., 2003; Nisbet et al., 1995). Additionally, positron emission tomography (PET) studies have identified enhanced opioidergic transmission in the striatum of PD patients with LID compared with non-dyskinetic patients (Piccini et al., 1997).

Given the localization of PPE-A to the indirect striatal output pathway and the understanding that enhanced GABAergic activity in the indirect striatal output pathway underlies parkinsonian symptoms, enhanced enkephalinergic transmission has been hypothesized to play a role in PD. Following long-term L-DOPA therapy and the development of LID, levels of PPE-A mRNA are further elevated beyond those seen in DA depletion alone, and levels of PPE-B mRNA are reversed from the level of reduction induced by DA denervation and elevated above normal. Levels of PPE-A and PPE-B are also elevated in the postmortem brains of patients with PD and LID (Calon et al., 2002; Henry et al., 2003; Nisbet et al., 1995).

Kappa (κ)-opioid receptors (KOR) control DA dynamics and KOR activation inhibits the release of DA in various midbrain efferents, including the nucleus accumbens (NAc) and the striatum (Di Chiara & Imperato, 1988; Gehrke et al., 2008). KOR inhibition increases DA release in the nucleus accumbens (Spanagel et al., 1992), yet it has not been investigated if the same is true in the dorsolateral striatum, the part of the striatum involved in motor control. The selective, long-acting (Kishioka et al., 2013) KOR antagonist nor-binaltorphimine (nor-BNI) has previously been shown to block L-DOPA-induced sensitization after nigral infusion in DA-depleted rats (Newman et al., 1997), and on the other hand activating KORs reduces LID (Ikeda et al., 2009), Kappa-opioid receptor agonists also increase locomotor activity in the monoamine-depleted rat model of parkinsonism (Hill & Brotchie, 1999; Hughes et al., 1998). These findings suggest that altered transmission of endogenous opioid neuropeptides, including KORs, plays a potentially important role in PD and LID, though further research is needed to accurately determine how these alterations relate to the pathophysiology of these disorders.

In the present study, we evaluated the modulatory effects of selective KOR antagonism in two experimental paradigms: 1) a functional restoration PD paradigm utilizing a mild unilateral striatal 6-hydroxydopamine (6-OHDA) rat model to model early PD, and 2) a prolonged LID development paradigm relying on chronic, escalating doses L-DOPA following a mild striatal 6-OHDA lesion. In addition, we then evaluated potential effects of nor-BNI on tonic DA levels in the dorsolateral striatum (DLS) using fast-scan controlled adsorption voltammetry (FSCAV) *in vivo*.

4.2 Methods & materials

4.2.1. Experimental Design

PD Restoration paradigm:

The design of the PD restoration experimental paradigm is diagramed in **Figure 1A.** In this paradigm, we evaluated the effects of nor-BNI (3 mg/kg; *s.c.*) to restore motor functionality in a mild unilateral striatal 6-OHDA lesion rat LID model (~75% DA depletion). Within this mild DA depletion paradigm, motor functionality was assessed in independent nor-BNI and vehicle-treated groups (n = 9/group; one animal in the nor-BNI group died of unrelated causes in week 7 post-treatment during this long-term study; some behavioral analysis videos for one animal in the control group were lost) using 1) amphetamine-induced rotations (AR), 2) cylinder, 3) forelimb-adjusting steps (FAS), and 4) vibrissae-stimulated forelimb placement (VSFP) tests in order to assess motor functionality.

LID development paradigm:

The design of the LID-development experimental paradigm is illustrated in the schematic diagram in **Figure 1B.** In this paradigm, we evaluated the effect of nor-BNI administration (3 mg/kg; *s.c.*) on the dose- and time-dependent development of abnormal voluntary movements (AIMs) in response to chronic escalating doses of L-DOPA treatment in a mild unilateral striatal 6-OHDA lesion rat LID model (~60% DA depletion). Animals (n = 14) with ≥ 1.0 amphetamine-induced rotations were included in the study (mean = 5.25, SEM = 1.30). Within this paradigm of prolonged LID development, independent nor-BNI and vehicle-treated groups (n = 7/group; one animal in the control group died of unrelated causes during this long-term study) were tested multiple times (2-4 times, at 2

testing sessions per week) across 5 escalating doses of L-DOPA (6, 12, 24, 48, 72 mg/kg; *i.p.*) and evaluated for total limb, axial, and orolingual (LAO) as well as locomotor AIMs scores under blinded conditions.

Tonic striatal DA levels:

In a third experiment (n =13) we evaluated tonic DA levels for 5 hours after systemic vehicle or nor-BNI administration in the DLS in anesthetized naïve rodents using *in vivo* FSCAV.

4.2.2 Animals

Male Sprague-Dawley rats (~225 g at arrival; Envigo RMC Inc., Indianapolis, IN), were used for Experiments 1 and 2. For the DA voltammetry measurement in Experiment 3 adult male Sprague-Dawley rats (258-310 g; Envigo) were housed two per cage. Animals from all studies were housed on a 12-hour light/dark cycle with food and water provided *ad libitum*. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Arizona and conformed to the guidelines of the NIH Guidelines for the Care and Use of Laboratory Animals, and with ARRIVE guidelines. All attempts were made to minimize the total number of animals used in these studies and their suffering.

4.2.3 Stereotaxic surgery for 6-hydroxydopamine striatal lesions

Two separate doses of 6-OHDA were used to induce chronic mild striatal lesions in animals—one for the functional restoration study and another for the LID-development study. For the functional restoration paradigm, a total amount of 15.0 µg 6-OHDA per animal was delivered into the striatum. For the for LID-development paradigm, a total amount of 12.0 µg 6-OHDA per animal was delivered

into the striatum. Prior to surgery, animals (n = 30 for the restoration paradigm and n = 18 for the LID development paradigm) were acclimated in the animal facility for approximately 2 weeks until they reached an average body mass of 300 g. In both experimental paradigms, a total volume of 8.0 µL of freshly prepared 6-hydroxydopamine hydrobromide solution (Sigma-Aldrich, St. Louis, MO; 1.875 and 1.5 µg per µL in 0.9 % sterile saline with 0.02% ascorbic acid to deliver total amounts of 15.0 and 12.0 µg via two injection sites in the DLS for the restoration and LID-development studies respectively). 6-OHDA was injected into two locations in the striatum with the following coordinates (1) AP +1.6; ML +2.2; DV −5.0; and (2) AP −0.4; ML +4.0; DV −5.0, according to the atlas of Paxinos & Watson (2007) as in (Yue et al., 2014). Injections were carried out at a rate of 1.0 µL per min using a 10.0 µL syringe and needle (26 gauge, 22 mm, 45° tip; Hamilton Company, Reno, NV) with a microinjector (Stoelting Co., Wood Dale, IL) attached to a stereotaxic micromanipulator (Narishige, Tokyo, Japan); the needle was left in place post-injection for a further 5 min to prevent any backflow of 6-OHDA solution upon withdrawal of needle. Given the established, standardized nature of the unilateral 6-OHDA model, no sham surgery groups were included.

4.2.4 Amphetamine-induced rotations (AR) test

In the functional restoration study, amphetamine-induced rotations were used to assess the severity/extent of dopaminergic denervation and asymmetric DA release occurring between the lesioned and intact hemispheres of the brain following development of unilateral 6-OHDA striatal lesions. Unilaterally-lesioned rats were injected with D-amphetamine (5.0 mg/kg, *i.p.*; Sigma-Aldrich) to induce dopamine release from dopaminergic neurons projecting to the striatum and were then placed in a plexiglass cylinder (38 cm diameter × 38 cm height). Rotational behavior was recorded by a camera placed above the cylinder for a total of 100 minutes following administration of amphetamine.

The average number of rotations per minute were counted and calculated by an individual blinded to experimental condition.

4.2.5. Cylinder test

Cylinder behavioral tests were conducted in plexiglass cylinders (14.5 cm diameter; 30.0 cm height). Forepaw touches on cylinder walls were counted by a blinded observer on a video for a minimum of 20 touches during a 5–10 minute recording period and calculated by a formula given in (Schallert, 2006): [(ipsi + 1/2 both)/(ipsi + contra + both)] × 100, which sets non-bias at 50%, and plotted as % contralateral forepaw contacts.

4.2.6 Forelimb-adjusting steps (FAS) test

The potential for restoration of motor functionality was assessed using a technique previously described (Chang et al., 1999; Eskow et al., 2007). Briefly, a 0.9-meter distance is marked off on a smooth tabletop surface. The animal is held by the experimenter with one hand supporting, but obstructing movement of the hindlimbs. The hind limbs are then slightly raised off the surface of the table. The experimenter's second hand is used to support and obstruct the movement of the forelimb not being assessed. The forelimb being assessed is allowed to touch the table and then moved sideways over the set distance at a rate of 0.1 cm/s. The experimenter then moves the forelimb first in the backhand direction, followed by the same motion in the forehand direction. Each forelimb was assessed three times per testing session. The total number of adjusting steps made in each direction during the three trials were recorded and later counted and used to calculate the average number of steps according to limb and direction (forehand vs. backhand). The proportion of the average number of steps taken by the motor defective left limb to the intact right limb for a given directionality (i.e.,

forehand vs. backhand) is then calculated. All tests were performed by a blinded observer. This test was utilized in both the LID-development and functional restoration paradigms.

4.2.7 Vibrissae-stimulated forepaw (VSFP) test

The VSFP test was used to assess sensorimotor deficits in parkinsonian animals of the functional restoration study. Animals were held parallel to the floor, with their hindlimbs supported and one forelimb restrained. The vibrissae on the side of the unrestrained limb were stimulated against the corner of a table in a single, constant motion. This normally causes the unrestrained forelimb to respond by reflexively touching the tabletop, unless a motor deficit is present in that limb due to the unilateral 6-OHDA lesion. Ten trials were performed for each forelimb and the proportion of touches on the motor defective left side to touches on the non-affected right side were calculated.

4.2.8 L-DOPA-induced AIMs

For the LID-development study, following development of a mild striatal lesion, animals were treated with chronic escalating doses L-DOPA. Within this mild-dyskinesia paradigm, independent *nor*-BNI and vehicle-treated groups were tested multiple times (2-4 times per week, depending on dose) across 5 escalating doses of L-DOPA (6, 12, 24, 48, 72 mg/kg; *i.p.*) and evaluated for total limb axial and orolingual (LAO), and locomotor AIMs scores. All injections were administered with the dopa decarboxylase inhibitor benserazide (14 mg/kg; *i.p.*) to prevent peripheral conversion of L-DOPA to DA and to mimic clinical therapy of human PD patients; injections were given once daily throughout the duration of the study. AIMs were tested twice per week (3 days between each testing session). AIMs were assessed by an observer blind to treatment condition, using an adapted version of the Abnormal Involuntary Movement Scale (Cenci et al., 1998), previously described by Paquette et al.

(2010) and in our previous publications (Bartlett et al., 2016, 2020; Flores et al., 2014, 2018). Briefly, each animal was rated on a scale of 0 (absent) to 4 (most severe) on each of four subscales (limb dyskinesia, axial dystonia, oral dyskinesia, and contraversive rotation). Limb, axial, oral, and locomotor type AIMs were based on 1-minute observation intervals conducted every 20 minutes for 180 minutes after administration of drugs. Specifically, for limb dyskinesia, a rating of 1 indicated 1 discrete period of abnormal movement, 2 indicated 3 or more discrete periods of abnormal movement, and 3-4 indicated continuous abnormal movement, with 4 indicating that AIMs could not be interrupted by a loud tap on the test cage. For axial dystonia, a rating of 1 indicated a contralateral bias in head orientation, 2 indicated a contralateral bias in head and upper body orientation, and 3-4 indicated a severe contralateral bias in head and upper body orientation (i.e., head above the tail in a four-paw stance), with 4 indicating loss of balance (i.e., falling). For oral dyskinesia, a rating of 1 indicated 2-3 bouts of mastication, 2 indicated more than 3 bouts of mastication, and 3-4 indicated continuous mastication, with 4 indicating the presence tongue protrusions. For L-DOPA-induced contraversive rotation, a rating of 1 indicated 2-3 contraversive turns, 2 indicated more than 3 contraversive turns, and 3-4 indicated continuous contraversive rotation, with a 4 indicating that these could not be interrupted by a loud tap on the cage. When ipsiversive rotation was observed, this was rated identically to contraversive rotation, except a negative score was provided to indicate contraversive directionality. Limb, axial, and oral subscale scores were summed to create a composite LAO AIMs score, while Locomotor AIMs scores were considered separately from LAO scores. Total scores for AIMs, as well as for each subscale, were summed over the entire duration of the experiment (180 minutes).

4.2.9 Drugs and administration protocols

In each study (functional restoration and LID-development), there were two independent groups (vehicle/control and nor-BNI). Assignment of animals to vehicle vs. experimental groups was based on post-lesion baseline performance in the AR test and also the cylinder and FAS test with the intention to balance the baseline motor functionality as much as possible between the groups prior to the commencement of testing (see **Figure 1**) with the intention of balancing the groups equally according to their level of motor deficit, which is in turn correlated to the severity of nigrostriatal degeneration and DA depletion. L-DOPA (levodopa; L-3,4-dihydroxyphenylalanine methyl ester hydrochloride), benserazide hydrochloride (D,L-serine 2-(2,3,4-trihydroxybenzyl) hydrazide hydrochloride) were purchased from Sigma-Aldrich, St. Louis, MO. Nor-binaltorphimine (*nor*-BNI) was purchased from Tocris Bioscience (Minneapolis, MN). In both the LID development and functional restoration paradigms, nor-BNI was administered subcutaneously (*s.c.*) at a concentration of 3 mg/kg three times per week, a dose that leads to long-term KOR antagonism in rodents (Navratilova et al., 2019). In the LID-development paradigm, nor-BNI was initially administered three days in advance of the commencement of L-DOPA administration, and three times per week to ensure complete ongoing blockade of KORs prior to AIMs testing. Nor-BNI was administered simultaneously with L-DOPA on testing days, L-DOPA and *nor*-BNI were administered at the beginning (t = 0 min) of testing.

4.2.10 Electrochemical measurement of DA and 5-HT and metabolites

Rat brains were placed in chilled Tris buffer (pH = 7.4, 15 mM Tris, 125 mM NaCl, 2.5 mM KCl, 2 mM $CaCl_2$) and gently agitated for approximately 30 s to remove any contaminants and then placed in a chilled brain block. Coronal brain slices were then collected and a 2 mm steel biopsy punch was

taken to sample tissue from the striatum. Samples from left and right hemispheres were collected and immediately flash frozen on an aluminum pan placed in liquid nitrogen -70 °C. Samples massed at 2.5 ± 0.5 mg, were then placed in 1.5 mL homogenization vials with 100 µL of 0.1 N $HClO_4$ (*aq*), manually homogenized (15 strokes) using a disposable pestle and stored at -80 °C for up to 2 weeks prior to analysis. High performance liquid chromatography with electrochemical detection (HPLC-EC) was subsequently used to separate and quantify DA, 3,4-dihydroxyphenylacetic acid (DOPAC), serotonin, and 5-hydroxyindoleacetic acid (5-HIAA).

4.2.11 Sectioning and immunohistochemical staining (functional restoration study)

Following behavioral analysis in the functional restoration study, immunohistochemical analysis was performed to verify the extent of the 6-OHDA lesioning in half of the animals in the Restoration study, as described in detail in prior work (Flores et al., 2018; X. Yue et al., 2011, 2014). Animals underwent perfusion-fixation using cold 4% paraformaldehyde, brains were dissected, and further fixed overnight in 4% paraformaldehyde. 40-micron sections through the substantia nigra and the striatum were obtained using a microtome (Leica Biosystems Nussloch GmbH) and mounted on standard glass slides (Fisher Scientific). Next, the sections were stained with a rabbit anti-TH primary antibody (1:10,000 for 24 hours at 4°C; Millipore) and a biotinylated donkey anti-rabbit immunoglobulin G secondary antibody (1:1,000 for 2 hours at RT; Millipore). To eliminate nonspecific binding prior to immunostaining endogenous peroxidase activity was blocked using 0.3% H_2O_2 for 30 min at room temperature (RT), the slides were then washed 3 times for 5 min with Tris buffer (pH = 7.6) and submerged in blocking solution (1% normal donkey serum, 0.1% Triton-X-100, Tris-HCl buffer, pH 7.6) for 2 hours at RT. The signal from the secondary antibody was amplified using the ABC reagent (Vectastain Elite ABC Kit, PK-6100) according to the manufacturer's protocol. We used DAB substrate

(Vector Laboratories, Burlingame, CA) as the chromogen for final visualization of TH immunoreactivity. Densitometry analysis of the total area of detected objects (total pixels^2) in a 480 × 480 pixel region of the mediolateral SN (3 sections/rat) was conducted by a blinded investigator with ImageJ software (vs.2.0.0-rc-69/1.52p, http://rsbweb.nih.gov/ij/) according to an established protocol (Bartlett et al., 2020; Kneynsberg et al., 2016).

4.2.12 Fast-ccan controlled adsorption voltammetry (FSCAV)

Electrodes: Carbon-fiber microelectrodes (CFMEs) were fabricated as previously described, with minor modifications (Gee et al., 2020). A single carbon fiber (7.1 μm diameter, AS4 12K, Hexcel, Stamford, CT) was aspirated into a glass capillary and pulled with a vertical micropipette puller (Narishige, Tokyo, Japan). The exposed carbon fiber was cut to a length of 70 μm with a scalpel under a Micromaster brightfield microscope (12−561B, Fisher Scientific, Hampton, NH). A silver wire (Kauffman Engineering, Cornelius, OR), coated with alcohol-based graphite conductive adhesive (42465, Alfa Aesar, Ward Hill, MA), was inserted into the back end of the glass capillary. Epoxy adhesive (Henkel Corp., Rocky Hill, CT) was applied to the wire-capillary interface and allowed to dry overnight. Each CFME was then coated with 3,4-ethylenedioxythiophene:Nafion (PEDOT:Nafion) to increase dopamine sensitivity and selectivity (Vreeland et al., 2015). Ag/AgCl-wire reference electrodes were prepared by soaking 0.25 mm-diameter silver wire (265578, Sigma-Aldrich, St. Louis, MO) in 8.25% sodium hypochlorite (The Clorox Company, Oakland, CA) for 24 hours.

Surgery: Rats were induced with 4% isoflurane gas and an air flow rate of 1.5 L/min. Rat body temperature was maintained with a water-circulating heating pad (T/Pump, Stryker, Portage, MI). A

bipolar stimulating electrode (Plastics One, Roanoke, VA) was implanted in the medial forebrain bundle (AP: -2.5 mm, ML: 1.7 mm, DV: -7.0 to -7.9 mm from bregma), a Ag/AgCl-wire reference electrode was implanted in the contralateral hemisphere, and a CFME working electrode was implanted in the dorsolateral striatum (DLS) (AP: 1.0 mm from bregma, ML: 3.2 mm from bregma, DV: -3.8 to -4.0 mm from brain surface). The final dorsoventral depth of the CFME was determined by maximizing stimulated dopamine release in the DLS. Following final electrode placement, tonic dopamine levels in the DLS were measured using fast-scan controlled adsorption voltammetry (FSCAV) (Atcherley et al., 2013). FSCAV was performed until a stable baseline was established (typically 30-60 minutes). Following baseline stability, FSCAV was performed for 10 minutes prior to injection of saline or nor-BNI (3 mg/kg) and continued for the remainder of the 5-hour experiment.

Histology: Placement of the CFME working electrode was confirmed according to Gee et al., 2020 with the addition of cresyl violet staining. Following experimentation, electrolytic lesions were made with the working electrode and the animals were euthanized via isoflurane overdose and cervical dislocation. Brains were immediately post-fixed in 10% formalin and sectioned at 40 microns through the DLS on a cryostat (CM1850, Leica Biosystems, Buffalo Grove, IL). Sections were then stained with cresyl violet to improve visualization. The CFME was located within the DLS in each experiment, therefore all animals were included in the analysis. Representative histological verification shown in **Figure 6A**. Lesioning was not required for verification of the stimulating electrode position in the MFB.

4.2.13 Statistical analysis

All statistical analyses were performed with GraphPad Prism 8 (GraphPad Software, La Jolla, CA). The TH-ir analysis was done using a one-way ANOVA with Tukey's multiple comparisons *post hoc* tests. Two-way ANOVA was used to compare striatal DA, DOPA, 5-HT, and 5-HIAA content in lesioned and intact hemispheres of nor-BNI and vehicle/control animals. For the AR, cylinder, FAS, and VSFP tests, two-way repeated measures ANOVA tests were used to compare the nor-BNI and vehicle/control groups across all postlesion experimental timepoints; separate two-way ANOVA tests were used to compare prelesion and postlesion baseline scores. Within the LID development paradigm, two-way repeated measures ANOVAs were used to compare AIMs scores between nor-BNI and vehicle control groups across all experimental timepoints. Cumulative AIMs scores for each of the L-DOPA concentrations (6, 12, 24, 48, 72 mg/kg) were compared between control and nor-BNI groups using two-tailed paired sample t-tests. For the FSCAV analysis, a repeated measures two-way ANOVA (with Bonferroni *post hoc* tests) was used. For all statistical analyses, two-tailed tests were performed to compare null and alternative hypotheses and the alternative hypothesis was rejected when $p < 0.05$. All graphs have plotted the sample mean ± SEM (std. error of mean).

4.3 Results

4.3.1 Functional restoration study: *Post hoc* analyses to verify unilateral 6-OHDA lesion

In *post hoc* analyses for the functional restoration study, a significant reduction in nigral TH immunoreactivity (**Figure 2A**) was observed in both vehicle/control ($n = 5$) and nor-BNI ($n = 4$) groups relative to the intact hemisphere, verifying successful development of a unilateral lesion of the nigrostriatal tract ($F_{[3, 14]} = 25.92$, two-tailed $p < 0.0001$, one-way ANOVA). Tukey's multiple comparisons *pos hoc* tests revealed significant reductions in TH-ir (vehicle intact vs. vehicle lesion:

mean diff. = 380.5, q = 7.656, df = 14, two-tailed $p < 0.0005$; nor-BNI intact vs. nor-BNI lesion: mean diff. = 533.7, q = 9.605, df = 14, two-tailed $p < 0.0001$) as shown in **Figure 2B**. There was no significant difference in TH-ir between vehicle and nor-BNI intact hemispheres nor was there a significant difference between vehicle and nor-BNI lesioned hemispheres (vehicle intact vs. nor-BNI intact: mean diff. = -157.1, q = 2.980, df = 14, two-tailed $p = 0.1985$; vehicle lesion vs. nor-BNI lesion: mean diff. = -3.870, q = 0.07341, df = 14, two-tailed $p > 0.9999$), indicating that the two groups were balanced in their levels of DA depletion prior to testing. Normalized TH-ir (% lesion/intact) was not significantly different between vehicle (mean = 28.086 , SEM = 7.570) and nor-BNI (mean = 20.9075, SEM = 3.518) groups, indicating that animals were assigned to control and nor-BNI groups such that the two groups were balanced in their average level of DA denervation prior to testing (unpaired t-test, $t = 0.7869$, df = 7, two-tailed $p = 0.4571$). The same was true for the remainder of the animals in the vehicle/control ($n = 4$) and nor-BNI ($n = 4$) groups where analysis of DA, DOPAC, 5-HT, and 5-HIAA content in lysates sampled *post hoc* from striatal tissue revealed significant reduction in levels of DA ($F_{[1,12]} = 8.192$, two-tailed $p = 0.0146$, two-way ANOVA) and DOPAC ($F_{[1, 12]} = 12.58$, two-tailed $p = 0.0040$, two-way ANOVA) levels between the lesioned and intact hemispheres (**Figures 2C** and **D**), while 5-HT ($F_{[1, 12]} = 2.553$, two-tailed $p = 0.1361$, two-way ANOVA) and 5-HIAA ($F_{[1, 12]} = 0.3154$, two-tailed $p = 0.5847$, two-way ANOVA) levels remained unaltered by the 6-OHDA lesion as shown in **Figures 2E** and **F**.

4.3.2 Treatment with nor-BNI did not improve motor deficits in the PD functional restoration paradigm

Analysis of the behavioral data from the AR, cylinder, FAS, and VSFP tests failed to detect any significant restorative effect of nor-BNI on parkinsonian motor deficits (**Figure 3**). For the AR, cylinder, FAS, and VSFP tests, two-way repeated measures ANOVA tests were used to compare the nor-BNI (n = 8) and vehicle/control (n = 8-9) groups across all postlesion experimental timepoints. For the assays that had prelesion baseline scores, separate two-way ANOVA tests were performed to compare prelesion and postlesion baseline scores, which, as expected, showed a marked reduction following the unilateral 6-OHDA lesion. Two-way repeated measures ANOVA was used to compare the average number of rotations per minute between control and nor-BNI groups in the AR test across all postlesion timepoints (**Figure 3C**). This analysis revealed that there was no significant difference between control and nor-BNI groups in the FAS test ($F_{[4, 56]}$ = 0.3432, two-tailed p = 0.8477). Two-way repeated measures ANOVA was used to compare forelimb touching bias scores from the cylinder test between vehicle/control (n = 8) and nor-BNI (n = 8) groups across all timepoints in the study. The analysis revealed that there was no significant difference in forelimb touching bias in the cylinder test between control and nor-BNI groups ($F_{[4, 56]}$ = 0.8483, two-tailed p = 0.5007) as shown in **Figure 3B**. Two-way repeated measures ANOVA was used to compare the proportion of motor-defective forelimb steps to total steps taken in the FAS test across all postlesion timepoints (**Figure 3C**). This analysis revealed that there was no significant difference between control and nor-BNI groups in the FAS test ($F_{[4, 56]}$ = 0.8609, two-tailed p = 0.4931). Finally, a two-way repeated measures ANOVA was used to compare the proportion of reflexive touch responses elicited on the motor-defective left side to the intact right side across all postlesion timepoints in the VSFP test (**Figure 3D**). This analysis revealed

that there was no significant difference between control and nor-BNI groups in the VSFP test ($F_{[4, 56]}$ = 1.456, two-tailed p = 0.2269).

4.3.3 LID development study: *Post hoc* analyses to verify unilateral 6-OHDA lesion

In the LID development study, *post hoc* HPLC-EC analysis was used to measure striatal concentrations of DA, DOPAC, 5-HT, and 5-HIAA in lysates derived from striatal tissue samples. Two-way ANOVA revealed that DA (**Figure 4A**) and DOPAC (**Figure 4B**) levels were significantly reduced in the lesioned versus the intact hemisphere (DA $_{\text{lesion vs. intact}}$: $F_{[1 ,22]}$ = 31.31, two-tailed p < 0.0001; DOPAC $_{\text{lesion vs. intact}}$: $F_{[1 ,22]}$ = 25.56, two-tailed p < 0.0001), thus verifying the successful development of the unilateral 6-OHDA lesion in animals, but that this reduction was not significantly different between nor-BNI and vehicle/control groups (DA $_{\text{vehicle vs. nor-BNI}}$: $F_{[1 ,22]}$ = 1.458, two-tailed p = 0.2400; DOPAC $_{\text{vehicle vs. nor-BNI}}$: $F_{[1 ,22]}$ = 9.876e-005, two-tailed p = 0.9922). This demonstrates that, as intended, animals were appropriately assigned to control and experimental groups, such that DA depletion was balanced between the two groups before commencing levodopa-therapy. By contrast, levels of 5-HT and 5-HIAA (**Figure 4C** and **4D**) between the lesioned and intact hemispheres were unaltered by the 6-OHDA lesion (5-HT: $F_{[1 ,22]}$ = 0.001239, two-tailed p = 0.9722; 5-HIAA: $F_{[1 ,22]}$ = 0.8918, two-tailed p = 0.3553) indicating that the lesion was specific to dopaminergic neurons.

4.3.4 Treatment with nor-BNI accelerated the development of L-DOPA-induced AIMs in the model of prolonged LID-development utilizing chronic escalating doses of L-DOPA

We evaluated the effect of *nor*-BNI administration (3 mg/kg; *s.c.*) on the dose- and time-dependent development of abnormal voluntary movements (AIMs) in response to chronic escalating doses of L-DOPA treatment in a mild unilateral striatal 6-OHDA lesion rat LID model (~60% DA depletion).

Within this mild-dyskinesia paradigm, independent nor-BNI and vehicle-treated groups (n = 6-7) were tested multiple times (2-4 times) across 5 escalating dose phases of L-DOPA (6, 12, 24, 48, 72 mg/kg; *i.p*) and evaluated for total limb axial and orolingual (LAO; **Figure 5A**), and locomotor (**Figure 5B**) AIMs scores under blinded conditions.

When cumulative AIMs scores were analyzed separately according to L-DOPA dose, we discovered that nor-BNI increased the expression of LAO AIMs (**Figure 5C**) at the 12 (paired sample t-test, t = 2.466, df = 23, two-tailed p = 0.0215) and 24 mg/kg (paired sample t-test, t = 2.473, df = 23, two-tailed p = 0.0366) L-DOPA doses. At the end of the escalation paradigm, after the 72 mg/kg L-DOPA dose, AIMs scores were the same in both groups (paired sample t-test, t = 0.1306, df = 11, p = 0.8985), suggesting that nor-BNI accelerates the rate of development of L-DOPA-induced AIMs, but does not increase the overall severity of AIMs once LID is fully established. When cumulative total locomotor AIMs scores were analyzed (**Figure 5D**) separately according to L-DOPA dose at the 12, 24 and 72 mg/kg doses locomotor AIMs were equal. In summary, nor-BNI significantly accelerated the rate of development of LAO but not locomotor AIMs induced by chronic, escalating doses of L-DOPA in a mild striatal lesion rat PD model, but did not increase either AIMs once LIDs were fully established.

4.3.5 Systemic treatment with nor-BNI did not alter tonic levels striatal dopamine

Tonic levels of DA were measured in the DLS using FSCAV following administration of nor-BNI (3 mg/kg, *s.c.*) in anesthetized naïve animals (**Figure 6A-C**). A two-way repeated measures analysis of data recorded from animals in the vehicle/control (n = 5) and nor-BNI (n = 8) groups revealed that nor-

BNI administration did not alter tonic DA levels ($F_{[1,9889]} = 0.05529$, two-tailed $p = 0.8184$). Bonferroni *post hoc* tests yielded $p > 0.05$ at all timepoints following nor-BNI administration.

