NON-DOPAMINERGIC SYSTEMS IN PARKINSON'S DISEASE

EDITED BY: Cristina Miguelez, Philippe De Deurwaerdere and Véronique Sgambato

PUBLISHED IN: Frontiers in Pharmacology and Frontiers in Neuroscience

frontiers Research Topics

frontiers

ISSN 1664-8714
ISBN 978-2-88966-144-2
DOI 10.3389/978-2-88966-144-2

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: researchtopics@frontiersin.org

NON-DOPAMINERGIC SYSTEMS IN PARKINSON'S DISEASE

Topic Editors:
Cristina Miguelez, University of the Basque Country, Spain
Philippe De Deurwaerdere, Université de Bordeaux, France
Véronique Sgambato, Institut National de la Santé et de la Recherche Médicale (INSERM), France

Citation: Miguelez, C., De Deurwaerdere, P., Sgambato, V., eds. (2020). Non-Dopaminergic Systems in Parkinson's Disease. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88966-144-2

Table of Contents

frontiers in Pharmacology

EDITORIAL
published: 11 September 2020
doi: 10.3389/fphar.2020.593822

Editorial: Non-Dopaminergic Systems in Parkinson's Disease

Cristina Miguelez[1,2]*, *Philippe De Deurwaerdère*[3] *and Véronique Sgambato*[4]

[1] Department of Pharmacology, Faculty of Medicine and Nursing, University of the Basque Country (UPV/EHU), Leioa, Spain, [2] Autonomic and Movement Disorders Unit, Neurodegenerative Diseases, Biocruces Health Research Institute, Barakaldo, Spain, [3] CNRS, Institut des Neurosciences Cognitives et Integratives d'Aquitaine, UMR 5287, Bordeaux, France, [4] University of Lyon, CNRS, Institut des Sciences Cognitives Marc Jeannerod, UMR 5229, Bron, France

Keywords: L-DOPA, serotonin, sleep, noradrenaline, GABA, non-motor symptoms, orphan receptor, prodromal Parkinson disease

Editorial on the Research Topic

Non-Dopaminergic Systems in Parkinson's Disease

This collection presents an array of articles addressing the non-dopaminergic mechanisms in Parkinson's disease (PD). The dopaminergic system has been the main focus for almost 70 years and dopaminergic-based strategies still remain the best symptomatic medication to improve quality of life of parkinsonian patients. However, the interest in non-dopaminergic systems, even beyond neurotransmission itself, has grown with the accumulation of data showing that PD cannot be considered as a pure motor disease and understood only through the prism of the nigrostriatal dopaminergic pathway deficiency. Indeed, the disease progresses through different stages and damages to other areas and neurochemical systems precede the degeneration of dopaminergic neurons in the substantia nigra compacta (SNc). The other affected systems participate in the so-called prodromal or pre-symptomatic phases and contribute to the malfunctioning of the motor and non-motor circuits. Meanwhile, the non-motor symptoms are increasingly recognized in PD. They severely alter the quality of life of the patients since they are often more debilitating than the motor ones and poorly respond to dopaminergic-based therapies. Finally, the studies addressing the mechanisms of action of antiparkinsonian drugs have revealed that their benefits/side effects involved mechanisms other than the dopaminergic ones.

Among the classical non-dopaminergic systems involved in PD, Paredes-Rodriguez et al. review the situation of the noradrenergic system in PD. The noradrenergic system issued from the locus coeruleus can display severe damages in the disease, presumably before the dopaminergic neurons according to the hypothesis that the disease progresses from the caudal to the rostral parts of the brainstem. In fact, the authors recall that the noradrenergic system exerts anti-inflammatory and neuroprotective effect on the dopaminergic degeneration, and noradrenergic damage can consequently favor the progress of the disease. Even though the noradrenergic system participates in the mechanism of action of L-DOPA, this interaction is still unclear and needs further investigation. Mallet et al. review another classical non-dopaminergic neurotransmitter that has been considered in PD even before dopamine, namely acetylcholine. The authors focus their analysis on the striatal cholinergic interneurons, which display specific electrophysiological features but are still difficult to apprehend due to their sparse distribution. Based on recent literature, the authors report the advantages of using optogenetic approaches possibly combined with computational studies to investigate the role of cholinergic interneurons in the physiology and pathophysiology of motor behavior and cognition. On the other hand, the serotonergic system is one of the most studied non-dopaminergic neurotransmitter systems during

OPEN ACCESS

Edited and reviewed by:
Nicholas M. Barnes, University of Birmingham, United Kingdom

***Correspondence:**
Cristina Miguelez
cristina.miguelez@ehu.eus

Specialty section:
This article was submitted to Neuropharmacology, a section of the journal Frontiers in Pharmacology

Received: *11 August 2020*
Accepted: *26 August 2020*
Published: *11 September 2020*

Citation:
Miguelez C, De Deurwaerdère P and Sgambato V (2020) Editorial: Non-Dopaminergic Systems in Parkinson's Disease. Front. Pharmacol. 11:593822. doi: 10.3389/fphar.2020.593822

the last decade in view of the demonstration of its primary participation in dyskinesia induced by L-DOPA. Schintu et al. rendered hemiparkinsonian inbred depression-like flinders sensitive line (FSL) rats and studied their motor responses to chronic L-DOPA. In contrast to control animals, the hemiparkinsonian FSL rats did not display sensitization in the turning response to apomorphine or L-DOPA and only a weak increase in dyskinesia. The marked differences in L-DOPA-induced dyskinesia were not associated with modification of the striatal expression of deltaFosB, a supposed marker of this side effect.

The imbalance of neurotransmitters in the brain is multiple in part due to degenerative processes away from the SNc. Chambers et al. recall that some neurons in the pedunculopontine nucleus, a complex structure housing GABAergic, glutamatergic, and cholinergic neurons, degenerate in PD. The neuronal degenerative process in the pedunculopontine nucleus likely participates in motor disability, notably gait control, and in non-motor symptoms including sleep deficits, likely due to the innervation of brain regions different from the SNc. Murueta-Goyena et al. cover the non-motor symptoms in PD and the GABAergic system alterations. They recall the participation of GABAergic mechanisms in various pre-symptomatic disturbances including olfactory loss, gastrointestinal abnormalities, visual alterations, cognitive and mood disorders, and make a special emphasis on rapid eye movement sleep behavior disorder. In this line, Meideiros et al. describe how neurotoxic and genetic manipulations of rodents have been utilized to reproduce some of the major sleep disturbances associated with PD. They further discuss how these abnormalities can be linked to noradrenaline, serotonin, and orexin transmission. Several neurotransmitter imbalances operate during the disease and may participate in the complexity and heterogeneity of the neuropsychiatric symptoms exhibited by PD patients. Dujardin and Sgambato propose a thorough examination of the neuropsychiatric symptoms, including depression, anxiety, apathy, psychosis, and impulse control disorders, their prevalence and pathophysiology. The authors deepened their analysis by looking at the relative contribution of the neurotransmitter systems dopamine, noradrenaline, serotonin, acetylcholine, GABA, and glutamate in each of the above-mentioned neuropsychiatric symptoms in both PD patients and animal models.

Beyond the neurotransmitter systems, there are some opportunities to develop treatments on specific targets, as the orphan receptors GPR88 and GPR143. Ingallinesi et al. focused on GPR88, almost exclusively expressed by the medium spiny GABAergic neurons of the striatum. They used a lentiviral-mediated knock-down approach of the receptor in the 6-hydroxydopamine neurotoxic rat model of PD and reported reduced amphetamine- but increased L-DOPA-induced turning behavior. These behavioral effects were paralleled by a normalization of some striatal tissue markers such as GAD67 expression, although the striatal expression of deltaFosB did not parallel L-DOPA-induced dyskinesia. Yoshio et al. also recall the interest of another orphan receptor, the GPR143. This receptor has been proposed as the target of L-DOPA, which would be its endogenous ligand at least in the retina. The authors review the possible contribution of GPR143 in PD with a special emphasis on its colocalization with the protein α-synuclein in Lewy bodies.

The availability of new animal models conditions the progress of research in the involvement of different neurotransmitters in the motor and non-motor symptoms of the disease. Gomez-Benito et al. discuss and provide detailed comparative analysis of two models based on α-synuclein: the α-synuclein pre-formed fibril model and the recombinant adeno-associated virus vector-mediated α-Synuclein overexpression models. The multiplicity and development of novel models are necessary for progressing in the understanding of the disease and help to decipher specific mechanisms. In any case, far beyond the consideration of dopaminergic neurons of the SNc, PD is a multifactorial disease evolving before the onset of motor symptoms.

AUTHOR CONTRIBUTIONS

All authors contributed to the article and approved the submitted version.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pharmacology

MINI REVIEW
published: 03 October 2019
doi: 10.3389/fphar.2019.01119

L-DOPA and Its Receptor GPR143: Implications for Pathogenesis and Therapy in Parkinson's Disease

Yoshio Goshima, Daiki Masukawa, Yuka Kasahara, Tatsuo Hashimoto and Aderemi Caleb Aladeokin*

Department of Molecular Pharmacology & Neurobiology, Yokohama City University Graduate School of Medicine, Yokohama, Japan

L-3,4-Dihydroxyphenylalanine (L-DOPA) is the most effective therapeutic agent for Parkinson's disease (PD). L-DOPA is traditionally believed to be an inert amino acid that exerts actions and effectiveness in PD through its conversion to dopamine. In contrast to this generally accepted idea, L-DOPA is proposed to be a neurotransmitter. Recently, GPR143 (OA1), the gene product of *ocular albinism 1* was identified as a receptor candidate for L-DOPA. GPR143 is widely expressed in the central and peripheral nervous system. GPR143 immunoreactivity was colocalized with phosphorylated α-synuclein in Lewy bodies in PD brains. GPR143 may contribute to the therapeutic effectiveness of L-DOPA and might be related to pathogenesis of PD.

Keywords: L-DOPA, neurotransmitter, G protein–coupled receptor, Parkinson's disease, dopamine, Lewy bodies

OPEN ACCESS

Edited by:
Philippe De Deurwaerdere, Université de Bordeaux, France

Reviewed by:
Erwan Bezard, UMR5293 Institut des Maladies Neurodégénératives (IMN), France
Clotilde Mannoury La Cour, Institut de Recherche International Servier, France

****Correspondence:***
Yoshio Goshima
goshima@med.yokohama-cu.ac.jp

Specialty section:
This article was submitted to Neuropharmacology, a section of the journal Frontiers in Pharmacology

Received: *13 April 2019*
Accepted: *30 August 2019*
Published: *03 October 2019*

Citation:
Goshima Y, Masukawa D, Kasahara Y, Hashimoto T and Aladeokin AC (2019) L-DOPA and Its Receptor GPR143: Implications for Pathogenesis and Therapy in Parkinson's Disease. Front. Pharmacol. 10:1119. doi: 10.3389/fphar.2019.01119

INTRODUCTION

Parkinson's disease (PD) results primarily from the degeneration of dopaminergic neurons in the substantia nigra. L-3,4-Dihydroxyphenylalanine (L-DOPA), a precursor of dopamine (DA), replenishes disease-related lower levels of DA by conversion of L-DOPA to DA in the brain by aromatic L-amino acid decarboxylase (AADC) and alleviates motor symptoms of PD. L-DOPA is mainly synthesized from tyrosine by tyrosine hydroxylase (TH), the rate-limiting enzyme for catecholamine synthesis. Synthetic DA agonists have been included in the treatment of PD for several decades, but L-DOPA is still the most effective therapeutic agent and widely considered the gold standard in PD treatment. However, L-DOPA therapy is associated with adverse effects, such as dyskinesias and impulse control disorders, the underlying mechanisms of which are largely unknown (Ahlskog, 2011; Gottwald and Aminoff, 2011; Rascol et al., 2011; Xie et al., 2015; Ivanova and Loonen, 2016; Voon et al., 2017).

In 1986, we first proposed that L-DOPA itself act a neurotransmitter. L-DOPA is released upon neuronal excitation, and L-DOPA exerts biological activity even with inhibition of AADC to prevent DA formation (Goshima et al., 2014). Most of these responses are antagonized by L-DOPA esters, compounds structurally related to L-DOPA. Recently, GPR143, a G protein–coupled receptor (GPCR), the gene product of *ocular albinism 1* (OA1) (Schiaffino et al., 1999), was reported to have binding activities for L-DOPA (Lopez et al., 2008). GPR143 was distributed in the central and peripheral nervous system and was further confirmed to play a role as an L-DOPA receptor *in vivo* (Hiroshima et al., 2014; Masukawa et al., 2014; Fukuda et al., 2015; Masukawa et al., 2017). GPR143 was also found to be localized in Lewy bodies, the histological hallmark of PD (Goshima et al., 2018). In this review, we revisit L-DOPA therapy and PD pathogenesis in light of L-DOPA action as a neurotransmitter.

EVIDENCE FOR L-DOPA AS A NEUROTRANSMITTER

L-DOPA has been believed to reside exclusively in the cytoplasm of catecholaminergic neurons only as a precursor of the neurotransmitter DA. Contrary to this generally accepted idea, we found that nerve stimulation elicited the release of L-DOPA *in vitro* and *in vivo* experiments (Goshima et al., 1988; Nakamura et al., 1992). The release was dependent on the extracellular Ca^{2+} and sensitive to tetrodotoxin, a voltage-dependent Na^{+}-channel blocker. The L-DOPA release was suppressed by inhibitors of P-type voltage-sensitive Ca^{2+} channels and of synaptobrevin (Zhu et al., 2004). These findings together suggest that L-DOPA is released *via* exocytosis (Misu and Goshima, 1993). By using specific antibodies, TH- and L-DOPA–positive but AADC- and DA-negative neurons were shown in some brain areas including the nucleus tractus solitarii (NTS), hypothalamic arcuate nucleus, and magnocellular neurosecretory system (Mons et al., 1988; Misu et al., 1996). Ultrastructurally, L-DOPA–positive signals were localized in the terminals with vesicle-like structures in the lateral habenular nucleus of the house-shrew brain (Karasawa et al., 1992). It is possible that L-DOPA release may arise from L-DOPA–containing vesicles, although vesicular L-DOPA transporter(s), if it exists, have not yet been identified. In addition, exogenous application of L-DOPA produced pharmacological actions in the presence of AADC inhibitor. L-DOPA facilitated the impulse-evoked noradrenaline (NA) release from superfused rat hypothalamic slices (Goshima et al., 1986; Goshima et al., 1991b). Structure–activity relationship study of DOPA-related compounds revealed that D-DOPA, the D-isomer of L-DOPA, did not mimic the effect of L-DOPA. In addition, L-DOPA methyl ester (L-DOPA ME) antagonized the action of L-DOPA in a competitive fashion. Thus, the L-DOPA recognition site(s) has high receptor-like specificity, being stereoselective and requiring specific structural features including the catechol moiety in addition to amino and carboxy groups. These findings support the existence of specific molecular recognition site(s) or receptor(s) for L-DOPA.

Previous reports suggested a role of L-DOPA as a neuromodulator in the regulation of motor function. Certain L-DOPA actions were observed only in PD model but not in normal control animals (Ueda et al., 1995a; Ueda et al., 1995b). Using *in vivo* microdialysis, L-DOPA decreased acetylcholine (ACh) from the striatum of rats lesioned with 6-hydroxydopamine (6-OHDA), but not from that of sham-operated rats. The L-DOPA–induced decrease was not affected by sulpiride, a D_2/D_3 antagonist. These findings suggest that L-DOPA by itself regulates the release of ACh. In addition, L-DOPA–sensitive mechanisms were supersensitized in the PD model (Ueda et al., 1995b). A similar supersensitization to L-DOPA was also observed in quinpirole-induced locomotor activity. In normal rats, a highest dose of quinpirole, a D_2/D_3 agonist, slightly increased the total accounts of locomotor activities. Concomitant treatment with noneffective dose of L-DOPA (30 mg/kg) potentiated hyperlocomotion induced by quinpirole. In 6-OHDA rats, a noneffective lower dose of L-DOPA (10 mg/kg) potentiated quinpirole-induced locomotor activities (Nakamura et al., 1994).

L-DOPA probably plays a role as a neurotransmitter of the primary baroreceptor afferents in the lower brain stem. A prominent effect of L-DOPA is its depressor and bradycardic actions when microinjected into the NTS of anesthetized rats (Kubo et al., 1992; Yue et al., 1994; Misu et al., 1996; Goshima et al., 2014). Phenylephrine-induced hypertension triggered L-DOPA release in the NTS and reflex bradycardia temporally associated with the rise and recovery of blood pressure. The L-DOPA release and bradycardia were abolished by denervation of bilateral carotid sinus and aortic nerves, which contained the baroreceptor afferents. Electrical stimulation of aortic nerve released L-DOPA and induced depressor and bradycardic responses, and these responses were antagonized by bilateral injection of L-DOPA ME into the NTS (Yue et al., 1994). The depressor response induced by L-DOPA might possibly be related to orthostatic hypotension, which is often encountered in PD patients on L-DOPA therapy (Connolly and Lang, 2014; De Pablo-Fernandez et al., 2017).

RECEPTOR CANDIDATES TARGETTED BY L-DOPA: THEIR LOCALIZATION AND PHARMACOLOGY IN NORMAL CONDITIONS

These findings together suggest that there is one or more specific receptors for L-DOPA. L-DOPA did not interact with cognate monoamine receptors (Goshima et al., 1991b; Misu et al., 1996). On the other hand, L-DOPA inhibited specific binding of [^{3}H]-AMPA in the brain membrane preparations, and L-DOPA induced current response in *Xenopus laevis* oocytes expressing AMPA receptors (Miyamae et al., 1999). However, L-DOPA interacted with AMPA receptors with a low affinity (ED_{50} of 2.2 mM). In addition, DOPA ME and L-DOPA cyclohexyl ester (CHE), L-DOPA antagonists, did not interact with AMPA receptors (Miyamae et al., 1999). These findings suggest that the L-DOPA CHE-sensitive actions of L-DOPA are mediated through recognition sites other than monoamine receptors and AMPA receptors. A clue to the identification of L-DOPA receptors involved genetic analysis of *oa1*, the gene responsible for ocular albinism type 1 (Schiaffino et al., 1999). The gene product of *oa1* referred to as GPR143 is critically involved in organogenesis of the melanosomes. Based on sequence similarities, the protein could not be assigned to any GPCR subfamily. Although binding activities of both DA and L-DOPA to GPR143 were detected, only L-DOPA induced intracellular Ca^{2+} response in cell lines expressing GPR143 (Lopez et al., 2008; Hiroshima et al., 2014). Dopamine also interacted with GPR143 with Ki of 2.4 μM, a comparable value to that for L-DOPA, but produced no functional response on intracellular Ca^{2+} levels in GPR143-expressing CHO cells. Dopamine may therefore

act as an antagonist or inverse agonist against GPR143, which is consistent with the structural features and the homology model for GPR143 (Ghosh et al., 2012). These findings indicate that L-DOPA may be an endogenous ligand for GPR143. The role of GPR143 in mediating the L-DOPA response was then examined in the NTS of anesthetized rats. The shRNA knockdown of GPR143 in the NTS abolished or attenuated the depressor response to L-DOPA but not to glutamate microinjected into the NTS. L-DOPA CHE also suppressed the L-DOPA response and displaced the specific binding of [^{3}H]-L-DOPA in CHO cells expressing GPR143. These findings suggest that GPR143 is involved in mediating the L-DOPA actions in the NTS (Hiroshima et al., 2014).

GPR143 is highly expressed in the retinal pigment epithelial cells (Schiaffino et al., 1999), and most studies have been conducted to delineate roles of GPR143 in melanogenesis (Schiaffino, 2010; De Filippo et al., 2017). Using specific antibodies against GPR143 and GPR143-KO mice, we investigated tissue distribution of GPR143. GPR143 immunoreactive cells were localized in the hippocampus, cerebral cortex, striatum, substantia nigra, hypothalamic median eminence and suprachiasmatic nucleus, NTS, and caudal ventrolateral medulla and olfactory bulb (Masukawa et al., 2014). A similar distribution pattern was confirmed in wild-type (*Wt*) mouse brains (Fukuda et al., 2015). Of note, GPR143 signals were observed in areas involving both hippocampal and cortical circuits as well as in basal ganglia and hypothalamic and brainstem neural networks. In line with GPR143 expression pattern, several observations reported an effect of L-DOPA in enhancing performances in executive verbal tasks and visuospatial working memory (Kulisevsky et al., 1996; Costa et al., 2003; Poletti and Bonuccelli, 2013), as well as in increasing secretion of growth hormone or cortisol (Biermasz et al., 2005; Muller et al., 2011; Marakaki et al., 2015), both of which being regulated by neurosecretory hypothalamic nuclei.

The downstream signaling pathways triggered by L-DOPA through GPR143 *in vivo* remain unknown (Schiaffino, 2010). Biochemical and morphological analysis suggest that Gai3, Go, and Gq are potential G protein partners for GPR143. Among them, Gai3 was shown to coprecipitate and colocalize with GPR143 (Young et al., 2008). In addition, the retinal pigment epithelial cells of Gai3-deficient mice contained fewer and larger melanosomes, and analysis of the optic pathways revealed a significant reduction of ipsilateral retinofugal projections, both of which were similar to the phenotypes of *Oa1/Gpr143*-deficient mice (Young et al., 2008). Although overall picture is far from understood (Schiaffino, 2010), it is likely that Gai3 plays a role at least in melanosome biogenesis and in the development of the optic tract.

There is additional evidence for the role of GPR143 as an L-DOPA receptor in the peripheral cardiovascular system (Masukawa et al., 2017). Phenotypic analysis of *Gpr143* gene-deficient (GPR143-KO) revealed that the pressor response to phenylephrine, an agonist of a_1-adrenoceptors (α_1ARs), was attenuated in GPR143-KO compared to that in *Wt* mice. The pressor response to vasopressin was not altered in GPR143-KO mice. In isolated GPR143-KO peripheral arteries, phenylephrine-induced contraction was reduced, indicating that GPR143 expressed in vascular smooth muscle cells could regulate α_1ARs -mediated arterial contraction. In GPR143-KO smooth muscle cells, phenylephrine-induced phosphorylation of myosin light chain 2 (MLC2) and ERK phosphorylation were attenuated compared to *Wt* smooth muscle cells. MLC2 and ERK phosphorylation are causally related to phenylephrine-induced contraction, an effect involving GPR143. Pretreatment with L-DOPA at low nanomolar concentrations augmented the vasoconstricting effect of phenylephrine in isolated arteries from *Wt* but not GPR143-KO mice. Consistently, exogenously applied L-DOPA at 1 to 10 nM augmented the contractile and intracellular Ca^{2+} responses to phenylephrine in smooth muscle cells. The binding affinity of phenylephrine against the specific binding of [^{3}H]-prazosin, an α_1ARs antagonist, was higher in HEK293 cells coexpressing both $\alpha1_B$AR-Myc and GPR143-EGFP than in cells coexpressing α_{1B}AR-Myc and free-EGFP. Immunoprecipitation assay also revealed that the interaction between GPR143 and α_{1B}AR was enhanced by L-DOPA. Importantly, the enhancement was observed at a concentration comparable to those of plasma L-DOPA (10–20 nM) (Chritton et al., 1997; Goldstein et al., 2003; Masukawa et al., 2017). GPR143, when functionally coupled with $\alpha1_B$AR, may have significantly more affinity for L-DOPA than when not forming heteromers with $\alpha1_B$AR (Masukawa et al., 2017). Förster resonance energy transfer signal was detected in live HEK293 cells expressing $\alpha1_B$AR-Venus and GPR143-CFP, but not in those expressing vasopressin receptor $AVPR1_A$ -CFP and GPR143-Venus (Masukawa et al., 2017). The direct interaction between GPR143 and α_{1B}AR was demonstrated in *Wt* tissue by *in situ* proximity ligation assay. Furthermore, the stress-induced pressor response and the rise in blood pressure in active phase in GPR143-KO were blunted when compared to *Wt* mice. Together, these findings indicate that L-DOPA sensitizes contractile response to sympathetic outflow through coupling of GPR143 with αARs in vascular smooth muscle cells (Masukawa et al., 2017; **Figure 1**) and further suggest the physiological relevance of GPR143 as an L-DOPA receptor.

Recent findings indicate that the interaction between distinct GPCRs, forming hetero-oligomers, can affect affinity for agonists, the performance, and range through which extracellular signals are transmitted to G protein molecules (Ferre et al., 2014; Ferre, 2015; Farran, 2017). The functional interaction of hetero-oligomer GPCRs was observed for DA D_1–D_3 heterodimer receptors. D_1–D_3 receptor heterodimer had greater affinity for DA than did DA_1 monomeric receptors without DA_3 receptors (Fiorentini et al., 2008). Coupling between GPR143 and $\alpha1_B$AR could also occur in the central nervous system, because $\alpha1_B$AR was present in the nucleus accumbens and colocalized with dopamine D_1 receptor (Mitrano et al., 2018). These finding suggest that some of the L-DOPA actions may be mediated through possible functional coupling between these GPCRs: D1, $\alpha1_B$AR and GPR143. L-DOPA itself did not interact with adrenergic α-, β-, D_1, and D_2 receptors (Goshima et al., 1991b). However, L-DOPA actions that were suppressed by antagonists for these receptors have been reported. For example, prazosin attenuated L-DOPA–induced

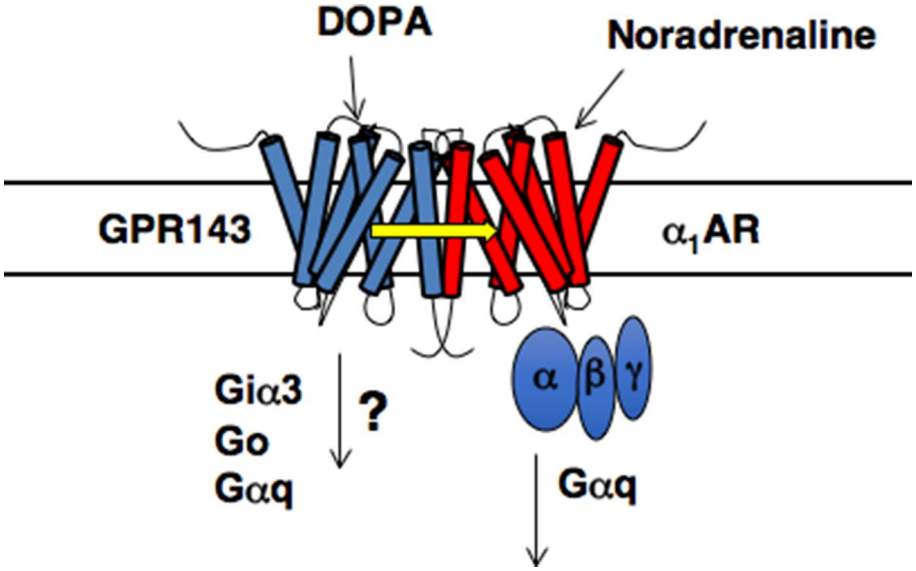


FIGURE 1 | Proposed model of GPR143 and $\alpha 1_B$AR heteromers (modified from Maurice et al., 2011).

hyperactivity without affecting its anti-PD actions or dyskinesia in 1-methyl-4-phenyl-1,2,3,6- tetrahydropyridine (MPTP)–lesioned macaques (Visanji et al., 2009). L-DOPA produced propranolol (nonselective β-adrenoceptor antagonist)–facilitatory action on the NA release from brain slices (Goshima et al., 1986). Propranolol attenuated L-DOPA–induced efflux of DA from the 6-OHDA–lesioned striatum and reduced L-DOPA–induced dyskinesia (Bhide et al., 2015).

Although GPR143 is the most plausible L-DOPA receptor candidate, we found that L-DOPA induced ptosis in GPR143-KO as well as *Wt* mice pretreated with a central AADC inhibitor (Ueda et al., 2016). Ptosis sometimes associates with short- or long-term L-DOPA treatment (Giladi et al., 1992). These findings raise the possibility that L-DOPA induces actions through both GPR143-dependent and GPR143-independent mechanisms as well as its conversion to DA.

PATHOPHYSIOLOGICAL SIGNIFICANCE OF L-DOPA/GPR143 INTERACTION IN PD

Parkinson's disease is characterized by neuronal loss of dopaminergic neurons, and enzymatic activities of TH and AADC decreased during the progression of the pathology (Lloyd et al., 1975; Nagatsu and Nagatsu, 2016). As mentioned, L-DOPA can induce orthostatic hypotension in the treatment of PD (Connolly and Lang, 2014; De Pablo-Fernandez et al., 2017). Thus, the reduction of TH levels and its subsequent decrease in the synthesis and release of endogenous L-DOPA may contribute to autonomic dysfunctions observed in PD (De Pablo-Fernandez et al., 2017). The possible reduction in endogenous levels of L-DOPA might be related to supersensitization of responses to L-DOPA or DA agonists in PD or experimental PD models (Ueda et al., 1995a; Shimamura et al., 2006; Bhide et al., 2015). On the other hand, long-term L-DOPA treatment could induce down-regulation or desensitization of the receptor(s) for L-DOPA. Thus, changes in GPR143 properties (expression, sensitization) may also arise in PD patients together with the progression of the pathology, which may account for the development of the side effects or loss of efficacy of L-DOPA.

The decrease in the AADC activity may also affect the pharmacological actions of L-DOPA. For example, in intact rat brain slices, L-DOPA increased the release of DA at 0.1 μM but produced no effect at 1 μM. In the presence of an AADC inhibitor, L-DOPA (1 μM) inhibited the release of DA (Goshima et al., 1986). Likewise, L-DOPA at 30 nM increased the DA release from intact slices, but decreased the release from the slices of MPTP-treated mice (Goshima et al., 1991a). These results imply that the action of L-DOPA can become predominant over the action of DA during the progression of PD. Another example is the ability of L-DOPA but not DA to induce the release of glutamate from the striatum (Goshima et al., 1993). The respective inhibitory and facilitatory effects of L-DOPA on the release of DA and glutamate seen under the decreased AADC activities (Goshima et al., 1986; Goshima et al., 1993) may be relevant to some adverse effects such as the decreased therapeutic efficacy, dyskinesia, or "on-off" encountered during long-term therapy (Marsden, 1994). The decreased AADC activities may further tip the balance in favor of the L-DOPA actions, because DA may act as an agonist or inverse agonist against GPR143 (Lopez et al., 2008).

Previous clinical data have suggested that L-DOPA either slows the progression of PD or has a prolonged effect on PD symptoms (Fahn et al., 2004). In contrast, a recent trial concluded that treatment with L-DOPA had no disease-modifying effect, either beneficial or detrimental, on early PD among patients who were evaluated over the course of 80 weeks (Verschuur et al., 2019). L-DOPA, DA, and related compounds have the potential to be cytotoxic, since free

radical and quinone metabolites of L-DOPA were shown to be toxic *in vitro* and *in vivo* (Graham et al., 1978; Cohen, 1987; Smith et al., 1994). Our demonstration of increased glutamate release by L-DOPA (Goshima et al., 1993) provides *in vivo* evidence for a mechanism that could accelerate the degeneration process. On the other hand, it was shown that chronic L-DOPA promoted recovery in remaining DA neurons, increased mRNA, and induced release of BDNF in the brain of PD models (Murer et al., 1998; Zhang et al., 2006). Thus, specific assays suggest the possibility that L-DOPA might have both beneficial and detrimental effects depending on various conditions such as therapeutic doses of L-DOPA, its metabolism, and the degree of degeneration of DA neurons. Whether GPR143 is required for any long-term effects of L-DOPA is an important issue to be answered in future studies.

In addition to a possible role of GPR143 in symptomatic-modifying effect of L-DOPA, GPR143 might also be involved in the pathogenesis of PD. To gain an insight into this issue, immunohistochemical analysis was conducted of PD brain tissues by using human anti-GPR143 antibody (Goshima et al., 2018). GPR143-immunoreactive neurons with large perikarya and neurites were observed in the midbrain of control and PD brains. The GPR143-immunoreactive signals showed a dot-like pattern in the perikarya, reflecting its localizations to melanosomes or late endosomes/lysosomes (Schiaffino, 2010). Consistently, unlike other GPCRs, an unconventional dileucine motif and a tryptophan-glutamic acid doublet motif were identified in GPR143 within its third cytosolic loop and the C-terminal tail, respectively, both of which may be responsible for driving its intracellular targeting (Piccirillo et al., 2006). In the PD brain tissue, GPR143 was colocalized with phosphorylated α-synuclein in Lewy bodies (Goshima et al., 2018) (**Figure 2**). It might be possible that accumulation and localization of GPCRs such as GPR143 are related to the pathogenesis of PD or to efficacy and/or untoward effects of L-DOPA. Interestingly, Parkin-associated endothelin receptor-like receptor (Pael-R) (GPR37) shared some properties common with those of GPR143. GPR37 showed overlapping expression pattern with GPR143 and poor trafficking to the plasma membrane. GPR37 was localized in Lewy bodies (Yang et al., 2003; Murakami et al., 2004) and, like GPR143, possessed high basal activities. Interestingly, overexpression of GPR37 resulted in death of dopaminergic neurons (Leinartaite and Svenningsson, 2017), while GPR37 was contributed to the signaling of certain neuroprotective factors (Meyer et al., 2013). Future studies should address whether GPR143 is also involved in the pathogenesis and the toward and/or untoward actions of L-DOPA in PD (**Figure 3**).

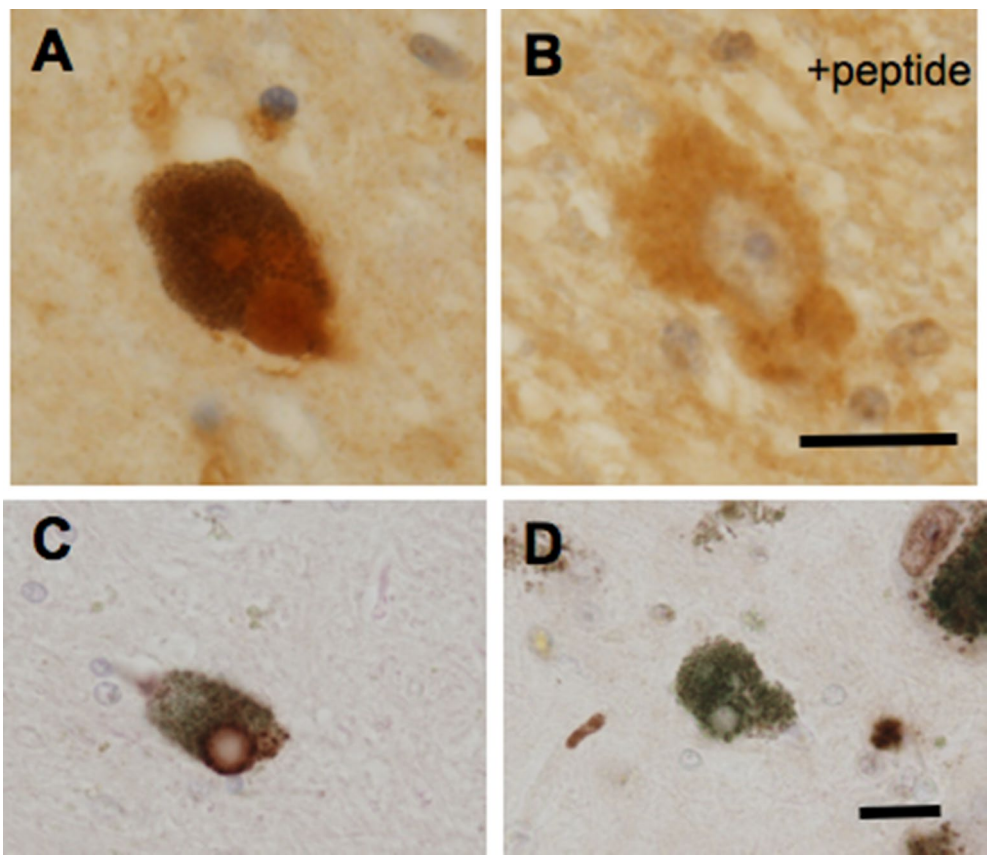


FIGURE 2 | GPR143-positive **(A**, **C)** and GPR143–negative **(D)** Lewy bodies in neurons in the substantia nigra compacta of PD brain tissue. GPR143-positive signals were blocked by the synthetic peptide **(B)**. Scale bar, 20 μm. (Goshima et al., 2018).

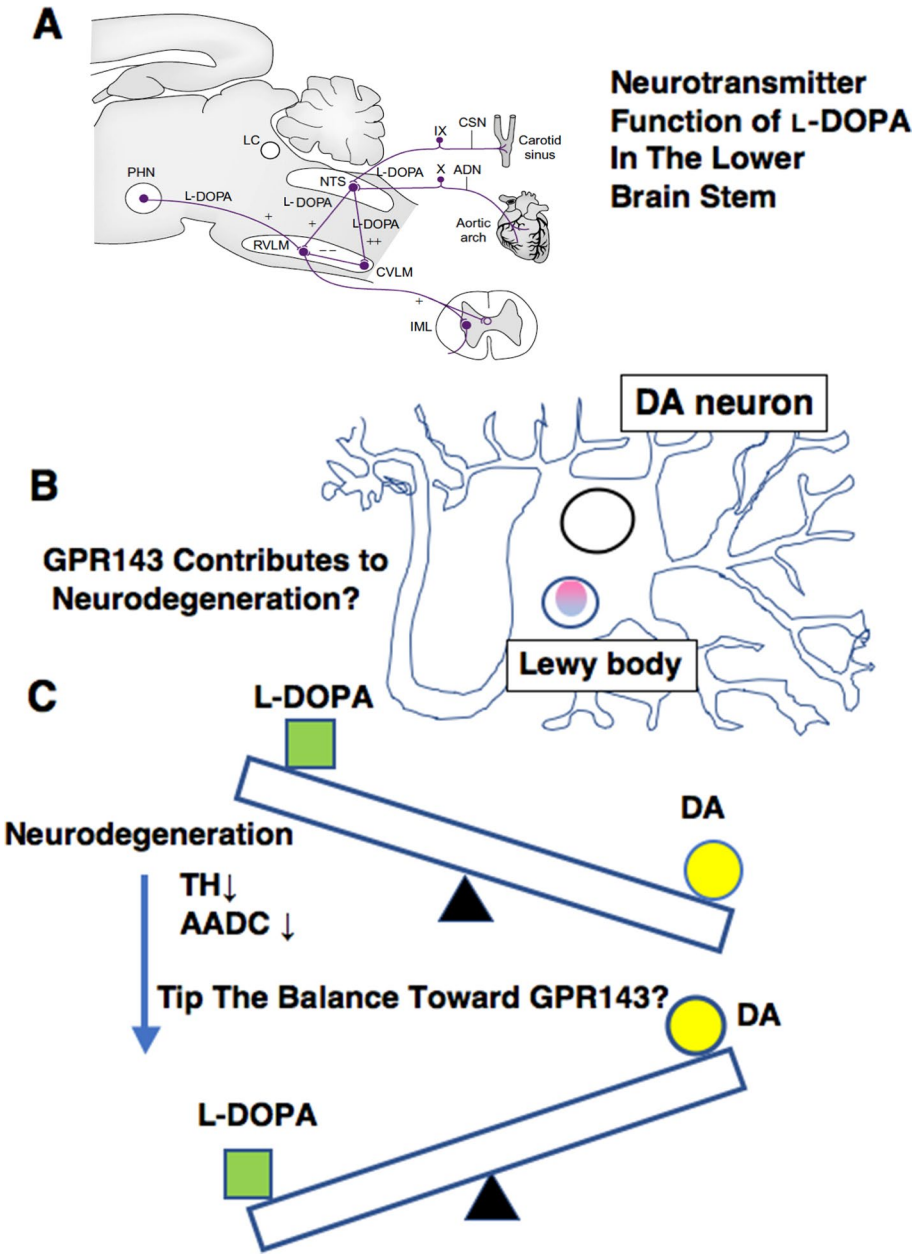


FIGURE 3 | Summary of various ways in which L-DOPA may act for normal physiology **(A)**, for PD pathology **(B)**, and for PD treatment **(C)**. **(A)** L-DOPA may play a role in carrying baroreceptor information as a neurotransmitter of the primary baroreceptor afferents terminating in the NTS. ADN, aortic depressor nerve; CSN, carotid sinus nerve; CVLM, caudal ventrolateral medulla; IML, intermediolateral cell column; LC, locus caeruleus; NTS, nucleus tractus solitarii; PHN, posterior hypothalamic nucleus; RVLM, rostral ventrolateral medulla. (See details in Misu et al., 2002) **(B)** GPR143 might be related to PD pathogenesis. **(C)** A possible impact on L-DOPA therapy. The decrease in the AADC activity may affect L-DOPA/DA receptor signaling balance in PD brains.

CONCLUSION

L-DOPA is likely to play a role as a neurotransmitter as well as a precursor of DA. Some critical questions, however, remain unanswered. Does L-DOPA release occur by a vesicular or nonvesicular mechanism? How does GPR143 mediate L-DOPA actions at a single-cell level? Is GPR143 involved in the pathogenesis of PD? Is GPR143 the only functional receptor for L-DOPA? This is the beginning of a new era for the mechanistic study of L-DOPA as a neurotransmitter, its neuronal network specificity, and its pathogenic role, all of which surely have a major impact on L-DOPA therapy in PD.

ETHICS STATEMENT

All the experiments were performed in accordance with the guidelines of ethical committees of Yokohama City University, Tokyo Metropolitan Neurological Hospital and Tokyo

Metropolitan Institute of Medical Sciences. Informed consent was obtained from all subjects.

AUTHOR CONTRIBUTIONS

YG, DM, YK, TH and AC all contributed to the writing and editing of this review.

FUNDING

YG is funded by a Grant-in-Aid for Scientific Research (B) (General) (no. 15H04687), Scientific Research (B) (General) (no. 18H02580), the Japanese SRF Grand for Biomedical Research (no. 1565), Uehara Memorial Foundation (no. 201320161 and 201720339), and the fund for Creation of Innovation Centers for Advanced Interdisciplinary Research Areas Program in the Project for Developing Innovation Systems from MEXT (no. 42890001). DM is funded by a Grant-in-Aid for Young Scientists (B) (no. 18K14923) and by the Uehara Memorial Foundation (201810115). TH is funded by a Grant-in-Aid for Scientific Research (C) (General) (no. 18K06896). YK is funded by a Grant-in-Aid for Young Scientists (no. 19K16375). AC is supported by Japanese government (MEXT) scholarship for PhD (no. 8513).

ACKNOWLEDGMENTS

We sincerely thank Drs M Koga, E Seki, N Arai, T Komori, Y Kamikubo, T Sakurai, K Tamura, H Wakui, U Yokoyama, and Y Ishikawa for collaboration. We also thank Dr SM Strittmatter for his critical reading of our manuscript.

REFERENCES

Ahlskog, J. E. (2011). Pathological behaviors provoked by dopamine agonist therapy of Parkinson's disease. *Physiol. Behav.* 104, 168–172. doi: 10.1016/j.physbeh.2011.04.055

Bhide, N., Lindenbach, D., Barnum, C. J., George, J. A., Surrena, M. A., and Bishop, C. (2015). Effects of the beta-adrenergic receptor antagonist propranolol on dyskinesia and L-DOPA–induced striatal DA efflux in the hemi-parkinsonian rat. *J. Neurochem.* 134, 222–232. doi: 10.1111/jnc.13125

Biermasz, N. R., Romijn, J. A., Pereira, A. M., and Roelfsema, F. (2005). Current pharmacotherapy for acromegaly: a review. *Expert Opin. Pharmacother* 6, 2393–2405. doi: 10.1517/14656566.6.14.2393

Chritton, S. L., Chinnow, S. L., Grabau, C., Dousa, M. K., Lucas, D., Roddy, D., et al. (1997). Adrenomedullary secretion of DOPA, catecholamines, catechol metabolites, and neuropeptides. *J. Neurochem.* 69, 2413–2420. doi: 10.1046/j.1471-4159.1997.69062413.x

Cohen, G. (1987). Monoamine oxidase, hydrogen peroxide, and Parkinson's disease. *Adv. Neurol.* 45, 119–125. doi: 10.1007/978-1-4613-0759-4_15

Connolly, B. S., and Lang, A. E. (2014). Pharmacological treatment of Parkinson disease: a review. *JAMA* 311, 1670–1683. doi: 10.1001/jama.2014.3654

Costa, A., Peppe, A., Dell'agnello, G., Carlesimo, G. A., Murri, L., Bonuccelli, U., et al. (2003). Dopaminergic modulation of visual-spatial working memory in Parkinson's disease. *Dement. Geriatr. Cogn. Disord.* 15, 55–66. doi: 10.1159/000067968

De Filippo, E., Manga, P., and Schiedel, A. C. (2017). Identification of novel G protein–coupled receptor 143 ligands as pharmacologic tools for investigating X-linked ocular albinism. *Invest. Ophthalmol. Vis. Sci.* 58, 3118–3126. doi: 10.1167/iovs.16-21128

De Pablo-Fernandez, E., Tur, C., Revesz, T., Lees, A. J., Holton, J. L., and Warner, T. T. (2017). Association of autonomic dysfunction with disease progression and survival in Parkinson disease. *JAMA Neurol.* 74, 970–976. doi: 10.1001/jamaneurol.2017.1125

Fahn, S., Oakes, D., Shoulson, I., Kieburtz, K., Rudolph, A., Lang, A., et al. (2004). Levodopa and the progression of Parkinson's disease. *N. Engl. J. Med.* 351, 2498–2508. doi: 10.1056/NEJMoa033447

Farran, B. (2017). An update on the physiological and therapeutic relevance of GPCR oligomers. *Pharmacol. Res.* 117, 303–327. doi: 10.1016/j.phrs.2017.01.008

Ferre, S. (2015). The GPCR heterotetramer: challenging classical pharmacology. *Trends Pharmacol. Sci.* 36, 145–152. doi: 10.1016/j.tips.2015.01.002

Ferre, S., Casado, V., Devi, L. A., Filizola, M., Jockers, R., Lohse, M. J., et al. (2014). G protein–coupled receptor oligomerization revisited: functional and pharmacological perspectives. *Pharmacol. Rev.* 66, 413–434. doi: 10.1124/pr.113.008052

Fiorentini, C., Busi, C., Gorruso, E., Gotti, C., Spano, P., and Missale, C. (2008). Reciprocal regulation of dopamine D1 and D3 receptor function and trafficking by heterodimerization. *Mol. Pharmacol.* 74, 59–69. doi: 10.1124/mol.107.043885

Fukuda, N., Naito, S., Masukawa, D., Kaneda, M., Miyamoto, H., Abe, T., et al. (2015). Expression of ocular albinism 1 (OA1), 3, 4- dihydroxy- L-phenylalanine (DOPA) receptor, in both neuronal and non-neuronal organs. *Brain Res.* 1602, 62–74. doi: 10.1016/j.brainres.2015.01.020

Ghosh, A., Sonavane, U., Andhirka, S. K., Aradhyam, G. K., and Joshi, R. (2012). Structural insights into human GPCR protein OA1: a computational perspective. *J. Mol. Model* 18, 2117–2133. doi: 10.1007/s00894-011-1228-8

Giladi, N., Mcmahon, D., Przedborski, S., Flaster, E., Guillory, S., Kostic, V., et al. (1992). Motor blocks in Parkinson's disease. *Neurology* 42, 333–339. doi: 10.1212/WNL.42.2.333

Goldstein, D. S., Eisenhofer, G., and Kopin, I. J. (2003). Sources and significance of plasma levels of catechols and their metabolites in humans. *J. Pharmacol. Exp. Ther.* 305, 800–811. doi: 10.1124/jpet.103.049270

Goshima, Y., Kubo, T., and Misu, Y. (1986). Biphasic actions of L-DOPA on the release of endogenous noradrenaline and dopamine from rat hypothalamic slices. *Br. J. Pharmacol.* 89, 229–234. doi: 10.1111/j.1476-5381.1986.tb11139.x

Goshima, Y., Kubo, T., and Misu, Y. (1988). Transmitter-like release of endogenous 3,4-dihydroxyphenylalanine from rat striatal slices. *J. Neurochem.* 50, 1725–1730. doi: 10.1111/j.1471-4159.1988.tb02470.x

Goshima, Y., Misu, Y., Arai, N., and Misugi, K. (1991a). Nanomolar L-DOPA facilitates release of dopamine *via* presynaptic beta-adrenoceptors: comparative studies on the actions in striatal slices from control and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)–treated C57 black mice, an animal model for Parkinson's disease. *Jpn J. Pharmacol.* 55, 93–100. doi: 10.1254/jjp.55.93

Goshima, Y., Nakamura, F., Masukawa, D., Chen, S., and Koga, M. (2014). Cardiovascular actions of DOPA mediated by the gene product of ocular albinism 1. *J. Pharmacol. Sci.* 126, 14–20. doi: 10.1254/jphs.14R03CR

Goshima, Y., Nakamura, S., and Misu, Y. (1991b). L-Dihydroxyphenylalanine methyl ester is a potent competitive antagonist of the L-dihydroxyphenylalanine–induced facilitation of the evoked release of endogenous norepinephrine from rat hypothalamic slices. *J. Pharmacol. Exp. Ther.* 258, 466–471.

Goshima, Y., Ohno, K., Nakamura, S., Miyamae, T., Misu, Y., and Akaike, A. (1993). L-DOPA induces Ca(2+)-dependent and tetrodotoxin-sensitive release of endogenous glutamate from rat striatal slices. *Brain Res.* 617, 167–170. doi: 10.1016/0006-8993(93)90631-V

Goshima, Y., Watanabe, S., Seki, E., Koga, M., Masukawa, D., Nakamura, F., et al. (2018). Immunoreactivity of a G protein–coupled l-DOPA receptor GPR143, in Lewy bodies. *Neurosci. Res.* 148, 49–53. doi: 10.1016/j.neures.2018.12.004

Gottwald, M. D., and Aminoff, M. J. (2011). Therapies for dopaminergic-induced dyskinesias in Parkinson disease. *Ann. Neurol.* 69, 919–927. doi: 10.1002/ana.22423

Graham, D. G., Tiffany, S. M., Bell, W. R., Jr., and Gutknecht, W. F. (1978). Autoxidation versus covalent binding of quinones as the mechanism of toxicity of dopamine, 6-hydroxydopamine, and related compounds toward C1300 neuroblastoma cells *in vitro. Mol. Pharmacol.* 14, 644–653.

Hiroshima, Y., Miyamoto, H., Nakamura, F., Masukawa, D., Yamamoto, T., Muraoka, H., et al. (2014). The protein Ocular albinism 1 is the orphan GPCR GPR143 and mediates depressor and bradycardic responses to DOPA in the nucleus tractus solitarii. *Br. J. Pharmacol.* 171, 403–414. doi: 10.1111/bph.12459

Ivanova, S. A., and Loonen, A. J. (2016). Levodopa-induced dyskinesia is related to indirect pathway medium spiny neuron excitotoxicity: a hypothesis based on an unexpected finding. *Parkinsons Dis.* 2016, 6461907. doi: 10.1155/2016/6461907

Karasawa, N., Isomura, G., and Nagatsu, I. (1992). Production of specific antibody against L-DOPA and its ultrastructural localization of immunoreactivity in the house-shrew (*Suncus murinus*) lateral habenular nucleus. *Neurosci. Lett.* 143, 267–270. doi: 10.1016/0304-3940(92)90280-K

Kubo, T., Yue, J. L., Goshima, Y., Nakamura, S., and Misu, Y. (1992). Evidence for L-DOPA systems responsible for cardiovascular control in the nucleus tractus solitarii of the rat. *Neurosci. Lett.* 140, 153–156. doi: 10.1016/0304-3940(92)90090-T

Kulisevsky, J., Avila, A., Barbanoj, M., Antonijoan, R., Berthier, M. L., and Gironell, A. (1996). Acute effects of levodopa on neuropsychological performance in stable and fluctuating Parkinson's disease patients at different levodopa plasma levels. *Brain* 119 (Pt 6), 2121–2132. doi: 10.1093/brain/119.6.2121

Leinartaite, L., and Svenningsson, P. (2017). Folding underlies bidirectional role of GPR37/Pael-R in Parkinson disease. *Trends Pharmacol. Sci.* 38, 749–760. doi: 10.1016/j.tips.2017.05.006

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

Lopez, V. M., Decatur, C. L., Stamer, W. D., Lynch, R. M., and Mckay, B. S. (2008). L-DOPA is an endogenous ligand for OA1. *PLoS Biol.* 6, e236. doi: 10.1371/journal.pbio.0060236

Marakaki, C., Papadimitriou, D. T., Kleanthous, K., Papadopoulou, A., and Papadimitriou, A. (2015). L-DOPA stimulates cortisol secretion through adrenocorticotropic hormone release in short children. *Horm. Res. Paediatr.* 84, 319–322. doi: 10.1159/000439532

Marsden, C. D. (1994). Problems with long-term levodopa therapy for Parkinson's disease. *Clin. Neuropharmacol.* 17 (Suppl), S32–S44.

Masukawa, D., Koga, M., Sezaki, A., Nakao, Y., Kamikubo, Y., Hashimoto, T., et al. (2017). L-DOPA sensitizes vasomotor tone by modulating the vascular alpha1-adrenergic receptor. *JCI Insight* 2, 17. doi: 10.1172/jci.insight.90903

Masukawa, D., Nakamura, F., Koga, M., Kamiya, M., Chen, S., Yamashita, N., et al. (2014). Localization of ocular albinism-1 gene product GPR143 in the rat central nervous system. *Neurosci. Res.* 88, 49–57. doi: 10.1016/j.neures.2014.07.008

Maurice, P., Kamal, M., and Jockers, R. (2011). Asymmetry of GPCR oligomers supports their functional relevance. *Trends Pharmacol. Sci.* 32, 514–520. doi: 10.1016/j.tips.2011.05.006

Meyer, R. C., Giddens, M. M., Schaefer, S. A., and Hall, R. A. (2013). GPR37 and GPR37L1 are receptors for the neuroprotective and glioprotective factors prosaptide and prosaposin. *Proc. Natl. Acad. Sci. U S A* 110, 9529–9534. doi: 10.1073/pnas.1219004110

Misu, Y., and Goshima, Y. (1993). Is L-DOPA an endogenous neurotransmitter? *Trends Pharmacol. Sci.* 14, 119–123. doi: 10.1016/0165-6147(93)90082-U

Misu, Y., Goshima, Y., Ueda, H., and Okamura, H. (1996). Neurobiology of L-DOPAergic systems. *Prog. Neurobiol.* 49, 415–454. doi: 10.1016/0301-0082(96)00025-1

Misu, Y., Goshima, Y., and Miyamae, T. (2002). Is DOPA a neurotransmitter? *Trends Pharmacol. Sci.* 23, 262–268. doi: 10.1016/S0165-6147(02)02013-8

Mitrano, D. A., Jackson, K., Finley, S., and Seeley, A. (2018). alpha1b-Adrenergic receptor localization and relationship to the D1-dopamine receptor in the rat nucleus accumbens. *Neuroscience* 371, 126–137. doi: 10.1016/j.neuroscience.2017.11.053

Miyamae, T., Goshima, Y., Shimizu, M., Shibata, T., Kawashima, K., Ohshima, E., et al. (1999). Some interactions of L-DOPA and its related compounds with glutamate receptors. *Life Sci.* 64, 1045–1054. doi: 10.1016/S0024-3205(99)00031-4

Mons, N., Danel, N., and Geffard, M. (1988). Visualization of L-dihydroxyphenylalanine in rat brain by using specific antibodies. *Brain Res.* 451, 403–407. doi: 10.1016/0006-8993(88)90793-7

Muller, T., Hellwig, R., and Muhlack, S. (2011). Levodopa induces synthesis of nerve growth factor and growth hormone in patients with Parkinson disease. *Clin. Neuropharmacol.* 34, 101–103. doi: 10.1097/WNF.0b013e318219dad0

Murakami, T., Shoji, M., Imai, Y., Inoue, H., Kawarabayashi, T., Matsubara, E., et al. (2004). Pael-R is accumulated in Lewy bodies of Parkinson's disease. *Ann. Neurol.* 55, 439–442. doi: 10.1002/ana.20064

Murer, M. G., Dziewczapolski, G., Menalled, L. B., Garcia, M. C., Agid, Y., Gershanik, O., et al. (1998). Chronic levodopa is not toxic for remaining dopamine neurons, but instead promotes their recovery, in rats with moderate nigrostriatal lesions. *Ann. Neurol.* 43, 561–575. doi: 10.1002/ana.410430504

Nagatsu, T., and Nagatsu, I. (2016). Tyrosine hydroxylase (TH), its cofactor tetrahydrobiopterin (BH4), other catecholamine-related enzymes, and their human genes in relation to the drug and gene therapies of Parkinson's disease (PD): historical overview and future prospects. *J. Neural Transm. (Vienna)* 123, 1255–1278. doi: 10.1007/s00702-016-1596-4

Nakamura, S., Goshima, Y., Yue, J. L., and Misu, Y. (1992). Transmitter-like basal and K(+)-evoked release of 3,4-dihydroxyphenylalanine from the striatum in conscious rats studied by microdialysis. *J. Neurochem.* 58, 270–275. doi: 10.1111/j.1471-4159.1992.tb09306.x

Nakamura, S., Yue, J. L., Goshima, Y., Miyamae, T., Ueda, H., and Misu, Y. (1994). Non-effective dose of exogenously applied L-DOPA itself stereoselectively potentiates postsynaptic D2 receptor–mediated locomotor activities of conscious rats. *Neurosci. Lett.* 170, 22–26. doi: 10.1016/0304-3940(94)90229-1

Piccirillo, R., Palmisano, I., Innamorati, G., Bagnato, P., Altimare, D., and Schiaffino, M. V. (2006). An unconventional dileucine-based motif and a novel cytosolic motif are required for the lysosomal and melanosomal targeting of OA1. *J. Cell Sci.* 119, 2003–2014. doi: 10.1242/jcs.02930

Poletti, M., and Bonuccelli, U. (2013). Acute and chronic cognitive effects of levodopa and dopamine agonists on patients with Parkinson's disease: a review. *Ther. Adv. Psychopharmacol.* 3, 101–113. doi: 10.1177/2045125312470130

Rascol, O., Lozano, A., Stern, M., and Poewe, W. (2011). Milestones in Parkinson's disease therapeutics. *Mov. Disord.* 26, 1072–1082. doi: 10.1002/mds.23714

Schiaffino, M. V. (2010). Signaling pathways in melanosome biogenesis and pathology. *Int. J. Biochem. Cell Biol.* 42, 1094–1104. doi: 10.1016/j.biocel.2010.03.023

Schiaffino, M. V., D'addio, M., Alloni, A., Baschirotto, C., Valetti, C., Cortese, K., et al. (1999). Ocular albinism: evidence for a defect in an intracellular signal transduction system. *Nat. Genet.* 23, 108–112. doi: 10.1038/12715

Shimamura, M., Shimizu, M., Yagami, T., Funabashi, T., Kimura, F., Kuroiwa, Y., et al. (2006). L-3,4-Dihydroxyphenylalanine–induced c-Fos expression in the CNS under inhibition of central aromatic L-amino acid decarboxylase. *Neuropharmacology* 50, 909–916. doi: 10.1016/j.neuropharm.2006.01.001

Smith, T. S., Parker, W. D., Jr., and Bennett, J. P., Jr. (1994). L-DOPA increases nigral production of hydroxyl radicals *in vivo*: potential L-DOPA toxicity? *Neuroreport* 5, 1009–1011. doi: 10.1097/00001756-199404000-00039

Ueda, H., Sato, K., Okumura, F., and Misu, Y. (1995b). L-DOPA inhibits spontaneous acetylcholine release from the striatum of experimental Parkinson's model rats. *Brain Res.* 698, 213–216. doi: 10.1016/0006-8993(95)00870-V

Ueda, H., Sato, K., Okumura, F., Inoue, A., Nakata, Y., Ozaki, N., et al. (1995a). Supersensitization of neurochemical responses by L-DOPA and dopamine receptor agonists in the striatum of experimental Parkinson's disease model rats. Biomed. Pharmacother. 49, 169–177. doi: 10.1016/0753-3322(96)82617-5

Ueda, S., Masukawa, D., Koga, M., and Goshima, Y. (2016). l-3,4-Dihydroxyphenylalanine induces ptosis through a GPR143-independent mechanism in mice. *J. Pharmacol. Sci.* 132, 109–112. doi: 10.1016/j.jphs.2016.08.005

Verschuur, C. V. M., Suwijn, S. R., Boel, J. A., Post, B., Bloem, B. R., Van Hilten, J. J., et al. (2019). Randomized delayed-start trial of levodopa in Parkinson's disease. *N. Engl. J. Med.* 380, 315–324. doi: 10.1056/NEJMoa1809983

Visanji, N. P., Fox, S. H., Johnston, T. H., Millan, M. J., and Brotchie, J. M. (2009). Alpha1-adrenoceptors mediate dihydroxyphenylalanine-induced activity in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine–lesioned macaques. *J. Pharmacol. Exp. Ther.* 328, 276–283. doi: 10.1124/jpet.108.144097

Voon, V., Napier, T. C., Frank, M. J., Sgambato-Faure, V., Grace, A. A., Rodriguez-Oroz, M., et al. (2017). Impulse control disorders and levodopa-induced dyskinesias in Parkinson's disease: an update. *Lancet Neurol.* 16, 238–250. doi: 10.1016/S1474-4422(17)30004-2

Xie, C. L., Zhang, Y. Y., Wang, X. D., Chen, J., Chen, Y. H., Pa, J. L., et al. (2015). Levodopa alone compared with levodopa-sparing therapy as initial treatment for Parkinson's disease: a meta-analysis. *Neurol. Sci.* 36, 1319–1329. doi: 10.1007/s10072-015-2253-7

Yang, Y., Nishimura, I., Imai, Y., Takahashi, R., and Lu, B. (2003). Parkin suppresses dopaminergic neuron-selective neurotoxicity induced by Pael-R in *Drosophila*. *Neuron* 37, 911–924. doi: 10.1016/S0896-6273(03)00143-0

Young, A., Powelson, E. B., Whitney, I. E., Raven, M. A., Nusinowitz, S., Jiang, M., et al. (2008). Involvement of OA1, an intracellular GPCR, and G alpha i3, its binding protein, in melanosomal biogenesis and optic pathway formation. *Invest. Ophthalmol. Vis. Sci.* 49, 3245–3252. doi: 10.1167/iovs.08-1806

Yue, J. L., Okamura, H., Goshima, Y., Nakamura, S., Geffard, M., and Misu, Y. (1994). Baroreceptor-aortic nerve-mediated release of endogenous L-3,4-dihydroxyphenylalanine and its tonic depressor function in the nucleus tractus solitarii of rats. *Neuroscience* 62, 145–161. doi: 10.1016/0306-4522(94)90321-2

Zhang, X., Andren, P. E., and Svenningsson, P. (2006). Repeated l-DOPA treatment increases c-fos and BDNF mRNAs in the subthalamic nucleus in the 6-OHDA rat model of Parkinson's disease. *Brain Res.* 1095, 207–210. doi: 10.1016/j.brainres.2006.04.019

Zhu, G., Okada, M., Yoshida, S., Hirose, S., and Kaneko, S. (2004). Determination of exocytosis mechanisms of DOPA in rat striatum using *in vivo* microdialysis. *Neurosci. Lett.* 367, 241–245. doi: 10.1016/j.neulet.2004.06.014

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pharmacology

ORIGINAL RESEARCH
published: 25 October 2019
doi: 10.3389/fphar.2019.01233

OPEN ACCESS

Edited by:
Philippe De Deurwaerdere, Université de Bordeaux, France

Reviewed by:
Michele Morari, University of Ferrara, Italy
Abdeslam Chagraoui, CHU de Rouen, France

***Correspondence:**
Rolando Meloni
rolando.meloni@upmc.fr

†These authors have contributed equally to this work

‡Present address:
Manuela Ingallinesi, Center for Therapeutic Innovation in Neuropsychiatry, Institut de Recherches Internationales Servier, Suresnes, France
Nicole Faucon Biguet, Anh Do Thi, Rolando Meloni, Laboratory of Gene Regulation and Adaptive Behaviors (GRAB) Neurosciences Paris Seine, CNRS UMR8246, UPMC UM119, INSERM UMRS1130, Institut de Biologie Paris Seine, 7 Quai Saint Bernard, Paris CEDEX 05, France

Specialty section:
This article was submitted to Neuropharmacology, a section of the journal Frontiers in Pharmacology

Received: *22 May 2019*
Accepted: *27 September 2019*
Published: *25 October 2019*

Citation:
Ingallinesi M, Galet B, Pegon J, Faucon Biguet N, Do Thi A, Millan MJ, Mannoury la Cour C and Meloni R (2019) Knock-Down of GPR88 in the Dorsal Striatum Alters the Response of Medium Spiny Neurons to the Loss of Dopamine Input and L-3-4-Dyhydroxyphenylalanine. Front. Pharmacol. 10:1233. doi: 10.3389/fphar.2019.01233

Knock-Down of GPR88 in the Dorsal Striatum Alters the Response of Medium Spiny Neurons to the Loss of Dopamine Input and L-3-4-Dyhydroxyphenylalanine

Manuela Ingallinesi[1†‡], Benjamin Galet[1†‡], Jonathan Pegon[1], Nicole Faucon Biguet[1‡], Anh Do Thi[1‡], Mark J Millan[2], Clotilde Mannoury la Cour[2] and Rolando Meloni[1*‡]

[1] Department of Biotechnology and Biotherapy, Institut du Cerveau et de la Moelle épinière (ICM) UPMC/INSERM U 1127/ CNRS UMR 7225, CHU Pitié-Salpêtrière, Paris, France, [2] Center for Innovation in Neuropsychiatry, Institut de Recherches Servier, Croissy sur Seine, France

The effects of L-3-4-dyhydroxyphenylalanine (L-DOPA) treatment for replacing the dopamine (DA) loss in Parkinson's disease (PD) progressively wear off and are hindered by the development of dyskinesia, prompting the search for new treatments. The orphan G protein-coupled receptor 88 (Gpr88) represents a potential new target, as it is highly and almost exclusively expressed in the projecting gamma-Aminobutyric Acid-ergic (GABAergic) medium spiny neurons of the striatum, is implicated in motor activity, and is downregulated by 6-hydroxydopamine (6-OHDA) lesions, an effect that is reversed by L-DOPA. Thus, to evaluate Gpr88 as a potential target for the management of PD and L-DOPA–induced dyskinesia (LID), we inactivated Gpr88 by lentiviral-mediated knock-down with a specifically designed microRNA (miR) (KD-Gpr88) in a 6-OHDA rat model of hemiparkinsonism. Then, we investigated the effects of the KD-Gpr88 in the DA-deprived dorsal striatum on circling behavior and LID as well as on specific markers of striatal neuron activity. The KD-Gpr88 reduced the acute amphetamine-induced and increased L-DOPA–induced turning behavior. Moreover, it normalized the upregulated expression of striatal *Gad67* and *proenkephalin* provoked by the 6-OHDA lesion. Finally, despite promoting ΔFosB accumulation, the KD-Gpr88 was associated neither with the upregulation of *prodynorphin*, which is causally linked to the severity of LID, nor with the aggravation of LID following chronic L-DOPA treatment in 6-OHDA–lesioned rats. These results thus justify further evaluation of Gpr88 as a potentially novel target for the management of PD as an alternative to L-DOPA therapy.

Keywords: Gpr88, 6-hydroxydopamine 6-OHDA, turning behavior, lentiviral vector, L-3-4-dyhydroxyphenylalanine–induced dyskinesia

INTRODUCTION

The loss of dopamine (DA) input to the GABAergic striatal medium spiny neurons (MSNs) provokes an imbalance between the direct stimulatory striato-nigral and the indirect inhibitory striato-pallidal pathways responsible for movement initiation, thereby inducing the motor symptoms of Parkinson's disease (PD) (Gerfen and Surmeier, 2011). The altered activation of these striatal outputs is associated with a net increase of the striatal glutamic acid decarboxylase 67 (*Gad67*) expression and the concomitant downregulation of prodynorphin (*Pdyn*) in the DA type 1 (D1) receptor regulated striato-nigral direct pathway as well as upregulation of proenkephalin (*Penk*) in the DA type 2 (D2) receptor regulated striato-pallidal indirect pathway (Carta et al., 2001). L-DOPA replacement therapy alleviates the motor symptoms but in the long term, by hyperactivating the sensitized D1 receptor in the direct stimulatory pathway, provokes L-DOPA–induced dyskinesia (LID) (Spigolon and Fisone, 2018) by a complex pattern of interaction with cellular substrates and neural networks (De Deurwaerdère et al., 2017) including the accumulation of ΔFosB (Nestler, 2015), which upregulates *Pdyn* in the MSN of the direct pathway (Andersson et al., 1999; Andersson et al., 2003). Thus, alternative antiparkinsonian targets, avoiding—or masking—the untoward effects of L-DOPA therapy and offering new therapeutic approaches, are needed for the treatment of PD (Huot et al., 2013; Fox et al., 2018).

Gpr88, an orphan G protein–coupled receptor almost exclusively expressed in the striatum (Mizushima et al., 2000), specifically in the MSN (Massart et al., 2009), displays several features of a potential target for the treatment of PD. Namely, Gpr88 knock-out (KO) mice display DA hypersensitivity, suggesting that Gpr88 may have an inhibitory influence on DA-dependent MSN activity (Logue et al., 2009; Quintana et al., 2012). Reciprocally, DA may modulate Gpr88 activity, since DA loss following 6-hydroxydopamine (6-OHDA) lesions of the DAergic nigrostriatal pathway downregulates *Gpr88* expression, which is thereafter restored by L-DOPA (Massart et al., 2009; Massart et al., 2012). However, the interplay between DA signaling and Gpr88 activity is not straightforward, since in basal conditions, the levels of Gpr88 expression are twofold higher in the MSN of the indirect pathway as compared to the MSN of the direct pathway (Massart et al., 2009). Moreover, the Gpr88 downregulation associated with DA loss is the net result of an increase of Gpr88 expression in the direct pathway and a decrease of its expression in the indirect pathway, hinting that Gpr88 responds differently to specific D1 or D2 receptor stimulation, while the L-DOPA treatment completely reverses the 6-OHDA–induced alterations of Gpr88 expression in both pathways (Massart et al., 2009). Finally, while the Gpr88 KO results in increased locomotion in response to DA stimulation (Logue et al., 2009), we have previously shown that the local inactivation of Gpr88 in the ventral striatum decreases the motor hyperactivity induced by amphetamine (Amph) (Ingallinesi et al., 2015). Thus, the precise role played by Gpr88 in motor regulation remains unclear, and its relevance as a target for PD treatment needs to be further evaluated.

To this end, using the unilateral 6-OHDA lesion as a model of PD, we locally inactivated Gpr88 in the dorsal DA-deprived striatum, a region that is associated with motor regulation (Do et al., 2013). We then assessed the impact of the Gpr88 knock-down (KD-Gpr88) on the turning behavior induced by Amph and L-DOPA, on the development of LID, and on the expression of striatal markers altered by the 6-OHDA lesion and the chronic L-DOPA treatment such as *Gad67* as a marker of general MSN activity (Cenci et al., 1998) as well as *Pdyn* and *Penk* as markers of direct and indirect pathway specific activity, respectively (Cenci et al., 1998; Steiner and Gerfen, 1998).

MATERIALS AND METHODS

Experimental Animals

Six-week-old male Sprague Dawley rats (Janvier Labs, Rte des chênes secs, 53940 Le Genest-Saint-Isle, France) weighing 250–270 g at the beginning of the experiments were housed on a 12 h dark/light cycle at 20–22°C with free access to food and water. Animal studies authorized by "Ministère de la Recherche" (APAFIS #3669-2016011817516297 v6) were conducted in an approved animal facility (agreement #B75-13-19). The animals were handled throughout the study in compliance with the European Union 2010 Animal Welfare Act and the 2010/63 French directive.

Drugs

6-Hydroxydopamine hydrobromide (6-OHDA), d-amphetamine sulfate (Amph), L-3,4-dihydroxyphenilalanine methyl ester hydrochloride (L-DOPA), and benserazide were purchased from Sigma-Aldrich. 6-OHDA was freshly prepared in physiological saline solution plus 0.02% ascorbic acid. The other drugs were freshly prepared in physiological saline solution only.

Lentiviral Vectors

Lentiviral (LV)-vectors (p24 of stock solutions ranging between 200 and 300 ng/μl and transducing units/ml around 2×10^8) co-expressing, under the drive of the ubiquitous phosphoglycerate kinase (PGK) promoter, the Emerald green fluorescent protein (EmGFP) and a designed microRNA (miR), either specifically directed against the Gpr88 mRNA (LV-miR-Gpr88) or without any target in the rat genome (LV-miR-control), were produced according to standard procedures (Zennou et al., 2001; Castaing et al., 2005).

