From the Department of Neuroscience,
Karolinska Institutet, Stockholm, Sweden

L-DOPA-induced Signaling Pathways and Neuroepigenetic Mechanisms in Experimental Parkinsonism and Dyskinesia

MICHAEL FEYDER

Karolinska Institutet

Stockholm 2014

Published by Karolinska Institutet.
Printed by Åtta.45 AB

ISBN 978-91-7549-736-5

Karolinska Institutet

Institutionen för Neurovetenskap

L-DOPA-induced Signaling Pathways and Neuroepigentic Mechanisms in Experimental Parkinsonism and Dyskinesia

AKADEMISK AVHANDLING
som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras i Föreläsningssal Farmakologi, Nanna Svartz väg 2

Fredagen den 28 november, 2014, kl 09.00

av
Michael Feyder

Huvudhandledare:
Prof. Gilberto Fisone
Karolinska Institutet
Department of Neuroscience

Bihandledare:
Dr. Mikael Altun
Karolinska Institutet
Department of Medical Biochemistry and Biophysics

Dr. Patrik Krieger
Ruhr University
Department of Systems Neuroscience

Fakultetsopponent:
Prof. Rosario Moratalla
Instituto Cajal
Department of Functional and Systems Neurobiology

Betygsnämnd:
Dr. Gunnar Schulte
Karolinska Institutet
Department of Physiology and Pharmacology

Prof. Cecilia Lundberg
Lund University
Department of Experimental Medical Science

Dr. Peter Wallen
Karolinska Institutet
Department of Neuroscience

Stockholm 2014

ABSTRACT

In patients with Parkinson's disease (PD), the restoration of depleted striatal dopamine by chronic administration of its precursor, L-DOPA, results in the emergence of debilitating involuntary movements. This complication, termed L-DOPA-induced dyskinesia (LID), represents a limitation to the most efficacious treatment for PD motor symptoms. LID progressively increases in severity despite the continual ability of L-DOPA to alleviate parkinsonian symptoms, suggesting divergent mechanisms of action and the potential for therapeutic intervention. Utilizing an experimental mouse model of PD, the work presented within this thesis investigates the molecular alterations underlying LID. These studies reveal that L-DOPA administration results in pathological intracellular signaling and gene expression within striatal medium spiny neurons (MSNs) expressing the dopamine D1 receptor (D1R). Following L-DOPA administration, sensitized D1R signaling results in hyperactivity of the cyclic 3'-5' adenosine monophosphate (cAMP)/ cAMP-dependent kinase (PKA)/ dopamine- and cAMP-regulated phosphoprotein of 32 kDA (DARPP-32) pathway. This exaggerated response results in excessive activation of the downstream extracellular-regulated kinases 1 and 2 (ERK1/2) and mammalian target of rapamycin complex 1 (mTORC1) cascades, both of which are implicated in LID. These data also demonstrate that nuclear events mediated by mitogen- and stress-activated kinase 1 (MSK1), a direct ERK1/2 substrate, promote the induction of the transcription factor ΔFosB, which exacerbates LID. Furthermore, the concerted activity of MSK1 and DARPP-32 promotes histone H3K27me3S28p and the dissociation from transcription start sites of Rnf2, a Polycomb group protein that represses gene expression. These events are associated with an increase in transcription. Taken together, these studies support the idea that sensitized striatal D1R signaling promotes LID by excessive activation of intracellular signaling pathways and nuclear events promoting gene expression.

LIST OF SCIENTIFIC PAPERS

Santini E*, Feyder M*, Gangarossa G, Bateup HS, Greengard P, Fisone G (2012) Dopamine- and cAMP-regulated phosphoprotein of 32-kDa (DARPP-32)-dependent activation of extracellular signal-regulated kinase (ERK) and mammalian target of rapamycin complex 1 (mTORC1) signaling in experimental parkinsonism. *J Biol Chem*. 287: 27806-27812. doi:10.1074/jbc.m112.388413

Feyder M, Södersten E, Santini E, Vialou V, LaPlant QC, Watts EL, Spigolon G, Hansen K, Caboche J, Nestler EJ and Fisone G. A role for mitogen- and stress-activated kinase 1 in L-DOPA-induced dyskinesia and ΔFosB expression. *Biol Psychiatry*. In press. doi:10.1016/j.biopsych.2014.07.019

Södersten E*, Feyder M*, Lerdrup M, Gomes A, Kryh H, Spigolon G, Caboche J, Fisone G, Hansen K (2014) Dopamine signaling leads to loss of polycomb repression and aberrant gene activation in experimental parkinsonism. *PLOS Genetics*. 10(9). doi:10.1371/journal.pgen.1004574

CONTENTS

LIST OF ABBREVIATIONS

6-OHDA	6-hydroxydopamine
AC5	adenylate cyclase
AP-1	activator protein-1
cAMP	cyclic 3'-5' adenosine monophosphate
ChIP	chromatin immunoprecipitation
COMT	catechol-O-methyltransferase
D1R	dopamine D1 receptor
D2R	dopamine D2 receptor
DARPP-32	dopamine- and cAMP-regulated phosphoprotein of 32 kDA
DAT	dopamine transporter
ERK	extracellular signal-regulated kinase
GABA	γ-aminobutyric acid
GPe	external segment of the globus pallidus
GPi	internal segment of the globus pallidus
L-DOPA	L-3,4-dihydroxyphenylalanine
MAO	monoamino oxidase
LID	L-DOPA-induced dyskinesia
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MSK	mitogen- and stress-activated kinase
MSN	medium spiny neuron
mTORC1	mechanistic target of rapamycin complex 1
NHP	non-human primate
PcG	Polycomb group
PD	Parkinson's disease
PKA	cAMP-dependent protein kinase
PP1	protein phosphatase 1
PRC	Polycomb repressive complex
PRE	Polycomb repressive element
qPCR	quantitative polymerase chain reaction
SNc	substantia nigra pars compacta
SNr	substantia nigra pars reticulata
STN	subthalamic nucleus
TSS	transcription start site

INTRODUCTION

In general, our everyday movements are so effortless and fluid, imparting us with the ability to seamlessly perform even complex actions, that we might take this ability for granted. It provides us with the very things that are necessary for our livelihood – essential resources such as food and water, mates for companionship and reproduction, and safety from environmental harms – that it would be difficult to image our lives without the ability to move. Furthermore, it imbues us with the notion of free will, and we feel that our movements are internally generated and with a conscious purpose. Consequently, movement impairments can alter fundamentally one's quality of life and sense of independence.

MOVEMENT DISORDERS

The central nervous system integrates sensory input and generates motor output. Clinically, its dysfunctions are categorized as either neurological or psychiatric, with movement disorders considered neurological. However, this strict segregation has begun to deteriorate with accumulating evidence of psychiatric disturbances experienced by patients with neurological disorders and with a re-conceptualization of psychiatric disorders within the context of movement[1,2]. Attention-deficit/hyperactivity disorder and bipolar disorder are considered psychiatric, but their definitions prominently reference movement. Likewise, autism spectrum disorder, obsessive-compulsive disorder and addictive behaviors include specific, repeated movements but are viewed as psychiatric. The conflation of neurology and psychiatry in the study of movement disorders is underscored by evidence for shared neural substrates, such as the basal ganglia. Therefore, an understanding of the neurobiology underlying movement and its disorders may also provide insight into psychiatric conditions.

Movement disorders can be broadly categorized as a reduction of movement, termed akinetic-rigid disorders, or as an excess of movement, termed hyperkinetic or dyskinetic disorders[3]. Idiopathic Parkinson's disease (PD), an akinetic-rigid disorder, afflicts approximately 0.5-3% of the population above the age of 65, increasing in prevalence with age[4].

PARKINSON'S DISEASE

Clinical Presentation

Modern diagnosis of PD relies on the observation of the same motor impairments extensively documented in 1817 by the physician James Parkinson[5-9]. Present early in its prognosis and initially affecting the limbs, the core PD motor symptoms include: bradykinesia, a reduced velocity of voluntary movement; hypokinesia, a reduced amplitude or poverty of voluntary movements; rigidity, a resistance to passive muscle extension; and resting temor. Additionally, axial motor symptoms, such as postural instability, and gait impairments emerge as the disease progresses. Cognitive, psychiatric and autonomic non-motor symptoms, including dementia, depression, hallucinations, sleep disorders and excessive drooling may appear early in PD or emerge in the later stages of the disease[10].

Neuroanatomical and Biochemical Pathology

PD symptoms result from an underlying progressive neurodegeneration apparent upon post-mortem analysis. While a variety of similar motor impairments resemble those of PD and are collectively referred to as parkinsonism, a diagnosis of PD requires the presence of brain protein aggregates, or inclusions, containing α-synuclein[11]. These cellular inclusions are termed Lewy bodies or Lewy neurites, depending on their localization in the cell body or neuronal processes, respectively. They are believed to progressively ascend from the brainstem, radiating out from the basal forebrain and eventually extending throughout the cortex in a stereotypical manner[12]. Although evidence conflicts concerning their pathological or beneficial effect, it is notable that these inclusions exist in nuclei known to degenerate in PD. These nuclei include the substantia nigra par compacta (SNc), the locus ceruleus, the nucleus basalis of Meynert and dorsal raphe, affecting the dopaminergic, noradrenergic, cholinergic and serotonergic systems, respectively[13].

In addition to these protein aggregates, the defining pathology of PD is the stereotypic degeneration of dopaminergic neurons in the SNc[14]. Extensive degeneration occurs in its caudolateral regions, progressing medially, dorsally and rostrally with less severity[15,16]. Histochemical subdivisions within the caudolateral region may show even greater susceptibility to degeneration[17]. Located within the mesencephalon, these degenerating neurons constitute, in part, the mesostriatal pathway, and their death is paralleled by a loss of dopaminergic terminals preferentially in the caudal putamen[18-20].

Consequently, striatal dopamine is diminished in PD patients[21-23]. Owing to the buffering capability of the mesostriatal pathway, substantial neuronal death, approximately 70% in the caudolateral SNc, occurs before motor symptoms appear[16]. This degeneration is sufficient to produce parkinsonian motor symptoms[24,25].

Pharmacotherapy for Motor Symptoms

The most efficacious and widely utilized anti-parkinsonian therapy is oral administration of the amino acid L-3,4-dihydroxyphenylalanine (L-DOPA), which is enzymatically converted to dopamine by aromatic L-amino acid decarboxylase (L-AADC, also known as DOPA decarboxylase) to reestablish striatal dopamine content[26-30]. Supplemented with a peripheral L-AADC inhibitor to limit adverse effects by preventing the accumulation of dopamine outside of the central nervous system, this combination provides acute improvement of PD motor symptoms[31,32]. This short-duration effect, lasting a few hours, mirrors the synthesis and metabolism of striatal dopamine[33-35]. Additionally, chronic treatment produces a long-duration effect which lasts approximately one to two weeks[29,31,36,37]. While L-DOPA continues to alleviate core motor impairments, the later emergence of postural instability and non-motor symptoms, both of which are resistant to L-DOPA therapy, severely reduce one's quality of life and may be attributed to the degeneration of non-dopaminergic neurons[7,9,38,39]. Indeed, while L-DOPA affords symptomatic relief for a subset of motor deficits, it does not modify the progressive neuronal degeneration.

L-DOPA-INDUCED DYSKINESIA

While maintaining an antiparkinsonian benefit, prolonged L-DOPA administration results in the emergence of a range of undesirable side-effects[40]. These may include an inability to control one's impulses, resulting in behaviors such as pathological gambling and hypersexuality. Psychiatric reactions, such as hallucinations or delusions, may result. Additionally, compulsive use of medication despite adverse reactions, such as L-DOPA induced dyskinesia (LID), may occur. For most patients, however, the emergence of LID represents a major complication and limits the utility of chronic L-DOPA treatment.

LID is a drug-induced disorder characterized by excessive movements, which can be broadly classified as either choreic or dystonic[3,41,42]. Choreic movements are small

amplitude, rapid, dance-like movement that appears to randomly flow throughout the body, resembling fidgeting in its mild form. Large amplitude chorea, termed ballism, can also occur. Chorea can co-occur with a writhing and twisting motion, termed choreoathetosis. Finally, dystonia is the involuntary contraction of opposing muscles, resulting in the twisting of the limbs and abnormal postures.

An earlier onset of PD, a longer duration of PD, a longer duration of L-DOPA therapy and a higher cumulative amount of L-DOPA are associated to the development of LID[42]. Taken together, these risk factors suggest that extensive loss of striatal dopamine and chronic L-DOPA therapy are both necessary for the development of LID, an idea reinforced by the inability of L-DOPA to promote dyskinesia in elderly, non-PD patients[43]. Peak-dose LID, which parallels blood plasma levels of L-DOPA following its acute administration, is dose-dependent when motor symptoms first appear[31,36,44-46]. In these early stages, reducing the dose of L-DOPA, which continues to maintain an antiparkinsonian effect, minimizes peak-dose LID. Unfortunately, however, the efficacy of this optimal dose is only temporary, and the therapeutic range between antiparkinsonian benefits and peak-dose LID narrows over time. While the dose of L-DOPA needed to alleviate PD symptoms remains comparatively constant, the threshold for the expression of peak-dose dyskinesia progressively diminishes. Ultimately, the two states coincide, reaching a point in which PD patients cannot maintain a therapeutic L-DOPA dose without experiencing concomitant dyskinesia[47] (Fig. 1).

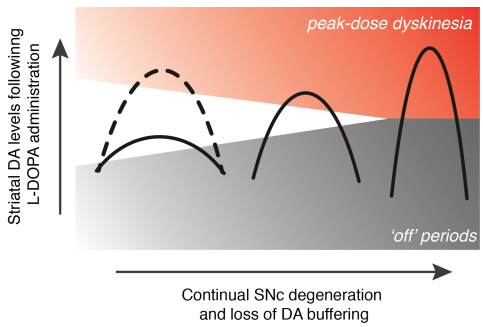

Figure 1. Early stages of PD are characterized by sufficient buffering capabilities of the remaining dopaminergic system, and the dose of L-DOPA can be adjusted for optimal symptomatic benefit without inducing peak-dose LID (left). However, continual dopaminergic loss and unregulated dopamine release result in large fluctuations of striatal dopamine, during which peak-dose LID occurs (right). Furthermore, the threshold for peak-dose LID coincides with the antiparkinsonian threshold, eliminating the possibility for a therapeutic dose without experiencing concomitant dyskinesia. Based on[41].

In most cases, LID appears approximately five years after the onset of motor symptoms and the consequential initiation of L-DOPA therapy. This delay is attributed to the ability of the remaining dopaminergic neurons to release L-DOPA-derived dopamine in a regulated, physiological manner[41,42]. However, as the progressive degeneration of the dopaminergic system continues at the expense of its buffering capabilities, unregulated dopamine release produces supraphysiological surges following L-DOPA administration. The result is a relatively short therapeutic window during which peak-dose choreic and dystonic movements occur, and afterwards striatal dopamine returns to subphysiological levels without subsequent L-DOPA administration[33-35]. This decline in striatal dopamine produces an 'off' medication state, a transition termed motor fluctuations, and necessitates repeated L-DOPA administration. Although less common, dyskinesia may be expressed while dopamine levels in the brain are rising or falling, a phenomenon termed diphasic dyskinesia. They may also be expressed in an unpredictable manner. Finally, dyskinesia, commonly dystonia, may occur when striatal dopamine returns to subphysiological levels, an effect defined as 'off' dyskinesia (Fig. 2)

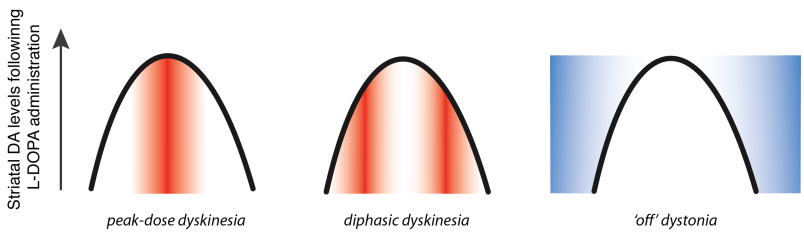

Figure 2. Following L-DOPA administration, peak-dose dyskinesia (left) occurs when striatal dopamine is maximal, while diphasic dyskinesia (center) presents during its rising or falling phase. 'Off' dystonia (right) occurs during periods of diminished striatal dopamine and the reemergence of parkinsonian symptoms. Based on[48].