4.4 Discussion

The opioid neurotransmission system is prominent in the basal ganglia. Endogenous opioid peptides and their precursors are highly expressed within the basal ganglia where they are modulators of GABA, DA, and glutamate neurotransmission (Fox et al., 2006; Fox & Brotchie, 2011). Several lines of evidence suggest that κ-mediated opioid signaling is overactive in LID. In the unilateral 6-OHDA lesioned rat model of LID, the severity of AIMs correlates strongly with levels of striatal PPE-B mRNA, which encodes endogenous ligands for KORs (Cenci et al., 1998). Another study, showed that striatal PPE-B mRNA levels were increased in both MPTP-lesioned macaques and PD patients with LID (Henry et al., 2003). Several other studies have shown that striatal levels of dynorphin are positively correlated with LID (Sgroi et al., 2016). Additionally, MALDI-TOF imaging mass spectrometry showed that levels of dynorphin B and alpha-neoendorphin in the SN are strongly correlated with the severity of AIMs in the 6-OHDA rat model of LID (Ljungdahl et al., 2011). Furthermore, [35S]GTPγS binding indicated hyperactive KOR signaling in the caudate nucleus and motor cortex in a nonhuman primate model of LID (Chen et al., 2005). Despite this evidence, however, antagonizing KORs with nor-BNI failed to reduce levodopa-induced abnormal involuntary movements in primates with established LID (Henry et al., 2001).

With regard to KORs, Aubert et al. (2007) found that they were unaltered in MPTP-lesioned primates, but that their expression was reduced in the globus pallidus exterior (GPe) and globus pallidus interior (GPi) following chronic L-DOPA administration. In contrast, κ-opioid receptor expression was decreased in the striatum and SN, but unaltered in the GP, of 6-OHDA-lesioned rats

that expressed AIMs in response to L-DOPA compared with non-dyskinetic animals (Johansson et al., 2001). Furthermore, activation of KORs with the selective κ-receptor agonist U50,488 reduced AIMs in the 6-OHDA rat model of LID and dyskinesia in MPTP-lesioned squirrel monkeys but also reversed the anti-parkinsonian effect of L-DOPA worsening motor behavior (Cox et al., 2007). On the basis of this evidence, we investigated effects of antagonizing KOR-opioid signaling in two rodent models of PD, testing the ability of the KOR-antagonist nor-BNI to a) functionally restore motor functionality in a PD restoration study and b) to explore the efficacy of nor-BNI in the 6-OHDA rat model of LID development, to test if nor-BNI either accelerates or attenuates the development of LID.

It has been established that nor-BNI selectively blocks KOR signaling, i.e. as KOR signaling is known to encode dysphoria, which nor-BNI was able to block in an experimental murine model (Land et al., 2008), and that KOR agonists induce dysphoric and aversive states (Xuei et al., 2006). Pain-induced negative affect is also mediated by activation of the kappa/dynorphin system in the NAc (Massaly et al., 2019). There has also been a great deal of work done testing the influence of KOR transmission associated with clinical depression, and within the context of withdrawal from opioid addiction and other substances of abuse (Chavkin & Koob, 2016; Knoll & Carlezon, 2010; Laurence Lalanne et al., 2014). Dynorphin is also thought to play a significant non-opioid role in response to injury, being upregulated and mediating a secondary damaging inflammatory response (Hauser et al., 2005). We found that nor-BNI accelerated the development of L-DOPA-induced AIMs using a paradigm of prolonged gradual LID development, yet it did not worsen LID once fully established, the latter replicating what has been shown in the primate model of established LID (Henry et al., 2001). Intrastriatal 6-OHDA administration induces damage of dopaminergic terminals, followed by a delayed, progressive loss of the dopaminergic neuron cell bodies in the SN, which are then secondarily affected through a dying-back phase. Taking this into account, we allowed 3-4 weeks for the striatal lesions to develop, to not interfere our assessment of nor-BNI's effects with this confounding variable.

Because terminals in the striatum are spatially diffuse relative to the densely bundled axons which travel between the SNc and striatum (included within the medial forebrain bundle tract), striatal administration of 6-OHDA causes a milder level of degeneration relative to more commonly-used lesions of the medial forebrain bundle, which cause severe (>90%) DA cell loss. For the functional Restoration study, we selected a lesion severity to realize ~75% DA depletion, which is generally accepted as the level of cell loss required for motor deficits to appear in human PD patients. This leads to a sufficient level of motor dysfunction in order to measure any acute motor-restorative effects of nor-BNI, and the 25% remaining DA terminals would allow for any possible effects of nor-BNI on striatal DA release, as it has been shown for NAc and dorsomedial striatum (Di Chiara & Imperato, 1988; Gehrke et al., 2008). In the experimental paradigms to test LID development, in addition to electing to utilize striatal lesions, we also used an even lower amount of 6-OHDA, to realize an even milder lesion of ~60% DA depletion, to assure enough functional DA activity remained. This is different from the severe MFB lesions, that model later PD stages and are the standard for the rat LID model, where development of LID can be induced by low L-DOPA doses, typically 6 mg/kg. Striatal lesions are typically not used for studies of LID because in this case very high doses of L-DOPA must be administered in order to induce AIMs. In paradigms using DA-depletion, L-DOPA doses as high as 50-100 mg/kg or more have been used to induce AIMs without toxicity (Engber et al., 1991; Ishida et al., 2000). The accelerated LID development seen could provide evidence in support of the hypothesis that nor-BNI causes a functional increase in DA levels within the dorsolateral striatum (DLS), as a rise in tonic DA within the motor part of the striatum would act to correct the imbalance between the direct and indirect striatal output pathways, which is a fundamental tenet of PD pathology. Alternatively, opioid modulation of signaling downstream of DA could underlie the effects of nor-BNI to accelerate development of LID. Our follow-up FSCAV experiment in naïve animals showed that systemic nor-BNI did not change tonic DA levels in the DLS over the course of the 5-hour experiment. This argues

against direct effects of nor-BNI on tonic DA levels in the DLS, but rather points to -opioid modulation of downstream signaling due to the block of KORs, which should be explored in detail in future studies. Our data further support involvement of KORs in LID, and indicate that KOR agonism could be especially effective early on in the beginning stages of the development of LID. On the other hand, the liability of KOR agonists is a concern, and might limit clinical use, specifically, in light of its role in driving aversion, negative affective states, stress-induced alterations in motivated behavior and cognition, as well as psychotomimetic effects (as reviewed in Tejeda & Bonci, 2019). Other recent studies have explored modulation of opioid signaling, including -opioid signaling, rather with dually acting compounds or within a combination treatment, i.e. the mixed κ-opioid receptor agonist/μ-opioid receptor antagonist nalbuphine did reduce LID in dyskinetic non-human LID primates (Potts et al., 2015). Further, a recent study did show that the novel molecule DPI-289, a mixed δ-opioid agonist/μ-opioid receptor antagonist, was anti-dyskinetic in both rodent and non-human primate LID models (Johnston et al., 2018). While a MOR antagonist with low selectivity reduced LID (Koprich et al., 2011), two highly-selective MOR antagonists did not (Bartlett et al., 2020; Bezard et al, 2020), and rather a MOR agonist showed anti-dyskinetic activity (Bezard et al., 2020). Together this indicates that more than one opioid receptor modulation might be needed for a successful anti-LID strategy.

In conclusion, our data indicate that while blocking KOR signaling did neither change PD motor dysfunction, nor established LID, it accelerated the development of LID in a rodent model of mild PD, further indicating -opioid signaling as an important player in the dysregulated neurotransmission within the basal ganglia in PD.

4.5 Acknowledgments

We would like to thank the ARCS Foundation (AJF) and The Jerry T. and Glenda G. Jackson Fellowship in Parkinson's Research to the University of Arizona (TF and SJS) for their support.

4.6 Figures

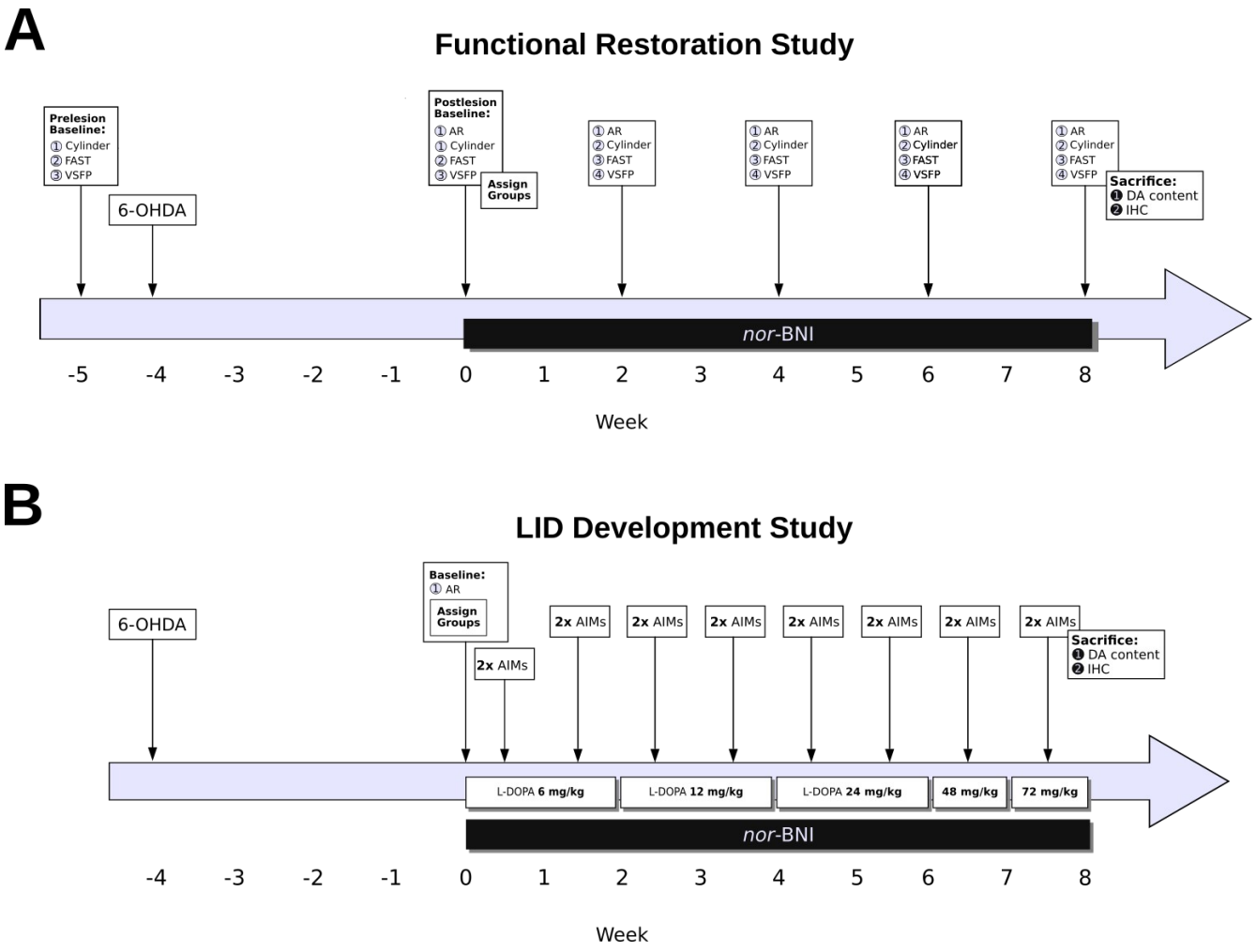

Figure 1. Experimental designs. (A) *Functional restoration paradigm:* We evaluated the potential of nor-BNI administration (3 mg/kg; s.c.) to restore motor functionality in a mild unilateral striatal 6-OHDA lesion rat model (~70% DA depletion). Within this paradigm, nor-BNI and vehicle-treated groups (n = 8-9) were tested multiple times using the amphetamine-induced rotations (AR) rotations test, the cylinder test, FAS test, and VSFP test to assess motor functionality. **(B)** *LID development paradigm:* We evaluated the effect of nor-BNI administration (3 mg/kg; s.c.) on the dose- and time-dependent development of abnormal involuntary movements (AIMs) in response to chronic escalating doses of L-DOPA treatment in a mild unilateral striatal 6-OHDA lesion rat LID model (~60% DA

depletion). Within this mild-dyskinesia paradigm, independent nor-BNI and vehicle/control groups (*n* = 6-7) were tested multiple times (2-4 times) across 5 escalating doses of L-DOPA (6, 12, 24, 48, 72 mg/kg; *i.p.*) and evaluated for total limb axial and orolingual (LAO), and locomotor AIMs scores under blinded conditions.

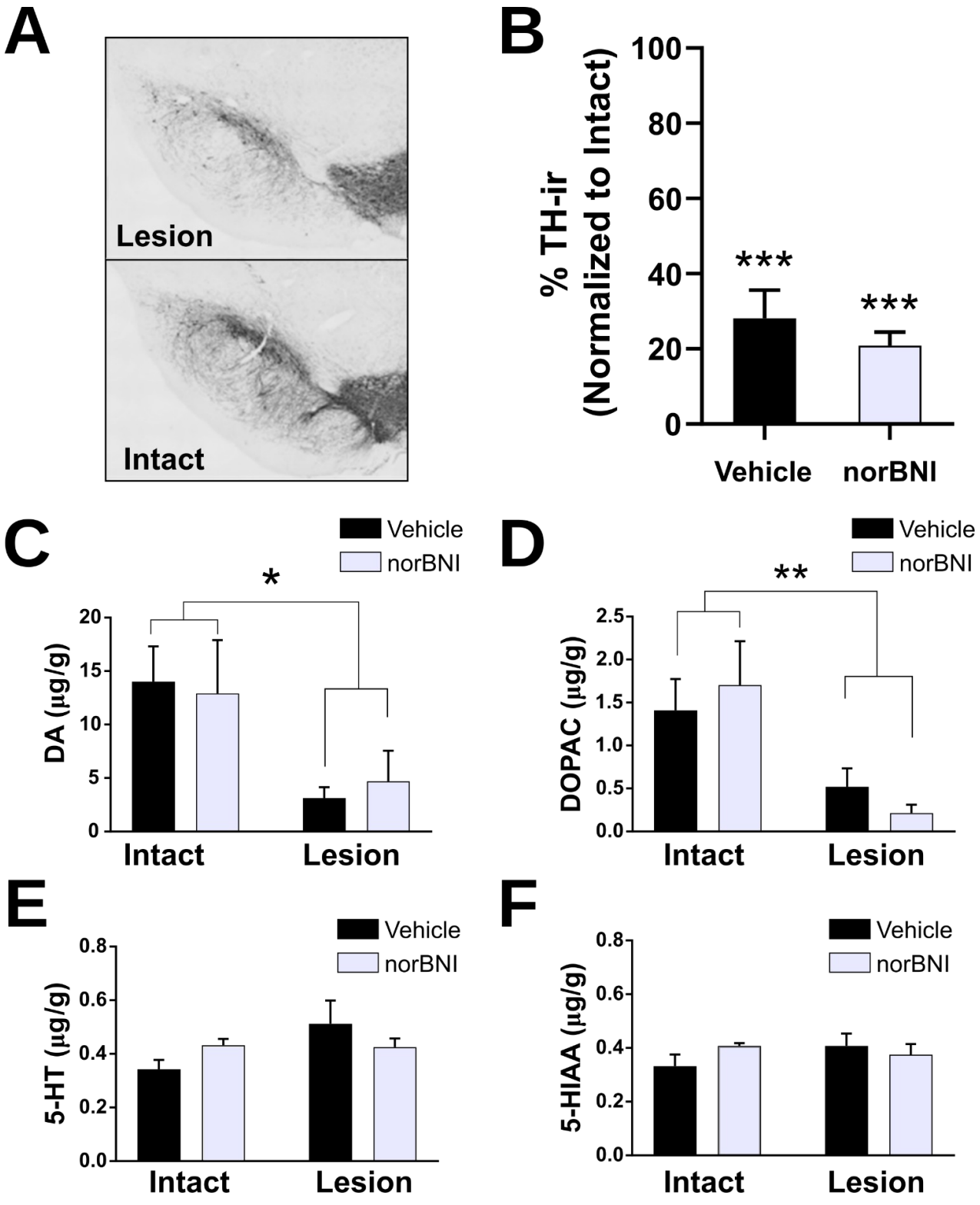
A
Lesion
Intact
B
% TH-ir
(Normalized to Intact)
100
80
60
40
20
0
***
***
Vehicle
norBNI
C
Vehicle
norBNI
*
DA (µg/g)
20
15
10
5
0
Intact
Lesion
D
Vehicle
norBNI
**
DOPAC (µg/g)
2.5
2.0
1.5
1.0
0.5
0.0
Intact
Lesion
E
Vehicle
norBNI
5-HT (µg/g)
0.8
0.6
0.4
0.2
0.0
Intact
Lesion
F
Vehicle
norBNI
5-HIAA (µg/g)
0.8
0.6
0.4
0.2
0.0
Intact
Lesion

Figure 2. Functional restoration study *post hoc* verification unilateral 6-OHDA-lesion. The presence of unilateral lesions was verified using tyrosine hydroxylase (TH) immunoreactivity (TH-ir) as a marker of dopaminergic neurons in the substantia nigra for a subset of animals (**A** and **B**), and electrochemical measurement of striatal DA and DOPAC concentrations for the remaining animals **(C-F)**. The photomicrograph in **(A)** shows representative TH-ir in the substantia nigra (*upper panel:* 6-OHDA Lesion; *lower panel:* Intact) used for image analysis. The graph in **(B)** has plotted normalized TH-ir (% lesion/intact) and shows ~75% reduction on the lesioned side (n = 4-5). In accordance with our mild unilateral 6-OHDA lesion paradigm, concentrations of striatal DA and its metabolite DOPAC measured with HPLC-EC (n = 4) were reduced by approximately 70 to 75% respectively in the lesioned striatum, while concentrations of serotonin (5-HT) and the serotonin metabolite 5-HIAA remained unaltered (**E** and **F**). Mean values ± SEM are plotted; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

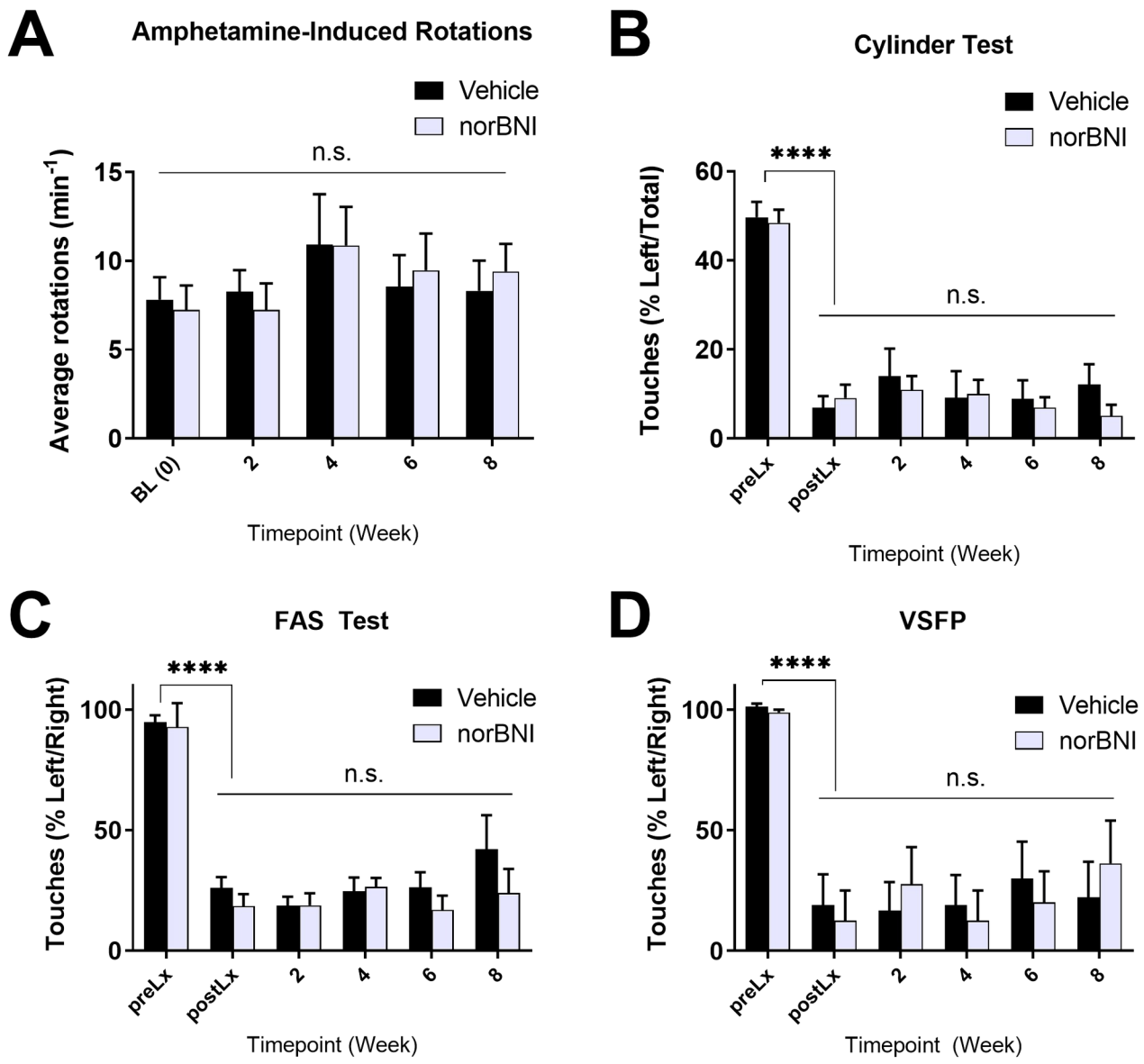

Figure 3. Functional restoration study: Behavioral analysis. (A) Amphetamine-induced rotations (AR) analysis failed to detect an effect of nor-BNI (3 mg/kg; *s.c.*) on the number of parkinsonian ipsilateral rotations per minute performed by the animals following amphetamine (5 mg/kg; *i.p.*) administration during timepoints following baseline (BL). Nor-BNI also did not show any motor-restorative effect in **(B)** FAS, **(C)** cylinder, nor **(D)** VSFP tests (preLx = prelesion; postLx = postlesion). Mean ± SEM, two-way repeated measures ANOVA, n = 8-9, **** $p < 0.0005$; n.s. = not significant.

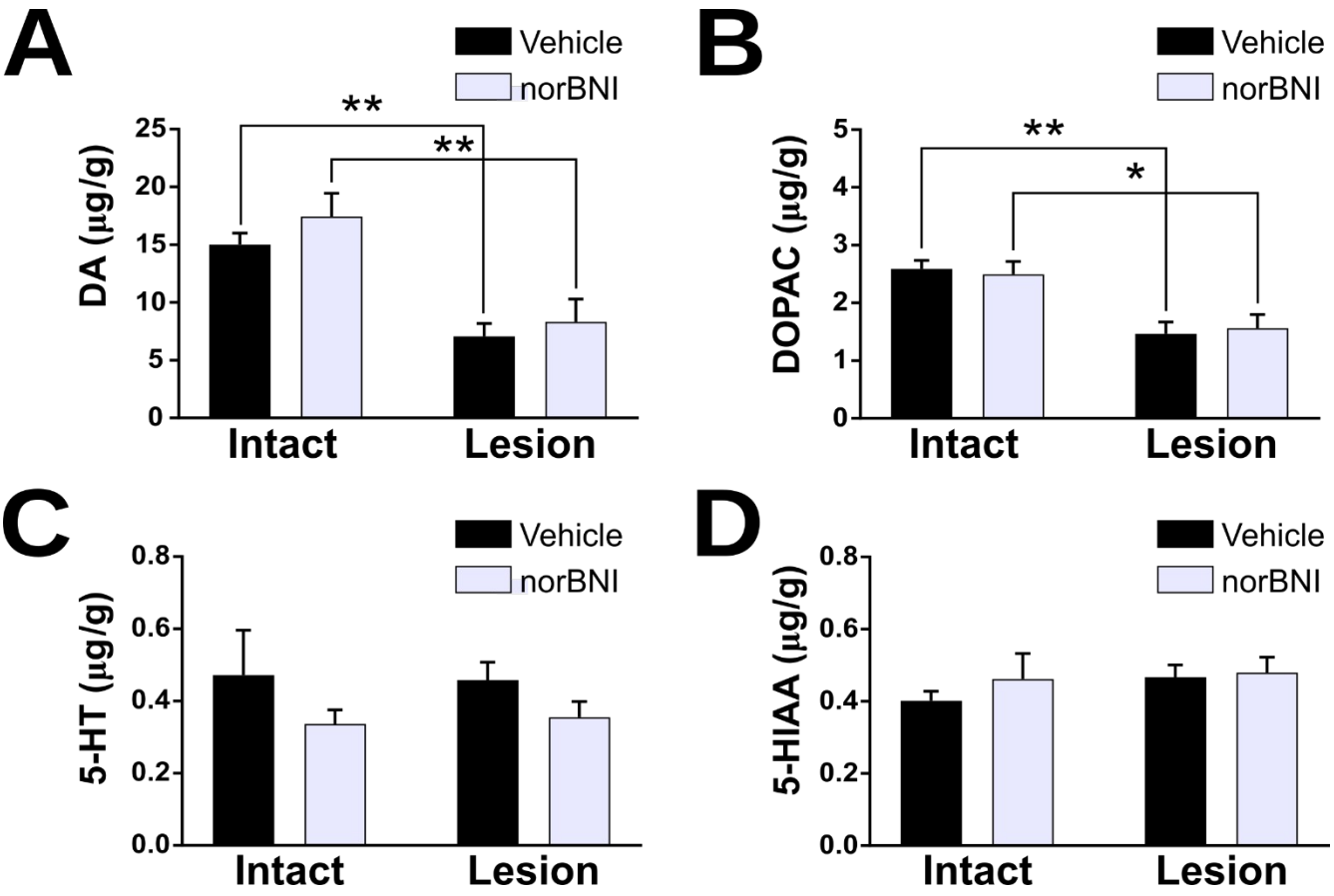

Figure 4. LID Development Study: *Post hoc* validation of extent of 6-OHDA-lesion.

Electrochemical measurement of striatal DA concentrations was performed to verify levels of DA depletion associated with the unilateral 6-OHDA lesion. In accordance with our mild unilateral 6-OHDA lesion paradigm, concentrations of striatal DA (**A**) and its metabolite DOPAC (**B**) measured with HPLC-EC were reduced by approximately 50-60% in the lesioned striatum. Concentrations of serotonin (5-HT) shown in (**C**) and the serotonin metabolite 5-HIAA (**D**) remained unaltered. Mean values ± SEM are plotted, n = 6-7; * $p < 0.05$, ** $p < 0.01$.

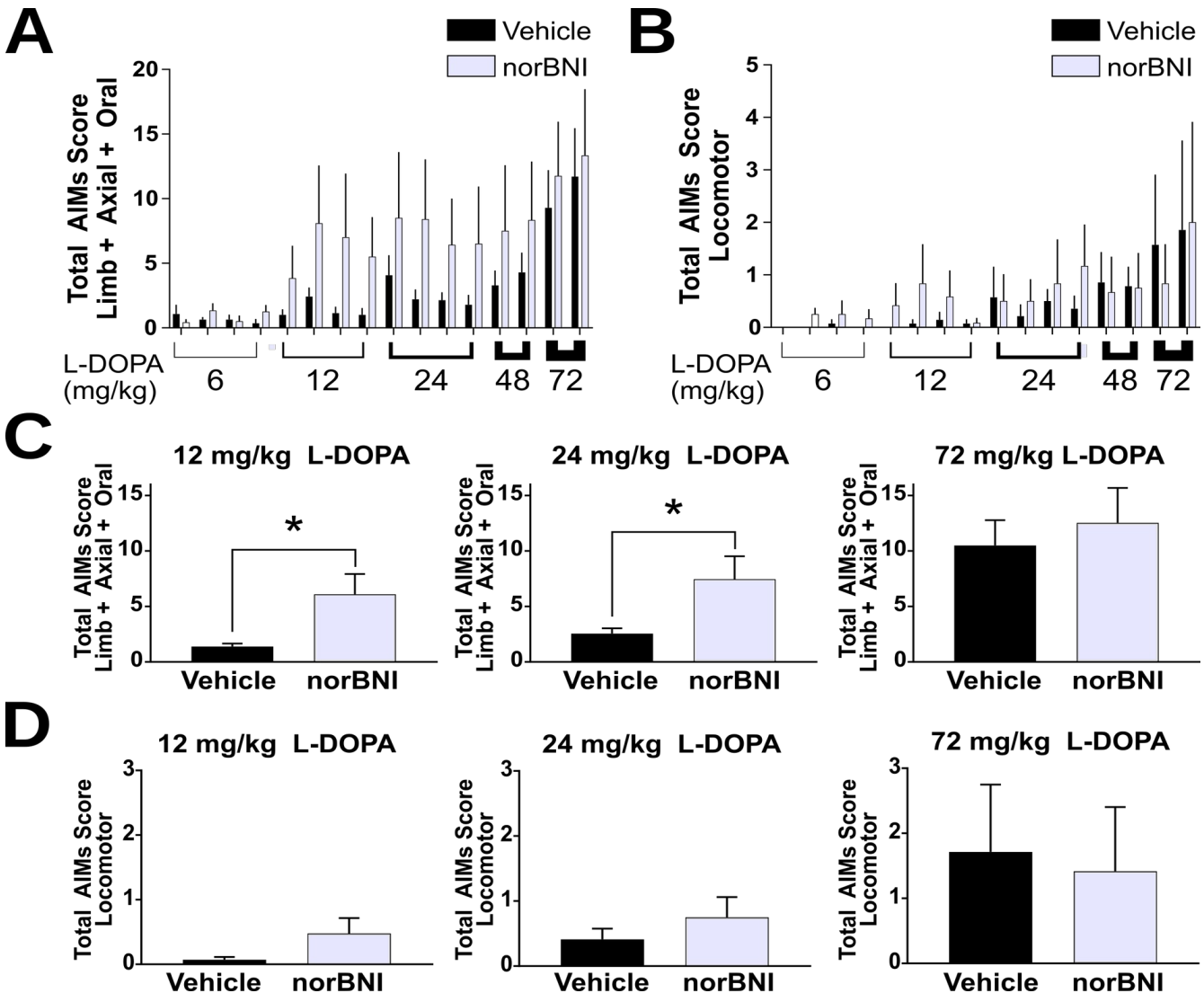

Figure 5. Treatment with nor-BNI accelerated the development of LAO and Locomotor AIMs. Animals were treated with gradually increasing doses of L-DOPA (6, 12, 24, 48, & 72 mg/kg). (**A**) Limb Axial Oral composite and (**B**) Locomotor AIMs scores obtained across the duration of the study. Two-way repeated measures ANOVA revealed that there was no significant effect of nor-BNI on single treatment days. Bar plots indicate mean values ± SEM; n = 6-7. (**C**) Cumulative total LAO AIMs scores were analyzed separately according to L-DOPA dose, and showed that nor-BNI increased the expression of LAO AIMs only at the 12 mg/kg and 24 mg/kg L-DOPA doses during LID development.