Experimental Design

The experimental design including the timeline, the different steps, and the number of animals involved is presented in **Figure 1**. Briefly, 6-week-old rats (n = 95) at the starting time (T-0) received stereotaxic 6-OHDA injections. Three weeks later (T-3), these rats were tested for acute Amph-induced turning behavior. The rats not showing individual means > 5 full turns per minute in the direction ipsilateral to the lesion were discarded (n = 15). At T-4, the remaining 6-OHDA–lesioned rats (n = 80) received stereotaxic injections of lentiviral vectors expressing

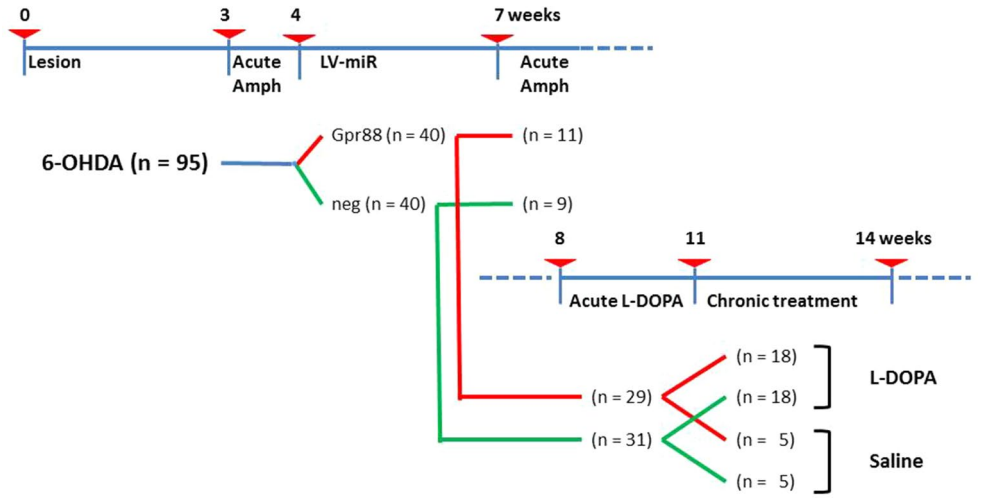


FIGURE 1 | Experimental design. Timeline in weeks showing the different experimental settings and animal groups. Number of animals used in each group is reported in parentheses.

either the designed miR directed against Gpr88 (n = 40) or the negative control miR (n = 40). Then, at T-7, a batch of miR-bearing rats (KD-Gpr88, n = 11; KD-neg, n = 9) were tested for Amph-induced turning behavior and immediately sacrificed. The remaining group of miR-bearing (KD-Gpr88, n = 29; KD-neg, n = 31) rats were tested at T-8 for acute L-DOPA–induced turning behavior. Thereafter, between T-11 and T-14, this batch of rats was chronically (21 days) treated with daily injections of either L-DOPA (KD-Gpr88, n = 18; KD-neg, n = 18) or saline solution (KD-Gpr88, n = 5; KD-neg, n = 5) and serially tested for the development of LID.

The rats were sacrificed by decapitation, and the brains were rapidly removed, frozen in isopentane (−45°C), and kept at −80°C for immunofluorescence and *in situ* hybridization (ISH) experiments.

Stereotaxic Surgery

The coordinates for stereotaxic surgery were calculated using the Paxinos and Watson rat brain atlas (Paxinos and Watson, 1997). Unilateral injections of 6-OHDA (8 µg in 4.0 µl 0.9% NaCl solution containing 0.02% ascorbic acid) (n = 95) were performed in the left medial forebrain bundle to lesion the nigrostriatal DAergic pathway at the following coordinates (in mm) relative to bregma and the dural surface: antero-posterior (AP) = −1.3, medio-lateral (ML) = +1.3, dorso-ventral (DV) = −8.0, tooth bar at +5. The delivery rate was set at 0.5 µl/min, and at the end of the injection, the needle was left in place for an additional 3 min.

LV-vectors were infused unilaterally in the dorsal striatum on the lesioned side at two dorsal–ventral levels over three sites (2 µl per site, 6 µl total) at the following coordinates (in mm) relative to bregma: (1) AP +1.8, ML +2.8, DV −5.5, −5.0; (2) AP +0.6, ML +2.5, DV −5.5, −5.0; (3) AP +0.6, ML +3.7, DV −5.5, −5.0. The delivery rate was set at 0.33 µl/min, and at the end of the injection, the needle was left in place for an additional 3 min at each site.

Behavioral Testing for Rotational Behavior

Rotation tests in response to Amph (5 mg/kg, i.p.) were performed in a batch of rats after both 6-OHDA and LV-vector stereotaxic injections by recording the number of turns in an automated rotometer over a 1 h period in time bins of 10 min.

Then, the net turning behavior was calculated for each animal at each 10 min time point by subtracting the number of ipsilateral turns after the 6-OHDA lesion from the number of ipsilateral turns after the lentiviral injection.

The rotational response to an acute injection of L-DOPA (10 mg/kg i.p.) and benserazide (15 mg/kg i.p.) was evaluated after the lentiviral injections. The doses of L-DOPA and benserazide were in the optimal range and proportions reported in the literature to obtain turning behavior (Tayarani-Binazir et al., 2012). Both ipsilateral and contralateral 360° turns were recorded over a 1 h period in time bins of 10 min, and the net rotational asymmetry (contralateral minus ipsilateral turns) was calculated.

Chronic L-DOPA Treatment

L-DOPA (6 mg/kg, i.p.) plus benserazide (15 mg/kg, i.p.) was injected daily for 21 days to induce a gradual development and a stable degree of LID. The doses of L-DOPA and benserazide were in the optimal range and proportions reported in protocols for developing LID (Cenci et al., 1998; Carta et al., 2006). Control animals received daily injections of saline solution.

Abnormal Involuntary Movement Rating

LID severity was evaluated by scoring L-DOPA–induced abnormal involuntary movements (AIMs) according to a rat's dyskinesia scale (Cenci et al., 1998; Cenci and Lundblad, 2007) by an experimenter blind to the type of LV-miR and pharmacological treatment received by the animal.

Rats were placed individually in a transparent plastic cylinder and observed every 20 min for a monitoring period

of 1 min, from 20 to 120 min after the injection of L-DOPA or physiological solution.

Ratings of dyskinesia were carried out at days 9, 11, 16, and 18 after the beginning of the chronic L-DOPA treatment. Four subtypes of AIMs were classified: locomotive AIMs (asymmetric locomotion with contralateral turning bias); axial AIMs (twisting of the neck and trunk toward the side contralateral to the lesion); limb AIMs (purposeless "grabbing" movements and/or dystonic posturing of the forelimb contralateral to the lesion); and orolingual AIMs (empty jaw movements and contralateral tongue protrusions). These movements can be clearly discerned from enhanced manifestations of normal motor activities (such as grooming, gnawing, rearing, and sniffing), and they have marked hyperkinetic and/or dystonic features.

Each AIM subtype was scored on a severity scale from 0 to 4 according to the proportion of time/monitoring period during which the AIM was present (0, absent; 1, occasional, i.e. present during less of 50% of the observation time; 2, frequent, i.e. present during more than 50% of the observation time; 3, continuous but interrupted by strong sensory stimuli; 4, continuous, not interrupted by strong sensory stimuli). In case of uncertainty between two consecutive grades of the scale, the corresponding half score was used (i.e. 1.5, 2.5, 3.5). The theoretical maximum sum of AIM scores reached by one rat in one testing session was 96, corresponding to the maximum score/monitoring period (i.e. 16) multiplied by the number of monitoring periods/testing sessions (i.e. 6). Thus, the maximum AIM score that can be attained at the end of the four testing sessions is $96 \times 4 = 384$.

Immunofluorescence

Immunofluorescence experiments were performed on 12-µm-thick striatal coronal cryosections spanning the lentivirus-injected area of diffusion. The sections were fixed in 4% paraformaldehyde in phosphate buffered saline (PBS) 1X for 30 min at 4°C, rinsed in PBS 1X, and micro-waved at 700 W for 2.5 min with antigen retrieval (DakoCytomation Dako Glostrup, Denmark). After rinsing in PBS-Triton wash buffer (PBS 1X, 0.2% Triton X-100), sections were blocked with 10% goat serum in PBS1X for 1 h at room temperature. The following primary antibodies were added to a base solution (2% goat serum in PBS1X) and incubated overnight at 4°C: tyrosine hydroxylase (TH) (Millipore MAB318, 1:400), ΔFosB (Abcam AB11959, 1:500), and Neuronal Nuclei (NeuN) (Chemicon MAB 377, 1:400). TH and NeuN immunolabeling was completed using a fluorophore-coupled secondary antibody (Alexa Fluor 647, Invitrogen A21235, 1:1000), while ΔFosB required 3,3′-Diaminobenzidine revelation for best results (BA-2000 secondary antibody, 1:250, and PK6100 kit from Vector Laboratories).

TH and NeuN immunolabeling was completed using a fluorophore-coupled secondary antibody (Alexa Fluor 647, Invitrogen A21235, 1:1000), incubated for 1 h at room temperature. For best results, ΔFosB immunofluorescence required tyramide signal amplification, according to the manufacturer protocol (PerkinElmer, TSA NEL702001KT). The sections were rinsed in PBS-Triton wash buffer three times before being stained with 4',6-diamidino-2-phenylindole (DAPI) for 10 min. Finally, the sections were rinsed in PBS and mounted using Fluorescent Mounting Medium (Dako Cytomation; Dako, Glostrup, Denmark).

In Situ Hybridization

ISH was performed with antisense digoxygenin-labeled complementary RNA probes designed to recognize *Th*, *Gpr88*, *Gad67*, *Penk*, and *Pdyn* mRNAs. The antisense riboprobes were transcribed from the pGEM®-T easy vector as previously described (Ingallinesi et al., 2015).

All plasmids (1 µg/probe) were linearized and used as templates for the synthesis by T7 or SP6 RNA polymerase (Promega, Madison, WI, USA) of the probe labeled with digoxygenin-11-UTP (Roche, Switzerland). The brain sections were processed for ISH as previously described (Ingallinesi et al., 2015).

Digitization and Semi-Quantitative Analysis

ISH and immunofluorescence slides were digitized using the Axio Scan.Z1 and ZEN software (Zeiss, Oberkochen, Germany). The resulting images were then processed in ImageJ (NIH, Bethesda, MD, USA). As fluorescence and colorimetric staining are not stoichiometrically related to biological content, the signal intensity was not quantified. Instead, a threshold was determined using control slides (secondary antibody alone/sense probe) and applied to all the images from the same experiment. A fixed-size region of interest was then drawn in the transduced areas, where the total signal-positive area was quantified. For each rat, the signal was measured over two to three anteroposterior locations between AP +0.2 mm and +1.8 mm, and averaged. The values from the lesioned/transduced side were then normalized to those obtained in the unaffected hemisphere.

Statistical Analysis

Rats with 6-OHDA lesion were included for turning behavior statistical analysis when showing individual means >5 full turns per minute in the direction ipsilateral to the lesion with the first Amph challenge. As reviewed by Bjorklund and Dunnet, literature data show that such rotation responses correspond to a permanent reduction in DA content in the striatum of greater than 90% (Bjorklund and Dunnett, 2019). Rats showing less than five full turns per minute (n = 15) were excluded from analysis and discarded from the experimental protocol.

Other inclusion criteria were the complete loss of TH immunoreactivity signal in the striatum or *Th* ISH signal in the *substantia nigra* after the 6-OHDA lesion and detectable EmGFP signals after lentiviral injections. Rats lacking one of these criteria (KD-neg, n = 8; KD-Gpr88, n = 6) were excluded from behavioral and molecular analyses. The number of rats at each experimental step presented in **Figure 1** takes

into account these exclusions. Data are presented as mean ± SEM. Statistics were performed using GRAPHPAD PRISM (GraphPad Software Inc., San Diego, CA, USA). One-way or two-way ANOVA followed by *post hoc* Tukey or Bonferroni tests was carried out for statistical analyses as indicated in the results. The distribution of the data from the AIM axial, limb, oral (ALO) locomotor (LOCO) and TOTAL scoring was tested for normality (Kolmogorov–Smirnov test) and then analyzed using a two-way ANOVA followed by Sidak's multiple comparisons test. The difference between comparisons was considered to be significant at $P < 0.05$.

RESULTS

6-OHDA–Induced Nigrostriatal Lesions and Gpr88 Knock-Down Extension

Th, the rate-limiting enzyme in the synthesis of DA and other catecholamines, was used to evaluate the efficacy of the unilateral 6-OHDA injections targeting the nigrostriatal pathway. The 6-OHDA lesion resulted in the complete loss of the specific signal for Th in the side of injection compared to the contralateral intact side, as assessed by immunoreactivity in the striatum and by ISH in the substantia nigra (**Figures 2A**, **B**).

The lentiviral vectors expressing either the miR-Gpr88 or the miR-neg were injected in the rat dorsal striatum ipsilateral to the 6-OHDA lesion. The diffusion of the lentiviral vector resulted in the localized loss of Gpr88 expression (**Figures 3A**, **B**) and the presence in the same region of the EmGFP marker (**Figure 3C**) that allowed for ascertaining the efficacy of the transduction. The ISH signal for Gpr88 was, on the contrary, present in the region transduced with the miR-neg (**Figure 3D**).

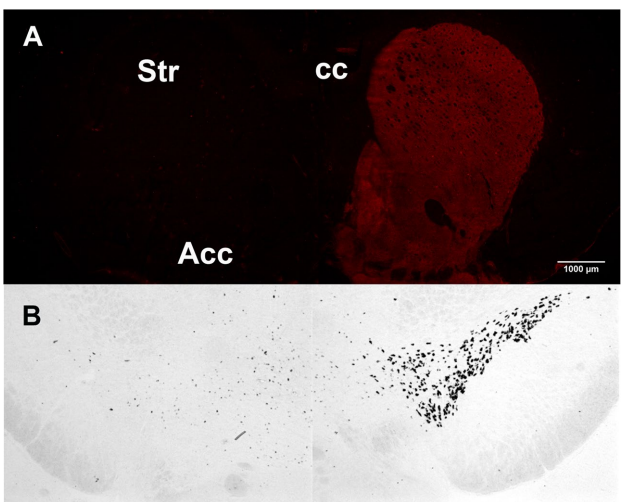


FIGURE 2 | Tyrosine hydroxylase unilateral loss after 6-hydroxydopamine (6-OHDA) lesion. **(A)** Tyrosine hydroxylase immunoreactivity in the 6-OHDA–lesioned (left) and in the intact striatum (right). **(B)** Tyrosine hydroxylase in situ hybridization in the 6-OHDA–lesioned (left) and in the intact substantia nigra (right).

The lentiviral-mediated transduction of striatal neurons did not produce any significant neuronal loss, since, as evidenced by the merging of *Gpr88* ISH and NeuN marking, the NeuN signal was uniformly distributed in the striatum without apparent differences between Gpr88-positive and -negative areas (**Figure 4**).

The KD-Gpr88 Improves Hemiparkisonian Turning Behavior

To ascertain the effects of the KD-Gpr88 on motor activity, we tested the 6-OHDA–lesioned rats, before and after intrastriatal injections of the lentiviral vectors, for Amph-induced turning behavior, which is a highly reliable test for evaluating the extent of DA loss and motor impairment (Iancu et al., 2005; Bjorklund and Dunnett, 2019). Three weeks after the 6-OHDA lesion, Amph elicited a strong turning behavior ipsilateral to the lesioned side (mean 11.7 ± 0.9 turns/min) with no appreciable contralateral turning. The Amph-induced turning behavior was then once again measured in a batch of rats 3 weeks after receiving lentiviral vectors expressing either the miR-neg (KD-neg) or the miR-Gpr88 (KD-Gpr88) in the dorsal region of the DA-depleted striatum. The net turning behavior resulted in positive values in KD-neg rats (n = 9), indicating that the effect of the 6-OHDA lesion increased over time. On the contrary, the net turning behavior resulted in negative values in the KD-Gpr88 rats (n = 11), showing that the Gpr88 inactivation partially reduced the motor imbalance induced by 6-OHDA lesions (**Figure 5A**).

Four weeks after lentiviral vector injections, turning behavior following an acute L-DOPA (10 mg/kg i.p.) plus benserazide (15 mg/kg i.p.) challenge was assessed in another batch of 6-OHDA–lesioned and lentiviral transduced rats that were not previously tested with a second Amph treatment. In the 6-OHDA–lesioned rats, initial turning toward the lesioned site was observed, which then switched to the contralateral side after half an hour, an effect of acute L-DOPA treatment that has been reported in the literature (Mura et al., 2002). The contralateral turning was increased in the KD-Gpr88 as compared to KD-neg rats, indicating that the Gpr88 inactivation enhances the effects of L-DOPA in the 6-OHDA–lesioned striatum (**Figure 5B**).

KD-Gpr88 and L-DOPA–Induced Dyskinesia

Two 6-OHDA–lesioned groups of rats, bearing either the KD-neg or the KD-Gpr88, were chronically treated for 21 days with L-DOPA (6 mg/kg) plus benserazide (15 mg/kg), while two corresponding groups of animals received an equal volume of saline solution. The 6-OHDA–lesioned rats chronically treated with saline (KD-neg, n = 5; KD-Gpr88, n = 5) did not develop LID. On the contrary and as expected according to the literature (Cenci et al., 1998), comprehensively over 50% (n = 20) of the 6-OHDA–lesioned rats chronically treated with L-DOPA developed LID, both in the KD-neg (n = 8 out of 18) and in the KD-Gpr88 (n = 12 out of 18) group. The distribution of the data from the AIM scoring (ALO, LOCO, and TOTAL scores) was

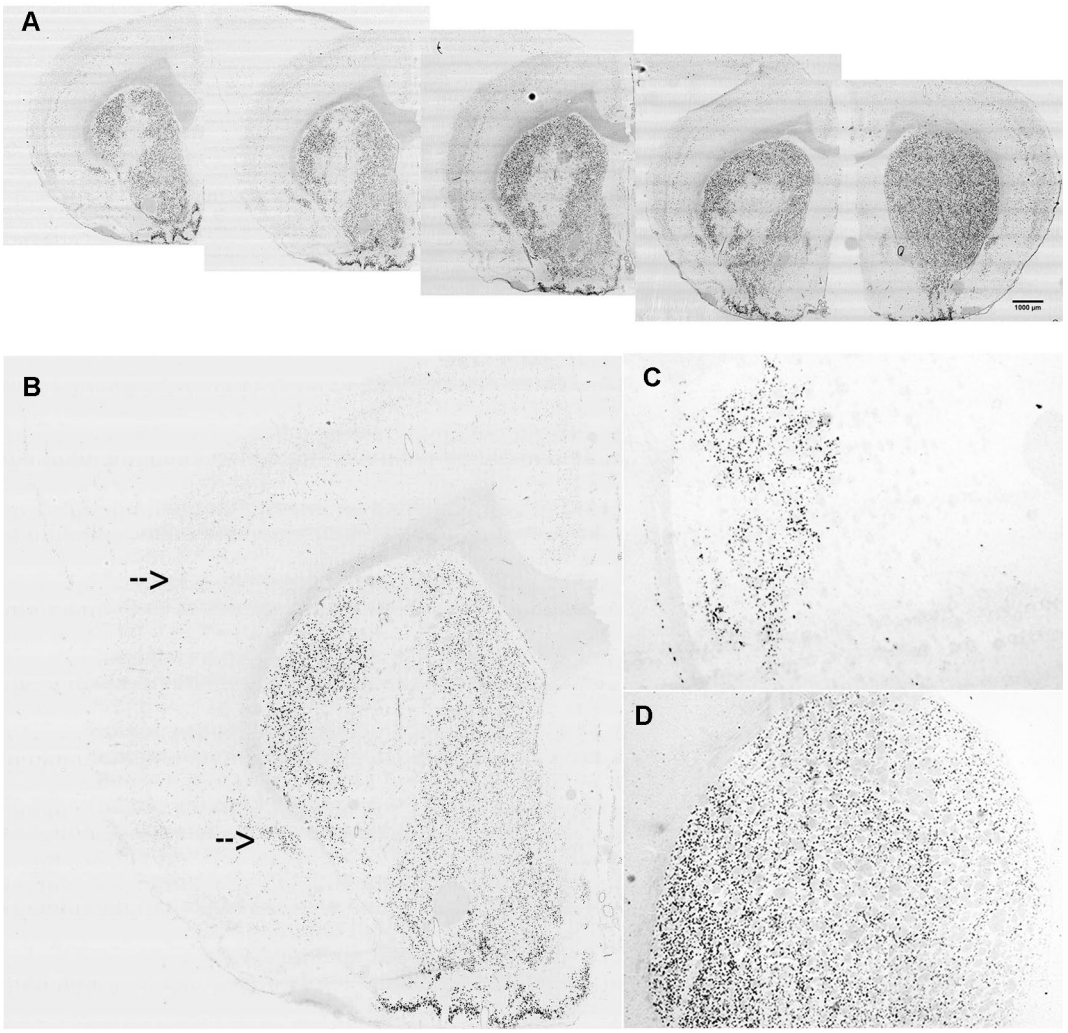


FIGURE 3 | *In situ* hybridization for lentiviral marker and Gpr88 expression. **(A)** Riboprobe against Gpr88 showing the loss of the Gpr88 signal in a series of anteroposterior sections in the region of the left striatum transduced with the specific miR-Gpr88. **(B)** Higher resolution showing the loss of the Gpr88 signal in a section of the left striatum transduced with the specific miR-Gpr88. Arrows indicate Gpr88 expression in the cortex and the piriform cortex. **(C)** Riboprobe against the Emerald green fluorescent protein (EmGFP) marker showing the extension of lentiviral-mediated striatal transduction in a section of the left striatum adjacent to the section reproduced in B. **(D)** Riboprobe against Gpr88 showing the uniform signal of Gpr88 expression in the striatum transduced with the miR-neg.

verified and passed the normality testing using the Kolmogorov–Smirnov test.

The total AIM score in the LID-positive animals was of about 55% of the maximal theoretical score of 384 (Cenci and Lundblad, 2007) (see *Materials and Methods*) and was not different between KD-neg (222.7 ± 27.9; n = 8) and KD-Gpr88 (206.4 ± 17.3; n = 12).

Also, the AIM score at each time point (9, 11, 16, and 18 days of L-DOPA treatment) calculated either as a whole or separating the ALO (axial, limb, oral) scores from the locomotor scores was not different between the two experimental groups (**Figure 6**). Thus, these results show that, notwithstanding the acute L-DOPA–elicited increase in contralateral turning behavior, the KD-Gpr88 does not worsen the severity of LID following a chronic L-DOPA treatment.

The KD-Gpr88 Normalizes the 6-OHDA–Induced *Gad67* and *Penk* Overexpression and Prevents LID-Associated *Pdyn* Upregulation

The chronic L-DOPA treatment associated with the development of LID increases the expression of *Gad67*, *Penk*, and *Pdyn*, with this latter being positively correlated with AIM scores (Cenci et al., 1998). Moreover, *Pdyn* upregulation associated with LID is promoted by ΔFosB (Andersson et al., 1999). Thus, we assessed the expression of *Gad67*, *Penk*, and *Pdyn* in the dorsal striatum of 6-OHDA–lesioned KD-neg and KD-Gpr88 rats that developed LID after 21 days' treatment with L-DOPA and their corresponding controls treated for 21 days with a saline solution.

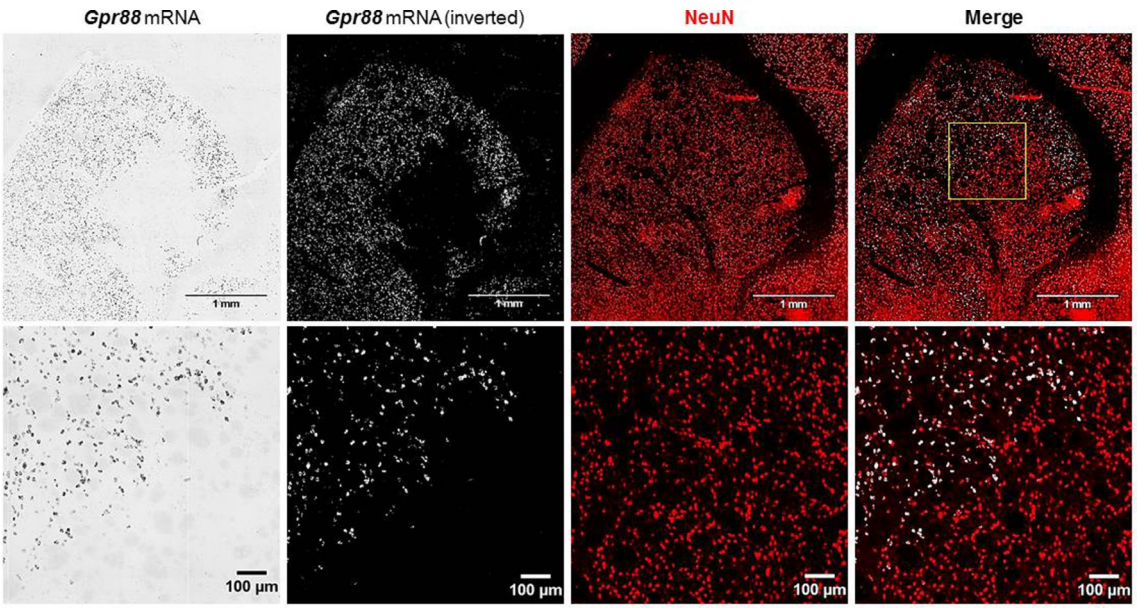


FIGURE 4 | *In situ* hybridization for Gpr88 expression and immunofluorescence for NEUronal Nuclei (NeuN) expression.

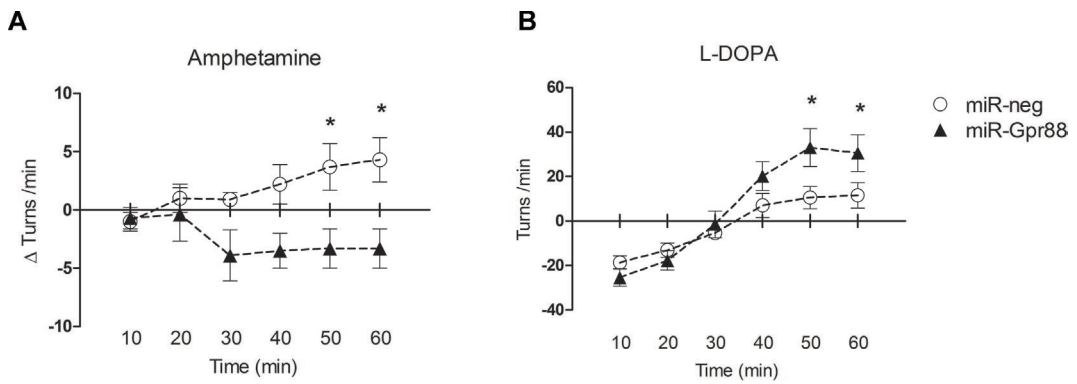


FIGURE 5 | Drug-elicited turning behavior. **(A)** Amphetamine (Amph) (5 mg/kg i.p.)–induced turning behavior: net turning behavior (Δturns) means of the differences calculated for each rat in 10 min interval deducting the number of turns recorded before from the number of turns recorded after lentiviral injection. KD-neg, n = 9; KD-Gpr88, n = 11. Two-way ANOVA: $F_{1, 108} = 20.26$, p = 0.0001. * p-value <0.05 (Bonferroni post-test). **(B)** L-DOPA (10 mg/kg i.p.) plus benserazide (15 mg/kg i.p.)–induced turning behavior: net rotational asymmetry (contralateral minus ipsilateral turns) after lentiviral vector injections. KD-neg, n = 22; KD-Gpr88, n = 17. Two-way ANOVA: $F_{5, 270} = 14.09$, p = 0.0001. * p-value <0.05 (Bonferroni post-test).

As expected according to the literature (Cenci et al., 1998), the 6-OHDA lesion increased *Gad67* and *Penk* and decreased *Pdyn* expression in KD-neg rats. However, the KD-Gpr88 reversed to baseline levels both *Gad67* and *Penk* but not *Pdyn* expression (**Figure 7A**). LID is associated with *Gad67*, *Penk*, and *Pdyn* overexpression. Accordingly, in LID-displaying KD-neg rats, the expression of *Gad67*, *Penk*, and *Pdyn* was concomitantly increased. However, in the KD-Gpr88 rats with LID, while *Gad67* and *Penk* were upregulated to the same extent as in their KD-neg counterparts, *Pdyn* expression remained inferior to the baseline and significantly different from *Pdyn* expression in the KD-neg rats (**Figure 7B**), indicating that the Gpr88 inactivation prevents the *Pdyn* upregulation that is associated with the development of LID (Cenci et al., 1998).

Hyperactivation of ΔFosb Following Gpr88 Inactivation

LID-associated *Pdyn* upregulation, which is positively correlated with AIM scores, is promoted by ΔFosB activation (Cenci et al., 1998). Actually, the L-DOPA chronic treatment after 6-OHDA lesions has been shown to increase the levels of ΔFosB, a member of the Fos family of transcription factors that is induced by repetitive stimulation and that accumulates in a stable form in the D1-expressing MSN of the direct pathway (Nestler, 2015). Since the accumulation of ΔFosB is also correlated with the severity of LID (Pavon et al., 2006; Engeln et al., 2016), we evaluated the effects of the Gpr88 inactivation on ΔFosB expression.

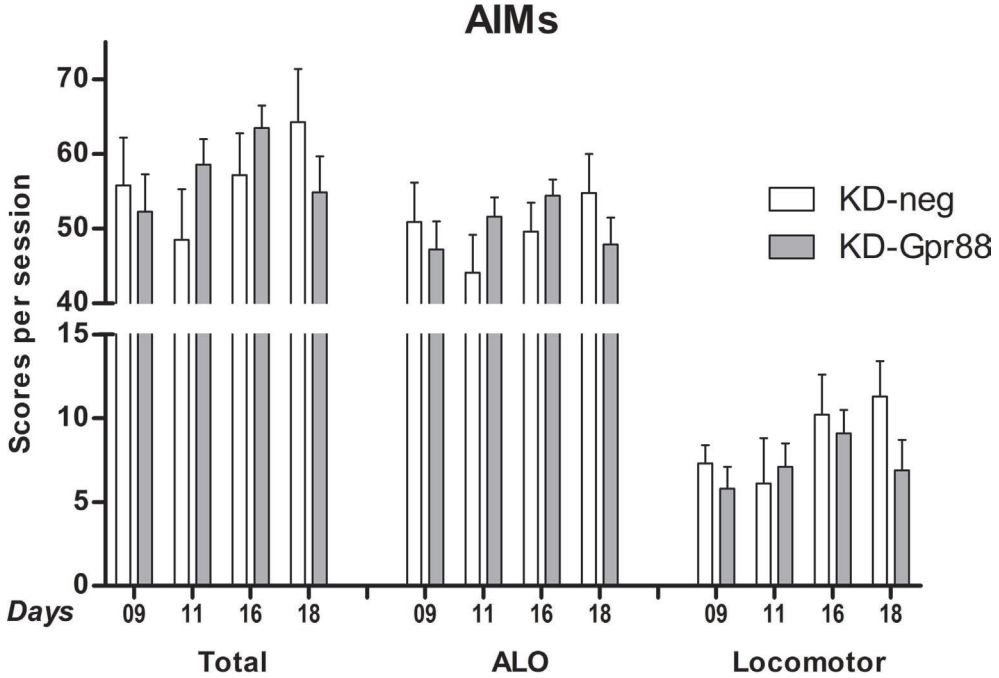


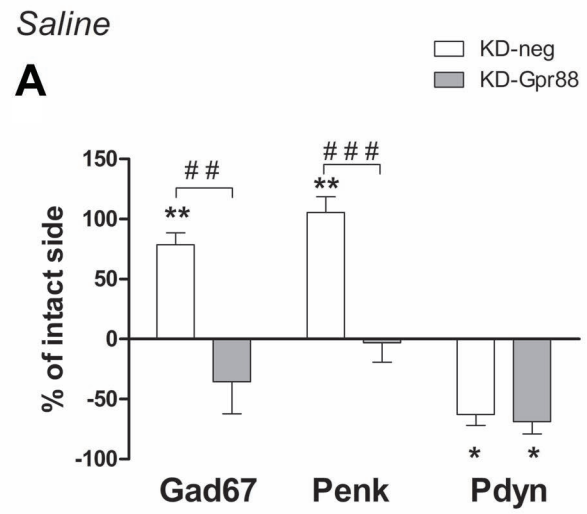
FIGURE 6 | L-DOPA–induced dyskinesia (LID): abnormal involuntary movement (AIM) scores. AIMs were scored at each time point (9, 11, 16, and 18 days of L-DOPA treatment) and calculated either as the total sum or separating the sum of the ALO (axial, limb, oral) scores from the locomotor scores. The data were analyzed using a two-way ANOVA followed by Sidak's multiple comparisons test. The analysis did not reveal a significant effect of the miR treatment ($F_{1, 57 =}$ 0.06, p = 0.81), time ($F_{3, 57 =}$ 1.02, p = 0.39), or their interaction ($F_{3, 57 =}$ 1.53, p = 0.22).

Strikingly, in the dorsal striatum of KD-Gpr88 rats, ΔFosB was strongly increased both in saline (n = 4) and in chronic L-DOPA (n = 4) treated rats as compared to KD-neg (**Figure 8**). Thus, the Gpr88 inactivation powerfully induced ΔFosB expression in the DA-deafferented striatum but was not associated with any further increase of ΔFosB following chronic L-DOPA treatment. Moreover, since the L-DOPA–induced *Pdyn* upregulation is inhibited, the increased accumulation of ΔFosB appeared to be unable to worsen LID in L-DOPA–treated KD-Gpr88 rats.

DISCUSSION

DA modulates MSN output by facilitating the activity of the D1-regulated striato-nigral pathway and inhibiting the activity of D2-regulated striato-pallidal pathway (Gerfen and Wilson, 1996). The imbalance between these striatal output pathways following unilateral DA loss can be evaluated measuring the turning behavior that results from the difference between the intact and lesioned striatum in the response to activation of the DA receptors. Turning behavior can thus be elicited by acute challenges with drugs such as Amph that, by increasing the release of DA in the intact striatum, provokes turning toward the lesioned side, or the DA precursor L-DOPA that, by promoting DA release in the DA-hypersensitive 6-OHDA–lesioned striatum, produces turning toward the intact side (Schwarting and Huston, 1996). We have found that the KD-Gpr88 in the dorsal DA-deafferented striatum produced a significant effect on motor activity in such a model of PD by attenuating the ipsilateral Amph- and enhancing the contralateral L-DOPA–induced turning behavior. This effect is consistent with an inhibitory role of Gpr88 on DA-dependent MSN activity, since Gpr88 KO mice display DA hypersensitivity, as shown by increased locomotion in basal conditions and in response to DA receptor stimulation with direct and indirect agonists (Logue et al., 2009; Quintana et al., 2012).

Using a threshold method for ISH analysis, we ascertained that, as already reported in the literature (Cenci et al., 1998), the DA loss following 6-OHDA lesions leads to hyperactivation of MSN with an increase in the expression of *Gad67*, coding for the rate-limiting enzyme in the synthesis of GABA, and also results in the decreased expression of *Pdyn* co-transmitter in the striato-nigral pathway and in the increased expression of *Penk* co-transmitter in the D2 striato-pallidal pathway. These modifications are the result of plastic changes triggered in the striatum by the loss of DA fibers, and reflect an imbalance in the activity of striatum output pathways, giving rise to motor deficits. The KD-Gpr88 normalized the expression of *Gad67* and *Penk*. However, it was unable to modify the decrease in *Pdyn* expression induced by the DA loss. Thus, in a well-established, unilateral, 6-OHDA lesion model of PD, the KD-Gpr88 appears to reduce the imbalance in motor responses to DA receptor stimulation essentially by normalizing the activity of the indirect inhibitory striato-pallidal pathway. This suggestion that KD-Gpr88 preferentially affects MSNs of the indirect pathway is consistent with the receptor's relative enrichment in this neuronal type (Massart et al., 2009) and the hypersensitivity to D2 agonists that has been consistently reported in Gpr88 KO mice (Logue et al., 2009; Quintana et al., 2012). As GPR88 is also known to modulate



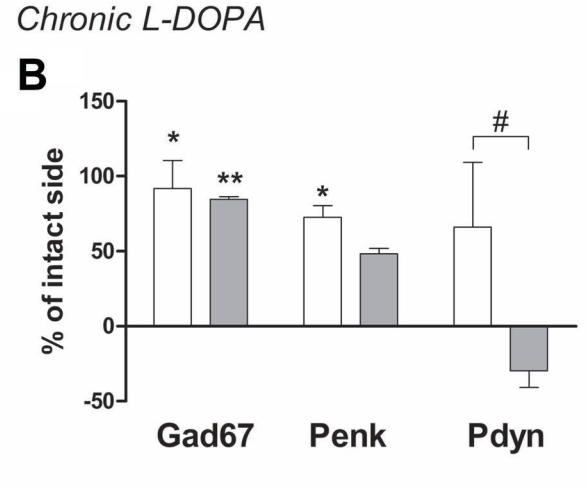


FIGURE 7 | *In situ* hybridization for striatal markers. *Gad67*, *Penk*, *Pdyn* mRNA expression. Evaluation of medium spiny neuron markers in the dorsal DA-deprived striatum transduced with a miR-neg or miR-Gpr88 expressed as percentage of change compared to the contralateral intact side in chronic saline control rats and chronic L-DOPA–treated rats developing LID. **(A)** Chronic saline: KD-neg, n = 4; KD-Gpr88, n = 3. **(B)** Chronic L-DOPA: KD-neg, n = 3; KD-Gpr88, n = 4. One-way ANOVA: control side v/s KD-neg v/s KD-Gpr88 followed by Tukey multiple comparison test. * 6-OHDA–lesioned v/s intact side—P: * < 0.05, ** < 0.01. # KD-neg v/s KD-Gpr88—P: # < 0.05, ## < 0.01, ### < 0.005.

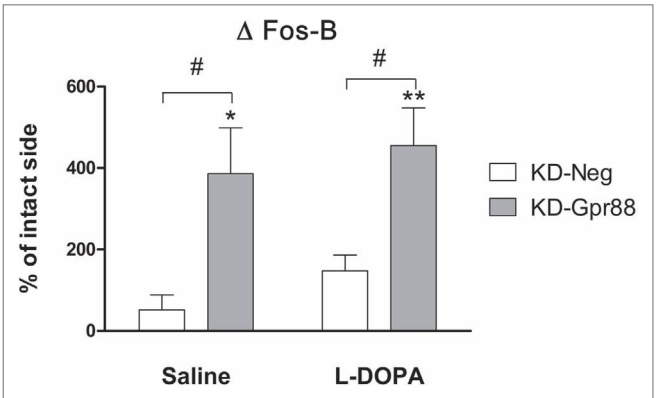


FIGURE 8 | ΔFosB protein expression. Immunofluorescence analysis of ΔFosB activation after chronic saline or L-DOPA treatment in the lentiviral vector transduced dorsal striatum of 6-OHDA–lesioned rats developing LID. Chronic saline: KD-neg, n = 4; KD-Gpr88, n = 4. Chronic L-DOPA: KD-neg, n = 3; KD-Gpr88, n = 4. One-way ANOVA: Control side v/s KD-neg v/s KD-Gpr88 followed by Tukey multiple comparison test. * 6-OHDA–lesioned v/s intact side—P: * < 0.05; ** < 0.01. # KD-neg v/s KD-Gpr88—P: # < 0.05.

enkephalin delta opioid receptor signaling (Meirsman et al., 2016), the reduction in Penk expression observed following KD-Gpr88 may partly result from a potentiation of neurotransmission at delta receptors. However, the KD-Gpr88, by not modifying the 6-OHDA–induced *Pdyn* downregulation, appears to not markedly impact the direct striato-nigral pathway that may remain hypersensitive upon D1 receptor stimulation. This may explain the lack of effects of Gpr88 long-term inactivation alone on the development of LID in contrast to a chronic treatment with L-DOPA. Actually, L-DOPA replacement therapy for the treatment of PD facilitates motor function by enhancing the activity of the D1-regulated striato-nigral pathway, an effect that, in the long term, leads to the development of LID (Santini et al., 2009). In this study, we found that the KD-Gpr88, even if it induced an increase of contralateral turning behavior after an acute challenge with L-DOPA, did not exacerbate the intensity of AIMs that are associated with the chronic L-DOPA–induced hyperactivation of the direct pathway (Santini et al., 2009).

Moreover, the development of LID is associated with an upregulation of *Gad67* and *Penk*, which is more pronounced in term of signal intensity than the upregulation induced by the DA deafferentiation, an upregulation of *Pdyn*, and the induction of ΔFosB in the direct pathway that is linked to the severity of LID (Pavon et al., 2006; Engeln et al., 2016). However, only *Gad67* and *Penk* were upregulated to the same extent in both KD-neg and KD-Gpr88 rats with LID, while the increase of *Pdyn* in KD-neg was not paralleled in KD-Gpr88 rats, where it remained below baseline. Furthermore, ΔFosB was significantly increased by about threefold both in saline- and L-DOPA–treated KD-Gpr88 as compared to KD-neg rats. Nevertheless, the sharp increase of ΔFosB in saline-treated KD-Gpr88 rats was not associated with the development of AIMs. Accordingly, it has been reported that high levels of ΔFosB expression obtained by viral vector transduction in the DA-denervated striatum are not *per se* associated with dyskinesia. However, the overexpression of ΔFosB results in the abrupt appearance of high-score AIMs immediately after an acute L-DOPA challenge, which are significantly increased compared to the AIM scores displayed after chronic L-DOPA treatment (Cao et al., 2010). This is not the case for the sharp increase in ΔFosB induced by the KD-Gpr88, which is not associated with the development of dyskinesia and with a significant increase in AIM scores after chronic L-DOPA. However, similarly to the KD-Gpr88, the overexpression of ΔFosB results in hypersensitivity to acute L-DOPA and increased contralateral turning (Cao et al., 2010). Thus, the lack of dyskinesia development and the lack of aggravation of AIMs after the dorso-striatal inactivation of Gpr88 suggest that, while the KD-Gpr88 may

act by hyperactivating the D1 direct pathway through the induction of ΔFosB, this effect is not directly coupled to dyskinetic effects even during a chronic L-DOPA treatment. Indeed, ΔFosB upregulates *Pdyn* (McClung et al., 2004), which is linked to both development and severity of LID (Andersson et al., 1999; Andersson et al., 2003). In this study, following chronic L-DOPA treatment, we observed a significant increase of *Pdyn* in KD-neg and a stable level of *Pdyn* in the KD-Gpr88 group, this latter group being also characterized by a striking increase in ΔFosB protein levels. Thus, this result suggests that the KD-Gpr88 may also affect the D1 direct pathway by inhibiting the upregulation of Pdyn following chronic L-DOPA and, therefore, that LID may not depend only on upregulated ΔFosB/*Pdyn* in the D1 direct pathway but also on a potential involvement of other cellular mechanisms in different neurotransmission systems such as the serotonergic system (De Deurwaerdère et al., 2017).

Taken together, our results indicate that the KD-Gpr88 may act on both the hyperactive indirect and hypoactive direct pathways following the 6-OHDA lesion and, by reducing the imbalance between them, may result in an antiparkinsonian-like effect not associated in the long term with the development of dyskinesia. However, the development of specific pharmacological antagonists will be crucial for further establishing whether GPR88 receptors genuinely represent an alternative target for the treatment of PD—alone or in association with other classes of agents—with a lower propensity to provoke motor side effects. Moreover, using specific D1 or D2 ligands in PD models combined with the KD-Gpr88 will be instrumental for parsing the specific contribution of Gpr88 to the direct and indirect pathways. However, to disentangle the relative contribution of each pathway in the effect of the Gpr88 KD would also require the utilization of lentiviral vectors with specific promoters. This will allow for dissecting more precisely the role of Gpr88 and for developing gene therapy tools that may offer alternative and possibly more efficient solutions than pharmacological interventions for treating the motor dysfunction of PD while avoiding the eventual emergence of dyskinesia.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The animal study was reviewed and approved by "Ministère de la Recherche" (APAFIS#3669-2016011817516297 v6).

AUTHOR CONTRIBUTIONS

MI, MM, CM, and RM conceived and organized the research project. MI, BG, JP, NF, ADT, and RM planned and executed the experimental work. MI, BG, and RM designed and executed the statistical analysis. MI wrote the first draft of the manuscript. MI, BG, NF, ADT, MM, and CM reviewed and provided scientific input to the manuscript. RM wrote the article.

ACKNOWLEDGMENTS

The lentiviral vectors were produced by the iVECTOR facility, and *in vivo* experiments were carried out in the Pheno-ICMice animal facility, both situated at ICM. We thank Annick Prigent, head of the HISTOMICS platform at ICM, for counseling in the histological procedures.

REFERENCES

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

Andersson, M., Westin, J. E., and Cenci, M. A. (2003). Time course of striatal DFosB-like immunoreactivity and prodynorphin mRNA levels after discontinuation of chronic dopaminomimetic treatment. *Eur. J. Neurosci.* 17, 661–666. doi: 10.1046/j.1460-9568.2003.02469.x

Bjorklund, A., and Dunnett, S. B. (2019). The amphetamine induced rotation test: a re-assessment of its use as a tool to monitor motor impairment and functional recovery in rodent models of parkinson's disease. *J. Parkinsons Dis.* 9, 17–29. doi: 10.3233/JPD-181525

Cao, X., Yasuda, T., Uthayathas, S., Watts, R. L., Mouradian, M. M., Mochizuki, H., et al. (2010). Striatal overexpression of DFosB reproduces chronic levodopa-induced involuntary movements. *J. Neurosci.* 30, 7335–7343. doi: 10.1523/JNEUROSCI.0252-10.2010

Carta, A., Fenu, S., and Morelli, M. (2001). Alterations in GAD67, dynorphin and enkephalin mRNA in striatal output neurons following priming in the 6-OHDA model of Parkinson's disease. *Neurol. Sci.* 22, 59–60. doi: 10.1007/s100720170046

Carta, M., Lindgren, H. S., Lundblad, M., Stancampiano, R., Fadda, F., and Cenci, M. A. (2006). Role of striatal l-DOPA in the production of dyskinesia in 6-hydroxydopamine lesioned rats. *J. Neurochem.* 96, 1718–1727. doi: 10.1111/j.1471-4159.2006.03696.x

Castaing, M., Guerci, A., Mallet, J., Czernichow, P., Ravassard, P., and Scharfmann, R. (2005). Efficient restricted gene expression in beta cells by lentivirus-mediated gene transfer into pancreatic stem/progenitor cells. *Diabetologia* 48, 709–719. doi: 10.1007/s00125-005-1694-6

Cenci, M. A., Lee, C. S., and Bjorklund, A. (1998). L-DOPA-induced dyskinesia in the rat is associated with striatal overexpression of prodynorphin- and glutamic acid decarboxylase mRNA. *Eur. J. Neurosci.* 10, 2694–2706. doi: 10.1046/j.1460-9568.1998.00285.x

Cenci, M. A., and Lundblad, M. (2007). Ratings of L-DOPA-induced dyskinesia in the unilateral 6-OHDA lesion model of Parkinson's disease in rats and mice. *Curr. Protoc. Neurosci.* 9, 25. doi: 10.1002/0471142301.ns0925s41

De Deurwaerdère, P., Di Giovanni, G., and Millan, M. J. (2017). Expanding the repertoire of L-DOPA's actions: a comprehensive review of its functional neurochemistry. *Prog. Neurobiol.* 151, 57–100. doi: 10.1016/j.pneurobio.2016.07.002

Do, J., Kim, J.-I., Bakes, J., Lee, K., and Kaang, B.-K. (2013). Functional roles of neurotransmitters and neuromodulators in the dorsal striatum. *Learn. Mem.* 20, 21–28. doi: 10.1101/lm.025015.111

Engeln, M., Bastide, M. F., Toulme, E., Dehay, B., Bourdenx, M., Doudnikoff, E., et al. (2016). Selective inactivation of striatal FosB/DeltaFosB-expressing neurons alleviates L-DOPA-induced dyskinesia. *Biol. Psychiatry* 79, 354–361. doi: 10.1016/j.biopsych.2014.07.007

Fox, S. H., Katzenschlager, R., Lim, S. Y., Barton, B., de Bie, R. M. A., Seppi, K., et al. (2018). International Parkinson and movement disorder society evidence-based medicine review: update on treatments for the motor symptoms of Parkinson's disease. *Mov. Disord.* 33, 1248–1266. doi: 10.1002/mds.27372

Gerfen, C. R., and Surmeier, D. J. (2011). Modulation of striatal projection systems by dopamine. *Annu. Rev. Neurosci.* 34, 441–466. doi: 10.1146/annurev-neuro-061010-113641

Gerfen, C. R., and Wilson, C. J. (1996). "Handbook of Chemical Neuroanatomy," in *Chapter II The basal ganglia*, vol. 12 . Eds. L. W. Swanson, A. B., and T. HÖkfelt (Amsterdam: Elsevier), 371–468. doi: 10.1016/S0924-8196(96)80004-2

Huot, P., Johnston, T. H., Koprich, J. B., Fox, S. H., and Brotchie, J. M. (2013). The pharmacology of l-DOPA-Induced dyskinesia in parkinson's disease. *Pharmacol. Rev.* 65, 171–222. doi: 10.1124/pr.111.005678

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

Ingallinesi, M., Le Bouil, L., Faucon Biguet, N., Do Thi, A., Mannoury la Cour, C., Millan, M. J., et al. (2015). Local inactivation of Gpr88 in the nucleus accumbens attenuates behavioral deficits elicited by the neonatal administration of phencyclidine in rats. *Mol. Psychiatry* 20, 951–958. doi: 10.1038/mp.2014.92

Logue, S. F., Grauer, S. M., Paulsen, J., Graf, R., Taylor, N., Sung, M. A., et al. (2009). The orphan GPCR, GPR88, modulates function of the striatal dopamine system: a possible therapeutic target for psychiatric disorders? *Mol. Cell Neurosci.* 42, 438–447. doi: 10.1016/j.mcn.2009.09.007

Massart, R., Guilloux, J.-P., Mignon, V., Pierre, S., and Jorge, D. (2009). Striatal GPR88 expression is confined to the whole projection neuron population and is regulated by dopaminergic and glutamatergic afferents. *Eur. J. Neurosci.* 30, 397–414. doi: 10.1111/j.1460-9568.2009.06842.x

Massart, R., Sokoloff, P., and Diaz, J. (2012). "Mechanisms in Parkinson's Disease - Models and Treatments," in *Distribution and Regulation of the G Protein-Coupled Receptor Gpr88 in the Striatum : relevance to Parkinson's Disease*. Ed. J. Dushanova (Rijeka, Croatia: InTech), 393–406. doi: 10.5772/21150

McClung, C. A., Ulery, P. G., Perrotti, L. I., Zachariou, V., Berton, O., and Nestler, E. J. (2004). DFosB: a molecular switch for long-term adaptation in the brain. *Mol. Brain Res.* 132, 146–154. doi: 10.1016/j.molbrainres.2004.05.014

Meirsman, A. C., Le Merrer, J., Pellissier, L. P., Diaz, J., Clesse, D., Kieffer, B. L., et al. (2016). Mice lacking GPR88 show motor deficit, improved spatial learning and low anxiety reversed by delta opioid antagonist. *Biol. Psychiatry.* 79, 917–927. doi: 10.1016/j.biopsych.2015.05.020

Mizushima, K., Miyamoto, Y., Tsukahara, F., Hirai, M., Sakaki, Y., and Ito, T. (2000). A Novel G-Protein-Coupled receptor gene expressed in striatum. *Genomics* 69, 314–321. doi: 10.1006/geno.2000.6340

Mura, A., Mintz, M., and Feldon, J. (2002). Behavioral and anatomical effects of long-term l-Dihydroxyphenylalanine (L-DOPA) administration in rats with unilateral lesions of the nigrostriatal system. *Exp. Neurol.* 177, 252–264. doi: 10.1006/exnr.2002.7976

Nestler, E. J. (2015). DFosB: a transcriptional regulator of stress and antidepressant responses. *Eur. J. Pharmacol.* 753, 66–72. doi: 10.1016/j.ejphar.2014.10.034

Pavon, N., Martin, A. B., Mendialdua, A., and Moratalla, R. (2006). ERK phosphorylation and FosB expression are associated with L-DOPA-induced dyskinesia in hemiparkinsonian mice. *Biol. Psychiatry* 59, 64–74. doi: 10.1016/j.biopsych.2005.05.044

Paxinos, G., and Watson, C. (1997). "stereotaxic coordinates," in *The rat brain* (San Diego: Academic Press).

Quintana, A., Sanz, E., Wang, W., Storey, G. P., Guler, A. D., Wanat, M. J., et al. (2012). Lack of GPR88 enhances medium spiny neuron activity and alters motor- and cue-dependent behaviors. *Nat. Neurosci.* 15, 1547–1555. doi: 10.1038/nn.3239

Santini, E., Alcacer, C., Cacciatore, S., Heiman, M., Herve, D., Greengard, P., et al. (2009). L-DOPA activates ERK signaling and phosphorylates histone H3 in the striatonigral medium spiny neurons of hemiparkinsonian mice. *J. Neurochem.* 108, 621–633. doi: 10.1111/j.1471-4159.2008.05831.x

Schwarting, R. K. W., and Huston, J. P. (1996). The unilateral 6-hydroxydopamine lesion model in behavioral brain research. Analysis of functional deficits, recovery and treatments. *Prog. Neurobiol.* 50, 275–331. doi: 10.1016/S0301-0082(96)00040-8

Spigolon, G., and Fisone, G. (2018). Signal transduction in l-DOPA-induced dyskinesia: from receptor sensitization to abnormal gene expression. *J. Neural Transm.* 125, 1171–1186. doi: 10.1007/s00702-018-1847-7

Steiner, H., and Gerfen, C. R. (1998). Role of dynorphin and enkephalin in the regulation of striatal output pathways and behavior. *Exp. Brain Res.* 123, 60–76. doi: 10.1007/s002210050545

Tayarani-Binazir, K. A., Jackson, M. J., Strang, I., Jairaj, M., Rose, S., and Jenner, P. (2012). Benserazide dosing regimen affects the response to L-3,4-dihydroxyphenylalanine in the 6-hydroxydopamine-lesioned rat. *Behav. Pharmacol.* 23, 126–133. doi: 10.1097/FBP.0b013e32834f9f82

Zennou, V., Serguera, C., Sarkis, C., Colin, P., Perret, E., Mallet, J., et al. (2001). The HIV-1 DNA flap stimulates HIV vector-mediated cell transduction in the brain. *Nat. Biotechnol.* 19, 446–450. doi: 10.1038/88115

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pharmacology

MINI REVIEW
published: 30 October 2019
doi: 10.3389/fphar.2019.01294

Contribution of the GABAergic System to Non-Motor Manifestations in Premotor and Early Stages of Parkinson's Disease

Ane Murueta-Goyena[1], Ane Andikoetxea[1], Juan Carlos Gómez-Esteban[1,2] and Iñigo Gabilondo[1,3]*

[1] Neurodegenerative Diseases Group, Biocruces Bizkaia Health Research Institute, Barakaldo, Spain, [2] Department of Neuroscience, University of the Basque Country (UPV/EHU), Leioa, Spain, [3] IKERBASQUE Basque Foundation for Science, Bilbao, Spain

Non-motor symptoms are common in Parkinson's disease (PD) and they represent a major source of disease burden. Several non-motor manifestations, such as rapid eye movement sleep behavior disorder, olfactory loss, gastrointestinal abnormalities, visual alterations, cognitive and mood disorders, are known to precede the onset of motor signs. Nonetheless, the mechanisms mediating these alterations are poorly understood and probably involve several neurotransmitter systems. The dysregulation of GABAergic system has received little attention in PD, although the spectrum of non-motor symptoms might be linked to this pathway. This Mini Review aims to provide up-to-date information about the involvement of the GABAergic system for explaining non-motor manifestations in early stages of PD. Therefore, special attention is paid to the clinical data derived from patients with isolated REM sleep behavior disorder or drug-naïve patients with PD, as they represent prodromal and early stages of the disease, respectively. This, in combination with animal studies, might help us to understand how the disturbance of the GABAergic system is related to non-motor manifestations of PD.

Keywords: GABA, REM sleep behavior disorder, hyposmia, visual alterations, gastrointestinal symptoms, neurotransmitters

OPEN ACCESS

Edited by:
Philippe De Deurwaerdere, Université de Bordeaux, France

Reviewed by:
Norbert Brüggemann, Universität zu Lübeck, Germany
Sulev Kõks, University of Tartu, Estonia

****Correspondence:***
Ane Murueta-Goyena
ane.muruetagoyena@osakidetza.eus

Specialty section:
This article was submitted to Neuropharmacology, a section of the journal Frontiers in Pharmacology

Received: *16 July 2019*
Accepted: *09 October 2019*
Published: *30 October 2019*

Citation:
Murueta-Goyena A, Andikoetxea A, Gómez-Esteban JC and Gabilondo I (2019) Contribution of the GABAergic System to Non-Motor Manifestations in Premotor and Early Stages of Parkinson's Disease. Front. Pharmacol. 10:1294. doi: 10.3389/fphar.2019.01294

INTRODUCTION

Parkinson's disease (PD) is the second most common neurodegenerative disorder with a prevalence of between 1% and 4% in over-60-year-olds (Tysnes and Storstein, 2017). The diagnosis of PD currently depends on the identification of motor clinical features, including rest tremor, rigidity and bradykinesia. In addition, patients with PD develop a wide range of non-motor manifestations including cognitive impairment and dementia, mood and sleep disturbances, sensory abnormalities and autonomic nervous system dysfunction (Poewe, 2008). It has been estimated that the first motor signs appear when 50% to 80% of dopaminergic neurons in the substantia nigra pars compacta have been lost (Cheng et al., 2010). Thus, by the time of diagnosis, brain injury has been ongoing for years and any attempt at neuroprotection at this stage might be unsuccessful. Great efforts are being made to detect markers of neuronal dysfunction early in the course of the disease (Postuma and Berg, 2019). In relation to this, it is increasingly recognized that non-motor symptoms not only accompany but also precede motor signs in PD (Poewe, 2008). This is consistent with the Braak PD

staging system, which suggests that α-synuclein deposition starts in areas involved in sleep regulation, olfaction or autonomic function before affecting the basal ganglia or cerebral cortex (Braak et al., 2003). The array of premotor symptoms might help to identify patients at high risk of developing α-synuclein-mediated neurodegenerative diseases, such as PD, dementia with Lewy bodies (DLB) or multiple system atrophy (MSA).

While the mechanisms for motor impairment are fairly well established, the neuroanatomical and molecular substrates for non-motor manifestations are far from clear. Current evidence suggests that neurotransmitters, such as acetylcholine, serotonin, noradrenaline, glutamate and gamma-aminobutyric acid (GABA), play an important role in the pathophysiology of PD (Sanjari Moghaddam et al., 2017). GABA is the main inhibitory neurotransmitter in the central nervous system (CNS), acts through $GABA_A$ and $GABA_B$ receptors, and is primarily released by local interneurons to regulate cortical and subcortical microcircuits (**Figures 1A, B**). GABAergic signaling modulates a wide range of physiological functions, including sensory perception, information processing and cognition. In patients with PD, GABAergic dysregulation has been observed in the basal ganglia *postmortem* and *in vivo* with magnetic resonance spectroscopy (Kish et al.,

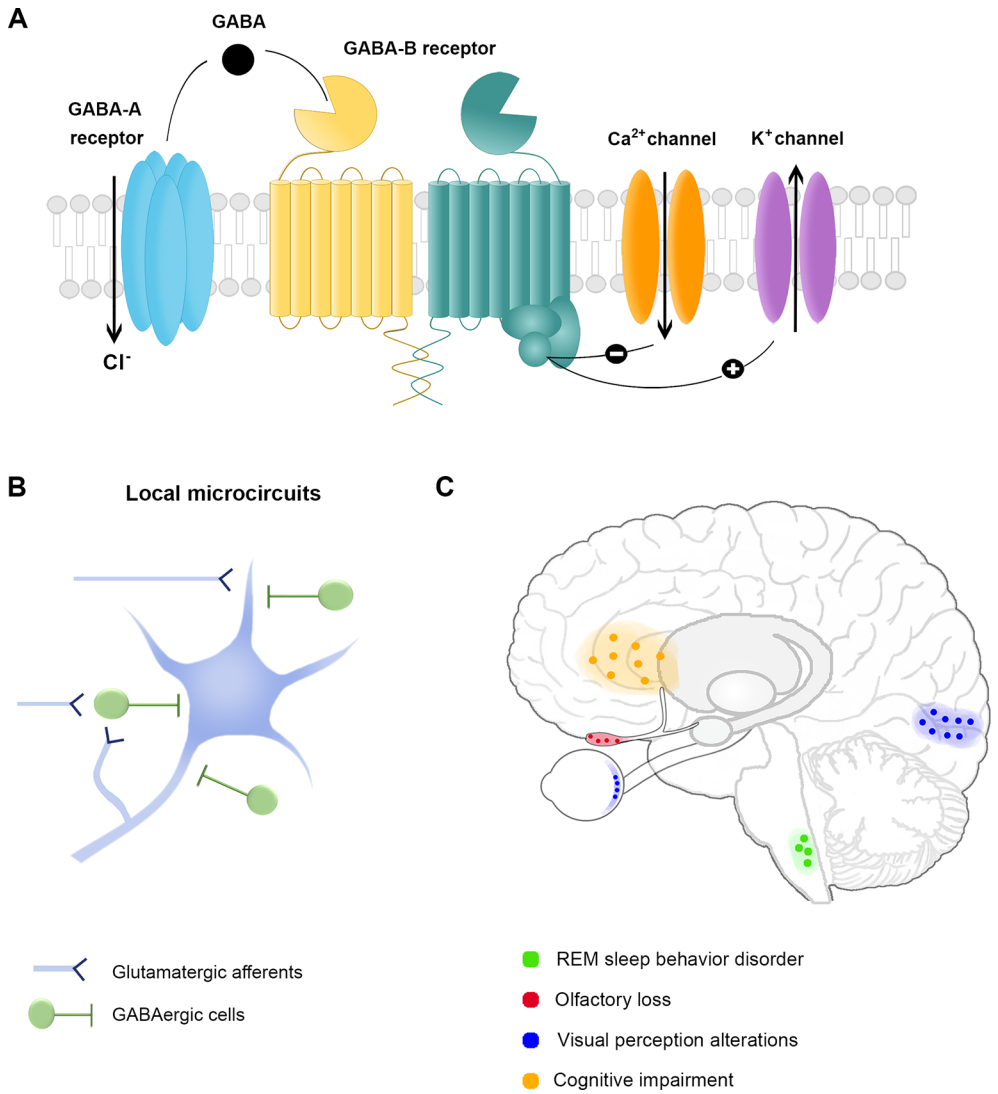


FIGURE 1 | The GABAergic system and non-motor symptoms in Parkinson's Disease. **(A)** GABA receptors. The inhibitory neurotransmitter GABA acts through ionotropic $GABA_A$ receptor or metabotropic $GABA_B$ receptor to reduce the membrane potential. The activation of $GABA_A$ receptors allows chloride (Cl^-) entry into the cytoplasm, while $GABA_B$ receptor activation leads to a cellular cascade resulting in calcium (Ca^{2+}) channel deactivation and potassium (K^+) channel opening. **(B)** Schematic representation of cortical and subcortical local microcircuit organization of GABAergic cells. Inhibitory GABAergic cells are primarily local projecting neurons with a broad array of anatomical and physiological properties. The effect resulting from the inhibition exerted by GABAergic cells depends on their sensitivity to incoming stimuli, their firing properties and the subcellular domain of excitatory cells targeted by each interneuron. The diversity of GABAergic cells provides the brain with extensive computational power to regulate sensory and cognitive processes. **(C)** Brain areas associated with non-motor symptoms in Parkinson's disease. Each color corresponds to a specific non-motor symptom and the associated area of the presumed GABAergic dysfunction.

1986; Emir et al., 2012; O'Gorman Tuura et al., 2018). Recently, it has been shown that striatal dopaminergic axons co-release GABA (Tritsch et al., 2012; Tritsch et al., 2014), which suggests that dopaminergic neurodegeneration could lead to GABA decline in basal ganglia circuits (O'Gorman Tuura et al., 2018). Considering that GABAergic networks regulate calcium-mediated mechanisms, like mitochondrial function and oxidative stress, loss of GABA inhibitory tone would facilitate accumulation of abnormal levels of intracellular calcium, triggering neurodegenerative processes. Consistent with this idea, it has been shown that GABA agonists, such as Baclofen or Bumetadine, relieve motor symptoms and protect dopaminergic cell bodies in mice models of PD (Hajj et al., 2015; Lozovaya et al., 2018). Nonetheless, GABAergic alterations might go beyond the basal ganglia. Unfortunately, few studies have investigated how GABAergic or other neurotransmitter systems may induce or modulate non-motor symptoms of PD. Identifying the separate role of each pathway may allow us to develop novel pharmacological compounds targeted to specific symptoms. Seeking to provide up-to-date information about the role of the GABAergic system, in this Mini Review, we focus on its ability to explain some of the non-motor manifestations that appear early in PD (**Table 1**).

TABLE 1 | Evidence of the involvement of GABAergic neurotransmission in early non-motor symptoms of Parkinson's disease.

Non-motor symptom	Evidence of GABAergic inhibitory deficit
REM sleep behavior disorder	GABAergic cells in the ventral medulla are dysfunctional † (Brooks and Peever, 2011)
Olfactory loss	Tonic inhibition exerted by interneurons regulates odor detection (Pirez and Wachowiak, 2008; Shao et al., 2009; Acebes et al., 2011)
Visual perception alterations	GABAergic depletion in the retina changes contrast sensitivity † (Hilgen et al., 2015) GABA antagonism in visual cortex decreases stimulus orientation and direction selectivity † (Katzner et al., 2011) GABA levels in visual cortex are predictive of visuospatial abilities * (Cook et al., 2016) Visual hallucinations are associated with decreased occipital GABA in PD (Firbank et al., 2018) and with the loss of postsynaptic GABA markers in DLB ** (Khundakar et al., 2016)
Cognitive impairment	GABA transcriptional changes in the frontal cortex ** (Santpere et al., 2018) Levels of PV and GAD67 mRNA expression are low in the frontal cortex ** (Lanoue et al., 2013; Lanoue et al., 2010)
Anxiety and depression	$GABA_A$ receptor positive modulators are anxiolytic and antidepressant, while negative modulators produce anxiogenic and depressive-like effects †,* (Kalueff and Nutt, 2007; Mohler, 2012) GABA receptor dysfunction is linked to anxiety and depression-like behaviors † (Kalueff and Nutt, 2007)
Gastrointestinal symptoms	GABA regulates the mobility and inflammatory responses of the gastrointestinal tract # (Auteri et al., 2005a; Auteri et al 2005b; Jin et al., 2013)

*†Studies conducted in animal models, #physiological function, * in healthy controls, ** in patients with Parkinson's disease (PD) or dementia with Lewy bodies (DLB).*

REM SLEEP BEHAVIOR DISORDER AND PONTINE GABAERGIC CELL DYSFUNCTION IN PD

Sleep disturbances are the most common non-motor manifestations of PD, with wide variability in the reported prevalence (66% to 98%) (Garcia-Borreguero et al., 2003). Sleep-related abnormalities in PD include insomnia, sleep fragmentation, restless legs syndrome, excessive daytime sleepiness and rapid eye movement (REM) sleep behavior disorder (RBD), among others. Some sleep abnormalities occur in early stages of the disease, even during the prodromal phase, including RBD (Iranzo et al., 2006), restless legs syndrome (Wong et al., 2014) and excessive daytime sleepiness (Abbott et al., 2005). Nonetheless, only idiopathic RBD (iRBD) has consistently shown to be an early predictor of the development of PD (Iranzo et al., 2017) (Postuma and Berg, 2019).

RBD is a parasomnia characterized by the loss of the normal muscle atonia of REM sleep. The diagnostic hallmark is excessive electromyographic activity during REM sleep as documented by polysomnography (Ferini-Strambi et al., 2016). Patients with RBD report the enactments of dreams, including kicking, punching or talking. In the absence of other neurological signs or CNS lesions, patients with iRBD are at high risk of developing α-synuclein-mediated neurodegenerative diseases in the years following the diagnosis (Hogl et al., 2018). It has also been suggested that the presence of RBD in PD patients is associated with an aggressive phenotype, these patients showing a higher density of α-synuclein aggregates (Knudsen et al., 2018).

Therefore, in recent years, special attention has been paid to PD-related non-motor manifestations and symptom progression in iRBD patients, seeking to find novel biomarkers of PD. Although the precise pathophysiological mechanisms for iRBD have not been fully determined, iRBD seems to be attributable to neurochemical imbalances in sleep regulatory systems (Boucetta et al., 2014). Previous studies have pointed toward a significant and specific neurodegeneration of GABA or glycine-containing neurons in the ventral medulla, such as in the nucleus raphe magnus and the ventral gigantocellular, alpha gigantocellular and lateral paragigantocellular reticular nuclei that directly project to spinal motor neurons to produce atonia during REM sleep (Iranzo, 2018) (**Figure 1C**). This hypothesis is supported by preclinical studies in transgenic mice that exhibit an RBD phenotype when glycine and GABA receptor function is impaired (Brooks and Peever, 2011). Moreover, allosteric agonists that bind at the α/γ subunit interface of $GABA_A$ receptors, i.e., benzodiazepines, including clonazepam, triazolam or alprazolam, are the first-line therapy in iRBD (Anderson and Shneerson, 2009), and the effectiveness of this treatment could be explained by GABAergic neurotransmission disruption in prodromal stages of PD.

OLFACTORY LOSS AND ITS RELATIONSHIP WITH GABAERGIC NEUROTRANSMISSION IN PD

Olfactory dysfunction is observed in more than 90% of PD patients (Doty, 2012), frequently precedes the onset of motor

symptoms (Fantini et al., 2006; Ross et al., 2008; Postuma et al., 2009), and predicts the early conversion of iRBD to PD or DLB (Mahlknecht et al., 2015; Fereshtehnejad et al., 2017). The mechanisms responsible for olfactory dysfunction in PD are currently unknown. Magnetic resonance imaging studies have shown significantly smaller olfactory bulb volumes in patients with PD than controls (Brodoehl et al., 2012; Li et al., 2016; Tanik et al., 2016), although other authors failed to find such differences (Altinayar et al., 2014). Axonal and myelin damage of olfactory tracts has also been observed using diffusor-tensor imaging (Scherfler et al., 2006; Scherfler et al., 2013). These results have been confirmed by *postmortem* analysis of olfactory bulbs, in which global glomerular voxel volume was found to be smaller in five PD cases than six healthy controls (Zapiec et al., 2017). Moreover, hyposmia has been related to pathological changes in other areas of the olfactory system, such as the anterior olfactory nucleus or basolateral nucleus of the amygdala (Pearce et al., 1995; Harding et al., 2002). On the other hand, sensory perception disturbances might represent subtle alterations of normal functioning that precede neuronal degeneration. Changes in network connectivity of brain structures related to olfaction have already been described (Westermann et al., 2008; Bohnen et al., 2010; Wen et al., 2017), and these functional abnormalities may arise from iron and sodium deposition (Gardner et al., 2017).

The limited literature about the precise anatomy and physiology of the human olfactory bulb makes it difficult to assess the mechanisms related to olfactory dysfunction in humans. In this regard, animal studies provide a wealth of knowledge, as the olfactory bulb of rodents has been well characterized. It has been shown that interneurons—GABA-releasing cells—are essential for odor detection, and functionally distinct GABAergic circuits within the olfactory bulb of rodents play different roles in olfactory coding. The tonic inhibition exerted by these cells is thought to regulate the sensitivity of odor detection and odor perception in the mammalian brain (Pirez and Wachowiak, 2008; Shao et al., 2009; Acebes et al., 2011) (**Figure 1C**). Even though animal findings suggest that interneuron connectivity is the major determinant of odor perception, whether the loss of inhibitory synapses contributes to olfactory changes in PD in humans needs further research.

VISUAL DISTURBANCES

Among primary visual functions, low-contrast visual acuity, contrast sensitivity and color vision are typically affected in PD (Weil et al., 2016). Patients with drug-naïve PD or iRBD also show decreased contrast sensitivity (Righi et al., 2007; Marques et al., 2010), and abnormal color vision discrimination has been described in iRBD, these patients having a 3-fold higher risk of conversion (Postuma et al., 2015). Nevertheless, color discrimination is not consistently impaired in the early stages of PD, indicating that color vision abnormalities may represent a specific PD phenotype (Vesela et al., 2001). Indeed, Postuma and colleagues reported that abnormal color vision in iRBD was a stronger predictor of primary dementia than parkinsonism (Postuma et al., 2015), which is in line with findings in PD, RBD increasing the risk of cognitive decline (Pagano et al., 2018). Patients with iRBD or *de novo* PD also display visuoconstructional and visuoperceptual disturbances that may be related to non-dopaminergic impairment (Ferini-Strambi et al., 2004; Gagnon et al., 2009; Aarsland et al., 2009a; Marques et al., 2010; Fantini et al., 2011; Kim et al., 2011; Ota et al., 2016).

In vivo neuroimaging studies in newly diagnosed and drug-naïve PD patients have detected structural alterations in the visual pathway, ranging from thinning of inner retinal layers to increased optic radiation mean diffusivity and reduced visual cortical volumes (Arrigo et al., 2017; Ahn et al., 2018; Murueta-Goyena et al., 2019), which might explain some visual disturbances. There is, however, a growing body of evidence highlighting the role of GABA in perceptual aspects of vision.

Retinal amacrine cells co-release dopamine and GABA and the degeneration of these specialized cells has been suggested to cause primary visual dysfunction, although this hypothesis has not been confirmed (Nguyen-Legros, 1988). In line with this, pharmacological depletion of endogenous retinal GABA with allylglycine induces changes in contrast sensitivity (Hilgen et al., 2015). On the other hand, animal studies have shown that $GABA_A$ receptor antagonist infusion in cat primary visual cortex decreases selectivity for stimulus orientation and direction, but not contrast sensitivity (Katzner et al., 2011). More recently, it has been observed that GABA levels measured by magnetic resonance spectroscopy are strong predictors of visuospatial abilities in healthy adults (Cook et al., 2016), and increasing GABA activity with systemic midazolam injections decreases visual sensitivity, preferentially affecting medium-to-high spatial frequencies and low temporal frequencies (Blin et al., 1993). Additionally, higher GABA concentrations in the visual cortex, as well as administration of the GABA agonist lorazepam, induce slower perceptual dynamics (van Loon et al., 2013).