CLINICAL MANAGEMENT OF LID

Once LID emerges, it persists and progressively worsens without therapeutic intervention. Peak-dose LID (herein referred to as LID) can be managed by surgical implantation of electrodes, programmed to deliver repetitive stimulation, into the subthalamic nuclei (STN). This procedure, termed deep brain stimulation, can produce a long-term improvement in LID for up to five years, an effect attributed to the reduction in the dose of L-

DOPA required for a therapeutic benefit[49]. Although its exact therapeutic mechanisms are unknown, it is thought to normalize pathological network activity or to promote movement-facilitating activity[50].

LID can also be managed by pharmacological means. Adjunct use of amantadine, a N-methyl-D-aspartate (NMDA) receptor antagonist, reduces LID for at least one year[51-58]. This therapeutic benefit may result from an attenuation of the exaggerated NMDA receptor activation in dyskinetic patients during LID[59]. Adjunct use of clozapine, which has a high affinity for a variety of serotonergic, dopaminergic, muscarinic and adrenergic receptors, also reduces LID for at least one year[60-65].

Another approach thought to delay the emergence of LID is to initiate treatment with dopamine receptor agonists, thereby minimizing the cumulative exposure to L-DOPA. Various agonists with a bias towards the dopamine D2 receptor, such as bromocriptine, pramipexol and ropinirole, reduce the severity of dyskinesia; however, these compounds also produce less antiparkinsonian benefit, an effect illustrated by the majority of PD patients that will eventually require supplemental L-DOPA to manage worsening symptoms[6,39,66-71]. Furthermore, the severity of dyskinesia when agonist-treated patients switch to L-DOPA does not differ from those who initiate therapy with L-DOPA[72,73]. As a result, a consensus has emerged whereby initiating treatment with L-DOPA provides superior initial symptomatic relief and confers no long-term harm in developing LID[74-78].

An emerging concept emphasizes the loss of striatal dopamine and a consequential priming of the striatum as a major determinant that, when stimulated by dopamine replacement therapy, promotes the development of LID[79]. Clinical data directly comparing the extent of SNc degeneration while controlling for the duration of L-DOPA therapy is impossible to assess, given the ethical considerations of withholding available therapy while degeneration persists. However, indirect evidence supports this hypothesis. Severe drug-induced parkinsonism occurring from the selective degeneration of the SNc produces a more rapid induction of LID when compared to idiopathic PD[25,80]. Therefore, the extent of dopaminergic degeneration, rather than the duration of L-DOPA therapy, may largely contribute to the development of LID. This idea is further supported by findings in underserved populations with varying stages of PD and where L-DOPA was recently introduced. In these patients, introduction of L-DOPA therapy later in the progression of PD led to a more rapid onset of dyskinesia[81,82]. These data suggest that the development of LID may be particularly influenced by the extent of dopamine loss and the consequential striatal priming, whose molecular effects are discussed in more detail later.

LID is expressed when the levels of striatal dopamine, derived from L-DOPA, are maximal and is exacerbated by the deteriorating buffering capability of the dopaminergic system. In line with these observations, interventions that attenuate the intermittent or pulsatile nature of L-DOPA administration is anticipated to reduce LID[83]. Pre-clinical evidence supports this hypothesis. Various strategies to establish continuous dopamine receptor stimulation, including gene therapy approaches introducing dopamine-synthesizing enzymes within the striatum or sustained delivery of L-DOPA, attenuate LID[84-88]. These approaches also prevent or revert the aberrant gene expression that occurs following pulsatile stimulation[84,88,89]. Clinical trials using similar strategies are being conducted and may represent a future avenue to alleviate LID[90-92].

EXPERIMENTAL MODELS OF PD AND LID

Experimental animal models provide a unique opportunity to investigate PD and LID in a more controlled and accessible manner than that afforded by clinical research. These models principally utilize 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or 6-hydroxydopamine (6-OHDA) toxins, whose reuptake and metabolism generate excessive oxidative stress to induce cell death[93-95]. The result is a selective and permanent lesion of the mesostriatal dopaminergic pathway and the emergence of parkinsonian motor symptoms.

Non-human primates (NHPs) and rodents are commonly used as experimental models of PD and LID. After repeated MPTP intoxication, NHPs mirror the clinical presentation and pattern of dopaminergic degeneration observed in PD[96]. Following chronic L-DOPA treatment, they display similar peak-dose choreoathetoid and dystonic movements as those observed in PD patients[80,97]. Models utilizing rodents afford a reduced ethical dilemma when compared to NHPs. When stereotaxically injected along the mesostriatal pathway, 6-OHDA also selectively and permanently eliminates striatal dopaminergic innervation and produces an akinetic state reminiscent of parkinsonian motor impairments[98]. Additionally, repeated administration of L-DOPA results in the appearance of peak-dose choreic and dystonic movements, similar to those observed in LID[99].

These models have provided the foundation for the pre-clinical data discussed throughout this thesis, and their utility in understanding these conditions and in discovering potential therapeutic strategies cannot be understated. They afford biochemical, electrophysiology and behavioral analysis in a controlled experimental environment, an impossible task with PD patients. The identification of L-DOPA as an anti-parkinsonian

therapy was preceded by pre-clinical animal research discovering the neurotransmitters present in the brain, their localization, and the behavioral consequences of their depletion and reestablishment. This foundational understanding provided the motivation for ethical and hypothesis-driven research utilizing human samples and for experimental therapeutic interventions[100]. The same argument can made for the discovery and clinical implementation of deep brain stimulation (DBS), and both examples serve as guiding principles in the use of animal models to investigate LID[101]. In the case of the research presented in this thesis, the 6-OHDA model has provided material for biochemical and behavioral analyses, thereby contributing to a body of knowledge guiding ethical and responsible therapeutic interventions.

Whereas the 6-OHDA rodent model reproduces the degeneration of the mesostriatal pathway, which is the principle feature of PD, it also has a number of limitations. Although the basal ganglia are though to be extensively conserved throughout evolution, the likely existence of biochemical and neuroanatomical species-specific differences may confound a translation to the human condition[102,103]. Additionally, the 6-OHDA lesion is made unilaterally to create a hemiparkinsonian state, which contrasts with the bilateral degeneration observed in PD. Furthermore, the degeneration occurs more rapidly when compared to PD. A final general observation regards the use of the 6-OHDA lesion model in combination with manipulations excising genes from or introducing transgenes into the mouse genome. Whereas this approach provides a unique opportunity to probe causal relationships, it may result in confounding effects arising from random insertion of the transgene within the genome, effects attributable to background strain or compensatory effects stemming from constitutive ablation[104,105].

THE MESOSTRIATAL DOPAMINE PATHWAY

The mesostriatal dopamine pathway is composed of cell bodies residing in the mesencephalon whose axons terminate in the dorsal striatum. Alternatively described as the nigrostriatal pathway to reflect the preponderance of neurons in the SNc contributing to this innervation, this terminology may represent an oversimplification of a more complex distribution of cell bodies throughout the mesencephalon[20]. In the context of PD, however, the degeneration of dopaminergic neurons in the SNc and their terminals in the striatum define its neuropathology[106].

Anatomy

Exemplifying the complexity of the mesostriatal dopaminergic pathway, the SNc can be divided into two-tiers: a dorsal tier, which has indistinguishable borders with the dopaminergic neurons of both the ventral tegmental area (VTA) and the retrorubral area, and a ventral tier, whose cell bodies reside immediately dorsal to or within the substantia nigra pars reticulata (SNr)[20,107]. The ventral tier projects to biochemically distinct striatal regions, termed striosomes, embedded within a surrounding matrix, which receives topographical projections from neurons of the dorsal tier. More medial regions of the dorsal tier preferentially innervate the ventral striatum, such as the nucleus accumbens, while more lateral regions preferentially innervate the dorsally located putamen, which is reflected in the pattern of degeneration in PD.

Synapses and Release

During development, mesencephalic dopaminergic neurons project rostrally in a large axonal tract, termed the medial forebrain bundle, where they innervate selected striatal regions that later develop into striosomes[108]. These axonal projections then extend throughout the striatum, terminating to form symmetric synapses on dendritic branches and the necks of dendritic spines[109]. However, approximately 60-70% of these terminals, or varicosities, lack an opposing post-synaptic structure, reflecting the diffuse nature of dopaminergic signaling[110]. Therefore, in contrast to glutamatergic signaling, where high-fidelity, point-to-point synaptic connections convey neurotransmission, dopaminergic signaling appears to have evolved an important spatial component, termed volume transmission[111]. Presumably, this extended 'sphere of influence' allows for extensive buffering to compensate for the degenerating dopaminergic system and for stable symptomatic benefit during initial L-DOPA therapy[16,112]. Additionally, dopamine is released from the cell body and dendrites of dopaminergic neurons, a process termed somatodendritic release, to modulate the surrounding extracellular environment[113,114]. Loss of this release after neuronal death may lack a similar buffering capability or compensatory mechanism, possibly explaining regional differences in the restoration of dopamine levels after L-DOPA administration[115].

The Serotonergic System and LID

In the PD brain, L-DOPA-derived dopamine continues to be released into the extracellular space despite the progressive degeneration of striatal dopaminergic innervation. This release is possible mainly through the serotonergic system[116]. This idea is supported by PD models in which serotonergic neurons, whose biochemical processes have accommodating substrate specificity, are capable of L-DOPA uptake, dopamine synthesis, vesicular packaging and axonal release[117,118]. In this scenario, the release of dopamine from serotonergic terminals is unregulated because of lack of dopamine D2 autoreceptors. Accordingly, in dyskinetic PD patients, an agonist of the serotonergic autoreceptor 5-HT1a reduces striatal dopamine release and attenuates LID[119].

Dopamine Receptors

Once released into the extracellular space, dopamine binds to heptahelical receptors, or G-protein coupled receptors, which transform this extracellular signal into intracellular output. These receptors are found on the cell body, dendrites and spines of MSNs[120]. Based on sequence homology and pharmacology, dopamine receptors are broadly categorized into two classes: D1-like and D2-like[121,122]. D1-like receptors include the dopamine D1 and D5 receptors, both of which promote cyclic 3'-5' adenosine monophosphate (cAMP) production by coupling to the G protein subunits G_{α_s} or $G_{\alpha_{olf}}$ and stimulating adenylate cyclase. The D2-like receptors include the dopamine D2, D3 and D4 receptors, all of which reduce cAMP synthesis by coupling to either the G protein subunits G_{α_i} or G_{α_o} and inhibiting the catalytic activity of adenylate cyclase. As discussed later, this coupling can initiate downstream events to modulate cellular properties.

Regulation of Extracellular Dopamine

Termination of extracellular striatal dopamine transmission occurs by reuptake through the dopamine transporter (DAT) or by enzymatic degradation. DAT is expressed exclusively within dopaminergic neurons, where it is found on unmyelinated axon segments or perisynaptic to axon terminals[123]. Perisynaptic DAT is situated to regulate dopamine

released within the synapse, while DAT located within the axon presumably functions to recycle diffuse, extrasynaptic dopamine.

Two intracellular monoamino oxidase (MAO) isoforms, MAO-A and -B, metabolize the majority of striatal dopamine and other catecholamines[124]. The striatum is enriched in MAO-B and, although primarily expressed in serotonergic neurons, inhibitors of this enzyme prolong the antiparkinsonian effects of L-DOPA and minimize motor fluctuations[32,125,126]. Additionally, MAO-A is present in the striatum, primarily in catecholaminergic neurons, and contributes to dopamine metabolism[124,126].

Striatal dopamine is also inactivated by the enzyme catechol-O-methyltransferase (COMT). COMT is absent from dopaminergic terminals but is instead localized intracellularly or membrane-bound in both glia and medium spiny neurons (MSNs)[127,128]. However, relative to MAOs, it metabolizes proportionally less dopamine[129]. Despite this reduced influence, COMT inhibitors minimize motor fluctuations, and it generates dopamine-derived metabolites associated with LID[32,130]. Thus, once dopamine is released into the extracellular space, a variety of mechanisms across a diverse number of cell-types can compensate for the neurodegenerative occurring in PD.

THE BASAL GANGLIA IN PD AND LID

Anatomically divided in primates by the axonal tracts of the internal capsule, the caudate nucleus and the putamen form the striatum. Because of its extensive dopaminergic innervation, the pathological consequences arising from the degeneration of this innervation and the motor complications following the prolonged restoration of dopamine, the striatum is well suited to be a major neural locus underlying LID. It is the largest in a collection of subcortical nuclei, termed the basal ganglia, whose anatomical connectivity creates parallel, or minimally integrating, open- and closed-circuit loops[131]. The striatum receives glutamatergic afferents from the thalamus and from the entire cerebral cortex and sends returning projections principally to the frontal lobe, thereby creating close-circuit cortical-basal ganglia-cortical loops with these regions. Depending on their inputs, these loops can be broadly categorized as limbic (or emotional), associative (or cognitive) or sensorimotor. Highlighting the segregated nature of these loops, the sensorimotor circuit can be sub-categorized into individual ‘channels’ specific for each bodily representation, suggesting that similar ‘channels’ may also exists in the limbic and associative loops[132]. The present discussion focuses on the sensorimotor loop because of the involvement of these

cortical regions in the control of motor output via the corticospinal and corticobulbar tracts and because the hyperactivation of these cortical areas is associated to LID[133]. However, the striatal integration of cortical input is thought to be independent of its origin, and analogous considerations could possibly be applied to the limbic and associative loops[131,134].

The 'Direct' and 'Indirect' Pathways and the Rate Model

The sensorimotor loop originates in cortical regions whose stimulation directly results in or is closely associated with motor output. These areas include the motor, premotor, supplementary and cingulate motor cortices, as well as the somatosensory cortex. They predominately project to the dorsolateral regions of the posterior putamen and, to a lesser extent, to the dorsolateral regions of the caudate nucleus[131,132]. Additional input to the striatum comes from the thalamus, particularly its intralaminar nucleus. An influential working model of the basal ganglia emphasizes two striatal projection pathways with opposing influences on motor output[135]. According to this model, MSNs constitute striatal output and can be segregated into a 'direct' or 'indirect' pathway, depending on their connectivity with basal ganglia output nuclei. MSNs of the 'direct' pathway form synapses onto neurons of the internal segment of the globus pallidus (GPi, or entopenducular nucleus in rodents), the primary nuclei for the sensorimotor loop, but also the SNr, which together create a somatotopically unified structure to form the output nuclei of the basal ganglia. Intermingled among neurons of the 'direct' pathway, MSNs of the 'indirect' pathways, however, project to neurons residing in the external segment of the globus pallidus (GPe). These neurons, in turn, project to the dorsolateral regions of the subthalamic nucleus (STN). Neurons of the STN form synapses onto those of the GPi/SNr, creating an indirect pathway of connectivity. Within the sensorimotor loop, the GPi/SNr projects to the ventral anterior and ventral lateral (VA/VL) nuclei of the thalamus, which complete the cortico-basal ganglia-cortical loop by projecting to motor cortices (Fig. 3). In addition, the neurons of the GPi/SNr also innervate brainstem sensorimotor nuclei, exemplified by projections to the superior colliculus to promote saccadic eye movements. Given the reciprocal superior colliculus-thalamic projections, this closed-loop may represent a phylogenetically older circuit and a fundamental neural unit repurposed in the basal ganglia for cortical input[136].