At the 72 mg/kg L-DOPA dose, LAO AIMs scores were not different between the two groups, suggesting that nor-BNI accelerates the rate of LID development but does not change LAO AIMs expression once LID is fully established. (**D**) Cumulative total Locomotor AIMs scores were analyzed separately according to L-DOPA dose. Locomotor AIMs were not significantly different between groups at 12, 24 and 72 mg/kg L-DOPA doses. Bar plots indicate mean values ± SEM, * $p < 0.05$.

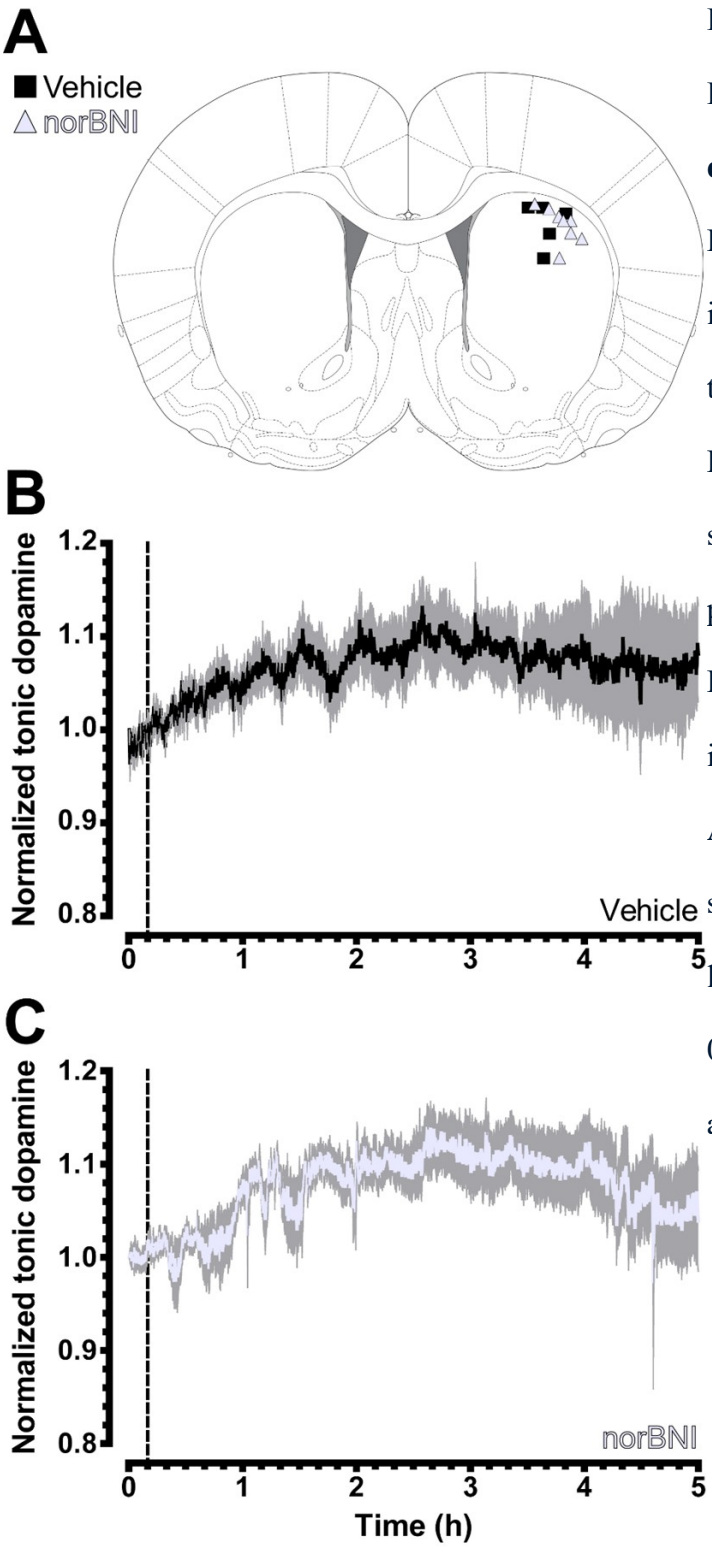

Figure 6. Subcutaneous injection of nor-BNI (3 mg/kg) does not alter tonic dopamine levels in the DLS. (**A**) Representative DLS recording locations included in dopamine analysis. Normalized tonic dopamine levels, determined using FSCAV, after subcutaneous injection of (**B**) saline or (**C**) nor-BNI. Mean ± SEM is plotted; n = 5 rats (saline), n = 8 rats (nor-BNI); dotted line at 10 min represents injection time. A two-way repeated measures ANOVA showed no significant effect of subcutaneous nor-BNI on tonic dopamine levels in the DLS ($F_{1,9889}$ = 0.05529, p = 0.8184). Bonferroni *post hoc* test: $p > 0.05$ at all timepoints.

4.7 References

Atcherley, C. W., Laude, N. D., Parent, K. L., & Heien, M. L. (2013). Fast-scan controlled-adsorption voltammetry for the quantification of absolute concentrations and adsorption dynamics. *Langmuir*, *29*(48), 14885–14892. https://doi.org/10.1021/la402686s

Aubert, I., Guigoni, C., Li, Q., Dovero, S., Bioulac, B. H., Gross, C. E., Crossman, A. R., Bloch, B., & Bezard, E. (2007). Enhanced Preproenkephalin-B-Derived Opioid Transmission in Striatum and Subthalamic Nucleus Converges Upon Globus Pallidus Internalis in L-3,4-dihydroxyphenylalanine-Induced Dyskinesia. *Biological Psychiatry*, *61*(7), 836–844. https://doi.org/10.1016/j.biopsych.2006.06.038

Bartlett, M. J., Flores, A. J., Ye, T., Smidt, S. I., Dollish, H. K., Stancati, J. A., Farrell, D. C., Parent, K. L., Doyle, K. P., Besselsen, D. G., Heien, M. L., Cowen, S. L., Steece-Collier, K., Sherman, S. J., & Falk, T. (2020). Preclinical evidence in support of repurposing sub-anesthetic ketamine as a treatment for L-DOPA-induced dyskinesia. *Experimental Neurology*, 113413. https://doi.org/10.1016/j.expneurol.2020.113413

Bartlett, M. J., Joseph, R. M., LePoidevin, L. M., Parent, K. L., Laude, N. D., Lazarus, L. B., Heien, M. L., Estevez, M., Sherman, S. J., & Falk, T. (2016). Long-term effect of sub-anesthetic ketamine in reducing l-DOPA-induced dyskinesias in a preclinical model. *Neuroscience Letters*, *612*, 121–125. https://doi.org/10.1016/j.neulet.2015.11.047

Calon, F., Birdi, S., Rajput, A. H., Hornykiewicz, O., Bédard, P. J., & Di Paolo, T. (2002). Increase of preproenkephalin mRNA levels in the putamen of Parkinson disease patients with levodopa-induced dyskinesias. *Journal of Neuropathology and Experimental Neurology*, *61*(2), 186–196. http://www.ncbi.nlm.nih.gov/pubmed/11853020

Cenci, M. A., Lee, C. S., & Björklund, A. (1998a). L-DOPA-induced dyskinesia in the rat is associated with striatal overexpression of prodynorphin- and glutamic acid decarboxylase mRNA. *The European Journal of Neuroscience*, *10*(8), 2694–2706. https://doi.org/10.1046/j.1460-9568.1998.00285.x

Cenci, M. A., Lee, C. S., & Björklund, A. (1998b). L-DOPA-induced dyskinesia in the rat is associated with striatal overexpression of prodynorphin- and glutamic acid decarboxylase mRNA. *European Journal of Neuroscience*, *10*(8), 2694–2706. https://doi.org/10.1046/j.1460-9568.1998.00285.x

Chang, J.-W., Wachtel, S. ., Young, D., & Kang, U.-J. (1999). Biochemical and anatomical characterization of forepaw adjusting steps in rat models of Parkinson's disease: studies on medial forebrain bundle and striatal lesions. *Neuroscience*, *88*(2), 617–628. https://doi.org/10.1016/S0306-4522(98)00217-6

Chavkin, C., & Koob, G. F. (2016). Dynorphin, dysphoria, and dependence: The stress of addiction. In *Neuropsychopharmacology* (Vol. 41, Issue 1, pp. 373–374). Nature Publishing Group. https://doi.org/10.1038/npp.2015.258

Chen, L., Togasaki, D. M., Langston, J. W., Di Monte, D. A., & Quik, M. (2005). Enhanced striatal opioid receptor-mediated G-protein activation in L-dopa-treated dyskinetic monkeys. *Neuroscience*, *132*(2), 409–420. https://doi.org/10.1016/j.neuroscience.2004.10.026

Cox, H., Togasaki, D. M., Chen, L., Langston, J. W., Di Monte, D. A., & Quik, M. (2007). The selective κ-opioid receptor agonist U50,488 reduces l-dopa-induced dyskinesias but worsens parkinsonism in MPTP-treated primates. *Experimental Neurology*, *205*(1), 101–107. https://doi.org/10.1016/j.expneurol.2007.01.024

Di Chiara, G., & Imperato, A. (1988). Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proceedings of the National Academy of Sciences of the United States of America*, *85*(14), 5274–5278. https://doi.org/10.1073/pnas.85.14.5274

Engber, T. M., Susel, Z., Kuo, S., Gerfen, C. R., & Chase, T. N. (1991). Levodopa replacement therapy alters enzyme activities in striatum and neuropeptide content in striatal output regions of 6-hydroxydopamine lesioned rats. *Brain Research*, *552*(1), 113–118. http://www.ncbi.nlm.nih.gov/pubmed/1717109

Eskow, K. L., Gupta, V., Alam, S., Park, J. Y., & Bishop, C. (2007). The partial 5-HT1A agonist buspirone reduces the expression and development of l-DOPA-induced dyskinesia in rats and improves l-DOPA efficacy. *Pharmacology Biochemistry and Behavior*, *87*(3), 306–314. https://doi.org/10.1016/J.PBB.2007.05.002

Flores, A. J., Bartlett, M. J., Root, B. K., Parent, K. L., Heien, M. L., Porreca, F., Polt, R., Sherman, S. J., & Falk, T. (2018). The combination of the opioid glycopeptide MMP-2200 and a NMDA receptor antagonist reduced L-DOPA-induced dyskinesia and MMP-2200 by itself reduced dopamine receptor 2-like agonist-induced dyskinesia. *Neuropharmacology*, *141*, 260–271. https://doi.org/10.1016/j.neuropharm.2018.09.005

Flores, A. J., Bartlett, M. J., So, L. Y., Laude, N. D., Parent, K. L., Heien, M. L., Sherman, S. J., & Falk, T. (2014). Differential effects of the NMDA receptor antagonist MK-801 on dopamine receptor D1- and D2-induced abnormal involuntary movements in a preclinical model. *Neuroscience Letters*, *564*, 48–52. https://doi.org/10.1016/j.neulet.2014.02.004

Fox, S. H., & Brotchie, J. M. (2011). Non-Dopaminergic Approaches to the Treatment of Parkinson's Disease. In W. C. Olanow, F. Stocchi, & A. E. Lang (Eds.), *Parkinson's Disease - Non-Motor and Non-Dopaminergic Features* (pp. 443–444). Wiley-Blackwell.

Fox, S. H., Lang, A. E., & Brotchie, J. M. (2006). Translation of nondopaminergic treatments for levodopa-induced dyskinesia from MPTP-lesioned nonhuman primates to phase IIa clinical studies: Keys to success and roads to failure. *Movement Disorders*, *21*(10), 1578–1594. https://doi.org/10.1002/mds.20936

Gee, T. A., Weintraub, N. C., Lu, D., Phelps, C. E., Navratilova, E., Heien, M. L., & Porreca, F. (2020). A pain-induced tonic hypodopaminergic state augments phasic dopamine release in the nucleus accumbens. *Pain*, *Publish ah*. https://doi.org/10.1097/j.pain.0000000000001925

Gehrke, B. J., Chefer, V. I., & Shippenberg, T. S. (2008). Effects of acute and repeated administration of salvinorin a on dopamine function in the rat dorsal striatum. *Psychopharmacology*, *197*(3), 509–517. https://doi.org/10.1007/s00213-007-1067-6

Hauser, K. F., Aldrich, J. V., Anderson, K. J., Bakalkin, G., Christie, M. J., Hall, E. D., Knapp, P. E., Scheff, S. W., Singh, I. N., Vissel, B., Woods, A. S., Yakovleva, T., & Shippenberg, T. S. (2005). Pathobiology of dynorphins in trauma and disease. In *Frontiers in Bioscience* (Vol. 10, Issue 1, pp. 216–235). Frontiers in Bioscience. https://doi.org/10.2741/1522

Henry, B., Duty, S., Fox, S. H., Crossman, A. R., & Brotchie, J. M. (2003). Increased striatal pre-proenkephalin B expression is associated with dyskinesia in Parkinson's disease. *Experimental Neurology*, *183*(2), 458–468. https://doi.org/10.1016/S0014-4886(03)00064-5

Henry, B., Fox, S. H., Crossman, A. R., & Brotchie, J. M. (2001). μ- and δ-Opioid Receptor Antagonists Reduce Levodopa-Induced Dyskinesia in the MPTP-Lesioned Primate Model of Parkinson's Disease. *Experimental Neurology*, *171*(1), 139–146. https://doi.org/10.1006/EXNR.2001.7727

Hill, M. P., & Brotchie, J. M. (1999). The adrenergic receptor agonist, clonidine, potentiates the anti-parkinsonian action of the selective κ-opioid receptor agonist, enadoline, in the monoamine-depleted rat. *British Journal of Pharmacology*, *128*(7), 1577–1585. https://doi.org/10.1038/sj.bjp.0702943

Hughes, N. R., McKnight, A. T., Woodruff, G. N., Hill, M. P., Crossman, A. R., & Brotchie, J. M. (1998). Kappa-opioid receptor agonists increase locomotor activity in the monoamine-depleted rat model of parkinsonism. *Movement Disorders*, *13*(2), 228–233. https://doi.org/10.1002/mds.870130206

Ikeda, K., Yoshikawa, S., Kurokawa, T., Yuzawa, N., Nakao, K., & Mochizuki, H. (2009). TRK-820, a selective kappa opioid receptor agonist, could effectively ameliorate L-DOPA-induced dyskinesia symptoms in a rat model of Parkinson's disease. *European Journal of Pharmacology*, *620*(1–3), 42–48. https://doi.org/10.1016/j.ejphar.2009.08.013

Ishida, Y., Hashiguchi, H., Todaka, K., Ishizuka, Y., & Mitsuyama, Y. (2000). Repeated administration of high dose levodopa enhances hydroxyl radical production in the rat striatum denervated with 6-hydroxydopamine. *Neuroscience Letters*, *290*(1), 33–36. https://doi.org/10.1016/S0304-3940(00)01320-3

Johansson, P. A., Andersson, M., Andersson, K. E., & Cenci, M. A. (2001). Alterations in Cortical and Basal Ganglia Levels of Opioid Receptor Binding in a Rat Model of l-DOPA-Induced Dyskinesia. *Neurobiology of Disease*, *8*(2), 220–239. https://doi.org/10.1006/nbdi.2000.0372

Johnston, T. H., Versi, E., Howson, P. A., Ravenscroft, P., Fox, S. H., Hill, M. P., Reidenberg, B. E., Corey, R., & Brotchie, J. M. (2018). DPI-289, a novel mixed delta opioid agonist / mu opioid antagonist (DAMA), has L-DOPA-sparing potential in Parkinson's disease. *Neuropharmacology*, *131*, 116–127. https://doi.org/10.1016/j.neuropharm.2017.11.046

Kishioka, S., Kiguchi, N., Kobayashi, Y., Yamamoto, C., Saika, F., Wakida, N., Ko, M. C., & Woods, J. H. (2013). Pharmacokinetic evidence for the long-lasting effect of nor-binaltorphimine, a potent kappa opioid receptor antagonist, in mice. *Neuroscience Letters*, *552*, 98–102. https://doi.org/10.1016/j.neulet.2013.07.040

Kneynsberg, A., Collier, T. J., Manfredsson, F. P., & Kanaan, N. M. (2016). Quantitative and semi-quantitative measurements of axonal degeneration in tissue and primary neuron cultures. *Journal of Neuroscience Methods*, *266*, 32–41. https://doi.org/10.1016/j.jneumeth.2016.03.004

Knoll, A. T., & Carlezon, W. A. (2010). Dynorphin, stress, and depression. *Brain Research*, *1314*, 56–73. https://doi.org/10.1016/j.brainres.2009.09.074

Koprich, J. B., Fox, S. H., Johnston, T. H., Goodman, A., Le Bourdonnec, B., Dolle, R. E., Dehaven, R. N., Dehaven-Hudkins, D. L., Little, P. J., & Brotchie, J. M. (2011). The selective mu-opioid

receptor antagonist adl5510 reduces levodopa-induced dyskinesia without affecting antiparkinsonian action in mptp-lesioned macaque model of Parkinson's disease. *Movement Disorders*, *26*(7), 1225–1233. https://doi.org/10.1002/mds.23631

Land, B. B., Bruchas, M. R., Lemos, J. C., Xu, M., Melief, E. J., & Chavkin, C. (2008). The Dysphoric Component of Stress Is Encoded by Activation of the Dynorphin κ-Opioid System. *Journal of Neuroscience*, *28*(2).

Laurence Lalanne, Gulebru Ayranci, Brigitte, L. K., & Lutz, P. E. (2014). The kappa opioid receptor: From addiction to depression, and back. In *Frontiers in Psychiatry* (Vol. 5, Issue DEC). Frontiers Media S.A. https://doi.org/10.3389/fpsyt.2014.00170

Ljungdahl, A., Hanrieder, J., Fälth, M., Bergquist, J., & Andersson, M. (2011). Imaging Mass Spectrometry Reveals Elevated Nigral Levels of Dynorphin Neuropeptides in L-DOPA-Induced Dyskinesia in Rat Model of Parkinson's Disease. *PLoS ONE*, *6*(9), e25653. https://doi.org/10.1371/journal.pone.0025653

Massaly, N., Copits, B. A., Wilson-Poe, A. R., Al-Hasani, R., Bruchas, M. R., & Moró, J. A. (2019). Pain-Induced Negative Affect Is Mediated via Recruitment of The Nucleus Accumbens Kappa Opioid System. *Neuron*, *102*, 564-573.e6. https://doi.org/10.1016/j.neuron.2019.02.029

Navratilova, E., Ji, G., Phelps, C., Qu, C., Hein, M., Yakhnitsa, V., Neugebauer, V., & Porreca, F. (2019). Kappa opioid signaling in the central nucleus of the amygdala promotes disinhibition and aversiveness of chronic neuropathic pain. *Pain*, *160*(4), 824–832. https://doi.org/10.1097/j.pain.0000000000001458

Newman, D. D., Rajakumar, N., Flumerfelt, B. A., & Stoessl, A. J. (1997). A kappa opioid antagonist blocks sensitization in a rodent model of Parkinson's disease. *NeuroReport*, *8*(3), 669–672. https://doi.org/10.1097/00001756-199702100-00018

Nisbet, A. P., Foster, O. J., Kingsbury, A., Eve, D. J., Daniel, S. E., Marsden, C. D., & Lees, A. J. (1995). Preproenkephalin and preprotachykinin messenger RNA expression in normal human basal ganglia and in Parkinson's disease. *Neuroscience*, *66*(2), 361–376. http://www.ncbi.nlm.nih.gov/pubmed/7477878

Paquette, M. A., Anderson, A. M., Lewis, J. R., Meshul, C. K., Johnson, S. W., & Paul Berger, S. (2010). MK-801 inhibits l-DOPA-induced abnormal involuntary movements only at doses that worsen parkinsonism. *Neuropharmacology*, *58*(7), 1002–1008. https://doi.org/10.1016/j.neuropharm.2010.01.003

Paxinos, G., & Watson, C. (2007). *The Rat Brain in Stereotaxic Coordinates* (6th ed.). Academic Press.

Piccini, P., Weeks, R. A., & Brooks, D. J. (1997). Alterations in opioid receptor binding in Parkinson's disease patients with levodopa-induced dyskinesias. *Annals of Neurology*, *42*(5), 720–726. https://doi.org/10.1002/ana.410420508

Potts, L. F., Park, E. S., Woo, J. M., Dyavar Shetty, B. L., Singh, A., Braithwaite, S. P., Voronkov, M., Papa, S. M., & Mouradian, M. M. (2015). Dual κ-agonist/μ-antagonist opioid receptor modulation reduces levodopa-induced dyskinesia and corrects dysregulated striatal changes in the nonhuman primate model of Parkinson disease. *Annals of Neurology*, *77*(6), 930–941. https://doi.org/10.1002/ana.24375

Schallert, T. (2006). Behavioral tests for preclinical intervention assessment. *NeuroRx : The Journal of*

the American Society for Experimental NeuroTherapeutics, *3*(4), 497–504. https://doi.org/10.1016/j.nurx.2006.08.001

Sgroi, S., Capper-Loup, C., Paganetti, P., & Kaelin-Lang, A. (2016). Enkephalin and dynorphin neuropeptides are differently correlated with locomotor hypersensitivity and levodopa-induced dyskinesia in parkinsonian rats. *Experimental Neurology*, *280*, 80–88. https://doi.org/10.1016/j.expneurol.2016.03.024

Spanagel, R., Herz, A., & Shippenberg, T. S. (1992). Opposing tonically active endogenous opioid systems modulate the mesolimbic dopaminergic pathway. *Proceedings of the National Academy of Sciences of the United States of America*, *89*(6), 2046–2050. https://doi.org/10.1073/pnas.89.6.2046

Tejeda, H. A., & Bonci, A. (2019). Dynorphin/kappa-opioid receptor control of dopamine dynamics: Implications for negative affective states and psychiatric disorders. In *Brain Research* (Vol. 1713, pp. 91–101). Elsevier B.V. https://doi.org/10.1016/j.brainres.2018.09.023

Vreeland, R. F., Atcherley, C. W., Russell, W. S., Xie, J. Y., Lu, D., Laude, N. D., Porreca, F., & Heien, M. L. (2015). Biocompatible PEDOT:Nafion composite electrode coatings for selective detection of neurotransmitters in vivo. *Analytical Chemistry*, *87*(5), 2600–2607. https://doi.org/10.1021/ac502165f

Xuei, X., Dick, D., Flury-Wetherill, L., Tian, H.-J., Agrawal, A., Bierut, L., Goate, A., Bucholz, K., Schuckit, M., Nurnberger, J., Tischfield, J., Kuperman, S., Porjesz, B., Begleiter, H., Foroud, T., & Edenberg, H. J. (2006). Association of the κ-opioid system with alcohol dependence. *Molecular Psychiatry*, *11*(11), 1016–1024. https://doi.org/10.1038/sj.mp.4001882

Yue, X., Hariri, D. J., Caballero, B., Zhang, S., Bartlett, M. J., Kaut, O., Mount, D. W., Wüllner, U., Sherman, S. J., & Falk, T. (2014). Comparative study of the neurotrophic effects elicited by VEGF-B and GDNF in preclinical in vivo models of Parkinson's disease. *Neuroscience*, *258*, 385–400. https://doi.org/10.1016/j.neuroscience.2013.11.038

Yue, Xu, Falk, T., Zuniga, L. A., Szabò, L., Porreca, F., Polt, R., & Sherman, S. J. (2011). Effects of the novel glycopeptide opioid agonist MMP-2200 in preclinical models of Parkinson's disease. *Brain Research*, *1413*, 72–83. https://doi.org/10.1016/j.brainres.2011.07.038

Chapter 5: Discussion and Conclusions

5.1 Introduction

Levodopa-induced dyskinesia (LID) remains a critical complication in the treatment of PD. Dopamine replacement therapy relying on the dopamine precursor levodopa is the most common and practical treatment for PD, however, chronic levodopa use is associated with the development of debilitating motor fluctuations and LID. PD affects 1 in every 100 people over the age of 60 (Tanner & Ben-Shlomo, 1999) and the number of afflicted individuals is set to rise as the population age continues to increase (Dorsey et al., 2007). Therefore, there is an important need for the development of novel pharmacological therapies for PD and LID.

The data presented in this dissertation were derived from preclinical neurobehavioral studies investigating the effects of drugs targeting NMDA and opioid receptors in the context of LID and PD utilizing the standard unilateral 6-OHDA lesion rat model of PD. A common, recurring theme in several of our studies was to take advantage of the differential expression of dopamine receptor subtypes and their co-expressed opioid peptides and receptors within the direct and indirect striatal output pathways in an attempt to identify the locus of drug action within these pathways. The activities of the direct and indirect striatal pathways have opposing effects on movement that are differentially dysregulated in PD and LID (Obeso, Rodriguez-Oroz, et al., 2000). Striatal MSNs belonging to the so-called 'direct' striatonigral output pathway selectively express D1 dopamine receptors and co-express opioid peptide co-transmitters dynorphin derived from PPE-B (Fallon et al., 1985), while those belonging to the 'indirect' striatopallidal output pathway express D2 dopamine receptors and enkephalins derived from PPE-A (Cuello & Paxinos, 1978; Gerfen & Young, 1988). Thus, in utilizing DA receptor agonists selective for D1 or D2 dopamine receptor subtypes, we were able to isolate AIMs

arising through independent activation the direct output pathway from those arising through inhibition the indirect output pathway.

5.2 NMDA neurotransmission in PD and LID

Preclinical studies utilizing rats and primates had previously shown that selective NMDA receptor antagonists, such as MK-801, reduce LID but worsen parkinsonism (Gomez-Mancilla & Bedard, 1993; Papa & Chase, 1996; Paquette et al., 2010; Rupniak et al., 1992). Amantadine is, among other activities, also a weak NMDA receptor antagonist that is already approved for the treatment of LID, however, it only helps some patients and is associated with inconvenient side effects including hallucinations and confusion (Paci et al., 2001). We also recently showed that ketamine has not only antidyskinetic long-term effects in the 6-OHDA rat preclinical model of LID, but also an acute antiparkinsonian activity (Bartlett et al., 2016; Bartlett, So, et al., 2020).

In Chapter 2, we present data showing differential effects of the highly-selective non-competitive NMDA receptor antagonist MK-801 on D1- and D2-receptor induced hyperkinetic AIMs in the unilateral 6-OHDA lesioned rat preclinical model of LID. Specifically, we show that AIMs induced by a selective D2 receptor agonist (quinpirole) could be suppressed by MK-801, while AIMs elicited by the selective D1 receptor agonist SKF85719 were actually increased by a small effect size by MK-801 administration (Flores et al., 2014). This finding is important because it investigated the role of NMDA receptor-mediated glutamate transmission in the expression of hyperkinetic AIMs induced separately by 1) activation of the ‘direct’ striatonigral output pathway, using a selective D1 receptor agonist (SKF85719), and 2) inhibition of the ‘indirect’ striatopallidal output pathway, using a selective D2 receptor agonist (quinpirole). Previously, in the same preclinical 6-OHDA rat model of LID, it had been shown that hyperkinetic AIMs induced by chronic administration of levodopa (i.e.,

LID), which is thought to arise through activation of both D1 and D2 receptor subtypes, could be suppressed by MK-801, but only at concentrations that were also pro-parkinsonian (Paquette et al., 2010). We were able to successfully replicate this same effect before investigating the effects of MK-801 on AIMs induced by selective DA receptor agonists independently (Flores et al., 2014). These results are presented in Chapter 2. Then, in Chapter 3, we searched for an interaction between glutamate and opioid transmission by studying the combined effects of MK-801 and a mixed δ/μ-activating glycopeptide MMP-2200 (lactomorphin) in the same preclinical model of LID as published in (Flores et al., 2018).

5.3 Opioid neurotransmission in PD and LID

We now turn to a discussion of altered opioid transmission in LID and PD as Chapters 2 and 3 present data from experiments that evaluated the effects of experimental opioid compounds in preclinical models of PD and LID. First, in Chapter 3, we present data from experiments evaluating the effects of MMP-2200 (lactomorphin), a synthetic glycopeptide with mixed δ/μ opioid receptor activity in a preclinical model of LID; and as mentioned above, we also tested MMP-2200 in combination with MK-801 (Flores et al., 2018). Then in Chapter 4, we present data from experiments that evaluated the effects of the κ-opioid receptor selective antagonist nor-BNI in two unique preclinical experimental paradigms: 1) an LID prevention paradigm and 2) a functional restoration paradigm.

Opioid neurotransmission is prominent in the basal ganglia. Endogenous opioid peptides and their precursors are widely expressed in the basal ganglia where they are modulators of GABA, DA, and glutamate neurotransmission (Fox et al., 2006). Additionally, all three subtypes of opioid receptor —μ (MORs), δ (DORs), and κ (KORs)—are expressed at high density in the striatum, and are co-localized on GABAergic striatal projection MSNs and are also expressed in nuclei receiving input from

the striatum. Striatal MSNs are segregated according to their differential expression of dopamine receptor subtype and corresponding opioid peptide co-transmitters. Specifically, the D2 receptor expressing indirect striatopallidal projection neurons co-express enkephalins (Leu- and Met-) derived from the precursor pre-proenkephalin A (PPE-A), which preferentially bind to DORs, whereas the D1 expressing direct striatonigral projection neurons co-express dynorphin and opioid peptides derived from pre-proenkephalin B (PPE-B), which predominantly activate KOR receptors (Breslin et al., 1993; Cuello & Paxinos, 1978; Gerfen et al., 1990; Gerfen & Young, 1988; Seizinger et al., 1984).