These findings suggest that GABA signaling plays a central role in visual perception and a disturbance of this circuit at any level of the visual pathway could influence proper sensory processing. Consistent with this, recent studies show that PD patients with visual hallucinations have low occipital GABA concentrations (Firbank et al., 2018), and complex visual hallucinations in DLB are associated with altered GABAergic synaptic activity (Khundakar et al., 2016), which further supports the view that dysregulation of GABAergic system is involved in the visual pathway of PD (**Figure 1C**). Whether this system is affected in the retina and visual cortex of all PD patients, and from early stages, remains to be determined.

COGNITIVE DYSFUNCTION AND GABAERGIC SIGNALING IN FRONTOSTRIATAL CIRCUITS

Cognitive manifestations are frequently reported in PD, with a prevalence of 20–25% for mild cognitive impairment and 30% for dementia. It is estimated that PD patients have a 3- to 6-fold higher risk of developing dementia than age-matched controls (Svenningsson et al., 2012). Cognitive dysfunction is thought to be one of the key premotor manifestations of PD. At diagnosis,

15–20% of PD patients have mild cognitive impairment (Aarsland et al., 2009b) and several studies in patients with iRBD have identified cognitive disturbances, including delayed verbal memory, poorer decision-making, worse attention and slower processing speed, these domains being predictive of future risk of developing PD or DLB (Fantini et al., 2011; Terzaghi et al., 2013; Youn et al., 2016; Genier Marchand et al., 2017). Thus, early onset cognitive abnormalities are mainly dependent on the frontal lobe (Ferini-Strambi et al., 2004; Massicotte-Marquez et al., 2008; Sasai et al., 2012; Chahine et al., 2018).

Despite the evidence of neuropathological abnormalities in frontal brain areas in PD, their molecular and cellular alterations are poorly understood. Several studies have suggested that cognitive impairment in PD is attributable to neurotransmitter dysregulation rather than frank neurodegeneration (Kehagia et al., 2010; Ray and Strafella, 2012). Dopamine and acetylcholine deficiencies in frontostriatal pathways play a major role in cognitive impairment in PD, but the contribution of the other neurotransmitter systems remains less certain. Regarding the role of GABA, in frontal cortex global transcriptional changes of GABAergic neurotransmission have been observed in DLB patients (Santpere et al., 2018). It has also been shown that mRNA expression of the GABA-synthesizing enzyme glutamic acid decarboxylase-67 (GAD67) (Lanoue et al., 2010) and the calcium-binding protein parvalbumin (PV) (Lanoue et al., 2013)—two key markers of GABAergic cells—is low in the dorsolateral prefrontal cortex of PD patients without evidence of cell loss, further suggesting the downregulation of inhibitory neurotransmission in the frontal cortex (**Figure 1C**). In basal ganglia, *in vivo* GABA concentration changes have been detected in PD patients performing cognitive tasks (Buchanan et al., 2015). Interestingly, boosting GABAergic neurotransmission by zolpidem administration in early stage PD patients modulates aberrant beta-frequency oscillations (Hall et al., 2014), and the desynchronization of low-frequency activity seems to restore cognitive functions (Hall et al., 2010). Although these findings point towards decreased GABAergic activity in frontostriatal circuits in PD and DLB, whether GABAergic neurotransmission is also perturbed in premotor stages of PD has not been established, and its contribution to cognitive dysfunction needs to be elucidated in future studies.

GABA IN ANXIETY AND DEPRESSION

Anxiety and depression are common non-motor symptoms of PD, with reported prevalence rates of 20–40% (Chen and Marsh, 2014) and 50% (Reijnders et al., 2008), respectively, and may precede motor signs (Jacob et al., 2010). Notably, RBD patients score worse on anxiety and depression scales than controls and even PD patients (Barber et al., 2017). Although the exact neurobiological mechanisms that underlie anxiety and depression have not been fully elucidated, they seem to be intrinsically interrelated.

Pharmacological studies in both humans and animals have revealed that positive modulators of $GABA_A$ receptors are anxiolytic and antidepressant, whereas negative modulators produce anxiogenic and depressive-like effects (Kalueff and Nutt, 2007; Mohler, 2012). Agents that enhance $GABA_A$ receptor conductance (e.g., benzodiazepines) and GABA metabolism (e.g., valproate, vigabatrin, and tiagabine) exert anxiolytic effects, and it seems that partial agonists of α_2/α_3 $GABA_A$ receptors, such as TPA-023, may also serve as antidepressants (Mohler, 2012). Furthermore, genetic studies implicate GABA-receptor dysfunction in the risk of developing anxiety and depression (Kalueff and Nutt, 2007). Recent evidence suggest that somatostatin contributes to the pathology of anxiety and depression (Fuchs et al., 2016; Fee et al., 2017), levels of this GABAergic marker being low in cerebrospinal fluid and induced pluripotent cells of PD patients (Dupont et al., 1982; Iwasawa et al., 2019). Nonetheless, there is still a lack of studies exploring the role of somatostatin in anxiety and depressive disorders in PD.

GASTROINTESTINAL SYMPTOMS AND GABA SIGNALING IN THE ENTERIC NERVOUS SYSTEM

Gastrointestinal disturbances fall within the spectrum of autonomic manifestations of PD patients. Hypersalivation, dysphagia, nausea, gastroparesis, small intestinal dysfunction, slow transit constipation and defecatory dysfunction have been attributed to α-synuclein-mediated small fiber neuropathy of the enteric nervous system (ENS) and to the neurodegeneration of the enteric branches of the vagus nerve in the brainstem (Pfeiffer, 2018). Among the gastrointestinal symptoms, constipation is the most frequent manifestation in PD and recent evidence suggests that it might also be one of the most common disturbances in prodromal PD (Stirpe et al., 2016). A multicenter study of 318 patients with polysomnography-confirmed iRBD concluded that they had substantially more autonomic symptoms than controls (SCOPA-AUT questionnaire), gastrointestinal symptoms being the most prominent domain (Ferini-Strambi et al., 2014). Nonetheless, gastric emptying measured with the 13C-octanoate breath test showed that only drug-naïve and early-stage Parkinson's disease patients had delayed gastric emptying, authors suggesting that changes in structures modulating gastric motility might not be sufficiently severe in iRBD (Unger et al., 2011).

The last three decades have seen an expansion in the literature on the role of GABA in the control of gastrointestinal function, including mobility and inflammatory responses (Auteri et al., 2015a; Auteri et al., 2015b). GABA has been identified as an important modulator of gastrointestinal tract function. This neurotransmitter can stimulate or inhibit the enteric neurons acting though $GABA_A$ or $GABA_B$ receptors (Auteri et al., 2015a). Its role is particularly important in the colon, where it modulates the peristatic reflex. On the other hand, enteric inflammation occurs in PD and has been related to the initiation and progression of the disease (Houser and Tansey, 2017). Nonetheless, it has yet to be determined why the production of pro-inflammatory cytokines takes place in the enteric tract. The purinergic system controls enteric inflammation, but GABA also has a major role in immune cell activity and inflammatory

events in the gastrointestinal tract (Jin et al., 2013). Topiramate—an anti-epileptic drug that acts as a $GABA_A$ agonist—reduces gastrointestinal inflammation in rats (Dudley et al., 2011), identifying GABA as a putative neuroimmune modulator. A better understanding of the relationship of GABA signaling with intestinal motility and inflammation is necessary, however, to reveal a possible functional link between this neurotransmitter, the ENS, and gastrointestinal symptoms of PD.

FINAL REMARKS AND CONCLUSIONS

Current evidence supports the view that PD is a degenerative disorder that affects multiple systems and presents with several non-motor symptoms. Over recent years, the importance of early, non-motor manifestations of PD has been increasingly recognized, as they may help to identify patients at high risk of developing α-synucleinopathies. Even though the neuronal circuits for motor symptoms are fairly well understood, the pathophysiological mechanisms for perceptual, cognitive, mood and autonomic disturbances of PD remain unclear.

Here, we report evidence consistent with the view that the GABAergic system is altered in PD and may contribute to non-motor symptoms that appear early in disease progression. Nonetheless, the literature in this field is dominated by non-placebo controlled and *postmortem* studies, generally based on small series and providing low-level evidence. To summarize, based on current findings, PD patients in premotor stages have anxiety and depression and alterations in the olfactory system, visual perception and visuospatial abilities, frontostriatal-related cognition, and gastrointestinal function. The neurobiological correlates of these deficits are unclear, in part because of the complex dynamic interactions between several neurotransmitter systems. Still, the dysfunction of GABAergic neurons in ventral medullary reticular formation seems to be linked to RBD. Moreover, preclinical studies show the relevance of interneurons in odor detection and the causal role of GABA in anxiety and depressive disorders, but we are far from establishing whether this also occurs in PD. On the other hand, disturbance of GABA signaling by pharmacological compounds affects visual processing and cognition, and GABA levels in the visual cortex are low in PD patients with visual hallucinations. It has been also shown that GABA controls gastrointestinal function, although it is not known whether this is associated with the gastrointestinal symptoms reported by PD patients. All these findings suggest that intervening in GABAergic signaling might modulate non-motor manifestations of PD and provide a novel avenue for non-dopaminergic therapy.

Nevertheless, there is a paucity of replication and large case-control studies. Future research should include *in vivo* longitudinal studies that examine the link between alterations in the GABAergic system and early non-motor symptoms by exploiting advances in PET ligands, magnetic resonance spectroscopy and CSF biomarkers. Preclinical studies might help to investigate the effects of GABA in the pathogenesis of non-motor symptoms, but we suggest that identifying the neurotransmitter deficits that correlate with clinical severity should be the mainstay for guiding future treatment studies.

AUTHOR CONTRIBUTIONS

AM-G conceptualized and wrote the manuscript. AA organized and prepared the manuscript. JG-E and IG contributed to writing and reviewing the manuscript.

FUNDING

This study was partially funded by the Michael J. Fox Foundation (2014 Rapid Response Innovation Awards; Grant 10189), the Carlos III Health Institute through Projects PI14/00679 and PI16/00005, and a Juan Rodes Grant (JR15/00008) (I.G.) (cofunded by the "Investing in Your Future" European Regional Development Fund/ European Social Fund programme), the Department of Health of the Basque Government through Project 2016111009, and EITB/ BIOEF telemarathon for Neurodegenerative Diseases (BIO17/ ND/010/BC).

REFERENCES

Aarsland, D., Bronnick, K., Larsen, J. P., Tysnes, O. B., and Alves, G. (2009a). Cognitive impairment in incident, untreated Parkinson disease: the Norwegian ParkWest study. *Neurol.* 72 (13), 1121–1126. doi: 10.1212/01.wnl.0000338632.00552.cb

Aarsland, D., Bronnick, K., Larsen, J. P., Tysnes, O. B., Alves, G., and Norwegian ParkWest Study, G. (2009b). Cognitive impairment in incident, untreated Parkinson disease: the Norwegian ParkWest study. *Neurol.* 72 (13), 1121–1126. doi: 10.1212/01.wnl.0000338632.00552.cb

Abbott, R. D., Ross, G. W., White, L. R., Tanner, C. M., Masaki, K. H., Nelson, J. S., et al. (2005). Excessive daytime sleepiness and subsequent development of Parkinson disease. *Neurol.* 65 (9), 1442–1446. doi: 10.1212/01.wnl.0000183056.89590.0d

Acebes, A., Martin-Pena, A., Chevalier, V., and Ferrus, A. (2011). Synapse loss in olfactory local interneurons modifies perception. *J. Neurosci.* 31 (8), 2734–2745. doi: 10.1523/jneurosci.5046-10.2011

Ahn, J., Lee, J. Y., Kim, T. W., Yoon, E. J., Oh, S., Kim, Y. K., et al. (2018). Retinal thinning associates with nigral dopaminergic loss in *de novo* Parkinson disease. *Neurol.* 91 (11), e1003–e1012. doi: 10.1212/wnl.0000000000006157

Altinayar, S., Oner, S., Can, S., Kizilay, A., Kamisli, S., and Sarac, K. (2014). Olfactory disfunction and its relation olfactory bulb volume in Parkinson's disease. *Eur. Rev. Med. Pharmacol. Sci.* 18 (23), 3659–3664.

Anderson, K. N., and Shneerson, J. M. (2009). Drug treatment of REM sleep behavior disorder: the use of drug therapies other than clonazepam. *J. Clin. Sleep. Med.* 5 (3), 235–239.

Arrigo, A., Calamuneri, A., Milardi, D., Mormina, E., Rania, L., Postorino, E., et al. (2017). Visual system involvement in patients with newly diagnosed parkinson disease. *Radiol.* 285 (3), 885–895. doi: 10.1148/radiol.2017161732

Auteri, M., Zizzo, M. G., and Serio, R. (2015a). GABA and GABA receptors in the gastrointestinal tract: from motility to inflammation. *Pharmacol. Res.* 93, 11–21. doi: 10.1016/j.phrs.2014.12.001

Auteri, M., Zizzo, M. G., and Serio, R. (2015b). The GABAergic system and the gastrointestinal physiopathology. *Curr. Pharm. Des.* 21 (34), 4996–5016. doi: 10.2174/1381612821666150914121518

Barber, T. R., Lawton, M., Rolinski, M., Evetts, S., Baig, F., Ruffmann, C., et al. (2017). Prodromal Parkinsonism and Neurodegenerative Risk Stratification in REM Sleep Behavior Disorder. *Sleep.* 40 (8), 1–10. doi: 10.1093/sleep/zsx071

Blin, O., Mestre, D., Paut, O., Vercher, J. L., and Audebert, C. (1993). GABA-ergic control of visual perception in healthy volunteers: effects of midazolam, a benzodiazepine, on spatio-temporal contrast sensitivity. *Br. J. Clin. Pharmacol.* 36 (2), 117–124. doi: 10.1111/j.1365-2125.1993.tb04206.x

Bohnen, N. I., Muller, M. L., Kotagal, V., Koeppe, R. A., Kilbourn, M. A., Albin, R. L., et al. (2010). Olfactory dysfunction, central cholinergic integrity and cognitive impairment in Parkinson's disease. *Brain.* 133 (Pt 6), 1747–1754. doi: 10.1093/brain/awq079

Boucetta, S., Cisse, Y., Mainville, L., Morales, M., and Jones, B. E. (2014). Discharge profiles across the sleep-waking cycle of identified cholinergic, GABAergic, and glutamatergic neurons in the pontomesencephalic tegmentum of the rat. *J Neurosci.* 34 (13), 4708–4727. doi: 10.1523/jneurosci.2617-13.2014

Braak, H., Del Tredici, K., Rub, U., de Vos, R. A., Jansen Steur, E. N., and Braak, E. (2003). Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiol. Aging* 24 (2), 197–211. doi: 10.1016/s0197-4580(02)00065-9

Brodoehl, S., Klingner, C., Volk, G. F., Bitter, T., Witte, O. W., and Redecker, C. (2012). Decreased olfactory bulb volume in idiopathic Parkinson's disease detected by 3.0-tesla magnetic resonance imaging. *Mov. Disord.* 27 (8), 1019–1025. doi: 10.1002/mds.25087

Brooks, P. L., and Peever, J. H. (2011). Impaired GABA and glycine transmission triggers cardinal features of rapid eye movement sleep behavior disorder in mice. *J Neurosci.* 31 (19), 7111–7121. doi: 10.1523/jneurosci.0347-11.2011

Buchanan, R. J., Gjini, K., Darrow, D., Varga, G., Robinson, J. L., and Nadasdy, Z. (2015). Glutamate and GABA concentration changes in the globus pallidus internus of Parkinson's patients during performance of implicit and declarative memory tasks: a report of two subjects. *Neurosci. Lett.* 589, 73–78. doi: 10.1016/j.neulet.2015.01.028

Chahine, L. M., Urbe, L., Caspell-Garcia, C., Aarsland, D., Alcalay, R., Barone, P., et al. (2018). Cognition among individuals along a spectrum of increased risk for Parkinson's disease. *PLoS One* 13 (8), e0201964. doi: 10.1371/journal.pone.0201964

Chen, J. J., and Marsh, L. (2014). Anxiety in Parkinson's disease: identification and management. *Ther. Adv. Neurol. Disord.* 7 (1), 52–59. doi: 10.1177/1756285613495723

Cheng, H. C., Ulane, C. M., and Burke, R. E. (2010). Clinical progression in Parkinson disease and the neurobiology of axons. *Ann. Neurol.* 67 (6), 715–725. doi: 10.1002/ana.21995

Cook, E., Hammett, S. T., and Larsson, J. (2016). GABA predicts visual intelligence. *Neurosci. Lett.* 632, 50–54. doi: 10.1016/j.neulet.2016.07.053

Doty, R. L. (2012). Olfactory dysfunction in Parkinson disease. *Nat Rev. Neurol.* 8 (6), 329–339. doi: 10.1038/nrneurol.2012.80

Dudley, J. T., Sirota, M., Shenoy, M., Pai, R. K., Roedder, S., Chiang, A. P., et al. (2011). Computational repositioning of the anticonvulsant topiramate for inflammatory bowel disease. *Sci. Transl. Med.* 3 (96), 96ra76. doi: 10.1126/scitranslmed.3002648

Dupont, E., Christensen, S. E., Hansen, A. P., de Fine Olivarius, B., and Orskov, H. (1982). Low cerebrospinal fluid somatostatin in Parkinson disease: an irreversible abnormality. *Neurol.* 32 (3), 312–314. doi: 10.1212/wnl.32.3.312

Emir, U. E., Tuite, P. J., and Oz, G. (2012). Elevated pontine and putamenal GABA levels in mild-moderate Parkinson disease detected by 7 tesla proton MRS. *PLoS One* 7 (1), e30918. doi: 10.1371/journal.pone.0030918

Fantini, M. L., Farini, E., Ortelli, P., Zucconi, M., Manconi, M., Cappa, S., et al. (2011). Longitudinal study of cognitive function in idiopathic REM sleep behavior disorder. *Sleep.* 34 (5), 619–625. doi: 10.1093/sleep/34.5.619

Fantini, M. L., Postuma, R. B., Montplaisir, J., and Ferini-Strambi, L. (2006). Olfactory deficit in idiopathic rapid eye movements sleep behavior disorder. *Brain. Res. Bull.* 70 (4-6), 386–390. doi: 10.1016/j.brainresbull.2006.07.008

Fee, C., Banasr, M., and Sibille, E. (2017). Somatostatin-positive gamma-aminobutyric acid interneuron deficits in depression: cortical microcircuit and therapeutic perspectives. *Biol. Psychiatry.* 82 (8), 549–559. doi: 10.1016/j.biopsych.2017.05.024

Fereshtehnejad, S. M., Montplaisir, J. Y., Pelletier, A., Gagnon, J. F., Berg, D., and Postuma, R. B. (2017). Validation of the MDS research criteria for prodromal Parkinson's disease: Longitudinal assessment in a REM sleep behavior disorder (RBD) cohort. *Mov. Disord.* 32 (6), 865–873. doi: 10.1002/mds.26989

Ferini-Strambi, L., Di Gioia, M. R., Castronovo, V., Oldani, A., Zucconi, M., and Cappa, S. F. (2004). Neuropsychological assessment in idiopathic REM sleep behavior disorder (RBD): does the idiopathic form of RBD really exist? *Neurol.* 62 (1), 41–45. doi: 10.1212/01.wnl.0000101726.69701.fa

Ferini-Strambi, L., Oertel, W., Dauvilliers, Y., Postuma, R. B., Marelli, S., Iranzo, A., et al. (2014). Autonomic symptoms in idiopathic REM behavior disorder: a multicentre case-control study. *J. Neurol.* 261 (6), 1112–1118. doi: 10.1007/s00415-014-7317-8

Ferini-Strambi, L., Rinaldi, F., Giora, E., Marelli, S., and Galbiati, A. (2016). REM sleep Behaviour Disorder. *Parkinsonism. Relat. Disord.* 22 Suppl 1, S69–S72. doi: 10.1016/j.parkreldis.2015.09.002

Firbank, M. J., Parikh, J., Murphy, N., Killen, A., Allan, C. L., Collerton, D., et al. (2018). Reduced occipital GABA in Parkinson disease with visual hallucinations. *Neurol.* 91 (7), e675–e685. doi: 10.1212/wnl.0000000000006007

Fuchs, T., Jefferson, S. J., Hooper, A., Yee, P. H., Maguire, J., and Luscher, B. (2016). Disinhibition of somatostatin-positive GABAergic interneurons results in an anxiolytic and antidepressant-like brain state. *Mol. Psychiatry.* 22, 920. doi: 10.1038/mp.2016.188

Gagnon, J. F., Vendette, M., Postuma, R. B., Desjardins, C., Massicotte-Marquez, J., Panisset, M., et al. (2009). Mild cognitive impairment in rapid eye movement sleep behavior disorder and Parkinson's disease. *Ann. Neurol.* 66 (1), 39–47. doi: 10.1002/ana.21680

Garcia-Borreguero, D., Larrosa, O., and Bravo, M. (2003). Parkinson's disease and sleep. *Sleep. Med. Rev.* 7 (2), 115–129. doi: 10.1053/smrv.2002.0229

Gardner, B., Dieriks, B. V., Cameron, S., Mendis, L. H. S., Turner, C., Faull, R. L. M., et al. (2017). Metal concentrations and distributions in the human olfactory bulb in Parkinson's disease. *Sci. Rep.* 7 (1), 10454. doi: 10.1038/s41598-017-10659-6

Genier Marchand, D., Montplaisir, J., Postuma, R. B., Rahayel, S., and Gagnon, J. F. (2017). Detecting the cognitive prodrome of dementia with lewy bodies: a prospective study of rem sleep behavior disorder. *Sleep.* 40 (1), 1–11. doi: 10.1093/sleep/zsw014

Hajj, R., Milet, A., Toulorge, D., Cholet, N., Laffaire, J., Foucquier, J., et al. (2015). Combination of acamprosate and baclofen as a promising therapeutic approach for Parkinson's disease. *Sci. Rep.* 5, 16084. doi: 10.1038/srep16084

Hall, S. D., Prokic, E. J., McAllister, C. J., Ronnqvist, K. C., Williams, A. C., Yamawaki, N., et al. (2014). GABA-mediated changes in inter-hemispheric beta frequency activity in early-stage Parkinson's disease. *Neurosci.* 281, 68–76. doi: 10.1016/j.neuroscience.2014.09.037

Hall, S. D., Yamawaki, N., Fisher, A. E., Clauss, R. P., Woodhall, G. L., and Stanford, I. M. (2010). GABA(A) alpha-1 subunit mediated desynchronization of elevated low frequency oscillations alleviates specific dysfunction in stroke–a case report. *Clin. Neurophysiol.* 121 (4), 549–555. doi: 10.1016/j.clinph.2009.11.084

Harding, A. J., Stimson, E., Henderson, J. M., and Halliday, G. M. (2002). Clinical correlates of selective pathology in the amygdala of patients with Parkinson's disease. *Brain.* 125 (Pt 11), 2431–2445. doi: 10.1093/brain/awf251

Hilgen, G., Softley, S., Pamplona, D., Kornprobst, P., Cessac, B., et al. (2015). The effect of retinal GABA Depletion by Allylglycine on mouse retinal ganglion cell responses to light. *European Retina Meeting*. Brigthon, United Kingdom. <hal-01235324>

Hogl, B., Stefani, A., and Videnovic, A. (2018). Idiopathic REM sleep behaviour disorder and neurodegeneration - an update. *Nat Rev. Neurol.* 14 (1), 40–55. doi: 10.1038/nrneurol.2017.157

Houser, M. C., and Tansey, M. G. (2017). The gut-brain axis: is intestinal inflammation a silent driver of Parkinson's disease pathogenesis? *NPJ. Parkinsons Dis.* 3, 3. doi: 10.1038/s41531-016-0002-0

Iranzo, A. (2018). The REM sleep circuit and how its impairment leads to REM sleep behavior disorder. *Cell. Tissue. Res.* 373 (1), 245–266. doi: 10.1007/s00441-018-2852-8

Iranzo, A., Molinuevo, J. L., Santamaria, J., Serradell, M., Marti, M. J., Valldeoriola, F., et al. (2006). Rapid-eye-movement sleep behaviour disorder as an early marker for a neurodegenerative disorder: a descriptive study. *Lancet. Neurol.* 5 (7), 572–577. doi: 10.1016/S1474-4422(06)70476-8

Iranzo, A., Stefani, A., Serradell, M., Marti, M. J., Lomena, F., Mahlknecht, P., et al. (2017). Characterization of patients with longstanding idiopathic REM sleep behavior disorder. *Neurol.* 89 (3), 242–248. doi: 10.1212/wnl.0000000000004121

Iwasawa, C., Kuzumaki, N., Suda, Y., Kagawa, R., Oka, Y., Hattori, N., et al. (2019). Reduced expression of somatostatin in GABAergic interneurons derived from induced pluripotent stem cells of patients with parkin mutations. *Mol. Brain.* 12 (1), 5. doi: 10.1186/s13041-019-0426-7

Jacob, E. L., Gatto, N. M., Thompson, A., Bordelon, Y., and Ritz, B. (2010). Occurrence of depression and anxiety prior to Parkinson's disease. *Parkinsonism. Relat. Disord.* 16 (9), 576–581. doi: 10.1016/j.parkreldis.2010.06.014

Jin, Z., Mendu, S. K., and Birnir, B. (2013). GABA is an effective immunomodulatory molecule. *Amino. Acids.* 45 (1), 87–94. doi: 10.1007/s00726-011-1193-7

Kalueff, A. V., and Nutt, D. J. (2007). Role of GABA in anxiety and depression. *Depress. Anxiety.* 24 (7), 495–517. doi: 10.1002/da.20262

Katzner, S., Busse, L., and Carandini, M. (2011). GABAA inhibition controls response gain in visual cortex. *J Neurosci.* 31 (16), 5931–5941. doi: 10.1523/jneurosci.5753-10.2011

Kehagia, A. A., Barker, R. A., and Robbins, T. W. (2010). Neuropsychological and clinical heterogeneity of cognitive impairment and dementia in patients with Parkinson's disease. *Lancet. Neurol.* 9 (12), 1200–1213. doi: 10.1016/s1474-4422(10)70212-x

Khundakar, A. A., Hanson, P. S., Erskine, D., Lax, N. Z., Roscamp, J., Karyka, E., et al. (2016). Analysis of primary visual cortex in dementia with Lewy bodies indicates GABAergic involvement associated with recurrent complex visual hallucinations. *Acta. Neuropathol. Commun.* 4 (1), 66. doi: 10.1186/s40478-016-0334-3

Kim, S. H., Park, J. H., Kim, Y. H., and Koh, S. B. (2011). Stereopsis in drug naive Parkinson's disease patients. *Can J. Neurol Sci* 38 (2), 299–302. doi: 10.1017/s0317167100011501

Kish, S. J., Rajput, A., Gilbert, J., Rozdilsky, B., Chang, L. J., Shannak, K., et al. (1986). Elevated gamma-aminobutyric acid level in striatal but not extrastriatal brain regions in Parkinson's disease: correlation with striatal dopamine loss. *Ann. Neurol.* 20 (1), 26–31. doi: 10.1002/ana.410200106

Knudsen, K., Fedorova, T. D., Hansen, A. K., Sommerauer, M., Otto, M., Svendsen, K. B., et al. (2018). In-vivo staging of pathology in REM sleep behaviour disorder: a multimodality imaging case-control study. *Lancet. Neurol.* 17 (7), 618–628. doi: 10.1016/s1474-4422(18)30162-5

Lanoue, A. C., Blatt, G. J., and Soghomonian, J. J. (2013). Decreased parvalbumin mRNA expression in dorsolateral prefrontal cortex in Parkinson's disease. *Brain. Res.* 1531, 37–47. doi: 10.1016/j.brainres.2013.07.025

Lanoue, A. C., Dumitriu, A., Myers, R. H., and Soghomonian, J. J. (2010). Decreased glutamic acid decarboxylase mRNA expression in prefrontal cortex in Parkinson's disease. *Exp. Neurol.* 226 (1), 207–217. doi: 10.1016/j.expneurol.2010.09.001

Li, J., Gu, C. Z., Su, J. B., Zhu, L. H., Zhou, Y., Huang, H. Y., et al. (2016). Changes in olfactory bulb volume in parkinson's disease: a systematic review and meta-analysis. *PLoS. One.* 11 (2), e0149286. doi: 10.1371/journal.pone.0149286

Lozovaya, N., Eftekhari, S., Cloarec, R., Gouty-Colomer, L. A., Dufour, A., Riffault, B., et al. (2018). GABAergic inhibition in dual-transmission cholinergic and GABAergic striatal interneurons is abolished in Parkinson disease. *Nat. Commun.* 9 (1), 1422. doi: 10.1038/s41467-018-03802-y

Mahlknecht, P., Iranzo, A., Hogl, B., Frauscher, B., Muller, C., Santamaria, J., et al. (2015). Olfactory dysfunction predicts early transition to a Lewy body disease in idiopathic RBD. *Neurol.* 84 (7), 654–658. doi: 10.1212/wnl.0000000000001265

Marques, A., Dujardin, K., Boucart, M., Pins, D., Delliaux, M., Defebvre, L., et al. (2010). REM sleep behaviour disorder and visuoperceptive dysfunction: a disorder of the ventral visual stream? *J. Neurol.* 257 (3), 383–391. doi: 10.1007/s00415-009-5328-7

Massicotte-Marquez, J., Decary, A., Gagnon, J. F., Vendette, M., Mathieu, A., Postuma, R. B., et al. (2008). Executive dysfunction and memory impairment in idiopathic REM sleep behavior disorder. *Neurol.* 70 (15), 1250–1257. doi: 10.1212/01.wnl.0000286943.79593.a6

Mohler, H. (2012). The GABA system in anxiety and depression and its therapeutic potential. *Neuropharmacol.* 62 (1), 42–53. doi: 10.1016/j.neuropharm.2011.08.040

Murueta-Goyena, A., Del Pino, R., Reyero, P., Galdos, M., Arana, B., Lucas-Jimenez, O., et al. (2019). Parafoveal thinning of inner retina is associated with visual dysfunction in Lewy body diseases. *Mov. Disord.* 34 (9), 1315–1324. doi: 10.1002/mds.27728

Nguyen-Legros, J. (1988). Functional neuroarchitecture of the retina: hypothesis on the dysfunction of retinal dopaminergic circuitry in Parkinson's disease. *Surg. Radiol. Anat.* 10 (2), 137–144. doi: 10.1007/BF02307822

O'Gorman Tuura, R. L., Baumann, C. R., and Baumann-Vogel, H. (2018). Beyond Dopamine: GABA, Glutamate, and the Axial Symptoms of Parkinson Disease. *Front. Neurol.* 9, 806. doi: 10.3389/fneur.2018.00806

Ota, K., Fujishiro, H., Kasanuki, K., Kondo, D., Chiba, Y., Murayama, N., et al. (2016). Prediction of later clinical course by a specific glucose metabolic pattern in non-demented patients with probable REM sleep behavior disorder admitted to a memory clinic: a case study. *Psychiatry. Res. Neuroimaging.* 248, 151–158. doi: 10.1016/j.pscychresns.2015.12.004

Pagano, G., De Micco, R., Yousaf, T., Wilson, H., Chandra, A., and Politis, M. (2018). REM behavior disorder predicts motor progression and cognitive decline in Parkinson disease. *Neurol.* 91 (10), e894–e905. doi: 10.1212/wnl.0000000000006134

Pearce, R. K., Hawkes, C. H., and Daniel, S. E. (1995). The anterior olfactory nucleus in Parkinson's disease. *Mov. Disord.* 10 (3), 283–287. doi: 10.1002/mds.870100309

Pfeiffer, R. F. (2018). Gastrointestinal dysfunction in Parkinson's Disease. *Curr. Treat. Options Neurol.* 20 (12), 54. doi: 10.1007/s11940-018-0539-9

Pirez, N., and Wachowiak, M. (2008). In vivo modulation of sensory input to the olfactory bulb by tonic and activity-dependent presynaptic inhibition of receptor neurons. *J Neurosci.* 28 (25), 6360–6371. doi: 10.1523/jneurosci.0793-08.2008

Poewe, W. (2008). Non-motor symptoms in Parkinson's disease. *Eur. J. Neurol.* 15 Suppl 1, 14–20. doi: 10.1111/j.1468-1331.2008.02056.x

Postuma, R. B., and Berg, D. (2019). Prodromal parkinson's disease: the decade past, the decade to come. *Mov. Disord.* 34 (5), 665–675. doi: 10.1002/mds.27670

Postuma, R. B., Gagnon, J. F., Bertrand, J. A., Genier Marchand, D., and Montplaisir, J. Y. (2015). Parkinson risk in idiopathic REM sleep behavior disorder: preparing for neuroprotective trials. *Neurol.* 84 (11), 1104–1113. doi: 10.1212/wnl.0000000000001364

Postuma, R. B., Gagnon, J. F., Vendette, M., and Montplaisir, J. Y. (2009). Markers of neurodegeneration in idiopathic rapid eye movement sleep behaviour disorder and Parkinson's disease. *Brain.* 132 (Pt 12), 3298–3307. doi: 10.1093/brain/awp244

Ray, N. J., and Strafella, A. P. (2012). The neurobiology and neural circuitry of cognitive changes in Parkinson's disease revealed by functional neuroimaging. *Mov. Disord.* 27 (12), 1484–1492. doi: 10.1002/mds.25173

Reijnders, J. S., Ehrt, U., Weber, W. E., Aarsland, D., and Leentjens, A. F. (2008). A systematic review of prevalence studies of depression in Parkinson's disease. *Mov. Disord.* 23 (2), 183–189, quiz 313. doi: 10.1002/mds.21803

Righi, S., Viggiano, M. P., Paganini, M., Ramat, S., and Marini, P. (2007). Recognition of category-related visual stimuli in Parkinson's disease: before and after pharmacological treatment. *Neuropsychologia.* 45 (13), 2931–2941. doi: 10.1016/j.neuropsychologia.2007.06.002

Ross, G. W., Petrovitch, H., Abbott, R. D., Tanner, C. M., Popper, J., Masaki, K., et al. (2008). Association of olfactory dysfunction with risk for future Parkinson's disease. *Ann. Neurol.* 63 (2), 167–173. doi: 10.1002/ana.21291

Sanjari Moghaddam, H., Zare-Shahabadi, A., Rahmani, F., and Rezaei, N. (2017). Neurotransmission systems in Parkinson's disease. *Rev. Neurosci.* 28 (5), 509–536. doi: 10.1515/revneuro-2016-0068

Santpere, G., Garcia-Esparcia, P., Andres-Benito, P., Lorente-Galdos, B., Navarro, A., and Ferrer, I. (2018). Transcriptional network analysis in frontal cortex in Lewy body diseases with focus on dementia with Lewy bodies. *Brain. Pathol.* 28 (3), 315–333. doi: 10.1111/bpa.12511

Sasai, T., Miyamoto, T., Miyamoto, M., Iwanami, M., Abe, T., Matsuura, M., et al. (2012). Impaired decision-making in idiopathic REM sleep behavior disorder. *Sleep. Med.* 13 (3), 301–306. doi: 10.1016/j.sleep.2011.09.012

Scherfler, C., Esterhammer, R., Nocker, M., Mahlknecht, P., Stockner, H., Warwitz, B., et al. (2013). Correlation of dopaminergic terminal dysfunction and microstructural abnormalities of the basal ganglia and the olfactory tract in Parkinson's disease. *Brain.* 136 (Pt 10), 3028–3037. doi: 10.1093/brain/awt234

Scherfler, C., Schocke, M. F., Seppi, K., Esterhammer, R., Brenneis, C., Jaschke, W., et al. (2006). Voxel-wise analysis of diffusion weighted imaging reveals disruption of the olfactory tract in Parkinson's disease. *Brain.* 129 (Pt 2), 538–542. doi: 10.1093/brain/awh674

Shao, Z., Puche, A. C., Kiyokage, E., Szabo, G., and Shipley, M. T. (2009). Two GABAergic intraglomerular circuits differentially regulate tonic and phasic presynaptic inhibition of olfactory nerve terminals. *J Neurophysiol.* 101 (4), 1988–2001. doi: 10.1152/jn.91116.2008

Stirpe, P., Hoffman, M., Badiali, D., and Colosimo, C. (2016). Constipation: an emerging risk factor for Parkinson's disease? *Eur. J. Neurol.* 23 (11), 1606–1613. doi: 10.1111/ene.13082

Svenningsson, P., Westman, E., Ballard, C., and Aarsland, D. (2012). Cognitive impairment in patients with Parkinson's disease: diagnosis,

biomarkers, and treatment. *Lancet. Neurol.* 11 (8), 697–707. doi: 10.1016/S1474-4422(12)70152-7

Tanik, N., Serin, H. I., Celikbilek, A., Inan, L. E., and Gundogdu, F. (2016). Associations of olfactory bulb and depth of olfactory sulcus with basal ganglia and hippocampus in patients with Parkinson's disease. *Neurosci. Lett.* 620, 111–114. doi: 10.1016/j.neulet.2016.03.050

Terzaghi, M., Zucchella, C., Rustioni, V., Sinforiani, E., and Manni, R. (2013). Cognitive performances and mild cognitive impairment in idiopathic rapid eye movement sleep behavior disorder: results of a longitudinal follow-up study. *Sleep.* 36 (10), 1527–1532. doi: 10.5665/sleep.3050

Tritsch, N. X., Ding, J. B., and Sabatini, B. L. (2012). Dopaminergic neurons inhibit striatal output through non-canonical release of GABA. *Nat.* 490 (7419), 262–266. doi: 10.1038/nature11466

Tritsch, N. X., Oh, W. J., Gu, C., and Sabatini, B. L. (2014). Midbrain dopamine neurons sustain inhibitory transmission using plasma membrane uptake of GABA, not synthesis. *Elife.* 3, e01936. doi: 10.7554/eLife.01936

Tysnes, O. B., and Storstein, A. (2017). Epidemiology of Parkinson's disease. *J. Neural. Transm. (Vienna)* 124 (8), 901–905. doi: 10.1007/s00702-017-1686-y

Unger, M. M., Moller, J. C., Mankel, K., Schmittinger, K., Eggert, K. M., Stamelou, M., et al. (2011). Patients with idiopathic rapid-eye-movement sleep behavior disorder show normal gastric motility assessed by the 13C-octanoate breath test. *Mov. Disord.* 26 (14), 2559–2563. doi: 10.1002/mds.23933

van Loon, A. M., Knapen, T., Scholte, H. S., St John-Saaltink, E., Donner, T. H., and Lamme, V. A. (2013). GABA shapes the dynamics of bistable perception. *Curr. Biol.* 23 (9), 823–827. doi: 10.1016/j.cub.2013.03.067

Vesela, O., Ruzicka, E., Jech, R., Roth, J., Stepankova, K., Mecir, P., et al. (2001). Colour discrimination impairment is not a reliable early marker of Parkinson's disease. *J. Neurol.* 248 (11), 975–978. doi: 10.1007/s004150170051

Weil, R. S., Schrag, A. E., Warren, J. D., Crutch, S. J., Lees, A. J., and Morris, H. R. (2016). Visual dysfunction in Parkinson's disease. *Brain.* 139 (11), 2827–2843. doi: 10.1093/brain/aww175

Wen, M. C., Xu, Z., Lu, Z., Chan, L. L., Tan, E. K., and Tan, L. C. S. (2017). Microstructural network alterations of olfactory dysfunction in newly diagnosed Parkinson's disease. *Sci. Rep.* 7 (1), 12559. doi: 10.1038/s41598-017-12947-7

Westermann, B., Wattendorf, E., Schwerdtfeger, U., Husner, A., Fuhr, P., Gratzl, O., et al. (2008). Functional imaging of the cerebral olfactory system in patients with Parkinson's disease. *J. Neurol. Neurosurg. Psychiatry* 79 (1), 19–24. doi: 10.1136/jnnp.2006.113860

Wong, J. C., Li, Y., Schwarzschild, M. A., Ascherio, A., and Gao, X. (2014). Restless legs syndrome: an early clinical feature of Parkinson disease in men. *Sleep.* 37 (2), 369–372. doi: 10.5665/sleep.3416

Youn, S., Kim, T., Yoon, I. Y., Jeong, J., Kim, H. Y., Han, J. W., et al. (2016). Progression of cognitive impairments in idiopathic REM sleep behaviour disorder. *J. Neurol. Neurosurg. Psychiatry* 87 (8), 890–896. doi: 10.1136/jnnp-2015-311437

Zapiec, B., Dieriks, B. V., Tan, S., Faull, R. L. M., Mombaerts, P., and Curtis, M. A. (2017). A ventral glomerular deficit in Parkinson's disease revealed by whole olfactory bulb reconstruction. *Brain.* 140 (10), 2722–2736. doi: 10.1093/brain/awx208

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pharmacology

REVIEW
published: 27 November 2019
doi: 10.3389/fphar.2019.01414

Sleep Disorders in Rodent Models of Parkinson's Disease

*Daniel de Castro Medeiros[1,2], Cleiton Lopes Aguiar[2], Márcio Flávio Dutra Moraes[2] and Gilberto Fisone[1]**

[1] Department of Neuroscience, Karolinska Institutet, Stockholm, Sweden, [2] Núcleo de Neurociências, Departamento de Fisiologia e Biofísica, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil

Sleep disorders are frequently diagnosed in Parkinson's disease and manifested in the prodromal and advanced stages of the disease. These conditions, which in some cases affect more than 50% of Parkinson's disease (PD) patients, include hypersomnia, often manifested as excessive daytime sleepiness, insomnia, characterized by delayed initiation and fragmentation of sleep at night, and disruption of rapid eye movement (REM) sleep, resulting in loss of atonia and dream enactment. Standard dopamine replacement therapies for the treatment of motor symptoms are generally inadequate to combat sleep abnormalities, which seriously affect the quality of life of PD patients. Rodent models still represent a major tool for the study of many aspects of PD. They have been primarily designed to eliminate midbrain dopamine neurons and elicit motor impairment, which are the traditional pathological features of PD. However, rodent models are increasingly employed to investigate non-motor symptoms, which are often caused by degenerative processes affecting multiple monoaminergic and peptidergic structures. This review describes how neurotoxic and genetic manipulations of rats and mice have been utilized to reproduce some of the major sleep disturbances associated with PD and to what extent these abnormalities can be linked to non-dopaminergic dysfunction, affecting for instance noradrenaline, serotonin, and orexin transmission. Strengths and limitations are discussed, as well as the consistency of results obtained so far, and the need for models that better reproduce the multisystemic neurodegenerative nature of PD, thereby allowing to replicate the complex etiology of sleep-related disorders.

Keywords: sleep, rapid eye movement sleep, slow wave sleep, rat, mouse, disease models, 6-hydroxydopamine, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

OPEN ACCESS

Edited by:
Philippe De Deurwaerdere, Université de Bordeaux, France

Reviewed by:
Karim Fifel, Université Claude Bernard Lyon 1, France
Micaela Morelli, University of Cagliari, Italy

***Correspondence:**
Gilberto Fisone
gilberto.fisone@ki.se

Specialty section:
This article was submitted to Neuropharmacology, a section of the journal Frontiers in Pharmacology

Received: *09 September 2019*
Accepted: *07 November 2019*
Published: *27 November 2019*

Citation:
Medeiros DdC, Lopes Aguiar C, Moraes MFD and Fisone G (2019) Sleep Disorders in Rodent Models of Parkinson's Disease. Front. Pharmacol. 10:1414. doi: 10.3389/fphar.2019.01414

INTRODUCTION

Since their first description two centuries ago, the symptoms of Parkinson's disease (PD) have been regularly re-assessed to include a large number of nonmotor conditions affecting both the peripheral and the central nervous system (Chaudhuri and Odin, 2010; Lang, 2011). This has occurred in parallel with the recognition of the complexity of the neurodegenerative processes at the basis of PD, whose

definition extends beyond that of a simple dopaminergic disorder. Indeed, accurate analyses of the spread of α-synuclein pathology from the peripheral nervous system, lower brainstem, and olfactory bulb, to the diencephalon, basal forebrain, and neocortex, show that PD affects not only dopamine, but also noradrenaline, serotonin, and acetylcholine containing structures (Braak et al., 2003).

This diversified pathology has been correlated to the gradual appearance of non-motor symptoms. For instance, gastrointestinal, bladder, and olfactory dysfunctions, which are common preclinical features of PD, have been linked to the initial stage of the disease, affecting the autonomic nervous system and olfactory nuclei. Other conditions, including sleep disturbances and affective disorders appear in conjunction with the caudal-rostral spread of α-synuclein deposition to the locus ceruleus, raphe, and lateral tegmental nuclei. Additional non-motor symptoms include cognitive dysfunction, often developing into dementia during the late stages of PD (Chaudhuri and Odin, 2010; Lang, 2011).

Sleep disorders are among the most frequent symptoms observed in PD patients, with a prevalence of 60–70% (De Cock et al., 2008; Barone et al., 2009; Neikrug et al., 2013). They often appear in the early stages of the disease and are considered as markers of prodromal parkinsonism (Schenck et al., 1996; Postuma et al., 2010; Lysen et al., 2019). Sleep-related symptoms in PD consist of a broad spectrum of conditions, which include hypersomnia, the difficulty in initiating and maintaining sleep and disturbances of rapid eye movement (REM) sleep. These disorders progressively worsen in the course of PD and represent a major factor affecting the patient's quality of life (Maetzler et al., 2009; Neikrug et al., 2013). They are generally refractory to, or exacerbated, by standard anti-parkinsonian medications. In this context, it should be mentioned that impaired synaptic downscaling associated with sleep disruption may promote the emergence of motor complications caused by standard pharmacological therapy (Galati et al., 2015), and that this idea is supported by clinical studies indicating a correlation between disrupted sleep and l-3,4-dihydroxyphenylalanine (L-DOPA)-induced dyskinesia (Mao et al., 2018).

Several studies show that surgical treatment, specifically deep brain stimulation targeting the subthalamic nucleus, ameliorates sleep quality in PD (Arnulf et al., 2000; Antonini et al., 2004; Cicolin et al., 2004; Hjort et al., 2004; Kharkar et al., 2018). This could be explained by the ability of this intervention to counteract motor disturbances (Arnulf et al., 2000), but additional effects possibly exerted on sleep structures cannot be excluded (Kharkar et al., 2018). In spite of these efforts, the progressive deterioration of sleep observed in a large proportion of patients still represents a major problem in the management of PD.

In this review, we describe and discuss current attempts to study PD-related sleep disorders in rodents, which represent the most common experimental animals used to model motor and non-motor symptoms of PD.

Sleep Physiology

Sleep is a natural and reversible state, defined by lack of mobility (or slight mobility) and relative unresponsiveness to internal and external stimuli (Carskadon and Dement, 2017), closely associated with the patient's general health condition and necessary for optimal cognitive functions (Scammell et al., 2017). In general, a typical sleep period encompasses recurrent cycle successions of two states with distinct physiological characteristics, as assessed by electroencephalography (EEG), and electromyography (EMG): non-REM (NREM) and REM sleep (Chokroverty, 2009). During NREM sleep, the EMG indicates a decrease of muscle activity and the EEG shows a predominance of slow oscillations (0.5–4 Hz). For this reason, NREM sleep is also referred to as slow-wave sleep (SWS). In contrast, the REM phase presents a desynchronized EEG pattern (similar to wakefulness) along with a drastic reduction of muscle tonus (Lee and Dan, 2012).

The sleep-wake brain states and muscle tonus are mainly regulated by the posterior hypothalamus and several nuclei in the brainstem, collectively named ascending activating (or arousal) system (AAS). This system projects to the thalamus, basal forebrain, neocortex, and to the spinal cord (Fuller et al., 2016) and is essential for arousal and wakefulness (Moruzzi and Magoun, 1949). The firing rate of the neural components in the AAS, which consist mainly of monoaminergic and cholinergic neurons, changes along NREM-REM sleep cycles (Jones, 2003). The sleep monoaminergic nuclei complex includes the noradrenergic locus coeruleus (LC) (Carter et al., 2010), the serotonergic dorsal and median raphe nuclei (Ito et al., 2013), the dopaminergic ventral periaqueductal gray matter (vPAG) (Lu et al., 2006), and the histaminergic tuberomammillary neurons (TMN) (Yu et al., 2014). These nuclei present high firing rates during the wake period, lower firing during NREM sleep and are almost silent throughout the REM state. Conversely, the cholinergic neurons, which are clustered in the pedunculopontine (PPT) and lateral dorsal tegmental nuclei, fire rapidly during wake and REM periods but slowly during NREM sleep (Boucetta et al., 2014).

The AAS receives inhibitory inputs from galanin and gamma-aminobutyric acid-ergic (GABAergic) neurons located in the ventrolateral preoptic area (VLPO) and median preoptic nucleus (Suntsova et al., 2007). These hypothalamic neurons present a faster firing rate during sleep and effectively shut down the AAS wake-promoting cells (Sherin et al., 1996; Lu et al., 2002). On the other hand, AAS afferents from the LC and dorsal raphe connect to VLPO neurons that are inhibited by norepinephrine and serotonin (Chou et al., 2002). This reciprocal inhibitory control acts as a feedback loop (neural flip-flop circuit), leading to the alternation and stability of sleep-wake competing states (Saper et al., 2001; Saper et al., 2005). In addition, the AAS includes afferent connections from GABAergic neurons in the substantia nigra pars reticulata and from orexins (orexin-A and orexin-B; also known as hypocretin-1 and 2) containing neurons in the lateral hypothalamus (LH) (de Lecea et al., 1998; Sakurai et al., 1998). While the former induces NREM-REM states *via* inhibition of dorsal raphe and LC (Liu and Dan, 2019), the later promotes wake and suppresses sleep (particularly REM sleep) (Scammell et al., 2017) by activating LC and TMN (Peyron et al., 1998).

REM sleep is also controlled by means of a mutual inhibition between neuronal circuits located in the mesopontine tegmentum. The sub-laterodorsal nucleus and precoeruleus region comprise the REM-promoting (REM-on) structures. Glutamatergic neurons projecting from these regions regulate the activity of the basal forebrain and medulla, thereby promoting cortical high-frequency paradoxical oscillations and muscle atonia typical of REM sleep. The REM-off nuclei, i.e., vPAG and lateral pontine tegmentum, provide a REM flip-flop switch arrangement *via* GABAergic inhibition of REM-on nuclei (Peever and Fuller, 2017).

Hypothalamic and brainstem neurotransmitter systems modulate the REM-switch structures and support the sleep cycles. LC noradrenergic and dorsal raphe serotonergic neurons suppress REM sleep by exciting REM-off and inhibiting REM-on areas, whereas lateral dorsal tegmental and PPT cholinergic neurons promote REM by opposite actions on the same REM-on/off populations. Additionally, orexin neurons excite REM-off structures and support sleep-wake stabilization, whereas the VLPO promotes the entry into REM sleep by inhibiting the same targets (Lu et al., 2006; Peever and Fuller, 2017).

Transitions between sleep and wake are thought to be regulated by two main processes—the homeostatic process (process S) and the circadian pacemaker (process C) (Borbély et al., 2016). Prolonged periods of wakefulness are followed by a corrective higher amount of sleep, referred to as sleep rebound. This homeostatic response is mediated by substances (somnogens) that accumulate during the wake periods and dissipate during sleep. One of the best-known somnogens is adenosine, a paracrine mediator produced by the degradation of ATP (Porkka-Heiskanen et al., 1997). Higher extracellular levels of adenosine promote sleep-state by inhibiting the AAS *via* adenosine A1 receptor (Strecker et al., 2000) and stimulating VLPO *via* A2 receptors (Scammell et al., 2001). The circadian pacemaker opposes the homeostatic process during the active period of the sleep-wake cycle, *via* the suprachiasmatic nucleus, which promotes wakefulness *via* excitation of LH orexin neurons and inhibition of VLPO neurons (Saper et al., 2005).

Sleep Disturbances in Parkinson's Disease

The progression of PD affects multiple neurotransmitter pathways that extend beyond dopaminergic degeneration in the substantia nigra pars compacta (SNc) (Braak et al., 2004; Surmeier et al., 2017), often comprising structures related to the sleep-wake cycle (French and Muthusamy, 2016). In fact, most PD patients present neuronal cell loss and Lewy bodies in the noradrenergic neurons of the locus coeruleus (Zarow et al., 2003), serotonergic and dopaminergic neurons in medial and dorsal raphe and vPAG (Halliday et al., 1990), as well as cholinergic, histaminergic, and orexinergic neurons in the pedunculopontine nuclei (PPN), TMN, and LH (Zweig et al., 1989; Fronczek et al., 2008; Shan et al., 2012; French and Muthusamy, 2018). Disruptions of these structures and connected circuits are likely to play an important role in sleep disturbances, such as insomnia, excessive daytime sleepiness (EDS), and REM sleep behavior disorder (RBD). In addition to these conditions, sleep in PD is influenced by motor abnormalities, such as restless legs syndrome, which can seriously compromise nocturnal sleep, and breathing disorders, leading to sleep apnea (Mery et al., 2017; Ferini-Strambi et al., 2018). Altogether, these disturbances seriously contribute to fragmented sleep-wake behavior observed in PD.

Insomnia is one of the most common sleep disorders in PD, affecting up to 60% of patients (Gjerstad et al., 2007). It is defined by a repeated difficulty in sleep initiation, duration, consolidation, or quality that occurs despite adequate time or opportunity for sleep (Thorpy, 2009). PD-related insomnia presents a multifactorial etiology and is often manifested as comorbidity rather than a single sleep problem (Ylikoski et al., 2015). The role played in PD-related insomnia by lesions affecting brain structures directly involved in arousal and sleep-wake behavior should not be overlooked. However, a number of motor conditions intrinsic to the disease process or induced by dopamine replacement therapy, such as tremor, restless legs syndrome, nighttime cramps, dystonia, and dyskinesia, play an essential role in the pathophysiology of PD-related insomnia (Arnulf et al., 2000). Similarly, non-motor symptoms including autonomic dysfunction and psychiatric symptoms have also been associated with both insomnia and hypersomnia (Kurtis et al., 2013). Drug-disease interaction is also associated with insomnia in PD patients. For example, D1 and D2 receptors activation by higher doses of dopaminergic medication at bedtime are correlated with poor sleep quality (Chahine et al., 2013).

EDS is diagnosed in 15–50% of PD patients (Ondo et al., 2001) and is characterized by a disabling urge to nod or fall asleep during different daily-life circumstances, with a severe negative impact on the overall quality of life (Arnulf, 2005). The damage in the AAS nuclei is an essential factor in PD related EDS (Arnulf and Leu-Semenescu, 2009), particularly the degeneration of hypothalamic orexin cells (Fronczek et al., 2008), whose activity is crucial for vigilance maintenance (Sakurai, 2007; Ono and Yamanaka, 2017) and for the suppression of pathological intrusions of REM sleep-related events during wakefulness (narcolepsy) (Hara et al., 2001). Additionally, EDS (somnolence) has direct correlation with the dosage of daytime dopamine agonist treatment (Avorn et al., 2005).

RBD is defined by the loss of normal REM atonia with prominent out-of-dreams muscle activation (Schenck et al., 2002), which may result in severe injuries to patients and their family. RBD affects approximately 30% of patients with PD (Barone et al., 2009) and is associated with loss of volume and neuronal degeneration in brainstem REM-on areas (Boeve et al., 2007; Garcia-Lorenzo et al., 2013; Boucetta et al., 2016), which may be caused by the pathological accumulation of α-synuclein (Braak et al., 2004). Additionally, REM-sleep stability may be compromised by multiple degeneration of serotonergic, noradrenergic, cholinergic, and orexinergic nuclei that support and modulate the REM-switching system (Boeve et al., 2007; Fronczek et al., 2007; Garcia-Lorenzo et al., 2013; Boucetta et al., 2016; French and Muthusamy, 2018).

Rodent Models of Parkinson's Disease for the Study of Non-Motor Symptoms

The multiple neurodegenerative processes potentially involved in non-motor symptoms contrast with the relatively well-established cause at the basis of the cardinal motor symptoms of PD, i.e., the progressive degeneration of dopamine neurons in the SNc, projecting to the dorsal striatum (DS). Because of the traditional view of PD as a motor disorder, the development of experimental models has been centered on the elimination of the dopamine nigrostriatal pathway. This is generally achieved by local or systemic administration of neurotoxins (Tieu, 2011) or by genetic manipulations (Dawson et al., 2010).

Neurotoxin Models of Parkinson's Disease

Neurotoxin models of PD are commonly based on the use of 6-hydroxydopamine (6-OHDA) (Simola et al., 2007) or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Meredith and Rademacher, 2011). These compounds act by inhibiting mitochondrial function and generating reactive oxygen species, ultimately leading to oxidative stress and cell death (Simola et al., 2007; Meredith and Rademacher, 2011). In the mouse, repeated systemic administration of MPTP leads to a substantial loss of dopamine neurons and fibers. Depending on dose and regimen, MPTP has been reported to produce from 20 to 70% neuronal loss in the SNc and from 50 to 90% decrease in dopamine levels in the DS (Jackson-Lewis and Przedborski, 2007; Meredith and Rademacher, 2011).

6-OHDA is typically administered *via* stereotaxic injection in the medial forebrain bundle (MFB), dopaminergic midbrain nuclei (e.g., SNc) or directly into the DS of rats and mice. The injection in the MFB produces a nearly complete loss of nigrostriatal dopamine, mimicking end-stage PD (Yuan et al., 2005). This approach is normally utilized in combination with a unilateral lesion since a total bilateral elimination of dopamine would result in high mortality. Bilateral lesions are most frequently performed by injecting 6-OHDA in the DS, which leads to a partial loss of dopamine neurons in the SNc and a reduction of striatal dopamine varying between 45 and 75% (Tadaiesky et al., 2008; Branchi et al., 2010; Bonito-Oliva et al., 2013). This latter approach is regarded as a model of early stage PD (Yuan et al., 2005).

Because of its similarity to endogenous catecholamines, 6-OHDA produces a combined degeneration of dopamine and noradrenaline neurons. This has been described in initial studies in rats (Breese and Traylor, 1971) and observed also in the mouse model of partial striatal lesion (Bonito-Oliva et al., 2014). In addition, both 6-OHDA and MPTP have been shown to decrease serotonin levels in the hippocampus (Reader and Gauthier, 1984; Santiago et al., 2010). The ability of these toxins to affect multiple neurotransmitter systems altered in PD, is especially important for the study of non-motor symptoms.

Sleep Disturbances in the 6-Hydroxydopamine Model of Parkinson's Disease

Rats with a unilateral 6-OHDA lesion of the MFB show decreased sleep time during the light-on, inactive phase of the 24-h light-dark cycle, and increased total wake time assessed during a 24-h recording (Vo et al., 2014). When compared to naïve animals, these animals also show increased muscle activity during REM sleep, which is indicative of RBD-like abnormalities (Vo et al., 2014). Sleep analysis has recently been performed in rats injected unilaterally with 6-OHDA in the SNc, which results in a substantial loss of dopamine neurons (Ciric et al., 2019). This intervention leads to increased number and mean duration of wake episodes assessed by cortical and hippocampal EEG during 6 h within the inactive, light-on period (Ciric et al., 2019). Increased wake time, particularly evident during the 12-h dark period, has also been observed in rats with a selective 6-OHDA lesion of the SNc (Qiu et al., 2016). Conversely, cortical EEG recording in rats injected with 6-OHDA in the lateral ventricle showed decreased wakefulness and augmented SWS (Monti et al., 1999). This difference is likely explained by the more generalized effect on multiple components of the catecholamine system exerted by intracerebroventricular (i.c.v.) administration of 6-OHDA, in comparison to selective stereotactic injections in MFB or SNc (Vo et al., 2014; Ciric et al., 2019).

Sakata et al. investigated the effect of bilateral 6-OHDA lesion of the ventral tegmental area (VTA) on sleep-wake cycles (Sakata et al., 2002). They found reduced REM sleep during the light-on period, in comparison with control rats. The 6-OHDA-lesion rats also displayed reduced wake duration and spontaneous activity accompanied by a significant increase of both REM and SWS (NREM) during the dark phase (Sakata et al., 2002). These findings are in line with some of the disturbances observed in PD patients, who are affected by insomnia at night and daytime sleepiness.

Further studies in rats showed that injection of 6-OHDA in the DS or MFB decreases the number of orexin neurons in the LH (Cui et al., 2010; Oliveira et al., 2018), thereby reproducing a similar damage described in PD (Maeda et al., 2006; Thannickal et al., 2007). This loss was paralleled by reduced baseline respiratory frequency during sleep in the light-on period (Oliveira et al., 2018), a condition reminiscent of breathing disturbances and sleep apnea observed in PD patients (Mery et al., 2017; Videnovic, 2017). The same 6-OHDA lesion did not affect the sleep-wake cycle (Oliveira et al., 2018). This contrasts with previous work in which LH orexinergic neurons were preferentially targeted using a neurotoxin acting through the ribosome-inactivating protein saporin (hypocretin2-saporin) (Gerashchenko et al., 2001). In this case, the authors found increased NREM and REM sleep during the dark, active phase, and sleep fragmentation leading to reduced number of REM sleep episodes during the light-on phase (Gerashchenko et al., 2001). Similar changes of REM and NREM sleep in the light-off period, were observed using a cycad toxin-based PD model (see below), which leads to a significant reduction in orexin neurons (McDowell et al., 2010). It should be noted however that in the 6-OHDA study EEG recordings were performed during the last 3 h of the light period and the first 3 h of the dark period (Oliveira et al., 2018), instead of the more appropriate 24-h continuous recordings, as

in the previous studies using saporin or cycad (Gerashchenko et al., 2001; McDowell et al., 2010).

Urethane anesthesia is characterized by alternations of oscillatory states which resemble REM-NREM cycling during natural sleep. Despite the obvious limitation of this approach, a number of studies have performed electrophysiological recordings in rodents under urethane to test mechanistic and functional hypothesis related to sleep-like states (Clement et al., 2008; Gonzalez-Rueda et al., 2018; Hauer et al., 2019). Resting-state functional magnetic resonance has been recently employed to examine modifications of functional connectivity in urethane-anesthetized rats with a unilateral partial striatal 6-OHDA-lesion (Zhurakovskaya et al., 2019). In this study, REM- and NREM-like sleep states were identified based on their association with high and low breathing rates, respectively (Pagliardini et al., 2012; Zhurakovskaya et al., 2019). Rats with a 6-OHDA lesion showed diminished intra-cortical, cortico-hippocampal, and striato-cortical functional connectivity, in comparison to naïve, or sham-lesion rats. Notably, these abnormalities were observed only during REM-like sleep (i.e., during states associated with high breathing rate), and may point toward changes in muscle tone suggestive of RBD (Zhurakovskaya et al., 2019). However, it remains to be established whether these alterations of connectivity can also be observed during sleep.

Sleep Disturbances in the 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine Model of Parkinson's Disease

Sleep disruptions have also been observed in the MPTP model of PD. However, these alterations are transitory, which could be ascribed to limited or reversible neuronal loss, as well as to parallel compensatory effects (Johannessen et al., 1985; Saitoh et al., 1987; Bezard et al., 2000). Mice treated with five daily injections of MPTP (25 mg/kg) showed increased REM sleep during the 24-h light/dark cycle, in comparison to control. However, this effect was observed only 20 days after toxin injection and disappeared 40 days post MPTP (Monaca et al., 2004; Laloux et al., 2008). Interestingly, the reduction of dopamine neurons produced by MPTP, which was restricted to the SNc and amounted to approximately 30%, remained constant for up to 60 days, suggesting the existence of mechanisms correcting for the neuronal loss produced by the toxin (Laloux et al., 2008). In contrast to the aforementioned work, EEG analysis performed in mice 14 days after a single injection of MPTP (40 mg/kg), reported increased wake, and decreased NREM sleep during the light-off period, but no change in REM sleep. In this study, however, MPTP produced a large reduction of dopamine neurons in both SNc (78%) and VTA (54%) (Revishchin et al., 2016).

A study in the rat, examined the effect on sleep patterns of local injection of MPTP in the SNc, leading to a temporary (24 h) 50% decrease of tyrosine hydroxylase (a marker of dopamine neurons) (Lima et al., 2007). The authors observed a decrease in the latency to SWS onset during both the light-on and light-off periods, which lasted for up to 5 days following MPTP administration. This change was accompanied by increased sleep efficiency and may represent a surrogate marker of daytime sleepiness in PD. The same study also reported a significant increase in the latency to REM sleep in the MPTP rats compared to control animals. However, this disturbance was transient and disappeared 3 days after the administration of MPTP (Lima et al., 2007).

Sleep Disturbances in Other Toxin-Based Models of Parkinson's Disease

Chronic administration of rotenone, a natural compound commonly used as a pesticide, leads to a significant degeneration of SNc dopamine neurons accompanied by α-synuclein inclusions (Betarbet et al., 2000; Alam and Schmidt, 2002). Working with a rotenone-based rat model of PD, Yi et al. reported increased SWS and REM sleep during the light-off active period and decreased SWS during the light-on period (Yi et al., 2007). These modifications, which are suggestive of PD-related daytime sleepiness and nighttime insomnia, were largely refractory to L-DOPA. Notably, the effect of rotenone was accompanied by increased hypothalamic levels of interleukin-1β, a cytokine previously shown to promote REM and NREM sleep (Krueger, 2008). In line with this observation, i.c.v. administration of the endogenous interleukin-1 receptor antagonist (IL-1RA) counteracted the changes in SWS produced by rotenone during both the active and inactive 12-h periods (Yi et al., 2007). A word of caution should be spent concerning the solution (1:1 DMSO/PEG) commonly used to dissolve rotenone. In one study, this vehicle precluded a conclusive interpretation of the results, since it produced sleep anomalies that occluded those possibly caused by the neurotoxin (Garcia-Garcia et al., 2005); but see (Yi et al., 2007). For this reason, particular care should be taken to include appropriate controls when employing the rotenone model.

In the rat, consumption of seeds from the plant *Cycas micronesica* (cycad) results in degeneration and accumulation of α-synuclein aggregates in dopaminergic and noradrenergic neurons of the SNc and LC (Shen et al., 2010). Sleep analysis performed in this model reported increased NREM and REM sleep during the active 12-h phase. These changes were associated with increased average duration of NREM sleep and increased number of REM episodes (McDowell et al., 2010). Notably, the same study also reported a reduction in the number of orexin neurons in the LH of cycad-fed rats (McDowell et al., 2010), a change which is in line with previous work in orexin depleted rats (Gerashchenko et al., 2001) and may contribute to the observed sleep abnormalities.

Gerashchenko et al. utilized the hypocretin2-saporin neurotoxin (see *Sleep Disturbances in the 6-Hydroxydopamine Model of Parkinson's Disease*) (Gerashchenko et al., 2001) to lesion substantia nigra and VTA, thereby eliminating a large proportion of neurons (dopaminergic and non-dopaminergic) in both regions (Gerashchenko et al., 2006). In the substantia nigra, but not in the VTA, this intervention led to increased wakefulness and reduced amounts of NREM and REM sleep during light-on and light-off periods. These results are indicative of insomnia and are in part consistent with findings in rats with a selective dopamine lesion of SNc (see *Sleep*

Disturbances in the 6-Hydroxydopamine Model of Parkinson's Disease and *Sleep Disturbances in the 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine Model of Parkinson's Disease*) (Lima et al., 2007; Ciric et al., 2019).

Genetic Models of Parkinson's Disease

Genetic models are commonly obtained by overexpressing genes linked to PD, or mutations implicated in autosomal-dominant or recessive forms of the disease. Additional approaches include interventions disrupting mitochondrial function or specific transcription factors (Dawson et al., 2010; Chesselet and Richter, 2011). These strategies allow the study of effects produced by molecular modifications implicated in familial forms of PD, and to reproduce the accumulation of α-synuclein, which is a hallmark of PD absent in 6-OHDA- or acute MPTP-based models (Dawson et al., 2010; Tieu, 2011). The neuronal loss observed in genetic models of PD is generally less pronounced in comparison to that achieved with neurotoxins, particularly 6-OHDA (Dawson et al., 2010; Chesselet and Richter, 2011). However, this does not necessarily represent a limitation to their use, since PD related sleep disorders are often manifested during the early phase of the disease.

In the mouse, overexpression of human α-synuclein driven by the Thy-1 promoter (Thy1-αSyn) leads to a progressive disruption of the nigrostriatal pathway, resulting in a 40% decrease of striatal dopamine (Chesselet et al., 2012). McDowell et al. examined sleep structure in Thy1-αSyn mice and found reduced REM sleep during the 24-h sleep-wake cycle, accompanied by increased NREM sleep during the light-on, inactive period (McDowell et al., 2014). The same authors also observed increased activity during the light-off, wake phase (McDowell et al., 2014). However, analysis of circadian rest-activity performed in Thy1-αSyn mice reported reduced wheel-running particularly pronounced during dark (Kudo et al., 2011). This discrepancy may be accounted for by the different parameters employed to assess the active state of the animals, i.e., whole-body movements detected by telemetry (McDowell et al., 2014) *vs.* wheel-running (Kudo et al., 2011). Notably, both EEG (McDowell et al., 2014) and visual (Kudo et al., 2011) analysis of Thy1-αSyn mice revealed a delay in sleep onset, although during different phases of the 24-h light-dark period.

Circadian rhythm has been also examined in the MitoPark mouse model of PD, in which dopamine neurons are progressively eliminated by selective impairment of mitochondrial function (Ekstrand and Galter, 2009). MitoPark mice showed a general reduction of locomotor activity in concomitance with the gradual loss of dopamine neurons, accompanied by a severe disruption of circadian rhythm in constant darkness (Fifel and Cooper, 2014). A similar loss of endogenous rhythmicity has been observed in neurotoxin models of PD based on striatal (Masini et al., 2017) or ventricular (Gravotta et al., 2011) injection of 6-OHDA. Further studies will be necessary to understand the mechanisms at the basis of these abnormalities as well as their impact on sleep function and architecture.

Mice with reduced (95%) levels of the vesicular monoamine transporter 2 (VMAT2) undergo nigrostriatal dysfunctions affecting the dopaminergic system, which replicate key pathologic features of PD (Caudle et al., 2007). This model displays a number of non-motor symptoms associated with PD, including anomalies of circadian and sleep function (Taylor et al., 2009). VMAT2-depleted mice show reduced ambulation during the active phase of the circadian period (Taylor et al., 2009). They also show a reduced latency to behavioral signs of sleep (Taylor et al., 2009), which contrasts with the delayed sleep onset observed in Thy1-αSyn mice (Kudo et al., 2011; McDowell et al., 2014). Notably, VMAT2-deficient mice show a concomitant reduction of dopamine, noradrenaline, and serotonin in striatum, hippocampus, and cortex (Taylor et al., 2009), which collectively may contribute to the wide range of non-motor symptoms observed in this model.

Altogether, the different findings reported in genetic models of PD fail to provide a coherent representation of one or more specific sleep alterations associated with PD. Aside from the contrasting effects on activity state observed in Thy1-αSyn mice, which may be attributable to different methodological approaches (see above), these inconsistencies are possibly a consequence of distinct genetic strategies (overexpression of α-synuclein as opposed to downregulation of VAMT2), which may produce unique alterations besides those affecting the dopaminergic system.

CONCLUSIONS AND PERSPECTIVES

Rodent models represent a simple and versatile tool to study not only the classic motor symptoms and complications (i.e., dyskinesia) of PD, but also a number of non-motor co-morbidities. Nonetheless, the development of rat and mouse models of PD-related sleep disturbances has lagged behind in comparison, for instance, to affective and cognitive symptoms. The reason for this lies in part in the complexity of sleep-related disorders, both with regard to their behavioral manifestation and their relationship to distinct cellular and anatomical substrates. The difference in sleep architecture between rodents and humans represents an additional obstacle when interpreting results. In this regard, modeling PD in non-human primates constitutes a clear advantage, since in these animals, sleep structure and EEG patterns closely reproduce the consolidated, monophasic organization observed in humans (Hsieh et al., 2008), which contrasts with the more dispersed distribution of sleep states in rodents (Fifel et al., 2016).

A critical issue related to rat and mouse models, and more in general to all current animal models, is the difficulty to recapitulate the diversified nature of the degenerative processes at the basis of PD, thereby offering the possibility of examining the concomitant involvement of multiple neurotransmitter systems in sleep alterations. Thus, it is not surprising that rodent models of PD do not reproduce the full spectrum of sleep abnormalities associated with PD. However, in spite of these limitations some of these ailments have been reproduced in rats (**Table 1**). For instance, decreased SWS during the quiescent period, suggestive of insomnia, has been described after 6-OHDA-lesion of the MFB (Vo et al., 2014) and in the rotenone

TABLE 1 | Sleep disturbances in neurotoxin and genetic rodent models of Parkinson's disease.