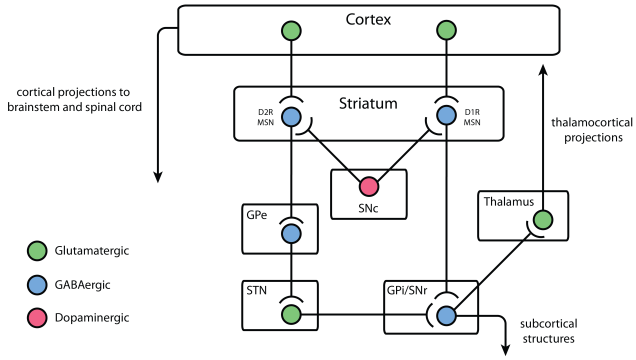

Figure 3. Simplified diagram emphasizing corticostriatal projections and unidirectional neurotransmission between basal ganglia nuclei. Glutamatergic projections from the thalamus (not shown) and cortex form synapses onto striatal MSNs expressing either D1Rs or D2Rs, which respond to dopamine released by neurons of the SNc to modulate MSN activity. These two populations of MSNs have divergent connectivity and opposing effects on motor output. Based on[89,135,136]

By integrating excitatory glutamateric input from the cortex and thalamus, MSNs of the 'direct' and 'indirect' pathway provide bidirectional regulation of the GPi/SNr. Activity of the 'direct' pathway stimulates motor output, while that of the 'indirect' pathway produces an opposite effect[135,137-140]. This difference is attributable to the neurotransmitters utilized by their projection neurons[141]. The projection neurons of the GPi/SNr tonically inhibit those of the thalamus by releasing the amino acid γ-aminobutyric acid (GABA). The thalamus, in turn, sends excitatory glutamatergic projections to motor cortices, ultimately generating motor output. An increase in GPi/SNr activity further suppresses thalamic output and, conversely, a decrease in GPi/SNr activity increases thalamic output. GABAergic, 'direct' pathway MSNs inhibit the GPi/SNr to disinhibit, or excite, the thalamus. Conversely, activation of GABAergic, 'indirect' pathway MSNs ultimately increase GPi/SNr activity, further suppressing thalamic output. This effect is achieved by inhibiting GABAergic neurons of the GPe, which in turn project to the STN. The net result is a disinhibition of the STN, whose excitatory, glutamatergic neurons project to the GPi/SNr. Increased excitatory input to the neurons of the GPi/SNr further suppresses thalamic output. Therefore, a balanced transmission, or rate of activity, between the two feed-forward pathways is proposed to generate optimal motor output, while an imbalance between the two is expected to generate pathological hypo- or hyper-activity[135].

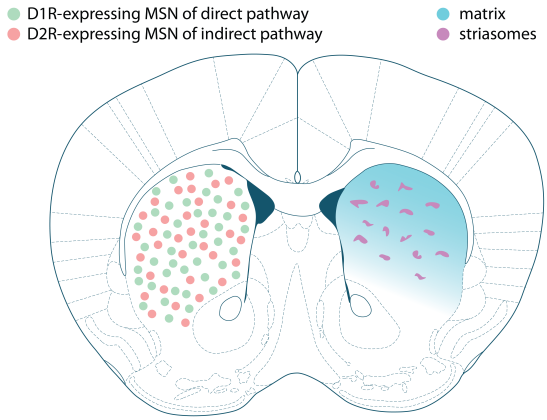

Figure 4. The 'direct' and 'indirect' pathways (left hemisphere) emphasize differences in anatomical connectivity and dopamine receptor expression between MSNs. Direct pathway MSNs express D1Rs and form synapses with the basal ganglia output nuclei, the GPi/SNr. Indirect pathway MSNs express D2Rs and indirectly influence GPi/SNr activity by intermediate synaptic connections. Superimposed upon these intermingled MSNs, striosomes and the surrounding matrix (right hemisphere) are enriched for a diverse array of molecules, endowing them with a unique biochemistry. These regions can also vary in their synaptic inputs. Striosomes receive projections from limbic regions, while the matrix is innervated by associative and sensorimotor regions. Striosomes are notable for their innervation from the SNc. Based on[142,143] and modified from[144].

Striatal dopamine modulates the activity of both 'direct' and 'indirect' pathway MSNs to promote motor output[141]. MSNs of the 'direct' pathway express the dopamine D1 receptor (D1R), while those of the 'indirect' pathway express the dopamine D2 receptor (D2R)[89] (Fig. 4). Their opposing actions on the generation of cAMP are paralleled by their effects on MSN excitability. In general terms, activation of D1Rs facilitates a transition to a spike-favorable 'up-state,' ultimately decreasing GPi/SNr activity and promoting motor output. Conversely, activation of D2Rs attenuates 'up-state' transitions, ultimately increasing GPi/SNr output and depressing motor output. Systemic stimulation with either a D1R- or D2R-specific agonist induces motor output, an effect that synergizes following striatal dopamine depletion[145-147].

In the context of this model, the pathologically low levels of striatal dopamine in PD promote excessive activation of the 'indirect' pathway and attenuate activation of the 'direct' pathway[135]. Electrophysiological recordings within the basal ganglia of PD patients

before and after dopamine agonist therapy broadly support this model[137,148,149]. Stimulation of dopamine receptors with apomorphine, an agonist with affinity towards both D1- and D2-like receptors, suppresses activity in the GPi and STN and increases activity in the GPe, in agreement with similar data obtained in NHP PD models[150-155]. Furthermore, in PD patients, LID is associated with an exaggerated suppression of GPi activity, and experimentally-induced STN inhibition can evoke choreic and dystonic movements[152,156].

The Rate Model: Growing Pains

Additional anatomical evidence reveals a more complex interrelationship between nuclei of the basal ganglia, with important implications for PD and LID[149,157,158]. For example, 'direct' and 'indirect' pathway MSNs are not functionally segregated but project reciprocating axon collaterals, whose strength is diminished following striatal dopamine depletion[159]. Furthermore, the 'hyperdirect' pathway from motor-related cortices to the STN, now redefined as a basal ganglia input nuclei, provides a possible mechanism to influence motor output independent from, or in combination with, corticostriatal innervation and may underlie the therapeutic effects of DBS within this nuclei[50,160].

Electrophysiological studies in PD models demonstrate bursting discharge and oscillatory activity, suggesting a greater complexity than simply the rate of action potentials[137,157,158]. Additionally, both 'direct' and 'indirect' pathways are simultaneously active during the initiation of locomotion, suggesting a model in which the patterned activity of 'direct' pathway activation promotes a specific motor output, while concurrent 'indirect' pathway activation suppresses competing or incongruent output[137,161,162].

Although enriched in the striatum, dopamine receptors within the basal ganglia are not limited to this nucleus or its MSNs. They are expressed by all striatal interneurons, which have been implicated in PD and LID, and by neurons of the GPe and GPi/SNr, both of which receive dopaminergic innervation[114,163-165].

Therefore, while the 'direct' and 'indirect' pathways of the basal ganglia provide a conceptually straightforward explanation to describe the motor output of PD and LID, as a model it reflects a simplification of a more complex biology. In the context of the data presented in this thesis, the exaggerated dopaminergic signaling implicated in LID predominately occurs in 'direct' pathway MSNs, in general agreement with the expected hyperactive motor output predicted by this model.

The Striosome and Matrix Organization

Superimposed upon the 'direct' and 'indirect' pathways, the dorsal striatum contains patch-like regions, termed striosomes, which are endowed with a unique biochemistry when compared to the surrounding matrix[142,166] (Fig. 4). MSNs of the 'direct' and 'indirect' pathway are found in both regions. Striosomes, for example, are enriched in μ-opioid receptors and the intracellular signaling molecule CalDAG-GEFII. Although not exhaustive, the matrix is enriched in molecules important for acetylcholine transmission, such as acetylcholinesterase and choline acetyltransferase, the calcium binding protein calbindin D28K and CalDAG-GEFI. Furthermore, these compartments differ in their afferent and efferent projections[142,167]. While topographic projections from associative and sensorimotor areas innervate the respective medial and lateral regions of the striatal matrix, limbic regions provide input to the striosomes[168,169]. Striosomes are notable for their projections to the SNc, which is thought to regulate dopamine release in the matrix, and for their specific dopaminergic innervation by neurons in the ventral tier of the SNc[107,170,171].

Imbalances in the activity between the two compartments are proposed to underlie dopamine-induced behaviors, including LID[142,170]. Chronic L-DOPA administration results in persistent immediate early gene induction in the striosomes, which absent in the ventromedial matrix[172,173]. Additionally, striatal dopamine depletion reduces CalDAG-GEFI expression in the striatum, an effect that is exacerbated by chronic L-DOPA administration, while CalDAG-GEFII is unaffected by the dopamine depletion but is up-regulated in both the matrix and striosomes following chronic L-DOPA administration[174]. The extent of their induction or repression correlates with LID. Finally, mRNA for the opioid precursor PPE-B is enriched in the striosomes of PD patients and NHPs with LID, and a μ-opioid receptor antagonist reduces dyskinesia in NHPs[175,176]. Taken together, these data suggest that excessive imbalance between MSNs of the striosome and matrix may promote LID.

INTRACELLULAR SIGNALING PATHWAYS

Because they are enriched in dopamine receptors, represent approximately 90% of all striatal neurons and compose striatal output, MSNs are well situated to underlie LID. In rodents, a unilateral lesion of the mesostriatal dopaminergic pathway results in spontaneous ipsilateral rotations, indicative of their hemi-parkinsonian condition and of an imbalance between the striatal outputs of each hemisphere. However, dopamine receptor stimulation, either with apomorphine or L-DOPA administration, produces contralateral

rotations. This behavior reflects a now inverted imbalance in striatal output between hemispheres and an underlying behavioral sensitization to dopamine receptor stimulation developed in response to dopamine depletion[177]. Chronic D2-like receptor stimulation produces a progressive increase in contralateral rotations and mild dyskinesia; however, chronic D1-like receptor stimulation results in a more pronounced increase in contralateral rotations and dyskinetic behavior[178-180]. These data are supported by a reduction of LID in rodent PD models following pre-treatment with D1R-like antagonists, ablation of D1R-expressing MSNs, or genetic deletion of the D1R [181-185].

In NHP PD models, individual stimulation of either the D1R or the D2R is sufficient to promote dyskinesia; however, D1R-like stimulation has a greater propensity to do so, and D1R-like stimulation induces established dyskinesia to a similar degree as L-DOPA in PD patients[186-188]. Therefore, while both receptors are sufficient to promote dyskinesia, synergizing to produce the superior antiparkinsonian and dyskinetic effects of L-DOPA, D1Rs appear to be prominently involved in the development and manifestation of LID. However, because D1R-like stimulation alleviates PD motor symptoms, its antagonism limits the antiparkinsonian effects of L-DOPA[189,190]. This dual nature of D1R stimulation suggests that divergent downstream mechanisms, one that is antiparkinsonian and another that is pro-dyskinetic, occur in the dopamine-depleted striatum.

SPECIFIC AIMS

The aim of this thesis is to investigate the molecular events occurring in D1R-expressing MSNs that promote LID. In an attempt to achieve this goal, the following studies are presented according to their general sequence following D1R stimulation, focusing first on the cAMP/PKA/DARPP-32 cascade (**PAPER I**). This pathway stimulates ERK1/2 and MSK1 to transmit cytoplasmic activity into a nuclear response promoting gene expression (**PAPER II**). Finally, specific molecular alterations involving repressive Polycomb group proteins, which are potentially responsible for induced transcription, are described (**PAPER III**).

cAMP/PKA/DARPP-32 pathway (PAPER I)

When bound to dopamine, the resulting conformational change of the D1R transforms this extracellular stimulus into an intracellular effect by coupling to heterotrimeric G proteins, consisting of an α subunit and a β and γ dimer. Following an exchange of guanosine-5'-diphosphate bound to the α subunit for a guanosine-5'-triphosphate, the α subunit and β and γ dimer dissociate to stimulate downstream effectors. In MSNs, the dopamine D1R stimulates cAMP production by promoting the dissociation of $G\alpha_{olf}$[191-195]. Release of $G\alpha_{olf}$ stimulates adenylyl cyclase 5 (AC5), which is highly expressed in the striatum, to increase cAMP synthesis[196,197]. Accumulating cAMP binds to the regulatory subunits of cAMP-dependent protein kinase (PKA), releasing its catalytic subunits to phosphorylate downstream substrates[198].

In PD models, D1R stimulation results in exaggerated downstream effects, which contribute to the development of LID. Increases in D1R surface membrane expression or impairments in D1R endocytosis, which can bias intracellular signaling outcomes, are associated to LID[199-204] (but see [205]). In dyskinetic NHPs, D1R stimulation results in a greater amount of $G\alpha$ bound with guanosine-5'-triphosphate, an effect that correlates with LID[199]. Striatal dopamine depletion increases the expression of $G\alpha_{olf}$, persisting during chronic L-DOPA administration to also correlate with LID[206,207] (but see [208]). This increase is observed in the putamen of PD patients[209]. Attenuation of $G\alpha_{olf}$, however, does not reduce LID, a result complicated by evidence that genetic ablation of AC5, which also increases following dopamine depletion and persists during L-DOPA administration, or inhibition of PKA reduce LID[84,206,208,210,211]. Finally, overexpression of G protein-coupled receptor kinase 6, which desensitizes D1R-mediated signaling by receptor phosphorylation, suppresses LID[212]. Taken together, these data reveal that sensitized canonical D1R-mediated signaling underlies the development and expression of LID.

In support of these data, exaggerated phosphorylation of the PKA substrate, dopamine- and cAMP-regulated phosphoprotein of 32 kDA (DARPP-32), is associated with LID. DARPP-32 is enriched in the striatum and integrates intracellular signaling pathways within MSNs[213]. By acting as a substrate for multiple upstream kinases, individual phosphorylation sites of DARPP-32 function to integrate intracellular signaling events, ultimately regulating protein phosphatase 1 (PP1). PKA phosphorylates DARPP-32 on threonine 34 (T34), converting DARPP-32 into a potent inhibitor of PP1 and increasing the phosphorylation state of downstream PP1 substrates[214]. In PD models, L-DOPA

administration results in exaggerated PKA-dependent phosphorylation of DARPP-32 at T34 in D1R-expressing MSNs[84,199,206,215-219]. This hypersensitivity is most pronounced after acute L-DOPA administration but also persists to a lesser degree following chronic administration. Accordingly, global genetic ablation of DARPP-32 or its selective elimination in D1R-expressing MSNs attenuates LID, further demonstrating an integral role of cAMP signaling to promote LID[140,215].

A similar pathological hyperactivation of the mitogen-activated protein kinases, extracellular signal-regulated kinases 1 and 2 (ERK1/2), promote LID[215,220]. ERK1/2 are found in the nucleus, cytoplasm and dendrites, and they have been implicated in regulating activity-dependent gene expression, synaptic plasticity and spine morphology[221]. Dual phosphorylation of threonine or tyrosine residues in the Thr-Glu-Tyr (TEY) motif of their activation loop stimulates ERK1/2 kinase activity[222]. Like cAMP signaling, this activation is more pronounced following acute L-DOPA administration but persists following chronic treatment[215]. In MSNs of the dopamine-depleted striatum, D1R stimulation promotes ERK1/2 activation [181,206,215,216,220,223-230]. Additionally, genetic ablation or pharmacological inhibition of upstream cAMP/PKA/DARPP-32 activation reduces ERK1/2 activation and attenuates LID[84,140,211,215]. Despite this evidence, some studies propose that ERK1/2 activation in the dopamine-depleted striatum switches from canonical cAMP/PKA/DARPP-32 activation to an alternative mechanism[206,231,232].

Furthermore, hyperactivity of the mammalian target of rapamycin complex 1 (mTORC1) pathway promotes LID[220,233,234]. Similar to ERK1/2, mTORC1 promotes cell growth and proliferation in mitotic cells and regulates synaptic plasticity in neurons[221,235]. Activation of this pathway is ERK1/2-dependent, but the necessity of upstream PKA-mediated DARPP-32 phosphorylation is unknown [220].

Given the diverse possibility of upstream signaling pathways integrated by the mTORC1 pathway, the experiments presented in **Paper I** were conducted to observe whether PKA-mediated DARPP-32 phosphorylation was necessary for ERK1/2 and mTORC1 activation[236].

Bitransgenic mice with a unique epitope tag genetically appended to DARPP-32 expressed under the promoter of either the D1R or D2R allow for its cell-type biochemical characterization[237]. Following striatal dopamine depletion, chronic L-DOPA administration produced an increase in DARPP-32 phosphorylation at T34 selectively in D1R-expressing MSNs, but remained unchanged in D2R-expressing MSNs. These results confirm previous

data demonstrating hyperactivity of D1R-mediated signaling and suggest that D2R-mediated inhibition of cAMP production does not alter DARPP-32 T34 phosphorylation in this model. DARPP-32 can be phosphorylated at threonine 75 (T75) by cyclin-dependent kinase 5, converting it to an inhibitor of PKA[238]. Inhibition of cyclin-dependent kinase 5, and the resulting decrease in T75 phosphorylation, increases PKA-mediated T34 phosphorylation of DARPP-32 and other PKA substrates, conceivably explaining the hyperactivity of PKA in this model. As no alterations in phosphorylation at T75 were observed, the increased T34 phosphorylation is therefore attributable to augmented PKA activity.