It has been established that expression levels of the opioid peptides and their precursors are dysregulated in PD and LID (Koprich et al., 2011), however, the role played by altered opioid transmission in PD and LID (compensatory vs. pathological) remains unresolved. In the DA-depleted striatum PPE-A mRNA and enkephalin peptide levels are upregulated (Cenci et al., 1998; Gerfen et al., 1990; Herrero et al., 1995; Nisbet et al., 1995; Sgroi et al., 2016), while striatal PPE-B mRNA and dynorphin are downregulated (Aubert et al., 2007; Cenci et al., 1998; Gerfen et al., 1990; Sgroi et al., 2016; Tel et al., 2002). Following chronic levodopa therapy, levels of PPE-A remain upregulated both in animal models (Westin et al., 2001) and in patients with LID (Calon et al., 2003), which indicates a persistent adaptive role for enkephalin in LID (Tamim et al., 2010). Levels of PPE-B, on the other hand, are reversed and upregulated following chronic levodopa therapy (Sgroi et al., 2016; Tamim et al., 2010; Westin et al., 2001). Furthermore, positron emission tomography (PET) studies have revealed abnormalities in opioid receptors related to enhanced opioid transmission in the striatum of patients with LID (Piccini et al., 1997), suggesting that increased opioid neurotransmission may be associated with the pathophysiology of LID.

Although there is a great deal of evidence implicating altered opioid neurotransmission in PD and LID, there are currently no opioid drugs that have been clinically approved for the treatment of PD nor LID. In review of the literature, one often finds that the results of studies evaluating the effects of

opioid compounds in models of PD and LID can vary substantially in consistency. This is true between studies using different experimental models and also between studies using the same experimental model. For example, results of preclinical studies evaluating the effects of the non-selective opioid receptor antagonists nalaxone and naltrexone have shown no effect (Gomez-Mancilla & Bedard, 1993), significant increase (Samadi, 2003), and significant decrease (Henry et al., 2001; Klintenberg et al., 2002) of LID respectively. The moderately-selective MOR antagonists cyprodine and ADL5510 reduced LID in MPTP-lesioned nonhuman primates without interfering with the antiparkinsonian action of L-DOPA (Henry et al., 2001; Koprich et al., 2011). These findings appear to make sense in light of the evidence which correlates enhanced opioid transmission with LID (Aubert et al., 2007; Johansson et al., 2001). Despite this, however, it was recently shown that a highly-selective MOR antagonist CTAP (which has >1,200-fold higher selectivity for MORs over DORs) did not affect the expression of levodopa-induced AIMs in the 6-OHDA rat preclinical model of LID (Bartlett, So, et al., 2020). ADL5510, by comparison, is only 15-fold selective for MORs over DORs, indicating that a combination of action on MOR and DOR are likely underlying the anti-dyskinetic effects of ADL5510, and not action on a single subtype of opioid receptors. An even more recent study by Bezard et al. (2020) has shown that MOR agonism, but not MOR antagonism, is antidyskinetic in MPTP-lesioned nonhuman primates. This study, which demonstrated that both oral and discrete intracerebral administration of a selective MOR agonist, but not an antagonist, ameliorates LID further calls into question the previous findings related to antidyskinetic effects of MOR antagonism.

Therefore, there are a number of factors that make it difficult to determine the role played by altered opioid transmission in LID. One relevant complicating factor is that opioid receptors are widely distributed in their expression throughout the brain, which makes it difficult to localize the site of action of a drug in correlation with a given behavioral response. The issue is further complicated by the fact that some opioid receptor-binding compounds can function as antagonists at certain

concentrations and as agonists at other concentrations, and may differentially activate and inhibit different receptor subtypes. Such is the case with the selective MOR antagonist ADL5510, which displays a 'U-shaped' dose response curve when tested in a nonhuman primate model of LID (Koprich et al., 2011).

Provided that activation of DORs in the GPe reduces GABAergic output, it has been suggested that upregulation of enkephalins may represent a compensatory mechanism to reduce hyperactivation of the indirect output pathway in PD (Brotchie et al., 1993; Maneuf et al., 1994). Furthermore, PPE-A mRNA precedes the appearance of PD motor deficits in MPTP treated nonhuman primates, which also supports a compensatory role (Bezard et al., 2001) and selective DOR agonists have demonstrated antiparkinsonian action in preclinical studies (Hille et al., 2001; Hudzik et al., 2000). In support of these data, a selective DOR antagonist naltrindole was anti-dyskinetic in non-human primates and in the 6-OHDA rat model (Billet et al., 2012; Henry et al., 2001). We previously showed that the mixed DOR/MOR glycopeptide agonist MMP-2200 (lactomorphin) has antiparkinsonian properties in two preclinical models of DA depletion (Xu Yue et al., 2011), which also supports that enhanced DOR activation is antiparkinsonian. However, it was later shown that MMP-2200 also has anti-dyskinetic properties in the 6-OHDA rat following priming with levodopa, thus making it difficult to determine the mechanism responsible for its antiparkinsonian properties beyond a reduction in the downstream effects of dopamine agonists in these models of DA depletion (Flores et al., 2018).

In Chapter 3, we have shown that MMP-2200 had differential effects on selective D1 and D2 receptor-activated hyperkinetic AIMs following chronic levodopa therapy to induce the expression of hyperkinetic AIMs. Then, as mentioned above, we also investigated the combined effects of MMP-2200 with the NMDA receptor antagonist MK-801 and found that MMP-2200 blocks the pro-parkinsonian effects of MK-801 while not interfering with its anti-dyskinetic properties. This finding, which was derived from testing the combined effects of an NMDA receptor antagonist and opioid drug

with dual μ/δ-agonist activity, emphasizes the potential use of other multi-ligand compounds and/or drug combinations. For example, work from our lab recently showed that showed that ketamine, which binds MORs and DORs with affinities similar to its affinity for NMDA receptors (Finck & Ngai 1982; Gupta et al., 2011), has lasting anti-dyskinetic properties in the same preclinical 6-OHDA rat model of LID (Bartlett et al., 2016; Bartlett, Flores, et al., 2020). And while an involvement of opioid activity in addition to the NMDA receptor antagonism of ketamine has not been investigated in a LID model, it is of interest that the anti-depressive activity of ketamine can be blocked by an opioid antagonist (Williams et al., 2018).

5.4 Kappa-mediated signaling in PD and LID

Several lines of evidence suggest that κ-mediated signaling is overactive in LID. In the 6-OHDA rat model of LID, the severity of AIMs correlates strongly with levels of striatal PPE-B mRNA, which encodes dynrophin, the endogenous ligand for KORs (Cenci et al., 1998). Another study, showed that striatal PPE-B mRNA levels were increased in both MPTP-lesioned macaques and PD patients with LID (Henry et al., 2003). Other studies have shown that striatal levels of dynorphin are positively correlated with LID (Sgroi et al., 2016). Additionally, MALDI-TOF imaging mass spectrometry showed that levels of dynorphin B and alpha-neoendorphin in the substantia nigra are strongly correlated with the severity of AIMs in the 6-OHDA rat model of LID (Ljungdahl et al., 2011). Furthermore, [^{35}S]GTPγS binding indicated hyperactive κ-mediated signaling in the caudate nucleus and motor cortex in a nonhuman primate model of LID (Chen et al., 2005).

With regard to KORs, (Aubert et al., 2007) found that they were unaltered in MPTP-lesioned primates, but that their expression was reduced in the GPe and GPi following chronic L-DOPA administration. In contrast, KOR expression was decreased in the striatum and substantia nigra, but

unaltered in the GP, of 6-OHDA-lesioned rats that expressed AIMs in response to L-DOPA compared with non-dyskinetic animals (Johansson et al., 2001).

Despite this evidence, however, antagonizing KORs with nor-BNI failed to reduce LID in primates with established LID (Henry et al., 2001). Furthermore, activation of KORs with the selective κ-receptor agonist U50,488 reduced AIMs in the 6-OHDA rat model of LID and dyskinesia in MPTP-lesioned squirrel monkeys (Cox et al., 2007). On the basis of this evidence, we decided to explore the efficacy of nor-BNI in the 6-OHDA rat model of LID development, to test if nor-BNI either accelerates or attenuates the development of LID. Both could have been possible outcomes, considering that also differences in alterations in KOR-mediated neurotransmission exist between the 6-OHDA rat and nonhuman primate models of LID. Therefore, although nor-BNI failed to reduce LID in nonhuman primates, we could not rule out it could have had anti-dyskinetic efficacy in the 6-OHDA rat model of LID. The results of our experiments are a further step toward furthering our understanding of kappa-mediated neurotransmission specifically in the 6-OHDA rat model of PD and LID.

In Chapter 4, we have shown that nor-BNI accelerates the development of LID in a 6-OHDA rat model that relied on a unique mild striatal lesion and a prolonged L-DOPA dose escalation paradigm that models gradual development of LID. Additionally, we found that nor-BNI had no motor restorative effects in a functional restoration PD paradigm. An important aspect of this study is that we utilized a mild striatal 6-OHDA lesion of approximately 60% DA depletion. It is important to realize that this is not typically done in studies of LID because it requires the use of extremely high doses of levodopa in order to induce AIMs.

We investigated the effects of the selective kappa-opioid receptor antagonist nor-BNI in 1) a preclinical LID prevention paradigm, in which we administered nor-BNI to block KORs during the priming phase of chronic levodopa treatment to prevent the plastic changes associated with the development of LID and 2) in a functional restoration paradigm to test for an acute antiparkinsonian

effect in a mild striatal lesion paradigm. We found that nor-BNI accelerated the development of levodopa-induced AIMs using a paradigm of prolonged LID development, however, it did not worsen LID once fully established, replicating what has been shown in the primate model (Henry et al., 2001). This indicates an effect of nor-BNI either directly on dopamine release, or downstream effects on dopaminergic signaling. However, we found no motor restorative effect of nor-BNI in a mild 6-OHDA unilateral striatal lesion model of PD, and we showed that nor-BNI did not change tonic DA levels in naïve animals, as measured with FSCAV. Both of these findings suggest that the effects of nor-BNI are not on dopamine release, but are rather downstream of the dopamine receptors.

5.5 Conclusions

It remains clear that alterations in the opioid neurotransmission system are closely associated with and occur in parallel with the pathological alterations in dopaminergic signaling occurring in PD and LID, though it is still unknown whether such alterations are pathological or compensatory. The data presented in this thesis showed that 1) the highly-selective non-competitive NMDA receptor antagonist MK-801 has differential effects on D2-like versus D1-like AIMs, 2) that the combined effects of MK-801 and the mixed MOR/DOR opioid agonist MMP-2200 suppressed levodopa-induced AIMs without inducing parkinsonism, 3) that MMP-2200 by itself has differential effects on D2-like AIMs vs D1-like AIMs, 4) that the selective KOR antagonist nor-BNI accelerates the development of levodopa-induced AIMs in a paradigm of prolonged chronic levodopa dose escalation in a mild striatal 6-OHDA lesion, and 5) that nor-BNI did not display motor restorative properties following a mild striatal 6-OHDA lesion. All of the studies utilized the preclinical 6-OHDA rat model of PD and LID, although we used specialized lesion parameters and experimental paradigms for each study. The preclinical studies presented here have provided important information about the role of altered opioid and NMDA

receptor-mediated neurotransmission in PD and LID that may help lead to the development of novel therapies for these disorders.

Since DA receptor agonists are used extensively in current clinical practice our data suggest that further exploration of the therapeutic potential of drugs structurally related to endogenous opioid peptides as adjuncts to established therapies could prove worthwhile. The mixed δ/μ opioid glycopeptide MMP-2200 reduced limb, axial, orolingual, and locomotor abnormal involuntary movements (AIMs) induced by a selective D2R agonist, possibly strengthening the homeostatic upregulation of enkepahlins in response to development of dyskinesia. In addition, MMP-2200 modified the effect of the NMDA receptor antagonist MK-801 to result in a potent reduction of L-DOPA-induced limb, axial and orolingual AIMs with reduced induction of parkinsonism, mitigating the pro-parkinsonian activity that MK-801 has by itself.

5.6 References

Aubert, I., Guigoni, C., Li, Q., Dovero, S., Bioulac, B. H., Gross, C. E., Crossman, A. R., Bloch, B., & Bezard, E. (2007). Enhanced Preproenkephalin-B-Derived Opioid Transmission in Striatum and Subthalamic Nucleus Converges Upon Globus Pallidus Internalis in L-3,4-dihydroxyphenylalanine-Induced Dyskinesia. *Biological Psychiatry*, *61*(7), 836–844. https://doi.org/10.1016/j.biopsych.2006.06.038

Bartlett, M. J., Flores, A. J., Ye, T., Smidt, S. I., Dollish, H. K., Stancati, J. A., Farrell, D. C., Parent, K. L., Doyle, K. P., Besselsen, D. G., Heien, M. L., Cowen, S. L., Steece-Collier, K., Sherman, S. J., & Falk, T. (2020). Preclinical evidence in support of repurposing sub-anesthetic ketamine as a treatment for L-DOPA-induced dyskinesia. *Experimental Neurology*, 113413. https://doi.org/10.1016/j.expneurol.2020.113413

Bartlett, M. J., Joseph, R. M., LePoidevin, L. M., Parent, K. L., Laude, N. D., Lazarus, L. B., Heien, M. L., Estevez, M., Sherman, S. J., & Falk, T. (2016). Long-term effect of sub-anesthetic ketamine in reducing l-DOPA-induced dyskinesias in a preclinical model. *Neuroscience Letters*, *612*, 121–125. https://doi.org/10.1016/j.neulet.2015.11.047

Bartlett, M. J., So, L. Y., Szabò, L., Skinner, D. P., Parent, K. L., Heien, M. L., Vanderah, T. W., Polt, R., Sherman, S. J., & Falk, T. (2020). Highly-selective μ-opioid receptor antagonism does not block L-DOPA-induced dyskinesia in a rodent model. *BMC Research Notes*, *13*(1), 1–6. https://doi.org/10.1186/s13104-020-04994-7

Bezard, E., Brotchie, J. M., & Gross, C. E. (2001). Pathophysiology of levodopa-induced dyskinesia: potential for new therapies. In *Nature Reviews Neuroscience* (Vol. 2, Issue 8, pp. 577–588). Nature Publishing Group. https://doi.org/10.1038/35086062

Bezard, E., Li, Q., Hulme, H., Fridjonsdottir, E., Nilsson, A., Pioli, E., Andren, P. E., & Crossman, A. R. (2020). μ Opioid receptor agonism for L-DOPA-induced dyskinesia in Parkinson's disease. *Journal of Neuroscience*, *40*(35), 6812–6819. https://doi.org/10.1523/JNEUROSCI.0610-20.2020

Billet, F., Costentin, J., & Dourmap, N. (2012). Influence of corticostriatal ??-opioid receptors on abnormal involuntary movements induced by L-DOPA in hemiparkinsonian rats. *Experimental Neurology*, *236*(2), 339–350. https://doi.org/10.1016/j.expneurol.2012.04.017

Breslin, M. B., Lindberg, I., Benjannet, S., Mathis, J. P., Lazure, C., & Seidah, N. G. (1993). Differential processing of proenkephalin by prohormone convertases 1(3) and 2 and furin. *The Journal of Biological Chemistry*, *268*(36), 27084–27093. http://www.ncbi.nlm.nih.gov/pubmed/8262946

Brotchie, J., Crossman, A., Mitchell, I., Duty, S., Carroll, C., Cooper, A., Henry, B., Hughes, N., & Maneuf, Y. (1993). Chapter 8 Chemical signalling in the globus pallidus in parkinsonism. *Progress in Brain Research*, *99*, 125–139. https://doi.org/10.1016/S0079-6123(08)61342-4

Calon, F., Rajput, A. H., Hornykiewicz, O., Bédard, P. J., & Di Paolo, T. (2003). Levodopa-induced motor complications are associated with alterations of glutamate receptors in Parkinson's disease. *Neurobiology of Disease*, *14*(3), 404–416. https://doi.org/10.1016/j.nbd.2003.07.003

Cenci, M. A., Lee, C. S., & Björklund, A. (1998). L-DOPA-induced dyskinesia in the rat is associated with striatal overexpression of prodynorphin- and glutamic acid decarboxylase mRNA. *European Journal of Neuroscience*, *10*(8), 2694–2706. https://doi.org/10.1046/j.1460-9568.1998.00285.x

Chen, L., Togasaki, D. M., Langston, J. W., Di Monte, D. A., & Quik, M. (2005). Enhanced striatal opioid receptor-mediated G-protein activation in L-dopa-treated dyskinetic monkeys. *Neuroscience*, *132*(2), 409–420. https://doi.org/10.1016/j.neuroscience.2004.10.026

Cox, H., Togasaki, D. M., Chen, L., Langston, J. W., Di Monte, D. A., & Quik, M. (2007). The selective κ-opioid receptor agonist U50,488 reduces l-dopa-induced dyskinesias but worsens parkinsonism in MPTP-treated primates. *Experimental Neurology*, *205*(1), 101–107. https://doi.org/10.1016/j.expneurol.2007.01.024

Cuello, A. C., & Paxinos, G. (1978). Evidence for a long Leu-enkephalin striopallidal pathway in rat brain. *Nature*, *271*(5641), 178–180. http://www.ncbi.nlm.nih.gov/pubmed/23498

Dorsey, E. R., Constantinescu, R., Thompson, J. P., Biglan, K. M., Holloway, R. G., Kieburtz, K., Marshall, F. J., Ravina, B. M., Schifitto, G., Siderowf, A., & Tanner, C. M. (2007). Projected number of people with Parkinson disease in the most populous nations, 2005 through 2030. *Neurology*, *68*(5), 384–386. https://doi.org/10.1212/01.wnl.0000247740.47667.03

Fallon, J. H., Leslie, F. M., & Cone, R. I. (1985). Dynorphin-containing pathways in the substantia nigra and ventral tegmentum: A double labeling study using combined immunofluorescence and retrograde tracing. *Neuropeptides*, *5*(4–6), 457–460. https://doi.org/10.1016/0143-4179(85)90053-8

Finck A. Donald, M. D., & Ngai S. H., M. D. (1982). Opiate Receptor Mediation of Ketamine Analgesia. *Anesthesiology*, *56*(4), 291–297. http://dx.doi.org/

Flores, A. J., Bartlett, M. J., Root, B. K., Parent, K. L., Heien, M. L., Porreca, F., Polt, R., Sherman, S. J., & Falk, T. (2018). The combination of the opioid glycopeptide MMP-2200 and a NMDA receptor antagonist reduced L-DOPA-induced dyskinesia and MMP-2200 by itself reduced dopamine receptor 2-like agonist-induced dyskinesia. *Neuropharmacology*, *141*, 260–271. https://doi.org/10.1016/j.neuropharm.2018.09.005

Flores, A. J., Bartlett, M. J., So, L. Y., Laude, N. D., Parent, K. L., Heien, M. L., Sherman, S. J., & Falk, T. (2014). Differential effects of the NMDA receptor antagonist MK-801 on dopamine receptor D1- and D2-induced abnormal involuntary movements in a preclinical model. *Neuroscience Letters*, *564*, 48–52. https://doi.org/10.1016/j.neulet.2014.02.004

Fox, S. H., Lang, A. E., & Brotchie, J. M. (2006). Translation of nondopaminergic treatments for levodopa-induced dyskinesia from MPTP-lesioned nonhuman primates to phase IIa clinical studies: Keys to success and roads to failure. *Movement Disorders*, *21*(10), 1578–1594. https://doi.org/10.1002/mds.20936

Gerfen, C R, Engber, T. M., Mahan, L. C., Susel, Z., Chase, T. N., Monsma, F. J., & Sibley, D. R. (1990). D1 and D2 dopamine receptor regulated gene expression of striatonigral and striatopallidal neurons. *Science*, *250*(1986), 1429–1432. https://doi.org/10.1126/science.2147780

Gerfen, Charles R., & Scott Young, W. (1988). Distribution of striatonigral and striatopallidal peptidergic neurons in both patch and matrix compartments: an in situ hybridization histochemistry and fluorescent retrograde tracing study. *Brain Research*, *460*(1), 161–167. https://doi.org/10.1016/0006-8993(88)91217-6

Gomez-Mancilla, B., & Bedard, P. J. (1993). Effect of nondopaminergic drugs on L-DOPA-induced dyskinesias in MPTP- treated monkeys. *Clinical Neuropharmacology*, *16*(5), 418–427. https://doi.org/10.1097/00002826-199310000-00004

Gupta, A., Devi, L. A., & Gomes, I. (2011). Potentiation of μ-opioid receptor-mediated signaling by ketamine. *Journal of Neurochemistry*, *119*(2), 294–302. https://doi.org/10.1111/j.1471-4159.2011.07361.x

Henry, B., Duty, S., Fox, S. H., Crossman, A. R., & Brotchie, J. M. (2003). Increased striatal pre-proenkephalin B expression is associated with dyskinesia in Parkinson's disease. *Experimental Neurology*, *183*(2), 458–468. https://doi.org/10.1016/S0014-4886(03)00064-5

Henry, B., Fox, S. H., Crossman, A. R., & Brotchie, J. M. (2001). μ- and δ-Opioid Receptor Antagonists Reduce Levodopa-Induced Dyskinesia in the MPTP-Lesioned Primate Model of Parkinson's Disease. *Experimental Neurology*, *171*(1), 139–146. https://doi.org/10.1006/EXNR.2001.7727

Herrero, M. T., Augood, S. J., Hirsch, E. C., Javoy-Agid, F., Luquin, M. R., Agid, Y., Obeso, J. A., & Emson, P. C. (1995). Effects of l-DOPA on preproenkephalin and preprotachykinin gene expression in the MPTP-treated monkey striatum. *Neuroscience*, *68*(4), 1189–1198. https://doi.org/10.1016/0306-4522(95)00120-8

Hille, C. J., Fox, S. H., Maneuf, Y. P., Crossman, A. R., & Brotchie, J. M. (2001). Antiparkinsonian action of a delta opioid agonist in rodent and primate models of Parkinson's disease. *Experimental Neurology*, *172*(1), 189–198. https://doi.org/10.1006/exnr.2001.7763

Hudzik, T. J., Howell, A., Payza, K., & Cross, A. J. (2000). Antiparkinson potential of delta-opioid receptor agonists. *European Journal of Pharmacology*, *396*(2–3), 101–107. http://www.ncbi.nlm.nih.gov/pubmed/10822062

Johansson, P. A., Andersson, M., Andersson, K. E., & Cenci, M. A. (2001). Alterations in Cortical and Basal Ganglia Levels of Opioid Receptor Binding in a Rat Model of l-DOPA-Induced Dyskinesia. *Neurobiology of Disease*, *8*(2), 220–239. https://doi.org/10.1006/nbdi.2000.0372

Klintenberg, R., Svenningsson, P., Gunne, L., & Andrén, P. E. (2002). Naloxone reduces levodopa-induced dyskinesias and apomorphine-induced rotations in primate models of parkinsonism. *Journal of Neural Transmission (Vienna, Austria : 1996)*, *109*(10), 1295–1307. https://doi.org/10.1007/s00702-002-0715-6

Koprich, J. B., Fox, S. H., Johnston, T. H., Goodman, A., Le Bourdonnec, B., Dolle, R. E., Dehaven, R. N., Dehaven-Hudkins, D. L., Little, P. J., & Brotchie, J. M. (2011). The selective mu-opioid receptor antagonist adl5510 reduces levodopa-induced dyskinesia without affecting antiparkinsonian action in mptp-lesioned macaque model of Parkinson's disease. *Movement Disorders*, *26*(7), 1225–1233. https://doi.org/10.1002/mds.23631

Ljungdahl, A., Hanrieder, J., Fälth, M., Bergquist, J., & Andersson, M. (2011). Imaging Mass Spectrometry Reveals Elevated Nigral Levels of Dynorphin Neuropeptides in L-DOPA-Induced

Dyskinesia in Rat Model of Parkinson's Disease. *PLoS ONE*, *6*(9), e25653. https://doi.org/10.1371/journal.pone.0025653

Maneuf, Y. P., Mitchell, I. J., Crossman, A. R., & Brotchie, J. M. (1994). On the Role of Enkephalin Cotransmission in the GABAergic Striatal Efferents to the Globus Pallidus. *Experimental Neurology*, *125*(1), 65–71. https://doi.org/10.1006/exnr.1994.1007

Nisbet, A. P., Foster, O. J., Kingsbury, A., Eve, D. J., Daniel, S. E., Marsden, C. D., & Lees, A. J. (1995). Preproenkephalin and preprotachykinin messenger RNA expression in normal human basal ganglia and in Parkinson's disease. *Neuroscience*, *66*(2), 361–376. http://www.ncbi.nlm.nih.gov/pubmed/7477878

Obeso, J. A., Rodriguez-Oroz, M. C., Rodriguez, M., Lanciego, J. L., Artieda, J., Gonzalo, N., & Olanow, C. W. (2000). Pathophysiology of the basal ganglia in Parkinson's disease. *Trends in Neurosciences*, *23*, S8–S19. https://doi.org/10.1016/S1471-1931(00)00028-8

Paci, C., Thomas, A., & Onofrj, M. (2001). Amantadine for dyskinesia in patients affected by severe Parkinson's disease. *Neurological Sciences*, *22*(1), 75–76. https://doi.org/10.1007/s100720170054

Papa, S. M., & Chase, T. N. (1996). Levodopa-induced dyskinesias improved by a glutamate antagonist in Parkinsonian monkeys. *Annals of Neurology*, *39*(5), 574–578. https://doi.org/10.1002/ana.410390505

Paquette, M. A., Anderson, A. M., Lewis, J. R., Meshul, C. K., Johnson, S. W., & Paul Berger, S. (2010). MK-801 inhibits l-DOPA-induced abnormal involuntary movements only at doses that worsen parkinsonism. *Neuropharmacology*, *58*(7), 1002–1008. https://doi.org/10.1016/j.neuropharm.2010.01.003

Piccini, P., Weeks, R. A., & Brooks, D. J. (1997). Alterations in opioid receptor binding in Parkinson's disease patients with levodopa-induced dyskinesias. *Annals of Neurology*, *42*(5), 720–726. https://doi.org/10.1002/ana.410420508

Rupniak, N. M. J., Boyce, S., Steventon, M. J., Iversen, S. D., & Marsden, C. D. (1992). Dystonia induced by combined treatment with L-dopa and MK-801 in parkinsonian monkeys. *Annals of Neurology*, *32*(1), 103–105. https://doi.org/10.1002/ana.410320118

Samadi, P. (2003). Opioid antagonists increase the dyskinetic response to dopaminergic agents in parkinsonian monkeys: interaction between dopamine and opioid systems. *Neuropharmacology*, *45*(7), 954–963. https://doi.org/10.1016/S0028-3908(03)00249-1

Seizinger, B. R., Grimm, C., Höllt, V., & Herz, A. (1984). Evidence for a selective processing of proenkephalin B into different opioid peptide forms in particular regions of rat brain and pituitary. *Journal of Neurochemistry*, *42*(2), 447–457. http://www.ncbi.nlm.nih.gov/pubmed/6141221

Sgroi, S., Capper-Loup, C., Paganetti, P., & Kaelin-Lang, A. (2016). Enkephalin and dynorphin neuropeptides are differently correlated with locomotor hypersensitivity and levodopa-induced dyskinesia in parkinsonian rats. *Experimental Neurology*, *280*, 80–88. https://doi.org/10.1016/j.expneurol.2016.03.024

Tamim, M. K., Samadi, P., Morissette, M., Grégoire, L., Ouattara, B., Lévesque, D., Rouillard, C., & Di Paolo, T. (2010). Effect of non-dopaminergic drug treatment on Levodopa induced dyskinesias in MPTP monkeys: Common implication of striatal neuropeptides. *Neuropharmacology*, *58*(1), 286–296. https://doi.org/10.1016/j.neuropharm.2009.06.030

Tanner, C. M., & Ben-Shlomo, Y. (1999). Epidemiology of Parkinson's disease. *Advances in Neurology*, *80*, 153–159. http://www.ncbi.nlm.nih.gov/pubmed/10410715

Tel, B. C., Zeng, B. Y., Cannizzaro, C., Pearce, R. K. B., Rose, S., & Jenner, P. (2002). Alterations in striatal neuropeptide mRNA produced by repeated administration of L-DOPA, ropinirole or bromocriptine correlate with dyskinesia induction in MPTP-treated common marmosets. *Neuroscience*, *115*(4), 1047–1058. https://doi.org/10.1016/S0306-4522(02)00535-3

Westin, J. E., Andersson, M., Lundblad, M., & Cenci, M. A. (2001). Persistent changes in striatal gene expression induced by long-term l -DOPA treatment in a rat model of Parkinson's disease. *European Journal of Neuroscience*, *14*(7), 1171–1176. https://doi.org/10.1046/j.0953-816x.2001.01743.x

Williams, N. R., Heifets, B. D., Blasey, C., Sudheimer, K., Pannu, J., Pankow, H., Hawkins, J., Birnbaum, J., Lyons, D. M., Rodriguez, C. I., & Schatzberg, A. F. (2018). Attenuation of antidepressant effects of ketamine by opioid receptor antagonism. *American Journal of Psychiatry*, *175*(12), 1205–1215. https://doi.org/10.1176/appi.ajp.2018.18020138

Yue, X., Falk, T., Zuniga, L. A., Szabò, L., Porreca, F., Polt, R., & Sherman, S. J. (2011). Effects of the novel glycopeptide opioid agonist MMP-2200 in preclinical models of Parkinson's disease. *Brain Research*, *1413*, 72–83. https://doi.org/10.1016/j.brainres.2011.07.038

Appendix: Curriculum Vitae

2020

Andrew J. Flores

Citizenship: United States of America

Telephone: + 1 520 354 9460

Email: ajf1@email.arizona.edu

Education

Ph.D. Physiological Sciences | University of Arizona, 2020
Dissertation research advisor: Torsten Falk, Ph.D.
minor in Molecular & Cellular Biology

M.S. Physiological Sciences | University of Arizona, 2013

Graduate Certificate in Statistics | University of Arizona, 2013

B.S. Psychology | University of Arizona, 2010
Cognition & Neural Systems emphasis
minors in Mathematics, Chemistry, and Molecular Biology
magna cum laude

Technical Skills

- Statistical analysis
- Stereotaxic surgery
- Behavioral analysis
- Perfusion fixation
- Cell culture
- Immunohistochemistry
- Immunocytochemistry
- Flow cytometry
- Western analysis
- ELISA

- Fluorescence microscopy
- Stereology

Computational / Programming Experience

- R
- Java
- MATLAB
- NetLogo
- ImageJ

Teaching

Fall 2013. PSIO 201 Lab, University of Arizona

- Taught two sections of introductory anatomy and physiology lab

Graduate Coursework

MCB 580 - Introduction to Systems Biology
MCB 572A - Cell Systems
MCB 588 - Cell & Molecular Neurobiology
PSIO 503 - Cell & Molecular Physiology
PSIO 603 - Human Physiology
CMM 510 - Human Histology
MATH 564 - Theory of Probability
MATH 566 - Theory of Statistics
MATH 574T - Time Series Analysis
SIE 531 - Simulation, Modeling, & Analysis

Verified Online Courses

- Introduction to Clinical Data Science, Coursera (University of Colorado)
- Introduction to Computer Science Using Java I, Harvard Extension School
- Introduction to Agent Based Modeling, Complexity Explorer (Santa Fe Institute)
- R programming, Coursera (Johns Hopkins University)
- Introduction to Interactive Programming in Python I & II, Coursera (Rice University)
- Dynamical Modeling Methods for Systems Biology, Coursera (SBCNY, Icahn School of Medicine)

Languages Spoken

- English
- Spanish

Undergraduate Research

August 2010-August 2011. Senior Thesis, Principal Investigator: Richard Lane, M.D., Ph.D. University of Arizona Department of Psychiatry

- Completed senior thesis on impairments in theory of mind and emotional awareness in patients with somatoform disorders.