Model	Procedure	Pathology	Wake	NREM/SWS sleep	REM sleep	Species	Reference
6-OHDA	Injection (200 µg in 20 µl) in the lateral ventricle	Decreased DA levels in DS (≈40%) ventral striatum (≈50%)	Decreased in light-on	Increased in light-on	Unaltered	Rat	Monti et al., 1999
6-OHDA	Bilateral injection in VTA (with desipramine)	Decreased DA neurons in VTA	Decreased in light-off	Increased in light-off	Increased in light-off and decreased in light-on	Rat	Sakata et al., 2002
6-OHDA	Unilateral injection in MFB (with desipramine)	Decreased DA neurons in SNc (unilateral, ≥95%)	Increased total time (24 h)	Decrease in light-on	Increased muscle activity	Rat	Vo et al., 2014
6-OHDA	Bilateral injections (12 µg/0.5 µl) in DS	Decreased (≈70%) DA neurons in SNc, reduced (27 to 39%) orexin neurons in LH	Unaltered	Unaltered duration (↓breathing frequency during sleep)	Unaltered duration (↓breathing frequency during sleep)	Rat	Oliveira et al., 2018
6-OHDA	Partial unilateral lesion of DS—sleep states identified by breathing rat fluctuations	Decreased levels of DA in the DS (unilateral, 51%)	NI	Duration unaltered	Duration unaltered	Rat	Zhurakovskaya et al., 2019
6-OHDA	Unilateral injection in SNc (12 or 24 µg)	Decreased DA neurons (56 to >92%) along the SNc rostro-caudal axis (unilateral)	Increased in light-on (↑bout number)	Unaltered	Unaltered	Rat	Ciric et al., 2019
MPTP	Injection (200 µg/2 µl) in the SNc	Transient (24 h) decrease (50%) of DA neurons in the SNc (unilateral)	NI	Decrease in latency of light-on and off (up to 5 days after lesion)	Transient (2 days) increase of latency in light-on and off	Rat	Lima et al., 2007
MPTP	Five daily systemic (i.p.) injections (25 mg/kg)—test 20 days after toxin treatment	Decreased DA neurons in SNc (30%)	Unchanged (↓bout number but ↑bout length)	Unchanged	Increased in light-on and off (↑bout length)	Mouse	Monaca et al., 2004
MPTP	Five daily systemic (i.p.) injections (25 mg/kg)—test 20 and 40 days after toxin treatment	Decreased DA neurons in SNc (30%) and terminals in DS (50%) up to 60 days after toxin treatment	Unaltered	Unaltered	Increased in dark (↑bout number) at day 20, but not at day 40, after toxin treatment	Mouse	Laloux et al., 2008
MPTP	One systemic (s.c.) injection (40 mg/kg)	Decreased DA neurons in SNc (78%) and VTA (54%) after 17 days	Increased in light-off at day 14 after toxin treatment	Decreased in light-off at day 14 after toxin treatment	No change	Mouse	Revishchin et al., 2016
Rotenone	Infusion (s.c.) 3 mg/kg/day for 28 days	Decrease (≈70%) of DA neurons in SNc	Decreased in dark and increased in light	Increased in dark (↑bout duration) and decreased in light (↓bout number)	Increased in dark (↑bout number)	Rat	Yi et al., 2007
Cycad	Feeding with pellet (1.25 g) for 22 weeks	α-syn aggregates in DA neurons, decreased DA neurons in the SNc and DA fibers in DS, reduced orexin neurons in the LH	Decreased in light-off (35% ↑bout number and 50% ↓bout duration)	Increased in light-off (↑bout duration)	Increased in light-off (↑bout number)	Rat	McDowell et al., 2010
Hypocretin2-saporin	Injection in SN and VTA	Non-selective neuronal depletion in both regions	Increased during light-on and off (only in SN-lesion)	Decreased during light-on and off (only in SN-lesion)	Decreased during light-on and off (only in SN-lesion)	Rat	Gerashchenko et al. (2006)

(Continued)

TABLE 1 | Continued

Model	Procedure	Pathology	Wake	NREM/SWS sleep	REM sleep	Species	Reference
Thy-1 α-Syn	Transgenic	α-syn aggregates in SNc and 40% reduction of striatal DA at 14 months	Increased active wake in light-off	Increased in light (↑bout length)—↑bout length in dark	Decreased in light-off (↓bout number) and light-on	Mouse	McDowell et al., 2014
VMAT2-deficiency	Transgenic	Decreased DA, NE, and 5-HT in DS, hippocampus, and cortex at 12–15 months	Decreased at 4–6 months	Decreased sleep latency		Mouse	Taylor et al., 2009

NI, not investigated; i.p., intraperitoneal; s.c., subcutaneous; SN, substantia nigra; DA, dopamine; NE, noradrenaline; 5-HT, serotonin; α-syn, α-synuclein; ↑, increased; ↓, decreased. See text for other abbreviations.

model (Yi et al., 2007). Moreover, 6-OHDA-lesion of the VTA (Sakata et al., 2002), as well as administration of rotenone (Yi et al., 2007), or cycad toxin (Shen et al., 2010) increase NREM and REM sleep during the active phase of the circadian cycle, which is indicative of EDS. These results are in line with the analysis of rest-wake circadian rhythm in mice with a bilateral striatal 6-OHDA-lesion, which show decreased activity during the active 12-h period (Masini et al., 2017).

Modifications in sleep architecture have also been observed in mice and rats intoxicated with MPTP (Monaca et al., 2004; Lima et al., 2007; Laloux et al., 2008; Revishchin et al., 2016) (**Table 1**). However, MPTP-based approaches have failed to provide consistent results and are often observed only during a limited period of time following administration of the toxin (Lima et al., 2007; Laloux et al., 2008). This limited efficacy is in line with the lack of alterations of circadian locomotor activity rhythm reported in mice following acute or chronic treatment with MPTP (Fifel et al., 2013).

How can rodent modeling of sleep disorders in PD be improved? Overall, a general effort should be made to refine and harmonize the different approaches used to generate neurotoxin-based models. In the case of 6-OHDA, for instance, rats have been treated with i.c.v. injection, unilateral and bilateral injections in MFB, distinct midbrain dopaminergic nuclei (i.e., SNc and VTA), or in the striatum (see **Table 1**). Moreover, some studies (Sakata et al., 2002; Vo et al., 2014) have used pretreatment with desipramine, a selective inhibitor of noradrenaline reuptake. The different degree of dopamine and noradrenaline depletion produced by these interventions may cause distinct effects in sleep behavior, thereby contributing to discrepancies and confounding interpretations.

Combined loss of dopamine, noradrenaline, and to some extent functional inactivation of serotonin has been achieved with neurotoxins (Breese and Traylor, 1971; Reader and Gauthier, 1984; Santiago et al., 2010; Bonito-Oliva et al., 2013). In addition, 6-OHDA has been found to reduce the number of orexin/hypocretin neurons in the LH (Cui et al., 2010; Oliveira et al., 2018). Therefore, some of the sleep abnormalities observed in rodent models of PD may depend on the loss, or impairment, of one or more of these components of the arousal system. However, several key structures implicated in sleep regulation, including cholinergic and histaminergic nuclei, are spared by neurotoxin or genetic interventions. Therefore it will be important to strengthen construct validity of rodent models by establishing a more stringent link between the observed sleep abnormalities and PD-related biological dysfunctions.

The limitation posed by the relatively restricted neurodegenerative effects observed in most experimental models has been in part circumvented by acting on specific targets. For instance, bilateral injection of ibotenic acid in the PPN has been proposed to represent a model of PD cholinopathy (Ciric et al., 2018). In the rat, this intervention results in altered microstructure of both NREM and REM sleep during the inactive phase (Ciric et al., 2018). However, the neurotoxic effect exerted by ibotenic acid in the PPN is likely to affect not only cholinergic neurons, but also glutamatergic and GABAergic cells.

Chemo- and optogenetics techniques offer a particularly attractive approach to parse the involvement of neuronal networks in PD-related sleep-wake disturbances. For instance, using selective expression of designer receptor exclusively activated by designer drugs (DREADD) (Alexander et al., 2009), Kroeger et al. showed that distinct neuronal populations in the PPN regulate different aspects of sleep-wake behavior (Kroeger et al., 2017). Similar approaches, including optogenetic manipulation, showed that the dopamine neurons of the VTA are required for the initiation and maintenance of wakefulness (Eban-Rothschild et al., 2016; Oishi et al., 2017). Notably, these findings are in line with results obtained in rats with a selective 6-OHDA lesion of the VTA, which display decreased wakefulness during the active, light-off phase (Sakata et al., 2002).

Opto- and chemogenetic interrogation has also been employed to investigate the role in sleep of a population of dopamine neurons located in the dorsal raphe. These cells degenerate in PD (Halliday et al., 1990), but their involvement in non-motor symptoms remains to be in large part assessed. Cho et al. showed that ontogenetic stimulation of these dopamine population induces and maintains wakefulness and that vice versa their chemogenetic inhibition counteracts wakefulness and increases NREM sleep (Cho et al., 2017). In another study, Qiu et al. clarified the contribution to sleep of the external globus pallidus, whose activity is reduced in PD. It was found that, in the rat, activation of this structure resulted in enhanced sleep, possibly mediated *via* inhibition

of cortical regions involved in the regulation of arousal nuclei (Qiu et al., 2016).

Recent evidence provides an attractive approach to reproduce the path of PD progression described by Braak et al. (2003). Injection of α-synuclein pathological fibrils in the gut muscle layer, leads to the spread of Lewy bodies from the peripheral nervous system, through the vagus nerve, to the brain, followed by loss of dopamine neurons (Kim et al., 2019). These effects are accompanied by motor impairment and by non-motor symptoms, including cognitive and affective deficits (Kim et al., 2019). The widespread distribution of α-synuclein fibrils, which are observed in several structures including the LC, suggests the potential use in the future of this or similar models for the study of sleep-related co-morbidities in PD.

One particularly interesting aspect of sleep disorders in PD is their potential impact on other frequent non-motor symptoms, such as cognitive and affective disorders. Indeed, cycling and coordinated transition of NREM and REM sleep are implicated in learning and memory processes (Moroni et al., 2014; Boyce et al., 2016). The synchronous activation of hippocampal pyramidal neurons during sleep results in the propagation of information throughout the neocortex, thereby leading to memory consolidation (Buzsaki, 1989). Depression has also been correlated to reduced SWS and increased duration and intensity of REM sleep (Riemann et al., 2001). The existence of a causative link between PD-related sleep disruptions and psychiatric co-morbidities remain to be further investigated. In this regard, it should be mentioned that memory deficit, depression, and anxiety are commonly observed in rodent models of PD, which may therefore represent valuable tools to help clarifying some of these questions.

AUTHOR CONTRIBUTIONS

GF conceived the presented idea and supervised the project. DM and GF wrote the manuscript. CA and MM provided critical feedback and helped shape the manuscript. All authors approved the final version for submission.

FUNDING

This work was supported by the Swedish Research Council (grant 2015-02886), the Swedish Brain Foundation and the Swedish Parkinson Foundation (GF). This work is part of a joint Brazilian-Swedish Research Collaboration supported by the Swedish Foundation for International Cooperation in Research and Higher Education (STINT) and by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brazil.

REFERENCES

Alam, M., and Schmidt, W. J. (2002). Rotenone destroys dopaminergic neurons and induces parkinsonian symptoms in rats. *Behav. Brain Res.* 136, 317–324. doi: 10.1016/S0166-4328(02)00180-8

Alexander, G. M., Rogan, S. C., Abbas, A. I., Armbruster, B. N., Pei, Y., Allen, J. A., et al. (2009). Remote control of neuronal activity in transgenic mice expressing evolved G protein-coupled receptors. *Neuron* 63, 27–39. doi: 10.1016/j.neuron.2009.06.014

Antonini, A., Landi, A., Mariani, C., DeNotaris, R., and Pezzoli, G. (2004). Deep brain stimulation and its effect on sleep in Parkinson's disease. *Sleep Med.* 5, 211–214. doi: 10.1016/j.sleep.2003.10.011

Arnulf, I., and Leu-Semenescu, S. (2009). Sleepiness in Parkinson's disease. *Parkinsonism Relat. Disord.* 15 Suppl 3, S101–S104. doi: 10.1016/S1353-8020(09)70792-8

Arnulf, I., Bejjani, B. P., Garma, L., Bonnet, A. M., Houeto, J. L., Damier, P., et al. (2000). Improvement of sleep architecture in PD with subthalamic nucleus stimulation. *Neurology* 55, 1732–1734. doi: 10.1212/wnl.55.11.1732

Arnulf, I. (2005). Excessive daytime sleepiness in parkinsonism. *Sleep Med. Rev.* 9, 185–200. doi: 10.1016/j.smrv.2005.01.001

Avorn, J., Schneeweiss, S., Sudarsky, L. R., Benner, J., Kiyota, Y., Levin, R., et al. (2005). Sudden uncontrollable somnolence and medication use in Parkinson disease. *Arch. Neurol.* 62, 1242–1248. doi: 10.1001/archneur.62.8.1242

Barone, P., Antonini, A., Colosimo, C., Marconi, R., Morgante, L., Avarello, T. P., et al. (2009). The PRIAMO study: a multicenter assessment of nonmotor symptoms and their impact on quality of life in Parkinson's disease. *Mov. Disord.* 24, 1641–1649. doi: 10.1002/mds.22643

Betarbet, R., Sherer, T. B., MacKenzie, G., Garcia-Osuna, M., Panov, A. V., and Greenamyre, J. T. (2000). Chronic systemic pesticide exposure reproduces features of Parkinson's disease. *Nat. Neurosci.* 3, 1301–1306. doi: 10.1038/81834

Bezard, E., Dovero, S., Imbert, C., Boraud, T., and Gross, C. E. (2000). Spontaneous long-term compensatory dopaminergic sprouting in MPTP-treated mice. *Synapse* 38, 363–368. doi: 10.1002/1098-2396(20001201)38:3<363::AID-SYN16>3.0.CO;2-A

Boeve, B. F., Silber, M. H., Saper, C. B., Ferman, T. J., Dickson, D. W., Parisi, J. E., et al. (2007). Pathophysiology of REM sleep behaviour disorder and relevance to neurodegenerative disease. *Brain* 130, 2770–2788. doi: 10.1093/brain/awm056

Bonito-Oliva, A., Pignatelli, M., Spigolon, G., Yoshitake, T., Seiler, S., Longo, F., et al. (2013). Cognitive impairment and dentate gyrus synaptic dysfunction in experimental parkinsonism. *Biol. Psychiatry* 75, 701–710. doi: 10.1016/j.biopsych.2013.02.015

Bonito-Oliva, A., Masini, D., and Fisone, G. (2014). A mouse model of non-motor symptoms in Parkinson's disease: focus on pharmacological interventions targeting affective dysfunctions. *Front. Behav. Neurosci.* 8, 290. doi: 10.3389/fnbeh.2014.00290

Borbély, A. A., Daan, S., Wirz-Justice, A., and Deboer, T. (2016). The two-process model of sleep regulation: a reappraisal. *J. Sleep Res.* 25, 131–143. doi: 10.1111/jsr.12371

Boucetta, S., Cisse, Y., Mainville, L., Morales, M., and Jones, B. E. (2014). Discharge profiles across the sleep-waking cycle of identified cholinergic, GABAergic, and glutamatergic neurons in the pontomesencephalic tegmentum of the rat. *J. Neurosci.* 34, 4708–4727. doi: 10.1523/JNEUROSCI.2617-13.2014

Boucetta, S., Salimi, A., Dadar, M., Jones, B. E., Collins, D. L., and Dang-Vu, T. T. (2016). Structural brain alterations associated with rapid eye movement sleep behavior disorder in Parkinson's disease. *Sci. Rep.* 6, 26782. doi: 10.1038/srep26782

Boyce, R., Glasgow, S. D., Williams, S., and Adamantidis, A. (2016). Causal evidence for the role of REM sleep theta rhythm in contextual memory consolidation. *Science* 352, 812–816. doi: 10.1126/science.aad5252

Braak, H., Del Tredici, K., Rub, U., de Vos, R. A., Jansen Steur, E. N., and Braak, E. (2003). Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiol. Aging* 24, 197–211. doi: S0197458002000659

Braak, H., Ghebremedhin, E., Rub, U., Bratzke, H., and Del Tredici, K. (2004). Stages in the development of Parkinson's disease-related pathology. *Cell Tissue Res.* 318, 121–134. doi: 10.1007/s00441-004-0956-9

Branchi, I., D'Andrea, I., Armida, M., Carnevale, D., Ajmone-Cat, M. A., Pezzola, A., et al. (2010). Striatal 6-OHDA lesion in mice: investigating early neurochemical changes underlying Parkinson's disease. *Behav. Brain Res.* 208, 137–143. doi: 10.1016/j.bbr.2009.11.020

Breese, G. R., and Traylor, T. D. (1971). Depletion of brain noradrenaline and dopamine by 6-hydroxydopamine. *Br. J. Pharmacol.* 42, 88–99. doi: 10.1111/j.1476-5381.1971.tb07089.x

Buzsaki, G. (1989). Two-stage model of memory trace formation: a role for "noisy" brain states. *Neuroscience* 31, 551–570. doi: 10.1016/0306-4522(89)90423-5

Carskadon, M. A., and Dement, W. C. (2017). Normal human sleep: an overview. *Principles and practice of sleep medicine.* 6th ed. 15–24.e13. doi: 10.1016/b978-0-323-24288-2.00002-7

Carter, M. E., Yizhar, O., Chikahisa, S., Nguyen, H., Adamantidis, A., Nishino, S., et al. (2010). Tuning arousal with optogenetic modulation of locus coeruleus neurons. *Nat. Neurosci.* 13, 1526–1533. doi: 10.1038/nn.2682

Caudle, W. M., Richardson, J. R., Wang, M. Z., Taylor, T. N., Guillot, T. S., McCormack, A. L., et al. (2007). Reduced vesicular storage of dopamine causes progressive nigrostriatal neurodegeneration. *J. Neurosci.* 27, 8138–8148. doi: 10.1523/JNEUROSCI.0319-07.2007

Chahine, L. M., Daley, J., Horn, S., Duda, J. E., Colcher, A., Hurtig, H., et al. (2013). Association between dopaminergic medications and nocturnal sleep in early-stage Parkinson's disease. *Parkinsonism Relat. Disord.* 19, 859–863. doi: 10.1016/j.parkreldis.2013.05.009

Chaudhuri, K. R., and Odin, P. (2010). The challenge of non-motor symptoms in Parkinson's disease. *Prog. Brain Res.* 184, 325–341. doi: 10.1016/S0079-6123(10)84017-8

Chesselet, M. F., and Richter, F. (2011). Modelling of Parkinson's disease in mice. *Lancet Neurol.* 10, 1108–1118. doi: 10.1016/S1474-4422(11)70227-7

Chesselet, M. F., Richter, F., Zhu, C., Magen, I., Watson, M. B., and Subramaniam, S. R. (2012). A progressive mouse model of Parkinson's disease: the Thy1-aSyn ("Line 61") mice. *Neurotherapeutics* 9, 297–314. doi: 10.1007/s13311-012-0104-2

Cho, J. R., Treweek, J. B., Robinson, J. E., Xiao, C., Bremner, L. R., Greenbaum, A., et al. (2017). Dorsal raphe dopamine neurons modulate arousal and promote wakefulness by salient stimuli. *Neuron* 941205-1219, e1208. doi: 10.1016/j.neuron.2017.05.020

Chokroverty, S. (2009). An Overview of Normal Sleep. *Sleep Disorders Medicine: Basic Science, Technical Considerations, and Clinical Aspects.* 3rd edition 5–21. doi: 10.1016/b978-0-7506-7584-0.00002-1

Chou, T. C., Bjorkum, A. A., Gaus, S. E., Lu, J., Scammell, T. E., and Saper, C. B. (2002). Afferents to the ventrolateral preoptic nucleus. *J. Neurosci.* 22, 977–990. doi: 10.1523/JNEUROSCI.22-03-00977.2002

Cicolin, A., Lopiano, L., Zibetti, M., Torre, E., Tavella, A., Guastamacchia, G., et al. (2004). Effects of deep brain stimulation of the subthalamic nucleus on sleep architecture in parkinsonian patients. *Sleep Med.* 5, 207–210. doi: 10.1016/j.sleep.2003.10.010

Ciric, J., Lazic, K., Kapor, S., Perovic, M., Petrovic, J., Pesic, V., et al. (2018). Sleep disorder and altered locomotor activity as biomarkers of the Parkinson's disease cholinopathy in rat. *Behav. Brain Res.* 339, 79–92. doi: 10.1016/j.bbr.2017.11.021

Ciric, J., Kapor, S., Perovic, M., and Saponjic, J. (2019). Alterations of sleep and sleep oscillations in the Hemiparkinsonian Rat. *Front. Neurosci.* 13, 148. doi: 10.3389/fnins.2019.00148

Clement, E. A., Richard, A., Thwaites, M., Ailon, J., Peters, S., and Dickson, C. T. (2008). Cyclic and sleep-like spontaneous alternations of brain state under urethane anaesthesia. *PloS One* 3 (4), e2004. doi: 10.1371/journal.pone.0002004

Cui, L. B., Li, B. W., Jin, X. H., Zhao, L., and Shi, J. (2010). Progressive changes of orexin system in a rat model of 6-hydroxydopamine-induced Parkinson's disease. *Neurosci. Bull.* 26, 381–387. doi: 10.1007/s12264-010-0410-9

Dawson, T. M., Ko, H. S., and Dawson, V. L. (2010). Genetic animal models of Parkinson's disease. *Neuron* 66, 646–661. doi: 10.1016/j.neuron.2010.04.034

De Cock, V. C., Vidailhet, M., and Arnulf, I. (2008). Sleep disturbances in patients with parkinsonism. *Nat. Clin. Pract. Neurol.* 4, 254–266. doi: 10.1038/ncpneuro0775

de Lecea, L., Kilduff, T. S., Peyron, C., Gao, X. B., Foye, P. E., Danielson, P. E., et al. (1998). The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. *Proc. Natl. Acad. Sci.* 95, 322–327. doi: 10.1073/pnas.95.1.322

Eban-Rothschild, A., Rothschild, G., Giardino, W. J., Jones, J. R., and de Lecea, L. (2016). VTA dopaminergic neurons regulate ethologically relevant sleep-wake behaviors. *Nat. Neurosci.* 19, 1356–1366. doi: 10.1038/nn.4377

Ekstrand, M. I., and Galter, D. (2009). The mitopark mouse - an animal model of Parkinson's disease with impaired respiratory chain function in dopamine neurons. *Parkinsonism Relat. Disord.* 15 Suppl 3, S185–S188. doi: 10.1016/S1353-8020(09)70811-9

Ferini-Strambi, L., Carli, G., Casoni, F., and Galbiati, A. (2018). Restless legs syndrome and Parkinson disease: a causal relationship between the two disorders? *Front. Neurol.* 9, 551. doi: 10.3389/fneur.2018.00551

Fifel, K., and Cooper, H. M. (2014). Loss of dopamine disrupts circadian rhythms in a mouse model of Parkinson's disease. *Neurobiol. Dis.* 71, 359–369. doi: 10.1016/j.nbd.2014.08.024

Fifel, K., Dkhissi-Benyahya, O., and Cooper, H. M. (2013). Lack of long-term changes in circadian, locomotor, and cognitive functions in acute and chronic MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) mouse models of Parkinson's disease. *Chronobiol. Int.* 30, 741–755. doi: 10.3109/07420528.2012.762011

Fifel, K., Piggins, H., and Deboer, T. (2016). Modeling sleep alterations in Parkinson's disease: how close are we to valid translational animal models? *Sleep Med. Rev.* 25, 95–111. doi: 10.1016/j.smrv.2015.02.005

French, I. T., and Muthusamy, K. A. (2016). A review of sleep and its disorders in patients with Parkinson's disease in relation to various brain structures. *Front. Aging Neurosci.* 8, 114. doi: 10.3389/fnagi.2016.00114

French, I. T., and Muthusamy, K. A. (2018). A review of the Pedunculopontine nucleus in Parkinson's disease. *Front. Aging Neurosci.* 10, 99. doi: 10.3389/fnagi.2018.00099

Fronczek, R., Overeem, S., Lee, S. Y., Hegeman, I. M., van Pelt, J., van Duinen, S. G., et al. (2007). Hypocretin (orexin) loss in Parkinson's disease. *Brain* 130, 1577–1585. doi: 10.1093/brain/awm090

Fronczek, R., Overeem, S., Lee, S. Y., Hegeman, I. M., van Pelt, J., van Duinen, S. G., et al. (2008). Hypocretin (orexin) loss and sleep disturbances in Parkinson's Disease. *Brain* 131, e88. doi: 10.1093/brain/awm222

Fuller, P. M., Gooley, J. J., and Saper, C. B. (2016). Neurobiology of the sleep-wake cycle: sleep architecture, circadian regulation, and regulatory feedback. *J. Biol. Rhythms* 21, 482–493. doi: 10.1177/0748730406294627

Galati, S., Salvade, A., Pace, M., Sarasso, S., Baracchi, F., Bassetti, C. L., et al. (2015). Evidence of an association between sleep and levodopa-induced dyskinesia in an animal model of Parkinson's disease. *Neurobiol. Aging* 36, 1577–1589. doi: 10.1016/j.neurobiolaging.2014.12.018

Garcia-Garcia, F., Ponce, S., Brown, R., Cussen, V., and Krueger, J. M. (2005). Sleep disturbances in the rotenone animal model of Parkinson disease. *Brain Res.* 1042, 160–168. doi: 10.1016/j.brainres.2005.02.036

Garcia-Lorenzo, D., Longo-Dos Santos, C., Ewenczyk, C., Leu-Semenescu, S., Gallea, C., Quattrocchi, G., et al. (2013). The coeruleus/subcoeruleus complex in rapid eye movement sleep behaviour disorders in Parkinson's disease. *Brain* 136, 2120–2129. doi: 10.1093/brain/awt152

Gerashchenko, D., Kohls, M. D., Greco, M., Waleh, N. S., Salin-Pascual, R., Kilduff, T. S., et al. (2001). Hypocretin-2-saporin lesions of the lateral hypothalamus produce narcoleptic-like sleep behavior in the rat. *J. Neurosci.* 21, 7273–7283. doi: 10.1523/JNEUROSCI.21-18-07273.2001

Gerashchenko, D., Blanco-Centurion, C. A., Miller, J. D., and Shiromani, P. J. (2006). Insomnia following hypocretin2-saporin lesions of the substantia nigra. *Neuroscience* 137, 29–36. doi: 10.1016/j.neuroscience.2005.08.088

Gjerstad, M. D., Wentzel-Larsen, T., Aarsland, D., and Larsen, J. P. (2007). Insomnia in Parkinson's disease: frequency and progression over time. *J. Neurol. Neurosurg. Psychiatry* 78, 476–479. doi: 10.1136/jnnp.2006.100370

Gonzalez-Rueda, A., Pedrosa, V., Feord, R. C., Clopath, C., and Paulsen, O. (2018). Activity-dependent downscaling of subthreshold synaptic inputs during slow-wave-sleep-like activity in vivo. *Neuron* 971244-1252, e1245. doi: 10.1016/j.neuron.2018.01.047

Gravotta, L., Gavrila, A. M., Hood, S., and Amir, S. (2011). Global depletion of dopamine using intracerebroventricular 6-hydroxydopamine injection disrupts normal circadian wheel-running patterns and PERIOD2 expression in the rat forebrain. *J. Mol. Neurosci.* 45, 162–171. doi: 10.1007/s12031-011-9520-8

Halliday, G. M., Li, Y. W., Blumbergs, P. C., Joh, T. H., Cotton, R. G., Howe, P. R., et al. (1990). Neuropathology of immunohistochemically identified brainstem neurons in Parkinson's disease. *Ann. Neurol.* 27, 373–385. doi: 10.1002/ana.410270405

Hara, J., Beuckmann, C. T., Nambu, T., Willie, J. T., Chemelli, R. M., Sinton, C. M., et al. (2001). Genetic ablation of orexin neurons in mice results in narcolepsy, hypophagia, and obesity. *Neuron* 30, 345–354. doi: 10.1016/s0896-6273(01)00293-8

Hauer, B. E., Pagliardini, S., and Dickson, C. T. (2019). The reuniens nucleus of the thalamus has an essential role in coordinating slow-wave activity between neocortex and hippocampus. *eNeuro* 6. doi: 10.1523/ENEURO.0365-19.2019

Hjort, N., Ostergaard, K., and Dupont, E. (2004). Improvement of sleep quality in patients with advanced Parkinson's disease treated with deep brain stimulation of the subthalamic nucleus. *Mov. Disord.* 19, 196–199. doi: 10.1002/mds.10639

Hsieh, K. C., Robinson, E. L., and Fuller, C. A. (2008). Sleep architecture in unrestrained rhesus monkeys (Macaca mulatta) synchronized to 24-hour light-dark cycles. *Sleep* 31, 1239–1250. doi: 10.5665/sleep/31.9.1239

Ito, H., Yanase, M., Yamashita, A., Kitabatake, C., Hamada, A., Suhara, Y., et al. (2013). Analysis of sleep disorders under pain using an optogenetic tool: possible involvement of the activation of dorsal raphe nucleus-serotonergic neurons. *Mol. Brain* 6, 59. doi: 10.1186/1756-6606-6-59

Jackson-Lewis, V., and Przedborski, S. (2007). Protocol for the MPTP mouse model of Parkinson's disease. *Nat. Protoc.* 2, 141–151. doi: 10.1038/nprot.2006.342

Johannessen, J. N., Chiueh, C. C., Burns, R. S., and Markey, S. P. (1985). Differences in the metabolism of MPTP in the rodent and primate parallel differences in sensitivity to its neurotoxic effects. *Life Sci.* 36, 219–224. doi: 10.1016/0024-3205(85)90062-1

Jones, B. E. (2003). Arousal systems. *Front. Biosci.* 8, s438–s451. doi: 10.2741/1074

Kharkar, S., Ellenbogen, J. R., Samuel, M., Rizos, A., Silverdale, M., Chaudhuri, K. R., et al. (2018). Changes in Parkinson's disease sleep symptoms and daytime somnolence after bilateral subthalamic deep brain stimulation in Parkinson's disease. *NPJ Parkinsons Dis.* 4, 16. doi: 10.1038/s41531-018-0053-5

Kim, S., Kwon, S. H., Kam, T. I., Panicker, N., Karuppagounder, S. S., Lee, S., et al. (2019). Transneuronal propagation of pathologic alpha-synuclein from the gut to the brain models Parkinson's disease. *Neuron* 103627-641, e627. doi: 10.1016/j.neuron.2019.05.035

Kroeger, D., Ferrari, L. L., Petit, G., Mahoney, C. E., Fuller, P. M., Arrigoni, E., et al. (2017). Cholinergic, glutamatergic, and gabaergic neurons of the pedunculopontine tegmental nucleus have distinct effects on sleep/wake behavior in mice. *J. Neurosci.* 37, 1352–1366. doi: 10.1523/JNEUROSCI.1405-16.2016

Krueger, J. M. (2008). The role of cytokines in sleep regulation. *Curr. Pharm. Des.* 14, 3408–3416. doi: 10.2174/138161208786549281

Kudo, T., Loh, D. H., Truong, D., Wu, Y., and Colwell, C. S. (2011). Circadian dysfunction in a mouse model of Parkinson's disease. *Exp. Neurol.* 232, 66–75. doi: 10.1016/j.expneurol.2011.08.003

Kurtis, M. M., Rodriguez-Blazquez, C., Martinez-Martin, P., and Group, E. (2013). Relationship between sleep disorders and other non-motor symptoms in Parkinson's disease. *Parkinsonism Relat. Disord.* 19, 1152–1155. doi: 10.1016/j.parkreldis.2013.07.026

Laloux, C., Derambure, P., Kreisler, A., Houdayer, E., Brueziere, S., Bordet, R., et al. (2008). MPTP-treated mice: long-lasting loss of nigral TH-ir neurons but not paradoxical sleep alterations. *Exp. Brain Res.* 186, 635–642. doi: 10.1007/s00221-008-1268-1

Lang, A. E. (2011). A critical appraisal of the premotor symptoms of Parkinson's disease: potential usefulness in early diagnosis and design of neuroprotective trials. *Mov. Disord.* 26, 775–783. doi: 10.1002/mds.23609

Lee, S.-H., and Dan, Y. (2012). Neuromodulation of Brain States. *Neuron* 76, 209–222. doi: 10.1016/j.neuron.2012.09.012

Lima, M. M., Andersen, M. L., Reksidler, A. B., Vital, M. A., and Tufik, S. (2007). The role of the substantia nigra pars compacta in regulating sleep patterns in rats. *PloS One* 2, e513. doi: 10.1371/journal.pone.0000513

Liu, D., and Dan, Y. (2019). A motor theory of sleep-wake control: arousal-action circuit. *Annu. Rev. Neurosci.* 42, 27–46. doi: 10.1146/annurev-neuro-080317-061813

Lu, J., Bjorkum, A. A., Xu, M., Gaus, S. E., Shiromani, P. J., and Saper, C. B. (2002). Selective activation of the extended ventrolateral preoptic nucleus during rapid eye movement sleep. *J. Neurosci.* 22, 4568–4576. doi: 20026455

Lu, J., Sherman, D., Devor, M., and Saper, C. B. (2006). A putative flip-flop switch for control of REM sleep. *Nature* 441, 589–594. doi: 10.1038/nature04767

Lysen, T. S., Darweesh, S. K. L., Ikram, M. K., Luik, A. I., and Ikram, M. A. (2019). Sleep and risk of parkinsonism and Parkinson's disease: a population-based study. *Brain* 142 (7), 2013–2022 doi: 10.1093/brain/awz113

Maeda, T., Nagata, K., Kondo, H., and Kanbayashi, T. (2006). Parkinson's disease comorbid with narcolepsy presenting low CSF hypocretin/orexin level. *Sleep Med.* 7, 662. doi: 10.1016/j.sleep.2006.05.017

Maetzler, W., Liepelt, I., and Berg, D. (2009). Progression of Parkinson's disease in the clinical phase: potential markers. *Lancet Neurol.* 8, 1158–1171. doi: 10.1016/S1474-4422(09)70291-1

Mao, C. J., Yang, Y. P., Chen, J. P., Wang, F., Chen, J., Zhang, J. R., et al. (2018). Poor nighttime sleep is positively associated with dyskinesia in Parkinson's disease patients. *Parkinsonism Relat. Disord.* 48, 68–73. doi: 10.1016/j.parkreldis.2017.12.022

Masini, D., Lopes-Aguiar, C., Bonito-Oliva, A., Papadia, D., Andersson, R., Fisahn, A., et al. (2017). The histamine H3 receptor antagonist thioperamide rescues circadian rhythm and memory function in experimental parkinsonism. *Transl. Psychiatry* 7, e1088. doi: 10.1038/tp.2017.58

McDowell, K. A., Hadjimarkou, M. M., Viechweg, S., Rose, A. E., Clark, S. M., Yarowsky, P. J., et al. (2010). Sleep alterations in an environmental neurotoxin-induced model of parkinsonism. *Exp. Neurol.* 226, 84–89. doi: 10.1016/j.expneurol.2010.08.005

McDowell, K. A., Shin, D., Roos, K. P., and Chesselet, M. F. (2014). Sleep dysfunction and EEG alterations in mice overexpressing alpha-synuclein. *J. Parkinsons Dis.* 4, 531–539. doi: 10.3233/JPD-140374

Meredith, G. E., and Rademacher, D. J. (2011). MPTP mouse models of Parkinson's disease: an update. *J. Parkinsons Dis.* 1, 19–33. doi: 10.3233/JPD-2011-11023

Mery, V. P., Gros, P., Lafontaine, A. L., Robinson, A., Benedetti, A., Kimoff, R. J., et al. (2017). Reduced cognitive function in patients with Parkinson disease and obstructive sleep apnea. *Neurology* 88, 1120–1128. doi: 10.1212/WNL.0000000000003738

Monaca, C., Laloux, C., Jacquesson, J. M., Gele, P., Marechal, X., Bordet, R., et al. (2004). Vigilance states in a parkinsonian model, the MPTP mouse. *Eur. J. Neurosci.* 20, 2474–2478. doi: 10.1111/j.1460-9568.2004.03694.x

Monti, J. M., Ponzoni, A., Jantos, H., Lagos, P., Silveira, R., and Banchero, P. (1999). Effects of accumbens m-chlorophenylbiguanide microinjections on sleep and waking in intact and 6-hydroxydopamine-treated rats. *Eur. J. Pharmacol.* 364, 89–98. doi: 10.1016/s0014-2999(98)00826-7

Moroni, F., Nobili, L., Iaria, G., Sartori, I., Marzano, C., Tempesta, D., et al. (2014). Hippocampal slow EEG frequencies during NREM sleep are involved in spatial memory consolidation in humans. *Hippocampus* 24, 1157–1168. doi: 10.1002/hipo.22299

Moruzzi, G., and Magoun, H. W. (1949). Brain stem reticular formation and activation of the EEG. *Electroencephalogr. Clin. Neurophysiol.* 1, 455–473. doi: 10.1016/0013-4694(49)90219-9

Neikrug, A. B., Maglione, J. E., Liu, L., Natarajan, L., Avanzino, J. A., Corey-Bloom, J., et al. (2013). Effects of sleep disorders on the non-motor symptoms of Parkinson disease. *J. Clin. Sleep Med.* 9, 1119–1129. doi: 10.5664/jcsm.3148

Oishi, Y., Suzuki, Y., Takahashi, K., Yonezawa, T., Kanda, T., Takata, Y., et al. (2017). Activation of ventral tegmental area dopamine neurons produces wakefulness through dopamine D2-like receptors in mice. *Brain Struct. Funct.* 222, 2907–2915. doi: 10.1007/s00429-017-1365-7

Oliveira, L. M., Falquetto, B., Moreira, T. S., and Takakura, A. C. (2018). Orexinergic neurons are involved in the chemosensory control of breathing during the dark phase in a Parkinson's disease model. *Exp. Neurol.* 309, 107–118. doi: 10.1016/j.expneurol.2018.08.004

Ondo, W. G., Dat Vuong, K., Khan, H., Atassi, F., Kwak, C., and Jankovic, J. (2001). Daytime sleepiness and other sleep disorders in Parkinson's disease. *Neurology* 57, 1392–1396. doi: 10.1212/wnl.57.8.1392

Ono, D., and Yamanaka, A. (2017). Hypothalamic regulation of the sleep/wake cycle. *Neurosci. Res.* 118, 74–81. doi: 10.1016/j.neures.2017.03.013

Pagliardini, S., Greer, J. J., Funk, G. D., and Dickson, C. T. (2012). State-dependent modulation of breathing in urethane-anesthetized rats. *J. Neurosci.* 32, 11259–11270. doi: 10.1523/JNEUROSCI.0948-12.2012

Peever, J., and Fuller, P. M. (2017). The Biology of REM Sleep. *Curr. Biol.* 27, R1237–R1248. doi: 10.1016/j.cub.2017.10.026

Peyron, C., Tighe, D. K., van den Pol, A. N., de Lecea, L., Heller, H. C., Sutcliffe, J. G., et al. (1998). Neurons containing hypocretin (Orexin) project to multiple neuronal systems. *J. Neurosci.* 18, 9996–10015. doi: 10.1523/jneurosci.18-23-09996.1998

Porkka-Heiskanen, T., Strecker, R. E., Thakkar, M., Bjorkum, A. A., Greene, R. W., and McCarley, R. W. (1997). Adenosine: a mediator of the sleep-inducing effects of prolonged wakefulness. *Science* 276, 1265–1268. doi: 10.1126/science.276.5316.1265

Postuma, R. B., Gagnon, J. F., Rompre, S., and Montplaisir, J. Y. (2010). Severity of REM atonia loss in idiopathic REM sleep behavior disorder predicts Parkinson disease. *Neurology* 74, 239–244. doi: 10.1212/WNL.0b013e3181ca0166

Qiu, M. H., Yao, Q. L., Vetrivelan, R., Chen, M. C., and Lu, J. (2016). Nigrostriatal Dopamine acting on globus pallidus regulates sleep. *Cereb. Cortex* 26, 1430–1439. doi: 10.1093/cercor/bhu241

Reader, T. A., and Gauthier, P. (1984). Catecholamines and serotonin in the rat central nervous system after 6-OHDA, 5-7-DHT and p-CPA. *J. Neural Transm.* 59, 207–227. doi: 10.1007/bf01250009

Revishchin, A., Moiseenko, L., Kust, N., Bazhenova, N., Teslia, P., Panteleev, D., et al. (2016). Effects of striatal transplantation of cells transfected with GDNF gene without pre- and pro-regions in mouse model of Parkinson's disease. *BMC Neurosci.* 17, 34. doi: 10.1186/s12868-016-0271-x

Riemann, D., Berger, M., and Voderholzer, U. (2001). Sleep and depression–results from psychobiological studies: an overview. *Biol. Psychol.* 57, 67–103. doi: 10.1016/s0301-0511(01)00090-4

Saitoh, T., Niijima, K., and Mizuno, Y. (1987). Long-term effect of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) on striatal dopamine content in young and mature mice. *J. Neurol. Sci.* 77, 229–235. doi: 10.1016/0022-510x(87)90125-0

Sakata, M., Sei, H., Toida, K., Fujihara, H., Urushihara, R., and Morita, Y. (2002). Mesolimbic dopaminergic system is involved in diurnal blood pressure regulation. *Brain Res.* 928, 194–201. doi: 10.1016/s0006-8993(01)03402-3

Sakurai, T., Amemiya, A., Ishii, M., Matsuzaki, I., Chemelli, R. M., Tanaka, H., et al. (1998). Orexins and orexin receptors: a family of hypothalamic neuropeptides and g protein-coupled receptors that regulate feeding behavior. *Cell* 92, 573–585. doi: 10.1016/s0092-8674(00)80949-6

Sakurai, T. (2007). The neural circuit of orexin (hypocretin): maintaining sleep and wakefulness. *Nat. Rev. Neurosci.* 8, 171–181. doi: 10.1038/nrn2092

Santiago, R. M., Barbieiro, J., Lima, M. M., Dombrowski, P. A., Andreatini, R., and Vital, M. A. (2010). Depressive-like behaviors alterations induced by intranigral MPTP, 6-OHDA, LPS and rotenone models of Parkinson's disease are predominantly associated with serotonin and dopamine. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 34, 1104–1114. doi: 10.1016/j.pnpbp.2010.06.004

Saper, C. B., Chou, T. C., and Scammell, T. E. (2001). The sleep switch: hypothalamic control of sleep and wakefulness. *Trends In Neurosci.* 24, 726–731. doi: 10.1016/s0166-2236(00)02002-6

Saper, C. B., Scammell, T. E., and Lu, J. (2005). Hypothalamic regulation of sleep and circadian rhythms. *Nature* 437, 1257–1263. doi: 10.1038/nature04284

Scammell, T. E., Gerashchenko, D. Y., Mochizuki, T., McCarthy, M. T., Estabrooke, I. V., Sears, C. A., et al. (2001). An adenosine A2a agonist increases sleep and induces Fos in ventrolateral preoptic neurons. *Neuroscience* 107, 653–663. doi: 10.1016/s0306-4522(01)00383-9

Scammell, T. E., Arrigoni, E., and Lipton, J. O. (2017). Neural circuitry of wakefulness and sleep. *Neuron* 93, 747–765. doi: 10.1016/j.neuron.2017.01.014

Schenck, C. H., Bundlie, S. R., and Mahowald, M. W. (1996). Delayed emergence of a parkinsonian disorder in 38% of 29 older men initially diagnosed with idiopathic rapid eye movement sleep behaviour disorder. *Neurology* 46, 388–393. doi: 10.1212/wnl.46.2.388

Schenck, C. H., Bundlie, S. R., Ettinger, M. G., and Mahowald, M. W. (2002). Chronic behavioral disorders of human REM sleep: a new category of parasomnia. 1986 [classical article]. *Sleep* 25, 293–308. doi: 10.1093/sleep/9.2.293

Shan, L., Liu, C. Q., Balesar, R., Hofman, M. A., Bao, A. M., and Swaab, D. F. (2012). Neuronal histamine production remains unaltered in Parkinson's disease despite the accumulation of Lewy bodies and Lewy neurites in the tuberomamillary nucleus. *Neurobiol. Aging* 33, 1343–1344. doi: 10.1016/j.neurobiolaging.2011.01.004

Shen, W. B., McDowell, K. A., Siebert, A. A., Clark, S. M., Dugger, N. V., Valentino, K. M., et al. (2010). Environmental neurotoxin-induced progressive model of parkinsonism in rats. *Ann. Neurol.* 68, 70–80. doi: 10.1002/ana.22018

Sherin, J. E., Shiromani, P. J., McCarley, R. W., and Saper, C. B. (1996). Activation of ventrolateral preoptic neurons during sleep. *Science* 271, 216–219. doi: 10.1126/science.271.5246.216

Simola, N., Morelli, M., and Carta, A. R. (2007). The 6-hydroxydopamine model of Parkinson's disease. *Neurotox. Res.* 11, 151–167. doi: 10.1007/bf03033565

Strecker, R. E., Morairty, S., Thakkar, M. M., Porkka-Heiskanen, T., Basheer, R., Dauphin, L. J., et al. (2000). Adenosinergic modulation of basal forebrain and preoptic/anterior hypothalamic neuronal activity in the control of behavioral state. *Behav. Brain Res.* 115, 183–204. doi: 10.1016/s0166-4328(00)00258-8

Suntsova, N., Guzman-Marin, R., Kumar, S., Alam, M. N., Szymusiak, R., and McGinty, D. (2007). The median preoptic nucleus reciprocally modulates activity of arousal-related and sleep-related neurons in the perifornical lateral hypothalamus. *J. Neurosci.* 27, 1616–1630. doi: 10.1523/jneurosci.3498-06.2007

Surmeier, D. J., Obeso, J. A., and Halliday, G. M. (2017). Selective neuronal vulnerability in Parkinson disease. *Nat. Rev. Neurosci.* 18, 101–113. doi: 10.1038/nrn.2016.178

Tadaiesky, M. T., Dombrowski, P. A., Figueiredo, C. P., Cargnin-Ferreira, E., Da Cunha, C., and Takahashi, R. N. (2008). Emotional, cognitive and neurochemical alterations in a premotor stage model of Parkinson's disease. *Neuroscience* 156, 830–840. doi: S0306-4522(08)01247-5 [pii] 10.1016/j.neuroscience.2008.08.035

Taylor, T. N., Caudle, W. M., Shepherd, K. R., Noorian, A., Jackson, C. R., Iuvone, P. M., et al. (2009). Nonmotor symptoms of Parkinson's disease revealed in an animal model with reduced monoamine storage capacity. *J. Neurosci.* 29, 8103–8113. doi: 10.1523/JNEUROSCI.1495-09.2009

Thannickal, T. C., Lai, Y. Y., and Siegel, J. M. (2007). Hypocretin (orexin) cell loss in Parkinson's disease. *Brain* 130, 1586–1595. doi: 10.1093/brain/awm097

Thorpy, M. J. (2009). Classification of sleep disorders. *Sleep Disorders Medicine: Basic Science, Technical Considerations, and Clinical Aspects*. 3rd ed. 275–278. doi: 10.1016/b978-0-7506-7584-0.00020-3

Tieu, K. (2011). A guide to neurotoxic animal models of Parkinson's disease. *Cold Spring Harb. Perspect. Med.* 1, a009316. doi: 10.1101/cshperspect.a009316

Videnovic, A. (2017). Management of sleep disorders in Parkinson's disease and multiple system atrophy. *Mov. Disord.* 32, 659–668. doi: 10.1002/mds.26918

Vo, Q., Gilmour, T. P., Venkiteswaran, K., Fang, J., and Subramanian, T. (2014). Polysomnographic features of sleep disturbances and REM sleep behavior disorder in the unilateral 6-OHDA lesioned hemiparkinsonian rat. *Parkinsons Dis.* 2014, 852965. doi: 10.1155/2014/852965

Yi, P. L., Tsai, C. H., Lu, M. K., Liu, H. J., Chen, Y. C., and Chang, F. C. (2007). Interleukin-1beta mediates sleep alteration in rats with rotenone-induced parkinsonism. *Sleep* 30, 413–425. doi: 10.1093/sleep/30.4.413

Ylikoski, A., Martikainen, K., Sieminski, M., and Partinen, M. (2015). Parkinson's disease and insomnia. *Neurol. Sci.* 36, 2003–2010. doi: 10.1007/s10072-015-2288-9

Yu, X., Zecharia, A., Zhang, Z., Yang, Q., Yustos, R., Jager, P., et al. (2014). Circadian factor BMAL1 in histaminergic neurons regulates sleep architecture. *Curr. Biol.* 24, 2838–2844. doi: 10.1016/j.cub.2014.10.019

Yuan, H., Sarre, S., Ebinger, G., and Michotte, Y. (2005). Histological, behavioural and neurochemical evaluation of medial forebrain bundle and striatal 6-OHDA lesions as rat models of Parkinson's disease. *J. Neurosci. Methods* 144, 35–45. doi: 10.1016/j.jneumeth.2004.10.004

Zarow, C., Lyness, S. A., Mortimer, J. A., and Chui, H. C. (2003). neuronal loss is greater in the locus coeruleus than nucleus basalis and substantia nigra in Alzheimer and Parkinson diseases. *Arch. Neurol.* 60, 337. doi: 10.1001/archneur.60.3.337

Zhurakovskaya, E., Leikas, J., Pirttimaki, T., Casas Mon, F., Gynther, M., Aliev, R., et al. (2019). Sleep-state dependent alterations in brain functional connectivity under urethane anesthesia in a rat model of early-stage Parkinson's disease. *eNeuro* 6. doi: 10.1523/ENEURO.0456-18.2019

Zweig, R. M., Jankel, W. R., Hedreen, J. C., Mayeux, R., and Price, D. L. (1989). The pedunculopontine nucleus in Parkinson's disease. *Ann. Neurol.* 26, 41–46. doi: 10.1002/ana.410260106

Conflict of Interest: The authors declare that the submitted work was carried out in the absence of any personal, professional or financial relationships that could potentially be construed as a conflict of interest.

frontiers in Pharmacology

PERSPECTIVE
published: 13 December 2019
doi: 10.3389/fphar.2019.01488

Striatal Cholinergic Interneurons: How to Elucidate Their Function in Health and Disease

Nicolas Mallet[1,2], *Arthur Leblois*[1,2], *Nicolas Maurice*[3] *and Corinne Beurrier*[3*]

[1] Université de Bordeaux, Institut des Maladies Neurodégénératives, Bordeaux, France, [2] CNRS UMR 5293, Institut des Maladies Neurodégénératives, Bordeaux, France, [3] Aix Marseille Univ, CNRS, IBDM, Marseille, France

Striatal cholinergic interneurons (CINs) are the main source of acetylcholine in the striatum and are believed to play an important role in basal ganglia physiology and pathophysiology. The role of CINs in striatal function is known mostly from extracellular recordings of tonically active striatal neurons in monkeys, which are believed to correspond to CINs. Because these neurons transiently respond to motivationally cues with brief pauses, flanked by bursts of increased activity, they are classically viewed as key players in reward-related learning. However, CIN modulatory function within the striatal network has been mainly inferred from the action of acetylcholine agonists/antagonists or through CIN activation. These manipulations are far from recapitulating CIN activity in response to behaviorally-relevant stimuli. New technical tools such as optogenetics allow researchers to specifically manipulate this sparse neuronal population and to mimic their typical pause response. For example, it is now possible to investigate how short inhibition of CIN activity shapes striatal properties. Here, we review the most recent literature and show how these new techniques have brought considerable insights into the functional role of CINs in normal and pathological states, raising several interesting and novel questions. To continue moving forward, it is crucial to determine in detail CIN activity changes during behavior, particularly in rodents. We will also discuss how computational approaches combined with optogenetics will contribute to further our understanding of the CIN role in striatal circuits.

Keywords: striatal cholinergic interneurons, basal ganglia, optogenetics, computational modelling, Parkinson's disease

OPEN ACCESS

Edited by:
Véronique Sgambato, Institut National de la Santé et de la Recherche Médicale (INSERM), France

Reviewed by:
Jill Crittenden, Massachusetts Institute of Technology, United States
John N. J. Reynolds, University of Otago, New Zealand

***Correspondence:**
Corinne Beurrier
corinne.beurrier@univ-amu.fr

Specialty section:
This article was submitted to Neuropharmacology, a section of the journal Frontiers in Pharmacology

Received: *10 September 2019*
Accepted: *15 November 2019*
Published: *13 December 2019*

Citation:
Mallet N, Leblois A, Maurice N and Beurrier C (2019) Striatal Cholinergic Interneurons: How to Elucidate Their Function in Health and Disease. Front. Pharmacol. 10:1488. doi: 10.3389/fphar.2019.01488

INTRODUCTION

The striatum is a brain region containing high levels of acetylcholine (ACh), muscarinic receptors, and other ACh-related markers (Weiner et al., 1990; Hersch et al., 1994). Cholinergic interneurons (CINs) are the main source of ACh in the striatum [but see (Dautan, 2014)]. Despite their small numbers (1–3% of all striatal cells) and scattered distribution throughout the striatum, CINs have dense terminal fields that overlap those of dopaminergic projections coming from the substantia

nigra pars compacta (Bolam et al., 1984). CINs contact the two populations of striatal output neurons (also called medium spiny neurons, MSNs) that express either the dopamine D1 or D2 receptors. While most striatal neurons are not autonomously active, CINs exhibit a regular spiking activity in absence of any synaptic inputs (Bennett et al., 2000). Extracellular recordings performed *in vivo* in the striatum of monkeys also reveal the presence of tonically active neurons (TANs), which are thought to correspond to CINs (Aosaki et al., 1995). Hence, the morphofunctional features of CINs—mainly their extensive arborization primarily directed to MSNs and their tonic activity—place them as potent modulators of striatal output. Striatal output regulation is a fundamental process of the basal ganglia functioning, as a balanced activity between D1 and D2 MSNs is required to ensure correct motor and cognitive behaviors.

The improvement of parkinsonian tremor by both dopaminergic agonists and anticholinergic drugs led to the dopamine (DA)-ACh balance hypothesis, where DA and ACh are believed to play opposite roles in the striatum (Barbeau, 1962). Even though the prescription of anticholinergic drugs has been phased out due to their side-effects, this long standing clinical observation underlines the functional impact of ACh as the level of DA falls and has often led to the consideration of Parkinson's disease (PD) as a hypercholinergic disorder [but see (McKinley, 2019)]. There is indeed compelling evidence showing that DA depletion triggers complex alterations in striatal cholinergic signaling, activity, and connectivity (Aosaki, 1994; Raz, 2001; Ding, 2006; Salin, 2009). However, there is no consensual view explaining how CINs contribute to motor symptoms and abnormal network dynamic in PD.

At the cellular level, CIN modulation of the striatal network has been mainly inferred from the action of ACh agonists or through CIN activation. While nicotinic receptors (nAChRs) are expressed by interneurons and extrinsic afferent terminals, MSNs respond to ACh exclusively *via* muscarinic receptors (mAChRs): M1 receptors are present on D1 and D2 MSNs and M4 receptors are preferentially expressed by D1 MSNs. Activation of mAChRs modulates an array of voltage-gated channels and intracellular pathways in MSNs. Determining the combinatorial effect of these actions, potentially even opposing each other, is highly challenging and has recently been covered at length by excellent recent reviews (Tanimura, 2018; Ztaou and Amalric, 2019; Abudukeyoumu et al., 2019). A hallmark of CINs is their continuous tonic activity, which is expected to lead to a high level of ACh in the striatum, and the stereotypical bursts and pauses activity that they acquire during sensorimotor learning (Apicella, 2007). We can assume that a drop in ACh release, as expected to happen after a brief decrease in firing, conveys meaningful information to the striatal network. A recent hypothesis proposes that the pause would open a permissive temporal window during which corticostriatal synaptic plasticity occurs (Deffains and Bergman, 2015). However, it is still unclear how inhibition of CIN activity shapes striatal properties. Here, we review the related literature and show how optogenetic and computational approaches may contribute to further our understanding of this topic.

CONSEQUENCES OF CHOLINERGIC INTERNEURON INHIBITION ON STRIATAL PROPERTIES

The widespread excitatory input from the cortex targeting D1 and D2 MSNs sets the activity of the direct and indirect striatofugal pathways which play a fundamental role in movement planning and action selection. Understanding how CINs modulate the dynamics of corticostriatal processing and MSN activity is therefore essential to uncover basal ganglia function. Cholinergic modulation of long-term corticostriatal plasticity has been addressed in excellent reviews (Lovinger, 2010; Lerner and Kreitzer, 2011) and will not be further discussed here.

The effects of cholinergic antagonists on corticostriatal transmission might provide interesting insights to predict how a pause in CIN firing impacts striatal output. It was reported that atropine, a broad mAChRs antagonist, or methoctramine, at a concentration that blocks M2 and M3 mAChRs, lead to a modest increase in corticostriatal transmission *via* the inhibition of mAChRs located on the glutamatergic terminals, suggesting the existence of tonic cholinergic presynaptic inhibition (Pakhotin and Bracci, 2007). On the other hand, pirenzepine, a blocker of M1 mAChRs, reduces corticostriatal transmission (Wang, 2006; Tozzi, 2011). In these last two studies, the authors suggest that lowering M1 mAChR activity in MSNs leads to the opening of L-type Ca^{2+} channels, which then triggers endocannabinoids release. Endocannabinoids are then able to reduce glutamate transmission by activating presynaptic CB1 receptors. Hence, mAChRs inhibition could exert opposite actions on basal corticostriatal transmission depending on their pre- or postsynaptic localization. Nicotinic $\alpha 7$ receptors have been described on cortical glutamatergic terminals but whether these receptors directly modulate corticostriatal transmission is still unclear (Howe et al., 2016).

One of the caveats of pharmacological experiments is that they do not allow to assess the effects of endogenous ACh that depends on the temporal dynamic of CIN firing. Moreover, CINs might co-release glutamate and GABA along with ACh, with effects that cannot be apprehended through this approach (Higley et al., 2009; Lozovaya, 2018). Optogenetic manipulations, enabling control of electrical activity in specific cell types with high temporal accuracy, can provide substantial insights into these issues. Here, we review the few studies showing the impact of optogenetic inhibition of CIN activity in the dorsal striatum. *In vitro*, we and others have shown that inhibition of CIN firing with halorhodopsin (eNpHR) is associated with a decrease in D1 and D2 MSN excitability that might involve a lowering of M1 mAChRs activation (Maurice, 2015; Zucca et al., 2018). In anaesthetized mice, opto-inhibition of eNpHR-expressing CINs was also reported to decrease MSN activity by hyperpolarizing their membrane potential and

increasing the duration of down states (Zucca et al., 2018). In contrast, eNpHR-induced inhibition of CIN firing in freely moving mice did not alter MSN activity (English, 2011). In this last study, it is the rebound of action potentials occurring at the end of the eNpHR-induced hyperpolarization that triggered a decrease in MSN firing. Interestingly, despite the cellular effect induced by this pause-rebound, it was not followed by any detectable behavioral responses (English, 2011). This is in agreement with other works showing that opto-inhibition of CIN firing does not affect locomotion, anxiety-like behavior, social memory recognition, and visuospatial object recognition (Maurice, 2015; Ztaou et al., 2018). In contrast, the behavior of PD mice, which perform poorly in all these tests, is improved by CIN silencing (Maurice, 2015). Restoring the balance between the striatofugal pathways at the level of the substantia nigra pars reticulata might be one component of the positive effect of CIN inhibition in parkinsonian condition (Maurice, 2015).

What conclusions can we draw from this brief overview? Obviously, more work is needed to understand how CIN inhibition shapes striatal output and modulates basal ganglia-related behavior. The conflicting effects of CIN firing inhibition on MSN activity and the lack of clear behavioral response in normal mice can be interpreted in different ways: (i) the light parameters (i.e. light duration, pattern or timing delivery) used to manipulate CIN firing are not physiologically relevant, (ii) CINs do not impact basal ganglia-related behaviors in physiological conditions and/or (iii) the behavioral tasks used are not appropriate to reveal CIN functions in rodents. What we know about the function of CINs comes from studies carried out in primates, describing the correlative changes in electrical activity of presumed-CINs during behavior. Optogenetics, mainly applied in rodents for technical reasons, is perfectly suited to go beyond correlational analysis and to investigate the causal implication of CINs in behavior. However, we first need to accurately describe the firing properties of these cells in rodents to be able to manipulate their activity in an appropriate way.

ACTIVITY OF CHOLINERGIC INTERNEURONS DURING BEHAVIOR

The identification of CINs in behaving animals is usually based on their unique *in vivo* extracellular firing activity (i.e. tonically active at 5 spikes/s, sometimes in a burst mode) and broad spike waveform (i.e. spike duration > 2 ms). These electrical properties are easily distinguishable from all the other striatal cell populations and represent a good signature of CINs as confirmed later by *in vivo* juxtacellular labelling (Inokawa et al., 2010; Sharott et al., 2012). Using these classification criteria, early studies first defined the pattern of CINs activity during classical conditioning. In these experiments, animals have to learn the association between a neutral stimulus (i.e. often a tone) and an unexpected reward. In this context, CINs classically respond with a pause in firing that occurs shortly after the conditioned stimulus and lasts around 200 ms. This pause can also be preceded and/or followed by excitatory burst responses (Kimura et al., 1984; Aosaki, 1994; Apicella, 2017). Interestingly, this stereotypical pause appears during learning (Aosaki, 1994) and is time-locked to the response of nigral dopaminergic neurons (Morris et al., 2004). It is also dependent on the integrity of both dopaminergic neurons (Aosaki et al., 1994; Raz et al., 1996) and glutamatergic inputs coming from the intralaminar thalamus (Matsumoto et al., 2001).

What is not yet clear is whether the pause and burst components carry different signals used to underlie specific functions (Apicella, 2002; Apicella, 2007). Importantly, these activity patterns are mostly synchronized in the CINs population (Raz et al., 1996) such that they might efficiently translate into global change of striatal ACh level, providing a temporal window for complex pre- and post-synaptic modifications of striatal network and plastic changes (Deffains and Bergman, 2015; Cox and Witten, 2019). As a consequence, the pause response of CINs is considered as a key cellular substrate for reward-based learning, and may be particularly important for stimulus-response and action-outcome associations. The exact cellular and network explanations underlying the generation of the pause/burst firing responses are not known precisely. Multiple mechanisms have been proposed to generate these responses. They all have in common the capacity to broadcast efficiently the information to spatially-distributed CINs (Goldberg and Reynolds, 2011; Schulz and Reynolds, 2013; Zhang and Cragg, 2017). Such broadcast mechanisms include:

a) a change in intrinsic excitability driven by excitatory synaptic inputs (Oswald et al., 2009; Ding et al., 2010; Doig et al., 2014; Zhang et al., 2018; Reynolds et al., 2004). This scenario has been well described for cortical and intralaminar nucleus thalamic inputs but whether it can occur from any other known glutamatergic sources [such as the one coming from the pedonculopontine nucleus (Assous et al., 2019)] remained to be addressed.
b) a putative effect of DA directly onto CINs (Yan et al., 1997; Maurice, 2004; Yan and Surmeier, 1997).
c) a cholinergic input coming from the pedunculopontine and laterodorsal tegmental nuclei that synapse preferentially with CINs and give rise to excitatory responses (Dautan, 2014; Dautan, 2018).
d) a direct inhibitory inputs coming from striatal GABAergic interneurons surrounding MSNs (Gonzales et al., 2013). Activation of one CIN is, for example, able to inhibit the firing of nearby CINs *via* nicotinic excitation of striatal GABAergic interneurons. This microcircuit allows a wide-spread inhibition of CINs by recurrent inhibition (Sullivan et al., 2008; Faust et al., 2016).

Also, external sources such as GABAergic neurons from the midbrain, or from the globus pallidus (GP), or from unknown origin might also synchronize CIN population (Zhang and Cragg, 2017). Among these GABAergic sources, the inhibitory inputs coming from GP neurons appear to be functionally efficient at inducing a pause in CINs (Klug et al., 2018). However, it is important to mention that the pallido-striatal inputs could originate from two main populations of GP neurons, namely the prototypic and the arkypallidal neurons

(Mallet, 2012), and that the respective contribution of arkypallidal or prototypic neurons in the CIN pause response have not yet been assessed. That being said, anatomical evidences would argue that arkypallidal neurons represent a good cellular substrate to generate a synchronized pause in CIN firing. Indeed, arkypallidal neurons provide widespread striatal GABAergic inhibition (Mallet, 2012; Mallet, 2016) that densely target, with "basket-like" perisomatic contacts, the soma and proximal dendrites of CINs (Mallet, 2012). It should also be noted that CINs represent preferential targets for arkypallidal neurons, as suggested by the larger number of apposition that a single-labeled arkypallidal cells make onto CINs (Mallet, 2012). Altogether, we propose that GABAergic arkypallidal neurons constitute a powerful mechanism to generate synchronized inhibitory responses in CINs population. Whether this arkypallidal-CINs circuit is part of a feed-back or a feed-forward loop is not known but should be addressed in future studies.

Apart from the classical conditioning experiments, the contribution of CIN activity has also been tested during operant tasks. In these experiments, the animal has to execute an action to obtain a reward. These studies have revealed the involvement of CINs in more complex behavioral aspects such as contextual (Apicella, 2007), temporal (Morris et al., 2004), goal-directed action (Bradfield et al., 2013), sensori-motor gating (Ding et al., 2010), movement control/modulation (Yarom and Cohen, 2011; Nougaret and Ravel, 2015; Lee et al., 2006), and action inhibition (Lee et al., 2006). Interestingly, the expression of the pause in CIN firing is largely dependent on the behavioral task paradigm (Benhamou et al., 2014). This might actually explain some of the discrepancies originally found between monkey and rodent recordings.

In addition, recent works have taken advantage of transgenic ChAT-Cre mice to genetically identify CINs and record their activity with two-photon calcium imaging and fiber photometry, during spontaneous locomotion in head-fixed animals (Gritton, 2019; Howe, 2019; Rehani, 2019). In doing so, novel features of CIN contributions to global locomotion control have been described. In particular, one study found that CINs increase their activity during behavioral state transition, and could thus favor the transition from one behavioral state to another (Howe, 2019). Alternatively, CINs activation can reduce ongoing movement while synchronizing the activity of MSNs (Gritton, 2019). This synchronizing effect on striatal neurons is a remarkable feature especially considering that excessive expression of synchronized oscillatory activity in the beta frequency band (12–30 Hz) is a hallmark associated with PD and possibly linked to akinesia/bradykinesia in PD patients (Brown, 2007) [but see (Nambu et al., 2015; McGregor and Nelson, 2019)]. This further adds to the view that CIN dysfunctional activity contributes to the pathophysiology of PD. Indeed, there is good evidence to suggest that the loss of DA in the striatum modifies the cholinergic signalling (Tanimura, 2018; McKinley, 2019; Ztaou and Amalric, 2019) and increases the correlated activity between CINs (Raz, 2001). Although the minimal neuronal circuit generating the parkinsonian beta synchronizations in basal ganglia circuits are not known, it is possible that CIN activity represents a good candidate to promote synchronized activity in these neuronal networks. Indeed, cholinergic agonist infusion in the striatum (McCarthy, 2011) and optogenetic excitation of CINs (Kondabolu, 2016) can induce an increase in the expression of beta oscillations. In addition, CINs opto-excitation in normal animals generates parkinsonian-like motor deficits (Kondabolu, 2016) while CINs opto-inhibition in PD mice decreases motor symptoms (Maurice, 2015).

EXPLORING CHOLINERGIC INTERNEURONS FUNCTIONS IN BASAL GANGLIA NETWORK: CONTRIBUTION OF COMPUTATIONAL MODELING

CINs modulate striatal activity during behavior. A theoretical study of the putative function of these neurons in motor learning and their possible role in pathophysiology through modeling could drive experimentally testable predictions and thereby guide further experimental investigation. Previous modeling efforts involving CINs remain relatively sparse. They range from simulating intracellular and ion-channel dynamics linked to cholinergic signaling to the effect of CIN activity modulation on behavior.

On the microscopic scale, two modeling studies have highlighted the tight coupling between DA neurons and CINs due to the dopaminergic modulation of both the intrinsic currents generating tonic firing (Aosaki et al., 1998; Maurice, 2004; Wilson and Goldberg, 2006; Deng et al., 2007) and the external inputs to CINs (Nicola et al., 2000; Pisani et al., 2000). Szalisznyó and Müller (2009) analyzed conductance-based changes in CIN subthreshold oscillations induced by DA and predicted that DA can switch CINs between stable oscillatory and fixed-point behaviors, with opposing effects of D1- and D2-type dopamine receptors. Tan and Bullock (2008) have shown that DA inputs robustly cooperate with thalamic inputs to control cue-dependent CIN pauses. Thereby, DA strongly affects performance- and learning-related dynamics in the striatum. The DA-CINs coupling could explain the adaptively scaled DA burst and the CIN burst and pause observed experimentally in response to reward-predicting cues. These changes would thus not necessarily require a modification in the weight of synapses onto CINs.

On the macroscopic scale, the influence of CINs on behavior can be either immediate, due to the modulation of striatal output by CINs, or delayed/persistent, due to ACh-dependent plasticity in the striatal network that leads to long-term changes in striatal response to its external inputs. A recent study by Vogt and Hofmann (2012) modeled the modulation in the activity of DA neurons and CINs in relation to external reward delivery and its internal expectation. They show that activity changes and their effect on learning outcome can be explained by a direct effect of neuromodulators (DA and ACh) on postsynaptic activity, even with unmodulated, two-factor spike timing-dependent plasticity (STDP). Obviously, it does not prohibit joint operation together with three-factor STDP rules. Interestingly, CIN pause could represent a time window to gate phasic DA release and "bracket"

the plasticity window, while DA variations reciprocally modulate the CIN pause duration to adjust this window (Kim, 2019). In the context of reward-based motor adaptation, phasic DA release could thereby deliver reward information for reinforcement learning in a timely manner. Changes in CIN-DA interactions due to DA depletion would then produce poor performance of motor adaptation. Alternatively, CINs could act mainly on MSNs to suppress their firing and regulate local inhibitory network (Ashby and Crossley, 2011; Franklin and Frank, 2015).

To go beyond this current state of theoretical investigations, one may ask the following questions. What are the respective/specific roles of DA and ACh during learning? How redundant are these signals? Are they separable in time or space? What is the specific motor impairment expected due to the abnormal CIN activity following DA depletion and could some PD symptoms be linked to CIN signaling dysfunction? These questions may be answered by integrating current experimental evidence and DA-ACh interactions and its effect on striatal dynamics revealed by previous theoretical work in a circuit model of the basal ganglia-thalamo-cortical loop. This model may display action selection properties and DA-driven reinforcement learning (Leblois et al., 2006; Guthrie et al., 2013), as well as PD-related dysfunction under DA depletion. The computational advantages brought by CINs and the neural mechanisms can be investigated in such a theoretical framework. Specific predictions can then be derived from model concerning the effects of manipulating CIN activity in a reinforcement learning protocol. These predictions could eventually be tested experimentally with physiological recordings performed in an operant conditioning task to ensure that the suggested mechanisms are indeed at play in the striatum during motor learning.

CONCLUDING REMARKS

Our current understanding of the role of CINs in striatal function derives mostly from extracellular recordings of TANs in monkeys. Because these neurons transiently respond to motivationally relevant cues with brief pauses, flanked by bursts of increased activity, they are classically viewed as key players in reward related learning. However, how CINs, and particularly the pause in their tonic firing, modulate striatal output has yet to be demonstrated. It is also undisputable that CINs play a key role in relation to dysfunctional aspect of basal ganglia information processing such as in PD and it seems important that future works keep dissecting the causal role of CINs in striatal circuits.

AUTHOR CONTRIBUTIONS

NMal, AL, NMau, and CB drafted, provided critical revision of the article, wrote, and approved the final version of the review.

ACKNOWLEDGMENTS

This work was supported by grants from the French Agence Nationale de la Recherche (ANR-2010-1416, ANR-14-CE13-0024-01, ANR-15-CE37-0006 and ANR-16-CE37-0020-01), Fondation de France (# 146280), France Parkinson nonprofit organization (OPE-2018-0459), and the LABEX BRAIN (ANR-10-LABX-43). We thank Nuno Miguel Luis for the manuscript proofreading.

REFERENCES

Abudukeyoumu, N., Hernandez-Flores, T., Garcia-Munoz, M., and Arbuthnott, G. W. (2019). Cholinergic modulation of striatal microcircuits. *Eur. J. Neurosci.* 49, 604–622. doi: 10.1111/ejn.13949

Aosaki, T., Tsubokawa, H., Ishida, A., Watanabe, K., Graybiel, A., and Kimura, M. (1994). Effect of the nigrostriatal dopamine system on acquired neural responses in the striatum of behaving monkeys. *Science* 265, 412–415. doi: 10.1126/science.8023166

Aosaki, T., Kimura, M., and Graybiel, A. M. (1995). Temporal and spatial characteristics of tonically active neurons of the primate's striatum. *J. Neurophysiol.* 73, 1234–1252. doi: 10.1152/jn.1995.73.3.1234

Aosaki, T., Kiuchi, K., and Kawaguchi, Y. (1998). Dopamine D1-like receptor activation excites rat striatal large aspiny neurons *in vitro*. *J. Neurosci.* 18, 5180–5190. doi: 10.1523/JNEUROSCI.18-14-05180.1998

Aosaki, T., Tsubokawa, H., Ishida, A., Watanabe, K., Graybiel, A. M., and Kimura, M. (1994). Responses of tonically active neurons in the primate's striatum undergo systematic changes during behavioral sensorimotor conditioning. *J. Neurosci.* 14, 3969–3984. doi: 10.1523/JNEUROSCI.14-06-03969.1994

Apicella, P. (2002). Tonically active neurons in the primate striatum and their role in the processing of information about motivationally relevant events. *Eur. J. Neurosci.* 16, 2017–2026. doi: 10.1046/j.1460-9568.2002.02262.x

Apicella, P. (2007). Leading tonically active neurons of the striatum from reward detection to context recognition. *Trends Neurosci.* 30, 299–306. doi: 10.1016/j.tins.2007.03.011

Apicella, P. (2017). The role of the intrinsic cholinergic system of the striatum: what have we learned from TAN recordings in behaving animals? *Neuroscience* 360, 81–94. doi: 10.1016/j.neuroscience.2017.07.060

Ashby, F. G., and Crossley, M. J. (2011). A computational model of how cholinergic interneurons protect striatal-dependent learning. *J. Cogn. Neurosci.* 23, 1549–1566. doi: 10.1162/jocn.2010.21523

Assous, M., Dautan, D., Tepper, J. M., and Mena-Segovia, J. (2019). Pedunculopontine glutamatergic neurons provide a novel source of feedforward inhibition in the striatum by selectively targeting interneurons. *J. Neurosci.* 39, 4727–4737. doi: 10.1523/JNEUROSCI.2913-18.2019

Barbeau, A. (1962). The pathogenesis of Parkinson's disease: a new hypothesis. *Can. Med. Assoc. J.* 87, 802–807.