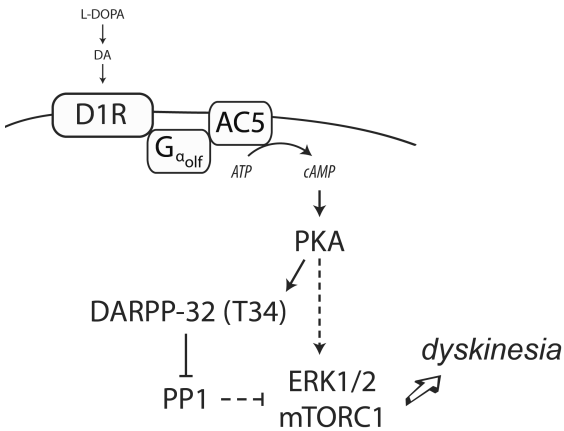

Figure 5. Summary of Paper I. D1R-mediated stimulation of cAMP/PKA/DARPP-32 pathway promotes the activation of downstream ERK1/2 and mTORC1, resulting in dyskinetic behavior

The DNA-cleaving enzyme cre recombinase excises genomic regions between two artificially introduced *loxP* (flanking *loxP* or 'floxed') sites, ligating the remaining DNA[239]. When these *loxP* sites are introduced within a protein-coding region and exogenous cre is expressed under the control of a gene promoter, the excision results in ablation of the protein product specifically in a genetically defined cell-type. Using this strategy, mice in which DARPP-32 was eliminated in either D1R- or D2R-expressing MSNs were depleted of striatal dopamine by a lesion with 6-OHDA and treated chronically with L-DOPA. Elimination of DARPP-32 in D1R-, but not D2R-, expressing MSNs attenuated the L-DOPA-induced phosphorylation of ERK1/2 and a downstream substrate of mTORC1, the ribosomal protein S6. Additionally histone phosphorylation at serine 10 in conjunction with acetylation at lysine 14, a post-translational modification generally associated with gene induction, was also reduced[240]. Furthermore, these effects were reproduced in mice in which

the T34 site of DARPP-32 was mutated to an alanine, a residue unable to be phosphorylated by PKA, reaffirming the necessity of this kinase and DARPP-32. Finally, these mice displayed reduced LID, a result similarly observed in mice lacking DARPP-32 in D1R-, but not D2R-, expressing MSNs or in global DARPP-32 KO[140,215]. In sum, these data support previous studies demonstrating an essential role for hypersensitized PKA-mediated activity and indicate that DARPP-32 stimulates ERK1/2 and mTORC1 signaling pathways downstream of the D1R to promote LID.

ERK1/2 and MSK1 pathway (PAPER II)

The numerous substrates of ERK1/2 obscure the precise molecular mechanisms underlying LID. Given the progressive development and persistence of LID, its nuclear substrates could be hypothesized to transform transient intracellular signaling events into more persistent cellular alterations by inducing gene expression[241]. ERK1/2 phosphorylation of mitogen- and stress-activated kinase 1 (MSK1) at threonine 581 (T581) is necessary to stimulate its activity and to promote gene expression concurrent to phosphorylation of histones or transcription factors[242-245]. Discussed in more detail below, histones efficiently structure nuclear DNA and are a platform for regulatory proteins, such as MSK1, to influence transcription[246]. MSK1 is enriched in the striatum and is expressed by MSNs[247]. While the closely related MSK2 is also present in the striatum and subserves similar functions to that of MSK1, its kinase activity is unaltered by increases in extracellular dopamine resulting from cocaine administration[248]. In the context of LID, phosphorylation of MSK1 at T581 occurs in the same D1R-expressing MSNs as increased ERK1/2 phosphorylation and is dependent on this upstream activity[181,215,220]. This association, however, has not been demonstrated to causally promote LID.

In D1R-expressing MSNs, chronic L-DOPA administration induces transcription of numerous genes, many of which are closely apposed to regulatory activator protein-1 (AP-1) sites[249]. AP-1 complexes are heterodimers composed of subunits from the Fos and Jun protein families (c-Fos, FosB, Fra1, Fra2 and c-Jun, JunB, JunD, respectively) that bind to AP-1 sites[250]. In LID models, these AP-1 complexes are predominately composed of FosB/ΔFosB and JunD[251]. L-DOPA administration induces expression of FosB, which can be alternatively spliced to excise two degradation domains, generating the truncated and uniquely long-lived transcription factor ΔFosB[172,224,252-254]. The protein product of this splice variant can persist up to sixteen days after chronic L-DOPA

administration, and FosB/ΔFosB mRNA induction continues after one year of chronic L-DOPA administration[254,255]. Within the sensorimotor striatum, FosB/ΔFosB densely accumulates in both striosomes and the matrix, but in medioventral regions of the dorsal striatum, it is primarily expressed within striosomes[172]. In contrast, JunD is constitutively expressed and comparatively unaffected by L-DOPA administration, suggesting that FosB/ΔFosB are rate-limiting in the formation of AP-1 complexes [253].

Indiscriminant striatal overexpression of ΔFosB exacerbates LID[256]. Furthermore, attenuation of FosB/ΔFosB accumulation or overexpression of a truncated JunD dominant-negative, which functionally antagonizes AP-1-dependent transcription, reduces LID[257,258]. Repressed excitability of brain-wide FosB/ΔFosB-expressing neurons reduces LID, possibly mediating its effect by also modulating the activity of striatal interneurons or neurons outside the striatum, both of which also expressed FosB when challenged with L-DOPA following chronic administration[224,259,260]. Finally, in agreement with pre-clinical data, ΔFosB is increased in the striatum of dyskinetic PD patients[261].

To investigate the involvement of MSK1 in LID, **Paper II** utilized mice in which MSK1 was constitutively ablated or knocked-out (MSK1 KO). When lesioned with 6-OHDA and treated chronically with L-DOPA, MSK1 KO mice displayed attenuated dyskinetic behavior. Furthermore, an absence of MSK1 did not impair the antiparkinsonian effect of L-DOPA, assessed by the ability of the drug to restore contralateral forelimb use. This reduction in LID was not a result of impaired upstream cAMP/PKA/DARPP-32 or ERK2 signaling. However, histone H3 phosphorylation at serine 10 (H3S10p), a previously demonstrated MSK1-dependent modification occurring after D1R stimulation, was attenuated following L-DOPA administration in MSK1 KO mice[248]. Apart from its specific co-occurrence with chromatin condensation during mitosis and meiosis, the H3S10p modification is commonly associated with gene expression[240]. Genetic deletion of MSK1 resulted in an attenuation of accumulated ΔFosB, which predominately, but not exclusively, localized to D1R-expressing MSNs. Accordingly, chromatin immunoprecipitation (ChIP) of the H3S10p modification and subsequent quantification of the precipitated FosB promoter by quantitative polymerase chain reaction (ChIP-qPCR) demonstrated that this induction occurred at the FosB promoter and was also reduced in MSK1 KO mice. Although ΔFosB accumulated in cells other than D1R-expressing MSNs, its specific overexpression in these neurons was sufficient to exacerbate LID. Conversely, functional antagonism of ΔFosB, achieved by overexpression of a truncated cJun dominant-negative, attenuated LID. Taken

together, these data suggest that LID results, in part, from MSK1-mediated FosB/ΔFosB induction in D1R-expressing MSNs (Fig. 6).

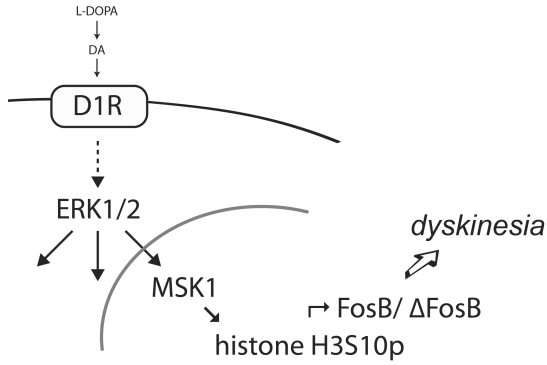

Figure 6. Summary of Paper II. Concurrent to H3S10p in D1R-expressing MSNs, MSK1 stimulates the expression of FosB/ΔFos to promote dyskinetic behavior.

These data provide evidence for nuclear events that underlie LID. Notable, they reflect only subset of possible mechanisms by which ERK1/2 signaling could promote LID. MSK1 represents just one nuclear substrate of ERK1/2, and its elimination produced only a mild reduction in LID, an effect not observed in another study[262]. Additional nuclear substrates, such as the transcription factor Elk1, are also phosphorylated by ERK1/2 and may mediate important transcriptional events in concert with or independent from MSK1 activity[241]. However, gene-specific targeting of MSK1 is sufficient to promote transcription in cell culture systems [263]. Furthermore, MSK1 can also phosphorylates cAMP-response element-binding protein, although immediate early gene induction in the dopamine depleted striatum differs from that of the intact striatum in that it appears to be independent of CREB activity[251].

Many of the molecular and behavioral effects of L-DOPA presented in Paper II are reminiscent of those following intermittent, elevated striatal dopamine due to chronic psychostimulant administration, suggesting a high degree of overlap between these two hyperdopaminergic conditions[264]. Repeated psychostimulant administration results in locomotor sensitization, which is diminished in MSK1 KO mice and exacerbated in the same ΔFosB-overexpressing used in this study[248,265]. While ΔcJun has no effect on locomotor sensitization, it prevents the induction of genes regulated by AP-1 complexes following

psychostimulant administration, emphasizing the mutual involvement of these promoter sites in both conditions but also divergent mechanisms between them[266].

Polycomb Target Gene Regulation (PAPER III)

Biochemical alterations of neuronal histones, or neuroepigenetic modifications, can integrate intracellular events to regulate gene expression[267]. Two copies of each histone protein (H2A, H2B, H3 and H4) create an octamer around which a segment of 147 base pairs of DNA wrap themselves to form a nucleosome[268]. When repeated across the entire genome to form chromatin, nucleosomes provide efficient spatial organization to the extensive amount of nuclear DNA.

Regulation of gene expression, however, requires efficient compaction of the genome but also sufficient physical access necessary for transcription[268]. In general terms, euchromatin is an 'open' chromatin state favorable to transcription, while heterochromatin is a 'closed' chromatin state, condensed and inaccessible to protein complexes necessary to transcription. Some histone modifications, such as phosphorylation and acetylation, are proposed to promote transcription by creating favorable electrostatic interactions and increasing accessibility[268,269]. Other modifications, such as methylation, do not impart any additional charge and may instead serve as binding sites for proteins to regulate the expression of accessible genes[268]. These modifications generally occur on histone termini, or tails, which protrude from the nucleosome core and are accessible to regulatory enzymes[270]. An extensive number of individual modifications exist, and their combinatorial possibilities may constitute a 'histone code' with predictive effects on chromatin structure and transcription[270].

These modifications can be conceptualized, in simplified terms, by the 'writers' that induce them, the 'readers' that interpret them, and the 'erasers' that remove them[271]. Within this framework, MSK1 functions as a 'writer,' generating H3S10p. Additionally, in cell culture systems, MSK1/2 transiently phosphorylates serine 28 of histone H3 (H3S28p) adjacent to a trimethylated lysine 27 (H3K27me3), generating a combinatorial code (H3K27me3S28p), which promotes gene expression[272]. During basal conditions, gene expression is repressed by the binding of a protein complex composed of Polycomb group (PcG) proteins to H3K27me3, thereby acting as a 'reader'[273]. The conventional view posits that they exist in two complexes, differing in subunit composition and varying in function. In broad terms, the Polycomb repressive complex 1 (PRC1) binds to the H3K27me3

modification catalyzed by PRC2. Upon the generation of H3K27me3S28p, PcG proteins are displaced from the chromatin to facilitate gene induction[272]. In cell culture systems, a similar mechanism occurs following induction of H3S10p adjacent to H3K9me3, resulting in the displacement of heterochromatin protein 1, which normally functions to promote heterochromatin formation and gene silencing[274-276].

PcG proteins are necessary for proper development, where they regulate cellular differentiation and maintain cell identity, and their dysfunction has been implicated in cancer [277-279]. Their plasticity in terminally differentiated, post-mitotic cells, such as neurons, is unknown. In **Paper III**, the data presented indicate that PcG genes are derepressed in D1R-expressing MSNs upon acute L-DOPA administration, suggesting a greater plasticity of PcG-repression than previously known.

Acute L-DOPA administration resulted in a prominent increase in global H3SK27me3S28p in the dopamine-depleted striatum when compared to the intact striatum, and this induction was principally localized to D1R-expressing MSNs. Repeated L-DOPA administration resulted in a progressive decrease in its induction, which failed to completely normalize. Sequencing of striatal RNA (RNA-seq) and ChIP followed by sequencing of precipitated genomic regions (ChIP-seq) revealed an association between transcription start sites (TSS) marked by H3K27me3S28p and increased mRNA expression. This association was greater than that found for TSS regardless of modifications or for TSS marked by H3K27me3 without an adjacent S28p. These findings are in agreement with similar correlations cell culture systems and with the proposal that this modification promotes transcription.

These genome-wide findings were confirmed by more conventional methods for four transcription factors: *Atf3, Klf4, Npas4 and Hoxa2*. ChIP-qPCR confirmed the presence of H3K27me3 at their TSS in both hemisphere, and a large increase in H3K27me3S28p in the dopamine-depleted hemisphere following L-DOPA administration. Furthermore, the presence of this combinatorial mark was accompanied by a reduction of Rnf2, a component of PRC1, reflecting a dissociation of PcG proteins from chromatin. The expected increase in mRNA resulting from this derepression was confirmed for *Atf3, Klf4 and Npas4*, but not for *Hoxa2*, suggesting that this mechanism functions in conjunction with other facilitating factors to influence gene expression. In support of this idea, the presence of trimethylated lysine 4 on histone H3 (H3K4me3), a modification associated with facilitating transcription, was observed at the TSS of the three expressed genes but not at that for *Hoxa2*.

The presence of both the facilitating H3K4me3 and the repressive H3K27me3 modifications constitutes a bivalent domain, which is proposed to prevent non-specific induction and to prime genes for transcription upon appropriate stimuli[280]. Interestingly, these bivalent domains are plastic and individual modifications can be lost or gained during cellular differentiation to influence gene expression[280-282]. Although not directly addressed in this study, it is tempting to speculate that these exist in mature MSNs and that a similar plasticity may occur upon L-DOPA administration. For example, after L-DOPA administration, H3K27me3S28p is found at TSS in the dopamine-depleted striatum that are not marked by H3K27me3 in the intact striatum, possibly attributable to the induction of both a H3K27me3 and H3S28p modification. Furthermore, after acute L-DOPA administration, a subset of genes contained both H3K4me3 and H3K27me3S28p at their TSS, although their mRNAs were not induced. However, the same mRNA were induced by chronic L-DOPA administration, perhaps resulting from an exchange of H3K27me3 for a Polycomb-antagonizing acetylation[263]. This influence of transient histone phosphorylation on more stable methylation may underlie long-term changes in cellular properties and serve as an index of cellular history.

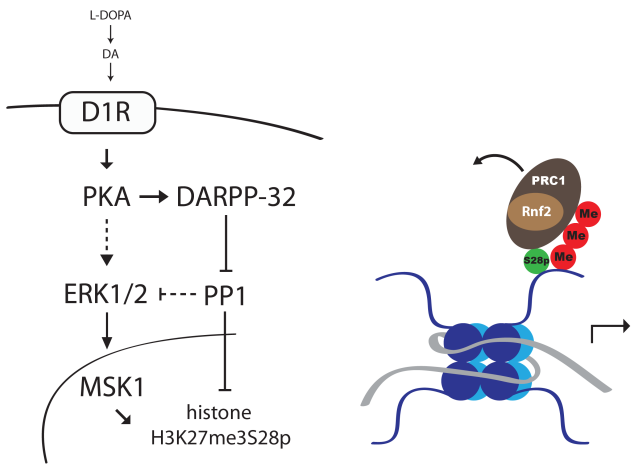

Figure 7. Summary of Paper III. Stimulation of the D1R promotes the induction of histone H3K27me3S28p by concerted activity of MSK1 and DARPP-32/PP1 (left). Concurrent to the presence of this mark is the displacement of Rnf2, a component of PRC1, and gene expression (right).