October 2007-December 2008. Principal Investigator: Michael J. Frank, Ph.D. Lab for Neural Computation and Cognition

- Completed an independent study on cognitive reinforcement learning and decision making, and obtained experience with human subjects research and electroencephalography.

Awards & Honors

- Achievement Rewards for College Scientists (ARCS) scholar award
  - 2015-2016 | 2016-2017 | 2017-2018
- Herbert E. Carter Travel Award, University of Arizona Graduate College, 2015
- National Institutes of Health (NIH)-IMSD Fellowship, 2013-2014 academic year
- University of Arizona College of Science Academic Distinction
  - 2007-2008, 2009-2010 academic years
- United Food & Commercial Workers Union scholarship, 2009
- Phi Beta Kappa honor society, University of Arizona, 2008

Program Committees

- Program Committee (Student Progress Commitee), Physiological Sciences Graduate Program, 2015-2016
- Seminar Committee, Physiological Sciences Graduate Program, 2016-2017
  - Organized student interactions with visiting faculty

Student Mentoring

- Mentor to high school students in the Summer Institute on Medical Ignorance (SIMI) research program at the University of Arizona College of Medicine. June 2013-Aug. 2013. Reference: Torsten Falk tfalk@email.arizona.edu

- Mentor to undergraduate student Michael Laswell in Arizona's Science, Engineering, and Math Scholars (ASEMS) program. Sept. 2014-Dec. 2014.
- Mentor to undergraduate student Grace Samtani in the Neuroscience and Cognitive Science undergraduate program. 2015-Present. Reference: Torsten Falk tfalk@email.arizona.edu
- Mentor to undergraduate student in the Minority Access to Research Careers (MARC) program

Professional Societies

- Society for Neuroscience (SfN)
- American Physiological Society (APS)

Peer-Reviewed Publications

1. Bartlett MJ, **Flores AJ**, Ye T, Smidt SI, Dollish HK, Stancati JA, Farrell DC, Parent KL, Doyle KP, Besselsen DG, Heien ML, Cowen SL, Steece-Collier K, Sherman SJ, Falk T. Preclinical evidence in support of repurposing sub-anesthetic ketamine as a treatment for L-DOPA-induced dyskinesia. *Experimental Neurology*, 2020. 113413. https://doi.org/10.1016/j.expneurol.2020.113413

2. **Flores AJ**, Bartlett MJ, So LY, Root BK, Parent KL, Heien ML, Porreca F, Polt R, Sherman SJ, Falk T. The combination of the opioid glycopeptide MMP-2200 and a NMDA receptor antagonist reduced L-DOPA-induced dyskinesia and MMP-2200 by itself reduced dopamine receptor 2-like agonist-induced dyskinesia. *Neuropharmacology,* 2018 Oct;141:260-271. Doi: 10.1016/j.neuropharm.2018.09.005. Epub 2018 Sep 7.

3. Teves JY, Bhargava V, Kirwan K, Corenblum MJ, Justiniano R, Wondrak GT, Annandurai A, **Flores AJ**, Schipper DA, Khalpey Z, Slight JE, Curiel-Lewandrowski C, Sherman SJ, Madhavan L. Signature cellular and molecular changes in sporadic Parkinson's disease fibroblasts. *Frontiers in Cellular Neuroscience*, 2018.

4. Corenblum MJ, **Flores AJ**, Badowski M, Harris DT, Madhavan L. Systemic human CD34(+) cells populate the brain and activate host mechanisms to counteract nigrostriatal degeneration. *Regenerative Medicine*. 2015;10(5):563-77.

5. **Flores AJ,** Bartlett MJ, So LY, Laude ND, Parent KL, Heien ML, Sherman SJ, Falk T. Differential effects of the NMDA receptor antagonist MK-801 on dopamine receptor D1- and D2-induced abnormal involuntary movements in a preclinical model. *Neuroscience Letters*, 2014 Apr 3;564:48-52. doi: 10.1016/j.neulet.2014.02.004. Epub 2014 Feb 11.

Publications in Preparation

1. **Flores AJ,** Bartlett MJ, Seaton BT, Samtani G, Sexauer MR, Siegenthaler JR, Weintraub NC, Lu D, Heien M, Porreca F, Sherman SJ, Falk T. The kappa opioid receptor antagonist nor-BNI accelerates development of L-DOPA-induced dyskinesia in a preclinical model of mild dopamine depletion. *Brain Research*. In preparation (**anticipated submission November 2020**).

2. Bartlett MJ, Mabrouk OS, Szabò L, **Flores AJ**, Parent KL, Heien ML, Kennedy RT, Polt R, Sherman SJ, Falk T; The delta-specific opioid glycopeptide BBI-11008: CNS penetration and behavioral analysis in a preclinical model of L-DOPA-induced dyskinesia. *International Journal of Molecular Sciences, Special Issue "Peptides for Health Benefits 2020"*. In preparation (**anticipated submission November 2020**).

Peer-Reviewed Abstracts & Conference Presentations

1. *Poster Presentation:* 5th World Parkinson Congress Meeting Kyoto, Japan, 2019. Long-term suppression of levodopa-induced dyskinesia by sub-anesthetic ketamine is mediated by BDNF and changes in striatal dendritic spine morphology. Bartlett MJ, **Flores AJ**, Dollish HK, Stancati J, Doyle KP, Heien ML, Steece-Collier K, Sherman SJ, Falk T.

2. *Poster Pr*esentation: 2nd Pan American Parkinson's Disease and Movement Disorders Congress in Miami, FL, 2018. Sub-anesthetic ketamine prevents levodopa-induced dyskinesia and improves motor function in a 6-OHDA rat model of Parkinson's disease. Bartlett MJ, **Flores AJ**, Dollish HK, Doyle KP, Steece-Collier K, Sherman SJ, Falk T.

3. *Poster* Presentation 2018 Society for Neuroscience Annual Meeting, San Diego, CA. The kappa opioid receptor antagonist nor-BNI accelerates development of L-DOPA-induced dyskinesia in a model of mild Parkinson's disease. Nava R, **Flores AJ**, Bartlett MJ, Sexauer MR*, Siegenthaler J, Heien ML, Sherman SJ, Porreca F, Falk T.

4. Poster Presentation: 2018 Society for Neuroscience Annual Meeting, San Diego, CA. Neuroplastic effects in the striatum contribute to the suppression of L-DOPA-induced dyskinesia by sub-anesthetic ketamine. Falk T, Bartlett MJ, **Flores AJ**, Dollish HK, Stancati JA, Doyle KP, Heien ML, Steece-Collier K, Sherman SJ.
5. *Poster presentation:* 2017 Society for Neuroscience Annual Meeting, Washington D.C. BDNF and mTOR contribute to the suppression of L-DOPA-induced dyskinesias by subanesthetic ketamine. **Flores AJ**, Bartlett MJ, Zehri AH, Parent KL, Heien ML, Sherman SJ, Falk T.

6. Bartlett MJ, **Flores AJ**, Dollish HK, Doyle KP, Pottenger AE, Morrison HL, Sherman SJ, Falk T; Neuroplastic effects contribute to the suppression of L-DOPA-induced dyskinesias by sub-anesthetic ketamine. AZ Commons - Neurobiology, Aging, Dementias and Movement Disorders Division Meeting, 2017.

7. *Poster presentation:* 2016 Society for Neuroscience Annual Meeting, San Diego, CA. Development of L-DOPA-induced dyskinesias in a rat model is reduced by subanesthetic

ketamine infusions. **Flores AJ**, Bartlett MJ, Zehri AH, Parent KL, Heien ML, Sherman SJ, Falk T.

8. *Poster presentation***:** 2015 Society for Neuroscience Annual Meeting, Chicago, IL. Analysis of cellular morphology, growth dynamics, and autophagic mechanisms in primary skin fibroblasts from individuals diagnosed with sporadic Parkinson's disease. **Flores AJ**, Corenblum MJ, Curiel C, Sherman SJ, Madhavan L.

9. *Poster presentation:* 2013 Society for Neuroscience Annual Meeting, San Diego, CA. The NMDA receptor antagonist MK-801 worsens dopamine 1-receptor-induced abnormal involuntary movements in a preclinical model. **Flores AJ**, Laude ND, Parent KL, Heien ML, Sherman SJ, Falk T.

10. *Poster presentation***:** 2013 Society for Neuroscience Annual Meeting, San Diego, CA. The opioid glycopeptide MMP-2200 blocks dopamine receptor 2-induced dyskinesia in a preclinical model. Falk T, **Flores AJ**, So LY, Porreca F, Polt R, Sherman SJ.

11. *Poster presentation:* 2012 Society for Neuroscience Annual Meeting, New Orleans, LA. The opioid glycopeptide MMP-2200: Analysis of CNS penetration and effects in a levodopa-induced dyskinesia model. **Flores AJ**, Mabrouk OS, Root BK, Yue X, Kennedy RT, Sherman SJ, Polt R, Falk T.

Other Presentations

1. **Flores AJ**, Laude ND, Parent KL, Heien ML, Sherman SJ, Falk T. The NMDA receptor antagonist MK-801 worsens dopamine 1-receptor-induced abnormal involuntary movements in a preclinical model. Poster presentation, *University of Arizona College of Medicine Frontiers in Biomedical Research.* October 30, 2013.

2. The opioid glycopeptide MMP-2200: Analysis of CNS penetration and effects in a levodopa-induced dyskinesia model. **Flores AJ**, Mabrouk OS, Root BK, Yue X, Kennedy RT, Sherman SJ, Polt R, Falk T. Poster presentation, *University of Arizona College of Medicine Frontiers in Biomedical Research.* October 7, 2012.

Employment History

- July 2011-Present. University of Arizona, Physiological Sciences Graduate Program & Department of Neurology, Graduate Research Assistant
- May 2011-August 2011. Easter Seals Foundation
  - Advocate for individuals with developmental disabilities

- January 2004-January 2009. Bashas' Supermarkets
  - Produce Clerk

Clinical Experience

October 2011-August 2012. University of Arizona Medical Center, Neurology Clinic, Movement Disorders Clinic, Neurology Outpatient Clinic

- Assisted in examinations of patients with Parkinson's disease and other movement disorders. Responsibilities included updating history of present illness, administering a symptom rating scale (UPDRS) and cognitive assessments, and completing medication charts. Total hours: 76. Supervisor: Scott J. Sherman, M.D., Ph.D.

April 2011-August 2012. Carondelet Hospice & Palliative Care, Tucson, AZ

- Provided comfort and support to help meet the needs of people living with a life-limiting illness. Training introduced fundamental practices in clinical patient care, OSHA and HIPAA guidelines, and knowledge of the dying process and bereavement.

Complete References

Ahmed, I., Bose, S. K., Pavese, N., Ramlackhansingh, A., Turkheimer, F., Hotton, G., Hammers, A., & Brooks, D. J. (2011). Glutamate NMDA receptor dysregulation in Parkinson's disease with dyskinesias. *Brain*, *134*(4), 979–986. https://doi.org/10.1093/brain/awr028

Albin, R. L., Young, A. B., & Penney, J. B. (1989). The functional anatomy of basal ganglia disorders. *Trends in Neurosciences*, *12*(10), 366–375. https://doi.org/10.1016/0166-2236(89)90074-X

Alexander, G. E., & Crutcher, M. D. (1990). Functional architecture of basal ganglia circuits: neural substrates of parallel processing. In *Trends in Neurosciences* (Vol. 13, Issue 7, pp. 266–271). Elsevier Current Trends. https://doi.org/10.1016/0166-2236(90)90107-L

Alves, G., Forsaa, E. B., Pedersen, K. F., Dreetz Gjerstad, M., & Larsen, J. P. (2008). Epidemiology of Parkinson's disease. *Journal of Neurology*, *255*(S5), 18–32. https://doi.org/10.1007/s00415-008-5004-3

Ambroziak, W., Koziorowski, D., Duszyc, K., Górka-Skoczylas, P., Potulska-Chromik, A., Sławek, J., & Hoffman-Zacharska, D. (2015). Genomic instability in the PARK2 locus is associated with Parkinson's disease. *Journal of Applied Genetics*, *56*(4), 451–461. https://doi.org/10.1007/s13353-015-0282-9

American Society of Anesthesiologists., A. D., & Ngai, S. H. (1982). Anesthesiology. In *Anesthesiology: The Journal of the American Society of Anesthesiologists* (Vol. 56, Issue 4). [American Society of Anesthesiologists, etc.]. http://anesthesiology.pubs.asahq.org/article.aspx?articleid=1956935

Andén, N.-E., Dahlström, A., Fuxe, K., & Larsson, K. (1965). Further evidence for the presence of nigro-neostriatal dopamine neurons in the rat. *American Journal of Anatomy*, *116*(1), 329–333. https://doi.org/10.1002/aja.1001160117

Andén, N. E., Carlsson, A., Dahlström, A., Fuxe, K., Hillarp, N. Å., & Larsson, K. (1964). Demonstration and mapping out of nigro-neostriatal dopamine neurons. *Life Sciences*, *3*(6), 523–530. https://doi.org/10.1016/0024-3205(64)90161-4

Andersson, M., Hilbertson, A., & Cenci, M. A. (1999). Striatal fosB expression is causally linked with l-DOPA-induced abnormal involuntary movements and the associated upregulation of striatal prodynorphin mRNA in a rat model of Parkinson's disease. *Neurobiology of Disease*, *6*(6), 461–474. https://doi.org/10.1006/nbdi.1999.0259

Atcherley, C. W., Laude, N. D., Parent, K. L., & Heien, M. L. (2013). Fast-scan controlled-adsorption voltammetry for the quantification of absolute concentrations and adsorption dynamics. *Langmuir*, *29*(48), 14885–14892. https://doi.org/10.1021/la402686s

Attwell, D., & Laughlin, S. B. (2001). An energy budget for signaling in the grey matter of the brain. *Journal of Cerebral Blood Flow and Metabolism : Official Journal of the International Society of Cerebral Blood Flow and Metabolism*, *21*(10), 1133–1145. https://doi.org/10.1097/00004647-200110000-00001

Aubert, I., Guigoni, C., Li, Q., Dovero, S., Bioulac, B. H., Gross, C. E., Crossman, A. R., Bloch, B., & Bezard, E. (2007). Enhanced Preproenkephalin-B-Derived Opioid Transmission in Striatum and Subthalamic Nucleus Converges Upon Globus Pallidus Internalis in L-3,4-dihydroxyphenylalanine-Induced Dyskinesia. *Biological Psychiatry*, *61*(7), 836–844. https://doi.org/10.1016/j.biopsych.2006.06.038

Bandy, B., & Davison, A. J. (1990). Mitochondrial mutations may increase oxidative stress: Implications for carcinogenesis and aging? *Free Radical Biology and Medicine*, *8*(6), 523–539. https://doi.org/10.1016/0891-5849(90)90152-9

Bartlett, M. J., Flores, A. J., Ye, T., Smidt, S. I., Dollish, H. K., Stancati, J. A., Farrell, D. C., Parent, K. L., Doyle, K. P., Besselsen, D. G., Heien, M. L., Cowen, S. L., Steece-Collier, K., Sherman, S. J., & Falk, T. (2020). Preclinical evidence in support of repurposing sub-anesthetic ketamine as a treatment for L-DOPA-induced dyskinesia. *Experimental Neurology*, 113413. https://doi.org/10.1016/j.expneurol.2020.113413

Bartlett, M. J., Joseph, R. M., LePoidevin, L. M., Parent, K. L., Laude, N. D., Lazarus, L. B., Heien, M. L., Estevez, M., Sherman, S. J., & Falk, T. (2016). Long-term effect of sub-anesthetic ketamine in reducing l-DOPA-induced dyskinesias in a preclinical model. *Neuroscience Letters*, *612*, 121–125. https://doi.org/10.1016/j.neulet.2015.11.047

Bartlett, M. J., So, L. Y., Szabò, L., Skinner, D. P., Parent, K. L., Heien, M. L., Vanderah, T. W., Polt, R., Sherman, S. J., & Falk, T. (2020). Highly-selective μ-opioid receptor antagonism does not block L-DOPA-induced dyskinesia in a rodent model. *BMC Research Notes*, *13*(1), 1–6. https://doi.org/10.1186/s13104-020-04994-7

Beardsley, P. M., Howard, J. L., Shelton, K. L., & Carroll, F. I. (2005). Differential effects of the novel kappa opioid receptor antagonist, JDTic, on reinstatement of cocaine-seeking induced by footshock stressors vs cocaine primes and its antidepressant-like effects in rats. *Psychopharmacology*, *183*(1), 118–126. https://doi.org/10.1007/s00213-005-0167-4

Bellou, V., Belbasis, L., Tzoulaki, I., Evangelou, E., & Ioannidis, J. P. A. (2016). Environmental risk factors and Parkinson's disease: An umbrella review of meta-analyses. *Parkinsonism & Related Disorders*, *23*, 1–9. http://linkinghub.elsevier.com/retrieve/pii/S1353802015300614

Belujon, P., Lodge, D. J., & Grace, A. A. (2010). Aberrant striatal plasticity is specifically associated with dyskinesia following levodopa treatment. *Movement Disorders*, *25*(11), 1568–1576. https://doi.org/10.1002/mds.23245

Benabid, A. L., Pollak, P., Hoffmann, D., Gervason, C., Hommel, M., Perret, J. E., de Rougemont, J., & Gao, D. M. (1991). Long-term suppression of tremor by chronic stimulation of the ventral intermediate thalamic nucleus. *The Lancet*, *337*(8738), 403–406. https://doi.org/10.1016/0140-6736(91)91175-T

Benazzouz, A., Gross, C., Féger, J., Boraud, T., & Bioulac, B. (1993). Reversal of Rigidity and Improvement in Motor Performance by Subthalamic High-frequency Stimulation in MPTP-treated Monkeys. *European Journal of Neuroscience*, *5*(4), 382–389. https://doi.org/10.1111/j.1460-9568.1993.tb00505.x

Berg, D., Schweitzer, K. J., Leitner, P., Zimprich, A., Lichtner, P., Belcredi, P., Brüssel, T., Schulte, C., Maass, S., Nägele, T., Wszolek, Z. K., & Gasser, T. (2005). Type and frequency of mutations in the LRRK2 gene in familial and sporadic Parkinson's disease*. *Brain*, *128*(12), 3000–3011. https://doi.org/10.1093/brain/awh666

Berger, K., Przedborski, S., & Cadet, J. L. (1991). Retrograde degeneration of nigrostriatal neurons induced by intrastriatal 6-hydroxydopamine injection in rats. *Brain Research Bulletin*, *26*(2), 301–307. https://doi.org/10.1016/0361-9230(91)90242-C

Bergman, H., Wichmann, T., & DeLong, M. R. (1990). Reversal of experimental parkinsonism by lesions of the subthalamic nucleus. *Science*, *249*(4975), 1436–1438. https://doi.org/10.1126/science.2402638

Betarbet, R. (2000). Chronic systemic pesticide exposure reproduces features of Parkinson's disease. *Nat. Neurosci.*, *3*.

Bezard, E., Brotchie, J. M., & Gross, C. E. (2001). Pathophysiology of levodopa-induced dyskinesia: potential for new therapies. In *Nature Reviews Neuroscience* (Vol. 2, Issue 8, pp. 577–588). Nature Publishing Group. https://doi.org/10.1038/35086062

Bezard, E., Li, Q., Hulme, H., Fridjonsdottir, E., Nilsson, A., Pioli, E., Andren, P. E., & Crossman, A. R. (2020). μ Opioid receptor agonism for L-DOPA-induced dyskinesia in Parkinson's disease. *Journal of Neuroscience*, *40*(35), 6812–6819. https://doi.org/10.1523/JNEUROSCI.0610-20.2020

Bezard, E., Ravenscroft, P., Gross, C. E., Crossman, A. R., & Brotchie, J. M. (2001). Upregulation of Striatal Preproenkephalin Gene Expression Occurs before the Appearance of Parkinsonian Signs in 1-Methyl-4-phenyl- 1,2,3,6-tetrahydropyridine Monkeys. *Neurobiology of Disease*, *8*(2), 343–350. https://doi.org/10.1006/nbdi.2000.0375

Billet, F., Costentin, J., & Dourmap, N. (2012). Influence of corticostriatal ??-opioid receptors on abnormal involuntary movements induced by L-DOPA in hemiparkinsonian rats. *Experimental Neurology*, *236*(2), 339–350. https://doi.org/10.1016/j.expneurol.2012.04.017

Bilsky, E. J., Egleton, R. D., Mitchell, S. A., Palian, M. M., Davis, P., Huber, J. D., Jones, H., Yamamura, H. I., Janders, J., Davis, T. P., Porreca, F., Hruby, V. J., & Polt, R. (2000). Enkephalin

glycopeptide analogues produce analgesia with reduced dependence liability. *Journal of Medicinal Chemistry*, *43*(13), 2586–2590. http://www.ncbi.nlm.nih.gov/pubmed/10891118

Birkmayer, W., & Hornykiewicz, O. (1961). [The L-3,4-dioxyphenylalanine (DOPA)-effect in Parkinson-akinesia]. *Wiener Klinische Wochenschrift*, *73*, 787–788. http://www.ncbi.nlm.nih.gov/pubmed/13869404

Blanchet, P. J., Konitsiotis, S., & Chase, T. N. (1998). Amantadine reduces levodopa-induced dyskinesias in parkinsonian monkeys. *Movement Disorders*, *13*(5), 798–802. https://doi.org/10.1002/mds.870130507

Block, M. L., Zecca, L., & Hong, J. S. (2007). Microglia-mediated neurotoxicity: Uncovering the molecular mechanisms. In *Nature Reviews Neuroscience* (Vol. 8, Issue 1, pp. 57–69). Nat Rev Neurosci. https://doi.org/10.1038/nrn2038

Blond, S., & Siegfried, J. (1991). Thalamic stimulation for the treatment of tremor and other movement disorders. *Acta Neurochirurgica. Supplementum*, *52*, 109–111. https://doi.org/10.1007/978-3-7091-9160-6_30

Bordet, R., Ridray, S., Carboni, S., Diaz, J., Sokoloff, P., & Schwartz, J. C. (1997). Induction of dopamine D3 receptor expression as a mechanism of behavioral sensitization to levodopa. *Proceedings of the National Academy of Sciences of the United States of America*, *94*(7), 3363–3367. https://doi.org/10.1073/PNAS.94.7.3363

Boveris, A., Oshino, N., & Chance, B. (1972). The cellular production of hydrogen peroxide. *The Biochemical Journal*, *128*(3), 617–630. https://doi.org/10.1042/bj1280617

Braak, H., & Del Tredici, K. (2004). Poor and protracted myelination as a contributory factor to neurodegenerative disorders. In *Neurobiology of Aging* (Vol. 25, Issue 1, pp. 19–23). Elsevier Inc. https://doi.org/10.1016/j.neurobiolaging.2003.04.001

Brar, S., Henderson, D., Schenck, J., & Zimmerman, E. A. (2009). Iron accumulation in the substantia nigra of patients with Alzheimer disease and parkinsonism. *Arch. Neurol.*, *66*.

Bratic, I., & Trifunovic, A. (2010). Mitochondrial energy metabolism and ageing. In *Biochimica et Biophysica Acta - Bioenergetics* (Vol. 1797, Issues 6–7, pp. 961–967). https://doi.org/10.1016/j.bbabio.2010.01.004

Breslin, M. B., Lindberg, I., Benjannet, S., Mathis, J. P., Lazure, C., & Seidah, N. G. (1993). Differential processing of proenkephalin by prohormone convertases 1(3) and 2 and furin. *The Journal of Biological Chemistry*, *268*(36), 27084–27093. http://www.ncbi.nlm.nih.gov/pubmed/8262946

Brice, A. (2005). Genetics of Parkinson's disease: LRRK2 on the rise. *Brain*, *128*(12), 2760–2762. https://doi.org/10.1093/brain/awh676

Brotchie, J., Crossman, A., Mitchell, I., Duty, S., Carroll, C., Cooper, A., Henry, B., Hughes, N., & Maneuf, Y. (1993). Chapter 8 Chemical signalling in the globus pallidus in parkinsonism. *Progress in Brain Research*, *99*, 125–139. https://doi.org/10.1016/S0079-6123(08)61342-4

Brown, P., Oliviero, A., Mazzone, P., Insola, A., Tonali, P., & Di Lazzaro, V. (2001). Dopamine dependency of oscillations between subthalamic nucleus and pallidum in Parkinson's disease. *Journal of Neuroscience*, *21*(3), 1033–1038. https://doi.org/10.1523/jneurosci.21-03-01033.2001

Bruchas, M. R., Land, B. B., Aita, M., Xu, M., Barot, S. K., Li, S., & Chavkin, C. (2007). Stress-Induced p38 Mitogen-Activated Protein Kinase Activation Mediates κ-Opioid-Dependent Dysphoria. *Journal of Neuroscience*, *27*(43).

Burns, R. S., Chiueh, C. C., Markey, S. P., Ebert, M. H., Jacobowitz, D. M., & Kopin, I. J. (1983). A primate model of parkinsonism: Selective destruction of dopaminergic neurons in the pars compacta of the substantia nigra by N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Proceedings of the National Academy of Sciences of the United States of America*, *80*(14 I), 4546–4550. https://doi.org/10.1073/pnas.80.14.4546

Buzsáki, G., & Draguhn, A. (2004). Neuronal olscillations in cortical networks. In *Science* (Vol. 304, Issue 5679, pp. 1926–1929). American Association for the Advancement of Science. https://doi.org/10.1126/science.1099745

Calne, D. B., & William Langston, J. (1983). AETIOLOGY OF PARKINSON'S DISEASE. *The Lancet*, *322*(8365–8366), 1457–1459. https://doi.org/10.1016/S0140-6736(83)90802-4

Calon, F., Birdi, S., Rajput, A. H., Hornykiewicz, O., Bédard, P. J., & Di Paolo, T. (2002). Increase of preproenkephalin mRNA levels in the putamen of Parkinson disease patients with levodopa-induced dyskinesias. *Journal of Neuropathology and Experimental Neurology*, *61*(2), 186–196. http://www.ncbi.nlm.nih.gov/pubmed/11853020

Calon, F., Rajput, A. H., Hornykiewicz, O., Bédard, P. J., & Di Paolo, T. (2003). Levodopa-induced motor complications are associated with alterations of glutamate receptors in Parkinson's disease. *Neurobiology of Disease*, *14*(3), 404–416. https://doi.org/10.1016/j.nbd.2003.07.003

Campbell, B. M., Kreipke, C. W., & Walker, P. D. (2006). Failure of MK-801 to suppress D1 receptor-mediated induction of locomotor activity and striatal preprotachykinin mRNA expression in the dopamine-depleted rat. *Neuroscience*, *137*(2), 505–517. https://doi.org/10.1016/j.neuroscience.2005.09.024

Carlsson, A., Lindqvist, M., & Magnusson, T. (1957). 3,4-Dihydroxyphenylalanine and 5-hydroxytryptophan as reserpine antagonists [16]. In *Nature* (Vol. 180, Issue 4596, p. 1200). Nature Publishing Group. https://doi.org/10.1038/1801200a0

Casals, J., Elizan, T. S., & Yahr, M. D. (1998). Postencephalitic parkinsonism - A review. In *Journal of Neural Transmission* (Vol. 105, Issues 6–7, pp. 645–676). https://doi.org/10.1007/s007020050086

Cassidy, M., Mazzone, P., Oliviero, A., Insola, A., Tonali, P., Lazzaro, V. Di, & Brown, P. (2002). Movement-related changes in synchronization in the human basal ganglia. *Brain*, *125*(6), 1235–1246. https://doi.org/10.1093/brain/awf135

Cenci, M. A., Lee, C. S., & Björklund, A. (1998). L-DOPA-induced dyskinesia in the rat is associated with striatal overexpression of prodynorphin- and glutamic acid decarboxylase mRNA. *European Journal of Neuroscience*, *10*(8), 2694–2706. https://doi.org/10.1046/j.1460-9568.1998.00285.x

Cenci, M. Angela, Whishaw, I. Q., & Schallert, T. (2002). Animal models of neurological deficits: How relevant is the rat? *Nature Reviews Neuroscience*, *3*(7), 574–579. https://doi.org/10.1038/nrn877

Chainani-Wu, N. (2003). Safety and anti-inflammatory activity of curcumin: A component of tumeric (Curcuma longa). *Journal of Alternative and Complementary Medicine*, *9*(1), 161–168. https://doi.org/10.1089/107555303321223035

Chan, C. S., Gertler, T. S., & Surmeier, D. J. (2009). Calcium homeostasis, selective vulnerability and Parkinson's disease. In *Trends in Neurosciences*. https://doi.org/10.1016/j.tins.2009.01.006

Chang, J.-W., Wachtel, S. ., Young, D., & Kang, U.-J. (1999). Biochemical and anatomical characterization of forepaw adjusting steps in rat models of Parkinson's disease: studies on medial forebrain bundle and striatal lesions. *Neuroscience*, *88*(2), 617–628. https://doi.org/10.1016/S0306-4522(98)00217-6

Charcot, J. M. (1872). Lecons sur les Maladies du Systeme Nerveux. In *Oeuvres completes de J.M. Charcot* (pp. 155–188). Bureaux du Progres Medical.