Benhamou, L., Kehat, O., and Cohen, D. (2014). Firing pattern characteristics of tonically active neurons in rat striatum: context dependent or species divergent? *J. Neurosci.* 34, 2299–2304. doi: 10.1523/JNEUROSCI.1798-13.2014

Bennett, B. D., Callaway, J. C., and Wilson, C. J. (2000). Intrinsic membrane properties underlying spontaneous tonic firing in neostriatal cholinergic interneurons. *J. Neurosci.* 20, 8493–8503. doi: 10.1523/JNEUROSCI.20-22-08493.2000

Bolam, J. P., Wainer, B. H., and Smith, A. D. (1984). Characterization of cholinergic neurons in the rat neostriatum. A combination of choline acetyltransferase immunocytochemistry, Golgi-impregnation and electron microscopy. *Neuroscience* 12, 711–718. doi: 10.1016/0306-4522(84)90165-9

Bradfield, L. A., Bertran-Gonzalez, J., Chieng, B., and Balleine, B. W. (2013). The thalamostriatal pathway and cholinergic control of goal-directed action: interlacing new with existing learning in the striatum. *Neuron* 79, 153–166. doi: 10.1016/j.neuron.2013.04.039

Brown, P. (2007). Abnormal oscillatory synchronisation in the motor system leads to impaired movement. *Curr. Opin. Neurobiol.* 17, 656–664. doi: 10.1016/j.conb.2007.12.001

Cox, J., and Witten, I. B. (2019). Striatal circuits for reward learning and decision-making. *Nat. Rev. Neurosci.* 20, 482–494. doi: 10.1038/s41583-019-0189-2

Dautan, D., Huerta-Ocampo, I., Valencia, M., Kondabolu, K. , Gerdjikov, T. V. , and Mena-Segovia, J. (2014). A major external source of cholinergic innervation of the striatum and nucleus accumbens originates in the brainstem. *J. Neurosci.* 34, 4509–4518. doi: 10.1523/JNEUROSCI.5071-13.2014

Dautan, D., Huerta-Ocampo, I., Valencia, M., Kondabolu, K., Gerdjikov, T. V., and Mena-Segovia, J. (2018). Cholinergic midbrain afferents modulate striatal circuits and shape encoding of action control. *bioRxiv*. doi: 10.1101/388223388223

Deffains, M., and Bergman, H. (2015). Striatal cholinergic interneurons and cortico-striatal synaptic plasticity in health and disease. *Mov. Disord.* 30, 1014–1025. doi: 10.1002/mds.26300

Deng, P., Zhang, Y., and Xu, Z. C. (2007). Involvement of Ih in dopamine modulation of tonic firing in striatal cholinergic interneurons. *J. Neurosci.* 27, 3148–3156. doi: 10.1523/JNEUROSCI.5535-06.2007

Ding, J. B., Guzman, J. N., Peterson, J. D., Goldberg, J. a., and Surmeier, D. J. (2010). Thalamic gating of corticostriatal signaling by cholinergic interneurons. *Neuron* 67, 294–307. doi: 10.1016/j.neuron.2010.06.017

Ding, J., Guzman, J. N., Tkatch, T., Chen, S., Goldberg, J. A., Erbert, P. J., et al. (2006). RGS4-dependent attenuation of M4 autoreceptor function in striatal cholinergic interneurons following dopamine depletion. *Nat. Neurosci.* 9, 832–842. doi: 10.1038/nn1700

Doig, N. M., Magill, P. J., Apicella, P., Bolam, J. P., and Sharott, A. (2014). Cortical and thalamic excitation mediate the multiphasic responses of striatal cholinergic interneurons to motivationally salient stimuli. *J. Neurosci.* 34, 3101–3117. doi: 10.1523/JNEUROSCI.4627-13.2014

English, D. F., Ibanez-Sandoval, O., Stark, E., Tecuapetla, F., Buzsáki, G., Deisseroth, K., et al. (2011). GABAergic circuits mediate the reinforcement-related signals of striatal cholinergic interneurons. *Nat. Neurosci.* 15, 123–130. doi: 10.1038/nn.2984

Faust, T. W., Assous, M., Tepper, J. M., and Koós, T. (2016). Neostriatal GABAergic interneurons mediate cholinergic inhibition of spiny projection neurons. *J. Neurosci.* 36, 9505–9511. doi: 10.1523/JNEUROSCI.0466-16.2016

Franklin, N. T., and Frank, M. J. (2015). A cholinergic feedback circuit to regulate striatal population uncertainty and optimize reinforcement learning. *Elife* 4, e12029. doi: 10.7554/eLife.12029

Goldberg, J. A., and Reynolds, J. N. J. (2011). Spontaneous firing and evoked pauses in the tonically active cholinergic interneurons of the striatum. *Neuroscience* 198, 27–43. doi: 10.1016/j.neuroscience.2011.08.067

Gonzales, K. K., Pare, J. F., Wichmann, T., and Smith, Y. (2013). GABAergic inputs from direct and indirect striatal projection neurons onto cholinergic interneurons in the primate putamen. *J. Comp. Neurol.* 521, 2502–2522. doi: 10.1002/cne.23295

Gritton, H. J., Howe, H. M., Romano, M. F., DiFeliceantonio, A. G., Kramer, M. A., Saligrama, V., et al. (2019). Unique contributions of parvalbumin and cholinergic interneurons in organizing striatal networks during movement. *Nat. Neurosci.* 22, 586–597. doi: 10.1038/s41593-019-0341-3

Guthrie, M., Leblois, A., Garenne, A., and Boraud, T. (2013). Interaction between cognitive and motor cortico-basal ganglia loops during decision making: a computational study. *J. Neurophysiol.* 109, 3025–3040. doi: 10.1152/jn.00026.2013

Hersch, S. M., Gutekunst, C. A., Rees, H. D., Heilman, C. J., and Levey, A. I. (1994). Distribution of m1-m4 muscarinic receptor proteins in the rat striatum: light and electron microscopic immunocytochemistry using subtype-specific antibodies. *J. Neurosci.* 14, 3351–3363. doi: 10.1523/JNEUROSCI.14-05-03351.1994

Higley, M. J., Soler-Llavina, G. J., and Sabatini, B. L. (2009). Cholinergic modulation of multivesicular release regulates striatal synaptic potency and integration. *Nat. Neurosci.* 12, 1121–1128. doi: 10.1038/nn.2368

Howe, W. M., Young, D. A., Bekheet, G., and Kozak, R. (2016). Nicotinic receptor subtypes differentially modulate glutamate release in the dorsal medial striatum. *Neurochem. Int.* 100, 30–34. doi: 10.1016/j.neuint.2016.08.009

Howe, M., Ridouh, I., Allegra Mascaro, A. L., Larios, A., Azcorra, M., Dombeck, D. A., et al. (2019). Coordination of rapid cholinergic and dopaminergic signaling in striatum during spontaneous movement. *Elife* 28 (8), e44903. doi: 10.7554/eLife.44903

Inokawa, H., Yamada, H., Matsumoto, N., Muranishi, M., and Kimura, M. (2010). Juxtacellular labeling of tonically active neurons and phasically active neurons in the rat striatum. *Neuroscience* 168, 395–404. doi: 10.1016/j.neuroscience.2010.03.062

Kim, T., Capps, R. A., Hamade, K. C., Barnett, W. H., Todorov, D. I., Latash, E. M., et al. (2019). The functional role of striatal cholinergic interneurons in reinforcement learning from computational perspective. *Front. Neural Circuits* 13, 10. doi: 10.3389/fncir.2019.00010

Kimura, M., Rajkowski, J., and Evarts, E. (1984). Tonically discharging putamen neurons exhibit set-dependent responses. *Proc. Natl. Acad. Sci. U. S. A.* 81, 4998–5001. doi: 10.1073/pnas.81.15.4998

Klug, J. R., Engelhardt, M. D., Cadman, C. N., Li, H., Smith, J. B., Ayala, S., et al. (2018). Differential inputs to striatal cholinergic and parvalbumin interneurons imply functional distinctions. *elife*, 7, e35657.

Kondabolu, K., Roberts, E. A., Bucklin, M., McCarthy, M. M., Kopell, N., and Han, X. (2016). Striatal cholinergic interneurons generate beta and gamma oscillations in the corticostriatal circuit and produce motor deficits. *Proc. Natl. Acad. Sci.* 113, E3159–E3168. doi: 10.1073/pnas.1605658113

Leblois, A., Boraud, T., Meissner, W., Bergman, H., and Hansel, D. (2006). Competition between feedback loops underlies normal and pathological dynamics in the Basal Ganglia. *J. Neurosci.* 26, 3567–3583. doi: 10.1523/JNEUROSCI.5050-05.2006

Lee, I. H., Seitz, A. R., and Assad, J. A. (2006). Activity of tonically active neurons in the monkey putamen during initiation and withholding of movement. *J. Neurophysiol.* 95, 2391–2403. doi: 10.1152/jn.01053.2005

Lerner, T. N., and Kreitzer, A. C. (2011). Neuromodulatory control of striatal plasticity and behavior. *Curr. Opin. Neurobiol.* 21, 322–327. doi: 10.1016/j.conb.2011.01.005

Lovinger, D. M. (2010). Neurotransmitter roles in synaptic modulation, plasticity and learning in the dorsal striatum. *Neuropharmacology* 58, 951–961. doi: 10.1016/j.neuropharm.2010.01.008

Lozovaya, N., Eftekhari, S., Cloarec, R., Gouty-Colomer, L. A., Dufour, A., Riffault, B., et al. (2018). GABAergic inhibition in dual-transmission cholinergic and GABAergic striatal interneurons is abolished in Parkinson disease. *Nat. Commun.* 9, 1422. doi: 10.1038/s41467-018-03802-y

Mallet, N., Micklem, B. R., Henny, P., Brown, M. T., Williams, C., Bolam, J. P., et al. (2012). Dichotomous organization of the external globus pallidus. *Neuron* 74, 1075–1086. doi: 10.1016/j.neuron.2012.04.027

Mallet, N., Schmidt, R., Leventhal, D., Chen, F., Amer, N., Boraud, T., et al. (2016). Arkypallidal cells send a stop signal to striatum. *Neuron* 89, 308–316. doi: 10.1016/j.neuron.2015.12.017

Matsumoto, N., Minamimoto, T., Graybiel, A. M., and Kimura, M. (2001). Neurons in the thalamic CM-Pf complex supply striatal neurons with information about behaviorally significant sensory events. *J. Neurophysiol.* 85, 960–976. doi: 10.1152/jn.2001.85.2.960

Maurice, N., Mercer, J., Chan, C. S., Hernandez-Lopez, S., Held, J., Tkatch, T., et al. (2004). D2 dopamine receptor-mediated modulation of voltage-dependent Na + channels reduces autonomous activity in striatal cholinergic interneurons. *J. Neurosci.* 24, 10289–10301. doi: 10.1523/JNEUROSCI.2155-04.2004

Maurice, N., Liberge, M., Jaouen, F., Ztaou, S., Hanini, M., Camon, J., et al. (2015). Striatal cholinergic interneurons control motor behavior and basal ganglia function in experimental parkinsonism. *Cell Rep.* 13, 657–666. doi: 10.1016/j.celrep.2015.09.034

McCarthy, M. M., Moore-Kochlacs, C., Gu, X., Boyden, E. S., Han, X., Kopell, N., et al. (2011). Striatal origin of the pathologic beta oscillations in Parkinson's disease. *Proc. Natl. Acad. Sci.* 108, 11620–11625. doi: 10.1073/pnas.1107748108

McGregor, M. M., and Nelson, A. B. (2019). Circuit Mechanisms of Parkinson's Disease. *Neuron* 101, 1042–1056. doi: 10.1016/j.neuron.2019.03.004

McKinley, J. W., Shi, Z., Kawikova, I., Hur, M., Bamford, U., Sudarsana Devi, S. P., et al. (2019). Dopamine deficiency reduces striatal cholinergic interneuron function in models of parkinson's disease. *Neuron* 103, 1056–1072.e6. doi: 10.1016/j.neuron.2019.06.013

Morris, G., Arkadir, D., Nevet, A., Vaadia, E., and Bergman, H. (2004). Coincident but distinct messages of midbrain dopamine and striatal tonically active neurons. *Neuron* 43, 133–143. doi: 10.1016/j.neuron.2004.06.012

Nambu, A., Tachibana, Y., and Chiken, S. (2015). Cause of parkinsonian symptoms: firing rate, firing pattern or dynamic activity changes? *Basal Ganglia* 5, 1–6. doi: 10.1016/j.baga.2014.11.001

Nicola, S. M., Surmeier, D. J., and Malenka, R. C. (2000). Dopaminergic modulation of neuronal excitability in the striatum and nucleus accumbens. *Annu. Rev. Neurosci.* 23, 185–215. doi: 10.1146/annurev.neuro.23.1.185

Nougaret, S., and Ravel, S. (2015). Modulation of tonically active neurons of the monkey striatum by events carrying different force and reward information. *J. Neurosci.* 35, 15214–15226. doi: 10.1523/JNEUROSCI.0039-15.2015

Oswald, M. J., Oorschot, D. E., Schulz, J. M., Lipski, J., and Reynolds, J. N. J. (2009). IH current generates the afterhyperpolarisation following activation of subthreshold cortical synaptic inputs to striatal cholinergic interneurons. *J. Physiol.* 587, 5879–5897. doi: 10.1113/jphysiol.2009.177600

Pakhotin, P., and Bracci, E. (2007). Cholinergic interneurons control the excitatory input to the striatum. *J. Neurosci.* 27, 391–400. doi: 10.1523/JNEUROSCI.3709-06.2007

Pisani, A., Bonsi, P., Centonze, D., Calabresi, P., and Bernardi, G. (2000). Activation of D2-like dopamine receptors reduces synaptic inputs to striatal cholinergic interneurons. *J. Neurosci.* 20, RC69 1–RC69 6. doi: 10.1523/JNEUROSCI.20-07-j0003.2000

Raz, A., Feingold, A., Zelanskaya, V., Vaadia, E., and Bergman, H. (1996). Neuronal synchronization of tonically active neurons in the striatum of normal and parkinsonian primates. *J. Neurophysiol.* 76, 2083–2088. doi: 10.1152/jn.1996.76.3.2083

Raz, A., Frechter-Mazar, V., Feingold, A., Abeles, M., Vaadia, E., Bergman, H., et al. (2001). Activity of pallidal and striatal tonically active neurons is correlated in mptp-treated monkeys but not in normal monkeys. *J. Neurosci.* 21, RC128 1–5. doi: 10.1523/JNEUROSCI.21-03-j0006.2001

Rehani, R., Atamna, Y., Tiroshi, L., Chiu, W. H., de Jesús Aceves Buendía, J., Martins, G. J., et al. (2019). Activity patterns in the neuropil of striatal cholinergic interneurons in freely moving mice represent their collective spiking dynamics. *Eneuro* 6 (1), ENEURO.0351–18.2018. doi: 10.1523/ENEURO.0351-18.2018

Reynolds, J. N. J., Hyland, B. I., and Wickens, J. R. (2004). Modulation of an afterhyperpolarization by the substantia nigra induces pauses in the tonic firing of striatal cholinergic interneurons. *J. Neurosci.* 24, 9870–9877. doi: 10.1523/JNEUROSCI.3225-04.2004

Salin, P., López, I. P., Kachidian, P., Barroso-Chinea, P., Rico, A. J., Gómez-Bautista, V., et al. (2009). Changes to interneuron-driven striatal microcircuits in a rat model of Parkinson's disease. *Neurobiol. Dis.* 34, 545–552. doi: 10.1016/j.nbd.2009.03.006

Schulz, J. M., and Reynolds, J. N. J. (2013). Pause and rebound: sensory control of cholinergic signaling in the striatum. *Trends Neurosci.* 36, 41–50. doi: 10.1016/j.tins.2012.09.006

Sharott, A., Doig, N. M., Mallet, N., and Magill, P. J. (2012). Relationships between the firing of identified striatal interneurons and spontaneous and driven cortical activities *in vivo*. *J. Neurosci.* 32, 13221–13236. doi: 10.1523/JNEUROSCI.2440-12.2012

Sullivan, M. A., Chen, H., and Morikawa, H. (2008). Recurrent inhibitory network among striatal cholinergic interneurons. *J. Neurosci.* 28, 8682–8690. doi: 10.1523/JNEUROSCI.2411-08.2008

Szalisznyó, K., and Müller, L. (2009). Dopamine induced switch in the subthreshold dynamics of the striatal cholinergic interneurons: a numerical study. *J. Theor. Biol.* 256, 547–560. doi: 10.1016/j.jtbi.2008.09.029

Tan, C. O., and Bullock, D. (2008). A dopamine–acetylcholine cascade: simulating learned and lesion-induced behavior of striatal cholinergic interneurons. *J. Neurophysiol.* 100, 2409–2421. doi: 10.1152/jn.90486.2008

Tanimura, A., Pancani, T., Lim, S. A. O., Tubert, C., Melendez, A. E., Shen, W., et al. (2018). Striatal cholinergic interneurons and Parkinson's disease. *Eur. J. Neurosci.* 47, 1148–1158. doi: 10.1111/ejn.13638

Tozzi, A., de Iure, A., Di Filippo, M., Tantucci, M., Costa, C., Borsini, F., et al. (2011). The distinct role of medium spiny neurons and cholinergic interneurons in the D2/A2A receptor interaction in the striatum: implications for Parkinson's Disease. *J. Neurosci.* 31, 1850–1862. doi: 10.1523/JNEUROSCI.4082-10.2011

Vogt, S. M., and Hofmann, U. G. (2012). Neuromodulation of STDP through short-term changes in firing causality. *Cogn. Neurodyn.* 6, 353–366. doi: 10.1007/s11571-012-9202-4

Wang, Z., Kai, L., Day, M., Ronesi, J., Yin, H. H., Ding, J., et al. (2006). Dopaminergic control of corticostriatal long-term synaptic depression in medium spiny neurons is mediated by cholinergic interneurons. *Neuron* 50, 443–452. doi: 10.1016/j.neuron.2006.04.010

Weiner, D. M., Levey, A. I., and Brann, M. R. (1990). Expression of muscarinic acetylcholine and dopamine receptor mRNAs in rat basal ganglia. *Proc. Natl. Acad. Sci.* 87, 7050–7054. doi: 10.1073/pnas.87.18.7050

Wilson, C. J., and Goldberg, J. A. (2006). Origin of the slow afterhyperpolarization and slow rhythmic bursting in striatal cholinergic interneurons. *J. Neurophysiol.* 95, 196–204. doi: 10.1152/jn.00630.2005

Yan, Z., and Surmeier, D. J. (1997). D5 dopamine receptors enhance Zn2+-sensitive GABA(A) currents in striatal cholinergic interneurons through a PKA/PP1 cascade. *Neuron* 19, 1115–1126. doi: 10.1016/S0896-6273(00)80402-X

Yan, Z., Song, W.-J., and Surmeier, D. J. D. (1997). 2 Dopamine receptors reduce N-Type Ca 2+ currents in rat neostriatal cholinergic interneurons through a membrane-delimited, protein-kinase-c-insensitive pathway. *J. Neurophysiol.* 77, 1003–1015. doi: 10.1152/jn.1997.77.2.1003

Yarom, O., and Cohen, D. (2011). Putative cholinergic interneurons in the ventral and dorsal regions of the striatum have distinct roles in a two choice alternative association task. *Front. Syst. Neurosci.* 5, 36. doi: 10.3389/fnsys.2011.00036

Zhang, Y.-F., and Cragg, S. J. (2017). Pauses in striatal cholinergic interneurons: what is revealed by their common themes and variations? *Front. Syst. Neurosci.* 11, 1–8. doi: 10.3389/fnsys.2017.00080

Zhang, Y. F., Reynolds, J. N. J., and Cragg, S. J. (2018). Pauses in cholinergic interneuron activity are driven by excitatory input and delayed rectification, with dopamine modulation. *Neuron* 98, 918–925.e3. doi: 10.1016/j.neuron.2018.04.027

Ztaou, S., and Amalric, M. (2019). Contribution of cholinergic interneurons to striatal pathophysiology in Parkinson's disease. *Neurochem. Int.* 126, 1–10. doi: 10.1016/j.neuint.2019.02.019

Ztaou, S., Lhost, J., Watabe, I., Torromino, G., and Amalric, M. (2018). Striatal cholinergic interneurons regulate cognitive and affective dysfunction in partially dopamine-depleted mice. *Eur. J. Neurosci.* 48, 2988–3004. doi: 10.1111/ejn.14153

Zucca, S., Zucca, A., Nakano, T., Aoki, S., and Wickens, J. (2018). Pauses in cholinergic interneuron firing exert an inhibitory control on striatal output *in vivo*. *Elife* 7, e32510. doi: 10.7554/eLife.32510

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pharmacology

MINI REVIEW
published: 15 January 2020
doi: 10.3389/fphar.2019.01494

Pedunculopontine Nucleus Degeneration Contributes to Both Motor and Non-Motor Symptoms of Parkinson's Disease

Nicole Elaine Chambers, Kathryn Lanza and Christopher Bishop *

Department of Psychology, Binghamton University, Binghamton, NY, United States

Parkinson's disease (PD) is a neurodegenerative disorder characterized by hypokinetic motor features; however, patients also display non-motor symptoms like sleep disorders. The standard treatment for PD is dopamine replacement with L-DOPA; however, symptoms including gait deficits and sleep disorders are unresponsive to L-DOPA. Notably, these symptoms have been linked to aberrant activity in the pedunculopontine nucleus (PPN). Of late, clinical trials involving PPN deep brain stimulation (DBS) have been employed to alleviate gait deficits. Although preclinical evidence implicating PPN cholinergic neurons in gait dysfunction was initially promising, DBS trials fell short of expected outcomes. One reason for the failure of DBS may be that the PPN is a heterogenous nucleus that consists of GABAergic, cholinergic, and glutamatergic neurons that project to a diverse array of brain structures. Second, DBS trials may have been unsuccessful because PPN neurons are susceptible to mitochondrial dysfunction, Lewy body pathology, and degeneration in PD. Therefore, pharmaceutical or gene-therapy strategies targeting specific PPN neuronal populations or projections could better alleviate intractable PD symptoms. Unfortunately, how PPN neuronal populations and their respective projections influence PD motor and non-motor symptoms remains enigmatic. Herein, we discuss normal cellular and neuroanatomical features of the PPN, the differential susceptibility of PPN neurons to PD-related insults, and we give an overview of literature suggesting a role for PPN neurons in motor and sleep deficits in PD. Finally, we identify future approaches directed towards the PPN for the treatment of PD motor and sleep symptoms.

Keywords: Parkinson's disease, pedunculopontine nucleus, neurodegeneration, gait deficits, non-motor symptoms, REM sleep behavior disorder, pharmacology

OPEN ACCESS

Edited by:
Cristina Miguelez, University of the Basque Country, Spain

Reviewed by:
Sulev Kõks, University of Tartu, Estonia
David Grabli, Sorbonne Universités, France

***Correspondence:**
Christopher Bishop
cbishop@binghamton.edu

Specialty section:
This article was submitted to Neuropharmacology, a section of the journal Frontiers in Pharmacology

Received: *21 July 2019*
Accepted: *19 November 2019*
Published: *15 January 2020*

Citation:
Chambers NE, Lanza K and Bishop C (2020) Pedunculopontine Nucleus Degeneration Contributes to Both Motor and Non-Motor Symptoms of Parkinson's Disease. Front. Pharmacol. 10:1494. doi: 10.3389/fphar.2019.01494

INTRODUCTION

Cellular, Molecular, and Neuroanatomical Properties of Pedunculopontine Tegmental Nucleus

The pedunculopontine tegmental nucleus (PPN), a heterogeneous brainstem structure, consists of glutamatergic, cholinergic, GABAergic, and glycinergic neurons (Mineff et al., 1998; Wang and Morales, 2009; Pienaar and van de Berg, 2013), which co-express neuropeptides including nitric oxide, substance P, atriopeptin, NADPH diaphorase, calcium binding proteins, and corticotropin-releasing factor

(Vincent et al., 1983; Standaert et al., 1986; Austin et al., 1995; Martinez-Gonzalez et al., 2012; D'Onofrio et al., 2015). Co-expression of acetylcholine (ACh) with GABA or glutamate has also been reported (Lavoie and Parent, 1994; Wang and Morales, 2009). The PPN is functionally divided into two components, a rostral portion containing GABAergic, glutamatergic, and sparse cholinergic neurons sending projections primarily to motor structures including the substantia nigra and thalamus, and a caudal portion consisting mainly of cholinergic and glutamatergic neurons projecting to structures involved in reward such as the nucleus accumbens (Dautan et al., 2016; Xiao et al., 2016). PPN neurons also send descending projections to cerebellar nuclei, brainstem structures like the pontinus oralis, gigantocellular nucleus, and the spinal cord to modulate movement and muscle tone (Rye et al., 1988; Spann and Grofova, 1989; Martinez-Gonzalez et al., 2014). See **Figures 1A, B** for PPN efferent projections and their involvement in Parkinson's disease (PD) symptoms.

PPN in PD

PD is associated with degeneration of nigrostriatal dopamine (DA) neurons; however, PPN cholinergic, glutamatergic, substance P, GABAergic, and glycinergic neurons also degenerate in PD (Hirsch et al., 1987; Jellinger, 1988; Gomez-Gallego et al., 2006; Rinne et al., 2008; Pienaar and van de Berg, 2013). Microglial activation and inflammatory responses occurring in PD likely contribute to PPN neurodegeneration (Elson et al., 2016). Cholinergic loss, in particular, is linked to PD symptom severity, especially akinesia and gait deficits (Pahapill and Lozano, 2000; Rinne et al., 2008; Bohnen et al., 2013). Multiple factors likely contribute to death of cholinergic PPN neurons in PD. For instance, mitochondrial DNA deletion occurs more often in PPN cholinergic neurons than in other PPN neurons (Bury et al., 2017). If substantial, this may inhibit protein translation and energy production. In addition, alpha synuclein aggregates may differentially affect PPN neuronal subtypes, as glutamatergic and cholinergic PPN neurons show more effects of alpha synuclein aggregates than GABAergic neurons (Heinrich et al., as cited by Surmeier, 2018).

Furthermore, it is unknown whether cholinergic loss is a downstream response to prior death of monoaminergic neurons, or whether death of PPN cholinergic neurons precipitates loss of DA neurons (Bensaid et al., 2016; MacLaren et al., 2018). Past

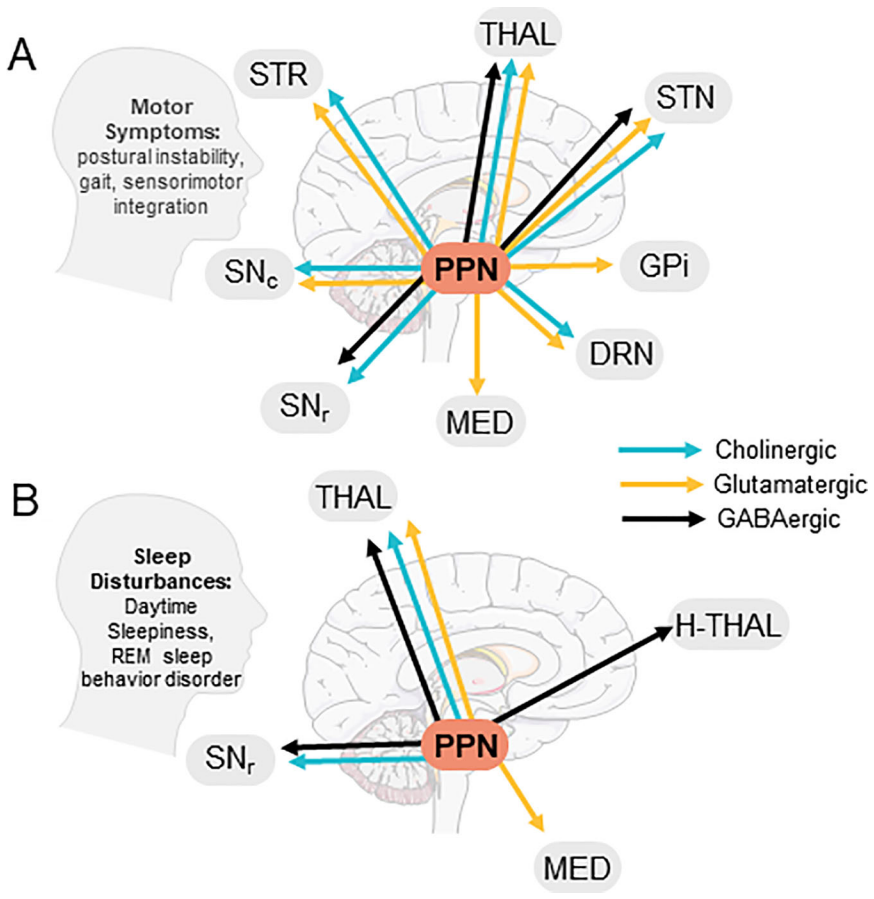


FIGURE 1 | Efferent PPN projections and putative involvement in PD symptoms. **(A)** PPN efferents to brain areas involved in PD motor symptoms, **(B)** PPN efferents contacting brain areas involved in PD-related sleep disturbances. STR, Striatum; THAL, Thalamus; STN, Sub-thalamic nucleus; GPi, Globus Pallidus Interna; DRN, Dorsal Raphe Nucleus; Med, Medulla; SNr, Substantia Nigra Pars Reticulata; SNc, Substantia Nigra Pars Compacta; H-Thal, Hypothalamus; (Images modified from serviermedical art https://smart.servier.com).

research in preclinical models indicates that both processes may occur. Additionally, lesioning both nigrostriatal dopamine and PPN cholinergic systems simultaneously results in more severe degeneration (Bensaid et al., 2016). These results imply that the reciprocal connections shared by PPN cholinergic and nigrostriatal DA neurons may be critical to their survival, and that simultaneous lesion alters cellular activity, potentially contributing to neurodegeneration. Accordingly, following nigrostriatal DA lesion, compensatory increases in activity occur in PPN neurons (Palombo et al., 1990; Carlson et al., 1999; Breit et al., 2001; Zhang et al., 2008). Initial or sustained hyperactivity of PPN glutamatergic or subthalamic nucleus glutamatergic neurons may accelerate nigrostriatal and PPN neurodegeneration and may be exacerbated by simultaneous loss of PPN cholinergic neurons. PPN hyperactivity may also arise due to loss of nigral DA and raphe serotonin afferents in PD, as PPN neurons contain the inhibitory DA D2R and 5HT1AR (Leonard and Llinás, 1994). Conversely, PPN hypoactivity (Jang et al., 2012) caused by increased SNr and GP inputs may lead to more severe PD symptoms (Nandi et al., 2002). Perhaps, PD heterogeneity could be partially explained by the order of cellular degeneration and by differential PPN activity. Future research should investigate the timing of neuronal loss, affected projections, and behaviors resulting from cell loss in these two lesion paradigms.

Although under-investigated, a recent study in preclinical models indicates that galanin may rescue PPN cholinergic neurons in PD. Following SNc lactacystin lesion, galanin is upregulated in rostral PPN cholinergic neurons (Elson et al., 2018). Galanin inhibits cholinergic transmission and may be neuroprotective, as it guards basal forebrain cholinergic neurons from amyloid beta toxicity in Alzheimer's disease (Ding et al., 2006; Pirondi et al., 2010; Elliot-Hunt et al., 2011). In PD, galanin could reduce the likelihood of apoptosis in response to Lewy bodies or neuroinflammation (however, see also, Crawley et al., 2002).

PPN Involvement in Movement

The role of the PPN in generating locomotion remains controversial; however, early studies suggest PPN involvement in gait, an assertion that has been partially supported and further refined based on recent studies. Early research shows that the PPN and cuneiform nucleus make up the mesencephalic locomotor region (MLR), a functional neurocircuit modulating movement, rhythm, and speed upon electrical stimulation (Garcia-Rill et al., 1987; Garcia-Rill and Skinner, 1988; see **Figure 1A** for PPN projections related to PD motor symptoms). MLR projections to the lateral paragigantocellular nucleus (LPGi) modulate activity of motorneurons and ventral spinal laminae "central pattern generators" that regulate patterns of locomotor activity (Grillner and Wallen, 1985). In support of the PPN's role in gait, fMRI studies demonstrate that PPN activity increases during fast imagined walking in healthy individuals (Bakker et al., 2008; Karachi et al., 2010); however, whether the PPN exerts its effects through ascending or descending pathways in actual fast walking is unknown. Although both PPN glutamatergic and cholinergic neurons may exert effects through LGPi efferents, recent data from preclinical models show that these neurons play different roles in locomotion. Based on these studies, glutamatergic neurons control flexor muscles to stop locomotion and are involved in slow-paced movement and exploratory behavior (Caggiano et al., 2018; Josset et al., 2018), whereas cholinergic neurons control extensor muscles and prolong the stance phase of locomotion and can also increase speed (Roseberry et al., 2016).

Ascending PPN efferents are also well-positioned to influence movement, although most existing data on this subject stem from research in preclinical models. Most importantly, the PPN projects to thalamic nuclei, which guide movements *via* thalamospinal projections. Additionally, although less direct, PPN glutamatergic and cholinergic inputs to SNc and striatum may affect both direct and indirect pathways. Relatedly, PPN cholinergic terminals in SNc drive locomotion (Xiao et al., 2016), whereas the motor outcomes of PPN afferents to striatum remain enigmatic, but are likely modulatory in nature (Dautan et al., 2016; Assous and Tepper, 2019). Finally, PPN cholinergic and GABAergic inputs to SNr exert influence over the nigrostriatal pathway. There, PPN ACh inhibits MSN terminals *via* M4R, inhibiting movement (Moehle et al., 2017), and PPN GABAergic projections improve motor learning through thalamic disinhibition (Li and Spitzer, 2019). Though PPN neurons likely contribute to normal gait, the role of neurons in the basal forebrain and cuneiform nucleus should also be carefully considered (Xiang et al., 2013; Sarter et al., 2014).

PPN Involvement in PD Gait and Motor Deficits

Nearly 20%–60% of PD patients experience gait dysfunction including freezing of gait (FOG) that is not consistently improved by DA replacement therapy (Giladi et al., 2001). FOG and falls are more common in PD patients exhibiting decreased ACh metabolism and reuptake in the thalamus (Bohnen et al., 2009; Bohnen and Albin, 2011; Bohnen et al., 2019), where the PPN is the main source of ACh. Cell death and synaptic inhibition from the GPi and SNr likely contribute to this decrease in cholinergic tone. In animal models, chemogenetic activation of PPN ACh neurons rescues motor deficits, indicating that cholinergic neurons remain which may be suitable therapeutic targets in PD (Pienaar et al., 2015). Similarly, cholinesterase inhibitors decrease falls in some PD patients (Chung et al., 2010).

Given its role in locomotion, the PPN has been considered as a site for deep brain stimulation (DBS) to improve otherwise intractable postural instability, FOG, and falling in PD and atypical Parkinsonism. Results of this approach have been varied. Multiple studies demonstrate that PPN-DBS can ameliorate FOG or general gait parameters in PD patients (Pereira et al., 2008; Ferraye et al., 2010; Khan et al., 2011; Thevathasan et al., 2011; Khan et al., 2012a; Khan et al., 2012b; Mazzone et al., 2014; Welter et al., 2015; Mestre et al., 2016). Although unilateral (Moro et al., 2010) and bilateral (Ferraye et al., 2010) approaches decrease falls, double-blind studies indicate the superiority of bilateral PPN stimulation for

improving PD-related gait symptoms (Thevathasan et al., 2010; Thevathasan et al., 2012). Conversely, postural instability is not consistently improved with PPN-DBS (Moro et al., 2010; Thevathasan et al., 2010). Despite symptomatic improvements in some studies, open questions remain on the clinical relevance of PPN-DBS, as it cannot eliminate PD gait symptoms, and exact mechanisms underlying PPN-DBS effects are unknown. Furthermore, side effects ranging from oscillopsia, paresthesia, and even worsening of FOG have occurred in some patients (Hamani et al., 2007).

Multiple factors explain inconsistent PPN-DBS clinical outcomes. First, electrode placements in the PPN of PD patients vary. MRI placements correlated with patient outcomes suggest bilateral lead placement in caudal PPN is most efficacious for gait improvement (Goetz et al., 2018; Khan et al., 2012b). Relatedly, preclinical data show that rostral PPN lead placement has deleterious effects on movement and that caudal PPN controls stepping behavior (Gut and Winn, 2015; Garcia-Rill, 1986; Garcia-Rill and Skinner, 1988; Garcia-Rill, 1991). Second, stimulation parameters for PPN-DBS differ from one study to another. Many paradigms employ frequencies within beta and gamma ranges, but some have also employed stimulation at very low frequencies. Finally, small sample sizes, differences in disease progression, and the heterogeneity of PD signs and symptoms likely contribute to differential results regarding PPN-DBS.

PPN Contribution to L-DOPA Response

DA replacement therapy with L-DOPA is standard treatment for PD. However, chronic use results in debilitating abnormal involuntary movements termed L-DOPA-induced dyskinesia (LID) in up to 90% of PD patients (Ahlskog and Muenter, 2001). Severe LID can lead to hospitalization and reduced quality of life (Péchevis et al., 2005; Lyoo and Lee, 2011). Although the PPN is not well investigated for its role in L-DOPA's motor efficacy and in LID, existing evidence from patients undergoing PPN-DBS and preclinical evidence on the PPN's involvement in motor stereotypy suggest that the PPN may contribute.

For instance, PD patients decrease their L-DOPA dose following long-term treatment with PPN-DBS (Mazzone et al., 2014), suggesting that PPN-DBS has pro-motor effects and may potentiate L-DOPA's motor efficacy. Prior combination of PPN-DBS with L-DOPA shows additional motor benefit beyond L-DOPA alone (Jenkinson et al., 2008; Stefani et al., 2007). Additionally, combining caudal PPN-DBS with L-DOPA in preclinical models results in attenuation of LID (Gut and Winn, 2015). Similarly, immediate early genes associated with development and expression of LID are upregulated in the PPN of dyskinetic rodents (Bastide et al., 2014), implicating the PPN in LID expression.

The PPN has long been known to contribute to motor stereotypy in preclinical models; contextualizing this literature may provide useful insights into the role of the PPN in LID, as research on the PPN's contribution to LID is sparse. PPN lesion produces orolingual stereotypy in response to d-amphetamine and apomorphine (Inglis et al., 1994), implying that PPN neurons suppress stereotyped movements in the normal brain. Furthermore, existing evidence shows that PPN GABAergic and cholinergic neurons have differing effects on motor stereotypy. PPN infusion of GABAergic antagonists reduces motor stereotypy produced by apomorphine and SNr microinfusion of muscimol (Childs and Gale, 1983), intimating that PPN GABA projections inhibit SNr neurons to promote motor stereotypy, or that SNr GABA projections to PPN promote motor stereotypy. Conversely, PPN infusion of mAChR antagonists increases motor stereotypy and exacerbates LID (Mathur et al., 1997; Chambers et al., 2019). Taken together, these results suggest that decreasing GABAergic tone or increasing cholinergic tone in the PPN may decrease LID, but this must be examined further. If true, this provides further support for hypoactivity of the PPN in PD. Future research should address specific contributions of PPN neuronal subtypes, and investigate the role of PPN efferents to the SNr and striatum.

PPN Involvement in Sleep

The PPN is part of the reticular activating system (RAS), a group of nuclei regulating consciousness and attention. The PPN receives lateral hypothalamus orexin projections and SNr GABA projections that regulate muscle tone during sleep and wakefulness (Takakusaki et al., 2011, see **Figure 1B**). As mentioned previously, SNr and GPi GABA transmission to PPN is enhanced in PD. Importantly, when GABA B receptors are bound, REM sleep is inhibited (Sakai and Koyama, 1996; Ulloor et al., 2004; Datta, 2007). This alone may account for a significant portion of REM sleep problems in PD.

PPN cholinergic neurons promote EEG desynchronization and changes in state of consciousness *via* thalamic projections which regulate cortical activity (Hallanger et al., 1987; Steriade et al., 1990; Kobayashi et al., 2002; Mena-Segovia et al., 2008; Kotagal et al., 2012). PPN cholinergic neurons are active during REM sleep and wakefulness, manifesting alpha activity at rest and gamma oscillations related to behavior during waking (Steriade et al., 1991; Steriade et al., 1996; Kezunovic et al., 2011). During REM sleep, cholinergic neurons promote muscle atonia through projections to the subcoeruleus dorsalis (Baghdoyan et al., 1984; Sanford et al., 1994), and nucleus reticularis gigantocellularis neurons which project to motorneurons (Takakusaki et al., 2011). Cholinergic neurons promote REM and pontogeniculooccipital waves characteristic of REM sleep *via* dorsolateral geniculate nucleus and frontal eye field efferents (Sakai et al., 1976; Callaway et al., 1987; Shouse and Siegel, 1992; Rye, 1997). During slow wave sleep cholinergic neurons also promote nested gamma oscillations associated with memory replay and neuronal plasticity in cortex and hippocampus (Lee and Wilson, 2002).

PPN Involvement in PD-Related Sleep Dysfunction

Sleep disturbances resulting in excessive daytime sleepiness occur in 98% of PD patients (for review see Comella, 2007). While prevalence of PD-related sleep disorders alone is concerning, sleep dysfunction, particularly REM sleep behavior

TABLE 1 | Potential targets for PPN-mediated improvement of PD motor and non-motor symptoms.

PPN Afferent/Efferent Projection	Symptom(s)	References
Intralaminar and Ventromedial Thalamus (Efferent)	Motor Deficits (Freezing of gait, falling) REM Sleep Behavior Disorder	(Bohnen et al., 2009; Hallanger et al., 1987; Bohnen and Albin, 2011; Brazhnik et al., 2016; Bohnen et al., 2019; Kezunovic et al., 2011; Steriade et al., 1990; Mena-Segovia et al., 2008)
Globus Pallidus Interna (Afferent)	Akinesia, freezing of gait	(Nandi et al., 2002; Zhang et al., 2012)
Lateral Paragigantocellular Nucleus (Efferent)	Trouble initiating movement	(Garcia-Rill et al., 1987; Garcia-Rill and Skinner, 1988; Roseberry et al., 2016; Josset et al., 2018)
Lateral Reticular Thalamic Nucleus, Superior Colliculus, Frontal Eye Fields (Efferent)	Visual Sensorimotor Integration/ Oscillopsia resulting from PPN-DBS	(Redgrave et al., 1987; Wallace and Fredens, 1988; Okada and Kobayashi, 2009)
Striatum (Efferent)	Motor Symptoms (akinesia) L-DOPA-induced dyskinesia	(Dautan et al., 2016; Assous and Tepper, 2019)
Substantia Nigra Pars Reticulata (Efferent)	L-DOPA-induced Dyskinesia REM Sleep Behavior Disorder	(Childs and Gale, 1983; Moehle et al., 2017)
Vestibular System (Afferent)	Postural Instability	(Cai et al., 2018; Vitale et al., 2011)

disorder (RBD), is linked to increased risk of cognitive impairment in PD patients (Marion et al., 2008; Gagnon et al., 2009). RBD is present in an estimated 50% of PD patients and is characterized by lack of muscle atonia during REM sleep which leads to patients engaging in complex motor behaviors during sleep. Intriguingly, diagnosis with idiopathic RBD often precedes PD and other synucleinopathies and may represent a prodromal phase of these illnesses (Boeve et al., 2001).

PPN ACh and substance-P-expressing neurons may contribute to the pathogenesis of RBD. Despite similar age of onset and disease duration, PD patients with RBD show decreased cholinergic transmission in cortex and thalamus relative to PD patients without RBD (Kotagal et al., 2012; however see also Bedard et al., 2019). Similarly, AChE inhibitors decrease symptoms of RBD, meaning that RBD may arise due to loss of cholinergic tone (Ringman and Simmons, 2000). Substance P is co-expressed in 27% of caudal PPN ACh neurons and is co-released onto the pontoreticular formation, where it has additive effects beyond those of ACh on initiation and maintenance of sleep (Kohlmeier et al., 2002). Substance P neurons degenerate in PD, which likely contributes to REM sleep atonia in PD patients. Congruent with PPN involvement in sleep dysfunction, PPN-DBS increases sleep efficiency, REM and stage 2 sleep, and decreases awakenings in PD patients (Romigi et al., 2008). Although understudied, these findings demonstrate that PPN-DBS could be used in the future to improve sleep for PD patients.

Therapeutic Strategies/Future Directions

Interpreting PPN-DBS outcomes is impeded by small sample sizes, differences in electrode placement, stimulation paradigms, and disease progression. Additionally, PPN-DBS paradigms employ open-loop DBS which does not consider the situation and/or symptoms the patient is experiencing, nor brain activity in other areas which influence PD symptoms. Employing closed-loop PPN-DBS may be more effective (Rosin et al., 2011; Hebb et al., 2014; Sun and Morrell, 2014), as different stimulation frequencies have varying effects on PD symptoms. Using machine learning strategies, clinicians could correlate specific patterns of EEG readout with problems with FOG or other symptoms, and then use this information to promote more efficacious PPN-DBS. Future research should also address effects of exercise on the PPN, as recent data show that similar to hippocampal and basal forebrain cholinergic neurons (Molteni et al., 2008; Hall and Savage, 2016), exercise may induce PPN neuroplasticity beneficial for motor learning (Li and Spitzer, 2018). Finally, gene-therapy strategies focusing on neuroprotection through galanin or modulating L-type calcium channels should be considered, as well as gene therapy strategies that target specific PPN projections for symptomatic relief (see **Table 1**).

CONCLUSION

Overall, through innervation of ascending and descending motor structures, RAS, and cortex, the PPN is well-positioned to modify PD motor and sleep symptoms. However, to develop effective PPN-centered treatment strategies, there is a need for cell-specific manipulations in the PPN. Currently, this is challenging, as the contribution of specific PPN pathways to gait and RBD symptoms, and pathways that govern PPN's modulation of L-DOPA's effects are not well understood. Additionally, receptor types specific to each PPN neuron and how they change in health and disease are not well-defined. Therefore, future research should elucidate the contribution of specific PPN afferent and efferent connections to behavior and identify receptors exclusive to each type of PPN neuron. Additionally, as PD is a heterogeneous disorder, it is especially important that the therapeutic strategies employed are custom-tailored to the patient based on his or her symptoms.

AUTHOR CONTRIBUTIONS

Contributed to writing of the manuscript: NC, CB Created figures: KL.

FUNDING

American Parkinson's Disease Association Research.

REFERENCES

Ahlskog, J. E., and Muenter, M. D. (2001). Frequency of levodopa-related dyskinesias and motor fluctuations as estimated from the cumulative literature. *Movement Disord.* 16, 448–458. doi: 10.1002/mds1090

Assous, M., and Tepper, J. M. (2019). Excitatory extrinsic afferents to striatal interneurons and interactions with striatal microcircuitry. *Eur. J. Neurosci.* 49 (5), 593–603. doi: 10.1111/ejn.13881

Austin, M., Rice, P., Mann, J., and Arango, V. (1995). Localization of corticotropin-releasing hormone in the human locus coeruleus and pedunculopontine tegmental nucleus: an immunocytochemical and *in situ* hybridization study. *Neuroscience* 64, 713–727. doi: 10.1016/0306-4522(94)00420-A

Baghdoyan, H. A., Rodrigo-Angulo, M. L., McCarley, R. W., and Hobson, J. A. (1984). Site-specific enhancement and suppression of desynchronized sleep signs following cholinergic stimulation of three brainstem regions. *Brain Res.* 306, 39–52. doi: 10.1016/0006-8993(84)90354-8

Bakker, M., De Lange, F. P., Helmich, R. C., Scheeringa, R., Bloem, B. R., and Toni, I. (2008). Cerebral correlates of motor imagery of normal and precision gait. *NeuroImage* 41, 998–1010. doi: 10.1016/j.neuroimage.2008.03.020

Bastide, M. F., Dovero, S., Charron, G., Porras, G., Gross, C. E., Fernagut, P.-O., et al. (2014). Immediate-early gene expression in structures outside the basal ganglia is associated to L-DOPA-induced dyskinesia. *Neurobiol. Dis.* 62, 179–192. doi: 10.1016/j.nbd.2013.09.020

Bedard, M.-A., Aghourian, M., Legault-Denis, C., Postuma, R. B., Soucy, J. P., Gagnon, J.-F., et al. (2019). Brain alterations in idiopathic REM sleep behavior disorder: a PET imaging study with 18F-FEOBV. *Sleep Med.* 58, 35–41. doi: 10.1016/j.sleep.2018.12.020

Bensaid, M., Michel, P. P., Clark, S. D., Hirsch, E. C., and François, C. (2016). Role of pedunculopontine cholinergic neurons in Parkinson's disease. *Exp. Neurol.* 275, 209–219. doi: 10.1016/j.expneurol.2015.11.004

Boeve, B. F., Silber, M. H., Ferman, T. J., Lucas, J. A., and Parisi, J. E. (2001). Association of REM sleep behavior disorder and neurodegenerative disease may reflect an underlying synucleinopathy. *Mov. Disord.* 16, 622–630. doi: 10.1002/mds1120

Bohnen, N. I., and Albin, R. L. (2011). The cholinergic system and Parkinson disease. *Behav. Brain Res.* 221 (2), 564–573. doi: 10.1016/j.bbr.2009.12.048

Bohnen, N. I., Frey, K. A., Studenski, S., Kotagal, V., Koeppe, R. A., Scott, P. J., et al. (2013). Gait speed in Parkinson disease correlates with cholinergic degeneration. *Neurology* 81 (18), 1611–1616. doi: 10.1212/WNL.0b013e3182a9f558

Bohnen, N. I., Kanel, P., Zhou, Z., Koeppe, R. A., Frey, K. A., Dauer, W. T., et al. (2019). Cholinergic system changes of falls and freezing of gait in Parkinson's disease. *Ann. Neurol.* 85 (4), 538–549. doi: 10.1002/ana.25430

Bohnen, N. I., Muller, M. L., Koeppe, R. A., Studenski, S. A., Kilbourn, M. A., Frey, K. A., et al (2009). History of falls in Parkinson's disease is associated with reduced cholinergic activity. *Neurology* 73, 1670–1676. doi: 10.1212/WNL.0b013e3181c1ded6

Brazhnik, E., McCoy, A. J., Novikov, N., Hatch, C. E., and Walters, J. R. (2016). Ventral medial thalamic nucleus promotes synchronization of increased high beta oscillatory activity in the basal ganglia—thalamocortical network of the hemiparkinsonian rat. *J. Neursci.* 361, 4196–4208. doi: 10.1523/JNEUROSCI.3582-152016

Breit, S., Bouali-Benazzouz, R., Benabid, A.-L., and Benazzouz, A. (2001). Unilateral lesion of the nigrostriatal pathway induces an increase of neuronal activity of the pedunculopontine nucleus, which is reversed by the lesion of the subthalamic nucleus in the rat. *Eur. J. Neurosci.* 14, 1833–1842. doi: 10.1046/j.0953-816x.2001.01800.x

Bury, A. G., Pyle, A., Elson, J. L., Greaves, L., Morris, C. M., Hudson, G., et al. (2017). Mitochondrial DNA changes in pedunculopontine cholinergic neurons in Parkinson disease. *Ann. Neurol.* 82 (6), 1016–1021. doi: 10.1002/ana.25099

Caggiano, V., Leiras, R., Goni-Erro, H., Masini, D., Bellardita, C., Bouvier, J., et al. (2018). Midbrain circuits that set locomotor speed and gait selection. *Nature* 553, 455–460. doi: 10.1038/nature25448

Cai, J., Lee, S., Ba, F., Garg, S., Kim, L. J., Liu, A., et al. (2018). Galvanic vestibular stimulation (GVS) augments deficient pedunculopontine nucleus connectivity in mild Parkinson's disease: fMRI effects of different stimuli. *Front. Neurosci.* 101, 1–13. doi: 10.3389/fnins.2018.00101

Callaway, C. W., Lydic, R., Baghdoyan, H. A., and Hobson, J. A. (1987). Pontogeniculooccipital waves: spontaneous visual system activity during rapid eye movement sleep. *Cell. Mol. Neurobiol.* 7, 105–149. doi: 10.1007/BF00711551

Carlson, J. D., Pearlsteinb, R. D., Buchholza, J., Iaconoc, R. P., and Maedaa, G. (1999). Regional metabolic changes in the pedunculopontine nucleus of unilateral 6-hydroxydopamine Parkinson's model rats. *Brain Res.* 828 (1-2), 12–19. doi: 10.1016/S0006-8993(99)01268-8

Chambers, N. E., Meadows, S. M., Taylor, A., Sheena, E., Lanza, K., Conti, M. M., et al (2019). Effects of muscarinic acetylcholine m1 and m4 receptor blockade on dyskinesia in the hemi-parkinsonian rat. *Neurosci* 409, 180–194. doi: 10.1016/j.neuroscience.2019.04.008

Childs, J. A., and Gale, K. (1983). Evidence that the nigrostriatal GABAergic projection mediates stereotypy induced by apomorphine and intranigral muscimol. *Life Sciences* 33, 1007–1010. doi: 10.1016/0024-3205(83)90758-0

Chung, K. A., Lobb, B. M., Nutt, J. G., and Horak, F. B. (2010). Effects of a central cholinesterase inhibitor on reducing falls in Parkinson disease. *Neurology* 75, 1263–1269. doi: 10.1212/WNL.0b013e3181f6128c

Comella, C. L. (2007). Sleep disorders in Parkinson's disease. *Movement Disord.* 22, S367–S373. doi: 10.1002/mds.21682

Crawley, J. N., Mufson, E. J., Hohmann, J. G., Teklemichael, D., Steiner, R. A., Holmbergy, K., et al. (2002). Galanin overexpressing transgenic mice. *Neuropeptides* 36, 145–156. doi: 10.1054/npep.20020891

D'Onofrio, S., Kezunovic, N., Hyde, J. R., Luster, B., Messias, E., Urbano, F. J., et al. (2015). Modulation of gamma oscillations in the pedunculopontine nucleus by neuronal calcium sensor protein-1: Relevance to schizophrenia and bipolar disorder. *J. Neurophysiol.* 113, 709–719. doi: 10.1152/jn.008282014

Datta, S. (2007). Activation of pedunculopontine tegmental PKA prevents GABAB receptor activation-mediated rapid eye movement sleep suppression in the freely moving rat. *J. Neurophysiol.* 97, 3841–3850. doi: 10.1152/jn.00263.2007

Dautan, D., Bay, H. H., Bolam, J. P., Gerdjikov, T. V., and Mena-Segovia, J. (2016). Extrinsic innervation of the striatal complex: a whole-brain mapping analysis. *Front. Neuroanat.* 10 (1), 1–10. doi: 10.3389/fnana.2016.00001

Ding, X., MacTavish, D., Kar, S., and Jhamandas, J. H. (2006). Galanin attenuates beta-amyloid (Abeta) toxicity in rat cholinergic basal forebrain neurons. *Neurobiol. Dis.* 21, 413–420. doi: 10.1016/j.nbd.2005.08.016

Elliot-Hunt, C. R., Holmes, F. E., Hartley, D. M., Perez, S., Mufson, E. J., and Wynick, D. (2011). Endogenous galanin protects mouse hippocampal neurons against amyloid beta toxicity *in vitro via* activation of galanin receptor-2. *J. Alzheimers Dis.* 25, 455–452. doi: 10.3233/JAD-2011-110011

Elson, J. L., Yates, A., and Pienaar, I. S. (2016). Pedunculopontine cell loss and protein aggregation direct microglia activation in parkinsonian rats. *Brain Struct. Funct.* 221, 2319–2341. doi: 10.1007/s00429-015-1045-4

Elson, J. L., Kochaj, R., Reynolds, R., and Pienaar, I. S. (2018). Temporal-spatial profiling of pedunculopontine galanin-cholinergic neurons in the lactacystin rat model of Parkinson's disease. *Neurotoxic. Res.* 34 (1), 16–31. doi: 10.1007/s12640-017-9846-2

Ferraye, M. U., Debû, B., Fraix, V., Goetz, L., Ardouin, C., Yelnik, J., et al. (2010). Effects of pedunculopontine nucleus area stimulation on gait disorders in Parkinson's disease. *Brain* 133 (1), 205–214. doi: 10.1093/brain/awp229

Gagnon, J. F., Vendette, M., Postuma, R. B., Desjardins, C., Massicotte-Marquez, J., Panisset, M., et al. (2009). Mild cognitive impairment in rapid eye movement sleep behavior disorder and Parkinson's disease. *Ann. Neurol.* 66, 39–47. doi: 10.1002/ana.21680

Garcia-Rill, E., and Skinner, R. D. (1988). Modulation of rhythmic function in the posterior midbrain. *Neurosciences* 17, 639–654. doi: 10.1016/0306-4522(88)90295-3

Garcia-Rill, E. (1986). The basal ganglia and the locomotor regions. *Brain Res. Rev.* 11, 47–63. doi: 10.1016/0165-0173(86)90009-3

Garcia-Rill, E., Houser, C. R., Skinner, R. D., Smith, W., and Woodward, D. J. (1987). Locomotion-inducing sites in the vicinity of the pedunculopontine nucleus. *Brain Res. Bull.* 18, 731– 738. doi: 10.1016/0361-9230(87)90208-5

Garcia-Rill, E. (1991). The pedunculopontine nucleus. *Prog. Neurobiol.* 36, 363–389. doi: 10.1016/0301-0082(91)90016-T

Giladi, N., Treves, T. A., Simon, E. S., Shabtai, H., Orlov, Y., Kandinov, B., et al. (2001). Freezing of gait in patients with advanced Parkinson's disease. *J. Neural Transm.* 108, 53–61. doi: 10.1007/s007020170096

Goetz, L., Bhattacharjee, M., Ferraye, M. U., Fraix, V., Maineri, C., Nosko, D., et al. (2018). Deep brain stimulation of the pedunculopontine nucleus area in Parkinson disease: MRI-based anatomoclinical correlations and optimal target. *Neurosurgery* 84, 506–518. doi: 10.1093/neuros/nyy151

Gomez-Gallego, M., Fernandez-Villalba, E., Fernandez-Barreiro, A., and Herrero, M. T. (2006). Changes in neuronal activity in the pedunculopontine nucleus in chronic MPTP-treated primates: an *in situ* hybridization study of cytochrome oxidase subunit I, choline acetyl transferase and substance P mRNA expression. *J. Neural Transm.* 114, 319–326. doi: 10.1007/s00702-006-0547-x

Grillner, S., and Wallen, P. (1985). Central pattern generators for locomotion, with special reference to vertebrates. *Annu. Rev. Neurosci.* 8 (1), 233–261. doi: 10.1146/annurev.ne.08.030185.001313

Gut, N. K., and Winn, P. (2015). Deep brain stimulation of different pedunculopontine targets in a novel rodent model of parkinsonism. *J. Neurosci.* 35, 4792–4803. doi: 10.1523/JNEUROSCI.3646-142015

Hall, J. M., and Savage, L. M. (2016). Exercise leads to the re-emergence of the cholinergic/nestin neuronal phenotype within the medial septum/diagonal band and subsequent rescue of both hippocampal ACh efflux and spatial behavior. *Exp. Neurol.* 278, 62–75. doi: 10.1016/j.expneurol.2016.01.018

Hallanger, A. E., Levey, A. I., Lee, H. J., Rye, D. B., and Wainer, B. H. (1987). The origins of cholinergic and other subcortical afferents to the thalamus in the rat. *J. Comp. Neurol.* 262 (1), 105–124. doi: 10.1002/cne.902620109

Hamani, C., Stone, S., Laxton, A., and Lozano, A. M. (2007). The pedunculopontine nucleus and movement disorders: Anatomy and the role for deep brain stimulation. *Parkinsonism Relat. Disord.* 13, S276–S280. doi: 10.1007/s00702-010-0547-8

Hebb, A. O., Zhang, J. J., Mahoor, M. H., Tsiokos, C., Matlack, C., Chizeck, H. J., et al. (2014). Creating the feedback loop: Closed-loop neurostimulation. *Neurosurg. Clin. N. Am.* 25, 187–204. doi: 10.1016/j.nec.2013.08.006

Hirsch, E. C., Graybiel, A. M., Duyckaerts, C., and Javoy-Agid, F. (1987). Neuronal loss in the pedunculopontine tegmental nucleus in Parkinson disease and in progressive supranuclear palsy. *Proc. Natl. Acad. Sci.* 84 (16), 5976–5980. doi: 10.1073/pnas.84.165976

Inglis, W. L., Allen, F., Whitelaw, R. B., Latimer, M. P., Brace, H. M., and Winn, P. (1994). An investigation into the role of the pedunculopontine tegmental nucleus in the mediation of locomotion and orofacial stereotypy induced by d-amphetamine and apomorphine in the rat. *Neuroscience* 58, 817–833. doi: 10.1016/0306-4522(94)90459-6

Inkster, L. M., and Eng, J. J. (2004). Postural control during a sit-to-stand task in individuals with mild Parkinson's disease. *Exp. Brain Res.* 154 (1), 33–38. doi: 10.1007/s00221-003-1629-8

Jang, D. P., Min, H.-K., Lee, S.-Y., Kim, I. Y., Park, H. W., Im, Y. H., et al. (2012). Functional neuroimaging of the 6-OHDA lesion rat model of Parkinson's disease. *Neurosci. Lett.* 513 (2), 187–192. doi: 10.1016/j.neulet.2012.02.034

Jellinger, K. (1988). The pedunculopontine nucleus in Parkinson's disease, progressive supranuclear palsy and Alzheimer's disease. *J. Neurol. Neurosurg. Psychiatry* 51 (4), 540–543. doi: 10.1136/jnnp.51.4.540

Jenkinson, N., Nandi, D., Oram, R., Stein, J. F., and Aziz, T. Z. (2008). Pedunculopontine nucleus electric stimulation alleviates akinesia independently of dopaminergic mechanisms. *Neuroreport* 17 (6), 639–641. doi: 10.1097/00001756-200604240-00016

Josset, N., Roussel, M., Lemieux, M., Lafrance-Zoubga, D., Rastqar, A., and Bretzner, F. (2018). Distinct contributions of mesencephalic locomotor region nuclei to locomotor control in the freely behaving mouse. *Curr. Biol.* 28, 884–901. doi: 10.1016/j.cub.2018.02.007

Karachi, C., Grabli, D., Bernard, F. A., Tandé, D., Wattiez, N., Belaid, H., et al. (2010). Cholinergic mesencephalic neurons are involved in gait and postural disorders in Parkinson disease. *J. Clin. Invest.* 120 (8), 2745–2754. doi: 10.1172/JCI42642

Kezunovic, N., Urbano, F. J., Simon, C., Hyde, J., Smith, K., and Garcia-Rill, E. (2011). Mechanism behind gamma band activity in the pedunculopontine nucleus (PPN). *Eur. J. Neurosci.* 34, 404–415. doi: 10.1111/j.1460-9568.2011.07766.x

Khan, S., Mooney, L., Plaha, P., Javed, S., White, P., Whone, A. L., et al. (2011). Outcomes from stimulation of the caudal zona incerta and pedunculopontine nucleus in patients with Parkinson's disease. *Br. J. Neurosurg.* 25 (2), 273–280. doi: 10.3109/02688697.2010.544790

Khan, S., Gill, S. S., Mooney, L., White, P., Whone, A., Brooks, D. J., et al. (2012a). Combined pedunculopontine-subthalamic stimulation in Parkinson disease. *Neurology* 78, 1090–1095. doi: 10.1212/WNL.0b013e31824e8e96

Khan, S., Javed, S., Mooney, L., White, P., Plaha, P., Whone, A., et al. (2012b). Clinical outcomes from bilateral versus unilateral stimulation of the pedunculopontine nucleus with and without concomitant caudal zona incerta region stimulation in Parkinson's disease. *Br. J. Neurosurg.* 26, 722–725. doi: 10.3109/02688697.2012.659297

Kobayashi, Y., Inoue, Y., Yamamoto, M., Isa, T., and Aizawa, H. (2002). Contribution of pedunculopontine tegmental nucleus neurons to performance of visually guided saccade task in monkeys. *J Neurophysiol.* 88, 715–731. doi: 10.1152/jn.2002.88.2.715

Kohlmeier, K. A., Burns, J., Reiner, P. B., and Semba, K. (2002). Substance P in the descending cholinergic projection to REM sleep-induction regions of the rat pontine reticular formation: anatomical and electrophysiological analyses. *Eur. J. Neurosci.* 15, 176–196. doi: 10.1046/j.0953-816x.2001.01829.x

Kotagal, V., Albin, R. L., Müller, M. L., Koeppe, R. A., Chervin, R. D., Frey, K. A., et al. (2012). Symptoms of rapid eye movement sleep behavior disorder are associated with cholinergic denervation in Parkinson disease. *Ann. Neurol.* 71, 560–568. doi: 10.1002/ana.22691

Lavoie, B., and Parent, A. (1994). Pedunculopontine nucleus in the squirrel monkey: cholinergic and glutamatergic projections to the substantia nigra. *J. Comp. Neurol.* 344 (2), 232–241. doi: 10.1002/cne.903440205

Lee, A. K., and Wilson, M. A. (2002). Memory of sequential experience in the hippocampus during slow wave sleep. *Neuron* 36, 1183–1194. doi: 10.1016/S0896-6273(02)01096-6

Leonard, C. S., and Llinás, R. (1994). Serotonergic and cholinergic inhibition of mesopontine cholinergic neurons controlling REM sleep: an *in vitro* electrophysiological study. *Neuroscience* 59, 309–330. doi: 10.1016/0306-4522(94)90599-1

Li, H.-Q., and Spitzer, N. C. (2018). Exercise enhances motor skill learning by neurotransmitter switching in the adult midbrain. *bioRxiv*. 1–34. doi: 10.1101/613539

Lyoo, H. C., and Lee, M. S. (2011). Rhabdomyolysis induced by severe levodopa induced dyskinesia in a patient with Parkinson's disease. *J. Neurol.* 258, 1893–1894. doi: 10.1007/s00415-011-6041-x

MacLaren, D. A., Ljungberg, T. L., Griffin, M. E., and Clark, S. D. (2018). Pedunculopontine tegmentum cholinergic loss leads to a progressive decline in motor abilities and neuropathological changes resembling progressive supranuclear palsy. *Eur. J. Neurosci.* 48 (12), 3477–3497. doi: 10.1111/ejn.14212

Marion, M. H., Qurashi, M., Marshall, G., and Foster, O. (2008). Is REM sleep behavior disorder (RBD) a risk factor of dementia in idiopathic Parkinson's disease? *J. Neurol.* 255, 192–196. doi: 10.1007/s00415-008-0629-9

Martinez-Gonzalez, C., Wang, H. L., Micklem, B. R., Bolam, J. P., and Mena-Segovia, J. (2012). Subpopulations of cholinergic, GABAergic and glutamatergic neurons in the pedunculopontine nucleus contain calcium-binding proteins and are heterogeneously distributed. *Eur. J. Neurosci.* 35, 723–734. doi: 10.1111/j.1460-9568.2012.08002.x

Martinez-Gonzalez, C., van Andel, J., Bolam, J. P., and Mena-Segovia, J. (2014). Divergent motor projections from the pedunculopontine nucleus are differentially regulated in Parkinsonism. *Brain Struct. Funct.* 219, 1415–1462. doi: 10.1007/s00429-013-0579-6

Mathur, A., Shandarin, A., LaViolette, S. R., Parker, J., and Yeomans, J. S. (1997). Locomotion and stereotypy induced by scopolamine: Contributions of muscarinic receptors near the pedunculopontine tegmental nucleus. *Brain Res.* 775, 144–155. doi: 10.1016/S0006-8993(97)00928-1

Mazzone, P., Paoloni, M., Mangone, M., Santilli, V., Insola, A., Fini, M., et al. (2014). Unilateral deep brain stimulation of the pedunculopontine tegmental nucleus in idiopathic Parkinson's disease: effects on gait initiation and performance. *Gait Posture* 40 (3), 357–362. doi: 10.1016/j.gaitpost.2014.05.002

Mena-Segovia, J., Sims, H. M., Magill, P. J., and Bolam, J. P. (2008). Cholinergic brainstem neurons modulate cortical gamma activity during slow oscillations. *J. Physiol.* 586, 2947–2960. doi: 10.1113/jphysiol.2008.153874

Mestre, T. A., Sidiropoulos, C., Hamani, C., Poon, Y. Y., Lozano, A. M., Lang, A. E., et al. (2016). Long-term double-blinded unilateral pedunculopontine area stimulation in Parkinson's disease. *Movement Disord.* 31 (10), 1570–1574. doi: 10.1002/mds.26710

Mineff, E. M., Popratiloff, A., Romansky, R., Kazakos, V., Kaimaktschieff, V., Usunoff, K. G., et al. (1998). Evidence for a possible glycinergic inhibitory neurotransmission in the midbrain and rostral pons of the rat studied by gethyrin. *Arch. Physiol. Biochem.* 106 (3), 210–220. doi: 10.1076/apab.106.3.2104377

Moehle, M. S., Pancani, T., Byun, N., Yohn, S. E., Wilson, G. H.III, Dickerson, J. W., et al. (2017). Cholinergic projections to the substantia nigra pars reticulata inhibit dopamine modulation of basal ganglia through the M4 muscarinic receptor. *Neuron* 96 (6), 1358–1372. doi: 10.1016/j.neuron.2017.12.008

Molteni, R., Ying, Z., and Gomez-Pinilla, F. (2008). Differential effects of acute and chronic exercise on plasticity-related genes in the rat hippocampus revealed by microarray. *Eur. J. Neurosci.* 16, 1107–1116. doi: 10.1046/j.1460-9568.2002.02158.x

Moro, E., Hamani, C., Poo, Y.-Y, Al-Khairallah, T., Dostrovsky, O., et al. (2010). Unilateral pedunculopontine stimulation improves falls in Parkinson's disease. *Brain* 133 (1), 215–224. doi: 10.1093/brain/awp261

Nandi, D., Aziz, T. Z., Giladi, N., Winter, J., and Stein, J. F. (2002). Reversal of akinesia in experimental parkinsonism by GABA antagonist microinjections in the pedunculopontine nucleus. *Brain* 125 (11), 2418–2430. doi: 10.1093/brain/awf259

Okada, K.-I., and Kobayashi, Y. (2009). Characterization of oculomotor and visual activities in the primate pedunculopontine tegmental nucleus during visually guided saccade tasks. *Eur. J. Neurosci.* 30, 2211–2223. doi: 10.1111/j.1460-9568.2009.07009

Pahapil, P. A., and Lozano, A. M. (2000). The pedunculopontine nucleus and Parkinson's disease. *Brain* 123, 1767–1783. doi: 10.1093/brain/123.9.1767

Palombo, E., Porrino, L. J., Bakiewicz, K. S., Crane, A. M., Sokoloff, L., and Kopin, L. J. (1990). Local cerebral glucose utilization in monkeys with hemiparkinsonism induced by intracaratoid infusion of the neurotoxin MPTP. *J. Neurosci.* 10 (3), 860–869. doi: 10.1523/JNEUROSCI.10-03-008601990

Péchevis, M., Clarke, C. E., Vieregge, P., Khoshnood, B., Deschaseaux-Voinet, C., Berdeaux, G., et al. (2005). Effects of dyskinesias in Parkinson's disease on quality of life and health-related costs: A prospective European study. *Eur. J. Neurol.* 12, 956–963. doi: 10.1111/j.1468-1331.2005.01096.x

Pereira, E. A., Muthusamy, K. A., De Pennington, N., Joint, C. A., and Aziz, T. Z. (2008). Deep brain stimulation of the pedunculopontine nucleus in Parkinson's disease. Preliminary experience at Oxford. *Br. J. Neurosurg.* 22 (sup1), S41–S44. doi: 10.1080/02688690802448335

Pienaar, I. S., and van de Berg, W. (2013). A non-cholinergic neuronal loss in the pedunculopontine nucleus of toxin-evoked Parkinsonian rats. *Exp. Neurol.* 248, 213–223. doi: 10.1016/j.expneurol.2013.06.008

Pienaar, I. S., Gartside, S. E., Sharma, P., De Paola, V., Gretenkord, S., Withers, D., et al. (2015). Pharmacogenetic stimulation of cholinergic pedunculopontine neurons reverses motor deficits in a rat model of Parkinson's disease. *Mol. Neurodegener.* 10, 1–22. doi: 10.1186/s13024-015-0044-5

Pirondi, S., Giuliani, A., Del, V. G., Giardino, L., Hokfelt, T., and Calza, L. (2010). The galanin receptor 2/3 agonist Gal2-11 protects the SN56 cells against beta-amyloid 25–35 toxicity. *J. Neurosci. Res.* 88 (5), 1064–1073. doi: 10.1002/jnr.22278

Redgrave, P., Mitchell, I. J., and Dean, P. (1987). Descending projections from the superior colliculus in rat: a study using orthograde transport of wheatgerm-agglutinin conjugated horseradish peroxidase. *Exp. Brain Res.* 68, 147–167. doi: 10.1007/BF00255241

Ringman, J. M., and Simmons, J. H. (2000). Treatment of REM sleep behavior disorder with donepezil: a report of three cases. *Neurology* 55 (6), 870–871. doi: 10.1212/WNL.55.6.870

Rinne, J. O., Ma, S. Y., Lee, M. S., Collan, Y., and Röyttä, M. (2008). Loss of cholinergic neurons in the pedunculopontine nucleus in Parkinson's disease is related to disability of the patients. *Parkinson. Relat. Disord.* 14 (7), 553–557. doi: 10.1016/j.parkreldis.2008.01.006

Romigi, A., Placidi, F., Peppe, A., Pierantozzi, M., Izzi, F., Brusa, L., et al (2008). Pedunculopontine nucleus stimulation influences REM sleep in Parkinson's disease. *Eur. J. Neurol.* 15, e64–e65. doi: 10.1111/j.1468-1331.2008.02167.x

Roseberry, T. K., Lee, A. M., Lalive, A. L., Wilbrecht, L., Bonci, A., and Kreitzer, A. C. (2016). Cell-type-specific control of brainstem locomotor circuits by basal ganglia. *Cell* 164 (3), 526–537. doi: 10.1016/j.cell.2015.12.037

Rosin, B., Slovik, M., Mitelman, R., Rivlin Etzion, M., Haber, S., Israel, Z., et al. (2011). Closed-loop deep brain stimulation is superior in ameliorating parkinsonism. *Neuron* 72, 370–384. doi: 10.1016/j.neuron.2011.08.023

Rye, D. B., Lee, H. J., Saper, C. B., and Wainer, B. H. (1988). Medullary and spinal efferents of the pedunculopontine tegmental nucleus and adjacent mesopontine tegmentum in the rat. *J. Comp. Neurol.* 269, 315–341. doi: 10.1002/cne.902690302

Rye, D. B. (1997). Contributions of the pedunculopontine region to normal and altered REM sleep. *Sleep* 20, 757–788. doi: 10.1093/sleep/20.9.757

Sakai, K., and Koyama, Y. (1996). Are there cholinergic and non-cholinergic paradoxical sleep-on neurons in the pons. *Neuroreport* 7, 2449–2453. doi: 10.1097/00001756-199611040-00009

Sakai, K., Petitjean, F., and Jouvet, M. (1976). Effects of ponto-mesencephalic lesions and electrical stimulation upon PGO waves and EMPs in unanesthetized cats. *Electroencephalogr. Clin. Neruophysiol.* 41, 49–63. doi: 10.1016/0013-4694(76)90214-5

Sanford, L. D., Morrison, A. R., Mann, G. I., Harris, J. S., Yoo, L., and Ross, R. J. (1994). Sleep patterning and behavior in cats with pontine lesions creating REM without atonia. *J. Sleep Res.* 527, 213–223. doi: 10.1111/j.1365-2869.1994.tb00136.x

Sarter, M., Albin, R. L., Kucinski, A., and Lustig, C. (2014). Where attention falls: increased risk of falls from the converging impact of cortical cholinergic and midbrain dopamine loss on striatal function. *Exp. Neurol.* 257, 120–129. doi: 10.1016/j.expneurol.2014.04.032

Shouse, M., and Siegel, J. M. (1992). Pontine regulation of REM sleep components in cats: Integrity of the pedunculopontine tegmentum (PPT) is important for phasic events but unnecessary for atonia during REM sleep. *Brain Res* 571, 50–63. doi: 10.1016/0006-8993(92)90508-7

Simon, C., Kezunovic, N., Ye, M., Hyde, J., Hayar, A., Williams, D. K., et al. (2010). Gamma band unit activity and population responses in the pedunculopontine nucleus. *J. Neurophys.* 104, 463–474. doi: 10.1152/jn.002422010

Spann, B. M., and Grofova, I. (1989). Origin of ascending and spinal pathways from the nucleus tegmenti pedunculopontinus in the rat. *J. Comp. Neurol.* 283, 13–27. doi: 10.1002/cne.902830103

Standaert, D., Saper, C., Rye, D., and Wainer, B. (1986). Colocalization of atriopeptin-like immunoreactivity with choline acetyltransferase- and substance—P-like immunoreactivity in the pedunculopontine and laterodorsal tegmental nuclei in the rat. *Neuroscience* 382, 163–168. doi: 10.1016/0006-8993(86)90126-5

Stefani, A., Lozano, A. M., Peppe, A., Stanzione, P., Galati, S., Tropepi, D., et al. (2007). Bilateral deep brain stimulation of the pedunculopontine and subthalamic nuclei in severe Parkinson's disease. *Brain* 130 (6), 1596–1607. doi: 10.1093/brain/awl346

Steriade, M., Datta, S., Paré, D., Oakson, G., and Dossi, C. (1990). Neuronal activities in brain-stem cholinergic nuclei related to tonic activation processes in thalamocortical systems. *J. Neurosci.* 10, 2541–2559. doi: 10.1523/JNEUROSCI.10-08-025411990

Steriade, M., Dossi, R. C., Paré, D., and Oakson, G. (1991). Fast oscillations (20-40 Hz) in thalamocortical systems and their potentiation by mesopontine cholinergic nuclei in the cat. *PNAS* 88, 4396–4400. doi: 10.1073/pnas.88.104396

Steriade, M., Amzica, F., and Contreras, D. (1996). Synchronization of Fast (30-40 Hz) spontaneous cortical rhythms during brain activation. *J. Neurosci.* 16, 392–417. doi: 10.1523/JNEUROSCI.16-01-003921996

Sun, F. T., and Morrell, M. J. (2014). Closed-loop neurostimulation: the clinical experience. *Neurotherapeutics* 11, 553–563. doi: 10.1007/s13311-014-0280-3

Surmeier, J. (2018). *Etiology of Parkinson"s disease: Cells and circuits* (San Diego, CA: Presentation at Neuroscience of Movement Disorders).