In cell culture systems, H3K27me3S28p is induced by MSK1/2[272]. Likewise, in the dopamine-depleted striatum, this modification was attenuated in MSK1 KO mice, confirming this kinase as a 'writer' of H3S28p. However, a greater reduction was observed in DARPP-32 T34A mutants, an effect larger than that expected based on its ability to attenuate ERK1/2, and therefore MSK1, activation. The nuclear accumulation of DARPP-32 promotes H3S10p in response to D1R stimulation, suggesting that a similar mechanism might promote the induction of H3K27me3S28p[283]. PP1 was capable of H3K27me3S28p dephosphorylation *in vitro*, and inhibition of PP1 in *ex vivo* striatal tissue was sufficient to promote its accumulation. These data suggest that PP1 acts as an 'eraser' of H3S28p and that PKA-mediated DARPP-32 phosphorylation, leading to inhibition of PP-1, act in concert with MSK1 to generate H3K27me3S28p (Fig. 7).

Taken together, the data in Paper III highlight that plasticity of PcG-repressed genes in terminally differentiated MSNs may contribute to the development of LID. They also indicate the existence of a dynamic epigenetic landscape that may affect the induction of genes following chronic L-DOPA administration.

CONCLUSION

The data presented within this thesis illustrate some of the molecular mechanisms by which L-DOPA administration interacts with sensitized D1Rs to promote LID. These events focus principally on intracellular signaling pathways regulating transcription; however, concurrent events implicated in LID also occur in the cytoplasm. Hyperactivity of mTORC1, a regulator of protein synthesis, promotes LID[220]. This effect could result from increased translation of currently present mRNA or translation of newly synthesized mRNA following L-DOPA administration. Additionally, possible alteration in the gene expression after chronic L-DOPA administration may result from stable neuroepigenetic changes and/or from the accumulation of long-lived transcription factors, such as ΔFosB. These transcriptional regulators may contribute to the basal collection of mRNA possibly translated following mTORC1 activation or may promote transcription following L-DOPA administration.

Additional cytoplasmic events result in abnormal corticostriatal transmission. Hyperactivity of the cAMP/DARPP-32/PP1 cascade aberrantly modulates glutamatergic

input, which may also be exaggerated by the increase in corticostriatal contacts associated with LID[284]. In LID, this sensitized pathway maintains a persistent potentiation of corticostiatal transmission despite de-potentiating stimuli[217,285]. This lack of plasticity may result in excessive integration or impaired segregation of corticostriatal transmission intended for motor learning[286]. Specifically, the sensorimotor striatum is necessary for habit formation and automatized behavior, which is absent in PD patients[287,288]. Interestingly, this form of learning is incremental and requires striatal dopaminergic transmission[289]. These characteristics mirror those of LID, which progresses in severity following L-DOPA administration. Therefore, sensitized D1R transmission may result from aberrant plasticity in MSNs intended for automatized motor output, potentially explaining the lack of voluntary control in LID.

Therapeutic interventions to alleviate LID may focus on both perturbed transcriptional and translational mechanisms. Pharmacological approaches targeted to epigenetic modifiers are promising therapeutic avenues for cancer could be repurposed for LID[290]. However, a greater understanding of the neuroepigenetic alterations, if any, is warranted. Additionally, systemic administration of these potential molecules is also likely to have unintended consequences on systems outside of select striatal MSNs, and their extended use may be limited. Ultimately, these maladaptive changes are a result of sensitized D1R signaling. Currently, the mechanism(s) behind this phenomenon remain to be fully elucidated, although the downstream consequences, such as those presented in this thesis, are actively investigated. It may be that events progressively further downstream of the D1R are proportionally less responsible for promoting LID, and interventions intending to prevent neurodegeneration or minimize the resulting D1R sensitization may represent additional effective therapies.

ACKNOWLEDGEMENTS

There are many people that I would like to acknowledge for helping me along the way. First and foremost is Gilberto, who has made this time an unforgettable experience, both scientifically and personally. It has been quite an adventure after jumping out into the unknown five years ago, and I could not have had such a supportive environment to develop and as great a time without you. To Mikael, thank you for being my co-supervisor, for your continuous advice and support, and for the hilarious conversations. Also to the numerous people, both past and present, that have spent time in the lab, thank you for creating such a dynamic environment in which to work. There are a number people to include in this group, and thank you Deb, Ale, Marion, Raj, Sophie, Giulia, Xiaochen, Helen, Emil, Emilie, Francesco and Francesca. Thank you Erik for initiating our collaboration, for introducing me to the world of epigenetics and for the stimulating scientific discussions. Thank you Simone for the enlightening conversations, about both science and life, and for the great times outside of the lab. Thank you Giada for tolerating my antics on a daily basis and for both the emotional and scientific support. And finally, thank you Caitlin for your patience and grounded perspective on life, without which this experience would certainly not have been the same.

Thank you to those at the NIH, including Andrew, Rosie, John and Marguerite, for introducing me to scientific research and for the continued support afterwards.

Thank you Kalle and Devesh for the numerous conversations over beers that probably did not help the body but eased the mind.

Lastly, an incredible thank you to my family for the encouragement to pursue my interests. Your lifelong support has made this moment possible, and although now is a celebration of my time here, it would have never been possible without your love from the beginning.

REFERENCES

1 Obeso, J. A., Rodriguez-Oroz, M. C., Stamelou, M., Bhatia, K. P. & Burn, D. J. The expanding universe of disorders of the basal ganglia. *Lancet*.

2 American Psychiatric Association: Diagnostic and Statistical Manual of Mental Disorders, F. E. A., VA, American Psychiatric Association, 2013. Web. [access date: 1 June 2013]. dsm.psychiatryonline.org.

3 Abdo, W. F., van de Warrenburg, B. P., Burn, D. J., Quinn, N. P. & Bloem, B. R. The clinical approach to movement disorders. *Nature Reviews Neurology* **6**, 29-37 (2010).

4 Nussbaum, R. L. & Ellis, C. E. Alzheimer's Disease and Parkinson's Disease. *New England Journal of Medicine* **348**, 1356-1364, (2003).

5 Parkinson, J. An essay on the shaking palsy. 1817. *J Neuropsychiatry Clin Neurosci* **14**, 223-236; discussion 222 (2002).

6 Nutt, J. G. & Wooten, G. F. Diagnosis and initial management of Parkinson's disease. *New England Journal of Medicine* **353**, 1021-1027 (2005).

7 Coelho, M. & Ferreira, J. J. Late-stage Parkinson disease. *Nature Reviews Neurology* **8**, 435-442 (2012).

8 Savitt, J. M., Dawson, V. L. & Dawson, T. M. Diagnosis and treatment of Parkinson disease: molecules to medicine. *Journal of Clinical Investigation* **116**, 1744-1754 (2006).

9 Klawans, H. L. Individual manifestations of Parkinson's disease after ten or more years of levodopa. *Movement Disorders* **1**, 187-192 (1986).

10 Chaudhuri, K. R., Healy, D. G., Schapira, A. H. & National Institute for Clinical, E. Non-motor symptoms of Parkinson's disease: diagnosis and management. *Lancet neurology* **5**, 235-245 (2006).

11 Goedert, M., Spillantini, M. G., Del Tredici, K. & Braak, H. 100 years of Lewy pathology. *Nature Reviews Neurology* **9**, 13-24 (2012).

12 Braak, H. *et al.* Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiology of aging* **24**, 197-211 (2003).

13 Forno, L. S. Neuropathology of Parkinson's disease. *J Neuropathol Exp Neurol* **55**, 259-272 (1996).

14 Trétiakoff, C. *Contribution a l'etude l'anatomie pathologique du locus Niger de soemmering: avec quelques déductions relatives à la pathogénie des troubles du tonus musculaire et de la maladie de Parkinson*, University of Paris, (1919).

15 Hassler, R. Zur Pathologie der Paralysis agitans und des postenzephalitischen Parkinsonismus. *J Psychol Neurol* **48**, 387-476 (1938).

16 Fearnley, J. M. & Lees, A. J. Ageing and Parkinson's disease: substantia nigra regional selectivity. *Brain : a journal of neurology* **114**, 2283-2301 (1991).

17 Damier, P., Hirsch, E., Agid, Y. & Graybiel, A. The substantia nigra of the human brain II. Patterns of loss of dopamine-containing neurons in Parkinson's disease. *Brain : a journal of neurology* **122**, 1437-1448 (1999).

18 Andén, N.-E. *et al.* Demonstration and mapping out of nigro-neostriatal dopamine neurons. *Life sciences* **3**, 523-530 (1964).

19 Kish, S. J., Shannak, K. & Hornykiewicz, O. Uneven pattern of dopamine loss in the striatum of patients with idiopathic Parkinson's disease. *New England Journal of Medicine* **318**, 876-880 (1988).

20 Björklund, A. & Dunnett, S. B. Dopamine neuron systems in the brain: an update. *Trends in neurosciences* **30**, 194-202 (2007).

21 Bertler, A. & Rosengren, E. Occurrence and distribution of dopamine in brain and other tissues. *Experientia* **15**, 10-11 (1959).

22 Ehringer, H. & Hornykiewicz, O. [Distribution of noradrenaline and dopamine (3-hydroxytyramine) in the human brain and their behavior in diseases of the extrapyramidal system]. *Klin Wochenschr* **38**, 1236-1239 (1960).

23 Ehringer, H. & Hornykiewicz, O. Distribution of noradrenaline and dopamine (3-hydroxytyramine) in the human brain and their behavior in diseases of the extrapyramidal system. *Parkinsonism Relat Disord* **4**, 53-57 (1998).

24 Langston, J. W., Ballard, P., Tetrud, J. W. & Irwin, I. Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. *Science* **219**, 979-980 (1983).

25 Ballard, P. A., Tetrud, J. W. & Langston, J. W. Permanent human parkinsonism due to 1-methy 1–4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) Seven cases. *Neurology* **35**, 949-949 (1985).

26 Birkmayer, W. & Hornykiewicz, O. [The L-3,4-dioxyphenylalanine (DOPA)-effect in Parkinson-akinesia]. *Wien Klin Wochenschr* **73**, 787-788 (1961).

27 Birkmayer, W. & Hornykiewicz, O. The effect of l-3,4-dihydroxyphenylalanine (=DOPA) on akinesia in parkinsonism. *Parkinsonism Relat Disord* **4**, 59-60 (1998).

28 Barbeau, A. The pathogenesis of Parkinson's disease: a new hypothesis. *Canadian Medical Association Journal* **87**, 802 (1962).

29 Cotzias, G. C., Van Woert, M. H. & Schiffer, L. M. Aromatic amino acids and modification of parkinsonism. *New England Journal of Medicine* **276**, 374-379 (1967).

30 Lloyd, K. G., Davidson, L. & Hornykiewicz, O. The neurochemistry of Parkinson's disease: effect of L-dopa therapy. *J Pharmacol Exp Ther* **195**, 453-464 (1975).

31 Cotzias, G. C., Papavasiliou, P. S. & Gellene, R. Modification of Parkinsonism—chronic treatment with L-dopa. *New England Journal of Medicine* **280**, 337-345 (1969).

32 Connolly, B. S. & Lang, A. E. Pharmacological Treatment of Parkinson Disease: A Review. *JAMA : the journal of the American Medical Association* **311**, 1670-1683 (2014).

33 de la Fuente-Fernández, R. *et al.* Levodopa-induced changes in synaptic dopamine levels increase with progression of Parkinson's disease: implications for dyskinesias. *Brain : a journal of neurology* **127**, 2747-2754 (2004).

34 Pavese, N. *et al.* Clinical correlates of levodopa-induced dopamine release in Parkinson disease A PET study. *Neurology* **67**, 1612-1617 (2006).

35 Tedroff, J. *et al.* Levodopa-induced changes in synaptic dopamine in patients with Parkinson's disease as measured by [11C] raclopride displacement and PET. *Neurology* **46**, 1430-1430 (1996).

36 Fahn, S. *et al.* Levodopa and the progression of Parkinson's disease. *The New England journal of medicine* **351**, 2498-2508 (2004).

37 Anderson, E. & Nutt, J. The long-duration response to levodopa: phenomenology, potential mechanisms and clinical implications. *Parkinsonism & related disorders* **17**, 587-592 (2011).

38 Bonnet, A.-M., Loria, Y., Saint-Hilaire, M.-H., Lhermitte, F. & Agid, Y. Does long-term aggravation of Parkinson's disease result from nondopaminergic lesions? *Neurology* **37**, 1539-1539 (1987).

39 Hely, M. A., Morris, J. G., Reid, W. G. & Trafficante, R. Sydney multicenter study of Parkinson's disease: Non-L-dopa–responsive problems dominate at 15 years. *Movement Disorders* **20**, 190-199 (2005).

40 Voon, V. *et al.* Chronic dopaminergic stimulation in Parkinson's disease: from dyskinesias to impulse control disorders. *The Lancet Neurology* **8**, 1140-1149 (2009).

41 Jankovic, J. Motor fluctuations and dyskinesias in Parkinson's disease: clinical manifestations. *Movement Disorders* **20**, S11-S16 (2005).

42 Fabbrini, G., Brotchie, J. M., Grandas, F., Nomoto, M. & Goetz, C. G. Levodopa-induced dyskinesias. *Movement Disorders* **22**, 1379-1389 (2007).

43 Newman, R. P., LeWitt, P. A., Jaffe, M., Calne, D. B. & Larsen, T. A. Motor function in the norimal aging population Treatment with levodopa. *Neurology* **35**, 571-571 (1985).

44 Tolosa, E. S., Martin, W. E., Cohen, H. P. & Jacobson, R. L. Patterns of clinical response and plasma dopa levels in Parkinson's disease. *Neurology* **25**, 177-177 (1975).

45 Barbeau, A. L-dopa therapy in Parkinson's disease: a critical review of nine years' experience. *Canadian Medical Association Journal* **101**, 59 (1969).

46 Godwin-Austen, R., Frears, C., Tomlinson, E. & Kok, H. Effects of L-dopa in Parkinson's disease. *The Lancet* **294**, 165-168 (1969).

47 Mouradian, M. *et al.* Motor fluctuations in Parkinson's disease: central pathophysiological mechanisms, part II. *Annals of neurology* **24**, 372-378 (1988).

48 Calabresi, P., Di Filippo, M., Ghiglieri, V., Tambasco, N. & Picconi, B. Levodopa-induced dyskinesias in patients with Parkinson's disease: filling the bench-to-bedside gap. *Lancet neurology* **9**, 1106-1117 (2010).

49 Munhoz, R. P., Cerasa, A. & Okun, M. S. Surgical treatment of dyskinesia in Parkinson's disease. *Frontiers in neurology* **5** (2014).

50 Gradinaru, V., Mogri, M., Thompson, K. R., Henderson, J. M. & Deisseroth, K. Optical deconstruction of parkinsonian neural circuitry. *Science* **324**, 354-359 (2009).

51 Metman, L. V. *et al.* Amantadine as treatment for dyskinesias and motor fluctuations in Parkinson's disease. *Neurology* **50**, 1323-1326 (1998).

52 Snow, B. J., Macdonald, L., Mcauley, D. & Wallis, W. The effect of amantadine on levodopa-induced dyskinesias in Parkinson's disease: a double-blind, placebo-controlled study. *Clinical neuropharmacology* **23**, 82-85 (2000).

53 Del Dotto, P. *et al.* Intravenous amantadine improves levadopa-induced dyskinesias: An acute double-blind placebo-controlled study. *Movement Disorders* **16**, 515-520 (2001).

54 Pereira da Silva-Júnior, F., Braga-Neto, P., Sueli Monte, F. & Meireles Sales de Bruin, V. Amantadine reduces the duration of levodopa-induced dyskinesia: a randomized, double-blind, placebo-controlled study. *Parkinsonism & related disorders* **11**, 449-452 (2005).

55 Luginger, E., Wenning, G., Bösch, S. & Poewe, W. Beneficial effects of amantadine on L-dopa-induced dyskinesias in Parkinson's disease. *Movement Disorders* **15**, 873-878 (2000).