Chase, T. N. (1998). Levodopa therapy: Consequences of the nonphysiologic replacement of dopamine. *Neurology*, *50*(5 SUPPL. 5), S17–S25. https://doi.org/10.1212/wnl.50.5_suppl_5.s17

Chavkin, C., & Koob, G. F. (2016). Dynorphin, dysphoria, and dependence: The stress of addiction. In *Neuropsychopharmacology* (Vol. 41, Issue 1, pp. 373–374). Nature Publishing Group. https://doi.org/10.1038/npp.2015.258

Chefer, V. I., Czyzyk, T., Bolan, E. A., Moron, J., Pintar, J. E., & Shippenberg, T. S. (2005). Endogenous κ-Opioid Receptor Systems Regulate Mesoaccumbal Dopamine Dynamics and Vulnerability to Cocaine. *Journal of Neuroscience*, *25*(20).

Chen, H., & Chan, D. C. (2009). Mitochondrial dynamics-fusion, fission, movement, and mitophagy-in neurodegenerative diseases. *Human Molecular Genetics*. https://doi.org/10.1093/hmg/ddp326

Chen, L., Togasaki, D. M., Langston, J. W., Di Monte, D. A., & Quik, M. (2005). Enhanced striatal opioid receptor-mediated G-protein activation in L-dopa-treated dyskinetic monkeys. *Neuroscience*, *132*(2), 409–420. https://doi.org/10.1016/j.neuroscience.2004.10.026

Clague, M. J., & Urbé, S. (2010). Ubiquitin: Same molecule, different degradation pathways. In *Cell* (Vol. 143, Issue 5, pp. 682–685). Cell Press. https://doi.org/10.1016/j.cell.2010.11.012

Clapham, D. E. (2007). Calcium Signaling. In *Cell* (Vol. 131, Issue 6, pp. 1047–1058). Cell. https://doi.org/10.1016/j.cell.2007.11.028

Clark, I. E., Dodson, M. W., Jiang, C., Cao, J. H., Huh, J. R., Seol, J. H., Yoo, S. J., Hay, B. A., & Guo, M. (2006). Drosophila pink1 is required for mitochondrial function and interacts genetically with parkin. *Nature*, *441*(7097), 1162–1166. https://doi.org/10.1038/nature04779

Collier, T. J., Kanaan, N. M., & Kordower, J. H. (2017). Aging and Parkinson's disease: Different sides of the same coin? In *Movement Disorders* (Vol. 32, Issue 7, pp. 983–990). John Wiley and Sons Inc. https://doi.org/10.1002/mds.27037

Collier, T. J., Lipton, J., Daley, B. F., Palfi, S., Chu, Y., Sortwell, C., Bakay, R. A. E., Sladek, J. R., & Kordower, J. H. (2007). Aging-related changes in the nigrostriatal dopamine system and the response to MPTP in nonhuman primates: Diminished compensatory mechanisms as a prelude to parkinsonism. *Neurobiology of Disease*, *26*(1), 56–65. https://doi.org/10.1016/j.nbd.2006.11.013

Connolly, B. S., & Lang, A. E. (2014). Pharmacological Treatment of Parkinson Disease. *JAMA*, *311*(16), 1670. https://doi.org/10.1001/jama.2014.3654

Correia Guedes, L., Ferreira, J. J., Rosa, M. M., Coelho, M., Bonifati, V., & Sampaio, C. (2010). Worldwide frequency of G2019S LRRK2 mutation in Parkinson's disease: A systematic review. In *Parkinsonism and Related Disorders* (Vol. 16, Issue 4, pp. 237–242). Parkinsonism Relat Disord. https://doi.org/10.1016/j.parkreldis.2009.11.004

Cotzias, G. C., Papavasiliou, P. S., & Gellene, R. (1969). Modification of Parkinsonism — Chronic Treatment with L-Dopa. *New England Journal of Medicine*, *280*(7), 337–345. https://doi.org/10.1056/NEJM196902132800701

Cotzias, G. C., Van Woert, M. H., & Schiffer, L. M. (1967). Aromatic Amino Acids and Modification of Parkinsonism. *New England Journal of Medicine*, *276*(7), 374–379. https://doi.org/10.1056/NEJM196702162760703

Cox, H., Togasaki, D. M., Chen, L., Langston, J. W., Di Monte, D. A., & Quik, M. (2007). The selective κ-opioid receptor agonist U50,488 reduces l-dopa-induced dyskinesias but worsens parkinsonism in MPTP-treated primates. *Experimental Neurology*, *205*(1), 101–107. https://doi.org/10.1016/j.expneurol.2007.01.024

Crews, K. R., Gaedigk, A., Dunnenberger, H. M., Leeder, J. S., Klein, T. E., Caudle, K. E., Haidar, C. E., Shen, D. D., Callaghan, J. T., Sadhasivam, S., Prows, C. A., Kharasch, E. D., & Skaar, T. C. (2014). Clinical Pharmacogenetics Implementation Consortium Guidelines for Cytochrome P450 2D6 Genotype and Codeine Therapy: 2014 Update. *Clinical Pharmacology & Therapeutics*, *95*(4), 376–382. https://doi.org/10.1038/clpt.2013.254

Cuello, A. C., & Paxinos, G. (1978). Evidence for a long Leu-enkephalin striopallidal pathway in rat brain. *Nature*, *271*(5641), 178–180. http://www.ncbi.nlm.nih.gov/pubmed/23498

Cui, G., Jun, S. B., Jin, X., Pham, M. D., Vogel, S. S., Lovinger, D. M., & Costa, R. M. (2013). Concurrent activation of striatal direct and indirect pathways during action initiation. *Nature*, *494*(7436), 238–242. https://doi.org/10.1038/nature11846

Dauer, W., & Przedborski, S. (2003). Parkinson's Disease: Mechanisms and Models. *Neuron*, *39*(6), 889–909. https://doi.org/10.1016/S0896-6273(03)00568-3

Davis, G. C., Williams, A. C., Markey, S. P., Ebert, M. H., Caine, E. D., Reichert, C. M., & Kopin, I. J. (1979). Chronic parkinsonism secondary to intravenous injection of meperidine analogues. *Psychiatry Research*, *1*(3), 249–254. https://doi.org/10.1016/0165-1781(79)90006-4

Day, M., Wang, Z., Ding, J., An, X., Ingham, C. A., Shering, A. F., Wokosin, D., Ilijic, E., Sun, Z., Sampson, A. R., Mugnaini, E., Deutcch, A. Y., Sesack, S. R., Arbuthnott, G. W., & Surmeier, D. J. (2006). Selective elimination of glutamatergic synapses on striatopallidal neurons in Parkinson disease models. *Nature Neuroscience*, *9*(2), 251–259. https://doi.org/10.1038/nn1632

De Coo, I., Renier, W., Ruitenbeek, W., Ter Laak, H., Bakker, M., Schägger, H., Van Oost, B., & Smeets, H. (1999). A 4-base Pair Deletion in the Mitochondrial Cytochrome B Gene Associated With parkinsonism/MELAS Overlap Syndrome. *Annals of Neurology*, *45*(1). https://doi.org/10.1002/1531-8249(199901)45:1<130::AID-ART21>3.3.CO;2-Q

De Hemptinne, C., Swann, N. C., Ostrem, J. L., Ryapolova-Webb, E. S., San Luciano, M., Galifianakis, N. B., & Starr, P. A. (2015). Therapeutic deep brain stimulation reduces cortical phase-amplitude coupling in Parkinson's disease. *Nature Neuroscience*, *18*(5), 779–786. https://doi.org/10.1038/nn.3997

de la Fuente-Fernandez, R., Schulzer, M., Mak, E., Calne, D. B., & Stoessl, A. J. (2004). Presynaptic mechanisms of motor fluctuations in Parkinson's disease: a probabilistic model. *Brain*, *127*(4), 888–899. https://doi.org/10.1093/brain/awh102

de Lau, L. M., & Breteler, M. M. (2006). Epidemiology of Parkinson's disease. In *Lancet Neurology* (Vol. 5, Issue 6, pp. 525–535). Elsevier. https://doi.org/10.1016/S1474-4422(06)70471-9

Deas, E., Wood, N. W., & Plun-Favreau, H. (2011). Mitophagy and Parkinson's disease: The PINK1-parkin link. In *Biochimica et Biophysica Acta - Molecular Cell Research*. https://doi.org/10.1016/j.bbamcr.2010.08.007

Dekundy, A., Lundblad, M., Danysz, W., & Cenci, M. A. (2007). Modulation of L-DOPA-induced abnormal involuntary movements by clinically tested compounds: further validation of the rat dyskinesia model. *Behavioural Brain Research*, *179*(1), 76–89. https://doi.org/10.1016/j.bbr.2007.01.013

DeLong, M. R. (1990). Primate models of movement disorders of basal ganglia origin. In *Trends in Neurosciences* (Vol. 13, Issue 7, pp. 281–285). Elsevier Current Trends. https://doi.org/10.1016/0166-2236(90)90110-V

DeMaagd, G., & Philip, A. (2015). Parkinson's disease and its management part 1: Disease entity, risk factors, pathophysiology, clinical presentation, and diagnosis. *P and T*, *40*(8), 504–532.

Di Chiara, G., & Imperato, A. (1988). Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proceedings of the National Academy of Sciences of the United States of America*, *85*(14), 5274–5278. https://doi.org/10.1073/pnas.85.14.5274

Di Monte, D. A., McCormack, A., Petzinger, G., Janson, A. M., Quik, M., & Langston, W. J. (2000). Relationship among nigrostriatal denervation, parkinsonism, and dyskinesias in the MPTP primate model. *Movement Disorders*, *15*(3), 459–466. https://doi.org/10.1002/1531-8257(200005)15:3<459::AID-MDS1006>3.0.CO;2-3

Dias, V., Junn, E., & Mouradian, M. M. (2013). The role of oxidative stress in parkinson's disease. In *Journal of Parkinson's Disease* (Vol. 3, Issue 4, pp. 461–491). I O S Press. https://doi.org/10.3233/JPD-130230

Diederich, N. J., Moore, C. G., Leurgans, S. E., Chmura, T. A., & Goetz, C. G. (2003). Parkinson disease with old-age onset: A comparative study with subjects with middle-age onset. *Archives of Neurology*, *60*(4), 529–533. https://doi.org/10.1001/archneur.60.4.529

Donatus, I. A., Sardjoko, & Vermeulen, N. P. E. (1990). Cytotoxic and cytoprotective activities of curcumin. Effects on paracetamol-induced cytotoxicity, lipid peroxidation and glutathione depletion in rat hepatocytes. *Biochemical Pharmacology*, *39*(12), 1869–1875. https://doi.org/10.1016/0006-2952(90)90603-I

Dorsey, E. R., Constantinescu, R., Thompson, J. P., Biglan, K. M., Holloway, R. G., Kieburtz, K., Marshall, F. J., Ravina, B. M., Schifitto, G., Siderowf, A., & Tanner, C. M. (2007). Projected number of people with Parkinson disease in the most populous nations, 2005 through 2030. *Neurology*, *68*(5), 384–386. https://doi.org/10.1212/01.wnl.0000247740.47667.03

Dupre, K. B., Eskow, K. L., Barnum, C. J., & Bishop, C. (2008). Striatal 5-HT1A receptor stimulation reduces D1 receptor-induced dyskinesia and improves movement in the hemiparkinsonian rat. *Neuropharmacology*, *55*(8), 1321–1328. https://doi.org/10.1016/j.neuropharm.2008.08.031

Dupre, K. B., Ostock, C. Y., Jaunarajs, K. L. E., Button, T., Savage, L. M., Wolf, W., & Bishop, C. (2011). *Local modulation of striatal glutamate efflux by serotonin 1A receptor stimulation in dyskinetic, hemiparkinsonian rats*. https://doi.org/10.1016/j.expneurol.2011.02.012

Ebadi, M., Srinivasan, S. K., & Baxi, M. D. (1996). Oxidative stress and antioxidant therapy in Parkinson's disease. In *Progress in Neurobiology* (Vol. 48, Issue 1, pp. 1–19). Elsevier Ltd. https://doi.org/10.1016/0301-0082(95)00029-1

Ehringer, H., & Hornykiewicz, O. (1960). [Distribution of noradrenaline and dopamine (3-hydroxytyramine) in the human brain and their behavior in diseases of the extrapyramidal system]. *Klinische Wochenschrift*, *38*, 1236–1239. https://doi.org/10.1007/bf01485901

Elmagbari, N. O., Egleton, R. D., Palian, M. M., Lowery, J. J., Schmid, W. R., Davis, P., Navratilova, E., Dhanasekaran, M., Keyari, C. M., Yamamura, H. I., Porreca, F., Hruby, V. J., Polt, R., & Bilsky, E. J. (2004). Antinociceptive structure-activity studies with enkephalin-based opioid glycopeptides. *The Journal of Pharmacology and Experimental Therapeutics*, *311*(1), 290–297. https://doi.org/10.1124/jpet.104.069393

Engber, T. M., Susel, Z., Kuo, S., Gerfen, C. R., & Chase, T. N. (1991). Levodopa replacement therapy alters enzyme activities in striatum and neuropeptide content in striatal output regions of 6-hydroxydopamine lesioned rats. *Brain Research*, *552*(1), 113–118. https://doi.org/10.1016/0006-8993(91)90667-K

Eskow, K. L., Gupta, V., Alam, S., Park, J. Y., & Bishop, C. (2007). The partial 5-HT1A agonist buspirone reduces the expression and development of l-DOPA-induced dyskinesia in rats and improves l-DOPA efficacy. *Pharmacology Biochemistry and Behavior*, *87*(3), 306–314. https://doi.org/10.1016/J.PBB.2007.05.002

Fahn, S., Jankovic, J., & Hallett, M. (2011). Principles and Practice of Movement Disorders: Second Edition. In *Principles and Practice of Movement Disorders: Second Edition*. Elsevier Inc. https://doi.org/10.1016/C2009-0-44357-5

Fallon, J. H., Leslie, F. M., & Cone, R. I. (1985). Dynorphin-containing pathways in the substantia nigra and ventral tegmentum: A double labeling study using combined immunofluorescence and retrograde tracing. *Neuropeptides*, *5*(4–6), 457–460. https://doi.org/10.1016/0143-4179(85)90053-8

Faucheux, B. A., Martin, M. E., Beaumont, C., Hauw, J. J., Agid, Y., & Hirsch, E. C. (2003). Neuromelanin associated redox-active iron is increased in the substantia nigra of patients with Parkinson's disease. *Journal of Neurochemistry*, *86*(5), 1142–1148. https://doi.org/10.1046/j.1471-4159.2003.01923.x

FEARNLEY, J. M., & LEES, A. J. (1991). AGEING AND PARKINSON'S DISEASE: SUBSTANTIA NIGRA REGIONAL SELECTIVITY. *Brain*, *114*(5), 2283–2301. https://doi.org/10.1093/brain/114.5.2283

Financial Burden of Parkinson Disease Is $52 Billion, More Than Double Previous Estimates. (n.d.). Retrieved May 10, 2020, from https://www.ajmc.com/newsroom/the-financial-burden-of-parkinson-disease-is-52-billion-more-than-double-previous-estimates-

Finck A. Donald, M. D., & Ngai S. H., M. D. (1982). Opiate Receptor Mediation of Ketamine Analgesia. *Anesthesiology*, *56*(4), 291–297. http://dx.doi.org/

Flores, A. J., Bartlett, M. J., Root, B. K., Parent, K. L., Heien, M. L., Porreca, F., Polt, R., Sherman, S. J., & Falk, T. (2018). The combination of the opioid glycopeptide MMP-2200 and a NMDA receptor antagonist reduced L-DOPA-induced dyskinesia and MMP-2200 by itself reduced dopamine receptor 2-like agonist-induced dyskinesia. *Neuropharmacology*, *141*, 260–271. https://doi.org/10.1016/j.neuropharm.2018.09.005

Flores, A. J., Bartlett, M. J., So, L. Y., Laude, N. D., Parent, K. L., Heien, M. L., Sherman, S. J., & Falk, T. (2014). Differential effects of the NMDA receptor antagonist MK-801 on dopamine receptor D1- and D2-induced abnormal involuntary movements in a preclinical model. *Neuroscience Letters*, *564*, 48–52. https://doi.org/10.1016/j.neulet.2014.02.004

Forno, L. S. (1996). Neuropathology of Parkinson's Disease. *Journal of Neuropathology and Experimental Neurology*, *55*(3), 259–272. https://doi.org/10.1097/00005072-199603000-00001

Fox, S. H., & Brotchie, J. M. (2011). Non-Dopaminergic Approaches to the Treatment of Parkinson's Disease. In W. C. Olanow, F. Stocchi, & A. E. Lang (Eds.), *Parkinson's Disease - Non-Motor and Non-Dopaminergic Features* (pp. 443–444). Wiley-Blackwell.

Fox, S. H., Brotchie, J. M., & Lang, A. E. (2008). Non-dopaminergic treatments in development for Parkinson's disease. In *The Lancet Neurology* (Vol. 7, Issue 10, pp. 927–938). https://doi.org/10.1016/S1474-4422(08)70214-X

Fox, S. H., & Lang, A. E. (2008). Levodopa-related motor complications-Phenomenology. *Movement Disorders*, *23*(S3), S509–S514. https://doi.org/10.1002/mds.22021

Fox, S. H., Lang, A. E., & Brotchie, J. M. (2006). Translation of nondopaminergic treatments for levodopa-induced dyskinesia from MPTP-lesioned nonhuman primates to phase IIa clinical studies: Keys to success and roads to failure. *Movement Disorders*, *21*(10), 1578–1594. https://doi.org/10.1002/mds.20936

Fox, S., Silverdale, M., Kellett, M., Davies, R., Steiger, M., Fletcher, N., Crossman, A., & Brotchie, J. (2004). Non-subtype-selective opioid receptor antagonism in treatment of levodopa-induced motor complications in Parkinson's disease. *Movement Disorders : Official Journal of the Movement Disorder Society*, *19*(5), 554–560. https://doi.org/10.1002/mds.10693

Frankel, P. S., Alburges, M. E., Bush, L., Hanson, G. R., & Kish, S. J. (2008). Striatal and ventral pallidum dynorphin concentrations are markedly increased in human chronic cocaine users. *Neuropharmacology*, *55*(1), 41–46. https://doi.org/10.1016/j.neuropharm.2008.04.019

Gardner, J. (2013). A history of deep brain stimulation: Technological innovation and the role of clinical assessment tools. *Social Studies of Science*, *43*(5), 707–728. https://doi.org/10.1177/0306312713483678

Gee, T. A., Weintraub, N. C., Lu, D., Phelps, C. E., Navratilova, E., Heien, M. L., & Porreca, F. (2020). A pain-induced tonic hypodopaminergic state augments phasic dopamine release in the nucleus accumbens. *Pain*, *Publish ah*. https://doi.org/10.1097/j.pain.0000000000001925

Gehrke, B. J., Chefer, V. I., & Shippenberg, T. S. (2008). Effects of acute and repeated administration of salvinorin a on dopamine function in the rat dorsal striatum. *Psychopharmacology*, *197*(3), 509–517. https://doi.org/10.1007/s00213-007-1067-6

Gems, D., & Partridge, L. (2013). Genetics of Longevity in Model Organisms: Debates and Paradigm Shifts. *Annual Review of Physiology*, *75*(1), 621–644. https://doi.org/10.1146/annurev-physiol-030212-183712

Gengo, P. J., & Chang, K.-J. (2003). Mixed Opioid Receptor Agonists as a New Class of Agents for the Treatment of Moderate to Severe Pain. In *The Delta Receptor* (pp. 231–244). CRC Press. https://doi.org/10.1201/9780203025765.ch14

Gerfen, C. R., Baimbridge, K. G., & Miller, J. J. (1985). The neostriatal mosaic: Compartmental distribution of calcium-binding protein and parvalbumin in the basal ganglia of the rat and monkey. *Proceedings of the National Academy of Sciences of the United States of America*, *82*(24), 8780–8784. https://doi.org/10.1073/pnas.82.24.8780

Gerfen, C R. (1990). D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. *Science*, *250*.

Gerfen, C R. (1992). The neostriatal mosaic: multiple levels of compartmental organization. *Trends in Neurosciences*, *15*(4), 133–139. http://www.ncbi.nlm.nih.gov/pubmed/1374971

Gerfen, C R, Engber, T. M., Mahan, L. C., Susel, Z., Chase, T. N., Monsma, F. J., & Sibley, D. R. (1990). D1 and D2 dopamine receptor regulated gene expression of striatonigral and striatopallidal neurons. *Science*, *250*(1986), 1429–1432. https://doi.org/10.1126/science.2147780

Gerfen, Charles R. (1984). The neostriatal mosaic: Compartmentalization of corticostriatal input and striatonigral output systems. *Nature*, *311*(5985), 461–464. https://doi.org/10.1038/311461a0

Gerfen, Charles R., & Scott Young, W. (1988). Distribution of striatonigral and striatopallidal peptidergic neurons in both patch and matrix compartments: an in situ hybridization histochemistry and fluorescent retrograde tracing study. *Brain Research*, *460*(1), 161–167. https://doi.org/10.1016/0006-8993(88)91217-6

Gerfen, Charles R., & Surmeier, D. J. (2011). Modulation of Striatal Projection Systems by Dopamine. *Annual Review of Neuroscience*, *34*(1), 441–466. https://doi.org/10.1146/annurev-neuro-061010-113641

Gerfen, Charles R, Herkenham, M., & Thibault, J. (1987). The Neostriatal Mosaic: II. Patch-and Matrix-Directed Mesostriatal Dopaminergic and Non-Dopaminergic Systems. In *The Journal of Neuroscience* (Vol. 7, Issue 12).

German, D. C., & Manaye, K. F. (1993). Midbrain dopaminergic neurons (nuclei A8, A9, and A10): Three-dimensional reconstruction in the rat. *Journal of Comparative Neurology*, *331*(3), 297–309. https://doi.org/10.1002/cne.903310302

Goetz, C. G., Poewe, W., Rascol, O., & Sampaio, C. (2005). Evidence-based medical review update: Pharmacological and surgical treatments of Parkinson's disease: 2001 to 2004. In *Movement Disorders* (Vol. 20, Issue 5, pp. 523–539). Mov Disord. https://doi.org/10.1002/mds.20464

Goldman, J. G., & Postuma, R. (2014). Premotor and nonmotor features of Parkinson's disease. *Current Opinion in Neurology*, *27*(4), 434–441. https://doi.org/10.1097/WCO.0000000000000112

Goldstein, M., Anagnoste, B., Owen, W. S., & Battista, A. F. (1966). The effects of ventromedial tegmental lesions on the biosynthesis of catecholamines in the striatum. *Life Sciences*, *5*(23), 2171–2176. https://doi.org/10.1016/0024-3205(66)90078-6

Gomez-Mancilla, B., & Bedard, P. J. (1993). Effect of nondopaminergic drugs on L-DOPA-induced dyskinesias in MPTP- treated monkeys. *Clinical Neuropharmacology*, *16*(5), 418–427. https://doi.org/10.1097/00002826-199310000-00004

Gong, S., Zheng, C., Doughty, M. L., Losos, K., Didkovsky, N., Schambra, U. B., Nowak, N. J., Joyner, A., Leblanc, G., Hatten, M. E., & Heintz, N. (2003). A gene expression atlas of the central nervous system based on bacterial artificial chromosomes. *Nature*, *425*(6961), 917–925. https://doi.org/10.1038/nature02033

Gradinaru, V., Mogri, M., Thompson, K. R., Henderson, J. M., & Deisseroth, K. (2009). Optical deconstruction of parkinsonian neural circuitry. *Science*, *324*(5925), 354–359. https://doi.org/10.1126/science.1167093

Grant, R. J., & Clarke, P. B. S. (2002). Susceptibility of ascending dopamine projections to 6-hydroxydopamine in rats: Effect of hypothermia. *Neuroscience*, *115*(4), 1281–1294. https://doi.org/10.1016/S0306-4522(02)00385-8

Graybiel, A. M., & Ragsdale, C. W. (1978). Histochemically distinct compartments in the striatum of human, monkey, and cat demonstrated by acetylthiocholinesterase staining. *Proceedings of the National Academy of Sciences of the United States of America*, *75*(11), 5723–5726. https://doi.org/10.1073/pnas.75.11.5723

Graybiel, Ann M., Canales, J. J., & Capper-Loup, C. (2000). Levodopa-induced dyskinesias and dopamine-dependent stereotypies: A new hypothesis. In *Trends in Neurosciences* (Vol. 23, Issue 10 SUPPL., pp. S71–S77). Elsevier Ltd. https://doi.org/10.1016/S1471-1931(00)00027-6

Greene, J. C., Whitworth, A. J., Kuo, I., Andrews, L. A., Feany, M. B., & Pallanck, L. J. (2003). Mitochondrial pathology and apoptotic muscle degeneration in Drosophila parkin mutants. *Proceedings of the National Academy of Sciences of the United States of America*, *100*(7), 4078–4083. https://doi.org/10.1073/pnas.0737556100

Greenfield, J. G., & Bosanquet, F. D. (1953). The brain-stem lesions in Parkinsonism. *Journal of Neurology, Neurosurgery, and Psychiatry*, *16*(4), 213–226. https://doi.org/10.1136/jnnp.16.4.213

Greengard, P. (2001). The neurobiology of slow synaptic transmission. In *Science* (Vol. 294, Issue 5544, pp. 1024–1030). https://doi.org/10.1126/science.294.5544.1024

Guigoni, C., Aubert, I., Li, Q., Gurevich, V. V., Benovic, J. L., Ferry, S., Mach, U., Stark, H., Leriche, L., Håkansson, K., Bioulac, B. H., Gross, C. E., Sokoloff, P., Fisone, G., Gurevich, E. V., Bloch, B., & Bezard, E. (2005). Pathogenesis of levodopa-induced dyskinesia: focus on D1 and D3 dopamine receptors. *Parkinsonism & Related Disorders*, *11*, S25–S29. https://doi.org/10.1016/J.PARKRELDIS.2004.11.005

Guillin, O., Diaz, J., Carroll, P., Griffon, N., Schwartz, J.-C., & Sokoloff, P. (2001). BDNF controls dopamine D3 receptor expression and triggers behavioural sensitization. *Nature*, *411*(6833), 86–89. https://doi.org/10.1038/35075076

Gupta, A., Devi, L. A., & Gomes, I. (2011). Potentiation of μ-opioid receptor-mediated signaling by ketamine. *Journal of Neurochemistry*, *119*(2), 294–302. https://doi.org/10.1111/j.1471-4159.2011.07361.x

Guzman, J. N., Sánchez-Padilla, J., Chan, C. S., & Surmeier, D. J. (2009). Robust pacemaking in substantia nigra dopaminergic neurons. *Journal of Neuroscience*, *29*(35), 11011–11019. https://doi.org/10.1523/JNEUROSCI.2519-09.2009

Hallman, H., Olson, L., & Jonsson, G. (1984). Neurotoxicity of the meperidine analogue N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine on brain catecholamine neurons in the mouse. *European Journal of Pharmacology*, *97*(1–2), 133–136. https://doi.org/10.1016/0014-2999(84)90521-1

Hammond, C., Ammari, R., Bioulac, B., & Garcia, L. (2008). Latest view on the mechanism of action of deep brain stimulation. *Movement Disorders*, *23*(15), 2111–2121. https://doi.org/10.1002/mds.22120

Hammond, C., Bergman, H., & Brown, P. (2007). Pathological synchronization in Parkinson's disease: networks, models and treatments. In *Trends in Neurosciences* (Vol. 30, Issue 7, pp. 357–364). Elsevier. https://doi.org/10.1016/j.tins.2007.05.004

Harris, J. J., Jolivet, R., & Attwell, D. (2012). Synaptic Energy Use and Supply. In *Neuron* (Vol. 75, Issue 5, pp. 762–777). Cell Press. https://doi.org/10.1016/j.neuron.2012.08.019

Hauser, K. F., Aldrich, J. V., Anderson, K. J., Bakalkin, G., Christie, M. J., Hall, E. D., Knapp, P. E., Scheff, S. W., Singh, I. N., Vissel, B., Woods, A. S., Yakovleva, T., & Shippenberg, T. S. (2005). Pathobiology of dynorphins in trauma and disease. In *Frontiers in Bioscience* (Vol. 10, Issue 1, pp. 216–235). Frontiers in Bioscience. https://doi.org/10.2741/1522

Hauser, R. A., Rascol, O., Korczyn, A. D., Jon Stoessl, A., Watts, R. L., Poewe, W., De Deyn, P. P., & Lang, A. E. (2007). Ten-year follow-up of Parkinson's disease patients randomized to initial therapy with ropinirole or levodopa. *Movement Disorders*, *22*(16), 2409–2417. https://doi.org/10.1002/mds.21743

Healy-Stoffel, M., Ahmad, S. O., Stanford, J. A., & Levant, B. (2014). Differential effects of intrastriatal 6-hydroxydopamine on cell number and morphology in midbrain dopaminergic subregions of the rat. *Brain Research*, *1574*, 113–119. https://doi.org/10.1016/j.brainres.2014.05.045

Hefti, F., Melamed, E., Sahakian, B. J., & Wurtman, R. J. (1980). Circling behavior in rats with partial, unilateral nigro-striatal lesions: Effect of amphetamine, apomorphine, and DOPA. *Pharmacology, Biochemistry and Behavior*, *12*(2), 185–188. https://doi.org/10.1016/0091-3057(80)90353-6

Heikkila, R. E., Hess, A., & Duvoisin, R. C. (1984). Dopaminergic neurotoxicity of 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine in mice. *Science*, *224*(4656), 1451–1453. https://doi.org/10.1126/science.6610213

Hely, M. A., Morris, J. G. L., Reid, W. G. J., O'Sullivan, D. J., Williamson, P. M., Broe, G. A., & Adena, M. A. (1995). Age at onset: the major determinant of outcome in Parkinson's disease. *Acta Neurologica Scandinavica*, *92*(6), 455–463. https://doi.org/10.1111/j.1600-0404.1995.tb00480.x

Henry, B, Crossman, A. R., & Brotchie, J. M. (1999). Effect of repeated L-DOPA, bromocriptine, or lisuride administration on preproenkephalin-A and preproenkephalin-B mRNA levels in the striatum of the 6-hydroxydopamine-lesioned rat. *Experimental Neurology*, *155*(2), 204–220. https://doi.org/10.1006/exnr.1998.6996

Henry, Brian, Duty, S., Fox, S. H., Crossman, A. R., & Brotchie, J. M. (2003). Increased striatal pre-proenkephalin B expression is associated with dyskinesia in Parkinson's disease. *Experimental Neurology*, *183*(2), 458–468. https://doi.org/10.1016/S0014-4886(03)00064-5

Henry, Brian, Fox, S. H., Crossman, A. R., & Brotchie, J. M. (2001). μ- and δ-Opioid Receptor Antagonists Reduce Levodopa-Induced Dyskinesia in the MPTP-Lesioned Primate Model of Parkinson's Disease. *Experimental Neurology*, *171*(1), 139–146. https://doi.org/10.1006/EXNR.2001.7727

Herkenham, M., & Pert, C. B. (1981). Mosaic distribution of opiate receptors, parafascicular projections and acetylcholinesterase in rat striatum. *Nature*, *291*(5814), 415–418. https://doi.org/10.1038/291415a0

Hernan, M. A., Takkouche, B., Caamano-Isorna, F., & Gestal-Otero, J. J. (2002). A meta-analysis of coffee drinking, cigarette smoking, and the risk of Parkinson's disease. *Ann. Neurol.*, *52*.