Takakusaki, K., Obara, K., Nozu, T., and Okumura, T. (2011). Modulatory effects of the GABAergic basal ganglia neurons on the PPN and the muscle tone inhibitory system in cats. *Arch. Italiennes Biol.* 149, 383–405. doi: 10.4449/aib.v149i4.1383

Thevathasan, W., Silburn, P. A., Brooker, H., Coyne, T. J., Khan, S., Gill, S. S., et al. (2010). The impact of low-frequency stimulation of the pedunculopontine nucleus region on reaction time in parkinsonism. *J. Neurol. Neurosurg. Psychiatry* 81 (10), 1099–1104. doi: 10.1136/jnnp.2009.189324

Thevathasan, W., Coyne, T. J., Hyam, J. A., Kerr, G., Jenkinson, N., Aziz, T. Z., et al. (2011). Pedunculopontine nucleus stimulation improves gait freezing in Parkinson disease. *Neurosurgery* 69 (6), 1248–1254. doi: 10.1227/NEU.0b013e31822b6f71

Thevathasan, W., Cole, M. H., Graepel, C. L., Hyam, J. A., Jenkinson, N., Brittain, J. S., et al. (2012). A spatiotemporal analysis of gait freezing and the impact of pedunculopontine nucleus stimulation. *Brain* 135 (5), 1446–1454. doi: 10.1093/brain/aws039

Ulloor, J., Mavanji, V., Saha, S., Siwek, D. F., and Datta, S. (2004). Spontaneous REM sleepis modulated by the activation of the pedunculopontine tegmental $GABA_B$ receptors in the freely moving rat. *J. Neurophysiol.* 91, 1822–1831. doi: 10.1152/jn.01104.2003

Vincent, S. R., Satoh, K., Armstrong, D. M., and Fibiger, H. C. (1983). Substance P in the ascending cholinergic reticular system. *Nature* 306, 688–691. doi: 10.1038/306688a0

Vitale, C., Marcelli, V., Furia, T., Santangelo, G., Cozzolino, A., Longo, K., et al. (2011). Vestibular impairment and adaptive postural imbalance in Parkinsonian patients with lateral trunk flexion. *Mov. Disord.* 26, 1458–1463. doi: 10.1002/mds.23657

Wallace, M. N., and Fredens, K. (1988). Origin of high acetylcholinesterase activity in the mouse superior colliculus. *Exp. Brain Res.* 72, 335–346. doi: 10.1007/BF00250255

Wang, H. I., and Morales, M. (2009). Pedunculopontine and laterodorsal tegmental nuclei contain distinct populations of cholinergic, glutamatergic, and GABAergic neurons in the rat. *Eur. J. Neurosci.* 29, 340–358. doi: 10.1111/j.1460-9568.2008.06576.x

Welter, M. L., Demain, A., Ewenczyk, C., Czernecki, V., Lau, B., El Helou, A., et al. (2015). PPNa-DBS for gait and balance disorders in Parkinson's disease: a double-blind, randomised study. *J. Neurol.* 262 (6), 1515–1525. doi: 10.1007/s00415-015-7744-1

Xiang, H.-B., Zhu, W.-Z., Guan, X.-H, and Ye, D. W. (2013). The cuneiform nucleus may be involved in the regulation of skeletal muscle tone by motor pathway: A virally mediated trans-synaptic tracing study in surgically sympathectomized mice. *Brain* 136, 1–4. doi: 10.1093/brain/awt123

Xiao, C., Cho, J. R., Zhou, C., Treweek, J. B., Chan, K., McKinney, S. L., et al. (2016). Cholinergic mesopontine signals govern locomotion and reward through dissociable midbrain pathways. *Neuron* 90, 333–347. doi: 10.1016/j.neuron.2016.03.028

Zhang, Q. J., Liu, J., Wang, Y., Wang, S., Wu, Z. H., Yan, W., et al. (2008). The firing activity of presumed cholinergic and non-cholinergic neurons of the pedunculopontine nucleus in 6-hydroxydopamine-lesioned rats: An *in vivo* electrophysiological study. *Brain Res.* 1243, 152–160. doi: 10.1016/j.brainres.2008.09.028

Zhang, J., Wang, Z. I., Baker, K. B., and Vitek, J. L. (2012). Effect of globus pallidus internus stimulation on neuronal activity in the pedunculopontine tegmental nucleus in the primate model of Parkinson's disease. *Exp. Neurol.* 233 (1), 575–580. doi: 10.1016/j.expneurol.2011.07.007

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neuroscience

REVIEW
published: 29 January 2020
doi: 10.3389/fnins.2020.00025

Neuropsychiatric Disorders in Parkinson's Disease: What Do We Know About the Role of Dopaminergic and Non-dopaminergic Systems?

Kathy Dujardin[1] and Véronique Sgambato[2]**

[1] Inserm U1171 Degenerative and Vascular Cognitive Disorders, Lille University Medical Center, Lille, France, [2] CNRS, Institut des Sciences Cognitives Marc Jeannerod, UMR 5229, Lyon University, Bron, France

Besides the hallmark motor symptoms (rest tremor, hypokinesia, rigidity, and postural instability), patients with Parkinson's disease (PD) have non-motor symptoms, namely neuropsychiatric disorders. They are frequent and may influence the other symptoms of the disease. They have also a negative impact on the quality of life of patients and their caregivers. In this article, we will describe the clinical manifestations of the main PD-related behavioral disorders (depression, anxiety disorders, apathy, psychosis, and impulse control disorders). We will also provide an overview of the clinical and preclinical literature regarding the underlying mechanisms with a focus on the role of the dopaminergic and non-dopaminergic systems.

Keywords: depression, anxiety, apathy, psychosis, impulse control disorders, Parkinson's disease, basal ganglia, animal models

OPEN ACCESS

Edited by:
Andrew C. McCreary, Uniqure B.V., Netherlands

Reviewed by:
Susan Fox, University Health Network (UHN), Canada
Philippe Huot, McGill University, Canada

****Correspondence:***
Kathy Dujardin
kathy.dujardin@univ-lille.fr
Véronique Sgambato
veronique.sgambato@inserm.fr

Specialty section:
This article was submitted to Neuropharmacology, a section of the journal Frontiers in Neuroscience

Received: *09 September 2019*
Accepted: *10 January 2020*
Published: *29 January 2020*

Citation:
Dujardin K and Sgambato V (2020) Neuropsychiatric Disorders in Parkinson's Disease: What Do We Know About the Role of Dopaminergic and Non-dopaminergic Systems? Front. Neurosci. 14:25. doi: 10.3389/fnins.2020.00025

INTRODUCTION

Parkinson's disease (PD) is a neurodegenerative disease resulting from progressive death of the dopaminergic neurons in the brainstem, particularly the substantia nigra pars compacta (SNc). Besides the hallmark motor symptoms (hypokinesia, rest tremor, rigidity, and postural instability), PD patients have non-motor symptoms including neuropsychiatric disorders (Chaudhuri et al., 2006). These behavioral disorders can be separated into two categories according to their pathogenesis. Depression, anxiety, apathy, and partly psychosis are consequences of the neurodegenerative process while some psychotic symptoms and impulse control disorders (ICDs) occur as adverse effects of some antiparkinsonian drugs. In this article, we will describe the clinical manifestations of these behavioral disorders and provide some elements of epidemiology. We will also provide an overview of the current literature regarding the underlying mechanisms with a focus on the role of the dopaminergic and non-dopaminergic systems at both the clinical and preclinical levels.

SEARCH STRATEGY

A structured search in PubMed for articles written in English up to September 2019 was performed. The following keywords and MeSH terms were used: Parkinson's disease, depression, depressive, anxiety, anxious, apathy, apathetic, psychosis, hallucination, hallucinatory, delusion, ICDs,

impulsivity, impulsive, compulsion, compulsive, gambling, hypersexuality, animal model, 6-OHDA, MPTP, alpha-synuclein, pathophysiology, pathophysiological mechanisms, neurotransmission, neuromodulation, catecholamine, dopamine, dopaminergic system, dopaminergic pathway, non-dopaminergic, non-dopaminergic pathway, serotonin, norepinephrine, noradrenaline, acetylcholine, glutamate, GABA.

DEPRESSION

The prevalence of clinically relevant depressive symptoms in patients with PD ranges from 30 to 35% according to most studies (Reijnders et al., 2008; Aarsland et al., 2011). A study on a very large sample of PD patients showed that this prevalence was higher in female than males, at advanced stages of the disease and in patients with dementia (Riedel et al., 2010).

It is noteworthy that 10 to 27% of early, untreated PD patients have significant depressive symptoms (Ravina et al., 2007b; Dujardin et al., 2014; Weintraub et al., 2015b). Moreover, depression may be a prodromal symptom of PD (Schapira et al., 2017) even though it is not a risk factor for the disease (Ascherio and Schwarzschild, 2016). In PD, there is an increased risk of depression among women, in the advanced stages of the disease and in patients with cognitive impairment (Aarsland et al., 2011).

Core symptoms of depression in PD are sadness, depressed mood, loss of pleasure, feelings of worthless, and guilt. Diagnosis is difficult because some symptoms of depression like slowness, loss of weight, sleep disturbances, or poor emotional expression are also common symptoms of PD and frequently occur in non-depressed PD patients (Okun and Watts, 2002; Brown et al., 2011). According to the last version of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5), patients with PD may present minor, major or persistent depressive disorders. Depression is often comorbid with anxiety in PD (Brown et al., 2011).

The pathophysiology of depression in PD remains largely unknown and multiple factors (genetic, inflammatory, cellular regulation, signaling pathways, . . .) are probably incriminated. The loss of dopamine (DA) in the mesocortical and mesolimbic pathways, leading to dysfunction of the orbito-frontal area, is likely involved (Vriend et al., 2014a), as well as the loss of striatal DA, particularly in the caudate (Vriend et al., 2014b). Molecular neuroimaging studies have shown that noradrenaline (NA) (Remy et al., 2005) and serotonin (5-HT) (Boileau et al., 2008; Politis et al., 2010; Ballanger et al., 2012; Maillet et al., 2016) also play a role. The severity of depression in early untreated PD patients correlates with the reduction of the serotonergic innervation in the anterior cingulate cortex (Maillet et al., 2016). Cholinergic deficits may also be involved in PD patients with depression and cognitive deficits (Bohnen et al., 2007). Looking for blood-based biomarkers in PD, one study reported that lower plasma levels of 5-HT and its metabolite 5HIAA correlate with more severe depression in PD patients (Tong et al., 2015). However, data are few and sometimes inconsistent (Aarsland et al., 2011). Further studies are needed to determine how DA and non-DA systems interact in the regulation of mood in PD.

Most of PD patients with depression are treated with selective serotonin reuptake inhibitors (SSRIs) (Starkstein and Brockman, 2017). The efficacy of dopamine agonists to counteract depression is still controversial as pramipexole induced positive results (Barone et al., 2010) but not rotigotine (Chung et al., 2016). One clinical trial has been completed on the safety and efficacy of pimavanserin, a selective 5-HT_{2A} antagonist/inverse agonist, to treat depression in PD but there are no results available (ClinicalTrials.gov Identifier: NCT03482882). Finally, there was a clinical trial on the use of atomoxetine, a potent NA reuptake inhibitor (ClinicalTrials.gov Identifier: NCT00304161) which has been completed and gave negative results as already shown by the same group previously (Weintraub et al., 2010a).

These clinical data are to be compared with experimental data obtained in rodents (rat and mouse) and non-human primate (NHP) models of PD (see **Table 1**). These models afford substantial resources to investigate the etiology and pathophysiology of PD as well as to develop therapeutic approaches. Animal models of PD include acute toxin models, such as 6-hydroxydopamine (6-OHDA) or 1-methyl-4phenyl-1,2,3,6-tertrahydropyridine (MPTP), as well as genetic models, such as α-synuclein, Parkin and monoamine-related alterations. Depressive-like behavior can be observed in animals using several behavioral paradigms, such as the tail suspension test (Cryan et al., 2005) and the forced swim test (Porsolt et al., 1977). These tests have been used in animal models of PD and most of the studies showed that partial and bilateral DA lesion of the nigrostriatal pathway induced depression-like behaviors, which were predominantly counteracted by dopaminergic medication, although some studies found positive effects of SSRIs. Results are far less strong for genetic studies, which showed either no difference or an increase of depression.

By looking more specifically at each of these models, MPTP-treated mice (with a moderate non-selective DA lesion) exhibited an increased depressive-like behavior (Mori et al., 2005; Vuckovic et al., 2008), which was counteracted by L-DOPA or a D2 agonist (Mori et al., 2005). However, these MPTP mice studies did not specifically target the nigrostriatal or mesostriatal dopaminergic pathways and 5-HT levels were also downregulated by MPTP.

The unilateral lesion of the substantia nigra pars compacta (SNc) with the toxin 6-OHDA also induced a depressive-like behavior and a hyperactivity in the lateral habenula. Interestingly, injection of muscimol (a $GABA_A$ agonist) in this area had antidepressant-like effects by decreasing the firing rate of the lateral habenula neurons and increasing serotonin release within the medial prefrontal cortex (Wang T. et al., 2017). Degeneration of the nigrostriatal pathway may impair GABA transmission in the lateral habenula, which play a role in regulating PD mood. Furthermore, the modulation of different 5-HT receptors within the lateral habenula also impacted depressive-like behavior in hemiparkinsonian rats (Han et al., 2015, 2016) and lesions of the lateral habenula cause a decrease in depressive-like behavior and an increase of 5-HT levels in the raphe nuclei (Luo et al., 2015).

TABLE 1 | Summary of the preclinical studies investigating the pathophysiology of PD-related neuropsychiatric-like disorders.

Behavior	Animal model	Target region (+ treatment if any)	Reduced/reversed by	Study
Depression-like	6-OHDA-lesioned mice	Bilateral dorsolateral striatum	Pramipexole, Reboxetine	Bonito-Oliva et al., 2014
		Bilateral SNc	L-DOPA, pramipexole	Chiu et al., 2015
	6-OHDA-lesioned rats	Bilateral dorsal striatum		Branchi et al., 2008
		Bilateral medial VTA		Drui et al., 2014
		Bilateral MFB		Faggiani et al., 2015
		Bilateral MFB and 5-HT/NA lesions		Faggiani et al., 2015
		Bilateral SNc	L-DOPA, Ropinirole, SKF38393, Sumanirole, PD-128907	Carnicella et al., 2014; Drui et al., 2014
		Bilateral SNc	Ketamine, Imipramine	Vecchia et al., 2018
		Bilateral SNc		Santiago et al., 2010
		Bilateral SNc and olfactory bulb		Ilkiw et al., 2019
		Bilateral ventrolateral dorsal striatum		Tadaiesky et al., 2008; da Silva et al., 2016
		Olfactory bulb		Ilkiw et al., 2019
		Unilateral MFB		Eskow Jaunarajs et al., 2010
		Unilateral MFB	Sarizotan	Zhang et al., 2011
		Unilateral MFB	5-HT_{1A}, 5-HT_6 agonist; 5-HT_7 agonist or antagonist	Hui et al., 2014; Liu et al., 2015; Zhang et al., 2015
		Unilateral SNc	Muscimol	Wang T. et al., 2017
		Unilateral SNc	5-HT_7 antagonist	Han et al., 2015, 2016
		Unilateral SNc and VTA	Citalopram, L-DOPA	Winter et al., 2007
	MPTP-intoxicated mice			Vuckovic et al., 2008
			L-DOPA and D2 agonist	Mori et al., 2005
	MPTP-intoxicated rats	Bilateral SNc		Santiago et al., 2010
	D3 dopamine receptor knockout mice			Moraga-Amaro et al., 2014
	Engrailed1 heterozygote mice			Le Pen et al., 2008
	Human α-synuclein overexpressing rats	Bilateral SNc		Caudal et al., 2015
	MitoPark mice			Cong et al., 2016
	VMAT2 low expression mice			Taylor et al., 2009
Anxiety-like	6-OHDA-lesioned mice	Bilateral dorsolateral striatum	Pramipexole, Reboxetine	Bonito-Oliva et al., 2014
	6-OHDA-lesioned rats	Bilateral MFB	L-DOPA	Faggiani et al., 2018
		Bilateral SNc	SKF-38193, Sumanirole, PD-1289907	Carnicella et al., 2014; Drui et al., 2014
		Bilateral SNc		Campos et al., 2013
		Bilateral SNc	L-DOPA	Loiodice et al., 2019
		Bilateral striatum	MPEP (mGluR5 antagonist)	Chen et al., 2011a
		Bilateral striatum		Tadaiesky et al., 2008
		Unilateral MFB		Eskow Jaunarajs et al., 2010
		Unilateral MFB	L-DOPA	Zhang et al., 2011; O'Connor et al., 2016
		Unilateral MFB	5-HT_{1A} agonist, 5-HT_6 agonist or antagonist	Sun et al., 2015
	MPTP-intoxicated mice			Gorton et al., 2010
	MPTP-intoxicated rats	Bilateral SNc		Wang et al., 2009; Sy et al., 2010
	Parkin exon 3 knockout mice			Zhu et al., 2007
	VMAT2 low expression mice			Taylor et al., 2009
	A53T α-synuclein transgenic rhesus macaques			Niu et al., 2015

(Continued)

TABLE 1 | Continued

Behavior	Animal model	Target region (+ treatment if any)	Reduced/reversed by	Study
Apathy-like	6-OHDA-lesioned rats	Bilateral SNc	Ropinirole, Pramipexole, PD-128907	Carnicella et al., 2014; Drui et al., 2014; Favier et al., 2014
	MPTP-intoxicated macaques			Brown et al., 2012; Tian et al., 2015
	MPTP-intoxicated macaques	(+ Bicuculline)		Sgambato-Faure et al., 2016
	VMAT2 low expression mice			Baumann et al., 2016
Psychosis-like	6-OHDA-lesioned rats	Bilateral SNc	Pimavanserin	McFarland et al., 2011; Hubbard et al., 2013
	MPTP-intoxicated macaques	(+ L-DOPA)	MDMA	Beaudoin-Gobert et al., 2015
	MPTP-intoxicated marmosets	(+ L-DOPA, Apomorphine, Pergolide, Ropinirole, Pramipexole)	Haloperidol, Clozapine, Quietapine	Fox et al., 2006; Visanji et al., 2006
Impulsive-like	6-OHDA-lesioned rats	Bilateral striatum		Tedford et al., 2015
	6-OHDA-lesioned rats	Bilateral striatum (+ Pramipexole)	Mirtazapine	Holtz et al., 2016
	Human A53T α-synuclein overexpressing rats	Bilateral SNc		Engeln et al., 2016; Jiménez-Urbieta et al., 2019
	Human A53T α-synuclein overexpressing rats	Bilateral SNc (+ Pramipexole)		Engeln et al., 2016; Jiménez-Urbieta et al., 2019
Compulsive-like	6-OHDA-lesioned rats	Bilateral SNc and VTA (+ Pramipexole)		Dardou et al., 2017
	MPTP-intoxicated macaques	(+ Bicuculline)		Sgambato-Faure et al., 2016
	MPTP-intoxicated African green monkeys			

In association to the substantia nigra and raphe, the lateral habenula then probably contributes to the pathophysiological mechanisms of depression. After a moderate bilateral DA lesion of the SNc (noradrenergic neurons were protected from 6-OHDA with desipramine), rats had no locomotion impairment but exhibited more depressive-like behaviors which were reversed by L-DOPA, ropinirole (a D2/D3 agonist), SKF-38393 (a selective D1 agonist), sumanirole (a selective D2 agonist) and PD-128907 (a preferred D3 agonist) but not citalopram (a SSRI) (Carnicella et al., 2014; Drui et al., 2014). This suggests that the injury of the DA nigrostriatal pathway is involved in the pathogenesis of depressive-like behavior in this PD animal model. In agreement with these data, both L-DOPA and pramipexole had anti-depressant effects in the forced swim test and both normalized decreased neurogenesis in the hippocampus in mice with bilateral 6-OHDA intra-nigral lesions (Chiu et al., 2015). Administration of ketamine (an antagonist of NMDA glutamatergic receptors) or imipramine (noradrenalin and serotonin reuptake inhibitor) can also improve depressive-like behavior in rats (Vecchia et al., 2018). Another study has shown that after bilateral intranigral injection of either MPTP or 6-OHDA (leading both to a 50% DA cell loss in the SNc), rats exhibited depressive-like behaviors which were associated to hippocampal reductions of DA, 5-HT and NA, suggesting an involvement of these monoamines in depression after nigral lesions (Santiago et al., 2010). Interestingly, after a partial (around 50%) 6-OHDA lesion of DA neurons from the olfactory bulb, rats exhibited an olfactory impairment, but also depressive-like behaviors (Ilkiw et al., 2019). Moreover, this lesion exacerbated the depressive-like behaviors, classically induced by the bilateral and partial lesion of SNc (Ilkiw et al., 2019), evidencing that the degeneration of the DA pathways within the olfactory bubble also impacts depression in PD.

Regarding the potential involvement of the ventral tegmental area (VTA), conflicting results have been obtained since rats with a bilateral but partial (around 50%) lesion of the medial part of VTA displayed no motor deficits, nor depressive-like behavior (Drui et al., 2014) but anhedonia, a major symptom of depression (Dardou et al., 2014). Another study showed that combined lesions of SNc and VTA led to no motor deficits but a depressive-like behavior, whose severity increased with nigral or the VTA lesions. It was reversed by both citalopram and L-DOPA (Winter et al., 2007). This suggests that SSRIs might be efficient at least partly by modulating the DA system (Ainsworth et al., 1998; Gambarana et al., 1999).

Unilateral 6-OHDA injections into the medial forebrain bundle (MFB) can also be used to model PD in rodents even though it leads to an extensive and not selective DA lesion. Several studies have reported depressive-like behaviors in such lesioned rats without any motor deficit (Winter et al., 2007; Zhang et al., 2011). L-DOPA failed to reverse these behaviors (Eskow Jaunarajs et al., 2010) while acute administration of sarizotan, an agonist at 5-HT receptors and partial agonist at D2 DA receptors had an antidepressant effect (Zhang et al., 2011). Again, these results fit well with the known involvement of the serotonin system in the modulation of depression. Activation of prelimbic 5-HT_{1A} receptors produced antidepressant effects while their blockade favored depressive-like behavior in the MFB-lesioned rats (Hui et al., 2014). It has also been shown that the modulation of 5-HT_6 or 5-HT_7 receptors within either the prelimbic cortex or the hippocampus had an impact on depressive-like behaviors (Liu et al., 2015; Zhang et al., 2015, 2016). After bilateral injection of 6-OHDA in the MFB, rats exhibited depressive-like behavior, which was dramatically exacerbated when lesions of serotonergic and noradrenergic neurons were combined with

the DA one (Faggiani et al., 2015). These data emphasize again the involvement of not only DA but also other monoamine pathways in the pathophysiology of depressive-like behavior in animal models of PD.

Intra-striatal 6-OHDA injections can also be used to model PD, especially at early stages. After bilateral lesion (around 70%) of the dorsolateral striatum (with or without using desipramine to protect noradrenergic neurons), mice displayed slight motor deficits and longer immobility times in forced swim test and tail suspension test (Bonito-Oliva et al., 2014). This depressive-like behavior could be reversed by pramipexole (D2/D3 agonist) as well as by a noradrenaline inhibitor but not by L-DOPA. In another study, rats injected with bilateral 6-OHDA into the dorsal striatum (no use of desipramine), exhibited a mild reduction (36%) of striatal DA associated with increased depressive-like behaviors (Branchi et al., 2008). Other studies have shown that the partial and restricted lesion (50%) of the ventrolateral area of the dorsal striatum triggered no motor deficits but increased depressive-like behavior with monoamines alterations (Tadaiesky et al., 2008; da Silva et al., 2016). Interestingly, while anhedonia-like behaviors were observed shortly after a partial bilateral 6-OHDA lesion of the dorsolateral striatum, rats exhibited depressive-like behavior later, when anhedonia was no more present, indicating a temporal dissociation between the dorsolateral striatum and the prefrontal cortex (Matheus et al., 2016). The dorsolateral part of the striatum has motor and non-motor functions and undergoes extensive DA depletion. This leads to dysfunction of other regions such as the prefrontal cortex which has been associated with the occurrence of anhedonia and depression at premotor stages of PD.

A few studies using genetic models of PD have reported depressive-like behaviors. Mice deficient for the DA D3 receptor do not show significant deficits in locomotion when tested in the open field but exhibit depressive-like behaviors (Moraga-Amaro et al., 2014). In the same line, heterozygote mice for Engrailed 1, a developmental gene controlling the survival of DA neurons, display a progressive loss of the mesencephalic DA neurons, motor deficits as well as depressive-like behaviors (Le Pen et al., 2008). Mice deficient for the vesicular monoamine transporter-2 (VMAT-2) display enhanced depression-like behaviors, which worsen with advancing age (Taylor et al., 2009). The MitoPark mouse is a genetic model of PD, replicating several essential features of PD, including adult onset of DA neuron loss, slow progressive neurodegeneration, formation of intra-neuronal inclusions albeit without α-synuclein, responsiveness to L-DOPA treatment and non-motor deficits. MitoPark mice show a depressive-like phenotype (Cong et al., 2016). This depressive-like phenotype is observed besides several other motor and behavioral deficits and brain structural changes. Several groups have overexpressed α-synuclein in animals as a progressive model of PD (Ulusoy et al., 2010). After bilateral injection of the wild type human α-synuclein in the substantia nigra, which produces a partial DA cell loss (43% in the SNc, 30% in the VTA, 31% in the striatum), rats display depressive-like behaviors, independently of mild locomotor deficits (Caudal et al., 2015).

Up to now, no study has measured or observed a depressive-like behavior in a NHP model of PD. Such studies would be interesting since ethological investigations have shown that some monkeys are more prone to depression than others (Camus et al., 2014).

ANXIETY

The prevalence of anxiety disorders in PD ranges from 25 to 43% (Dissanayaka et al., 2014). It is higher than in other diseases causing similar disability. A recent systematic review considering data from a total of 2399 patients found that the point prevalence of anxiety disorders in PD is 31% (Broen et al., 2016). When referring to the DSM-IV-R diagnostic criteria, generalized anxiety is the most frequent, followed by agoraphobia, social phobia and panic disorder (see **Figure 1**). A large proportion of patients have multiple anxiety disorders (Leentjens et al., 2011). Moreover, a recurrent observation of the studies is the large proportion of patients (13.3% according to the systematic review) with significant anxiety symptoms that do not fit usual criteria for anxiety disorders (Dissanayaka et al., 2014; Broen et al., 2016). It is considered as "not otherwise specified" (NOS) anxiety disorder (Pontone et al., 2009). It refers to PD-specific anxiety, like phobia of falling, of driving, social phobia related to potentially embarrassing symptoms (drooling, dysarthria, . . .), anxiety related to withdrawal of DA medication or to wearing-off of medication in patients with fluctuations, panic-like disorder related to OFF periods, among others. Adverse effects of DA medication can also generate significant anxiety as in patients with ICD, DA dysregulation syndrome, or hallucinations. By consequence, anxiety disorders are probably largely underestimated in PD as triggers (predisposing and precipitating factors) are multiple.

The main manifestations of anxiety in PD are inability to relax, feeling tense, excessive concern, restlessness. Somatic symptoms may also be observed as palpitations, shortness of breath, sweating, digestive upset, etc. Usually, all the parkinsonian symptoms worsen with anxiety. Moreover, anxiety frequently implies emotion regulation deficits, irritability, excessive tiredness and difficulties falling asleep.

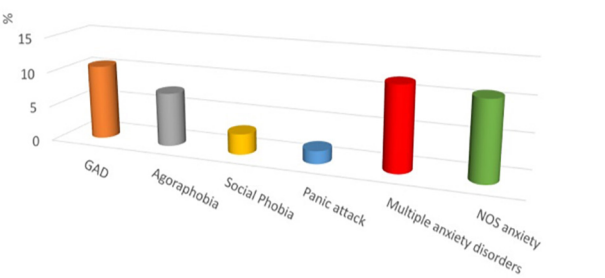


FIGURE 1 | Prevalence of anxiety disorders in Parkinson's disease (adapted from Leentjens et al., 2011). GAD, generalized anxiety disorder; NOS, not otherwise specified.

The association between anxiety and motor fluctuations, the significant exacerbation of anxiety provoked by DA withdrawal suggest that neurodegeneration of the DA pathways is involved in anxiety in PD. This is also supported by a study showing that in *de novo* patients, lower DA uptake at the caudate was associated with more severe trait anxiety (Picillo et al., 2017). However, the pathophysiology of PD-related anxiety disorders is complex and remains to be elucidated. In humans, several neuroimaging studies tried to decipher the mechanisms underlying anxiety in PD. Few specifically focused on anxiety. Most were PET or SPECT studies (Remy et al., 2005; Weintraub et al., 2005; Moriyama et al., 2011; Erro et al., 2012; Ceravolo et al., 2013; Huang et al., 2013; Maillet et al., 2016; Picillo et al., 2017; Wang X. et al., 2017; Joling et al., 2018). Three were volumetric studies (Tinaz et al., 2011; Vriend et al., 2016; Wee et al., 2016). Overall, the functional studies showed the involvement of the striatum and of the DA, 5-HT and NA pathways in the occurrence of anxiety manifestations in PD. The anatomical studies showed reductions in the volume of several brain areas, namely the amygdala, the anterior cingulate cortex and the orbito-frontal cortex. However, most of these studies were correlation studies including PD patients whatever their status in terms of anxiety disorders. Moreover, the anxiety measures used were not always optimal. There is thus a real need of further investigations.

It is absolutely necessary to continue studies to decipher the mechanisms of anxiety in PD. To date, no interventional clinical trial specifically targeting PD-related anxiety has been published. Two trials have just ended but their results are not published. One concerns rotigotine, a D2–D3 agonist (ClinicalTrials.gov Identifier: NCT02365870). The other concerns buspirone, a 5-HT_{A1} agonist (ClinicalTrials.gov Identifier: NCT02803749).

Anxiety-like behaviors can be observed in animals using several behavioral paradigms such as the elevated plus maze, the Light/Dark box and the open field. These tests have been used in animal models of PD and most of the literature report an increased anxiety-like behavior in 6-OHDA- or MPTP-lesioned animals (**Table 1**). In contrast, genetic models of the disease either show no difference or a decrease of anxiety-like behavior.

Specifically, after a MPTP intoxication that causes an almost complete depletion of striatal DA, mice exhibited motor deficits and an increased anxiety-like behavior, concomitant with a reduction of 5-HT levels in the basolateral nucleus of the amygdala (BLA) (Gorton et al., 2010). Several studies also reported an increase in anxiety-like behavior after bilateral intranigral injection of MPTP in rats (Wang et al., 2009; Sy et al., 2010).

After 6-OHDA bilateral lesion of nigral neurons within the SNc (inducing a maximal DA loss of about 70% in the dorsal striatum, noradrenergic neurons being protected with desipramine), rats exhibited no motor deficits but an increased anxiety-like behavior (Carnicella et al., 2014; Drui et al., 2014). A significant correlation was found between the latency of response and the striatal DA loss, suggesting that the increased anxiety-like response was related to the degree of striatal DA depletion (Drui et al., 2014). However, no similar correlations were found in the elevated plus-maze. DA agonists (SKF-38193, Sumanirole, and PD-128907) all reduced anxiety-like behavior in these 6-OHDA bilaterally lesioned rats (Carnicella et al., 2014). Another study showed that after a partial (less than 45%) and bilateral 6-OHDA lesion of the SNc (desipramine use), rats displayed an increased anxiety-like behavior as well as motor deficits (Campos et al., 2013). Bilateral partial (48%) lesion of the SNc induces significant deficits in the elevated plus maze, which were not reversed by either acute or chronic treatment with L-DOPA (Loiodice et al., 2019). This lesion was not associated to motor impairment.

The partial and bilateral lesion of the medial VTA failed to induce anxiety (Drui et al., 2014). On the contrary, after unilateral 6-OHDA MFB lesion, rats exhibited a mild increase of anxiety-like behavior, which could not be improved by chronic L-DOPA (Eskow Jaunarajs et al., 2010). However, other studies using the same type of lesion have shown anxiety-like behaviors, which could be improved by chronic L-DOPA or diazeapam (O'Connor et al., 2016), but not by sarizotan (Zhang et al., 2011) or diazepam (O'Connor et al., 2016). Several studies have shown that acting on 5-HT_{1A}, 5-HT_6 or 5-HT_7 receptor subtypes within the amygdala, the hippocampus or the prelimbic cortex can modulate anxiety-like behavior (Sun et al., 2015, 2018; Du et al., 2018; Zhang et al., 2018; Liu et al., 2019), underlying the major role of the serotonergic system in anxiety-like behavior in PD animal models. However, another study has shown, in 6-OHDA bilaterally MFB lesioned rats, that the additional depletion of serotonin or of noradrenalin had no further effects on anxiety-like behavior (Faggiani et al., 2015). However, L-DOPA enhanced the firing rate of amygdala and significantly decreased anxiety in these animals (Faggiani et al., 2018). This suggests that the increased activity of serotonin neurons may enhance the anxiolytic action of L-DOPA.

In mice, bilateral 6-OHDA lesion (75%) of the dorsolateral striatum also led to an increased anxiety-like behavior with slight motor deficits (Bonito-Oliva et al., 2014). This anxiety-like behavior was corrected by pramipexole (but not by L-DOPA) and reboxetine (a selective noradrenaline reuptake inhibitor), and was independent of noradrenaline depletion as the use of desipramine to protect noradrenergic neurons from the toxicity of 6-OHDA did not modify this anxiety-like behavior (Bonito-Oliva et al., 2014). Again, this suggests that the depletion of DA caused by 6-OHDA is sufficient to induce affective-like symptoms. Other studies showed increased anxiety in rats with a bilateral and partial (less than 50%) lesion of the striatum inducing slight motor deficits as well (Tadaiesky et al., 2008; Chen et al., 2011a; da Silva et al., 2016). The administration of an antagonist of metabotropic receptors was efficient to counteract both behavioral and neuronal changes (Tadaiesky et al., 2008; Chen et al., 2011a,b; da Silva et al., 2016).

A few studies have reported anxiety-like symptoms in genetic mouse models of PD. For instance, Zhu and colleagues reported increased anxiety-like behavior in Parkin deficient mice (Zhu et al., 2007). VMAT-2 deficient mice display an enhanced anxiety-like behavior, worsening with aging (Taylor et al., 2009). Compared to α-synuclein deficient mice and wild-type controls, mice overexpressing the human mutated form A53T of α-synuclein exhibited, besides early and late stage cognitive and sensorimotor deficits, a reduced anxiety-like

behavior (George et al., 2008). These results indicate a possible role for α-synuclein in anxiety-like behavior.

Regarding studies performed on monkey models of PD, only one study mentioned the case of a transgenic macaque monkey expressing the mutated form of α-synuclein and exhibiting an enhanced anxiety-like behavior (Niu et al., 2015).

APATHY

Clinically, apathy refers to a set of behavioral, emotional and cognitive manifestations, such as reduced interest and participation in the main activities of daily life, loss of initiative, lack of perseverance, indifference, and flattening of affect. As noted by Marin, apathy can exist *per se* as a syndrome. It is not just a symptom of depression or dementia (Marin, 1991). The definition of Marin was anchored on the motivational component of apathy: "*diminished motivation not attributable to diminished level of consciousness, cognitive impairment, or emotional distress*" (Marin, 1996). Due to the difficulty of addressing the concept of motivation, a more operational definition of apathy is currently preferred. According to the recent revision of the clinical diagnosis criteria (see **Table 2**), apathy corresponds to a quantitative reduction of goal-directed activity either in behavioral, cognitive, emotional, or social dimensions in comparison to the patient's previous level of functioning in these areas (Robert et al., 2018).

According to a meta-analysis on a large set of data, the mean prevalence of apathy in PD is 39.8% (n = 5,388; 95% CI 34.6–45.0%) (den Brok et al., 2015). Patients with apathy are on average older than the non-apathetic patients, have more impaired cognition in general and executive function in particular, have more severe motor symptoms and an increased risk of co-morbid depression. Apathy may be a predictive factor for dementia and cognitive decline over time (Dujardin et al., 2009; Pedersen et al., 2009).

Regarding the mechanisms underlying apathy, most neuropathological and neuroimaging studies in apathetic patients show abnormalities in a network of brain regions involved in motivated behavior, especially the dorsal part of the anterior cingulate cortex, the medial orbitofrontal cortex and the ventral striatum, under the influence of the mesolimbic DA system originating in the VTA (Heron et al., 2018). However, only few studies have examined the pathophysiology of apathy in PD patients. Reijnders et al. (2010) used voxel-based morphometry to identify the anatomical correlates of apathy in a group of 60 PD patients (16% were apathetic). High apathy scores were correlated with lower cognitive efficiency, more depressive symptoms and low gray matter density in the posterior cingulate, precuneus, insula and inferior parietal gyrus. Carriere et al. (2014) compared with shape analysis the volume of the striatum in apathetic vs. non-apathetic PD patients and showed a remodeling of the left caudate and left accumbens in apathetic patients. Most other studies in PD have used functional imaging. Remy et al. (2005) used [11C]-RTI-32 Positron emission tomography (PET), an *in vivo* marker of both DA and NA membrane transporters, with low affinity for 5-HT transporters. They found that apathy scores were negatively correlated with binding potential values in the ventral striatum bilaterally, suggesting that catecholamine denervation of this

TABLE 2 | Criteria for clinical diagnosis of apathy.

Criterion A: A quantitative reduction of goal-directed activity either in behavioral, cognitive, emotional, or social dimensions in comparison to the patient's previous level of functioning in these areas. These changes may be reported by the patient themselves or by observation of others.

Criterion B: The presence of at least 2 of the 3 following dimensions for a period of at least 4 weeks and present most of the time.

B1. BEHAVIOR AND COGNITION

Loss of, or diminished, goal-directed behavior or cognitive activity as evidenced by at least one of the following:
– reduced level of activity either at home or work, makes less effort or needs to be prompted to perform activities;
– less persistence in maintaining an activity or conversation, finding solutions to problems or alternative ways;
– less interest in or reaction to news, or less interest in doing new things;
– less interest in their own health and well-being or personal image;

B2. EMOTION

Loss of, or diminished, emotion as evidenced by at least one of the following:
– less spontaneous (self-generated) emotions regarding their own affairs;
– less emotional reaction in response to positive or negative events in the environment,
– less concern about the impact of their actions or feelings on the people around him/her;
– less empathy to the emotions or feelings of others;
– less verbal or physical reactions that reveal his/her emotional states;

B3. SOCIAL INTERACTION

Loss of, or diminished engagement in social interaction as evidenced by at least one of the following:
– less initiative in spontaneously proposing social or leisure activities to family or others;
– less participation in social or leisure activities suggested by people around them;
– less interest in family members;
– less likely to initiate a conversation, or early withdrawal from it;
– less interest in getting out to meet people;

Criterion C: These symptoms (A–B) cause clinically significant impairment in personal, social, occupational, or other important areas of functioning.

Criterion D: The symptoms (A–B) are not exclusively explained or due to physical disabilities (e.g., blindness and loss of hearing), to motor disabilities, to a diminished level of consciousness, to the direct physiological effects of a substance (e.g., drug of abuse, medication), or to major changes in the patient's environment.

area has a role in apathy. We used [11C]DASB and [11C]PE2I PET, radioligands, binding to the serotonergic and dopaminergic transporters, respectively (Maillet et al., 2016) and found that the severity of apathy in 12 *de novo* PD patients was mainly related to specific serotonergic lesions within the right-sided anterior caudate nucleus and the orbitofrontal cortex. Robert et al. (2012) used ^{18}FDG PET to measure glucose metabolism in 45 patients with PD and reported a correlation between the severity of apathy symptoms and cerebral metabolism in the inferior frontal gyrus, middle frontal gyrus, cuneus and anterior insula, at the right side. As apathy is a possible complication of the treatment of PD with deep brain stimulation of the subthalamic nucleus (STN-DBS), several studies have used the model of STN stimulation to investigate the pathophysiology of apathy in PD. Jeune et al. (2009) observed a worsening of apathetic symptoms after surgery and showed that this change was correlated with greater glucose metabolism in the right frontal middle gyrus (Brodmann area 10) and right inferior frontal gyrus (Brodmann areas 46 and 47) and lower glucose metabolism in the right posterior cingulate gyrus (Brodmann area 31) and left medial frontal lobe (Brodmann area 9). According to Jeune et al. (2009), stimulation of the STN could have a negative effect on the limbic territory of the STN, thus destabilizing the limbic circuit. The same team showed that reduced preoperative glucose metabolism within the right ventral striatum was associated with the occurrence of post-surgery apathy (Robert et al., 2014).

Thobois et al. (2010) used PET with an antagonist of DA D2 receptors to investigate the role of DA denervation in post-surgery apathy. They compared 12 patients who had developed apathy after initiation of DBS and 13 control PD patients who had not developed apathy after stimulation. Binding potential values were higher in apathetic patients compared with non-apathetic patients, in the orbito-frontal, dorsolateral and posterior cingulate cortex, bilaterally, as well as in the left striatum and right amygdala. After a challenge with methylphenidate, a drug that inhibits the reuptake of DA and NA and increases DA concentration at dopaminergic terminals, they observed that apathetic patients had lower reserve of endogenous DA. Although these results are consistent with a hypodopaminergic etiology of apathy in PD, other neurotransmitter systems are probably involved. As already mentioned, we found a prominent role of serotonergic degeneration in the occurrence of apathy at early stage of the disease (Maillet et al., 2016). This was confirmed by a complementary study by Prange et al. (2019) showing microstructural changes specifically associated with apathy in the limbic system in *de novo* patients with apathy compared with non-apathetic patients and healthy controls. These changes overlapped with the functional alteration of the serotoninergic terminals but not with the dopaminergic abnormalities (Prange et al., 2019). Given the links between apathy and cognitive decline in PD patients, a role of cholinergic denervation is also possible. The reduction of apathy symptoms by administration of rivastigmine, an inhibitor of acetylcholinesterase, reinforced such an assumption (Devos et al., 2013). Further confirmation of the role of non-dopaminergic systems in the PD apathy is still needed.

The first studies searching for a pharmacological treatment of apathy targeted the dopaminergic pathway. Czernecki et al. (2008) were the first to describe an improvement of post-surgery apathy after administration of ropinirole in a small group of patients. However, the level of evidence was very low. Later, Thobois et al. (2013) tested the effectiveness of piribedil to reduce post-surgery apathy in PD. They observed a significant reduction of the symptoms after 12 weeks of pharmacotherapy (Thobois et al., 2013). The effectiveness of rotigotine, another D2/D3 dopamine agonist, on apathy was also tested but there was no effect on the symptom severity (Hauser et al., 2016). Methylphenidate, a drug enhancing mesolimbic dopaminergic stimulation, was shown to improve apathy in an 82-year-old patient. Its effectiveness was not explored further in PD (Chatterjee and Fahn, 2002). As stated above, the efficacy of rivastigmine, an acetylcholinesterase inhibitor, on apathy in non-demented, non-depressed patients was also tested (Devos et al., 2013). Compared with placebo, there was a significant improvement of apathy in the rivastigmine group, particularly on the cognitive and initiation components of apathy.

Apathy is difficult to assess in animals. Some behavioral conditions considered as "activities of daily life" can be used in rodents such as the burrowing test or the nest building test. Other tests can also be used such as the runway task for food, operant sucrose self-administration and the saccharin preference test. There are very few studies on apathy-like behavior in PD animal models (**Table 1**). Three studies from the same group have evidenced motivational deficits after the selective, partial (less than 70%) and bilateral 6-OHDA lesion of the SNc in rats (Carnicella et al., 2014; Drui et al., 2014; Favier et al., 2014). But these animals also exhibited depressive-like and anxious-like behaviors. This apathy-like behavior, which was detected with the operant sucrose self-administration, was only improved by drugs preferentially acting on D3 type of DA receptors such as ropinirole, pramipexole, and PD-128907. Lesion of the medial part of VTA did not induce such a behavior, indicating that damage of the nigrostriatal pathway is a key risk factor to develop apathy-like behavior in the 6-OHDA rat model of PD. Another study did report behavioral signs of apathy in aged mice deficient for the vesicular monoamine transporter 2 (Baumann et al., 2016).

We have shown that apathy-like behavior (a hypoactive state with loss of motivation for food) can be induced by non-DA pharmacological dysfunction of the primate ventral striatum (Sgambato-Faure et al., 2016; Voon et al., 2017; Sgambato and Tremblay, 2018). This behavior involved a circuit including the orbital and medial prefrontal cortex, anterior insula, and lateral parts of medial output basal ganglia regions. This lack of motivation was induced by bicuculline (a $GABA_A$ antagonist) in moderately DA-depleted monkeys, suggesting that dopamine might only modulate the expression of apathy rather than cause it. However, another group has shown in MPTP-treated monkeys that the dopaminergic pathways play a key role in apathy-like behavior (Brown et al., 2012). Specifically, they tested the impact of MPTP on the monkeys' willingness to attempt goal-directed behaviors (Brown et al., 2012). Using PET imaging and post-mortem analysis, they showed that apathy-like scores correlated

with the degree of lesion of the DA mesostriatal pathway, and that dysfunction of the mesostriatal pathway predicted apathy-like behavior better than DA nigrostriatal dysfunction. More recently, the same group has extended these findings by showing that DA dysfunction in cortical regions also contributed to the development of apathetic behavior in NHP (Tian et al., 2015). Apathy scores correlated with DA injury (detected by PET tracers) in the dorsolateral prefrontal cortex, ventromedial prefrontal cortex, and insular cortex. Furthermore, only VTA cell counts could predict DAT changes in the insular cortex, suggesting a particular role for this pathway in the manifestation of apathy in this monkey model of PD. Contrary to the results in rodents, these data indicate a key role for the VTA involving both subcortical and cortical regions in the pathophysiology of apathy. Further studies investigating the role of both DA and non-DA pathways in apathy-like behavior in PD animal models are thus warranted.

PSYCHOSIS

In PD, psychosis encompasses a set of symptoms including illusions, hallucinations, delusions, and related symptoms. These symptoms are typically visual, more rarely in other modalities. They usually form a continuum progressing with the course of the disease (Ffytche and Aarsland, 2017). Passage (feeling like something is passing at the outer visual field) and presence (feeling that someone is close by) hallucinations as well as illusions (mis- or distorted perception of an actual stimulus) are the most common symptoms at early stage of the disease. Passage and presence hallucinations may concern 50% of the patients (Wood et al., 2015). As the disease progresses, visual hallucinations in PD may be complex and formed, usually vision of animals, people or objects. The scene is often stereotyped. They tend to occur in a low sensory environment. The hallucinations usually ignore the patient and disappear when approaching. Initially, insight is preserved but later, it is lost and contribute to the development of delusions.

There are few prevalence studies and frequencies vary widely. Point prevalence of complex hallucinations ranges from 22 to 38% (Fenelon and Alves, 2010). The prevalence of minor psychotic symptoms is much more variable. Fénelon et al. (2000) reported that 26% of a sample of 216 consecutive patients taking medication had minor hallucinations. In early diagnosed untreated patients, we observed that 6.32% had minor hallucinations (Dujardin et al., 2014) while another study reported a much higher prevalence of 42% (Pagonabarraga et al., 2016). The risk of developing visual hallucinations increases with age, severity of motor and cognitive symptoms, disease duration and medication (Fenelon and Alves, 2010). Moreover, visual hallucinations have been shown to be the main risk factor for nursing home placement (Aarsland et al., 2000). It increases the risk of cognitive impairment and dementia (Guo et al., 2019) and mortality (Goetz and Stebbins, 1995).

The mechanisms behind visual hallucinations (VH) remain largely unknown. Changes in visual information processing are incriminated. For example, compared with patients without visual hallucinations and healthy controls, PD patients with visual hallucinations had a thinning of the retinal nerve fiber layer, as measured by optical coherence tomography (Lee et al., 2014). Some studies have shown that PD patients with visual hallucinations have lower performance in visuospatial tasks, but findings are not consistent probably due to a lack of sensitivity of the tests and variability of the populations (for a review, see Ffytche and Aarsland, 2017). However, changes in the networks involved in perceptual and cognitive processing of visual information have been shown by anatomical and functional neuroimaging studies in patients with visual hallucinations compared with those without visual hallucinations. Most anatomical studies reported an atrophy in areas corresponding to dorsal and ventral visual streams (for a review, see Ffytche and Aarsland, 2017). Functional MRI (fMRI) studies reported reduced activity of the visual areas compensated by an increased activity in the associative cortical areas (Diederich et al., 2009; Shine et al., 2012; Yao et al., 2014, 2015, 2016; Shine et al., 2015). However, a better understanding of the pathophysiology of chronic visual hallucinations in PD lacks a direct exploration of the brain changes at the time of hallucination (i.e., capture studies). Because of the challenging nature of state explorations of hallucinations, only two studies were in PD. One single-case study reported the results of an fMRI scan performed in a 66-year old PD patient at time of hallucinations (Goetz et al., 2014). When comparing epochs with and without VH, there was an increased activation in a large attentional network and decreased activation in the right primary visual cortex. Recently, we measured brain functional changes when VH occurred in seven PD patients. We observed increased connectivity in visual networks concomitant to VH (Dujardin et al., 2019).

According to clinical experience, DA agonists may cause the onset of hallucinations, suggesting a role of the dopaminergic pathways in the occurrence of psychosis. However, the role of medication is very controversial since visual hallucinations are observed even in drug-naïve patients. As suggested by a work group on psychosis in PD, anti-parkinson medication is more to consider as a modifier than a trigger of hallucinations (Ravina et al., 2007a; Dujardin et al., 2014; Weintraub et al., 2015a). Regarding the other neuromodulation systems, the benefit of acetylcholinesterase inhibitors on symptoms suggests an involvement of the cortical cholinergic pathway (Burn et al., 2006; Oh et al., 2015). Moreover, an atrophy of the substantia innominate has been observed in PD patients with hallucinations (Shin et al., 2012). A contribution of 5-HT has also been suggested since a 5-HT depletion in the ventral occipitotemporal regions and bilateral frontal cortex was found in PD patients with visual hallucinations (Ballanger et al., 2010). However, further investigations are really needed to better understand the respective role of DA and non-DA pathways in the occurrence of psychosis in PD.

Pharmacological treatments currently available to reduce psychosis in PD patients have an action on these DA and 5-HT pathways. Benefits on psychotic symptoms have been reported in several placebo-controlled trials with clozapine and pimavanserin while quietapine and olanzapine provided no significant benefit (Wilby et al., 2017).

The number of studies in which psychosis-like symptoms have been observed in animal models of PD is still limited (**Table 1**). Behavioral assays such as head switches, hallucinatory-like responses, amphetamine-induced locomotor activity and disrupted prepulse inhibition are frequently used to assess antipsychotic activity. These tests have been used in animal models of PD.

Animals with bilateral 6-OHDA lesions of the substantia nigra display motor impairments as well as increased head twitches, locomotor activity, and disrupted prepulse inhibition (McFarland et al., 2011; Hubbard et al., 2013). Pimavanserin, a selective 5-HT_{2A} antagonist/inverse agonist, reverse psychosis-like behaviors, suggesting that $5HT_{2A}$ antagonism/inverse agonism may treat psychosis in PD.

A psychosis-like behavior rating scale has been developed and used in MPTP-treated marmosets (Fox et al., 2006; Visanji et al., 2006). This scale allows to assess four types of psychosis-like behaviors: agitation, hallucinatory-like responses to non-apparent stimuli, obsessive grooming, and stereotypies. It has been shown that L-DOPA, apomorphine, pramipexole, pergolide, ropinirole reversed motor symptoms and induced psychosis-like behaviors in MPTP-intoxicated marmosets, and that amantadine exacerbated them (Fox et al., 2006; Visanji et al., 2006). Finally, while haloperidol reduced those behaviors but increases motor symptoms, clozapine and quietapine reduced them without exacerbating parkinsonian disability. We have shown that L-DOPA triggered such psychosis-like behaviors in moderately-lesioned macaque monkeys and that these psychosis-like behaviors were reduced following lesion of serotonergic fibers by MDMA (Beaudoin-Gobert et al., 2015). Altogether, these data suggest both DA, 5-HT and glutamatergic mechanisms are involved in the pathophysiology of psychosis-like behavior.

IMPULSE CONTROL DISORDERS

Impulse control disorders refer to a class of psychiatric disorders characterized by impulsivity, i.e., an urge or failure to resist to temptation. In the DSM-5, it is included in a new section labeled "disruptive, impulse control, and conduct disorders." ICDs result in behaviors performed repetitively, excessively, and compulsively to an extent that interferes with major areas of daily life (Weintraub and Claassen, 2017). The individual pursues certain reward-based activities without taking account of the potentially deleterious consequences of these repetitive activities. The semiology is diverse. In PD, four major forms of ICDs have been described: pathological gambling, compulsive buying, pathological sexual behavior, and compulsive eating. Other impulsive/compulsive behaviors are linked, namely DA dysregulation syndrome (Giovannoni et al., 2000), an addiction to DA medication, particularly high-potency and short-acting drugs (e.g., subcutaneous apomorphine or dispersible formulation of levodopa). Related phenomena have also been identified as punding (repetitive, purposeless, and stereotyped behaviors) (Spencer et al., 2011), hobbyism (excessive exercise or creative activities) and hoarding (acquisition and keeping of a large number of items with little or no objective value, e.g., used gloves) (O'Sullivan et al., 2010). Very often, those patients also develop hypomania which interferes at lot with their familial and social life.

Regarding prevalence, the main available data come from the DOMINION study, which included 3090 PD patients from movement disorders clinics in North America and assessed the frequency of the four main ICDs (Weintraub et al., 2010b). It revealed that 13.6% of patients had one or more ICDs (compulsive buying in 5.7%, gambling in 5%, binge eating disorders in 4.3% and compulsive sexual behavior in 3.5%) and 3.9% had two or more ICDs. Similar prevalence data have been reported in studies conducted in different countries around the world (for a review, see Weintraub and Claassen, 2017). The Italian multi-center prospective ICARIUS study reported a higher point prevalence with 28.6% of the 1069 patients having at least one ICD (Antonini et al., 2017). The 2-year incidence was of 20.6% (Barone et al., 2019). The prevalence of the other impulsive/compulsive disorders is not well-documented.

Dopamine replacement therapy, namely DA agonist use, is the main risk factor for ICDs. In the DOMINION study, ICDs were more frequent in patients receiving DA agonists (17.1%) than in patients only treated by levodopa (6.9%). An analysis on the baseline data of the Parkinson's Progression Markers Initiative (PPMI) cohort, including 168 untreated PD patients and 143 healthy control subjects, revealed that the frequencies of ICDs and related symptoms did not differ in both groups (Weintraub et al., 2013). After 24-month follow-up, there was no increase of ICDs prevalence in the PD patient group, overall. However, in those who were on DA replacement therapy for at least 1 year, the incidence of ICDs was significantly higher than in patients still untreated or who initiate DA replacement therapy for less than 1 year (de la Riva et al., 2014). Hence, these results suggest that PD itself does not increase the risk to develop ICDs but reinforce the hypothesis that DA medication plays a major role in the occurrence of such disorders. The risk is greater with DA agonists having a preferential selectivity for D3 and D2 receptors, suggesting that the mesocortical and mesolimbic dopaminergic pathways are most likely involved. These pathways play a key role in reward-based learning and decision-making (Ballard et al., 2011; Haber, 2014; Hauser et al., 2017). Dysfunction of this DA system was confirmed by several neuroimaging studies (Cilia et al., 2008; Steeves et al., 2009; Voon et al., 2010, 2014). However, ICDs seem to be multi-determined and dysfunction of the mesocorticolimbic system due to DA replacement therapy is not the only underlying mechanism. Other clinical features (early onset disease, comorbid depression, and anxiety), premorbid (personality traits, genetic polymorphisms, past history of addiction), environmental (ease to access to the internet, pornography, casino, etc.), demographical (higher frequency of hypersexuality in men than women while the inverse is observed for compulsive buying) and cultural factors, cognitive bias (altered executive functions and decision-making) also influence the occurrence of ICDs (Weintraub et al., 2015b). Other neuromodulation systems are probably also involved, as suggested by studies in animal models of PD but further investigations are needed in patients.

To date, there is no available treatment for ICDs (Gatto and Aldinio, 2019). One clinical trial should recruit PD patients soon. It concerns pimavanserin, a selective 5-HT_{2A} inverse agonist (ClinicalTrials.gov Identifier: NCT03947216). Another trial has started recruiting PD patients. It will test the efficacy of clonidine, an α2 noradrenergic agonist (ClinicalTrials.gov Identifier: NCT03552068).

The number of studies in which impulsive/compulsive-like behavior has been modeled in animal models of PD is still quite limited (**Table 1**). Several models try to recapitulate the DA deficit of early or more advanced PD and the impulsive or compulsive traits provoked by DA loss in association with dopatherapy, in order to better understand pathophysiological mechanisms. Impulsive or compulsive behaviors can be observed in animals using several behavioral tasks, such as food-related instrumental learning tasks (5-choice reaction time task, delay-discounting task, rat gambling task, rodent betting task, etc.). Some of these tests have been used in animal models of PD.

Specifically, the lesion of the DA pathway seems to be necessary but not always sufficient to induce impulsive/compulsive-like behaviors, in agreement with the clinical study mentioned above, showing that the DA lesion by itself does not confer any risk for ICDs in *de novo* PD patients (Weintraub et al., 2013). On the 6-OHDA rat model of PD, it has been shown that bilateral dopaminergic lesions (use of desipramine) of the dorsolateral striatum increase impulsivity using a delay-discounting task (Tedford et al., 2015). Delivery of pramipexole via osmotic pumps enhanced impulsivity (risk-taking), which is reduced by mirtazapine, a NA and 5-HT antidepressant in those moderately-lesioned rats (Holtz et al., 2016). Moreover, the administration of chronic pramipexole to bilaterally 6-OHDA-lesioned rats (75% of DA lesion in the SNc and 50% in lateral part of VTA; with desipramine pretreatment; minor motor impairment) was required to induce a compulsive-like behavior (using a lever-pressing task), the DA lesion by itself being without any effects (Dardou et al., 2017). Furthermore, mapping performed with the expression of the immediate early gene c-*fos* suggests that the behavior is supported by the activation of the orbitofrontal cortex and the dorsal striatum (Dardou et al., 2017). This is consistent with previous studies showing that activation (Ahmari et al., 2013) or inhibition (Burguière et al., 2013) of the orbitofrontal cortex–striatal pathway modulates repetitive grooming in mice. In normal unlesioned rats, chronic pramipexole failed to induce compulsive behavior (Dardou et al., 2017). By contrast, quinpirole administration does induce compulsive checking behavior and is used as a rat model of obsessive-compulsive disorders (Winter et al., 2008). Compulsive behavior may share similarities with habit formation (Graybiel and Rauch, 2000) and lesion of the striatum is known to increase perseverative behavior in rodents on a 5-choice serial reaction time task in rodents (Baunez and Robbins, 1999).

Regarding genetic models of PD, it has been shown recently that the bilateral SNc lesion (64% of loss considered as mild) in rats overexpressing human A53T mutated α-synuclein, enhanced waiting impulsivity (increases of the premature response rate using the 5-choice serial reaction time task) and that this behavior was further exacerbated with long-term administration

TABLE 3 | Overview on the involvement of DA and non-DA systems in the pathophysiology of neuropsychiatric disorders through the different species used.

	Depression or depression-like behavior							Anxiety or anxiety-like behavior							Apathy or apathy-like behavior							Psychosis or psychosis-like behavior							ICDs or ICDs-like behaviors						
	DA	5-HT	NA	Ach	Glu	GABA		DA	5-HT	NA	Ach	Glu	GABA		DA	5-HT	NA	Ach	Glu	GABA		DA	5-HT	NA	Ach	Glu	GABA		DA	5-HT	NA	Ach	Glu	GABA
Mice																																		
Rats																																		
Monkeys																																		
Patients																																		

Yellow, gray, blue, and red colors respectively refer to studies performed on mice, rats, monkeys, or patients.

of pramipexole in both OFF and ON states (Jiménez-Urbieta et al., 2019). By contrast, pramipexole was not associated with changes in compulsivity. Similarly, α-synuclein-induced nigrostriatal neurodegeneration increases impulsivity by itself, subsequent chronic pramipexole administration exacerbating it (Engeln et al., 2016). Impulsive behavior does not develop in 6-OHDA-lesioned rodents (Baunez and Robbins, 1999; Carvalho et al., 2017). Differences may rely on the severity and topography of the dopaminergic lesions and on the tests used.

We have shown that impulsive and compulsive behaviors can be induced by pharmacological perturbation of different basal ganglia territories in NHP (for a review, see Sgambato and Tremblay, 2018). Compulsive behaviors were exhibited by both normal and DA-moderately lesioned monkeys. They involved a circuit including the lateral orbitofrontal cortex and limbic parts of the basal ganglia (Sgambato-Faure et al., 2016). Further studies are needed to investigate pathophysiological mechanisms of such behavioral disorders in parkinsonian monkeys.

LIMITATIONS OF THE EXISTING ANIMAL MODELS OF PD-RELATED NEUROPSYCHIATRIC DISORDERS AND FUTURE DIRECTIONS

Most of the existing animal models on PD rely on the use of neurotoxins (Cenci and Sgambato, 2019). 6-OHDA is used in mice and rats, while MPTP is mainly used in mice and monkeys (although it can also be used in rats). These neurotoxic models have been used extensively in the past to study motor aspects related to PD. As research now also aims at studying non-motor symptoms, these models have important limitations because the DA lesion of the nigrostriatal system often induces a decrease in motor abilities. Moreover, these neurotoxic models do not reproduce all the pathological features of the disease such as the formation of protein aggregates resembling Lewy bodies, or the involvement of brain regions outside of the nigrostriatal DA system. The same models are used to study the pathophysiological mechanisms of different neuropsychiatric-like disorders, without any specificity. There is therefore a real need to evolve these animal models, with more specific tools and/or combined lesions or impairments, to better understand the dysfunctions associated to each neuropsychiatric symptom. An important advance has been made with the development of genetic models of PD, including those based on the use of alpha-synuclein. This abnormally folded protein is enriched in Lewy bodies, the pathological sign of the disease. The injection and overexpression of this protein (in its native or mutated form) in the substantia nigra induces a more progressive loss of DA neurons. This offers the possibility of a longer asymptomatic motor phase to evaluate neuropsychiatric symptoms, without interference with motor signs. This is interesting in the case of depression and anxiety, for example, which may occur during the prodromal phase of PD. Moreover, we must really diversify our models to be able to mimic different stages of progression of the disease and the associated symptoms.

Finally, we have summarized in **Table 3** the current knowledge regarding the potential involvement of the neurotransmission systems in the pathophysiology of neuropsychiatric disorders through the different species used (rodent and primate). It comes out that, although the DA and 5-HT systems seem to be involved in the pathophysiology of all these symptoms, the other systems have been much less studied. For example, in PD patients, there is evidence for an involvement of the cholinergic system in depression, apathy and psychosis but, to our knowledge, no animal study has yet shown causal links between an alteration of this system and the expression (or the modulation) of a neuropsychiatric-like symptom. Furthermore, there is a real need for new preclinical studies on the pathophysiological substrates of apathy, psychosis and ICD as the arsenal of drugs to treat these disorders in patients is limited or non-existent (for ICD).

CONCLUSION

Neuropsychiatric disorders are among the most common and disabling non-motor manifestations of PD. Their negative impact on quality of life of both the patients and caregivers is indubitable. They also result in a heavy socio-economic cost. In spite of that, the underlying mechanisms remain largely unknown. Available data suggest that both dopaminergic and non-dopaminergic (serotonergic, noradrenergic, cholinergic, glutamatergic, and GABAergic) systems are involved in the expression or modulation of these disorders. Translational studies with valid animal models of PD and well-characterized group of patients are needed to continue deciphering the affected processes and propose efficient therapeutic strategies.

AUTHOR CONTRIBUTIONS

Both authors wrote the draft and reviewed the manuscript.