56 Metman, L. V. *et al.* Amantadine for levodopa-induced dyskinesias: a 1-year follow-up study. *Archives of neurology* **56**, 1383-1386 (1999).

57 Thomas, A. *et al.* Duration of amantadine benefit on dyskinesia of severe Parkinson's disease. *Journal of Neurology, Neurosurgery & Psychiatry* **75**, 141-143 (2004).

58 Wolf, E. *et al.* Long-term antidyskinetic efficacy of amantadine in Parkinson's disease. *Movement Disorders* **25**, 1357-1363 (2010).

59 Ahmed, I. *et al.* Glutamate NMDA receptor dysregulation in Parkinson's disease with dyskinesias. *Brain : a journal of neurology*, awr028 (2011).

60 Roth, B. L., Sheffler, D. J. & Kroeze, W. K. Magic shotguns versus magic bullets: selectively non-selective drugs for mood disorders and schizophrenia. *Nature Reviews Drug Discovery* **3**, 353-359 (2004).

61 Durif, F. *et al.* Clozapine improves dyskinesias in Parkinson disease A double-blind, placebo-controlled study. *Neurology* **62**, 381-388 (2004).

62 Durif, F. *et al.* Low-dose clozapine improves dyskinesias in Parkinson's disease. *Neurology* **48**, 658-662 (1997).

63 Bennett, J. P., Landow, E. R., Dietrich, S. & Schuh, L. A. Suppression of dyskinesias in advanced Parkinson's disease: Moderate daily clozapine doses provide long-term dyskinesia reduction. *Movement Disorders* **9**, 409-414 (1994).

64 Bennett, J. P., Landow, E. R. & Schuh, L. A. Suppression of dyskinesias in advanced Parkinson's disease II. Increasing daily clozapine doses suppress dyskinesias and improve parkinsonism symptoms. *Neurology* **43**, 1551-1551 (1993).

65 Pierelli, F. *et al.* Low dosage clozapine effects on L-dopa induced dyskinesias in parkinsonian patients. *Acta neurologica scandinavica* **97**, 295-299 (1998).

66 Group, P. S. Pramipexole vs levodopa as initial treatment for Parkinson disease: a randomized controlled trial. *JAMA : the journal of the American Medical Association* **284**, 1931-1938 (2000).

67 Wooten, G. Agonists vs levodopa in PD The thrilla of whitha. *Neurology* **60**, 360-362 (2003).

68 Lees, A., Katzenschlager, R., Head, J. & Ben–Shlomo, Y. Ten-year follow-up of three different initial treatments in de-novo PD A randomized trial. *Neurology* **57**, 1687-1694 (2001).

69 Rascol, O. *et al.* 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**, 1484-1491 (2000).

70 Holloway, R. *et al.* Pramipexole vs levodopa as initial treatment for Parkinson disease: a 4-year randomized controlled trial. *Archives of neurology* **61**, 1044-1053 (2004).

71 Katzenschlager, R. *et al.* Fourteen-year final report of the randomized PDRG-UK trial comparing three initial treatments in PD. *Neurology* **71**, 474-480 (2008).

72 Rascol, O. *et al.* Development of dyskinesias in a 5-year trial of ropinirole and L-dopa. *Movement Disorders* **21**, 1844-1850 (2006).

73 Constantinescu, R., Romer, M., McDermott, M. P., Kamp, C. & Kieburtz, K. Impact of pramipexole on the onset of levodopa-related dyskinesias. *Movement Disorders* **22**, 1317-1319 (2007).

74 Kurlan, R. "Levodopa phobia": A new iatrogenic cause of disability in Parkinson disease. *Neurology* **64**, 923-924 (2005).

75 Weiner, W. J. & Reich, S. G. Agonist or levodopa for Parkinson disease? Ultimately, it doesn't matter; neither is good enough. *Neurology* **71**, 470-471 (2008).

76 Vlaar, A., Hovestadt, A., van Laar, T. & Bloem, B. R. The treatment of early Parkinson's disease: levodopa rehabilitated. *Practical neurology* **11**, 145-152 (2011).

77 Lang, A. E. & Marras, C. Initiating dopaminergic treatment in Parkinson's disease. *The Lancet* (2014).

78 Fox, S. H. & Lang, A. E. 'Don't delay, start today': delaying levodopa does not delay motor complications. *Brain : a journal of neurology* **137**, 2628-2630 (2014).

79 Nadjar, A., Gerfen, C. R. & Bezard, E. Priming for l-dopa-induced dyskinesia in Parkinson's disease: a feature inherent to the treatment or the disease? *Progress in neurobiology* **87**, 1-9 (2009).

80 Langston, J. & Ballard, P. Parkinsonism induced by 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP): implications for treatment and the pathogenesis of Parkinson's disease. *The Canadian journal of neurological sciences. Le journal canadien des sciences neurologiques* **11**, 160-165 (1984).

81 Cilia, R. *et al.* The modern pre-levodopa era of Parkinson's disease: insights into motor complications from sub-Saharan Africa. *Brain : a journal of neurology*, awu195 (2014).

82 Kostić, V., Marinković, J., Svetel, M., Stefanova, E. & Przedborski, S. The effect of stage of Parkinson's disease at the onset of levodopa therapy on development of motor complications. *European Journal of Neurology* **9**, 9-14 (2002).

83 Olanow, C. W., Obeso, J. A. & Stocchi, F. Drug insight: Continuous dopaminergic stimulation in the treatment of Parkinson's disease. *Nature clinical practice. Neurology* **2**, 382-392 (2006).

84 Lebel, M., Chagniel, L., Bureau, G. & Cyr, M. Striatal inhibition of PKA prevents levodopa-induced behavioural and molecular changes in the hemiparkinsonian rat. *Neurobiology of disease* **38**, 59-67 (2010).

85 Bjorklund, T., Carlsson, T., Cederfjall, E. A., Carta, M. & Kirik, D. Optimized adeno-associated viral vector-mediated striatal DOPA delivery restores sensorimotor function and prevents dyskinesias in a model of advanced Parkinson's disease. *Brain : a journal of neurology* **133**, 496-511 (2010).

86 Jarraya, B. *et al.* Dopamine gene therapy for Parkinson's disease in a nonhuman primate without associated dyskinesia. *Sci Transl Med* **1** (2009).

87 Papathanou, M., van der Laan, R., Jenner, P., Rose, S. & McCreary, A. C. Levodopa infusion does not decrease the onset of abnormal involuntary movements in parkinsonian rats. *Mov Disord* **28**, 1072-1079 (2013).

88 Carlsson, T. *et al.* Reversal of dyskinesias in an animal model of Parkinson's disease by continuous L-DOPA delivery using rAAV vectors. *Brain : a journal of neurology* **128**, 559-569 (2005).

89 Gerfen, C. R. *et al.* D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. *Science* **250**, 1429-1432 (1990).

90 Olanow, C. W. *et al.* Continuous intrajejunal infusion of levodopa-carbidopa intestinal gel for patients with advanced Parkinson's disease: a randomised, controlled, double-blind, double-dummy study. *The Lancet Neurology* **13**, 141-149 (2014).

91 Palfi, S. *et al.* Long-term safety and tolerability of ProSavin, a lentiviral vector-based gene therapy for Parkinson's disease: a dose escalation, open-label, phase 1/2 trial. *The Lancet* **383**, 1138-1146 (2014).

92 Eberling, J. *et al.* Results from a phase I safety trial of hAADC gene therapy for Parkinson disease. *Neurology* **70**, 1980-1983 (2008).

93 Kopin, I. & Markey, S. MPTP toxicity: implications for research in Parkinson's disease. *Annual review of neuroscience* **11**, 81-96 (1988).

94 Kostrzewa, R. M. & Jacobowitz, D. M. Pharmacological actions of 6-hydroxydopamine. *Pharmacological reviews* **26**, 199-288 (1974).

95 Blum, D. *et al.* Molecular pathways involved in the neurotoxicity of 6-OHDA, dopamine and MPTP: contribution to the apoptotic theory in Parkinson's disease. *Progress in neurobiology* **65**, 135-172 (2001).

96 Moratalla, R. *et al.* Differential vulnerability of primate caudate-putamen and striosome-matrix dopamine systems to the neurotoxic effects of 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine. *Proceedings of the National Academy of Sciences* **89**, 3859-3863 (1992).

97 Clarke, C., Sambrook, M., Mitchell, I. & Crossman, A. Levodopa-induced dyskinesia and response fluctuations in primates rendered parkinsonian with 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP). *Journal of the neurological sciences* **78**, 273-280 (1987).

98 Ungerstedt, U. 6-Hydroxy-dopamine induced degeneration of central monoamine neurons. *European journal of pharmacology* **5**, 107-110 (1968).

99 Cenci, M. A. & Lundblad, M. Ratings of L-DOPA-Induced Dyskinesia in the Unilateral 6-OHDA Lesion Model of Parkinson's Disease in Rats and Mice. *Current protocols in Neuroscience*, 9.25. 21-29.25. 23 (2007).

100 Hornykiewicz, O. L-DOPA: from a biologically inactive amino acid to a successful therapeutic agent. *Amino acids* **23**, 65-70 (2002).

101 Wichmann, T., DeLong, M. R., Guridi, J. & Obeso, J. A. Milestones in research on the pathophysiology of Parkinson's disease. *Movement Disorders* **26**, 1032-1041 (2011).

102 Stephenson-Jones, M., Samuelsson, E., Ericsson, J., Robertson, B. & Grillner, S. Evolutionary conservation of the basal ganglia as a common vertebrate mechanism for action selection. *Current Biology* **21**, 1081-1091 (2011).

103 Smith, Y., Wichmann, T. & DeLong, M. R. Corticostriatal and mesocortical dopamine systems: do species differences matter? *Nature Reviews Neuroscience* (2013).

104 Kramer, P. F. *et al.* Dopamine D2 receptor overexpression alters behavior and physiology in Drd2-EGFP mice. *The Journal of Neuroscience* **31**, 126-132 (2011).

105 Gerlai, R. Gene-targeting studies of mammalian behavior: is it the mutation or the background genotype? *Trends in neurosciences* **19**, 177-181 (1996).

106 Perl, D. P. Neuropathologic Involvement of the Dopaminergic Neuronal Systems in Parkinson's Disease. *Parkinson's Disease: Non-Motor and Non-Dopaminergic Features*, 7-14 (2011).

107 Gerfen, C. R. 2.1 Functional Neuroanatomy of Dopamine in the Striatum. *Dopamine handbook*, 11 (2010).

108 den Heuvel, V., Dianne, M. & Pasterkamp, R. J. Getting connected in the dopamine system. *Progress in neurobiology* **85**, 75-93 (2008).

109 Wilson, C. J. Basal ganglia. *The synaptic organization of the brain* **5**, 361-414 (2004).

110 Descarries, L., Watkins, K. C., Garcia, S., Bosler, O. & Doucet, G. Dual character, asynaptic and synaptic, of the dopamine innervation in adult rat neostriatum: A quantitative autoradiographic and immunocytochemical analysis. *The Journal of Comparative Neurology* **375**, 167-186 (1996).

111 Agnati, L., Zoli, M., Strömberg, I. & Fuxe, K. Intercellular communication in the brain: wiring versus volume transmission. *Neuroscience* **69**, 711-726 (1995).

112 Rice, M. E., Patel, J. C. & Cragg, S. J. Dopamine release in the basal ganglia. *Neuroscience* **198**, 112-137 (2011).

113 Cheramy, A., Leviel, V. & Glowinski, J. Dendritic release of dopamine in the substantia nigra. *Nature* **289**, 537-543 (1981).

114 Dupuis, J. P. *et al.* Dopamine-dependent long-term depression at subthalamo-nigral synapses is lost in experimental parkinsonism. *The Journal of Neuroscience* **33**, 14331-14341 (2013).

115 Navailles, S., Bioulac, B., Gross, C. & De Deurwaerdère, P. Chronic L-DOPA therapy alters central serotonergic function and L-DOPA-induced dopamine release in a region-dependent manner in a rat model of Parkinson's disease. *Neurobiology of disease* **41**, 585-590 (2011).

116 Carta, M. & Tronci, E. Serotonin system implication in L-DOPA-induced dyskinesia: from animal models to clinical investigations. *Frontiers in neurology* **5** (2014).

117 Ng, K., Chase, T., Colburn, R. & Kopin, I. Dopamine: stimulation-induced release from central neurons. *Science* **172**, 487-489 (1971).

118 Carta, M., Carlsson, T., Kirik, D. & Björklund, A. Dopamine released from 5-HT terminals is the cause of L-DOPA-induced dyskinesia in parkinsonian rats. *Brain : a journal of neurology* **130**, 1819-1833 (2007).

119 Politis, M. *et al.* Serotonergic mechanisms responsible for levodopa-induced dyskinesias in Parkinson's disease patients. *The Journal of clinical investigation* **124**, 1340-1349 (2014).

120 Yung, K. K. *et al.* Immunocytochemical localization of D1 and D2 dopamine receptors in the basal ganglia of the rat: light and electron microscopy. *Neuroscience* **65**, 709-730 (1995).

121 Sibley, D. R. & Monsma Jr, F. J. Molecular biology of dopamine receptors. *Trends in pharmacological sciences* **13**, 61-69 (1992).

122 RANKIN, M. L. *et al.* 3.1 Molecular Pharmacology of the Dopamine Receptors. *Dopamine handbook*, 63 (2010).

123 Caron, M. G. & Gainetdinov, R. R. 3.2 Role of Dopamine Transporters in Neuronal Homeostasis. *Dopamine handbook*, 88 (2010).

124 Youdim, M. B. H., Edmondson, D. & Tipton, K. F. The therapeutic potential of monoamine oxidase inhibitors. *Nat Rev Neurosci* **7**, 295-309 (2006).

125 Fowler, J. *et al.* Mapping human brain monoamine oxidase A and B with 11C-labeled suicide inactivators and PET. *Science* **235**, 481-485 (1987).

126 Westlund, K., Denney, R., Kochersperger, L., Rose, R. & Abell, C. Distinct monoamine oxidase A and B populations in primate brain. *Science* **230**, 181-183 (1985).

127 Männistö, P. T. & Kaakkola, S. Catechol-O-methyltransferase (COMT): biochemistry, molecular biology, pharmacology, and clinical efficacy of the new selective COMT inhibitors. *Pharmacological reviews* **51**, 593-628 (1999).

128 Kastner, A. *et al.* Immunohistochemical study of catechol-O-methyltransferase in the human mesostriatal system. *Neuroscience* **62**, 449-457 (1994).

129 Karoum, F., Chrapusta, S. J. & Egan, M. F. 3-Methoxytyramine Is the Major Metabolite of Released Dopamine in the Rat Frontal Cortex: Reassessment of the Effects of Antipsychotics on the Dynamics of

Dopamine Release and Metabolism in the Frontal Cortex, Nucleus Accumbens, and Striatum by a Simple Two Pool Model. *Journal of Neurochemistry* **63**, 972-979 (1994).

130 Rajput, A. H. *et al.* Human brain dopamine metabolism in levodopa-induced dyskinesia and wearing-off. *Parkinsonism & related disorders* **10**, 221-226 (2004).

131 Alexander, G. E., DeLong, M. R. & Strick, P. L. Parallel organization of functionally segregated circuits linking basal ganglia and cortex. *Annual review of neuroscience* **9**, 357-381 (1986).

132 Middleton, F. A. & Strick, P. L. Basal ganglia and cerebellar loops: motor and cognitive circuits. *Brain research. Brain research reviews* **31**, 236-250 (2000).

133 Rascol, O. *et al.* Cortical motor overactivation in parkinsonian patients with L-dopa-induced peak-dose dyskinesia. *Brain : a journal of neurology* **121**, 527-533 (1998).

134 Redgrave, P. *et al.* Goal-directed and habitual control in the basal ganglia: implications for Parkinson's disease. *Nature Reviews Neuroscience* **11**, 760-772 (2010).

135 Albin, R. L., Young, A. B. & Penney, J. B. The functional anatomy of basal ganglia disorders. *Trends in neurosciences* **12**, 366-375 (1989).

136 McHaffie, J. G., Stanford, T. R., Stein, B. E., Coizet, V. & Redgrave, P. Subcortical loops through the basal ganglia. *Trends in neurosciences* **28**, 401-407 (2005).