Herrero, M. T., Augood, S. J., Hirsch, E. C., Javoy-Agid, F., Luquin, M. R., Agid, Y., Obeso, J. A., & Emson, P. C. (1995). Effects of l-DOPA on preproenkephalin and preprotachykinin gene expression in the MPTP-treated monkey striatum. *Neuroscience*, *68*(4), 1189–1198. https://doi.org/10.1016/0306-4522(95)00120-8

Hill, M. P., & Brotchie, J. M. (1999). The adrenergic receptor agonist, clonidine, potentiates the anti-parkinsonian action of the selective κ-opioid receptor agonist, enadoline, in the monoamine-depleted rat. *British Journal of Pharmacology*, *128*(7), 1577–1585. https://doi.org/10.1038/sj.bjp.0702943

Hille, C. J., Fox, S. H., Maneuf, Y. P., Crossman, A. R., & Brotchie, J. M. (2001). Antiparkinsonian action of a delta opioid agonist in rodent and primate models of Parkinson's disease. *Experimental Neurology*, *172*(1), 189–198. https://doi.org/10.1006/exnr.2001.7763

Hirsch, E., Graybiel, A. M., & Agid, Y. A. (1988). Melanized dopaminergic neurons are differentially susceptible to degeneration in Parkinson's disease. *Nature*, *334*(6180), 345–348. https://doi.org/10.1038/334345a0

Hokfelt, T., & Ungerstedt, U. (1973). Specificity of 6-hydroxydopamine induced degeneration of central monoamine neurones: an electron and fluorescence microscopic study with special reference to intracerebral injection on the nigro-striatal dopamine system. *Brain Res.*, *60*.

Holloway, R. G. (2004). Pramipexole vs levodopa as initial treatment for Parkinson disease: a 4-year randomized controlled trial. *Arch. Neurol.*, *61*.

Holloway, R., Marek, K., Biglan, K., Dick, A., Fahn, S., Julian-Baros, E., Kamp, C., Kieburtz, K., Lang, A., McDermott, M., Seibyl, J., Shinaman, A., Shoulson, I., Weiner, W., Pahwa, R., Grimes, D., Miyasaki, J., Johnston, L., Panisset, M., … Hunter, C. W. (2009). Long-term Effect of Initiating Pramipexole vs Levodopa in Early Parkinson Disease. *Archives of Neurology*, *66*(5), 563–570. https://doi.org/10.1001/archneur.66.1.nct90001

Holm, S. (1979). A Simple Sequentially Rejective Multiple Test Procedure. In *Scandinavian Journal of Statistics* (Vol. 6, pp. 65–70). WileyBoard of the Foundation of the Scandinavian Journal of Statistics. https://doi.org/10.2307/4615733

Hudzik, T. J., Howell, A., Payza, K., & Cross, A. J. (2000). Antiparkinson potential of delta-opioid receptor agonists. *European Journal of Pharmacology*, *396*(2–3), 101–107. http://www.ncbi.nlm.nih.gov/pubmed/10822062

Hughes, N. R., McKnight, A. T., Woodruff, G. N., Hill, M. P., Crossman, A. R., & Brotchie, J. M. (1998). Kappa-opioid receptor agonists increase locomotor activity in the monoamine-depleted rat

model of parkinsonism. *Movement Disorders*, *13*(2), 228–233. https://doi.org/10.1002/mds.870130206

Huot, P., Johnston, T. H., Koprich, J. B., Fox, S. H., & Brotchie, J. M. (2013). The Pharmacology of l-DOPA-Induced Dyskinesia in Parkinson's Disease. *Pharmacological Reviews*, *65*(1).

Iancu, R., Mohapel, P., Brundin, P., & Paul, G. (2005). Behavioral characterization of a unilateral 6-OHDA-lesion model of Parkinson's disease in mice. *Behavioural Brain Research*, *162*(1), 1–10. https://doi.org/10.1016/j.bbr.2005.02.023

Ikeda, K., Yoshikawa, S., Kurokawa, T., Yuzawa, N., Nakao, K., & Mochizuki, H. (2009). TRK-820, a selective kappa opioid receptor agonist, could effectively ameliorate L-DOPA-induced dyskinesia symptoms in a rat model of Parkinson's disease. *European Journal of Pharmacology*, *620*(1–3), 42–48. https://doi.org/10.1016/j.ejphar.2009.08.013

Irwin, I., DeLanney, L. E., McNeill, T., Chan, P., Forno, L. S., Murphy, G. M., Di Monte, D. A., Sandy, M. S., & Langston, J. W. (1994). Aging and the nigrostriatal dopamine system: a non-human primate study. *Neurodegeneration : A Journal for Neurodegenerative Disorders, Neuroprotection, and Neuroregeneration*, *3*(4), 251–265.

Ishida, Y., Hashiguchi, H., Todaka, K., Ishizuka, Y., & Mitsuyama, Y. (2000). Repeated administration of high dose levodopa enhances hydroxyl radical production in the rat striatum denervated with 6-hydroxydopamine. *Neuroscience Letters*, *290*(1), 33–36. https://doi.org/10.1016/S0304-3940(00)01320-3

Jankovic, J., & Aguilar, L. G. (2008). Current approaches to the treatment of Parkinson's disease. *Neuropsychiatric Disease and Treatment*, *4*(4), 743–757. http://www.ncbi.nlm.nih.gov/pubmed/19043519

Jankovic, J., & Kapadia, A. S. (2001). Functional decline in Parkinson disease. *Archives of Neurology*, *58*(10), 1611–1615. https://doi.org/10.1001/archneur.58.10.1611

Jenner, P. (2005). *Principles of Treatment in Parkinson's Disease* (Anthony H. V. Schapira & C. W. Olanow (Eds.)).

Jenner, Peter, Hunot, Olanow, Beal, Kordower, Tatton, & Schapira. (2003). Oxidative stress in Parkinson's disease. In *Annals of Neurology* (Vol. 53, Issue SUPPL. 3, pp. S26–S36). https://doi.org/10.1002/ana.10483

Jenner, Peter, & Olanow, C. W. (2006). The pathogenesis of cell death in Parkinson's disease. In *Neurology* (Vol. 66, Issue 10 SUPPL. 4). Lippincott Williams and Wilkins. https://doi.org/10.1212/wnl.66.10_suppl_4.s24

Jeon, B. S., Jackson-Lewis, V., & Burke, R. E. (1995). 6-Hydroxydopamine Lesion of the Rat Substantia Nigra: Time Course and Morphology of Cell Death. *Neurodegeneration*, *4*(2), 131–137. https://doi.org/10.1006/neur.1995.0016

Johansson, P. A., Andersson, M., Andersson, K. E., & Cenci, M. A. (2001). Alterations in Cortical and Basal Ganglia Levels of Opioid Receptor Binding in a Rat Model of l-DOPA-Induced Dyskinesia. *Neurobiology of Disease*, *8*(2), 220–239. https://doi.org/10.1006/nbdi.2000.0372

Johnston, T. H., Versi, E., Howson, P. A., Ravenscroft, P., Fox, S. H., Hill, M. P., Reidenberg, B. E., Corey, R., & Brotchie, J. M. (2018). DPI-289, a novel mixed delta opioid agonist / mu opioid antagonist (DAMA), has L-DOPA-sparing potential in Parkinson's disease. *Neuropharmacology*, *131*, 116–127. https://doi.org/10.1016/j.neuropharm.2017.11.046

Jonkers, N., Sarre, S., Ebinger, G., & Michotte, Y. (2002). MK801 suppresses the L-DOPA-induced increase of glutamate in striatum of hemi-Parkinson rats. In *Brain Research* (Vol. 926). www.elsevier.com/locate/bres

Katzenschlager, R., Head, J., Schrag, A., Ben-Shlomo, Y., Evans, A., Lees, A. J., & Parkinson's Disease Research Group of the United Kingdom. (2008). Fourteen-year final report of the randomized PDRG-UK trial comparing three initial treatments in PD. *Neurology*, *71*(7), 474–480. https://doi.org/10.1212/01.wnl.0000310812.43352.66

Kelloff, G. J., Crowell, J. A., Hawk, E. T., Steele, V. E., Lubet, R. A., Boone, C. W., Covey, J. M., Doody, L. A., Omenn, G. S., Greenwald, P., Hong, W. K., Parkinson, D. R., Bagheri, D., Baxter, G. T., Blunden, M., Doeltz, M. K., Eisenhauer, K. M., Johnson, K., Knapp, G. G., … Sigman, C. C. (1996). Strategy and planning for chemopreventive drug development: Clinical development plans II. *Journal of Cellular Biochemistry*, *63*(S26), 54–71. https://doi.org/10.1002/jcb.240630705

Kirik, D., Rosenblad, C., & Björklund, A. (1998). Characterization of behavioral and neurodegenerative changes following partial lesions of the nigrostriatal dopamine system induced by intrastriatal 6-hydroxydopamine in the rat. *Experimental Neurology*, *152*(2), 259–277. https://doi.org/10.1006/exnr.1998.6848

Kirkwood, T. B. L. (2005). Understanding the odd science of aging. In *Cell* (Vol. 120, Issue 4, pp. 437–447). Cell Press. https://doi.org/10.1016/j.cell.2005.01.027

Kishioka, S., Kiguchi, N., Kobayashi, Y., Yamamoto, C., Saika, F., Wakida, N., Ko, M. C., & Woods, J. H. (2013). Pharmacokinetic evidence for the long-lasting effect of nor-binaltorphimine, a potent kappa opioid receptor antagonist, in mice. *Neuroscience Letters*, *552*, 98–102. https://doi.org/10.1016/j.neulet.2013.07.040

Kitada, T., Asakawa, S., Hattori, N., Matsumine, H., Yamamura, Y., Minoshima, S., Yokochi, M., Mizuno, Y., & Shimizu, N. (1998). Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature*, *392*(6676), 605–608. https://doi.org/10.1038/33416

Klintenberg, R., Svenningsson, P., Gunne, L., & Andrén, P. E. (2002). Naloxone reduces levodopa-induced dyskinesias and apomorphine-induced rotations in primate models of parkinsonism. *Journal of Neural Transmission (Vienna, Austria : 1996)*, *109*(10), 1295–1307. https://doi.org/10.1007/s00702-002-0715-6

Kneynsberg, A., Collier, T. J., Manfredsson, F. P., & Kanaan, N. M. (2016). Quantitative and semi-quantitative measurements of axonal degeneration in tissue and primary neuron cultures. *Journal of Neuroscience Methods*, *266*, 32–41. https://doi.org/10.1016/j.jneumeth.2016.03.004

Knoll, A. T., & Carlezon, W. A. (2010). Dynorphin, stress, and depression. *Brain Research*, *1314*, 56–73. https://doi.org/10.1016/j.brainres.2009.09.074

Kobylecki, C., Hill, M. P., Crossman, A. R., & Ravenscroft, P. (2011). Synergistic antidyskinetic effects of topiramate and amantadine in animal models of Parkinson's disease. *Movement Disorders*, *26*(13), 2354–2363. https://doi.org/10.1002/mds.23867

Koprich, J. B., Fox, S. H., Johnston, T. H., Goodman, A., Le Bourdonnec, B., Dolle, R. E., Dehaven, R. N., Dehaven-Hudkins, D. L., Little, P. J., & Brotchie, J. M. (2011). The selective mu-opioid receptor antagonist adl5510 reduces levodopa-induced dyskinesia without affecting antiparkinsonian action in mptp-lesioned macaque model of Parkinson's disease. *Movement Disorders*, *26*(7), 1225–1233. https://doi.org/10.1002/mds.23631

Krack, P., Pollak, P., Limousin, P., Benazzouz, A., & Benabid, A. (1997). Stimulation of subthalamic nucleus alleviates tremor in Parkinson's disease. *The Lancet*, *350*(9092), 1675. https://doi.org/10.1016/S0140-6736(97)24049-3

Kravitz, A. V. (2010). Regulation of parkinsonian motor behaviours by optogenetic control of basal ganglia circuitry. *Nature*, *466*.

Kreitzer, A. C., & Malenka, R. C. (2007). Endocannabinoid-mediated rescue of striatal LTD and motor deficits in Parkinson's disease models. *Nature*, *445*(7128), 643–647. https://doi.org/10.1038/nature05506

Kühn, A. A., Trottenberg, T., Kivi, A., Kupsch, A., Schneider, G. H., & Brown, P. (2005). The relationship between local field potential and neuronal discharge in the subthalamic nucleus of patients with Parkinson's disease. *Experimental Neurology*, *194*(1), 212–220. https://doi.org/10.1016/j.expneurol.2005.02.010

Laitinen, L. V., Bergenheim, A. T., & Hariz, M. I. (1992). Leksell's posteroventral pallidotomy in the treatment of Parkinson's disease. *Journal of Neurosurgery*, *76*(1), 53–61. https://doi.org/10.3171/jns.1992.76.1.0053

Land, B. B., Bruchas, M. R., Lemos, J. C., Xu, M., Melief, E. J., & Chavkin, C. (2008). The Dysphoric Component of Stress Is Encoded by Activation of the Dynorphin κ-Opioid System. *Journal of Neuroscience*, *28*(2).

Langston, J., Ballard, P., Tetrud, J., & Irwin, I. (1983). Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. *Science*, *219*(4587), 979–980. https://doi.org/10.1126/science.6823561

Lanza, K., Meadows, S. M., Chambers, N. E., Nuss, E., Deak, M. M., Ferré, S., & Bishop, C. (2018). Behavioral and cellular dopamine D1 and D3 receptor-mediated synergy: Implications for L-DOPA-induced dyskinesia. *Neuropharmacology*, *138*, 304–314. https://doi.org/10.1016/J.NEUROPHARM.2018.06.024

Laurence Lalanne, Gulebru Ayranci, Brigitte, L. K., & Lutz, P. E. (2014). The kappa opioid receptor: From addiction to depression, and back. In *Frontiers in Psychiatry* (Vol. 5, Issue DEC). Frontiers Media S.A. https://doi.org/10.3389/fpsyt.2014.00170

Lee, C. S., Cenci, M. A., Schulzer, M., & Björklund, A. (2000). Embryonic ventral mesencephalic grafts improve levodopa-induced dyskinesia in a rat model of Parkinson's disease. *Brain : A Journal of Neurology*, *123 (Pt 7*, 1365–1379. http://www.ncbi.nlm.nih.gov/pubmed/10869049

Lees, A. J., Hardy, J., & Revesz, T. (2009). Parkinson's disease. *Lancet (London, England)*, *373*(9680), 2055–2066. https://doi.org/10.1016/S0140-6736(09)60492-X [doi]

Levy, R., Ashby, P., Hutchison, W. D., Lang, A. E., Lozano, A. M., & Dostrovsky, J. O. (2002). Dependence of subthalamic nucleus oscillations on movement and dopamine in Parkinson's disease. *Brain*, *125*(6), 1196–1209. https://doi.org/10.1093/brain/awf128

Lewy, F. H. (1914). Zur pathologischen Anatomie der Paralysis agitans. *Dtsch Ztschr Nervenheilkunde*, *50*, 50–55.

Li, Y., Lefever, M. R., Muthu, D., Bidlack, J. M., Bilsky, E. J., & Polt, R. (2012). Opioid glycopeptide analgesics derived from endogenous enkephalins and endorphins. *Future Medicinal Chemistry*, *4*(2), 205–226. https://doi.org/10.4155/fmc.11.195

Limousin, P., Pollak, P., Benazzouz, A., Hoffmann, D., Le Bas, J.-F., Perret, J. E., Benabid, A.-L., & Broussolle, E. (1995). Effect on parkinsonian signs and symptoms of bilateral subthalamic nucleus stimulation. *The Lancet*, *345*(8942), 91–95. https://doi.org/10.1016/S0140-6736(95)90062-4

Limousin, Patricia, & Martinez-Torres, I. (2008). Deep Brain Stimulation for Parkinson's Disease. *Neurotherapeutics*, *5*(2), 309–319. https://doi.org/10.1016/j.nurt.2008.01.006

Lindskog, M., Svenningsson, P., Fredholm, B. B., Greengard, P., & Fisone, G. (1999). Activation of dopamine D2 receptors decreases DARPP-32 phosphorylation in striatonigral and striatopallidal projection neurons via different mechanisms. *Neuroscience*, *88*(4), 1005–1008. https://doi.org/10.1016/S0306-4522(98)00411-4

Ljungdahl, A., Hanrieder, J., Fälth, M., Bergquist, J., & Andersson, M. (2011). Imaging Mass Spectrometry Reveals Elevated Nigral Levels of Dynorphin Neuropeptides in L-DOPA-Induced

Dyskinesia in Rat Model of Parkinson's Disease. *PLoS ONE*, *6*(9), e25653. https://doi.org/10.1371/journal.pone.0025653

López-Otín, C., Blasco, M. A., Partridge, L., Serrano, M., & Kroemer, G. (2013). The hallmarks of aging. In *Cell* (Vol. 153, Issue 6, p. 1194). Cell Press. https://doi.org/10.1016/j.cell.2013.05.039

Lotharius, J., & Brundin, P. (2002). Pathogenesis of parkinson's disease: Dopamine, vesicles and α-synuclein. *Nature Reviews Neuroscience*, *3*(12), 932–942. https://doi.org/10.1038/nrn983

Lowery, J. J., Raymond, T. J., Giuvelis, D., Bidlack, J. M., Polt, R., & Bilsky, E. J. (2011). In vivo characterization of MMP-2200, a mixed δ/μ opioid agonist, in mice. *The Journal of Pharmacology and Experimental Therapeutics*, *336*(3), 767–778. https://doi.org/10.1124/jpet.110.172866

Lundblad, M., Andersson, M., Winkler, C., Kirik, D., Wierup, N., Cenci Nilsson, M. A., & Cenci, M. A. (2002). Pharmacological validation of behavioural measures of akinesia and dyskinesia in a rat model of Parkinson's disease. *The European Journal of Neuroscience*, *15*(1), 120–132. https://doi.org/10.1046/j.0953-816x.2001.01843.x

Lundblad, M., Usiello, A., Carta, M., Håkansson, K., Fisone, G., & Cenci, M. A. (2005). Pharmacological validation of a mouse model of l-DOPA-induced dyskinesia. *Experimental Neurology*, *194*(1), 66–75. https://doi.org/10.1016/j.expneurol.2005.02.002

Luthman, J., Fredriksson, A., Sundström, E., Jonsson, G., & Archer, T. (1989). Selective lesion of central dopamine or noradrenaline neuron systems in the neonatal rat: motor behavior and monoamine alterations at adult stage. *Behavioural Brain Research*, *33*(3), 267–277. https://doi.org/10.1016/S0166-4328(89)80121-4

Mabrouk, O. S., Falk, T., Sherman, S. J., Kennedy, R. T., & Polt, R. (2012). CNS penetration of the opioid glycopeptide MMP-2200: a microdialysis study. *Neuroscience Letters*, *531*(2), 99–103. https://doi.org/10.1016/j.neulet.2012.10.029

Madabhushi, R., Pan, L., & Tsai, L. H. (2014). DNA damage and its links to neurodegeneration. In *Neuron* (Vol. 83, Issue 2, pp. 266–282). Cell Press. https://doi.org/10.1016/j.neuron.2014.06.034

Maneuf, Y. P., Mitchell, I. J., Crossman, A. R., & Brotchie, J. M. (1994). On the Role of Enkephalin Cotransmission in the GABAergic Striatal Efferents to the Globus Pallidus. *Experimental Neurology*, *125*(1), 65–71. https://doi.org/10.1006/exnr.1994.1007

Mann, V. M., Cooper, J. M., Krige, D., Daniel, S. E., Schapira, A. H. V, & Marsden, C. D. (1992). Brain, skeletal muscle and platelet homogenate mitochondrial function in Parkinson's disease. *Brain*, *115*(2), 333–342. https://doi.org/10.1093/brain/115.2.333

Manson, A. J., Katzenschlager, R., Hobart, J., & Lees, A. J. (2001). High dose naltrexone for dyskinesias induced by levodopa. *Journal of Neurology, Neurosurgery, and Psychiatry*, *70*(4),

554–556. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1737317&tool=pmcentrez&rendertype=abstract

Marley, P. D., Emson, P. C., & Rehfeld, J. F. (1982). Effect of 6-hydroxydopamine lesions of the medial forebrain bundle on the distribution of cholecystokinin in rat forebrain. *Brain Research*, *252*(2), 382–385. https://doi.org/10.1016/0006-8993(82)90407-3

Marras, C., Beck, J. C., Bower, J. H., Roberts, E., Ritz, B., Ross, G. W., Abbott, R. D., Savica, R., Van Den Eeden, S. K., Willis, A. W., & Tanner, C. (2018). Prevalence of Parkinson's disease across North America. *Npj Parkinson's Disease*, *4*(1), 1–7. https://doi.org/10.1038/s41531-018-0058-0

Marsden, C. D. (1983). Neuromelanin and Parkinson's disease. *J. Neural Transm. Suppl.*, *19*.

Martin, L. J. (2008). DNA damage and repair: Relevance to mechanisms of neurodegeneration. In *Journal of Neuropathology and Experimental Neurology* (Vol. 67, Issue 5, pp. 377–387). https://doi.org/10.1097/NEN.0b013e31816ff780

Martínez-Rumayor, A., Arrieta, O., Sotelo, J., & García, E. (2009). Female gender but not cigarette smoking delays the onset of Parkinson's disease. *Clinical Neurology and Neurosurgery*, *111*(9), 738–741. https://doi.org/10.1016/j.clineuro.2009.07.012

Massaly, N., Copits, B. A., Wilson-Poe, A. R., Al-Hasani, R., Bruchas, M. R., & Moró, J. A. (2019). Pain-Induced Negative Affect Is Mediated via Recruitment of The Nucleus Accumbens Kappa Opioid System. *Neuron*, *102*, 564-573.e6. https://doi.org/10.1016/j.neuron.2019.02.029

Matsuda, W., Furuta, T., Nakamura, K. C., Hioki, H., Fujiyama, F., Arai, R., & Kaneko, T. (2009). Single nigrostriatal dopaminergic neurons form widely spread and highly dense axonal arborizations in the neostriatum. *Journal of Neuroscience*, *29*(2), 444–453. https://doi.org/10.1523/JNEUROSCI.4029-08.2009

Mefford, I. N., Gilberg, M., & Barchas, J. D. (1980). Simultaneous determination of catecholamines and unconjugated 3,4-dihydroxyphenylacetic acid in brain tissue by ion-pairing reverse-phase high-performance liquid chromatography with electrochemical detection. *Analytical Biochemistry*, *104*(2), 469–472. https://doi.org/10.1016/0003-2697(80)90101-3

Millan, M. J., Maiofiss, L., Cussac, D., Audinot, V., Boutin, J.-A., Newman-Tancredi, A., Lejeune, F., Rivet, J., Brocco, M., Duqueyroix, D., Nicolas, J., Boutin, J., & Newman-Tancredi, A. (2002). Differential actions of antiparkinson agents at multiple classes of monoaminergic receptor. I. A multivariate analysis of the binding profiles of 14 drugs at 21 native and cloned human receptor subtypes. *The Journal of Pharmacology and Experimental Therapeutics*, *303*(2), 791–804. https://doi.org/10.1124/jpet.102.039867

Miller, I. N., & Cronin-Golomb, A. (2010). Gender differences in Parkinson's disease: Clinical characteristics and cognition. In *Movement Disorders* (Vol. 25, Issue 16, pp. 2695–2703). https://doi.org/10.1002/mds.23388

Moustafa, A. A., Sherman, S. J., & Frank, M. J. (2008). A dopaminergic basis for working memory, learning and attentional shifting in Parkinsonism. *Neuropsychologia*, *46*(13), 3144–3156. https://doi.org/10.1016/J.NEUROPSYCHOLOGIA.2008.07.011

Müller-Nedebock, A. C., Brennan, R. R., Venter, M., Pienaar, I. S., van der Westhuizen, F. H., Elson, J. L., Ross, O. A., & Bardien, S. (2019). The unresolved role of mitochondrial DNA in Parkinson's disease: An overview of published studies, their limitations, and future prospects. In *Neurochemistry International* (Vol. 129). Elsevier Ltd. https://doi.org/10.1016/j.neuint.2019.104495

Nambu, A. (2004). A new dynamic model of the cortico-basal ganglia loop. *Progress in Brain Research*, *143*, 461–466. https://doi.org/10.1016/S0079-6123(03)43043-4

Narendra, D. P. (2010). PINK1 is selectively stabilized on impaired mitochondria to activate Parkin. *PLoS Biol.*, *8*.

Navratilova, E., Ji, G., Phelps, C., Qu, C., Hein, M., Yakhnitsa, V., Neugebauer, V., & Porreca, F. (2019). Kappa opioid signaling in the central nucleus of the amygdala promotes disinhibition and aversiveness of chronic neuropathic pain. *Pain*, *160*(4), 824–832. https://doi.org/10.1097/j.pain.0000000000001458

Newman, D. D., Rajakumar, N., Flumerfelt, B. A., & Stoessl, A. J. (1997). A kappa opioid antagonist blocks sensitization in a rodent model of Parkinson's disease. *NeuroReport*, *8*(3), 669–672. https://doi.org/10.1097/00001756-199702100-00018

Nieuwenhuys, R., Geeraedts, L. M. G., & Veening, J. G. (1982). The medial forebrain bundle of the rat. I. General introduction. *Journal of Comparative Neurology*, *206*(1), 49–81. https://doi.org/10.1002/cne.902060106

Nisbet, A. P., Foster, O. J., Kingsbury, A., Eve, D. J., Daniel, S. E., Marsden, C. D., & Lees, A. J. (1995). Preproenkephalin and preprotachykinin messenger RNA expression in normal human basal ganglia and in Parkinson's disease. *Neuroscience*, *66*(2), 361–376. http://www.ncbi.nlm.nih.gov/pubmed/7477878

Noble, F., & Cox, B. M. (2002). Differential Regulation of D1 Dopamine Receptor- and of A2a Adenosine Receptor-Stimulated Adenylyl Cyclase by μ-, δ1-, and δ2-Opioid Agonists in Rat Caudate Putamen. *Journal of Neurochemistry*, *65*(1), 125–133. https://doi.org/10.1046/j.1471-4159.1995.65010125.x

Nussbaum, R. L., & Ellis, C. E. (2003). Alzheimer's Disease and Parkinson's Disease. *New England Journal of Medicine*, *348*(14), 1356–1364. https://doi.org/10.1056/NEJM2003ra020003

Nutt, J. G., Obeso, J. A., & Stocchi, F. (2000). Continuous dopamine-receptor stimulation in advanced Parkinson's disease. In *Trends in Neurosciences* (Vol. 23, Issue 10 SUPPL., pp. S109–S115). Elsevier Ltd. https://doi.org/10.1016/S1471-1931(00)00029-X

Obeso, J A, Rodriguez-Oroz, M. C., Rodriguez, M., DeLong, M. R., & Olanow, C. W. (2000). Pathophysiology of levodopa-induced dyskinesias in Parkinson's disease: problems with the current model. *Annals of Neurology*, *47*(4 Suppl 1), S22-32; discussion S32-4. http://www.ncbi.nlm.nih.gov/pubmed/10762129

Obeso, José A., Olanow, C. W., & Nutt, J. G. (2000). Levodopa motor complications in Parkinson's disease. *Trends in Neurosciences*, *23*, S2–S7. https://doi.org/10.1016/S1471-1931(00)00031-8

Obeso, Jose A., Rodríguez-Oroz, M. C., Benitez-Temino, B., Blesa, F. J., Guridi, J., Marin, C., & Rodriguez, M. (2008). Functional organization of the basal ganglia: Therapeutic implications for Parkinson's disease. *Movement Disorders*, *23*(SUPPL. 3). https://doi.org/10.1002/mds.22062

Obeso, José A., Rodriguez-Oroz, M. C., Rodriguez, M., Lanciego, J. L., Artieda, J., Gonzalo, N., & Olanow, C. W. (2000). Pathophysiology of the basal ganglia in Parkinson's disease. *Trends in Neurosciences*, *23*, S8–S19. https://doi.org/10.1016/S1471-1931(00)00028-8

Olanow, C W. (2009). A double-blind, delayed-start trial of rasagiline in Parkinson's disease. *N. Engl. J. Med.*, *361*.