REFERENCES

Aarsland, D., Larsen, J., Tandberg, E., and Laake, K. (2000). Predictors of nursing home placement in Parkinson's disease: a population-based, prospective study. *J. Am. Geriatr. Soc.* 48, 938–942. doi: 10.1111/j.1532-5415.2000.tb06891.x

Aarsland, D., Påhlhagen, S., Ballard, C. G., Ehrt, U., and Svenningsson, P. (2011). Depression in Parkinson disease–epidemiology, mechanisms and management. *Nat. Rev. Neurol.* 8, 35–47. doi: 10.1038/nrneurol.2011.189

Ahmari, S. E., Spellman, T., Douglass, N. L., Kheirbek, M. A., Simpson, B. H., Deisseroth, K., et al. (2013). Repeated cortico-striatal stimulation generates persistent OCD-like behavior. *Science* 340, 1234–1239. doi: 10.1126/science.1234733

Ainsworth, K., Smith, S., Zetterström, T., Pei, Q., Franklin, M., and Sharp, T. (1998). Effect of antidepressant drugs on dopamine D1 and D2 receptor expression and dopamine release in the nucleus accumbens of the rat. *Psychopharmacology* 140, 470–477. doi: 10.1007/s002130050791

Antonini, A., Barone, P., Bonuccelli, U., Annoni, K., Asgharnejad, M., and Stanzione, P. (2017). ICARUS study: prevalence and clinical features of impulse control disorders in Parkinson's disease. *J. Neurol. Neurosurg. Psychiatry* 88, 317–324. doi: 10.1136/jnnp-2016-315277

Ascherio, A., and Schwarzschild, M. A. (2016). The epidemiology of Parkinson's disease: risk factors and prevention. *Lancet Neurol.* 15, 1257–1272. doi: 10.1016/s1474-4422(16)30230-7

Ballanger, B., Klinger, H., Eche, J., Lerond, J., Vallet, A., Bars, D., et al. (2012). Role of serotonergic 1A receptor dysfunction in depression associated with Parkinson's disease. *Mov. Disord.* 27, 84–89. doi: 10.1002/mds.23895

Ballanger, B., Strafella, A. P., van Eimeren, T., Zurowski, M., Rusjan, P. M., Houle, S., et al. (2010). Serotonin 2A receptors and visual hallucinations in Parkinson disease. *Arch. Neurol.* 67, 416–421. doi: 10.1001/archneurol.2010.35

Ballard, I. C., Murty, V. P., Carter, M. R., MacInnes, J. J., Huettel, S. A., and Adcock, A. R. (2011). Dorsolateral prefrontal cortex drives mesolimbic dopaminergic regions to initiate motivated behavior. *J. Neurosci.* 31, 10340–10346. doi: 10.1523/jneurosci.0895-11.2011

Barone, P., Antonini, A., Stanzione, P., Annoni, K., Asgharnejad, M., and Bonuccelli, U. (2019). Risk factors for impulse control disorders and related behaviors in Parkinson's disease: secondary analyses of the ICARUS study. *J. Drug Assess.* 8, 159–166. doi: 10.1080/21556660.2019.1675670

Barone, P., Poewe, W., Albrecht, S., Debieuvre, C., Massey, D., Rascol, O., et al. (2010). Pramipexole for the treatment of depressive symptoms in patients with Parkinson's disease: a randomised, double-blind, placebo-controlled trial. *Lancet Neurol.* 9, 573–580. doi: 10.1016/s1474-4422(10)70106-x

Baumann, A., Moreira, C. G., Morawska, M. M., Masneuf, S., Baumann, C. R., and Noain, D. (2016). Preliminary evidence of apathetic-like behavior in aged vesicular monoamine transporter 2 deficient mice. *Front. Hum. Neurosci.* 10:587. doi: 10.3389/fnhum.2016.00587

Baunez, C., and Robbins, T. (1999). Effects of dopamine depletion of the dorsal striatum and further interaction with subthalamic nucleus lesions in an attentional task in the rat. *Neuroscience* 92, 1343–1356. doi: 10.1016/s0306-4522(99)00065-2

Beaudoin-Gobert, M., Epinat, J., Météreau, E., Duperrier, S., Neumane, S., Ballanger, B., et al. (2015). Behavioural impact of a double dopaminergic and serotonergic lesion in the non-human primate. *Brain J. Neurol.* 138, 2632–2647. doi: 10.1093/brain/awv183

Bohnen, N., Kaufer, D., Hendrickson, R., Constantine, G., Mathis, C., and Moore, R. (2007). Cortical cholinergic denervation is associated with depressive symptoms in Parkinson's disease and parkinsonian dementia. *J. Neurol. Neurosurg. Psychiatry* 78, 641–643. doi: 10.1136/jnnp.2006.100073

Boileau, I., Warsh, J. J., Guttman, M., Saint-Cyr, J. A., McCluskey, T., Rusjan, P., et al. (2008). Elevated serotonin transporter binding in depressed patients with Parkinson's disease: a preliminary PET study with [11C]DASB. *Mov. Disord.* 23, 1776–1780. doi: 10.1002/mds.22212

Bonito-Oliva, A., Masini, D., and Fisone, G. (2014). A mouse model of non-motor symptoms in Parkinson's disease: focus on pharmacological interventions targeting affective dysfunctions. *Front. Behav. Neurosci.* 8:290. doi: 10.3389/fnbeh.2014.00290

Branchi, I., D'Andrea, I., Armida, M., Cassano, T., Pèzzola, A., Potenza, R., et al. (2008). Nonmotor symptoms in Parkinson's disease: investigating early-phase onset of behavioral dysfunction in the 6-hydroxydopamine-lesioned rat model. *J. Neurosci. Res.* 86, 2050–2061. doi: 10.1002/jnr.21642

Broen, M. P., Narayen, N. E., Kuijf, M. L., Dissanayaka, N. N., and Leentjens, A. F. (2016). Prevalence of anxiety in Parkinson's disease: a systematic review and meta-analysis. *Mov. Disord.* 31, 1125–1133. doi: 10.1002/mds.26643

Brown, C., Campbell, M., Karimi, M., Tabbal, S., Loftin, S., Tian, L., et al. (2012). Dopamine pathway loss in nucleus accumbens and ventral tegmental area predicts apathetic behavior in MPTP-lesioned monkeys. *Exp. Neurol.* 236, 190–197. doi: 10.1016/j.expneurol.2012.04.025

Brown, R. G., Landau, S., Hindle, J. V., Playfer, J., Samuel, M., Wilson, K. C., et al. (2011). Depression and anxiety related subtypes in Parkinson's disease. *J. Neurol. Neurosurg. Psychiatry* 82, 803–809. doi: 10.1136/jnnp.2010.213652

Burguière, E., Monteiro, P., Feng, G., and Graybiel, A. M. (2013). Optogenetic stimulation of lateral orbitofronto-striatal pathway suppresses compulsive behaviors. *Science* 340, 1243–1246. doi: 10.1126/science.1232380

Burn, D., Emre, M., McKeith, I., Deyn, P., Aarsland, D., Hsu, C., et al. (2006). Effects of rivastigmine in patients with and without visual hallucinations in dementia associated with Parkinson's disease. *Mov. Disord.* 21, 1899–1907. doi: 10.1002/mds.21077

Campos, F. L., Carvalho, M. M., Cristovão, A. C., Je, G., Baltazar, G., Salgado, A. J., et al. (2013). Rodent models of Parkinson's disease: beyond the motor symptomatology. *Front. Behav. Neurosci.* 7:175. doi: 10.3389/fnbeh.2013.00175

Camus, S. M., Rochais, C., Blois-Heulin, C., Li, Q., Hausberger, M., and Bezard, E. (2014). Depressive-like behavioral profiles in captive-bred single- and socially-housed rhesus and cynomolgus macaques: a species comparison. *Front. Behav. Neurosci.* 8:47. doi: 10.3389/fnbeh.2014.00047

Carnicella, S., Drui, G., Boulet, S., Carcenac, C., Favier, M., Duran, T., et al. (2014). Implication of dopamine D3 receptor activation in the reversion of Parkinson's disease-related motivational deficits. *Transl. Psychiatry* 4:e401. doi: 10.1038/tp.2014.43

Carriere, N., Besson, P., Dujardin, K., Duhamel, A., Defebvre, L., Delmaire, C., et al. (2014). Apathy in Parkinson's disease is associated with nucleus accumbens atrophy: a magnetic resonance imaging shape analysis. *Mov. Disord.* 29, 897–903. doi: 10.1002/mds.25904

Carvalho, M. M., Campos, F. L., Marques, M., Soares-Cunha, C., Kokras, N., Dalla, C., et al. (2017). Effect of levodopa on reward and impulsivity in a rat model of Parkinson's disease. *Front. Behav. Neurosci.* 11:145. doi: 10.3389/fnbeh.2017.00145

Caudal, D., Alvarsson, A., Björklund, A., and Svenningsson, P. (2015). Depressive-like phenotype induced by AAV-mediated overexpression of human α-synuclein in midbrain dopaminergic neurons. *Exp. Neurol.* 273, 243–252. doi: 10.1016/j.expneurol.2015.09.002

Cenci, M., and Sgambato, V. (2019). Toxin-based rodent models of PD. *Neuromethods Ser.* (in press).

Ceravolo, R., Frosini, D., Poletti, M., Kiferle, L., Pagni, C., Mazzucchi, S., et al. (2013). Mild affective symptoms in de novo Parkinson's disease patients: relationship with dopaminergic dysfunction. *Eur. J. Neurol.* 20, 480–485. doi: 10.1111/j.1468-1331.2012.03878.x

Chatterjee, A., and Fahn, S. (2002). Methylphenidate treats apathy in Parkinson's disease. *J. Neuropsychiatry Clin. Neurosci.* 14, 461–462. doi: 10.1176/jnp.14.4.461

Chaudhuri, R. K., Healy, D. G., Schapira, A. H., and National Institute for Clinical Excellence. (2006). Non-motor symptoms of Parkinson's disease: diagnosis and management. *Lancet Neurol.* 5, 235–245. doi: 10.1016/s1474-4422(06)70373-8

Chen, L., Liu, J., Ali, U., Gui, Z., Hou, C., Fan, L., et al. (2011a). Chronic, systemic treatment with a metabotropic glutamate receptor 5 antagonist produces anxiolytic-like effects and reverses abnormal firing activity of projection neurons in the basolateral nucleus of the amygdala in rats with bilateral 6-OHDA lesions. *Brain Res. Bull.* 84, 215–223. doi: 10.1016/j.brainresbull.2011.01.005

Chen, L., Liu, J., Zhang, Q., Feng, J., Gui, Z., Ali, U., et al. (2011b). Alterations of emotion, cognition and firing activity of the basolateral nucleus of the amygdala after partial bilateral lesions of the nigrostriatal pathway in rats. *Brain Res. Bull.* 85, 329–338. doi: 10.1016/j.brainresbull.2011.05.009

Chiu, W.-H., Depboylu, C., Hermanns, G., Maurer, L., Windolph, A., Oertel, W., et al. (2015). Long-term treatment with L-DOPA or pramipexole affects adult neurogenesis and corresponding non-motor behavior in a mouse model of Parkinson's disease. *Neuropharmacology* 95, 367–376. doi: 10.1016/j.neuropharm.2015.03.020

Chung, S., Asgharnejad, M., Bauer, L., Ramirez, F., and Jeon, B. (2016). Evaluation of rotigotine transdermal patch for the treatment of depressive symptoms in patients with Parkinson's disease. *Expert Opin. Pharmacol.* 17, 1453–1461. doi: 10.1080/14656566.2016.1202917

Cilia, R., Siri, C., Marotta, G., Isaias, I. U., Gaspari, D., Canesi, M., et al. (2008). Functional abnormalities underlying pathological gambling in Parkinson disease. *Arch. Neurol.* 65, 1604–1611. doi: 10.1001/archneur.65.12.1604

Cong, L., Muir, E. R., Chen, C., Qian, Y., Liu, J., Biju, K., et al. (2016). Multimodal MRI evaluation of the MitoPark mouse model of Parkinson's disease. *PLoS One* 11:e0151884. doi: 10.1371/journal.pone.0151884

Cryan, J. F., Mombereau, C., and Vassout, A. (2005). The tail suspension test as a model for assessing antidepressant activity: review of pharmacological and genetic studies in mice. *Neurosci. Biobehav. Rev.* 29, 571–625. doi: 10.1016/j.neubiorev.2005.03.009

Czernecki, V., Schüpbach, M., Yaici, S., Lévy, R., Bardinet, E., Yelnik, J., et al. (2008). Apathy following subthalamic stimulation in Parkinson disease: a dopamine responsive symptom. *Mov. Disord.* 23, 964–969. doi: 10.1002/mds.21949

da Silva, T., Poli, A., Hara, D., and Takahashi, R. (2016). Time course study of microglial and behavioral alterations induced by 6-hydroxydopamine in rats. *Neurosci. Lett.* 622, 83–87. doi: 10.1016/j.neulet.2016.04.049

Dardou, D., Chassain, C., and Durif, F. (2014). Chronic pramipexole treatment increases tolerance for sucrose in normal and ventral tegmental lesioned rats. *Front. Neurosci.* 8:437. doi: 10.3389/fnins.2014.00437

Dardou, D., Reyrolle, L., Chassain, C., and Durif, F. (2017). Chronic pramipexole treatment induces compulsive behavior in rats with 6-OHDA lesions of the substantia nigra and ventral tegmental area. *Behav. Brain Res.* 332, 327–336. doi: 10.1016/j.bbr.2017.06.016

de la Riva, P., Smith, K., Xie, S. X., and Weintraub, D. (2014). Course of psychiatric symptoms and global cognition in early Parkinson disease. *Neurology* 83, 1096–1103. doi: 10.1212/wnl.0000000000000801

den Brok, M. G., van Dalen, J., van Gool, W. A., van Charante, E. P., de Bie, R. M., and Richard, E. (2015). Apathy in Parkinson's disease: a systematic review and meta-analysis. *Mov. Disord.* 30, 759–769. doi: 10.1002/mds.26208

Devos, D., Moreau, C., Maltête, D., Lefaucheur, R., Kreisler, A., Eusebio, A., et al. (2013). Rivastigmine in apathetic but dementia and depression-free patients with Parkinson's disease: a double-blind, placebo-controlled, randomised clinical trial. *J. Neurol. Neurosurg. Psychiatry* 85, 668–674. doi: 10.1136/jnnp-2013-306439

Diederich, N. J., Fenelon, G., Stebbins, G., and Goetz, C. G. (2009). Hallucinations in Parkinson disease. *Nat. Rev. Neurol.* 5, 331–342. doi: 10.1038/nrneurol.2009.62

Dissanayaka, N. N., White, E., O'Sullivan, J. D., Marsh, R., Pachana, N. A., and Byrne, G. J. (2014). The clinical spectrum of anxiety in Parkinson's disease. *Mov. Disord.* 29, 967–975. doi: 10.1002/mds.25937

Drui, G., Carnicella, S., Carcenac, C., Favier, M., Bertrand, A., Boulet, S., et al. (2014). Loss of dopaminergic nigrostriatal neurons accounts for the motivational and affective deficits in Parkinson's disease. *Mol. Psychiatry* 19, 358–367. doi: 10.1038/mp.2013.3

Du, C.-X., Guo, Y., Zhang, Q., Zhang, J., Lv, S., and Liu, J. (2018). Involvement of prelimbic 5-HT7 receptors in the regulation of anxiety-like behaviors in hemiparkinsonian rats. *Neurol. Res.* 109, 1–9. doi: 10.1080/01616412.2018.1493962

Dujardin, K., Langlois, C., Plomhause, L., Carette, A.-S., Delliaux, M., Duhamel, A., et al. (2014). Apathy in untreated early-stage Parkinson disease: relationship with other non-motor symptoms. *Mov. Disord.* 29, 1796–1801. doi: 10.1002/mds.26058

Dujardin, K., Roman, D., Baille, G., Pins, D., Lefebvre, S., Delmaire, C., et al. (2019). What can we learn from fMRI capture of visual hallucinations in Parkinson's disease? *Brain Imaging Behav.* [Epub ahead of print]. doi: 10.1007/s11682-019-00185-6

Dujardin, K., Sockeel, P., Delliaux, M., Destée, A., and Defebvre, L. (2009). Apathy may herald cognitive decline and dementia in Parkinson's disease. *Mov. Disord.* 24, 2391–2397. doi: 10.1002/mds.22843

Engeln, M., Ansquer, S., Dugast, E., Bezard, E., Belin, D., and Fernagut, P.-O. (2016). Multi-facetted impulsivity following nigral degeneration and dopamine replacement therapy. *Neuropharmacology* 109, 69–77. doi: 10.1016/j.neuropharm.2016.05.013

Erro, R., Pappatà, S., Amboni, M., Vicidomini, C., Longo, K., Santangelo, G., et al. (2012). Anxiety is associated with striatal dopamine transporter availability in newly diagnosed untreated Parkinson's disease patients. *Parkinson. Relat. Disord.* 18, 1034–1038. doi: 10.1016/j.parkreldis.2012.05.022

Eskow Jaunarajs, K. L., Dupre, K. B., Ostock, C. Y., Button, T., Deak, T., and Bishop, C. (2010). Behavioral and neurochemical effects of chronic L-DOPA treatment on nonmotor sequelae in the hemiparkinsonian rat. *Behav. Pharmacol.* 21, 627–637. doi: 10.1097/FBP.0b013e32833e7e80

Faggiani, E., Delaville, C., and Benazzouz, A. (2015). The combined depletion of monoamines alters the effectiveness of subthalamic deep brain stimulation. *Neurobiol. Dis.* 82, 342–348. doi: 10.1016/j.nbd.2015.07.010

Faggiani, E., Naudet, F., Janssen, M. L., Temel, Y., and Benazzouz, A. (2018). Serotonergic neurons mediate the anxiolytic effect of l-DOPA: neuronal correlates in the amygdala. *Neurobiol. Dis.* 110, 20–28. doi: 10.1016/j.nbd.2017.11.001

Favier, M., Duran, T., Carcenac, C., Drui, G., Savasta, M., and Carnicella, S. (2014). Pramipexole reverses Parkinson's disease-related motivational deficits in rats. *Mov. Disord.* 29, 912–920. doi: 10.1002/mds.25837

Fenelon, G., and Alves, G. (2010). Epidemiology of psychosis in Parkinson's disease. *J. Neurol. Sci.* 289, 12–17. doi: 10.1016/j.jns.2009.08.014

Fénelon, G., Mahieux, F., Huon, R., and Ziégler, M. (2000). Hallucinations in Parkinson's disease: prevalence, phenomenology and risk factors. *Brain J. Neurol.* 123(Pt 4), 733–745. doi: 10.1093/brain/123.4.733

Ffytche, D. H., and Aarsland, D. (2017). Psychosis in Parkinson's Disease. *Int. Rev. Neurobiol.* 133, 585–622. doi: 10.1016/bs.irn.2017.04.005

Fox, S. H., Visanji, N. P., Johnston, T. H., Gomez-Ramirez, J., Voon, V., and Brotchie, J. M. (2006). Dopamine receptor agonists and levodopa and inducing psychosis-like behavior in the MPTP primate model of Parkinson disease. *Arch. Neurol.* 63, 1343–1344. doi: 10.1001/archneur.63.9.1343

Gambarana, C., Masi, F., Tagliamonte, A., Scheggi, S., Ghiglieri, O., and Montis, D. M. (1999). A chronic stress that impairs reactivity in rats also decreases dopaminergic transmission in the nucleus accumbens: a microdialysis study. *J. Neurochem.* 72, 2039–2046. doi: 10.1046/j.1471-4159.1999.0722039.x

Gatto, E. M., and Aldinio, V. (2019). Impulse control disorders in Parkinson's disease. a brief and comprehensive review. *Front. Neurol.* 10:351. doi: 10.3389/fneur.2019.00351

George, S., van den Buuse, M., Mok, S., Masters, C. L., Li, Q.-X., and Culvenor, J. G. (2008). Alpha-synuclein transgenic mice exhibit reduced anxiety-like behaviour. *Exp. Neurol.* 210, 788–792. doi: 10.1016/j.expneurol.2007.12.017

Giovannoni, G., O'Sullivan, J., Turner, K., Manson, A., and Lees, A. (2000). Hedonistic homeostatic dysregulation in patients with Parkinson's disease on dopamine replacement therapies. *J. Neurol. Neurosurg. Psychiatry* 68, 423–428. doi: 10.1136/jnnp.68.4.423

Goetz, C., and Stebbins, G. (1995). Mortality and hallucinations in nursing home patients with advanced Parkinson's disease. *Neurology* 45, 669–671. doi: 10.1212/wnl.45.4.669

Goetz, C. G., Vaughan, C. L., Goldman, J. G., and Stebbins, G. T. (2014). I finally see what you see: Parkinson's disease visual hallucinations captured with functional neuroimaging. *Mov. Disord.* 29, 115–117. doi: 10.1002/mds.25554

Gorton, L. M., Vuckovic, M. G., Vertelkina, N., Petzinger, G. M., Jakowec, M. W., and Wood, R. I. (2010). Exercise effects on motor and affective behavior and catecholamine neurochemistry in the MPTP-lesioned mouse. *Behav. Brain Res.* 213, 253–262. doi: 10.1016/j.bbr.2010.05.009

Graybiel, A., and Rauch, S. (2000). Toward a neurobiology of obsessive-compulsive disorder. *Neuron* 28, 343–347. doi: 10.1016/s0896-6273(00)00113-6

Guo, Y., Xu, W., Liu, F.-T., Li, J.-Q., Cao, X.-P., Tan, L., et al. (2019). Modifiable risk factors for cognitive impairment in Parkinson's disease: a systematic review and meta-analysis of prospective cohort studies. *Mov. Disord.* 34, 876–883. doi: 10.1002/mds.27665

Haber, S. (2014). The place of dopamine in the cortico-basal ganglia circuit. *Neuroscience* 282, 248–257. doi: 10.1016/j.neuroscience.2014.10.008

Han, L.-N., Zhang, L., Li, L.-B., Sun, Y.-N., Wang, Y., Chen, L., et al. (2015). Activation of serotonin(2C) receptors in the lateral habenular nucleus increases the expression of depression-related behaviors in the hemiparkinsonian rat. *Neuropharmacology* 93, 68–79. doi: 10.1016/j.neuropharm.2015.01.024

Han, L.-N., Zhang, L., Sun, Y.-N., Du, C.-X., Zhang, Y.-M., Wang, T., et al. (2016). Serotonin7 receptors in the lateral habenular nucleus regulate depressive-like behaviors in the hemiparkinsonian rats. *Brain Res.* 1644, 79–87. doi: 10.1016/j.brainres.2016.05.016

Hauser, R. A., Slawek, J., Barone, P., Dohin, E., Surmann, E., Asgharnejad, M., et al. (2016). Evaluation of rotigotine transdermal patch for the treatment of apathy and motor symptoms in Parkinson's disease. *BMC Neurol.* 16:90. doi: 10.1186/s12883-016-0610-7

Hauser, T. U., Eldar, E., and Dolan, R. J. (2017). Separate mesocortical and mesolimbic pathways encode effort and reward learning signals. *Proc. Natl. Acad. Sci. U.S.A.* 114, E7395–E7404. doi: 10.1073/pnas.1705643114

Heron, L. C., Apps, M., and Husain, M. (2018). The anatomy of apathy: a neurocognitive framework for amotivated behaviour. *Neuropsychologia* 118, 54–67. doi: 10.1016/j.neuropsychologia.2017.07.003

Holtz, N. A., Tedford, S. E., Persons, A. L., Grasso, S. A., and Napier, C. T. (2016). Pharmacologically distinct pramipexole-mediated akinesia vs. risk-taking in a rat model of Parkinson's disease. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 70, 77–84. doi: 10.1016/j.pnpbp.2016.05.004

Huang, C., Ravdin, L. D., Nirenberg, M. J., Piboolnurak, P., Severt, L., Maniscalco, J. S., et al. (2013). Neuroimaging markers of motor and nonmotor features of Parkinson's disease: an 18f fluorodeoxyglucose positron emission computed

tomography study. *Dement. Geriatr. Cogn. Disord.* 35, 183–196. doi: 10.1159/000345987

Hubbard, D., Hacksell, U., and McFarland, K. (2013). Behavioral effects of clozapine, pimavanserin, and quetiapine in rodent models of Parkinson's disease and Parkinson's disease psychosis: evaluation of therapeutic ratios. *Behav. Pharmacol.* 24, 628–632. doi: 10.1097/fbp.0b013e3283656db6

Hui, Y., Zhang, Q., Zhang, L., Chen, L., Guo, Y., Qiao, H., et al. (2014). Activation of prelimbic 5-HT1A receptors produces antidepressant-like effects in a unilateral rat model of Parkinson's disease. *Neuroscience* 268, 265–275. doi: 10.1016/j.neuroscience.2014.03.033

Ilkiw, J. L., Kmita, L. C., Targa, A. D., Noseda, A. D., Rodrigues, L. S., Dorieux, F. W., et al. (2019). Dopaminergic lesion in the olfactory bulb restores olfaction and induces depressive-like behaviors in a 6-OHDA model of Parkinson's disease. *Mol. Neurobiol.* 56, 1082–1095. doi: 10.1007/s12035-018-1134-5

Jeune, L. F., Drapier, D., Bourguignon, A., Péron, J., Mesbah, H., Drapier, S., et al. (2009). Subthalamic nucleus stimulation in Parkinson disease induces apathy: a PET study. *Neurology* 73, 1746–1751. doi: 10.1212/wnl.0b013e3181c34b34

Jiménez-Urbieta, H., Gago, B., Quiroga-Varela, A., Rodríguez-Chinchilla, T., Merino-Galán, L., Oregi, A., et al. (2019). Pramipexole-induced impulsivity in mildparkinsonian rats: a model of impulse control disorders in Parkinson's disease. *Neurobiol. Aging* 75, 126–135. doi: 10.1016/j.neurobiolaging.2018.11.021

Joling, M., van den Heuvel, O. A., Berendse, H. W., Booij, J., and Vriend, C. (2018). Serotonin transporter binding and anxiety symptoms in Parkinson's disease. *J. Neurol. Neurosurg. Psychiatry* 89, 89–94. doi: 10.1136/jnnp-2017-316193

Lee, J.-Y., Kim, J., Ahn, J., Kim, H.-J., Jeon, B. S., and Kim, T. (2014). Retinal nerve fiber layer thickness and visual hallucinations in Parkinson's Disease. *Mov. Disord.* 29, 61–67. doi: 10.1002/mds.25543

Leentjens, A. F., Dujardin, K., Marsh, L., Martinez-Martin, P., Richard, I. H., and Starkstein, S. E. (2011). Symptomatology and markers of anxiety disorders in Parkinson's disease: a cross-sectional study. *Mov. Disord.* 26, 484–492. doi: 10.1002/mds.23528

Le Pen, G., Sonnier, L., Hartmann, A., Bizot, J.C., Trovero, F., Krebs, M. O., et al. (2008). Progressive loss of dopaminergic neurons in the ventral midbrain of adult mice heterozygote for *Engrailed1*: a new genetic model for Parkinson's disease? *Parkinsonism Relat. Disord.* 14, S107–S111. doi: 10.1016/j.parkreldis.2008.04.007

Liu, K., Guo, Y., Zhang, J., Chen, L., Liu, Y., Lv, S., et al. (2019). Activation and blockade of dorsal hippocampal Serotonin6 receptors regulate anxiety-like behaviors in a unilateral 6-hydroxydopamine rat model of Parkinson's disease. *Neurol. Res.* 41, 791–801. doi: 10.1080/01616412.2019.1611204

Liu, K., Li, J.-Y., Tan, H.-H., Du, C.-X., Xie, W., Zhang, Y.-M., et al. (2015). Serotonin6 receptors in the dorsal hippocampus regulate depressive-like behaviors in unilateral 6-hydroxydopamine-lesioned Parkinson's rats. *Neuropharmacology* 95, 290–298. doi: 10.1016/j.neuropharm.2015.03.031

Loiodice, S., Young, H., Rion, B., Méot, B., Montagne, P., Denibaud, A.-S., et al. (2019). Implication of nigral dopaminergic lesion and repeated L-dopa exposure in neuropsychiatric symptoms of Parkinson's disease. *Behav. Brain Res.* 360, 120–127. doi: 10.1016/j.bbr.2018.12.007

Luo, X. F., Zhang, B. L., Li, J. C., Yang, Y. Y., Sun, Y. F., and Zhao, H. (2015). Lateral habenula as a link between dopaminergic and serotonergic systems contributes to depressive symptoms in Parkinson's disease. *Brain Res. Bull.* 110, 40–46. doi: 10.1016/j.brainresbull.2014.11.006

Maillet, A., Krack, P., Lhommée, E., Météreau, E., Klinger, H., Favre, E., et al. (2016). The prominent role of serotonergic degeneration in apathy, anxiety and depression in de novo Parkinson's disease. *Brain J. Neurol.* 139, 2486–2502. doi: 10.1093/brain/aww162

Marin, R. (1991). Apathy: a neuropsychiatric syndrome. *J. Neuropsychiatry Clin. Neurosci.* 3, 243–254. doi: 10.1176/jnp.3.3.243

Marin, R. (1996). Apathy: concept, syndrome, neural mechanisms, and treatment. *Semin. Clin. Neuropsychiatry* 1, 304–314. doi: 10.1053/scnp00100304

Matheus, F. C., Rial, D., Real, J. I., Lemos, C., Takahashi, R. N., Bertoglio, L. J., et al. (2016). Temporal dissociation of striatum and prefrontal cortex uncouples anhedonia and defense behaviors relevant to depression in 6-OHDA-lesioned rats. *Mol. Neurobiol.* 53, 3891–3899. doi: 10.1007/s12035-015-9330-z

McFarland, K., Price, D. L., and Bonhaus, D. W. (2011). Pimavanserin, a 5-HT2A inverse agonist, reverses psychosis-like behaviors in a rodent model of Parkinson's disease. *Behav. Pharmacol.* 22, 681–692. doi: 10.1097/fbp.0b013e32834aff98

Moraga-Amaro, R., Gonzalez, H., Pacheco, R., and Stehberg, J. (2014). Dopamine receptor D3 deficiency results in chronic depression and anxiety. *Behav. Brain Res.* 274, 186–193. doi: 10.1016/j.bbr.2014.07.055

Mori, A., Ohashi, S., Nakai, M., Moriizumi, T., and Mitsumoto, Y. (2005). Neural mechanisms underlying motor dysfunction as detected by the tail suspension test in MPTP-treated C57BL/6 mice. *Neurosci. Res.* 51, 265–274. doi: 10.1016/j.neures.2004.11.008

Moriyama, T. S., Felicio, A. C., Chagas, M. H., Tardelli, V. S., Ferraz, H., Tumas, V., et al. (2011). Increased dopamine transporter density in Parkinson's disease patients with social anxiety disorder. *J. Neurol. Sci.* 310, 53–57. doi: 10.1016/j.jns.2011.06.056

Niu, Y., Guo, X., Chen, Y., Wang, C.-E., Gao, J., Yang, W., et al. (2015). Early Parkinson's disease symptoms in α-synuclein transgenic monkeys. *Hum. Mol. Genet.* 24, 2308–2317. doi: 10.1093/hmg/ddu748

O'Connor, K. A., Feustel, P. J., Ramirez-Zamora, A., Molho, E., Pilitsis, J. G., and Shin, D. S. (2016). Investigation of diazepam efficacy on anxiety-like behavior in hemiparkinsonian rats. *Behav. Brain Res.* 301, 226–237. doi: 10.1016/j.bbr.2015.12.045

Oh, Y.-S., Kim, J.-S., and Lee, P. (2015). Effect of rivastigmine on behavioral and psychiatric symptoms of Parkinson's disease dementia. *J. Mov. Disord.* 8, 98–102. doi: 10.14802/jmd.15041

Okun, M. S., and Watts, R. L. (2002). Depression associated with Parkinson's disease: clinical features and treatment. *Neurology* 58, S63–S70.

O'Sullivan, S. S., Djamshidian, A., Evans, A. H., Loane, C. M., Lees, A. J., and Lawrence, A. D. (2010). Excessive hoarding in Parkinson's disease. *Mov. Disord.* 25, 1026–1033. doi: 10.1002/mds.23016

Pagonabarraga, J., Martinez-Horta, S., de Bobadilla, R., Pérez, J., Ribosa-Nogué, R., Marín, J., et al. (2016). Minor hallucinations occur in drug-naive Parkinson's disease patients, even from the premotor phase. *Mov. Disord.* 31, 45–52. doi: 10.1002/mds.26432

Pedersen, K., Alves, G., Brønnick, K., Aarsland, D., Tysnes, O.-B., and Larsen, J. (2009). Apathy in drug-naïve patients with incident Parkinson's disease: the Norwegian ParkWest study. *J. Neurol.* 257, 217–223. doi: 10.1007/s00415-009-5297-x

Picillo, M., Santangelo, G., Erro, R., Cozzolino, A., Amboni, M., Vitale, C., et al. (2017). Association between dopaminergic dysfunction and anxiety in de novo Parkinson's disease. *Parkinson. Relat. Disord.* 37, 106–110. doi: 10.1016/j.parkreldis.2017.02.010

Politis, M., Wu, K., Loane, C., Turkheimer, F., Molloy, S., Brooks, D., et al. (2010). Depressive symptoms in PD correlate with higher 5-HTT binding in raphe and limbic structures. *Neurology* 75, 1920–1927. doi: 10.1212/wnl.0b013e3181feb2ab

Pontone, G. M., Williams, J. R., Anderson, K. E., Chase, G., Goldstein, S. A., Grill, S., et al. (2009). Prevalence of anxiety disorders and anxiety subtypes in patients with Parkinson's disease. *Mov. Disord.* 24, 1333–1338. doi: 10.1002/mds.22611

Porsolt, R., Pichon, L. M., and Jalfre, M. (1977). Depression: a new animal model sensitive to antidepressant treatments. *Nature* 266, 730–732. doi: 10.1038/266730a0

Prange, S., Metereau, E., Maillet, A., Lhommée, E., Klinger, H., Pelissier, P., et al. (2019). Early limbic microstructural alterations in apathy and depression in de novo Parkinson's disease. *Mov. Disord.* 34, 1644–1654. doi: 10.1002/mds.27793

Ravina, B., Camicioli, R., Como, P., Marsh, L., Jankovic, J., Weintraub, D., et al. (2007a). The impact of depressive symptoms in early Parkinson disease. *Neurology* 69, 342–347. doi: 10.1212/01.wnl.0000268695.63392.10

Ravina, B., Marder, K., Fernandez, H. H., Friedman, J. H., McDonald, W., Murphy, D., et al. (2007b). Diagnostic criteria for psychosis in Parkinson's disease: report of an NINDS, NIMH work group. *Mov. Disord.* 22, 1061–1068. doi: 10.1002/mds.21382

Reijnders, J. S., Ehrt, U., Weber, W. E., Aarsland, D., and Leentjens, A. F. (2008). A systematic review of prevalence studies of depression in Parkinson's disease. *Mov. Disord.* 23, 183–189. doi: 10.1002/mds.21803

Reijnders, J. S., Scholtissen, B., Weber, W. E., Aalten, P., Verhey, F. R., and Leentjens, A. F. (2010). Neuroanatomical correlates of apathy in Parkinson's disease: a magnetic resonance imaging study using voxel-based morphometry. *Mov. Disord.* 25, 2318–2325. doi: 10.1002/mds.23268

Remy, P., Doder, M., Lees, A., Turjanski, N., and Brooks, D. (2005). Depression in Parkinson's disease: loss of dopamine and noradrenaline innervation in the limbic system. *Brain J. Neurol.* 128, 1314–1322. doi: 10.1093/brain/awh445

Riedel, O., Klotsche, J., Spottke, A., Deuschl, G., Förstl, H., Henn, F., et al. (2010). Frequency of dementia, depression, and other neuropsychiatric symptoms in 1,449 outpatients with Parkinson's disease. *J. Neurol.* 257, 1073–1082. doi: 10.1007/s00415-010-5465-z

Robert, G., Jeune, F., Lozachmeur, C., Drapier, S., Dondaine, T., Péron, J., et al. (2012). Apathy in patients with Parkinson disease without dementia or depression: a PET study. *Neurology* 79, 1155–1160. doi: 10.1212/wnl.0b013e3182698c75

Robert, G. H., Jeune, F., Lozachmeur, C., Drapier, S., Dondaine, T., Péron, J., et al. (2014). Preoperative factors of apathy in subthalamic stimulated Parkinson disease: a PET study. *Neurology* 83, 1620–1626. doi: 10.1212/wnl.0000000000000941

Robert, P., Lanctôt, K., Agüera-Ortiz, L., Aalten, P., Bremond, F., Defrancesco, M., et al. (2018). Is it time to revise the diagnostic criteria for apathy in brain disorders? The 2018 international consensus group. *Eur. Psychiatry J. Assoc. Eur. Psychiatr.* 54, 71–76. doi: 10.1016/j.eurpsy.2018.07.008

Santiago, R., Barbieiro, J., Lima, M., Dombrowski, P. A., Andreatini, R., and Vital, M. A. (2010). Depressive-like behaviors alterations induced by intranigral MPTP, 6-OHDA, LPS and rotenone models of Parkinson's disease are predominantly associated with serotonin and dopamine. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 34, 1104–1114. doi: 10.1016/j.pnpbp.2010.06.004

Schapira, A. H., Chaudhuri, R. K., and Jenner, P. (2017). Non-motor features of Parkinson disease. *Nat. Rev. Neurosci.* 18, 435–450. doi: 10.1038/nrn.2017.62

Sgambato, V., and Tremblay, L. (2018). Pathophysiology of dyskinesia and behavioral disorders in non-human primates: the role of serotonergic fibers. *J. Neural Trans.* 125, 1145–1156. doi: 10.1007/s00702-018-1871-7

Sgambato-Faure, V., Worbe, Y., Epinat, J., Féger, J., and Tremblay, L. (2016). Cortico-basal ganglia circuits involved in different motivation disorders in non-human primates. *Brain Struct. Funct.* 221, 345–364. doi: 10.1007/s00429-014-0911-9

Shin, S., Lee, J., Hong, J., Sunwoo, M.-K., Sohn, Y., and Lee, P. (2012). Neuroanatomical substrates of visual hallucinations in patients with non-demented Parkinson's disease. *J. Neurol. Neurosurg. Psychiatry* 83, 1155–1161. doi: 10.1136/jnnp-2012-303391

Shine, J., Halliday, G. H., Carlos, M., Naismith, S. L., and Lewis, S. J. (2012). Investigating visual misperceptions in Parkinson's disease: a novel behavioral paradigm. *Mov. Disord.* 27, 500–505. doi: 10.1002/mds.24900

Shine, J., Muller, A. J., O'Callaghan, C., Hornberger, M., Halliday, G. M., and Lewis, S. (2015). Abnormal connectivity between the default mode and the visual system underlies the manifestation of visual hallucinations in Parkinson's disease: a task-based fMRI study. *NPJ Parkinsons Dis.* 1:15003. doi: 10.1038/npjparkd.2015.3

Spencer, A. H., Rickards, H., Fasano, A., and Cavanna, A. E. (2011). The prevalence and clinical characteristics of punding in Parkinson's disease. *Mov. Disord.* 26, 578–586. doi: 10.1002/mds.23508

Starkstein, S. E., and Brockman, S. (2017). Management of depression in Parkinson's disease: a systematic review. *Mov. Disord. Clin. Pract.* 4, 470–477. doi: 10.1002/mdc3.12507

Steeves, T., Miyasaki, J., Zurowski, M., Lang, A., Pellecchia, G., Eimeren, V. T., et al. (2009). Increased striatal dopamine release in Parkinsonian patients with pathological gambling: a [11C] raclopride PET study. *Brain J. Neurol.* 132, 1376–1385. doi: 10.1093/brain/awp054

Sun, Y.-N., Wang, T., Wang, Y., Han, L.-N., Li, L.-B., Zhang, Y.-M., et al. (2015). Activation of 5-HT1A receptors in the medial subdivision of the central nucleus of the amygdala produces anxiolytic effects in a rat model of Parkinson's disease. *Neuropharmacology* 95, 181–191. doi: 10.1016/j.neuropharm.2015.03.007

Sun, Y.-N., Yao, L., Li, L.-B., Wang, Y., Du, C.-X., Guo, Y., et al. (2018). Activation and blockade of basolateral amygdala 5-HT6 receptor produce anxiolytic-like behaviors in an experimental model of Parkinson's disease. *Neuropharmacology* 137, 275–285. doi: 10.1016/j.neuropharm.2018.05.016

Sy, H.-N., Wu, S.-L., Wang, W.-F., Chen, C.-H., Huang, Y.-T., Liou, Y.-M., et al. (2010). MPTP-induced dopaminergic degeneration and deficits in object recognition in rats are accompanied by neuroinflammation in the hippocampus. *Pharmacol. Biochem. Behav.* 95, 158–165. doi: 10.1016/j.pbb.2009.12.020

Tadaiesky, M., Dombrowski, P., Figueiredo, C., Cargnin-Ferreira, E., Cunha, D. C., and Takahashi, R. (2008). Emotional, cognitive and neurochemical alterations in a premotor stage model of Parkinson's disease. *Neuroscience* 156, 830–840. doi: 10.1016/j.neuroscience.2008.08.035

Taylor, T. N., Caudle, M. W., Shepherd, K. R., Noorian, A., Jackson, C. R., Iuvone, M. P., et al. (2009). Nonmotor symptoms of Parkinson's disease revealed in an animal model with reduced monoamine storage capacity. *J. Neurosci.* 29, 8103–8113. doi: 10.1523/jneurosci.1495-09.2009

Tedford, S. E., Persons, A. L., and Napier, C. T. (2015). Dopaminergic lesions of the dorsolateral striatum in rats increase delay discounting in an impulsive choice task. *PLoS One* 10:e0122063. doi: 10.1371/journal.pone.0122063

Thobois, S., Ardouin, C., Lhommée, E., Klinger, H., Lagrange, C., Xie, J., et al. (2010). Non-motor dopamine withdrawal syndrome after surgery for Parkinson's disease: predictors and underlying mesolimbic denervation. *Brain J. Neurol.* 133, 1111–1127. doi: 10.1093/brain/awq032

Thobois, S., Lhommée, E., Klinger, H., Ardouin, C., Schmitt, E., Bichon, A., et al. (2013). Parkinsonian apathy responds to dopaminergic stimulation of D2/D3 receptors with piribedil. *Brain J. Neurol.* 136, 1568–1577. doi: 10.1093/brain/awt067

Tian, L., Xia, Y., Flores, H. P., Campbell, M. C., Moerlein, S. M., and Perlmutter, J. S. (2015). Neuroimaging analysis of the dopamine basis for apathetic behaviors in an MPTP-lesioned primate model. *PLoS One* 10:e0132064. doi: 10.1371/journal.pone.0132064

Tinaz, S., Courtney, M. G., and Stern, C. E. (2011). Focal cortical and subcortical atrophy in early Parkinson's disease. *Mov. Disord.* 26, 436–441. doi: 10.1002/mds.23453

Tong, Q., Zhang, L., Yuan, Y., Jiang, S., Zhang, R., Xu, Q., et al. (2015). Reduced plasma serotonin and 5-hydroxyindoleacetic acid levels in Parkinson's disease are associated with nonmotor symptoms. *Parkinson. Relat. Disord.* 21, 882–887. doi: 10.1016/j.parkreldis.2015.05.016

Ulusoy, A., Decressac, M., Kirik, D., and Björklund, A. (2010). Viral vector-mediated overexpression of α-synuclein as a progressive model of Parkinson's disease. *Prog. Brain Res.* 184, 89–111. doi: 10.1016/s0079-6123(10)84005-1

Vecchia, D., Kanazawa, L., Wendler, E., de Hocayen, P., Bruginski, E., Campos, F., et al. (2018). Effects of ketamine on vocal impairment, gait changes, and anhedonia induced by bilateral 6-OHDA infusion into the substantia nigra pars compacta in rats: therapeutic implications for Parkinson's disease. *Behav. Brain Res.* 342, 1–10. doi: 10.1016/j.bbr.2017.12.041

Visanji, N. P., Gomez-Ramirez, J., Johnston, T. H., Pires, D., Voon, V., Brotchie, J. M., et al. (2006). Pharmacological characterization of psychosis-like behavior in the MPTP-lesioned nonhuman primate model of Parkinson's disease. *Mov. Disord.* 21, 1879–1891. doi: 10.1002/mds.21073

Voon, V., Napier, C. T., Frank, M. J., Sgambato-Faure, V., Grace, A. A., Rodriguez-Oroz, M., et al. (2017). Impulse control disorders and levodopa-induced dyskinesias in Parkinson's disease: an update. *Lancet Neurol.* 16, 238–250. doi: 10.1016/s1474-4422(17)30004-2

Voon, V., Pessiglione, M., Brezing, C., Gallea, C., Fernandez, H. H., Dolan, R. J., et al. (2010). Mechanisms underlying dopamine-mediated reward bias in compulsive behaviors. *Neuron* 65, 135–142. doi: 10.1016/j.neuron.2009.12.027

Voon, V., Rizos, A., Chakravartty, R., Mulholland, N., Robinson, S., Howell, N. A., et al. (2014). Impulse control disorders in Parkinson's disease: decreased striatal dopamine transporter levels. *J. Neurol. Neurosurg. Psychiatry* 85, 148–152. doi: 10.1136/jnnp-2013-305395

Vriend, C., Boedhoe, P. S., Rutten, S., Berendse, H. W., van der Werf, Y. D., and van den Heuvel, O. A. (2016). A smaller amygdala is associated with anxiety in Parkinson's disease: a combined FreeSurfer-VBM study. *J. Neurol. Neurosurg. Psychiatry* 87, 493–500. doi: 10.1136/jnnp-2015-310383

Vriend, C., Pattij, T., van der Werf, Y. D., Voorn, P., Booij, J., Rutten, S., et al. (2014a). Depression and impulse control disorders in Parkinson's disease: two sides of the same coin? *Neurosci. Biobehav. Rev.* 38, 60–71. doi: 10.1016/j.neubiorev.2013.11.001

Vriend, C., Raijmakers, P., Veltman, D. J., van Dijk, K. D., van der Werf, Y. D., Foncke, E. M., et al. (2014b). Depressive symptoms in Parkinson's disease are related to reduced [123I]FP-CIT binding in the caudate nucleus. *J. Neurol. Neurosurg. Psychiatry* 85, 159–164. doi: 10.1136/jnnp-2012-304811

Vuckovic, M. G., Wood, R. I., Holschneider, D. P., Abernathy, A., Togasaki, D. M., Smith, A., et al. (2008). Memory, mood, dopamine, and serotonin in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-lesioned mouse model of basal ganglia injury. *Neurobiol. Dis.* 32, 319–327. doi: 10.1016/j.nbd.2008.07.015

Wang, T., Zhang, L., Zhang, Q., Wang, Y., Du, C.-X., Sun, Y.-N., et al. (2017). Involvement of lateral habenula α1 subunit-containing GABAA receptor-mediated inhibitory transmission in the regulation of depression-related behaviors in experimental Parkinson's disease. *Neuropharmacology* 116, 399–411. doi: 10.1016/j.neuropharm.2017.01.015

Wang, X., Zhang, J., Yuan, Y., Li, T., Zhang, L., Ding, J., et al. (2017). Cerebral metabolic change in Parkinson's disease patients with anxiety: a FDG-PET study. *Neurosci. Lett.* 653, 202–207. doi: 10.1016/j.neulet.2017.05.062

Wang, W.-F., Wu, S.-L., Liou, Y.-M., Wang, A.-L., Pawlak, C., and Ho, Y.-J. (2009). MPTP lesion causes neuroinflammation and deficits in object recognition in Wistar rats. *Behav. Neurosci.* 123, 1261–1270. doi: 10.1037/a0017401

Wee, N., Wen, M.-C., Kandiah, N., Chander, R. J., Ng, A., Au, W., et al. (2016). Neural correlates of anxiety symptoms in mild Parkinson's disease: a prospective longitudinal voxel-based morphometry study. *J. Neurol. Sci.* 371, 131–136. doi: 10.1016/j.jns.2016.10.021

Weintraub, D., and Claassen, D. O. (2017). Impulse control and related disorders in Parkinson's disease. *Int. Rev. Neurobiol.* 133, 679–717. doi: 10.1016/bs.irn.2017.04.006

Weintraub, D., David, A. S., Evans, A. H., Grant, J. E., and Stacy, M. (2015a). Clinical spectrum of impulse control disorders in Parkinson's disease. *Mov. Disord.* 30, 121–127. doi: 10.1002/mds.26016

Weintraub, D., Koester, J., Potenza, M. N., Siderowf, A. D., Stacy, M., Voon, V., et al. (2010a). Impulse control disorders in Parkinson disease: a cross-sectional study of 3090 patients. *Arch. Neurol.* 67, 589–595. doi: 10.1001/archneurol.2010.65

Weintraub, D., Mavandadi, S., Mamikonyan, E., Siderowf, A. D., Duda, J. E., Hurtig, H. I., et al. (2010b). Atomoxetine for depression and other neuropsychiatric symptoms in Parkinson disease. *Neurology* 75, 448–455. doi: 10.1212/wnl.0b013e3181ebdd79

Weintraub, D., Newberg, A. B., Cary, M. S., Siderowf, A. D., Moberg, P. J., Kleiner-Fisman, G., et al. (2005). Striatal dopamine transporter imaging correlates with anxiety and depression symptoms in Parkinson's disease. *J. Nucl. Med.* 46, 227–232.

Weintraub, D., Papay, K., Siderowf, A., and Initiative, P. (2013). Screening for impulse control symptoms in patients with de novo Parkinson disease: a case-control study. *Neurology* 80, 176–180. doi: 10.1212/wnl.0b013e31827b915c

Weintraub, D., Simuni, T., Caspell-Garcia, C., Coffey, C., Lasch, S., Siderowf, A., et al. (2015b). Cognitive performance and neuropsychiatric symptoms in early, untreated Parkinson's disease. *Mov. Disord.* 30, 919–927. doi: 10.1002/mds.26170

Wilby, K., Johnson, E. G., Johnson, H. E., and Ensom, M. H. (2017). Evidence-based review of pharmacotherapy used for Parkinson's disease psychosis. *Ann. Pharmacother.* 51, 682–695. doi: 10.1177/1060028017703992

Winter, C., Mundt, A., Jalali, R., Joel, D., Harnack, D., Morgenstern, R., et al. (2008). High frequency stimulation and temporary inactivation of the subthalamic nucleus reduce quinpirole-induced compulsive checking behavior in rats. *Exp. Neurol.* 210, 217–228. doi: 10.1016/j.expneurol.2007.10.020

Winter, C., von Rumohr, A., Mundt, A., Petrus, D., Klein, J., Lee, T., et al. (2007). Lesions of dopaminergic neurons in the substantia nigra pars compacta and in the ventral tegmental area enhance depressive-like behavior in rats. *Behav. Brain Res.* 184, 133–141. doi: 10.1016/j.bbr.2007.07.002

Wood, R. A., Hopkins, S. A., Moodley, K. K., and Chan, D. (2015). Fifty percent prevalence of extracampine hallucinations in Parkinson's disease patients. *Front. Neurol.* 6:263. doi: 10.3389/fneur.2015.00263

Yao, N., Chang, R., Cheung, C., Pang, S., Lau, K., Suckling, J., et al. (2014). The default mode network is disrupted in Parkinson's disease with visual hallucinations. *Hum. Brain Mapp.* 35, 5658–5666. doi: 10.1002/hbm.22577

Yao, N., Cheung, C., Pang, S., Chang, R., Lau, K., Suckling, J., et al. (2016). Multimodal MRI of the hippocampus in Parkinson's disease with visual hallucinations. *Brain Struct. Funct.* 221, 287–300. doi: 10.1007/s00429-014-0907-5

Yao, N., Pang, S., Cheung, C., Chang, R., Lau, K., Suckling, J., et al. (2015). Resting activity in visual and corticostriatal pathways in Parkinson's disease with hallucinations. *Parkinson. Relat. Disord.* 21, 131–137. doi: 10.1016/j.parkreldis.2014.11.020

Zhang, Q., Du, C., Tan, H., Zhang, L., Li, L., Zhang, J., et al. (2015). Activation and blockade of serotonin7 receptors in the prelimbic cortex regulate depressive-like behaviors in a 6-hydroxydopamine-induced Parkinson's disease rat model. *Neuroscience* 311, 45–55. doi: 10.1016/j.neuroscience.2015.10.016

Zhang, X., Egeland, M., and Svenningsson, P. (2011). Antidepressant-like properties of sarizotan in experimental Parkinsonism. *Psychopharmacology* 218, 621–634. doi: 10.1007/s00213-011-2356-7

Zhang, Y.-M., Guo, Y., Du, C.-X., Zhang, J., Lv, S., and Liu, J. (2018). Serotonin6 receptors in the prelimbic cortex are involved in the regulation of anxiety-like behaviors in the rat 6-hydroxydopamine Parkinson's disease model. *Chin. J. Physiol.* 61, 201–209. doi: 10.4077/cjp.2018.bah588

Zhang, Y.-M., Zhang, L., Wang, Y., Sun, Y.-N., Guo, Y., Du, C.-X., et al. (2016). Activation and blockade of prelimbic 5-HT6 receptors produce different effects on depressive-like behaviors in unilateral 6-hydroxydopamine-induced Parkinson's rats. *Neuropharmacology* 110, 25–36. doi: 10.1016/j.neuropharm.2016.07.014

Zhu, X.-R., Maskri, L., Herold, C., Bader, V., Stichel, C. C., Güntürkün, O., et al. (2007). Non-motor behavioural impairments in parkin-deficient mice. *Eur. J. Neurosci.* 26, 1902–1911. doi: 10.1111/j.1460-9568.2007.05812.x

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pharmacology

ORIGINAL RESEARCH
published: 20 March 2020
doi: 10.3389/fphar.2020.00304

Non-dopaminergic Alterations in Depression-Like FSL Rats in Experimental Parkinsonism and L-DOPA Responses

Nicoletta Schintu[1†], Xiaoqun Zhang[1†], Nikolas Stroth[1], Aleksander A. Mathé[1], Per E. Andrén[2,3] and Per Svenningsson[1]*

[1] Department of Clinical Neuroscience, Karolinska University Hospital, Karolinska Institutet, Stockholm, Sweden, [2] Medical Mass Spectrometry Imaging, Department of Pharmaceutical Biosciences, Uppsala University, Uppsala, Sweden, [3] Science for Life Laboratory, Uppsala University, Uppsala, Sweden

Depression is a common comorbid condition in Parkinson's disease (PD). Patients with depression have a two-fold increased risk to develop PD. Further, depression symptoms often precede motor symptoms in PD and are frequent at all stages of the disease. However, the influence of a depressive state on the responses to antiparkinson treatments is largely unknown. In this study, the genetically inbred depression-like flinders sensitive line (FSL) rats and control flinders resistant line (FRL) rats were studied in models of experimental parkinsonism. FSL rats showed a potentiated tremorgenic response to tacrine, a cholinesterase inhibitor used experimentally to induce 6 Hz resting tremor reminiscent of parkinsonian tremor. We also studied rats lesioned with 6-OHDA to induce hemiparkinsonism. No baseline differences in dopaminergic response to acute apomorphine or L-DOPA was found. However, following chronic treatment with L-DOPA, FRL rats developed sensitization of turning and abnormal involuntary movements (AIMs); these effects were counteracted by the anti-dyskinetic 5-HT_{1A} agonist/D_2 partial agonist sarizotan. In contrast, FSL rats did not develop sensitization of turning and only minor AIMs in response to L-DOPA treatment. The roles of several non-dopamine systems underlying this discrepancy were studied. Unexpectedly, no differences of opioid neuropeptides or serotonin markers were found between FRL and FSL rats. The marked behavioral difference between the FRL and FSL rats was paralleled with the striatal expression of the established marker, c-fos, but also the GABAergic transporter (vGAT), and a hitherto unknown marker, tamalin, that is known to regulate mGluR5 receptor function and postsynaptic organization. This study demonstrates that behavioral and transcriptional responses of non-dopaminergic systems to experimental parkinsonism and L-DOPA are modified in a genetic rat model of depression.

Keywords: flinders sensitive line, Parkinson's disease, L-DOPA, dyskinesia, tremor, tamalin, GRASP

OPEN ACCESS

Edited by:
Philippe De Deurwaerdere, Université de Bordeaux, France

Reviewed by:
Christopher R. Bishop, Binghamton University, United States
Gilberto Fisone, Karolinska Institutet (KI), Sweden

***Correspondence:**
Per Svenningsson
per.svenningsson@ki.se

[†] These authors have contributed equally to this work

Specialty section:
This article was submitted to Neuropharmacology, a section of the journal Frontiers in Pharmacology

Received: *19 July 2019*
Accepted: *28 February 2020*
Published: *20 March 2020*

Citation:
Schintu N, Zhang X, Stroth N, Mathé AA, Andrén PE and Svenningsson P (2020) Non-dopaminergic Alterations in Depression-Like FSL Rats in Experimental Parkinsonism and L-DOPA Responses. Front. Pharmacol. 11:304. doi: 10.3389/fphar.2020.00304

INTRODUCTION

Parkinson's disease (PD) is the second most common neurodegenerative disorder and is diagnosed based on the presence of bradykinesia, rigidity and tremor (Kalia and Lang, 2015). PD patients often have comorbid depression that may precede the onset of motor signs, but is frequently present at all stages of the disease (Aarsland et al., 2012). When comparing PD patients to patients suffering

from other chronic motor disabilities, parkinsonian individuals score higher in rating scales for depression (Robins, 1976).Thus, depression symptoms appear to be a part of PD pathology and not only a reaction to the somatic disease manifestations. In fact, unipolar depressed patients have a two-fold increased risk of being diagnosed with PD when compared to other groups with chronic illness that also need continuous treatment (Nilsson et al., 2001; Leentjens et al., 2003). Despite the clinical importance, few preclinical or clinical studies have examined how a depressive state affects the progression of PD or the response to antiparkinson treatments.

The flinders sensitive line (FSL) of rats is commonly used as a genetic animal model of depression with the flinders resistant line (FRL) serving as control (Overstreet et al., 2005). The FSL rat strain was established from Sprague Dawley rats through a selective breeding program for increased sensitivity to the cholinergic compound di-isopropyl fluorophosphates (DFP). FRL rats exhibit low sensitivity to DFP. FSL rats have good face validity and exhibit increased immobility in the forced swim test. The construct validity of this genetic model is reflected by the similarities with the human pathology affecting neurochemical systems including the serotonergic, dopaminergic and glutamatergic systems. In particular, studies have reported altered 5-HT_{1A}, D_1, mGluR5 receptor levels in FSL rats (Bjørnebekk et al., 2007; Eriksson et al., 2012; Kovačević et al., 2012; Shrestha et al., 2014). The FSL rats also possess predictive validity, since all so far tested antidepressant therapies show antidepressant-like effects in these rats (Overstreet and Wegener, 2013).

L-DOPA is the most efficacious medication for the treatment of PD. Unfortunately, the therapeutic response to L-DOPA is wearing off over time, while abnormal involuntary movements (AIMs), such as dyskinesias, emerge as prominent side-effects in approximately 50% of all treated patients within 5–10 years (Obeso et al., 2000). Several molecular and cellular mechanisms involving non-dopaminergic systems have been proposed to explain the development of dyskinesias in PD (Cenci et al., 2011; Huot et al., 2013). One possibility is that in the advanced stage of dopaminergic cell loss, remaining serotonergic neurons in the basal ganglia complex can specifically take up L-DOPA and convert it to dopamine. In contrast to the normal situation, release of dopamine occurs in this setting when the serotonergic neurons are activated and dopamine acts as a false transmitter. Drugs that act on the 5-HT_{1A} autoreceptor, which regulates the firing of serotonergic neurons, can antagonize L-DOPA-induced dyskinesia, as shown in preclinical and clinical studies (Bibbiani et al., 2001; Tomiyama et al., 2005; Carta et al., 2007; Svenningsson et al., 2015). Sarizotan is a 5-HT_{1A} receptor agonist and dopamine D2-like partial agonist that reduces dyskinesia in PD animal models (Gregoire et al., 2009; Zhang et al., 2011a). Sarizotan has also been shown to have some anti-dyskinetic effect in PD patients (Bara-Jimenez et al., 2005; Goetz et al., 2007), but reduces also beneficial effects of L-DOPA (Goetz et al., 2007) and has not been approved for the treatment of dyskinesas. In addition to serotonin, studies have implicated the glutamatergic, cholinergic, opioidergic and GABAergic systems in L-DOPA-induced dyskinesias (Cenci et al., 2011).

The aim of the present study was to increase understanding of the influence of a depression-like genotype on experimental parkinsonism using tacrine-induced tremor and hemiparkinsonian FSL and FRL rats at baseline and upon chronic treatment with L-DOPA. A special emphasis was put on study non-dopaminergic alterations under these conditions.

MATERIALS AND METHODS

Animals, Surgery and Pharmacological Treatment

Male FRL and FSL rats (290–390 g) were housed in air-conditioned rooms (12-h dark/light cycle) at 20°C and a humidity of 53%. Experiments were performed in agreement with the European Communities Council Directive of 24 November 1986 (86/609/EEC) on the ethical use of animals and were approved by the local ethical committee at Karolinska Institutet.

Tacrine-Induced Jaw Movements

Flinders sensitive line ($n = 12$) and FRL ($n = 10$) rats were treated with tacrine (2.5 mg/kg, i.p., Sigma) to induce jaw movements which were then manually scored for 5 min after the 10-min habituation (Salamone et al., 1998).

Unilateral 6-OHDA Lesion

As shown in **Figure 1**, another group of FRL ($n = 14$) and FSL ($n = 22$) rats were anesthetized with ketamine (100 mg/kg, i.p.; Intervet)/xylazine (5 mg/kg, i.p.; Bayer, Kiel, Germany), pretreated with desipramine (25 mg/kg, i.p.; Sigma, St Louis, MO, United States)/pargyline (5 mg/kg, i.p.; Sigma), placed in a stereotaxic instrument and injected with 6-OHDA (2.5 μl of a 5 mg/ml solution; Sigma) into the median forebrain bundle (MFB) of the right hemisphere (AP −2.8 mm, ML −2.0 mm, and V −9.0 mm). Two weeks after the unilateral 6-OHDA lesion, rats were injected with apomorphine (1 mg/kg, i.p.; Sigma) and their contralateral rotations were measured to determine the degree of nigrostriatal denervation. Only rats rotating >100 turns over 30 min were included in further experiments.

Pharmacological Treatment and Behavioral Evaluation

Four weeks after surgery, rats were divided in groups according their rotation upon apomorphine so that they were similar in terms of anticipated dopamine lesion (**Figure 1**). They were treated with saline (FRL, $n = 3$; FSL, $n = 5$), sarizotan (2.5 mg/kg, i.p., Merck KGA, Darmstadt, Germany) (FRL, $n = 3$; FSL, $n = 3$), L-DOPA/benserazide (10/7.5 mg/kg, i.p., Sigma), alone (FRL, $n = 4$; FSL, $n = 6$) or in combination (FRL, $n = 4$; FSL, $n = 5$) once daily for 23 days. Once per week, rotational behavior and AIMs were measured. The number of contralateral rotations was manually counted for 2 h following drug administration. The incidence of AIMs was scored during turning behavior. AIMs were classified into three subtypes according to their topographic distribution, as previously described (Lundblad et al., 2002): axial,

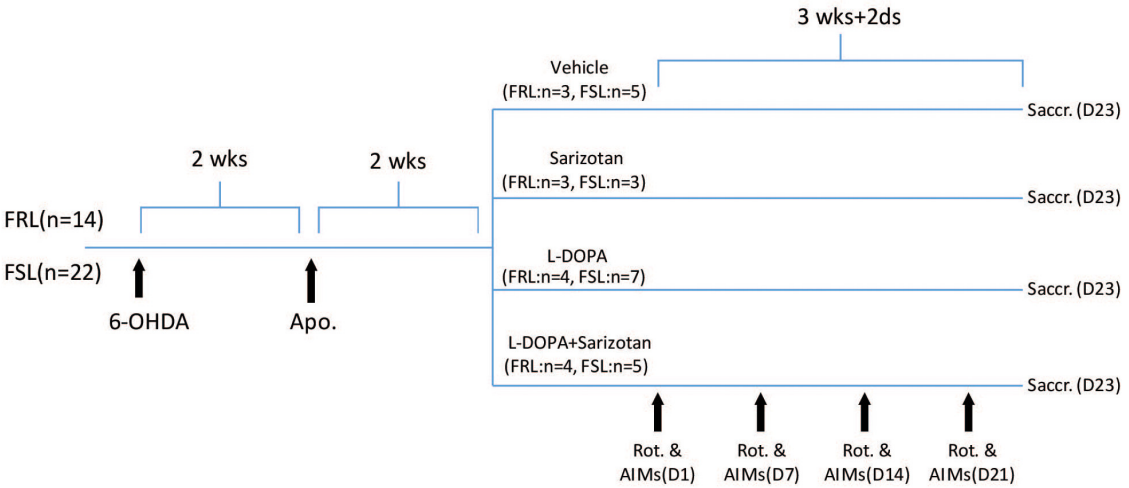


FIGURE 1 | Schematic representation of the study design. FRL (n = 14) and FSL (n = 22) rats were injected with 6-OHDA (2.5 μl of a 5 mg/ml solution) into MFB of the right hemisphere. Two weeks after the unilateral 6-OHDA lesion, rats were injected with apomorphine (1 mg/kg, i.p.) and their contralateral rotations were measured to determine the degree of nigrostriatal denervation. Four weeks after surgery, rats were treated with saline (FRL, n = 3; FSL, n = 5), sarizotan (2.5 mg/kg, i.p.) (FRL, n = 3; FSL, n = 3), L-DOPA/benserazide (10/7.5 mg/kg, i.p.), alone (FRL, n = 4; FSL, n = 6) or in combination (FRL, n = 4; FSL, n = 5) once daily for 23 days. Rotational behavior and AIMs were measured on Day 1, Day 7, Day 14, and Day 21. Animals were sacrificed 30 min after the last drug administration.

limb, and orolingual AIMs. Each AIM was quantified in a 5-min testing period every 10 min (starting 15 min after L-DOPA administration). The severity of each AIM subtype was assessed using scores from 0 to 4 (0: absent, 1: occasional, i.e., present less than 50% of the time; 2: frequent, i.e., present more than 50% of the time; 3: continuous, but interrupted by strong sensory stimuli, and 4: continuous, not interrupted by strong sensory stimuli).

Animals were sacrificed 30 min after the last drug administration. Brains were rapidly removed and frozen in dry ice-cooled isopentane. Some brains were cut in a cryostat at −20°C into 12 μm coronal sections and stored at −80°C until use.

Explorative Microarray Gene Expression Analysis

Total RNA was extracted from the 6-OHDA-lesioned striatum of vehicle- or L-DOPA-treated FRL and FSL rats using Trizol (Sigma) followed by RNeasy (Qiagen, Hilden, Germany) clean-up according to manufacturer's instructions. After quality control (Bioanalyzer), RNA samples were submitted to the Gene Expression Service Workflow of Roche NimbleGen, which included hybridization with a Rat Gene Expression 12 × 135K Array (Design Name 100718_Rat_HX12_expr), array scanning, data extraction and processing. Normalized gene expression data ("Normalized Calls") provided by Roche NimbleGen were analyzed using ANAIS (Simon and Biot, 2010) via the "Norm genes" function. Genes were considered differentially expressed if their transcript abundance (scanned signal intensity) was ≥1.5-fold higher or lower in at least one experimental group compared to striatum of vehicle-treated FRL rats. The resulting list of 6040 genes was imported into Microsoft Excel, where only those genes with ANAIS-computed ANOVA p-values <0.05 were retained. At this stage, the list of candidates was subjected to manual annotation using NCBI's Gene database, such that updated GeneIDs could be assigned to all candidates.

In situ Hybridization Studies

Radioactive complementary probe for the vesicular GABAergic transporter (vGAT) and tamalin (also called GRP1-associated scaffold protein, GRASP) were made (**Supplementary Table S1**) and used along with probes for c-fos, enkephalin and dynorphin (Zhang et al., 2006, 2011b). Briefly, sections were pretreated with 4% paraformaldehyde for 5 min at room temperature, rinsed twice in 4 × sodium chloride–sodium citrate (SSC) buffer and placed into 0.25% acetic anhydride in 0.1 M triethanolamine/4 × SSC (pH 8) for 10 min at room temperature. After dehydration in graded alcohols, the sections were hybridized overnight at 55°C with 10^6 c.p.m. of ^{35}S-labeled probe in 50 μl of hybridization solution (20 mM Tris–HCl/1 mM EDTA/300 mM NaCl/50% formamide/10% dextran sulfate/1× Denhardt's solution/250 g/ml yeast tRNA/100 g/ml salmon sperm DNA/0.1% SDS/0.1% sodium thiosulphate). The slides were washed in 4 × SSC (5 min, four times), RNAse A (20 g/ml) (20 min, at 37°C), 2 × SSC (5 min, twice), 1 × SSC (5 min), 0.5 × SSC (5 min) at room temperature, and rinsed in 0.1 × SSC at 65°C (30 min, twice), before being dehydrated in graded alcohols. The slides were then exposed to X-ray films for 5–21 days.

Autoradiographic Studies

The radioligands [^{125}I] RTI-55 (serotonin and dopamine transporter binding) and [^{125}I] MPPI [4-(2′methoxyphenyl)-1-2′[2′-pyridinyl-)-iodo-benzomido] piperazine (5-HT_{1A} binding) were purchased from Perkin Elmer Life Sciences Inc. (Boston, MA, United States).

Section for detection of DAT (dopamine transporter) and serotonin transporter (SERT) (serotonin transporter) were preincubated in 50 mM Tris–HCl/120 mM NaCl (pH 7.5) for 20 min, incubated for 1 h in the same buffer supplemented with 50 pM [^{125}I] RTI-55 in the presence of either 1 μM fluoxetine (selective serotonin reuptake inhibitor; Sigma) to label the DAT or 1 μM nomifensine (dopamine reuptake inhibitor; Sigma) to label the SERT. For non-specific binding, 100 μM nomifensine or 100 μM fluoxetine were added to the assay. The slides were washed 2 × 10 s in ice-cold binding buffer, rapidly dipped in deionized water and dried.

Sections for detection of 5-HT$_{1A}$ receptors were preincubated with 50 mM Tris–HCl containing 2 mM $MgCl_2$ (pH 7.4) for 30 min, incubated for 2 h in the same buffer together with 0.01 nM [^{125}I] MPPI. For non-specific binding, 10 μM serotonin was added. The slides were washed 2 × 15 min in ice-cold binding buffer, quickly dipped in deionized water and dried. The sections were then exposed to Kodak BioMax MR films (Sigma) for 2 days.

Data Analysis of Autoradiograms From Ligand-Binding or *in situ* Hybridization Experiments

For ligand-binding and *in situ* hybridization experiments, autoradiograms were digitized using a Dia-Scanner (Epson Perfection 4870 PHOTO). Optical density values were measured using Image J. For the analysis of the striatum, measurements in the 6-OHDA-lesioned hemisphere are given as ratios of the corresponding area from the intact hemisphere. For analysis in the raphe nuclei, data are expressed as percentage of the vehicle-treated FRL rats group.

Experiment With Sprague Dawley Rats

Male Sprague Dawley rats have been injected with 6-OHDA following exactly the same protocol described for the FSL and FRL experiment. After injecting the rats with apomorphine (1 mg/kg, i.p.), to evaluate the efficacy of the 6-OHDA lesioning, rats were treated with vehicle or L-DOPA/benserazide (10/7.5 mg/kg, i.p.) for 1 day (acute L-DOPA) or 4 weeks (chronic L-DOPA). 30 min after the last injection, brains have been removed and stored as described above for FRL and FSL rats.

Statistics

Behavioral and biochemical results were analyzed with two-way ANOVA followed by Fisher *post hoc* test for comparison between experimental groups. In the analysis of the contralateral turning behavior, time and treatment were used as independent factors; genotype and treatment were the independent factors for the analysis of AIMs and *in situ* hybridization data. In the experiment with Sprague-Dawley rats treated with saline, acute or chronic L-DOPA, one-way ANOVA was used followed by Fisher *post hoc* test. In the tacrine-induced jaw movement's experiment, where only two groups were compared, Student *t*-test was used for statistical analysis.

RESULTS

Tacrine-Induced Jaw Movements

Tremulous jaw movements induced by acute administration of the cholinesterase inhibitor tacrine (2.5 mg/kg, i.p.) were more frequent in FSL than in FRL rats ($p < 0.05$, **Figure 2**). This result indicates that movement responses reminiscent of parkinsoniam 6 Hz tremor are exaggerated in FSL compared to FRL rats.

Efficacy of Unilateral 6-OHDA Lesion

6-OHDA-induced lesioning of the MFB induces a near-complete degeneration of the nigrostriatal dopamine neurons. Supersensitization of dopaminergic receptors in the lesioned hemisphere results in induction of contralateral turning behavior after administration of dopaminergic drugs. Thus, to assess the degree of dopaminergic lesion, we measured the number of contralateral rotations after administration of the dopaminergic D_1/D_2 receptors agonist apomorphine (1 mg/kg, i.p.). No difference was found between FRL and FSL rats (**Figure 3A**). Likewise, [125I]RTI-55 autoradiography of DAT in striatum post-mortem did not detect any change in the degree of the dopaminergic lesioning between FRL and FSL rats (**Figure 3B**).

Contralateral Turning Behavior and Abnormal Involuntary Movements After L-DOPA and Sarizotan Treatment

Chronic treatment with L-DOPA elicits locomotion and also abnormal motor responses such as sensitization of contralateral turning behavior and AIMs in unilaterally 6-OHDA-lesioned rodents (Lundblad et al., 2002).

The first administration of L-DOPA induced similar contralateral turning behavior in both FRL and FSL rats (**Figure 4A**). However, the response to chronic L-DOPA treatment differed markedly between the two genotypes. In FRL rats, significant effects of treatment ($F_{1,12} = 15.18$; $p < 0.01$),

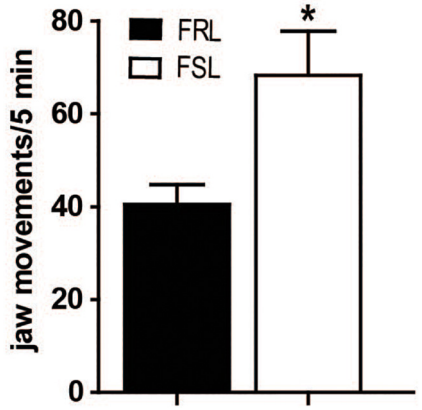


FIGURE 2 | FSL rats showed increased tacrine-induced jaw movements. Effect of tacrine (2.5 mg/kg, i.p.) on jaw movement in FRL and FSL rats. Data represent mean ± SEM for 10–12 animals per group. *$p < 0.01$ vs FRL group, accordingly to student *t*-test.

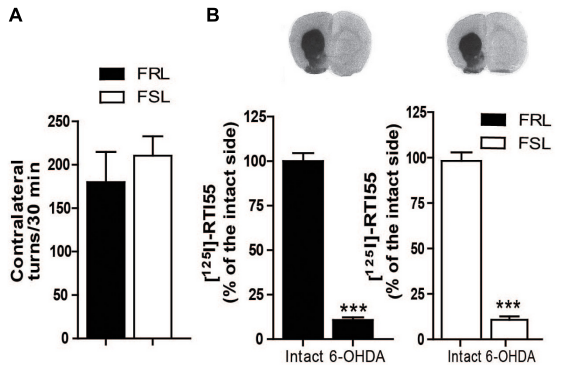


FIGURE 3 | Efficacy of 6-OHDA lesion is shown by apomorphine-induced contralateral turning behavior and dopamine transporter binding. **(A)** Shows the number of contralateral turns in FRL and FSL rats treated with apomorphine (1 mg/kg, i.p.). **(B)** Shows autoradiograms and histograms of [^{125}I] RTI-55 binding for analysis of the dopamine transporter in FRL and FSL rats. Data were generated as optical density measures. Data represent mean ± SEM of the values expressed as a percentage of the intact hemisphere for three to seven animals per group. ***$p < 0.001$ vs correspondent intact hemisphere.

time ($F_{1,12} = 4.64$; $p < 0.05$) and their interaction ($F_{1,12} = 8.79$; $p < 0.05$) were found. *Post hoc* test showed a significant increase in the number of rotations observed after chronic treatment with L-DOPA compared to the first administration ($p < 0.001$, **Figure 4A**). In contrast, in FSL rats, chronic L-DOPA treatment did not induce a significant change in the number of rotations observed, compared to acute administration (**Figure 4A**).