137 Nelson, A. B. & Kreitzer, A. C. Reassessing Models of Basal Ganglia Function and Dysfunction. *Annual review of neuroscience* **37**, 117-135 (2014).

138 Kravitz, A. V. *et al.* Regulation of parkinsonian motor behaviours by optogenetic control of basal ganglia circuitry. *Nature* **466**, 622-626 (2010).

139 Durieux, P. F., Schiffmann, S. N. & de Kerchove d'Exaerde, A. Differential regulation of motor control and response to dopaminergic drugs by D1R and D2R neurons in distinct dorsal striatum subregions. *The EMBO journal* **31**, 640-653 (2012).

140 Bateup, H. S. *et al.* Distinct subclasses of medium spiny neurons differentially regulate striatal motor behaviors. *Proceedings of the National Academy of Sciences* **107**, 14845-14850 (2010).

141 Gerfen, C. R. & Surmeier, D. J. Modulation of striatal projection systems by dopamine. *Annual review of neuroscience* **34**, 441 (2011).

142 Crittenden, J. R. & Graybiel, A. M. Basal Ganglia disorders associated with imbalances in the striatal striosome and matrix compartments. *Frontiers in neuroanatomy* **5** (2011).

143 Kreitzer, A. C. Physiology and pharmacology of striatal neurons. *Annual review of neuroscience* **32**, 127-147 (2009).

144 Paxinos, G. & **Franklin, K. B.** *The mouse brain in stereotaxic coordinates*. (Gulf Professional Publishing, 2004).

145 Eilam, D. & Szechtman, H. Biphasic effect of D-2 agonist quinpirole on locomotion and movements. *European journal of pharmacology* **161**, 151-157 (1989).

146 Arnt, J., Hyttel, J. & Sánchez, C. Partial and full dopamine D1 receptor agonists in mice and rats: relation between behavioural effects and stimulation of adenylate cyclase activity in vitro. *European journal of pharmacology* **213**, 259-267 (1992).

147 Robertson, G. S. & Robertson, H. A. Synergistic effects of D1 and D2 dopamine agonists on turning behaviour in rats. *Brain research* **384**, 387-390 (1986).

148 Boraud, T., Bezard, E., Bioulac, B. & Gross, C. E. From single extracellular unit recording in experimental and human Parkinsonism to the development of a functional concept of the role played by the basal ganglia in motor control. *Progress in neurobiology* **66**, 265-283 (2002).

149 Wichmann, T. & DeLong, M. R. Pathophysiology of Parkinson's disease: the MPTP primate model of the human disorder. *Annals of the New York Academy of Sciences* **991**, 199-213 (2003).

150 Hutchison, W., Levy, R., Dostrovsky, J., Lozano, A. & Lang, A. Effects of apomorphine on globus pallidus neurons in parkinsonian patients. *Annals of neurology* **42**, 767-775 (1997).

151 Stefani, A. *et al.* Effects of increasing doses of apomorphine during stereotaxic neurosurgery in Parkinson's disease: clinical score and internal globus pallidus activity. *Journal of neural transmission* **104**, 895-904 (1997).

152 Lozano, A. M., Lang, A. E., Levy, R., Hutchison, W. & Dostrovsky, J. Neuronal recordings in Parkinson's disease patients with dyskinesias induced by apomorphine. *Annals of neurology* **47**, S141-146 (2000).

153 Filion, M. & Tremblay, L. Abnormal spontaneous activity of globus pallidus neurons in monkeys with MPTP-induced parkinsonism. *Brain research* **547**, 140-144 (1991).

154 Filion, M., Tremblay, L. & Bédard, P. J. Effects of dopamine agonists on the spontaneous activity of globus pallidus neurons in monkeys with MPTP-induced parkinsonism. *Brain research* **547**, 145-149 (1991).

155 Miller, W. C. & DeLong, M. R. in *The basal ganglia II*, 415-427 (Springer, 1987).

156 Levy, R. *et al.* Lidocaine and muscimol microinjections in subthalamic nucleus reverse Parkinsonian symptoms. *Brain : a journal of neurology* **124**, 2105-2118 (2001).

157 Obeso, J. A. *et al.* The basal ganglia in Parkinson's disease: current concepts and unexplained observations. *Annals of neurology* **64**, S30-S46 (2008).

158 Obeso, J. A. *et al.* Pathophysiology of the basal ganglia in Parkinson's disease. *Trends in neurosciences* **23**, S8-S19 (2000).

159 Taverna, S., Ilijic, E. & Surmeier, D. J. Recurrent collateral connections of striatal medium spiny neurons are disrupted in models of Parkinson's disease. *The Journal of Neuroscience* **28**, 5504-5512 (2008).

160 Nambu, A., Tokuno, H. & Takada, M. Functional significance of the cortico–subthalamo–pallidal 'hyperdirect'pathway. *Neuroscience research* **43**, 111-117 (2002).

161 Cui, G. *et al.* Concurrent activation of striatal direct and indirect pathways during action initiation. *Nature* **494**, 238-242 (2013).

162 Calabresi, P., Picconi, B., Tozzi, A., Ghiglieri, V. & Di Filippo, M. Direct and indirect pathways of basal ganglia: a critical reappraisal. *Nature neuroscience* **17**, 1022-1030 (2014).

163 Lindvall, O. & Björklund, A. Dopaminergic innervation of the globus pallidus by collaterals from the nigrostriatal pathway. *Brain research* **172**, 169-173 (1979).

164 Tritsch, N. X. & Sabatini, B. L. Dopaminergic modulation of synaptic transmission in cortex and striatum. *Neuron* **76**, 33-50 (2012).

165 Levey, A. I. *et al.* Localization of D1 and D2 dopamine receptors in brain with subtype-specific antibodies. *Proceedings of the National Academy of Sciences* **90**, 8861-8865 (1993).

166 Prensa, L., Giménez-Amaya, J. M. & Parent, A. Chemical heterogeneity of the striosomal compartment in the human striatum. *Journal of Comparative Neurology* **413**, 603-618 (1999).

167 Graybiel, A. M. Neurotransmitters and neuromodulators in the basal ganglia. *Trends in neurosciences* **13**, 244-254 (1990).

168 Eblen, F. & Graybiel, A. M. Highly restricted origin of prefrontal cortical inputs to striosomes in the macaque monkey. *J Neurosci* **15**, 5999-6013 (1995).

169 Donoghue, J. P. & Herkenham, M. Neostriatal projections from individual cortical fields conform to histochemically distinct striatal compartments in the rat. *Brain Res* **365**, 397-403 (1986).

170 Graybiel, A. M., Canales, J. J. & Capper-Loup, C. Levodopa-induced dyskinesias and dopamine-dependent stereotypies: a new hypothesis. *Trends in neurosciences* **23**, S71-S77 (2000).

171 Gerfen, C. R. The neostriatal mosaic: compartmentalization of corticostriatal input and striatonigral output systems. *Nature* **311**, 461-464 (1984).

172 Cenci, M., Tranberg, A., Andersson, M. & Hilbertson, A. Changes in the regional and compartmental distribution of FosB-and JunB-like immunoreactivity induced in the dopamine-denervated rat striatum by acute or chronic L-dopa treatment. *Neuroscience* **94**, 515-527 (1999).

173 Saka, E., Elibol, B., Erdem, S. & Dalkara, T. Compartmental changes in expression of c-Fos and FosB proteins in intact and dopamine-depleted striatum after chronic apomorphine treatment. *Brain research* **825**, 104-114 (1999).

174 Crittenden, J. R. *et al.* Dysregulation of CalDAG-GEFI and CalDAG-GEFII predicts the severity of motor side-effects induced by anti-parkinsonian therapy. *Proceedings of the National Academy of Sciences* **106**, 2892-2896 (2009).

175 Henry, B., Duty, S., Fox, S. H., Crossman, A. R. & Brotchie, J. M. Increased striatal pre-proenkephalin B expression is associated with dyskinesia in Parkinson's disease. *Experimental neurology* **183**, 458-468 (2003).

176 Koprich, J. B. *et al.* 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**, 1225-1233 (2011).

177 Thornburg, J. & Moore, K. Supersensitivity to dopamine agonists following unilateral, 6-hydroxydopamine-induced striatal lesions in mice. *Journal of Pharmacology and Experimental Therapeutics* **192**, 42-49 (1975).

178 Silverman, P. B. Sensitization and conditioned rotation: apomorphine, quinpirole and SKF-38393 compared. *Neuroreport* **2**, 669-672 (1991).

179 Lundblad, M. *et al.* Pharmacological validation of a mouse model of l-DOPA-induced dyskinesia. *Experimental neurology* **194**, 66-75 (2005).

180 Carta, A. R. *et al.* Behavioral and biochemical correlates of the dyskinetic potential of dopaminergic agonists in the 6-OHDA lesioned rat. *Synapse* **62**, 524-533 (2008).

181 Westin, J. E., Vercammen, L., Strome, E. M., Konradi, C. & Cenci, M. A. Spatiotemporal pattern of striatal ERK1/2 phosphorylation in a rat model of L-DOPA–induced dyskinesia and the role of dopamine D1 receptors. *Biological psychiatry* **62**, 800-810 (2007).

182 Darmopil, S., Martín, A. B., De Diego, I. R., Ares, S. & Moratalla, R. Genetic inactivation of dopamine d1 but not d2 receptors inhibits l-dopa–induced dyskinesia and histone activation. *Biological psychiatry* **66**, 603-613 (2009).

183 Monville, C., Torres, E. M. & Dunnett, S. B. Validation of the l-dopa-induced dyskinesia in the 6-OHDA model and evaluation of the effects of selective dopamine receptor agonists and antagonists. *Brain research bulletin* **68**, 16-23 (2005).

184 Taylor, J. L., Bishop, C. & Walker, P. D. Dopamine D1 and D2 receptor contributions to L-DOPA-induced dyskinesia in the dopamine-depleted rat. *Pharmacology Biochemistry and Behavior* **81**, 887-893 (2005).

185 Révy, D. *et al.* Cellular and Behavioral Outcomes of Dorsal Striatonigral Neuron Ablation: New Insights into Striatal Functions. *Neuropsychopharmacology: official publication of the American College of Neuropsychopharmacology* (2014).

186 Rascol, O. *et al.* Induction by dopamine D1 receptor agonist ABT-431 of dyskinesia similar to levodopa in patients with Parkinson disease. *Archives of neurology* **58**, 249-254 (2001).

187 Calon, F. *et al.* Chronic D1 and D2 dopaminomimetic treatment of MPTP-denervated monkeys: effects on basal ganglia $GABA_A$/benzodiazepine receptor complex and GABA content. *Neurochemistry international* **35**, 81-91 (1999).

188 Luquin, M. R., Laguna, J. & Obeso, J. A. Selective D2 receptor stimulation induces dyskinesia in parkinsonian monkeys. *Annals of neurology* **31**, 551-554 (1992).

189 Rascol, O. *et al.* ABT-431, a D1 receptor agonist prodrug, has efficacy in Parkinson's disease. *Annals of neurology* **45**, 736-741 (1999).

190 Grondin, R., Grégoire, L. & Bédard, P. J. D1 receptor blockade improves L-dopa–induced dyskinesia but worsens parkinsonism in MPTP monkeys. *Neurology* **52**, 771-771 (1999).

191 Monsma, F. J., Mahan, L. C., McVittie, L. D., Gerfen, C. R. & Sibley, D. R. Molecular cloning and expression of a D1 dopamine receptor linked to adenylyl cyclase activation. *Proceedings of the National Academy of Sciences* **87**, 6723-6727 (1990).

192 Drinnan, S., Hope, B., Snutch, T. & Vincent, S. G olf in the basal ganglia. *Molecular and Cellular Neuroscience* **2**, 66-70 (1991).

193 Herve, D. *et al.* G (olf) and Gs in rat basal ganglia: possible involvement of G (olf) in the coupling of dopamine D1 receptor with adenylyl cyclase. *The Journal of Neuroscience* **13**, 2237-2248 (1993).

194 Corvol, J., Studler, J., Schonn, J., Girault, J. & Herve, D. Gαolf is necessary for coupling D1 and A2a receptors to adenylyl cyclase in the striatum. *Journal of Neurochemistry* **76**, 1585-1588 (2001).

195 Zhuang, X., Belluscio, L. & Hen, R. Golf mediates dopamine D1 receptor signaling. *J Neurosci* **20**, 1-5 (2000).

196 Glatt, C. E. & Snyder, S. H. Cloning and expression of an adenylyl cyclase localized to the corpus striatum. (1993).

197 Lee, K.-W. *et al.* Impaired D2 dopamine receptor function in mice lacking type 5 adenylyl cyclase. *The Journal of Neuroscience* **22**, 7931-7940 (2002).

198 Brandon, E. P., Idzerda, R. L. & McKnight, G. S. PKA isoforms, neural pathways, and behaviour: making the connection. *Current opinion in neurobiology* **7**, 397-403 (1997).

199 Aubert, I. *et al.* Increased D1 dopamine receptor signaling in levodopa-induced dyskinesia. *Annals of neurology* **57**, 17-26 (2005).

200 Irannejad, R. & von Zastrow, M. GPCR signaling along the endocytic pathway. *Current opinion in cell biology* **27**, 109-116 (2014).

201 Porras, G. *et al.* PSD-95 expression controls L-DOPA dyskinesia through dopamine D1 receptor trafficking. *J Clin Invest* **122**, 3977-3989 (2012).

202 Guigoni, C., Doudnikoff, E., Li, Q., Bloch, B. & Bezard, E. Altered D(1) dopamine receptor trafficking in parkinsonian and dyskinetic non-human primates. *Neurobiol Dis* **26**, 452-463 (2007).

203 Berthet, A. *et al.* Pharmacological analysis demonstrates dramatic alteration of D1 dopamine receptor neuronal distribution in the rat analog of L-DOPA-induced dyskinesia. *J Neurosci* **29**, 4829-4835 (2009).

204 Fiorentini, C. *et al.* Loss of synaptic D1 dopamine/N-methyl-D-aspartate glutamate receptor complexes in L-DOPA-induced dyskinesia in the rat. *Mol Pharmacol* **69**, 805-812 (2006).

205 Turjanski, N., Lees, A. & Brooks, D. In vivo studies on striatal dopamine D1 and D2 site binding in L-dopa-treated Parkinson's disease patients with and without dyskinesias. *Neurology* **49**, 717-723 (1997).

206 Alcacer, C. *et al.* Gαolf Mutation Allows Parsing the Role of cAMP-Dependent and Extracellular Signal-Regulated Kinase-Dependent Signaling in l-3, 4-Dihydroxyphenylalanine-Induced Dyskinesia. *The Journal of Neuroscience* **32**, 5900-5910 (2012).

207 Penit-Soria, J., Durand, C., Besson, M. & Herve, D. Levels of stimulatory G protein are increased in the rat striatum after neonatal lesion of dopamine neurons. *Neuroreport* **8**, 829-833 (1997).

208 Rangel-Barajas, C. *et al.* l-DOPA-induced dyskinesia in hemiparkinsonian rats is associated with up-regulation of adenylyl cyclase type V/VI and increased GABA release in the substantia nigra reticulata. *Neurobiology of disease* **41**, 51-61 (2011).

209 Corvol, J.-C. *et al.* Persistent increase in olfactory type G-protein α subunit levels may underlie D1 receptor functional hypersensitivity in Parkinson disease. *The Journal of Neuroscience* **24**, 7007-7014 (2004).

210 Pifl, C., Nanoff, C., Schingnitz, G., Schütz, W. & Hornykiewicz, O. Sensitization of Dopamine-Stimulated Adenylyl Cyclase in the Striatum of 1-Methyl-4-Phenyl-1, 2, 3, 6-Tetrahydropyridine-Treated Rhesus Monkeys and Patients with Idiopathic Parkinson's Disease. *Journal of Neurochemistry* **58**, 1997-2004 (1992).

211 Park, H.-Y. *et al.* Inhibition of Adenylyl Cyclase Type 5 Prevents l-DOPA-Induced Dyskinesia in an Animal Model of Parkinson's Disease. *The Journal of Neuroscience* **34**, 11744-11753 (2014).

212 Ahmed, M. R. *et al.* Lentiviral Overexpression of GRK6 Alleviates l-Dopa–Induced Dyskinesia in Experimental Parkinson's Disease. *Science translational medicine* **2**, 28ra28-28ra28 (2010).