Olanow, C Warren, Obeso, J. A., & Stocchi, F. (2006). Continuous dopamine-receptor treatment of Parkinson's disease: scientific rationale and clinical implications. *The Lancet Neurology*, *5*(8), 677–687. https://doi.org/10.1016/S1474-4422(06)70521-X

Olanow, C Warren, Stern, M. B., & Sethi, K. (2009). The scientific and clinical basis for the treatment of Parkinson disease (2009). *Neurology*, *72*(21 Suppl 4), S1-136. https://doi.org/10.1212/WNL.0b013e3181a1d44c

Paci, C., Thomas, A., & Onofrj, M. (2001). Amantadine for dyskinesia in patients affected by severe Parkinson's disease. *Neurological Sciences*, *22*(1), 75–76. https://doi.org/10.1007/s100720170054

Pahwa, R., Factor, S. A., Lyons, K. E., Ondo, W. G., Gronseth, G., Bronte-Stewart, H., Hallett, M., Miyasaki, J., Stevens, J., Weiner, W. J., & Quality Standards Subcommittee of the American Academy of Neurology. (2006). Practice Parameter: Treatment of Parkinson disease with motor fluctuations and dyskinesia (an evidence-based review): [RETIRED]. *Neurology*, *66*(7), 983–995. https://doi.org/10.1212/01.wnl.0000215250.82576.87

Papa, S. M., & Chase, T. N. (1996). Levodopa-induced dyskinesias improved by a glutamate antagonist in Parkinsonian monkeys. *Annals of Neurology*, *39*(5), 574–578. https://doi.org/10.1002/ana.410390505

Papavasiliou, P. S., Cotzias, G. C., Düby, S. E., Steck, A. J., Fehling, C., & Bell, M. A. (1972). Levodopa in Parkinsonism: Potentiation of Central Effects with a Peripheral Inhibitor. *New England Journal of Medicine*, *286*(1), 8–14. https://doi.org/10.1056/NEJM197201062860102

Paquette, M. A., Anderson, A. M., Lewis, J. R., Meshul, C. K., Johnson, S. W., & Paul Berger, S. (2010). MK-801 inhibits l-DOPA-induced abnormal involuntary movements only at doses that worsen parkinsonism. *Neuropharmacology*, *58*(7), 1002–1008. https://doi.org/10.1016/j.neuropharm.2010.01.003

Paquette, M. A., Martinez, A. A., Macheda, T., Meshul, C. K., Johnson, S. W., Berger, S. P., & Giuffrida, A. (2012). Anti-dyskinetic mechanisms of amantadine and dextromethorphan in the 6-OHDA rat model of Parkinson's disease: Role of NMDA vs. 5-HT1A receptors. *European Journal of Neuroscience*, *36*(9), 3224–3234. https://doi.org/10.1111/j.1460-9568.2012.08243.x

Park, J. (2006). Mitochondrial dysfunction in Drosophila PINK1 mutants is complemented by parkin. *Nature*, *441*.

Parkinson, J. (1817). *An Essay on the Shaking Palsy*. Wittingham and Rowland.

Paxinos, G., & Watson, C. (2007). *The Rat Brain in Stereotaxic Coordinates* (6th ed.). Academic Press.

Paxinos, G., & Watson, C. (2017). *The Rat Brain in Stereotaxic Coordinates* (7th ed.). Academic Press.

Pellerin, L., & Magistretti, P. J. (1994). Glutamate uptake into astrocytes stimulates aerobic glycolysis: A mechanism coupling neuronal activity to glucose utilization. *Proceedings of the National Academy of Sciences of the United States of America*, *91*(22), 10625–10629. https://doi.org/10.1073/pnas.91.22.10625

Piccini, P., Weeks, R. A., & Brooks, D. J. (1997). Alterations in opioid receptor binding in Parkinson's disease patients with levodopa-induced dyskinesias. *Annals of Neurology*, *42*(5), 720–726. https://doi.org/10.1002/ana.410420508

Pissadaki, E., & Bolam, J. P. (2013). The energy cost of action potential propagation in dopamine neurons: clues to susceptibility in Parkinson's disease . In *Frontiers in Computational Neuroscience* (Vol. 7, p. 13). https://www.frontiersin.org/article/10.3389/fncom.2013.00013

Plenz, D., & Kital, S. T. (1999). A basal ganglia pacemaker formed by the subthalamic nucleus and external globus pallidus. *Nature*, *400*(6745), 677–682. https://doi.org/10.1038/23281

Poirier, L. J., & Sourkes, T. L. (1965). Influence of the substantia nigra on the catecholamine content of the striatum. *Brain*, *88*(1), 181–192. https://doi.org/10.1093/brain/88.1.181

Pollack, A. E., & Haisley, E. C. (2001). (No Title). In *Brain Research* (Vol. 897). www.elsevier.com/locate/bres

Pollack, P., Benabid, A. L., Gross, C., Gao, D. M., Laurent, A., Benazzoiz, A., Hoffmann, D., Gentil, M., & Perret, J. (1993). Effects of the stimulation of the subthalamic nucleus in Parkinson disease. *Rev Neurol (Paris)*, *149*(3), 175–176.

Portoghese, P. S., Lipkowski, A. W., & Takemori, A. E. (1987). Binaltorphimine and nor-binaltorphimine, potent and selective k-opioid receptor antagonists. *Life Sciences*, *40*(13), 1287–1292. https://doi.org/10.1016/0024-3205(87)90585-6

Potts, L. F., Park, E. S., Woo, J. M., Dyavar Shetty, B. L., Singh, A., Braithwaite, S. P., Voronkov, M., Papa, S. M., & Mouradian, M. M. (2015). Dual κ-agonist/μ-antagonist opioid receptor modulation reduces levodopa-induced dyskinesia and corrects dysregulated striatal changes in the nonhuman primate model of Parkinson disease. *Annals of Neurology*, *77*(6), 930–941. https://doi.org/10.1002/ana.24375

Prager, E. M., & Plotkin, J. L. (2019). Compartmental function and modulation of the striatum. In *Journal of Neuroscience Research* (Vol. 97, Issue 12, pp. 1503–1514). John Wiley and Sons Inc. https://doi.org/10.1002/jnr.24522

Pringsheim, T., Jette, N., Frolkis, A., & Steeves, T. D. L. (2014). The prevalence of Parkinson's disease: A systematic review and meta-analysis. In *Movement Disorders* (Vol. 29, Issue 13, pp. 1583–1590). John Wiley and Sons Inc. https://doi.org/10.1002/mds.25945

Przedbroski, S., Leviver, M., Jiang, H., Ferreira, M., Jackson-Lewis, V., Donaldson, D., & Togasaki, D. M. (1995). Dose-dependent lesions of the dopaminergic nigrostriatal pathway induced by instrastriatal injection of 6-hydroxydopamine. *Neuroscience*, *67*(3), 631–647. https://doi.org/10.1016/0306-4522(95)00066-R

Puopolo, M., Raviola, E., & Bean, B. P. (2007). Roles of subthreshold calcium current and sodium current in spontaneous firing of mouse midbrain dopamine neurons. *Journal of Neuroscience*, *27*(3), 645–656. https://doi.org/10.1523/JNEUROSCI.4341-06.2007

Rascol, O, Fabre, N., Blin, O., Poulik, J., Sabatini, U., Senard, J. M., Ané, M., Montastruc, J. L., & Rascol, A. (1994). Naltrexone, an opiate antagonist, fails to modify motor symptoms in patients with Parkinson's disease. *Movement Disorders : Official Journal of the Movement Disorder Society*, *9*(4), 437–440. https://doi.org/10.1002/mds.870090410

Rascol, Olivier, Brooks, D. J., Korczyn, A. D., De Deyn, P. P., Clarke, C. E., & Lang, A. E. (2000). A Five-Year Study of the Incidence of Dyskinesia in Patients with Early Parkinson's Disease Who Were Treated with Ropinirole or Levodopa. *New England Journal of Medicine*, *342*(20), 1484–1491. https://doi.org/10.1056/NEJM200005183422004

Redila, V. A., & Chavkin, C. (2008). Stress-induced reinstatement of cocaine seeking is mediated by the kappa opioid system. *Psychopharmacology*, *200*(1), 59–70. https://doi.org/10.1007/s00213-008-1122-y

Robelet, S., Melon, C., Guillet, B., Salin, P., & Kerkerian-Le Goff, L. (2004). Chronic L-DOPA treatment increases extracellular glutamate levels and GLT1 expression in the basal ganglia in a

rat model of Parkinson's disease. *European Journal of Neuroscience*, *20*(5), 1255–1266. https://doi.org/10.1111/j.1460-9568.2004.03591.x

Roberts, S. B., & Rosenberg, I. (2006). Nutrition and aging: Changes in the regulation of energy metabolism with aging. In *Physiological Reviews* (Vol. 86, Issue 2, pp. 651–667). Physiol Rev. https://doi.org/10.1152/physrev.00019.2005

Rosin, B., Slovik, M., Mitelman, R., Rivlin-Etzion, M., Haber, S. N., Israel, Z., Vaadia, E., & Bergman, H. (2011). Closed-loop deep brain stimulation is superior in ameliorating parkinsonism. *Neuron*, *72*(2), 370–384. https://doi.org/10.1016/j.neuron.2011.08.023

Rupniak, N. M. J., Boyce, S., Steventon, M. J., Iversen, S. D., & Marsden, C. D. (1992). Dystonia induced by combined treatment with L-dopa and MK-801 in parkinsonian monkeys. *Annals of Neurology*, *32*(1), 103–105. https://doi.org/10.1002/ana.410320118

Samadi, P. (2003). Opioid antagonists increase the dyskinetic response to dopaminergic agents in parkinsonian monkeys: interaction between dopamine and opioid systems. *Neuropharmacology*, *45*(7), 954–963. https://doi.org/10.1016/S0028-3908(03)00249-1

Sauer, H., & Oertel, W. H. (1994). Progressive degeneration of nigrostriatal dopamine neurons following intrastriatal terminal lesions with 6-hydroxydopamine: A combined retrograde tracing and immunocytochemical study in the rat. *Neuroscience*, *59*(2), 401–415. https://doi.org/10.1016/0306-4522(94)90605-X

Schallert, T. (2006). Behavioral tests for preclinical intervention assessment. *NeuroRx : The Journal of the American Society for Experimental NeuroTherapeutics*, *3*(4), 497–504. https://doi.org/10.1016/j.nurx.2006.08.001

Schapira, Anthony H., & Jenner, P. (2011). Etiology and pathogenesis of Parkinson's disease. In *Movement Disorders* (Vol. 26, Issue 6, pp. 1049–1055). Mov Disord. https://doi.org/10.1002/mds.23732

Schapira, A. H. V., & Obeso, J. (2006). Timing of treatment initiation in Parkinson's disease: A need for reappraisal? In *Annals of Neurology* (Vol. 59, Issue 3, pp. 559–562). https://doi.org/10.1002/ana.20789

Schapira, A H. (1990). Anatomic and disease specificity of NADH CoQ1 reductase (complex I) deficiency in Parkinson's disease. *J. Neurochem.*, *55*.

Schapira, A. H. V., Cooper, J. M., Dexter, D., Clark, J. B., Jenner, P., & Marsden, C. D. (1990). Mitochondrial Complex I Deficiency in Parkinson's Disease. *Journal of Neurochemistry*, *54*(3), 823–827. https://doi.org/10.1111/j.1471-4159.1990.tb02325.x

Scheller, D., Ullmer, C., Berkels, R., Gwarek, M., & Lübbert, H. (2009). The in vitro receptor profile of rotigotine: a new agent for the treatment of Parkinson's disease. *Naunyn-Schmiedeberg's Archives of Pharmacology*, *379*(1), 73–86. https://doi.org/10.1007/s00210-008-0341-4

Schon, E A, & Przedborski, S. (2011). Mitochondria: the next (neurode)generation. *Neuron*, *70*.

Schumacher, B., Garinis, G. A., & Hoeijmakers, J. H. J. (2008). Age to survive: DNA damage and aging. In *Trends in Genetics* (Vol. 24, Issue 2, pp. 77–85). https://doi.org/10.1016/j.tig.2007.11.004

Schwalb, J. M., & Hamani, C. (2008). The History and Future of Deep Brain Stimulation. *Neurotherapeutics*, *5*(1), 3–13. https://doi.org/10.1016/j.nurt.2007.11.003

Seizinger, B. R., Grimm, C., Höllt, V., & Herz, A. (1984). Evidence for a selective processing of proenkephalin B into different opioid peptide forms in particular regions of rat brain and pituitary. *Journal of Neurochemistry*, *42*(2), 447–457. http://www.ncbi.nlm.nih.gov/pubmed/6141221

Sgroi, S., Capper-Loup, C., Paganetti, P., & Kaelin-Lang, A. (2016). Enkephalin and dynorphin neuropeptides are differently correlated with locomotor hypersensitivity and levodopa-induced dyskinesia in parkinsonian rats. *Experimental Neurology*, *280*, 80–88. https://doi.org/10.1016/j.expneurol.2016.03.024

Sgroi, S., & Tonini, R. (2018). Opioidergic Modulation of Striatal Circuits, Implications in Parkinson's Disease and Levodopa Induced Dyskinesia. *Frontiers in Neurology*, *9*, 524. https://doi.org/10.3389/fneur.2018.00524

Sharott, A., Gulberti, A., Hamel, W., Köppen, J. A., Münchau, A., Buhmann, C., Pötter-Nerger, M., Westphal, M., Gerloff, C., Moll, C. K. E., & Engel, A. K. (2018). Spatio-temporal dynamics of cortical drive to human subthalamic nucleus neurons in Parkinson's disease. *Neurobiology of Disease*, *112*, 49–62. https://doi.org/10.1016/j.nbd.2018.01.001

Shen, W., Flajolet, M., Greengard, P., & Surmeier, D. J. (2008). Dichotomous dopaminergic control of striatal synaptic plasticity. *Science*, *321*(5890), 848–851. https://doi.org/10.1126/science.1160575

Shimohama, S., Sawada, H., Kitamura, Y., & Taniguchi, T. (2003). Disease model: Parkinson's disease. In *Trends in Molecular Medicine* (Vol. 9, Issue 8, pp. 360–365). Elsevier Ltd. https://doi.org/10.1016/S1471-4914(03)00117-5

Shippenberg, T. S., Chefer, V. I., Zapata, A., & Heidbreder, C. A. (2006). Modulation of the Behavioral and Neurochemical Effects of Psychostimulants by κ-Opioid Receptor Systems. *Annals of the New York Academy of Sciences*, *937*(1), 50–73. https://doi.org/10.1111/j.1749-6632.2001.tb03558.x

Sian, J., Dexter, D. T., Lees, A. J., Daniel, S., Agid, Y., Javoy-Agid, F., Jenner, P., & Marsden, C. D. (1994). Alterations in glutathione levels in Parkinson's disease and other neurodegenerative

disorders affecting basal ganglia. *Annals of Neurology*, *36*(3), 348–355. https://doi.org/10.1002/ana.410360305

Siegfried, J., & Lippitz, B. (1994). Bilateral chronic electrostimulation of ventroposterolateral pallidum: A new therapeutic approach for alleviating all parkinsonian symptoms. *Neurosurgery*, *35*(6), 1126–1130. https://doi.org/10.1227/00006123-199412000-00016

Smith, Y., Bevan, M. D., Shink, E., & Bolam, J. P. (1998). Microcircuitry of the direct and indirect pathways of the basal ganglia. In *Neuroscience* (Vol. 86, Issue 2, pp. 353–387). Elsevier Ltd. https://doi.org/10.1016/S0306-4522(98)00004-9

Smith, Y., Wichmann, T., Factor, S. A., & DeLong, M. R. (2012). Parkinson's disease therapeutics: new developments and challenges since the introduction of levodopa. *Neuropsychopharmacology*, *37*.

Solís, O., Garcia-Montes, J. R., González-Granillo, A., Xu, M., & Moratalla, R. (2017). Dopamine D3 Receptor Modulates l-DOPA-Induced Dyskinesia by Targeting D1 Receptor-Mediated Striatal Signaling. *Cerebral Cortex*, *27*(1), bhv231. https://doi.org/10.1093/cercor/bhv231

Sonsalla, P. K., & Heikkila, R. E. (1986). The influence of dose and dosing interval on MPTP-induced dopaminergic neurotoxicity in mice. *European Journal of Pharmacology*, *129*(3), 339–345. https://doi.org/10.1016/0014-2999(86)90444-9

Spanagel, R., Herz, A., & Shippenberg, T. S. (1992). Opposing tonically active endogenous opioid systems modulate the mesolimbic dopaminergic pathway. *Proceedings of the National Academy of Sciences of the United States of America*, *89*(6), 2046–2050. https://doi.org/10.1073/pnas.89.6.2046

Spanagel, Rainer, Almeida, O. F. X., Bartl, C., & Shippenberg, T. S. (1994). Endogenous κ-opioid systems in opiate withdrawal: role in aversion and accompanying changes in mesolimbic dopamine release. *Psychopharmacology*, *115*(1–2), 121–127. https://doi.org/10.1007/BF02244761

Spillantini, M. G., Schmidt, M. L., Lee, V. M. Y., Trojanowski, J. Q., Jakes, R., & Goedert, M. (1997). α-synuclein in Lewy bodies [8]. In *Nature* (Vol. 388, Issue 6645, pp. 839–840). Nature Publishing Group. https://doi.org/10.1038/42166

Sulzer, D. (2007). Multiple hit hypotheses for dopamine neuron loss in Parkinson's disease. In *Trends in Neurosciences* (Vol. 30, Issue 5, pp. 244–250). https://doi.org/10.1016/j.tins.2007.03.009

Surmeier, D. J., Guzman, J. N., Sanchez-Padilla, J., & Schumacker, P. T. (2011). The role of calcium and mitochondrial oxidant stress in the loss of substantia nigra pars compacta dopaminergic neurons in Parkinson's disease. In *Neuroscience* (Vol. 198, pp. 221–231). https://doi.org/10.1016/j.neuroscience.2011.08.045

Swerdlow, R. H. (2011). Brain aging, Alzheimer's disease, and mitochondria. In *Biochimica et Biophysica Acta - Molecular Basis of Disease* (Vol. 1812, Issue 12, pp. 1630–1639). Biochim Biophys Acta. https://doi.org/10.1016/j.bbadis.2011.08.012

Tamim, M. K., Samadi, P., Morissette, M., Grégoire, L., Ouattara, B., Lévesque, D., Rouillard, C., & Di Paolo, T. (2010). Effect of non-dopaminergic drug treatment on Levodopa induced dyskinesias in MPTP monkeys: Common implication of striatal neuropeptides. *Neuropharmacology*, *58*(1), 286–296. https://doi.org/10.1016/j.neuropharm.2009.06.030

Tanner, C. M., & Aston, D. A. (2000). Epidemiology of Parkinson's disease and akinetic syndromes [In Process Citation]. *Current Opinion in Neurology*, *13*(4), 427–430. https://journals.lww.com/co-neurology/Abstract/2000/08000/Epidemiology_of_Parkinson_s_disease_and_akinetic.10.aspx

Tanner, C. M., & Ben-Shlomo, Y. (1999). Epidemiology of Parkinson's disease. *Advances in Neurology*, *80*, 153–159. http://www.ncbi.nlm.nih.gov/pubmed/10410715

Taylor, J. L., Bishop, C., Ullrich, T., Rice, K. C., & Walker, P. D. (2006). Serotonin 2A receptor antagonist treatment reduces dopamine D1 receptor-mediated rotational behavior but not l-DOPA-induced abnormal involuntary movements in the unilateral dopamine-depleted rat. *Neuropharmacology*, *50*(6), 761–768. https://doi.org/10.1016/j.neuropharm.2005.12.004

Tecuapetla, F., Jin, X., Lima, S. Q., & Costa, R. M. (2016). Complementary Contributions of Striatal Projection Pathways to Action Initiation and Execution. *Cell*, *166*(3), 703–715. https://doi.org/10.1016/j.cell.2016.06.032

Tejeda, H. A., & Bonci, A. (2019). Dynorphin/kappa-opioid receptor control of dopamine dynamics: Implications for negative affective states and psychiatric disorders. In *Brain Research* (Vol. 1713, pp. 91–101). Elsevier B.V. https://doi.org/10.1016/j.brainres.2018.09.023

Tel, B. C., Zeng, B. Y., Cannizzaro, C., Pearce, R. K. B., Rose, S., & Jenner, P. (2002). Alterations in striatal neuropeptide mRNA produced by repeated administration of L-DOPA, ropinirole or bromocriptine correlate with dyskinesia induction in MPTP-treated common marmosets. *Neuroscience*, *115*(4), 1047–1058. https://doi.org/10.1016/S0306-4522(02)00535-3

Thiruchelvam, M, Brockel, B. J., Richfield, E. K., Baggs, R. B., & Cory-Slechta, D. A. (2000). Potentiated and preferential effects of combined paraquat and maneb on nigrostriatal dopamine systems: environmental risk factors for Parkinson's disease? *Brain Res.*, *873*.

Thiruchelvam, Mona, McCormack, A., Richfield, E. K., Baggs, R. B., Tank, A. W., Di Monte, D. A., & Cory-Slechta, D. A. (2003). Age-related irreversible progressive nigrostriatal dopaminergic neurotoxicity in the paraquat and maneb model of the Parkinson's disease phenotype. *European Journal of Neuroscience*, *18*(3), 589–600. https://doi.org/10.1046/j.1460-9568.2003.02781.x

Thomas, A., Iacono, D., Luciano, A. L., Armellino, K., Di Iorio, A., & Onofrj, M. (2004). Duration of amantadine benefit on dyskinesia of severe Parkinson's disease. *Journal of Neurology, Neurosurgery and Psychiatry*, *75*(1), 141–143. www.jnnp.com

Trietiakoff, C. (1919). *Contribution a L'étude de L'anatomie Pathologique de Locus Niger de Soemmerling*. Universite de Paris.

Ungerstedt, U, & Arbuthnott, G. (1970). Quantitative recording of rotational behaviour in rats after 6-hydroxydopamine lesions of the nigrostriatal dopamine system. *Brain Res.*, *24*.

Ungerstedt, Urban. (1968). 6-hydroxy-dopamine induced degeneration of central monoamine neurons. *European Journal of Pharmacology*, *5*(1), 107–110. https://doi.org/10.1016/0014-2999(68)90164-7

Ungerstedt, Urban. (1976). 6-Hydroxydopamine-induced degeneration of the nigrostriatal dopamine pathway: The turning syndrome. *Pharmacology And Therapeutics: Part B: General And Systematic*, *2*(1), 37–40. https://doi.org/10.1016/0306-039X(76)90016-7

Valente, E. M., Abou-Sleiman, P. M., Caputo, V., Muqit, M. M. K., Harvey, K., Gispert, S., Ali, Z., Del Turco, D., Bentivoglio, A. R., Healy, D. G., Albanese, A., Nussbaum, R., González-Maldonado, R., Deller, T., Salvi, S., Cortelli, P., Gilks, W. P., Latchman, D. S., Harvey, R. J., … Wood, N. W. (2004). Hereditary early-onset Parkinson's disease caused by mutations in PINK1. *Science*, *304*(5674), 1158–1160. https://doi.org/10.1126/science.1096284

Valko, M., Leibfritz, D., Moncol, J., Cronin, M. T. D., Mazur, M., & Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. In *International Journal of Biochemistry and Cell Biology* (Vol. 39, Issue 1, pp. 44–84). Int J Biochem Cell Biol. https://doi.org/10.1016/j.biocel.2006.07.001

Van De Witte, S. V., Groenewegen, H. J., & Voorn, P. (2002). MK-801 alters the effects of priming with L-DOPA on dopamine D1 receptor-induced changes in neuropeptide mRNA levels in the rat striatal output neurons. *Synapse*, *43*(1), 1–11. https://doi.org/10.1002/syn.1113

Van Laar, V. S., & Berman, S. B. (2009). Mitochondrial dynamics in Parkinson's disease. In *Experimental Neurology*. https://doi.org/10.1016/j.expneurol.2009.03.019

Venero, J. L., Revuelta, M., Cano, J., & Machado, A. (2002). Time Course Changes in the Dopaminergic Nigrostriatal System Following Transection of the Medial Forebrain Bundle: Detection of Oxidatively Modified Proteins in Substantia Nigra. *Journal of Neurochemistry*, *68*(6), 2458–2468. https://doi.org/10.1046/j.1471-4159.1997.68062458.x

Vijg, J., & Campisi, J. (2008). Puzzles, promises and a cure for ageing. In *Nature* (Vol. 454, Issue 7208, pp. 1065–1071). Nature Publishing Group. https://doi.org/10.1038/nature07216

Vives-Bauza, C. (2010). PINK1-dependent recruitment of Parkin to mitochondria in mitophagy. *Proc. Natl Acad. Sci. USA*, *107*.

Voorn, P., Vanderschuren, L. J. M. J., Groenewegen, H. J., Robbins, T. W., & Pennartz, C. M. A. (2004). Putting a spin on the dorsal-ventral divide of the striatum. In *Trends in Neurosciences* (Vol. 27, Issue 8, pp. 468–474). Elsevier Current Trends. https://doi.org/10.1016/j.tins.2004.06.006

Vreeland, R. F., Atcherley, C. W., Russell, W. S., Xie, J. Y., Lu, D., Laude, N. D., Porreca, F., & Heien, M. L. (2015). Biocompatible PEDOT:Nafion composite electrode coatings for selective detection of neurotransmitters in vivo. *Analytical Chemistry*, *87*(5), 2600–2607. https://doi.org/10.1021/ac502165f

Warren Olanow, C., Kieburtz, K., Rascol, O., Poewe, W., Schapira, A. H., Emre, M., Nissinen, H., Leinonen, M., & Stocchi, F. (2013). Factors predictive of the development of Levodopa-induced dyskinesia and wearing-off in Parkinson's disease. *Movement Disorders*, *28*(8), 1064–1071. https://doi.org/10.1002/mds.25364

Wellstead, P., & Cloutier, M. (2011). An energy systems approach to Parkinson's disease. *Wiley Interdisciplinary Reviews: Systems Biology and Medicine*, *3*(1), 1–6. https://doi.org/10.1002/wsbm.107

Westin, J. E., Andersson, M., Lundblad, M., & Cenci, M. A. (2001). Persistent changes in striatal gene expression induced by long-term l -DOPA treatment in a rat model of Parkinson's disease. *European Journal of Neuroscience*, *14*(7), 1171–1176. https://doi.org/10.1046/j.0953-816x.2001.01743.x

William Langston, J., Forno, L. S., Rebert, C. S., & Irwin, I. (1984). Selective nigral toxicity after systemic administration of 1-methyl-4-phenyl-1,2,5,6-tetrahydropyrine (MPTP) in the squirrel monkey. *Brain Research*, *292*(2), 390–394. https://doi.org/10.1016/0006-8993(84)90777-7

Williams, N. R., Heifets, B. D., Blasey, C., Sudheimer, K., Pannu, J., Pankow, H., Hawkins, J., Birnbaum, J., Lyons, D. M., Rodriguez, C. I., & Schatzberg, A. F. (2018). Attenuation of antidepressant effects of ketamine by opioid receptor antagonism. *American Journal of Psychiatry*, *175*(12), 1205–1215. https://doi.org/10.1176/appi.ajp.2018.18020138

Willis, M. W., Ketter, T. A., Kimbrell, T. A., George, M. S., Herscovitch, P., Danielson, A. L., Benson, B. E., & Post, R. M. (2002). Age, sex and laterality effects on cerebral glucose metabolism in healthy adults. *Psychiatry Research - Neuroimaging*, *114*(1), 23–37. https://doi.org/10.1016/S0925-4927(01)00126-3

Winkler, C., Kirik, D., Björklund, A., & Cenci, M. A. (2002). L-DOPA-induced dyskinesia in the intrastriatal 6-hydroxydopamine model of parkinson's disease: relation to motor and cellular parameters of nigrostriatal function. *Neurobiology of Disease*, *10*(2), 165–186. http://www.ncbi.nlm.nih.gov/pubmed/12127155

Wright Willis, A., Evanoff, B. A., Lian, M., Criswell, S. R., & Racette, B. A. (2010). Geographic and ethnic variation in Parkinson disease: A population-based study of us medicare beneficiaries. *Neuroepidemiology*, *34*(3), 143–151. https://doi.org/10.1159/000275491

Xuei, X., Dick, D., Flury-Wetherill, L., Tian, H.-J., Agrawal, A., Bierut, L., Goate, A., Bucholz, K., Schuckit, M., Nurnberger, J., Tischfield, J., Kuperman, S., Porjesz, B., Begleiter, H., Foroud, T., & Edenberg, H. J. (2006). Association of the κ-opioid system with alcohol dependence. *Molecular Psychiatry*, *11*(11), 1016–1024. https://doi.org/10.1038/sj.mp.4001882

Yahr, M. D., Duvoisin, R. C., Schear, M. J., Barrett, R. E., & Hoehn, M. M. (1969). Treatment of Parkinsonism With Levodopa. *Archives of Neurology*, *21*(4), 343–354. https://doi.org/10.1001/archneur.1969.00480160015001

Yeomans, J. S., Maidment, N. T., & Bunney, B. S. (1988). Excitability properties of medial forebrain bundle axons of A9 and A10 dopamine cells. *Brain Research*, *450*(1–2), 86–93. https://doi.org/10.1016/0006-8993(88)91547-8

Yue, X., Hariri, D. J., Caballero, B., Zhang, S., Bartlett, M. J., Kaut, O., Mount, D. W., Wüllner, U., Sherman, S. J., & Falk, T. (2014). Comparative study of the neurotrophic effects elicited by VEGF-B and GDNF in preclinical in vivo models of Parkinson's disease. *Neuroscience*, *258*, 385–400. https://doi.org/10.1016/j.neuroscience.2013.11.038

Yue, Xu, Falk, T., Zuniga, L. A., Szabò, L., Porreca, F., Polt, R., & Sherman, S. J. (2011). Effects of the novel glycopeptide opioid agonist MMP-2200 in preclinical models of Parkinson's disease. *Brain Research*, *1413*, 72–83. https://doi.org/10.1016/j.brainres.2011.07.038