213 Svenningsson, P. *et al.* DARPP-32: an integrator of neurotransmission. *Annu. Rev. Pharmacol. Toxicol.* **44**, 269-296 (2004).

214 Hemmings, H. C., Greengard, P., Tung, H. L. & Cohen, P. DARPP-32, a dopamine-regulated neuronal phosphoprotein, is a potent inhibitor of protein phosphatase-1. (1984).

215 Santini, E. *et al.* Critical involvement of cAMP/DARPP-32 and extracellular signal-regulated protein kinase signaling in L-DOPA-induced dyskinesia. *The Journal of Neuroscience* **27**, 6995-7005 (2007).

216 Santini, E. *et al.* Distinct changes in cAMP and extracellular signal-regulated protein kinase signalling in L-DOPA-induced dyskinesia. *PLoS One* **5**, e12322 (2010).

217 Picconi, B. *et al.* Loss of bidirectional striatal synaptic plasticity in L-DOPA–induced dyskinesia. *Nature neuroscience* **6**, 501-506 (2003).

218 Cao, X., Guan, Q., Xu, Y., Wang, L. & Sun, S. Mechanism of over-activation in direct pathway mediated by dopamine D_1 receptor in rats with levodopa-induced dyskinesia. *Neuroscience bulletin* **22**, 159-164 (2006).

219 Guan, Q., Zhan, Q., He, Y. & Tan, L. Changes in the prodynorphin gene and DARPP-32 state in 6-OHDA-lesioned rats following long-term treatment with l-dopa. *Neuroscience letters* **426**, 64-68 (2007).

220 Santini, E., Heiman, M., Greengard, P., Valjent, E. & Fisone, G. Inhibition of mTOR signaling in Parkinson's disease prevents L-DOPA-induced dyskinesia. *Science signaling* **2** (2009).

221 Thomas, G. M. & Huganir, R. L. MAPK cascade signalling and synaptic plasticity. *Nature Reviews Neuroscience* **5**, 173-183 (2004).

222 Roux, P. P. & Blenis, J. ERK and p38 MAPK-activated protein kinases: a family of protein kinases with diverse biological functions. *Microbiology and molecular biology reviews* **68**, 320-344 (2004).

223 Fasano, S. *et al.* Inhibition of Ras-guanine nucleotide-releasing factor 1 (Ras-GRF1) signaling in the striatum reverts motor symptoms associated with L-dopa–induced dyskinesia. *Proceedings of the National Academy of Sciences* **107**, 21824-21829 (2010).

224 Pavón, N., Martín, A. B., Mendialdua, A. & Moratalla, R. ERK phosphorylation and FosB expression are associated with L-DOPA-induced dyskinesia in hemiparkinsonian mice. *Biological psychiatry* **59**, 64-74 (2006).

225 Ding, Y. *et al.* Enhanced striatal cholinergic neuronal activity mediates l-DOPA–induced dyskinesia in parkinsonian mice. *Proceedings of the National Academy of Sciences* **108**, 840-845 (2011).

226 Nicholas, A. P. *et al.* Striatal histone modifications in models of levodopa-induced dyskinesia. *Journal of Neurochemistry* **106**, 486-494 (2008).

227 Bezard, E. *et al.* L-DOPA reverses the MPTP-induced elevation of the arrestin2 and GRK6 expression and enhanced ERK activation in monkey brain. *Neurobiology of disease* **18**, 323-335 (2005).

228 Fiorentini, C., Savoia, P., Savoldi, D., Barbon, A. & Missale, C. Persistent activation of the D1R/Shp-2/Erk1/2 pathway in l-DOPA-induced dyskinesia in the 6-hydroxy-dopamine rat model of Parkinson's disease. *Neurobiology of disease* **54**, 339-348 (2013).

229 Fieblinger, T. *et al.* Mechanisms of Dopamine D1 Receptor-Mediated ERK1/2 Activation in the Parkinsonian Striatum and Their Modulation by Metabotropic Glutamate Receptor Type 5. *The Journal of Neuroscience* **34**, 4728-4740 (2014).

230 Errico, F. *et al.* Higher free d-aspartate and n-methyl-d-aspartate levels prevent striatal depotentiation and anticipate l-DOPA-induced dyskinesia. *Experimental neurology* **232**, 240-250 (2011).

231 Gerfen, C. R., Miyachi, S., Paletzki, R. & Brown, P. D1 dopamine receptor supersensitivity in the dopamine-depleted striatum results from a switch in the regulation of ERK1/2/MAP kinase. *The Journal of Neuroscience* **22**, 5042-5054 (2002).

232 Gerfen, C. R., Paletzki, R. & Worley, P. Differences between dorsal and ventral striatum in Drd1a dopamine receptor coupling of dopamine-and cAMP-regulated phosphoprotein-32 to activation of extracellular signal-regulated kinase. *The Journal of Neuroscience* **28**, 7113-7120 (2008).

233 Subramaniam, S. *et al.* Rhes, a striatal-enriched small G protein, mediates mTOR signaling and L-DOPA-induced dyskinesia. *Nature neuroscience* **15**, 191-193 (2012).

234 Decressac, M. & Björklund, A. mTOR Inhibition Alleviates L-DOPA-Induced Dyskinesia in Parkinsonian Rats. *Journal of Parkinson's disease* **3**, 13-17 (2013).

235 Hoeffer, C. A. & Klann, E. mTOR signaling: at the crossroads of plasticity, memory and disease. *Trends in neurosciences* **33**, 67-75 (2010).

236 Laplante, M. & Sabatini, D. M. mTOR signaling in growth control and disease. *Cell* **149**, 274-293 (2012).

237 Bateup, H. S. *et al.* Cell type–specific regulation of DARPP-32 phosphorylation by psychostimulant and antipsychotic drugs. *Nature neuroscience* **11**, 932-939 (2008).

238 Bibb, J. A. *et al.* Phosphorylation of DARPP-32 by Cdk5 modulates dopamine signalling in neurons. *Nature* **402**, 669-671 (1999).

239 Kilby, N. J., Snaith, M. R. & Murray, J. A. Site-specific recombinases: tools for genome engineering. *TRENDS in Genetics* **9**, 413-421 (1993).

240 Nowak, S. J. & Corces, V. G. Phosphorylation of histone H3: a balancing act between chromosome condensation and transcriptional activation. *TRENDS in Genetics* **20**, 214-220 (2004).

241 Brami-Cherrier, K., Roze, E., Girault, J. A., Betuing, S. & Caboche, J. Role of the ERK/MSK1 signalling pathway in chromatin remodelling and brain responses to drugs of abuse. *Journal of Neurochemistry* **108**, 1323-1335 (2009).

242 Deak, M., Clifton, A. D., Lucocq, J. M. & Alessi, D. R. Mitogen-and stress-activated protein kinase-1 (MSK1) is directly activated by MAPK and SAPK2/p38, and may mediate activation of CREB. *The EMBO journal* **17**, 4426-4441 (1998).

243 Soloaga, A. *et al.* MSK2 and MSK1 mediate the mitogen-and stress-induced phosphorylation of histone H3 and HMG-14. *The EMBO journal* **22**, 2788-2797 (2003).

244 Thomson, S. *et al.* The nucleosomal response associated with immediate-early gene induction is mediated via alternative MAP kinase cascades: MSK1 as a potential histone H3/HMG-14 kinase. *The EMBO journal* **18**, 4779-4793 (1999).

245 Wiggin, G. R. *et al.* MSK1 and MSK2 are required for the mitogen-and stress-induced phosphorylation of CREB and ATF1 in fibroblasts. *Molecular and cellular biology* **22**, 2871-2881 (2002).

246 Badeaux, A. I. & Shi, Y. Emerging roles for chromatin as a signal integration and storage platform. *Nature reviews Molecular cell biology* **14**, 211-224 (2013).

247 Heffron, D. & Mandell, J. W. Differential localization of MAPK-activated protein kinases RSK1 and MSK1 in mouse brain. *Molecular brain research* **136**, 134-141 (2005).

248 Brami-Cherrier, K. *et al.* Parsing molecular and behavioral effects of cocaine in mitogen-and stress-activated protein kinase-1-deficient mice. *The Journal of Neuroscience* **25**, 11444-11454 (2005).

249 Heiman, M. *et al.* Molecular adaptations of striatal spiny projection neurons during levodopa-induced dyskinesia. *Proceedings of the National Academy of Sciences* **111**, 4578-4583 (2014).

250 Nestler, E. J. Transcriptional mechanisms of addiction: role of ΔFosB. *Philosophical Transactions of the Royal Society B: Biological Sciences* **363**, 3245-3255 (2008).

251 Andersson, M., Konradi, C. & Cenci, M. A. cAMP response element-binding protein is required for dopamine-dependent gene expression in the intact but not the dopamine-denervated striatum. *The Journal of Neuroscience* **21**, 9930-9943 (2001).

252 Vallone, D. *et al.* Behavioural sensitization in 6-hydroxydopamine-lesioned rats is related to compositional changes of the AP-1 transcription factor: evidence for induction of FosB-and JunD-related proteins. *Molecular brain research* **52**, 307-317 (1997).

253 Valastro, B., Andersson, M., Lindgren, H. & Cenci, M. Expression pattern of JunD after acute or chronic l-DOPA treatment: Comparison with ΔFosB. *Neuroscience* **144**, 198-207 (2007).

254 Westin, J., Andersson, M., Lundblad, M. & Cenci, M. 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**, 1171-1176 (2001).

255 Andersson, M., Westin, J. & Cenci, M. Time course of striatal ΔFosB-like immunoreactivity and prodynorphin mRNA levels after discontinuation of chronic dopaminomimetic treatment. *European journal of neuroscience* **17**, 661-666 (2003).

256 Cao, X. *et al.* Striatal overexpression of ΔFosB reproduces chronic levodopa-induced involuntary movements. *The Journal of Neuroscience* **30**, 7335-7343 (2010).

257 Andersson, M., Hilbertson, A. & Cenci, M. 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**, 461-474 (1999).

258 Berton, O. *et al.* Striatal overexpression of ΔJunD resets L-DOPA-induced dyskinesia in a primate model of Parkinson disease. *Biological psychiatry* **66**, 554-561 (2009).

259 Engeln, M. *et al.* Selective inactivation of striatal FosB/ΔFosB-expressing neurons alleviates L-Dopa-induced dyskinesia. *Biological psychiatry* (2014).

260 Bastide, M. F. *et al.* Immediate-early gene expression in structures outside the basal ganglia is associated to L-DOPA-induced dyskinesia. *Neurobiology of disease* **62**, 179-192 (2014).

261 Lindgren, H. S. *et al.* Putaminal Upregulation of FosB/ΔFosB-Like Immunoreactivity in Parkinson's Disease Patients with Dyskinesia. *Journal of Parkinson's disease* **1**, 347-357 (2011).

262 Alcacer, C., Charbonnier-Beaupel, F., Corvol, J.-C., Girault, J.-A. & Hervé, D. Mitogen-and stress-activated protein kinase 1 is required for specific signaling responses in dopamine-denervated mouse striatum, but is not necessary for l-DOPA-induced dyskinesia. *Neuroscience letters* (2014).

263 Lau, P. N. I. & Cheung, P. Histone code pathway involving H3 S28 phosphorylation and K27 acetylation activates transcription and antagonizes polycomb silencing. *Proceedings of the National Academy of Sciences* **108**, 2801-2806 (2011).

264 Murer, M. G. & Moratalla, R. Striatal signaling in L-DOPA-induced dyskinesia: common mechanisms with drug abuse and long term memory involving D1 dopamine receptor stimulation. *Frontiers in neuroanatomy* **5** (2011).

265 Kelz, M. B. *et al.* Expression of the transcription factor ΔFosB in the brain controls sensitivity to cocaine. *Nature* **401**, 272-276 (1999).

266 Peakman, M.-C. *et al.* Inducible, brain region-specific expression of a dominant negative mutant of c-Jun in transgenic mice decreases sensitivity to cocaine. *Brain research* **970**, 73-86 (2003).

267 Sweatt, J. D. The emerging field of neuroepigenetics. *Neuron* **80**, 624-632 (2013).

268 Zentner, G. E. & Henikoff, S. Regulation of nucleosome dynamics by histone modifications. *Nature structural & molecular biology* **20**, 259-266 (2013).

269 Banerjee, T. & Chakravarti, D. A peek into the complex realm of histone phosphorylation. *Molecular and cellular biology* **31**, 4858-4873 (2011).

270 Jenuwein, T. & Allis, C. D. Translating the histone code. *Science* **293**, 1074-1080, doi:10.1126/science.1063127 (2001).

271 Borrelli, E., Nestler, E. J., Allis, C. D. & Sassone-Corsi, P. Decoding the epigenetic language of neuronal plasticity. *Neuron* **60**, 961-974 (2008).

272 Gehani, S. S. *et al.* Polycomb group protein displacement and gene activation through MSK-dependent H3K27me3S28 phosphorylation. *Molecular cell* **39**, 886-900 (2010).

273 Simon, J. A. & Kingston, R. E. Mechanisms of polycomb gene silencing: knowns and unknowns. *Nature reviews Molecular cell biology* **10**, 697-708 (2009).

274 Hirota, T., Lipp, J. J., Toh, B.-H. & Peters, J.-M. Histone H3 serine 10 phosphorylation by Aurora B causes HP1 dissociation from heterochromatin. *Nature* **438**, 1176-1180 (2005).

275 Fischle, W. *et al.* Regulation of HP1–chromatin binding by histone H3 methylation and phosphorylation. *Nature* **438**, 1116-1122 (2005).

276 Fanti, L. & Pimpinelli, S. HP1: a functionally multifaceted protein. *Current opinion in genetics & development* **18**, 169-174 (2008).

277 Bracken, A. P. & Helin, K. Polycomb group proteins: navigators of lineage pathways led astray in cancer. *Nature Reviews Cancer* **9**, 773-784 (2009).

278 Di Croce, L. & Helin, K. Transcriptional regulation by Polycomb group proteins. *Nat Struct Mol Biol* **20**, 1147-1155 (2013).

279 Lanzuolo, C. & Orlando, V. Memories from the polycomb group proteins. *Annual review of genetics* **46**, 561-589 (2012).

280 Bernstein, B. E. *et al.* A bivalent chromatin structure marks key developmental genes in embryonic stem cells. *Cell* **125**, 315-326 (2006).

281 Mohn, F. *et al.* Lineage-specific polycomb targets and de novo DNA methylation define restriction and potential of neuronal progenitors. *Molecular cell* **30**, 755-766 (2008).

282 Pan, G. *et al.* Whole-genome analysis of histone H3 lysine 4 and lysine 27 methylation in human embryonic stem cells. *Cell stem cell* **1**, 299-312 (2007).

283 Stipanovich, A. *et al.* A phosphatase cascade by which rewarding stimuli control nucleosomal response. *Nature* **453**, 879-884 (2008).

284 Zhang, Y. *et al.* Aberrant restoration of spines and their synapses in L-DOPA-induced dyskinesia: involvement of corticostriatal but not thalamostriatal synapses. *The Journal of Neuroscience* **33**, 11655-11667 (2013).

285 Thiele, S. L. *et al.* Selective loss of bi-directional synaptic plasticity in the direct and indirect striatal output pathways accompanies generation of parkinsonism and l-DOPA induced dyskinesia in mouse models. *Neurobiology of disease* (2014).

286 Pisani, A., Centonze, D., Bernardi, G. & Calabresi, P. Striatal synaptic plasticity: implications for motor learning and Parkinson's disease. *Movement Disorders* **20**, 395-402 (2005).

287 Yin, H. H. & Knowlton, B. J. The role of the basal ganglia in habit formation. *Nature Reviews Neuroscience* **7**, 464-476 (2006).

288 Knowlton, B. J., Mangels, J. A. & Squire, L. R. A neostriatal habit learning system in humans. *Science* **273**, 1399-1402 (1996).

289 Faure, A., Haberland, U., Condé, F. & El Massioui, N. Lesion to the nigrostriatal dopamine system disrupts stimulus-response habit formation. *The Journal of Neuroscience* **25**, 2771-2780 (2005).

290 Dawson, M. A. & Kouzarides, T. Cancer epigenetics: from mechanism to therapy. *Cell* **150**, 12-27, doi:10.1016/j.cell.2012.06.013 (2012).