Chronic treatment with L-DOPA induced AIMs in both strains of rats (**Figure 4B**). However, there were significant effects of treatment ($F_{1,15} = 37.85$; $p < 0.005$). *Post hoc* test showed that FRL rats treated with L-DOPA spent more time in AIMs than FSL rats ($p < 0.05$). For the limb AIMs score, there were significant effects of treatment ($F_{1,15} = 41.07$; $p < 0.005$) and genotype ($F_{1,15} = 13.48$; $p < 0.05$). *Post hoc* test showed that FRL rats treated with L-DOPA spent more time in limb AIMs than FSL rats ($p < 0.01$).

The first co-administration of sarizotan/L-DOPA induced similar contralateral rotations in FRL and FSL rats (**Figure 4A**). Furthermore, chronic co-administration of sarizotan/L-DOPA did not induce any change in contralateral rotations compared to the first day of treatment. Accordingly, the number of contralateral turns in chronically treated sarizotan/L-DOPA FRL rats was significantly lower ($p < 0.001$) than in chronically L-DOPA-treated FRL (**Figure 4A**). No difference in the number of contralateral turns was found between sarizotan/L-DOPA- and L-DOPA-treated FSL rats. Likewise, co-administration with sarizotan significantly ($p < 0.01$) reduced L-DOPA-induced AIMs in FRL, but not in FSL, rats (**Figure 4B**).

Biochemical Mechanisms Underlying Dyskinesia

c-fos

c-fos is an inducible transcription factor that is rapidly transcribed in response to a variety of stimuli often related to increased neuronal activity (Morgan and Curran, 1989). c-fos and related Fos/Jun members, particularly deltaFosB protein, have been linked to the development of AIMs in several animal models of PD (Cenci et al., 2011). In our model of comorbid depression and PD, genotype ($F_{1,25} = 4.80$; $p < 0.05$), treatment ($F_{3,25} = 19.76$; $p < 0.001$) and their interaction ($F_{3,25} = 3.14$; $p < 0.05$) had significant effects on c-fos expression. *Post hoc* test showed that chronic treatment with L-DOPA significantly increased c-fos mRNA compared to vehicle- and sarizotan-treated rats, in both FRL and FSL groups ($p < 0.001$; **Figure 5**). However, c-fos mRNA in L-DOPA-treated FSL rats was significantly lower than in FRL rats ($p < 0.001$; **Figure 5**). c-fos mRNA in sarizotan/L-DOPA-treated FRL rats was significantly lower than in the L-DOPA treated group ($p < 0.001$; **Figure 5**), whereas no significant difference between these treatments was found in FSL rats.

Opioid Neuropeptides

Increased striatal levels of enkephalin and dynorphin are associated with the development of dyskinesia in parkinsonian animals treated with L-DOPA (Sgroi and Tonini, 2018).

Surprisingly, neither treatment nor genotype (**Figure 6A**) modified enkephalin mRNA. Dynorphin mRNA revealed a significant difference for treatment ($F_{3,25} = 11.76$; $p < 0.001$). *Post hoc* test showed that dynorphin mRNA was significantly increased ($p < 0.05$) in the L-DOPA and sarizotan/L-DOPA groups compared with vehicle and sarizotan alone in both genotypes (**Figure 6B**).

Serotonergic System

As mentioned above, there is accumulating evidence for the involvement of serotonergic neurons in the development of AIMs. In particular, stimulation of 5-HT$_{1A}$ autoreceptors located at the cell body reduces the firing of serotonergic neurons and thus the release of "false" dopamine formed from L-DOPA.

In our study, [^{125}I]MPPI binding to 5-HT$_{1A}$ receptor in the raphe nuclei of vehicle and L-DOPA treated rats revealed a significant effect of genotype ($F_{1,14} = 11.60$; $p < 0.01$) with lower levels in FSL than in FRL rats. In L-DOPA treated rats, levels of [^{125}I]MPPI were lower in FSL compared to the FRL group (*post hoc* test $p < 0.01$; **Figure 7**). The decreased level of 5-HT$_{1A}$ receptor in the raphe nuclei unlikely explains the low dyskinetic potential of L-DOPA in FSL rats, but may relate to the fact that sarizotan is less antidyskinetic in FSL compared to FRL rats. There was no significant differences in [^{125}I]MPPI binding in the striatum between hemiparkinsonian FSL and FRL rats (**Figure 8**).

Since a correlation between striatal SERT and severity of AIMs has been reported in 6-OHDA-lesioned rats, MPTP-treated macaque monkeys and in PD patients (Rylander et al., 2010), we also performed radioligand binding experiments for SERT. However, no significant differences in [^{125}I]RTI-55 binding in the striatum or raphe nuclei between hemiparkinsonian FSL and FRL rats were found (**Figures 9A,B**).

Explorative Microarray Study

The aforementioned analyses of opioid neuropeptides and serotonin systems previously linked to AIMs did not yield

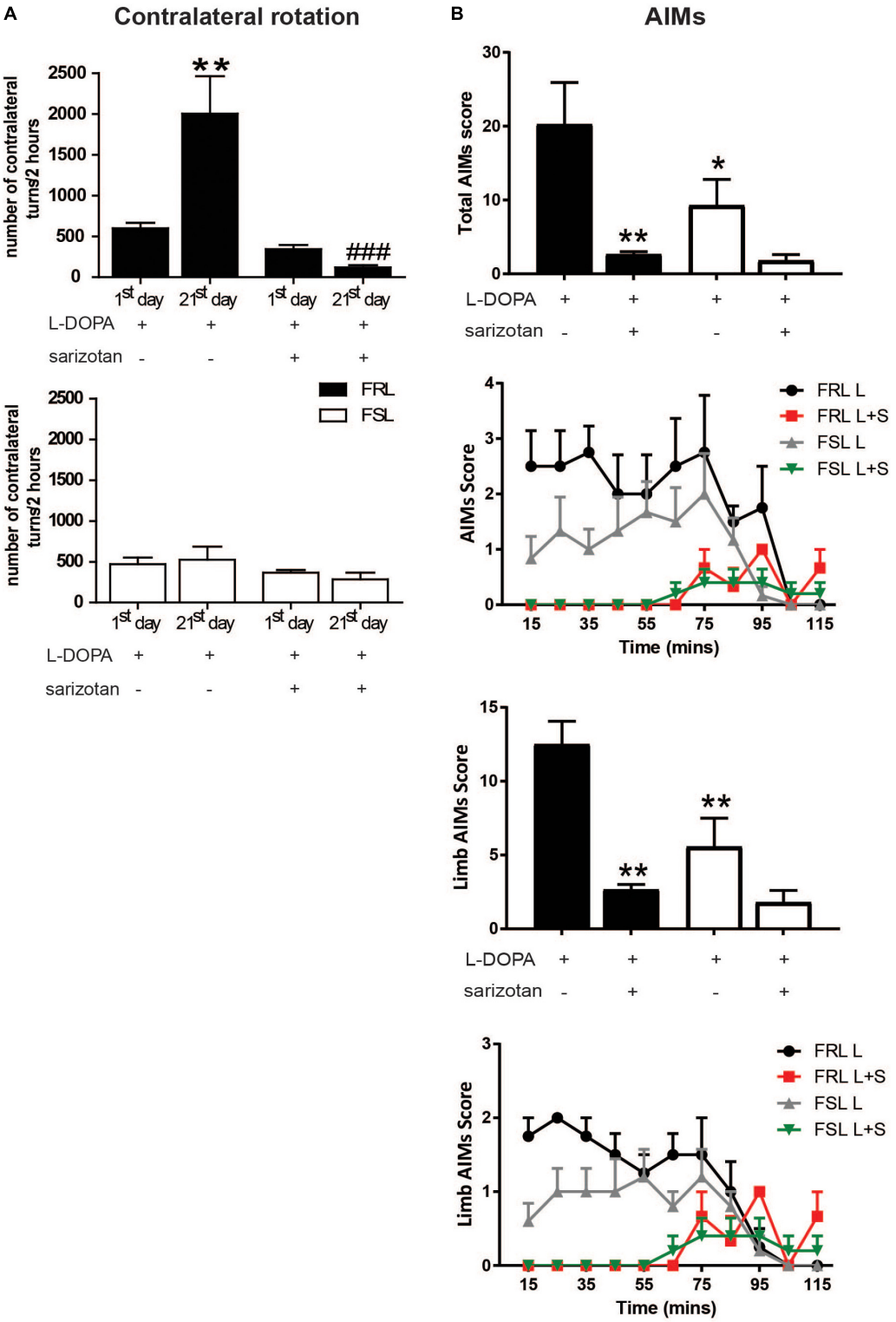


FIGURE 4 | Unilaterally 6-OHDA-lesioned FSL and FRL rats respond differently to chronic treatment with L-DOPA and sarizotan/L-DOPA in terms of turning behavior and abnormal involuntary movement. Effect of chronic treatment with L-DOPA (10 mg/kg, i.p.) and sarizotan/L-DOPA (2.5/10/mg/kg, i.p.) on **(A)** the number of contralateral turns at the 1st and 21st day of treatment and (B) Total sum of each AIMs scores, limb AIMs score and the time course at the 21st day of treatment in FRL and FSL rats. Data represent mean ± SEM for four to seven animals per group. **(A)** $^{**}p < 0.01$ vs FRL group at the 1st day of L-DOPA treatment, $^{###}p < 0.001$ vs FRL group at the 21st day of L-DOPA treatment. **(B)** $^{*}p < 0.05$, $^{**}p < 0.01$ vs FRL group treated with chronic L-DOPA accordingly to two-way ANOVA followed by Fisher *post hoc* test.

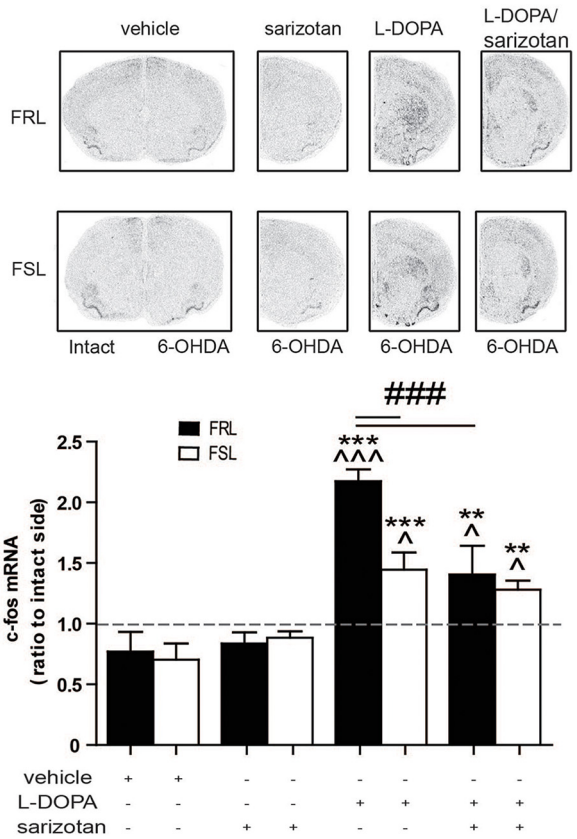


FIGURE 5 | Regulation of c-fos mRNA levels in unilaterally 6-OHDA-lesioned FRL and FSL rats treated in response to chronic treatment with L-DOPA and sarizotan/L-DOPA. Autoradiograms and histograms of *in situ* hybridization experiment against c-fos mRNA in the striatum of FRL and FSL rats chronically treated with vehicle, sarizotan (2.5 mg/kg, i.p.), L-DOPA (10 mg/kg, i.p.) and sarizotan/L-DOPA (2.5/10 mg/kg, i.p.). Data represent mean ± SEM of the ratio between the lesioned and the intact hemisphere for three to seven animals per group. $^{**}p < 0.01$, $^{***}p < 0.001$ vs correspondent vehicle-treated group; $^{\wedge}p < 0.05$, $^{\wedge\wedge\wedge}p < 0.001$ vs correspondent sarizotan-treated group, $^{\#\#\#}p < 0.001$ vs indicated groups, accordingly to two-way ANOVA followed by Fisher *post hoc* test.

correlates to the behavioral findings in our model of comorbid depression and PD. Therefore we decided to perform an explorative microarray analysis with 6-OHDA-lesioned striata collected from FRL and FSL rats (three animals per group) chronically treated with vehicle or L-DOPA. After selecting only genes that were 1.5-fold statistically different from the vehicle-treated FRL group, a list of 856 genes was obtained. Interestingly, in the chronic L-DOPA-treated FRL rats, 224 genes were found to be upregulated compared to the vehicle-treated FRL group, while only 68 were found in the L-DOPA-treated FSL group, indicating a generally lower response in the depression-like genotype (**Supplementary Tables S2** and **S3**). The list of candidate genes was manually investigated looking for genes potentially linked to dyskinesia. c-fos was found to be in the list and already validated by our previous *in situ* hybridization result (**Figure 4**). Some other genes were chosen based on a

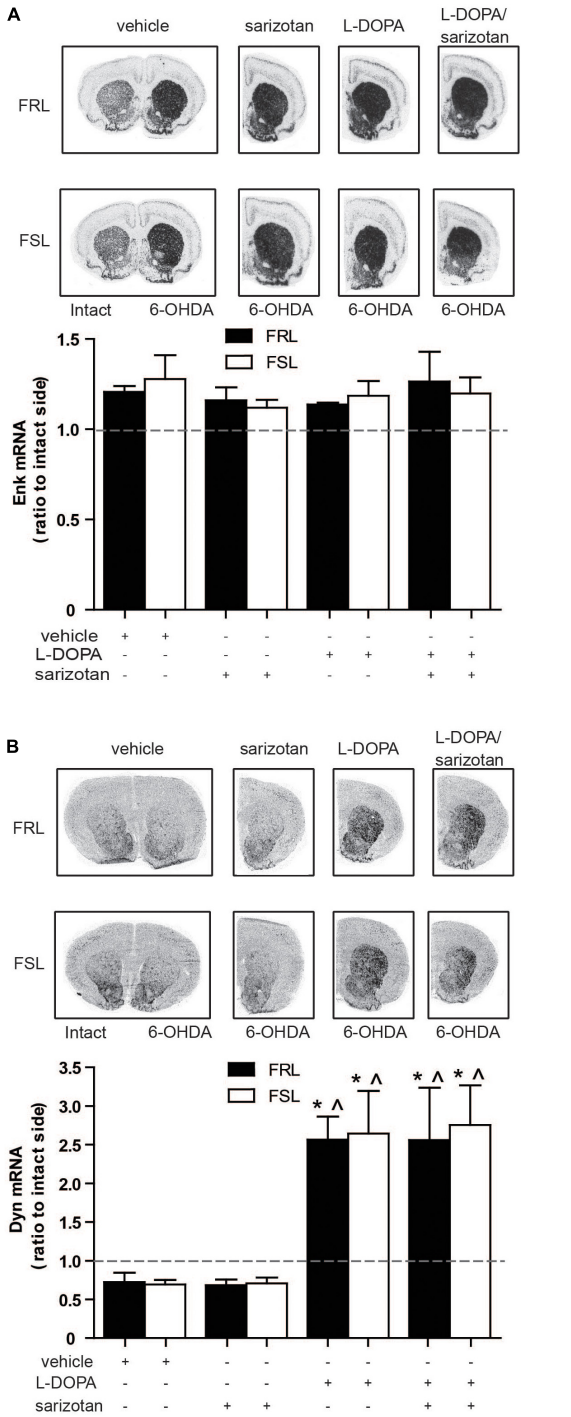


FIGURE 6 | Effect of chronic treatment with L-DOPA and sarizotan/L-DOPA on the enkephalin and dynorphin mRNA levels in 6-OHDA-lesioned FRL and FSL rat. Autoradiograms and histograms of *in situ* hybridization experiment against **(A)** enkephalin and **(B)** dynorphin mRNAs in the striatum of FRL and FSL rats chronically treated with vehicle, sarizotan (2.5 mg/kg, i.p.), L-DOPA (10 mg/kg, i.p.) and sarizotan/L-DOPA (2.5/10 mg/kg, i.p.). Data represent mean ± SEM of the ratio between the lesioned and the intact hemisphere for three to seven animals per group. **(B)** $^{*}p < 0.05$ vs correspondent vehicle-treated group; $^{\wedge}p < 0.05$ vs correspondent sarizotan-treated group, accordingly to two-way ANOVA followed by Fisher *post hoc* test.

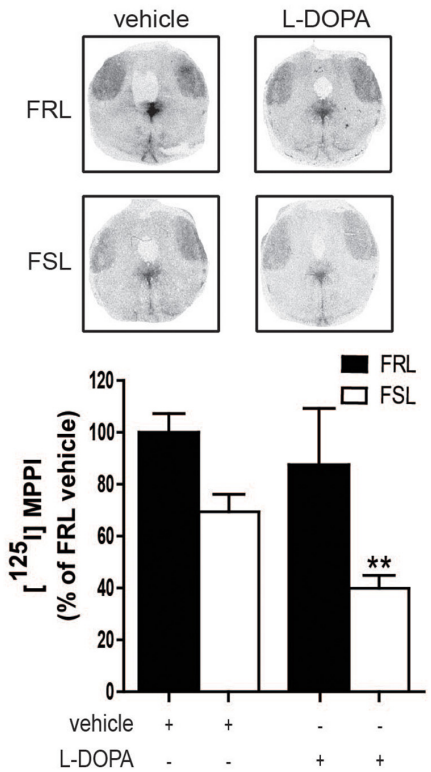


FIGURE 7 | 5-HT$_{1A}$ levels in the raphe nuclei of 6-OHDA-lesioned FRL and FSL rat chronically treated with L-DOPA. Autoradiograms and histograms of [^{125}I] MPPI binding experiment in the raphe nuclei of FRL and FSL rats chronically treated with vehicle and L-DOPA (10 mg/kg, i.p.). Data were generated as optical density measures. Data represent mean ± SEM of the percentage of the vehicle-treated FRL group, for three to seven animals per group. $^{**}p < 0.001$ vs L-DOPA-treated FRL group, accordingly to two-way ANOVA followed by Fisher *post hoc* test.

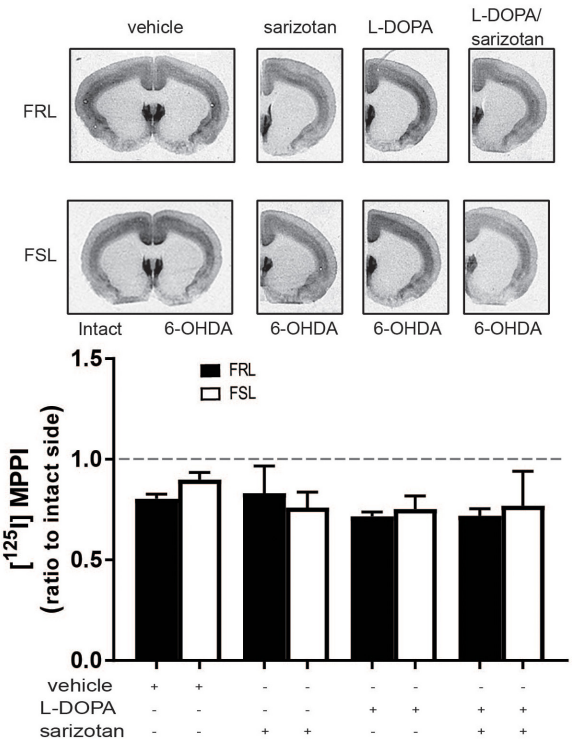


FIGURE 8 | 5-HT$_{1A}$ levels in the striatum of 6-OHDA-lesioned FRL and FSL rat chronically treated with L-DOPA. Autoradiograms and histograms of [^{125}I] MPPI binding experiment in the striatum of FRL and FSL rats chronically treated with vehicle, sarizotan (2.5 mg/kg, i.p.), L-DOPA (10 mg/kg, i.p.) and sarizotan/L-DOPA (2.5/10 mg/kg, i.p.). Data were generated as optical density measures. Data represent mean ± SEM of the ratio between the lesioned and the intact hemisphere for three to seven animals per group.

possible correlation with dyskinesias for further validation by *in situ* hybridization.

Vesicular GABA Transporter, Previously Known to Correlated With AIMs

GABA-related genes have previously been linked with AIMs (Wang et al., 2007). Among the genes found via microarray analysis, vGAT (Slc32a1) expression correlated well with the behavioral data. *In situ* hybridization studies revealed a significant effect of treatment ($F_{3,25} = 3.72$; $p < 0.05$). *Post hoc* test showed that chronic treatment with L-DOPA increased ($p < 0.01$) vGAT mRNA in FRL but not FSL rats when compared to vehicle- or sarizotan-treated groups (**Figure 10**). FRL rats treated with sarizotan/L-DOPA showed a significantly lower vGAT mRNA level than FRL rats treated with L-DOPA ($p < 0.05$; **Figure 10**).

Tamalin, Previously Unknown to Correlate With AIMs

Within the list of genes that were found through the microarray analysis, tamalin, was chosen for further analyses with *in situ* hybridization although it has never been linked to AIMs before. However, the important role of tamalin in controlling the function of mGluR5 (Sugi et al., 2007) attracted our attention. Significant effects of genotype ($F_{1,25} = 6.56$; $p < 0.05$) and treatment ($F_{3,25} = 5.69$; $p < 0.01$) were found. *Post hoc* test showed that chronic treatment with L-DOPA increased ($p < 0.01$) tamalin mRNA in FRL but not FSL rats when compared to vehicle- or sarizotan-treated groups (**Figure 11A**). Moreover, sarizotan/L-DOPA-treated FRL rats had a significantly lower tamalin mRNA level than L-DOPA-treated FRL ($p < 0.05$; **Figure 11A**).

To confirm that tamalin expression gene is paralleled by L-DOPA responses not only in FRL rats, tamalin mRNA was measured also in unilaterally 6-OHDA-lesioned Sprague Dawley rats treated with vehicle, or with acute or chronic (4 weeks) L-DOPA. An overall significant treatment effect was found ($F_{2,15} = 14.0$; $p < 0.001$). *Post hoc* test showed that rats chronically treated with L-DOPA had a significantly higher tamalin mRNA both when compared to rats treated with vehicle and acute L-DOPA ($p < 0.001$ and $p < 0.05$, respectively; **Figure 11B**).

DISCUSSION

Depressed patients have a two-fold increased risk to develop PD when compared to healthy siblings and the general

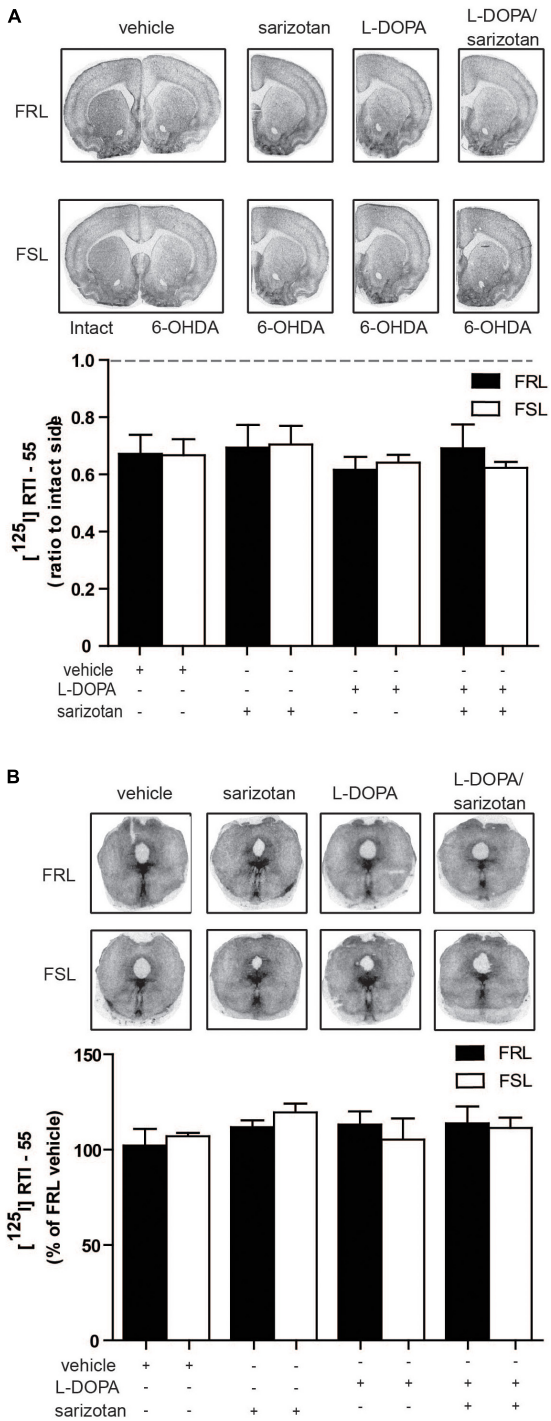


FIGURE 9 | Serotonin transporter levels in the striatum and raphe nuclei of 6-OHDA-lesioned FRL and FSL rat chronically treated with L-DOPA and sarizotan/L-DOPA. Autoradiograms and histograms of [^{125}I] RTI-55 binding experiments in **(A)** the striatum and **(B)** raphe nuclei of FRL and FSL rats chronically treated with vehicle, sarizotan (2.5 mg/kg, i.p.), L-DOPA (10 mg/kg, i.p.) and sarizotan/L-DOPA (2.5/10 mg/kg, i.p.). Data were generated as optical density measures. Data represent mean ± SEM of **(A)** the ratio between the lesioned and the intact hemisphere and **(B)** the percentage of the vehicle-treated FRL group, for three to seven animals per group, accordingly to two-way ANOVA followed by Fisher *post hoc* test.

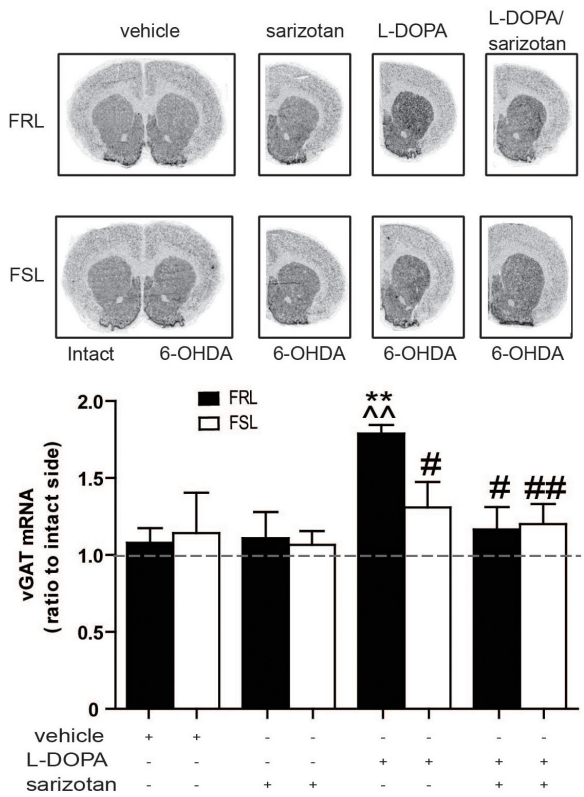


FIGURE 10 | Different effect of chronic treatment with L-DOPA and sarizotan/L-DOPA on the vGAT mRNA levels in unilaterally 6-OHDA-lesioned FRL and FSL rats. Autoradiograms and histograms of *in situ* hybridization experiment against vGAT mRNA in the striatum of FRL and FSL rats chronically treated with vehicle, sarizotan (2.5 mg/kg, i.p.), L-DOPA (10 mg/kg, i.p.) and sarizotan/L-DOPA (2.5/10 mg/kg, i.p.). Data represent mean ± SEM of the ratio between the lesioned and the intact hemisphere for three to seven animals per group. $^{**}p < 0.01$ vs correspondent vehicle-treated group; $^{\wedge}p < 0.01$ vs correspondent sarizotan-treated group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs FRL group chronically treated with L-DOPA, accordingly to two-way ANOVA followed by Fisher *post hoc* test.

population. Tacrine induces jaw movements in rodents that have been suggested to model parkinsonian tremor (Salamone et al., 1998). FSL rats were found to be more responsive to tacrine-induced tremorous jaw movements which is consistent with their supersensitivity toward cholinergic compounds (Overstreet et al., 2005). In a clinical perspective it is interesting to note that both parkinsonian tremor and depressive symptomatology can be counteracted by cholinergic muscarine receptor antagonists (Drevets et al., 2013; Kalia and Lang, 2015). A speculation derived from the data on tacrine actions in FSL rats is that precautions should be taken to prescribe cholinesterase inhibitors to depressed parkinsonian patients with a tremor-dominant type. Vice versa, such patients may have antitremor as well as antidepressant responses to anti-cholinergic compounds. Our data also suggest that a depression-like genetic background combined with hemiparkinsonism results in a reduced response to antiparkinson treatments compared to control "non-depressed" FRL rats. Thus, chronic treatment

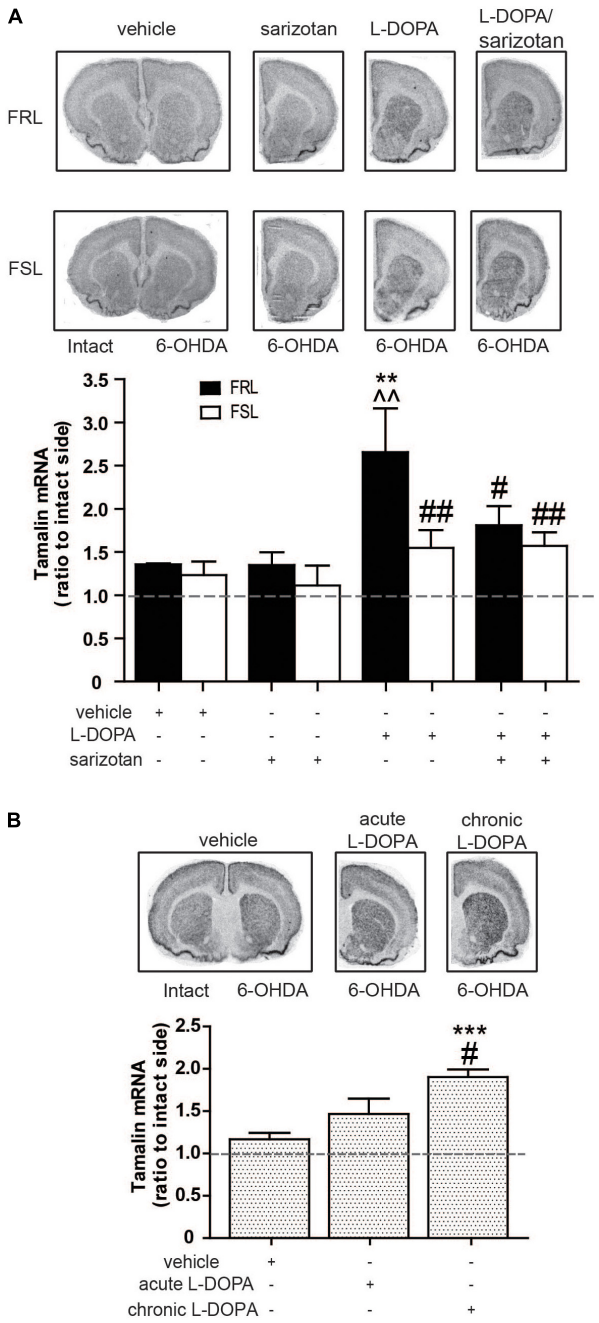


FIGURE 11 | Chronic L-DOPA treatment increases in tamalin mRNA levels in unilaterally 6-OHDA-lesioned FRL and Sprague Dawley, but not FSL, rats. Autoradiograms and histograms of *in situ* hybridization experiment against tamalin mRNA in the striatum of **(A)** FRL and FSL rats chronically treated with vehicle, sarizotan (2.5 mg/kg, i.p.), L-DOPA (10 mg/kg, i.p.), and sarizotan/L-DOPA (2.5/10 mg/kg, i.p.) and **(B)** Spraque Dawley rats treated with vehicle, acute and chronic L-DOPA (10 mg/kg, i.p.). Data represent mean ± SEM of the ratio between the lesioned and the intact hemisphere for three to eight animals per group. **(A)** $^{**}p < 0.01$ vs correspondent vehicle-treated group, $^{\wedge}p < 0.01$ vs correspondent sarizotan-treated group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs FRL group chronically treated with L-DOPA, accordingly to two-way ANOVA followed by Fisher *post hoc* test; **(B)** $^{***}p < 0.001$ vs vehicle-treated group, $^{\#}p < 0.05$ vs the group acutely treated with L-DOPA.

with L-DOPA caused development of supersensitization and dyskinetic behaviors in FRL, but not in FSL, rats. To our knowledge, hemiparkinsonian FSL rats represent the first rat strain that have reduced AIMs upon chronic L-DOPA treatment. In the clinical setting, it is known that patients which develops supersentization and dyskinesia toward L-DOPA have significant problems with depression and anxiety (van der Velden et al., 2018). However, to our knowledge, it remains to be studied whether parkinsonian patients with depression are more likely to develop L-DOPA supersensitization and dyskinesia when compared to matched patients without depression. Effects of the antidyskinetic agent sarizotan were attenuated in FSL rats. Co-administration of sarizotan and L-DOPA prevented dyskinesia, both in terms of sensitization to turning and AIMs, in FRL, but not FSL, rats. A possible explanation is the finding of decreased levels of 5-HT_{1A} receptor in the raphe nuclei of FSL rats chronically treated with L-DOPA.

The reduced L-DOPA response in FSL rats provides an interesting opportunity to learn more about molecular mechanisms underlying the development of L-DOPA-induced sensitization and AIMs. For this reason, a series of experiments were performed to identify parallels between gene expression markers previously linked to dyskinesia and the dramatic different behavioral response found in the hemiparkinsonian FSL and FRL rats. As expected, we found that striatal c-fos mRNA was increased in FSL, compared to FRL, rats. We did not find any genotype difference to an acute challenge with Apomorphine, a D_1/D_2 receptor agonist, or L-DOPA. These data, together with the previous finding that D_1 receptors are actually increased in FSL rats (Bjørnebekk et al., 2007) argue against a prominent role of alterations in the dopaminergic system underlying the lack of supersentization toward L-DOPA in FSL rats. As mentioned above, several non-dopaminergic systems have been implicated in L-DOPA-induced dyskinesias and we studied several of them. No parallels between the behavioral responses and the levels of striatal opioid neuropeptides or serotonin markers were found. This discrepancy could be due to several contributes. Importantly, FRL and FSL rats are selectively bred strains and differ from Sprague Dawley rats which are normally used in preclinical studies of L-DOPA induced dyskinesia. Moreover, our data could further indicate that the interval between the last administration of L-DOPA and the time of death has to be taken into account when interpreting results on the level of opioid peptides (Sgroi and Tonini, 2018). Furthermore, there are contradictory reports when it comes to alterations in SERT in dyskinetic PD patients (Kish et al., 2008; Rylander et al., 2010).

Since the difference between the FRL and FSL rats in response to L-DOPA did not correlate to changes in opioid peptidergic or serotonergic correlates of dyskinesia, we decided to search for other biochemical correlates to elucidate this difference. We therefore performed an exploratory microarray experiment with the striata of FRL and FSL animals chronically treated with L-DOPA and found a parallel behavioral responses and c-fos [along with other Fos/Jun members including fosB (**Supplementary Table S3**)]. Moreover, a correlation between increased vGAT and behavioral responses was also found. An alteration of vesicular GABA release in L-DOPA-treated animals

was previously reported (Wang et al., 2007) and our result further supports a role of the GABAergic system in the modulation of L-DOPA supersentization and dyskinesia.

An additional novel finding is the parallel alterations between an increased tamalin mRNA expression and L-DOPA-induced sensitization and AIMs. Tamalin is a scaffold protein highly expressed in the brain that interacts with metabotropic groups 1 and 2 glutamate receptors and $GABA_{B2}$ receptors along with specific guanine nucleotide exchange factors (Nevrivy et al., 2000; Sugi et al., 2007). Tamalin has been shown to have an autoinhibiting conformation when its concentration is low and when the concentration increases, the binding with mGluR5 receptors stabilizes the active conformation (Sugi et al., 2007). mGluR5 receptors are critically implicated in the development of L-dopa-induced dyskinesias (Sgambato-Faure and Cenci, 2012). Tamalin also interacts with scaffolding proteins, including PSD-95, S-SCAM, SAPAP1/3, Mint2, and CASK, that are involved in postsynaptic organization and protein trafficking in neuronal cells. Thus, through these multiple interactions, tamalin is positioned to control a variety of signaling pathways that could be involved in the mediation of AIMs. In this context, it is intriguing that mGluR5 receptors are reduced in FSL rats (Kovačević et al., 2012). It is possible that a reduced upregulation of mGluR5/Tamalin signaling contributes to the reduced L-DOPA responses in FSL rats.

In conclusion, our study suggests that a genetic depression-like rat model have altered behavioral and transcriptional responses in experimental parkinsonism and to antiparkinson drugs. Several adaptations in non-dopaminergic systems appears to underlie this differential responsivity and, in particular, induction of the mGluR5 adaptor protein, tamalin, is a novel correlate to L-DOPA-induced supersentization and dyskinesia.

DATA AVAILABILITY STATEMENT

The data generated for this study can be found in NCBI using the accession numbers MN474033–MN475147.

ETHICS STATEMENT

The experiments were performed in agreement with the European Communities Council Directive of 24 November 1986 (86/609/EEC) on the ethical use of animals and were approved by the local Ethical Committee at Karolinska Institutet.

AUTHOR CONTRIBUTIONS

NSc performed the research, analyzed the data, and wrote and edited the manuscript. XZ performed the research, analyzed the data, and edited the manuscript. NSt analyzed the data and edited the manuscript. AM contributed with essential reagents and edited the manuscript. PA edited the manuscript. PS designed the study and wrote the manuscript.

FUNDING

This study was supported by the Swedish Parkinson Fund, the Fisher Foundation, the Swedish Research Council, the Swedish Brain Foundation, the Swedish Foundation for Strategic Research, and the Science for Life Laboratory.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2020.00304/full#supplementary-material

TABLE S1 | Primers used to generate riboprobes.

TABLE S2 | Genes expressed in the 6-OHDA-lesioned striata of FRL and FSL chronically treated with vehicle. List of genes obtained from the microarray analysis of the RNA extracted from 6-OHDA-lesioned striata of FRL and FSL chronically treated with vehicle. Selecting criteria was made in the way that only genes that were found to be 1.5-fold statistically different at least in one experimental group from the vehicle-treated FRL group were listed.

TABLE S3 | Genes expressed in the 6-OHDA-lesioned striata of FRL and FSL chronically treated with L-DOPA. List of genes obtained from the microarray analysis of the RNA extracted from 6-OHDA-lesioned striata of FRL and FSL chronically treated with L-DOPA/benserazide (10/7.5 mg/kg, i.p.). Selecting criteria was made in the way that only genes that were found to be 1.5-fold statistically different at least in one experimental group from the vehicle-treated FRL group were listed.

REFERENCES

Aarsland, D., Pahlhagen, S., Ballard, C. G., Ehrt, U., and Svenningsson, P. (2012). Depression in Parkinson disease–epidemiology, mechanisms and management. *Nat. Rev. Neurol.* 8, 35–47. doi: 10.1038/nrneurol.2011.189

Bara-Jimenez, W., Bibbiani, F., Morris, M. J., Dimitrova, T., Sherzai, A., Mouradian, M. M., et al. (2005). Effects of serotonin 5-HT1A agonist in advanced Parkinson's disease. *Mov. Disord.* 20, 932–936. doi: 10.1002/mds.20370

Bibbiani, F., Oh, J. D., and Chase, T. N. (2001). Serotonin 5-HT1A agonist improves motor complications in rodent and primate parkinsonian models. *Neurology* 57, 1829–1834. doi: 10.1212/wnl.57.10.1829

Bjørnebekk, A., Mathé, A. A., and Brené, S. (2007). Isolated flinders sensitive line rats have decreased dopamine D2 receptor mRNA. *Neuroreport* 18, 1039–1043. doi: 10.1097/wnr.0b013e3281668bf7

Carta, M., Carlsson, T., Kirik, D., and Bjorklund, A. (2007). Dopamine released from 5-HT terminals is the cause of L-DOPA-induced dyskinesia in parkinsonian rats. *Brain* 130, 1819–1833. doi: 10.1093/brain/awm082

Cenci, M. A., Ohlin, K. E., and Odin, P. (2011). Current options and future possibilities for the treatment of dyskinesia and motor fluctuations in Parkinson's disease. *CNS Neurol. Disord. Drug Targets* 10, 670–684. doi: 10.2174/187152711797247885

Drevets, W. C., Zarate, C. A. Jr., and Furey, M. L. (2013). Antidepressant effects of the muscarinic cholinergic receptor antagonist scopolamine: a review. *Biol. Psychiatry* 73, 1156–1163. doi: 10.1016/j.biopsych.2012.09.031

Eriksson, T. M., Delagrange, P., Spedding, M., Popoli, M., Mathe, A. A., Ogren, S. O., et al. (2012). Emotional memory impairments in a genetic rat model of depression: involvement of 5-HT/MEK/Arc signaling in restoration. *Mol. Psychiatry* 17, 173–184. doi: 10.1038/mp.2010.131

Goetz, C. G., Damier, P., Hicking, C., Laska, E., Müller, T., Olanow, C. W., et al. (2007). Sarizotan as a treatment for dyskinesias in Parkinson's disease: a double-blind placebo-controlled trial. *Mov. Disord.* 22, 179–186. doi: 10.1002/mds.21226

Gregoire, L., Samadi, P., Graham, J., Bedard, P. J., Bartoszyk, G. D., and Di Paolo, T. (2009). Low doses of sarizotan reduce dyskinesias and maintain antiparkinsonian efficacy of L-Dopa in parkinsonian monkeys. *Parkinsonism Relat. Disord.* 15, 445–452. doi: 10.1016/j.parkreldis.2008.11.001

Huot, P., Johnston, T. H., Koprich, J. B., Fox, S. H., and Brotchie, J. M. (2013). The pharmacology of L-DOPA- induced dyskinesia in Parkinson's disease. *Pharmacol. Rev.* 65, 171–222. doi: 10.1124/pr.111.005678

Kalia, L. V., and Lang, A. E. (2015). Parkinson's disease. *Lancet* 386, 896–912. doi: 10.1016/S0140-6736(14)61393-3

Kish, S. J., Tong, J., Hornykiewicz, O., Rajput, A., Chang, L. J., Guttman, M., et al. (2008). Preferential loss of serotonin markers in caudate versus putamen in Parkinson's disease. *Brain* 131, 120–131.

Kovačević, T., Skelin, I., Minuzzi, L., Rosa-Neto, P., and Diksic, M. (2012). Reduced metabotropic glutamate receptor 5 in the Flinders Sensitive Line of rats, an animal model of depression: an autoradiographic study. *Brain Res. Bull.* 87, 406–412. doi: 10.1016/j.brainresbull.2012.01.010

Leentjens, A. F., Van den Akker, M., Metsemakers, J. F., Lousberg, R., and Verhey, F. R. (2003). Higher incidence of depression preceding the onset of Parkinson's disease: a register study. *Mov. Disord.* 18, 414–418. doi: 10.1002/mds.10387

Lundblad, M., Andersson, M., Winkler, C., Kirik, D., Wierup, N., and Cenci, M. A. (2002). Pharmacological validation of behavioural measures of akinesia and dyskinesia in a rat model of Parkinson's disease. *Eur. J. Neurosci.* 15, 120–132. doi: 10.1046/j.0953-816x.2001.01843.x

Morgan, J. I., and Curran, T. (1989). Stimulus-transcription coupling in neurons: role of cellular immediate- early genes. *Trends Neurosci.* 12, 459–462. doi: 10.1016/0166-2236(89)90096-9

Nevrivy, D. J., Peterson, V. J., Avram, D., Ishmael, J. E., Hansen, S. G., Dowell, P., et al. (2000). Interaction of GRASP, a protein encoded by a novel retinoic acid-induced gene, with members of the cytohesin family of guanine nucleotide exchange factors. *J. Biol. Chem.* 275, 16827–16836. doi: 10.1074/jbc.275.22.16827

Nilsson, F. M., Kessing, L. V., and Bolwig, T. G. (2001). Increased risk of developing Parkinson's disease for patients with major affective disorder: a register study. *Acta Psychiatr. Scand.* 104, 380–386. doi: 10.1111/j.1600-0447.2001.00372.x

Obeso, J. A., Olanow, C. W., and Nutt, J. G. (2000). Levodopa motor complications in Parkinson's disease. *Trends Neurosci.* 23 (10 Suppl), S2–S7.

Overstreet, D. H., Friedman, E., Mathe, A. A., and Yadid, G. (2005). The Flinders Sensitive Line rat: a selectively bred putative animal model of depression. *Neurosci. Biobehav. Rev.* 29, 739–759. doi: 10.1016/j.neubiorev.2005.03.015

Overstreet, D. H., and Wegener, G. (2013). The flinders sensitive line rat model of depression–25 years and still producing. *Pharmacol. Rev.* 65, 143–155. doi: 10.1124/pr.111.005397

Robins, A. H. (1976). Depression in patients with Parkinsonism. *Br. J. Psychiatry* 128, 141–145.

Rylander, D., Parent, M., O'Sullivan, S. S., Dovero, S., Lees, A. J., Bezard, E., et al. (2010). Maladaptive plasticity of serotonin axon terminals in levodopa-induced dyskinesia. *Ann. Neurol.* 68, 619–628. doi: 10.1002/ana.22097

Salamone, J. D., Mayorga, A. J., Trevitt, J. T., Cousins, M. S., Conlan, A., and Nawab, A. (1998). Tremulous jaw movements in rats: a model of parkinsonian tremor. *Prog. Neurobiol.* 56, 591–611. doi: 10.1016/s0301-0082(98)00053-7

Sgambato-Faure, V., and Cenci, M. A. (2012). Glutamatergic mechanisms in the dyskinesias induced by pharmacological dopamine replacement and deep brain stimulation for the treatment of Parkinson's disease. *Prog. Neurobiol.* 96, 69–86. doi: 10.1016/j.pneurobio.2011.10.005

Sgroi, S., and Tonini, R. (2018). Opioidergic modulation of striatal circuits, implications in Parkinson's disease and levodopa induced Dyskinesia. *Front. Neurol.* 9:524. doi: 10.3389/fneur.2018.00524

Shrestha, S. S., Pine, D. S., Luckenbaugh, D. A., Varnäs, K., Henter, I. D., Innis, R. B., et al. (2014). Antidepressant effects on serotonin 1A/1B receptors in the rat brain using a gene x environment model. *Neurosci. Lett.* 559, 163–168. doi: 10.1016/j.neulet.2013.11.034

Simon, A., and Biot, E. (2010). ANAIS: analysis of NimbleGen arrays interface. *Bioinformatics* 26, 2468–2469. doi: 10.1093/bioinformatics/btq410

Sugi, T., Oyama, T., Muto, T., Nakanishi, S., Morikawa, K., and Jingami, H. (2007). Crystal structures of autoinhibitory PDZ domain of Tamalin: implications for metabotropic glutamate receptor trafficking regulation. *EMBO J.* 26, 2192–2205. doi: 10.1038/sj.emboj.7601651

Svenningsson, P., Rosenblad, C., Af Edholm Arvidsson, K., Wictorin, K., Keywood, C., Shankar, B., et al. (2015). Eltoprazine counteracts l-DOPA-induced dyskinesias in Parkinson's disease: a dose-finding study. *Brain* 138, 963–973. doi: 10.1093/brain/awu409

Tomiyama, M., Kimura, T., Maeda, T., Kannari, K., Matsunaga, M., and Baba, M. (2005). A serotonin 5-HT1A receptor agonist prevents behavioral sensitization to L-DOPA in a rodent model of Parkinson's disease. *Neurosci. Res.* 52, 185–194. doi: 10.1016/j.neures.2005.03.004

van der Velden, R. M. J., Broen, M. P. G., Kuijf, M. L., and Leentjens, A. F. G. (2018). Frequency of mood and anxiety fluctuations in Parkinson's disease patients with motor fluctuations: a systematic review. *Mov. Disord.* 33, 1521–1527. doi: 10.1002/mds.27465

Wang, H., Katz, J., Dagostino, P., and Soghomonian, J. J. (2007). Unilateral 6-hydroxydopamine lesion of dopamine neurons and subchronic L-DOPA administration in the adult rat alters the expression of the vesicular GABA transporter in different subsets of striatal neurons and in the substantia nigra, pars reticulata. *Neuroscience* 145, 727–737. doi: 10.1016/j.neuroscience.2006.12.001

Zhang, X., Andren, P. E., Glennon, R. A., and Svenningsson, P. (2011a). Distribution, level, pharmacology, regulation, and signaling of 5-HT6 receptors in rats and marmosets with special reference to an experimental model of parkinsonism. *J. Comp. Neurol.* 519, 1816–1827. doi: 10.1002/cne.22605

Zhang, X., Egeland, M., and Svenningsson, P. (2011b). Antidepressant-like properties of sarizotan in experimental Parkinsonism. *Psychopharmacology* 218, 621–634. doi: 10.1007/s00213-011-2356-7

Zhang, X., Andren, P. E., and Svenningsson, P. (2006). Repeated l-DOPA treatment increases c-fos and BDNF mRNAs in the subthalamic nucleus in the 6-OHDA rat model of Parkinson's disease. *Brain Res.* 1095, 207–210. doi: 10.1016/j.brainres.2006.04.019

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer, GF, declared a shared affiliation, though no other collaboration, with several of the authors, PS, XZ, NS, and AM, to the handling Editor.

frontiers in Pharmacology

REVIEW
published: 08 April 2020
doi: 10.3389/fphar.2020.00435

The Noradrenergic System in Parkinson's Disease

Elena Paredes-Rodriguez[1,2], *Sergio Vegas-Suarez*[1,2], *Teresa Morera-Herreras*[1,2], *Philippe De Deurwaerdere*[3] *and Cristina Miguelez*[1,2]*

[1] Department of Pharmacology, Faculty of Medicine and Nursing, University of the Basque Country (UPV/EHU), Leioa, Spain, [2] Autonomic and Movement Disorders Unit, Neurodegenerative Diseases, Biocruces Health Research Institute, Barakaldo, Spain, [3] Centre National de la Recherche scientifique, Institut des Neurosciences Cognitives et Intégratives d'Aquitaine (INCIA UMR 5287), Bordeaux, France

Nowadays it is well accepted that in Parkinson's disease (PD), the neurodegenerative process occurs in stages and that damage to other areas precedes the neuronal loss in the substantia nigra pars compacta, which is considered a pathophysiological hallmark of PD. This heterogeneous and progressive neurodegeneration may explain the diverse symptomatology of the disease, including motor and non-motor alterations. In PD, one of the first areas undergoing degeneration is the locus coeruleus (LC). This noradrenergic nucleus provides extensive innervation throughout the brain and plays a fundamental neuromodulator role, participating in stress responses, emotional memory, and control of motor, sensory, and autonomic functions. Early in the disease, LC neurons suffer modifications that can condition the effectiveness of pharmacological treatments, and importantly, can lead to the appearance of common non-motor symptomatology. The noradrenergic system also exerts anti-inflammatory and neuroprotective effect on the dopaminergic degeneration and noradrenergic damage can consequently condition the progress of the disease. From the pharmacological point of view, it is also important to understand how the noradrenergic system performs in PD, since noradrenergic medication is often used in these patients, and drug interactions can take place when combining them with the gold standard drug therapy in PD, L-3,4-dihydroxyphenylalanine (L-DOPA). This review provides an overview about the functional status of the noradrenergic system in PD and its contribution to the efficacy of pharmacological-based treatments. Based on preclinical and clinical publications, a special attention will be dedicated to the most prevalent non-motor symptoms of the disease.

Keywords: Parkinson's disease, noradrenaline, locus coeruleus, non-motor symptoms, neuroprotection, prodromic, L-DOPA, neuroinflammation

OPEN ACCESS

Edited by:
Mahmoud Iravani, University of Hertfordshire, United Kingdom

Reviewed by:
Grzegorz Kreiner, Polish Academy of Sciences, Poland
Anna Maria Pittaluga, University of Genoa, Italy

***Correspondence:**
Cristina Miguelez
cristina.miguelez@ehu.eus

Specialty section:
This article was submitted to Neuropharmacology, a section of the journal Frontiers in Pharmacology

Received: *08 October 2019*
Accepted: *20 March 2020*
Published: *08 April 2020*

Citation:
Paredes-Rodriguez E, Vegas-Suarez S, Morera-Herreras T, De Deurwaerdere P and Miguelez C (2020) The Noradrenergic System in Parkinson's Disease. Front. Pharmacol. 11:435. doi: 10.3389/fphar.2020.00435

INTRODUCTION

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the presence of α-synuclein protein aggregates in the form of Lewy bodies in specific brain regions. These aggregates may be responsible for the onset and progression of the disease likely by promoting mitochondrial dysfunction, microglial activation, and neuroinflammatory responses, but they do

not appear all over the brain at the same time. Indeed, recent publications have suggested that the α-synuclein pathology begins in the gut and travels *via* the vagal nerve up to the brain where it spreads following the six stages defined by Braak and colleagues (Ulusoy et al., 2013; Kim et al., 2019). Although historically the hallmark of the disease has been focused on the degeneration of the substantia nigra pars compacta (SNc), it is now well accepted that the spread of α-synuclein in the brain occurs in stages and that damage to other areas precedes the degeneration of SNc neurons, affecting glutamatergic, noradrenergic, serotonergic, histaminergic, and cholinergic projection cells (Del Tredici et al., 2002; Braak et al., 2003). This heterogeneous, progressive neurodegeneration may explain the diverse symptomatology of PD, which includes motor and non-motor alterations (Chaudhuri and Schapira, 2009). Indeed, PD is more likely to be a multisystem disorder rather than a pure motor disease.

According to Braak's theory (Braak et al., 2004), the first α-synuclein aggregates in the central nervous system appear in the anterior olfactory structures and the dorsal motor nucleus of the vagus nerve, following by lower raphe system and the locus coeruleus (LC) in stage 2. It is not until stage 3 that the SNc is affected together with the amygdala, tegmental pedunculopontine nucleus, and the higher raphe nuclei, among others. During stage 4, α-synuclein spreads to the hippocampal formation and specific cortical areas and finally, in the last two stages (5 and 6), almost the whole cortex is damaged. This pattern of α-synuclein propagation between interconnected nuclei has also been mimicked in animal models in which α-synuclein was overexpressed by means of viral vector administration in peripheral structures (Rey et al., 2013; Ulusoy et al., 2013; Ulusoy et al., 2017; Rusconi et al., 2018). The pathological process underlying PD would consist of a prodromal period followed by a symptomatic one when the disease is often diagnosed. The presymptomatic or prodromal phase (stages 1–3) is often characterized by olfactory dysfunction, autonomic dysregulation, pain, sleep, and mood disorders while the symptomatic phase (stages 4–6) is accompanied by the classical somatomotor symptoms and impaired cognitive functioning (Chaudhuri and Schapira, 2009; Braak and Del Tredici, 2016).

Among the brain areas that undergo degeneration in the prodromal phase, the LC deserves special attention for being one of the first nuclei to develop Lewy bodies, and because LC dysfunction may be related to several of the non-motor symptoms observed in the disease. Here, we will review the functional status of the LC in PD using data from experimental models and patients. We will also analyze the potential role of the LC in PD-associated neuroinflammation, the appearance of non-motor complications, and the pharmacological therapies.

THE LOCUS COERULEUS

The LC is a bilateral nucleus located in the upper dorsolateral pontine tegmentum and is considered the principal noradrenergic nucleus in the central nervous system (Amaral and Sinnamon, 1977). Although noradrenergic neurons are the biggest cell population, GABAergic interneurons also inhabit the LC making synapses and efficiently inhibit the noradrenergic neurons (Aston-Jones et al., 2004; Jin et al., 2016; Breton-Provencher and Sur, 2019). Neurochemical content and receptor expression are also very heterogeneous containing adrenergic, GABAergic, serotonergic, glutamatergic, μ-opioid, orexin/hypocreatin, nicotinic acetylcholine, and cannabinoid receptors (reviewed in Berridge and Waterhouse, 2003; Schwarz and Luo, 2015). LC noradrenergic cells, as happens with SNc neurons, also contains neuromelanin which makes them specially vulnerable to neurodegeneration in PD (reviewed in Martin-Bastida et al., 2017 and Vila, 2019).

Despite being a tiny nucleus, the LC shows an enormous projecting network, influencing the activity of nuclei all over the brain. It sends descending projections to the spinal cord (Westlund et al., 1983) and densely innervates ascending areas of the CNS as the amygdala, superior colliculus, paraventricular thalamic nucleus, hippocampus, olfactory bulb, dorsal raphe, and cortex, including prefrontal, orbitofrontal, anterior cingulate, and primary motor cortices (Fallon et al., 1978; Shipley et al., 1985; Loughlin et al., 1986; Kim et al., 2004; Chandler et al., 2014; Schwarz et al., 2015; Kempadoo et al., 2016; Takeuchi et al., 2016; McCall et al., 2017; Beas et al., 2018; Li L. et al., 2018). The SNc and the ventral tegmental area also receive modest noradrenergic innervation from the LC (Baldo et al., 2003; Mejías-Aponte et al., 2009). By contrast, those areas with intense dopaminergic innervation as the nucleus accumbens or the striatum show discrete noradrenergic innervation (Mason and Fibiger, 1979; Berridge et al., 1997; Delfs et al., 1998; Fitoussi et al., 2013). As for the afferences, the LC also receives a large variety of inputs including those from the paragigantocellularis, prepositus hypoglossi, dorsal raphe, superior colliculus, prefrontal cortex, or the SNc (Aston-Jones et al., 1986; Devoto et al., 2005b; Delaville et al., 2011; Lu et al., 2012; Breton-Provencher and Sur, 2019).

It is interesting to mention that although the LC does not project to nuclei highly innervated by the dopaminergic system, it can still influence dopaminergic transmission distally. Devoto and collaborators have extensively characterized that LC-tyrosine hydroxylase positive fibers can co-release not only noradrenaline (NA) but also dopamine (DA) in the cortex, including prefrontal, parietal, and occipital cortices involving α_2-adrenoceptor-mediated mechanisms (Devoto et al., 2001; Devoto et al., 2003; Devoto et al., 2004; Devoto et al., 2005a; Devoto et al., 2005b). More recently, other authors have also supported that LC activation promotes DA release in the thalamus and hippocampus, contributing to stress and cognitive functions (Smith and Greene, 2012; Kempadoo et al., 2016; Yamasaki and Takeuchi, 2017; Beas et al., 2018).

In view of the dense noradrenergic projection network, it is easy to understand the implication of this nucleus in many physiological functions and pathological conditions. Experimental preclinical models have demonstrated the implication of the LC in arousal, cognition, anxiety, depression, pain, attention, and locomotor control (Aston-Jones and Bloom, 1981; Carter et al., 2010; Curtis et al., 2012; Sara and Bouret, 2012; Chandler et al., 2014; McCall

et al., 2015; Szot et al., 2016; Benarroch, 2017; Hirschberg et al., 2017; McCall et al., 2017; Beas et al., 2018; Breton-Provencher and Sur, 2019; Llorca-Torralba et al., 2019). The availability of new technologies, as opto- and chemogenetics, that allow efficient activation/inhibition of specific anatomical projections or cellular subtypes, has provided a better understanding of those functions and unraveled that the LC is a more heterogeneous nucleus than previously proposed. Interestingly many of the pathological situations triggered by the dysfunction of the LC are present in PD, stressing the role of this nucleus in the development and management of the non-motor complications of the disease.

NORADRENERGIC DYSFUNCTION IN PARKINSON'S DISEASE

Preclinical Evidence

In the last decades, researchers have shown increasing interest in further understanding the pathophysiological basis of the non-motor symptoms present in PD with special focus on the noradrenergic system. In the preclinical studies, viral-vector-induced, gene-mutated, or neurotoxin-based animal models are regularly used although the vast majority of the data come from these latter ones.

Anatomical studies using the unilaterally 6-hydroxydopamine (6-OHDA)-lesioned rat model show that the number of LC neurons is not affected by the DA loss (Miguelez et al., 2011b; Ostock et al., 2018) but NA levels in different projection areas are variably decreased. In the prefrontal cortex of the lesioned hemisphere, some authors found unchanged (Delaville et al., 2012a; Delaville et al., 2012b) or reduced NA concentrations (Shin et al., 2014; Ostock et al., 2018). Similarly, other areas with sparse noradrenergic innervation, as the striatum, show unchanged or lower NA levels (Shin et al., 2014; Ostock et al., 2018). Bilateral models of 6-OHDA show, however, more robust NA deficits in the cortex and striatum (Vieira et al., 2019). 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated monkeys exhibit clear noradrenergic damage, including LC cell loss, lower NA concentrations in several brain regions, and reduced noradrenergic innervation of the SNc and the subthalamic nucleus (Pifl et al., 1991; Masilamoni et al., 2017). Some publications have also reported low NA striatal and cortical tissue content in MPTP mice (Luchtman et al., 2009; Nayyar et al., 2009; Ando et al., 2018). Indeed, MPTP reproduces better than 6-OHDA the heterogeneous neuronal damage produced in PD, as recently shown by a publication using matrix-assisted laser desorption-ionization mass spectrometry (Kadar et al., 2014). Evidence regarding the integrity of the noradrenergic system in transgenic mice models of PD is more scarce but also stresses noradrenergic impairment. Thus, reduced tyrosine hydroxylase positive cells and α-synuclein aggregations in the LC have been demonstrated in PINK1 knockout rats (Grant et al., 2015; Cullen et al., 2018), LRRK, and parkin knockout mice (Von Coelln et al., 2004; Giaime et al., 2017). However, in transgenic mice overexpressing human A53T α-synuclein, although having lower NA levels at the level of the striatum, olfactory bulb, and spinal cord, TH positive cells in the LC expressed modest α-synuclein aggregates but remained intact in number (Giasson et al., 2002; Sotiriou et al., 2010). These noradrenergic dysfunctions are accompanied by behavioral deficits, such as early vocalization and swallowing deficits.

It is also interesting to stress that in parkinsonian conditions, the noradrenergic system may contribute in some extent to the loss of dopaminergic function. In animals lesioned with 6-OHDA, noradrenergic transporter (NET) are increased possibly for compensating for the severe DA loss (Chotibut et al., 2012). Indeed, NET can reuptake not only NA but also DA in those regions with sparse DA innervation (Morón et al., 2002). In this line, some publications support that in absence of DA transporters, NET reuptakes L-DOPA-derived DA in the striatum and other areas, playing a possible role in L-DOPA induced dyskinesia (LID), as later discussed.

Apart from the anatomical and neurochemical changes, few electrophysiological studies using anaesthetized 6-OHDA lesioned animals, have revealed that experimental DA degeneration also impacts LC neuron basal activity and its response to antidepressant agents in parkinsonian rodents. Regarding the electrophysiological changes, increased and decreased activity was reported by different authors (Wang et al., 2009; Miguelez et al., 2011a; Miguelez et al., 2011b). This discrepancy may rely in methodological differences that could imply direct noradrenergic damage produced by the neurotoxins (Szot et al., 2016). On the other hand, in control rats, serotonergic and noradrenergic antidepressants interact with L-DOPA when administered together (see below). Using the forced swimming test, control rats subchronically treated with L-DOPA and fluoxetine showed reduced efficacy of the antidepressant drug, while coadministration of the NET blocker reboxetine and L-DOPA provided the opposite effect (Miguelez et al., 2013). At the behavioral level, regardless inconsistencies found through the scientific publications, parkinsonian animals tend to mimic the human symptomatology showing motor but also non-motor impairments (Titova et al., 2017). An array of studies report that rodents lesioned with 6-OHDA or MPTP show anxious and depressive behavior, pain, cognitive, and sleep disturbances (Monaca et al., 2004; Pérez et al., 2009; Berghauzen-Maciejewska et al., 2014; Vo et al., 2014; Kamińska et al., 2017; Charles et al., 2018; Campos et al., 2019; Domenici et al., 2019), more notably in bilateral models of the disease (Ferro et al., 2005; Tadaiesky et al., 2008; Santiago et al., 2010; Bonito-Oliva et al., 2014; Vieira et al., 2019). Although the participation of other nuclei cannot be ruled out, the role of the LC in the mentioned functions is widely accepted.

Although preclinical data to large extent support the noradrenergic affection in parkinsonian models, several discrepancies exist, which may be due to methodological variations. In this regard, especially when using the neurotoxin 6-OHDA, it should be considered that lesion protocols vary considerably among the studies, including key steps like animal age, toxin dose and injection site, magnitude of the lesions, and administration of the non-selective NET blocker desipramine to protect noradrenergic neurons from the 6-OHDA toxicity.

Genetic and α-synuclein based models are less often used with considerable results variation among the studies. The use of other neurotoxins as MPTP seems to provide results that are more consistent.

Clinical Evidence

In line with the aforementioned findings reported in animal models of PD, there is substantial evidence showing degeneration of the noradrenergic system in PD patients. Numerous anatomical *post mortem* studies in PD brains have documented a moderate to severe cell loss (around 30–90%) and Lewy body pathology in the LC, equal in magnitude throughout the rostral-caudal parts of the nucleus (Gaspar and Gray, 1984; Chan-Palay and Asan, 1989; German et al., 1992; Bertrand et al., 1997; Zarow et al., 2003; McMillan et al., 2011). Specifically, neuromelanin-containing medium-size LC neurons present somatic and dendritic alterations, whereas smaller non-noradrenergic LC cells do not show severe pathological changes (Patt and Gerhard, 1993).

Although *in vivo* positron emission tomography imaging studies using non-specific ligands failed to identify the noradrenergic damage, more recent neuromelanin-sensitive magnetic resonance studies have found progressive loss of the LC signal in both idiopathic and genetic PD patients and even a lower signal in those PD patients with depressive symptoms (Sasaki et al., 2006; Castellanos et al., 2015; Schwarz et al., 2017; Wang et al., 2018). ^{18}F-dopa positron emission tomography imaging, as an index of monoaminergic nerve terminal function, have also demonstrated a reduced uptake in the LC, indicating progressive loss of noradrenergic terminal function (Pavese et al., 2011).

While the relationship between α-synuclein accumulation and neuronal death is not fully understood, it has been proposed that this protein burden may lead to neuronal dysfunction/degeneration and, therefore, impair neurotransmission (Espay et al., 2014). As explained before, Braak and colleagues established six levels of degeneration over the course of the disease where the noradrenergic impairment would occur earlier than the dopaminergic one and the subsequent primary motor symptoms. It has further been proposed that α-synuclein pathology in the LC not only precedes, but may also be of greater magnitude than that occurring in the SNc, a finding that persists across disease stages (Zarow et al., 2003). These results suggest that LC dysfunction may directly contribute to disease onset and progression rather than be a collateral consequence.

Consistent with LC neuron loss and degeneration, there is a decreased noradrenergic innervation of LC target structures, including the prefrontal and motor cortex, striatum, thalamus, hypothalamus, and cerebellum (Kish et al., 1984; Shannak et al., 1994; Pavese et al., 2011; Pifl et al., 2012; Sommerauer et al., 2018b). The atrophy of tyrosine hydroxylase-containing axons is not restricted to the central nervous system, and a prominent loss of noradrenergic innervations of the peripheral autonomic system has been demonstrated, including in the left cardiac ventricle (Hakusui et al., 1994; Takatsu et al., 2000; Slaets et al., 2015).

Although plasma NA levels are elevated in *de novo* PD patients (Ahlskog et al., 1996), neurochemical studies have reported lower levels of the neuronal NA metabolite dihydroxyphenylglycol in the cerebrospinal fluid (Goldstein et al., 2012), as well as marked reduction of DA-beta-hydroxylase activity, an enzyme responsible for hydroxylation of DA to NA, in parkinsonian patients (Hurst et al., 1985; O'Connor et al., 1994). Regarding changes in adrenergic receptors in PD, an *in vitro* autoradiographic study showed upregulation of α_1- and β_1- and reduced density of α_2-adrenoceptors in the prefrontal cortex of *post mortem* parkinsonian patients (Cash et al., 1984).

Despite some discrepancies in animal models, both preclinical and clinical studies support the notion that the noradrenergic system is impaired in parkinsonism. This is important for understanding the complexity of the neurodegenerative process and should be taken into account when administering drugs whose pharmacological effect relies on the integrity of this system.

CLINICAL IMPLICATIONS OF NORADRENERGIC DYSFUNCTION IN PD

The degeneration of the noradrenergic system in the CNS and periphery occurring in PD is associated with a broad spectrum of non-motor symptoms that encompass autonomic, behavioral, and cognitive parameters. The appearance of these symptoms and signs cannot be just attributed to an alteration in the functioning of the noradrenergic system, as they are also known to be associated with deficits in other neurotransmission systems such as cholinergic, serotonergic, GABAergic, or glutamatergic (Schapira et al., 2017). However, in this review, we will focus on non-motor complications appearing in the prodromal phase of the disease that, apart from other neurotransmitters' abnormalities, implicate malfunctioning of LC neurons.

In accordance with predicted Braak's stages, the different clinical features due to noradrenergic dysfunction can be observed along the progression of the disease (Halliday et al., 2011) and, often appear before motor symptoms onset. Detecting noradrenergic impairment could be used as a diagnostic biomarker for early detection of the neurodegeneration, providing an opportunity for intervention with disease-modifying therapies (Betts et al., 2019).

Autonomic Disturbances

Sympathetic autonomic dysfunction is a common clinical feature of PD and may precede motor symptomatology, becoming more prevalent as the disease progresses (Schapira et al., 2017). The most common dysautonomic symptoms are orthostatic hypotension, urogenital dysfunction, and constipation (Martinez-Martin et al., 2015), but PD patients can also suffer fatigue, thermoregulatory dysregulation, excessive perspiration, or postural light-headedness. Autonomic dysfunction has a heterogeneous manifestation and its progression is not predictable, however, it is associated with reduced autonomy and a decline in quality of life,

regardless of severity or duration of the disease (Leclair-Visonneau et al., 2018). The orthostatic hypotension affects 30–58% of PD patients (Goldstein, 2006) and has been linked to peripheral sympathetic cardiovascular denervation and also in a certain degree to central autonomic involvement. PD patients with defined symptomatic orthostatic hypotension exhibit decreased LC neuromelanin signal on magnetic resonance studies (Sommerauer et al., 2018a) and low plasma levels of NA, which is associated with both supersensitivity of vascular adrenergic receptors and an up-regulation of platelet α_2-adrenoceptors (Senard et al., 1990). As a consequence of a reduced capacity to adapt the peripheral vasculature and cerebral perfusion pressure PD patients can often manifest postural light-headedness and syncope (Sharabi et al., 2008). Constipation and prolonged gastrointestinal transit time affect more than 80% of PD patients and, in some cases, may lead to megacolon (for review see Cersosimo and Benarroch, 2008). Although defecation dysfunction seems to be multifactorial, one of the proposed pathophysiological mechanisms is the accumulation of α-synuclein immunoreactive Lewy bodies in sympathetic ganglia (Wakabayashi and Takahashi, 1997). Urinary and sexual dysfunctions are also late features of PD related to degeneration of brain regions that innervate the bladder, among them the LC (Micieli et al., 2003; Park and Stacy, 2009).

Sleep Disorders

Sleep disturbances are among the most frequent PD symptoms, affecting some 60–98% of patients (Stacy, 2002). Common sleep disorders include excessive daytime somnolence, nocturnal wakefulness, sleep attacks, REM sleep behavior disorder, or restless leg syndrome. All these sleep impairments can be early premotor manifestations and related to LC dysfunction as underlying mechanism, since this nucleus contributes to the control of arousal and sleep-wake cycle (Carter et al., 2010). In fact, *post mortem* examinations of patients with REM sleep behavior disorder without motor symptoms revealed neuronal loss and Lewy bodies in the LC (Uchiyama et al., 1995). A recent magnetic resonance study further linked LC neuromelanin levels with amount of REM sleep without atonia in PD patients (Sommerauer et al., 2018a). Clinical management of sleep disorders in PD is complex because most of the antiparkinsonian drugs can alter sleep architecture and induce sleepiness as a side effect. Nevertheless clonazepam or melatonin are often prescribed (Gagnon et al., 2006).

Depression

Depression affects up to 40% of PD patients and may precede the onset of motor symptomatology (Cummings, 1992; Shiba et al., 2000; Cummings et al., 2019). There is strong evidence for a correlation between noradrenergic function and depression in PD patients. In fact, neuroimaging and neuropathological studies have demonstrated reduced LC projections to limbic brain areas (cingulate cortex, thalamus, ventral striatum, or amygdala), as well as gliosis and cell loss at LC level, which was more pronounced in patients with higher frequency of depression or anxiety (Remy et al., 2005; Frisina et al., 2009). As in major depression, selective serotonin reuptake inhibitors, tricyclic antidepressants, serotonin and NA reuptake inhibitors, and monoamine oxidase inhibitors are used in the pharmacotherapy, with partial effect, probably influenced by the neurodegeneration of the serotonergic and noradrenergic systems (Ryan et al., 2019). The use of antidepressant that do not target monoaminergic systems may potentially offer benefit in these patients (Vanle et al., 2018).

Cognitive Manifestations

The loss of LC neurons and decreased noradrenergic innervation of forebrain targets are associated with cognitive dysfunction in PD (Cash et al., 1987; Rommelfanger and Weinshenker, 2007; Vazey and Aston-Jones, 2012; Sommerauer et al., 2018a). In early PD, subtle cognitive deficits include difficulty in executing functioning, particularly cognitive flexibility, which is the capacity to update and redirect attention when the environmental or homeostatic conditions change. Flexibility in cognitive processing is an essential function of prefrontal cortex and it has been proposed that loss of prefrontal noradrenergic input may contribute to this prodromal cognitive deficit (Vazey and Aston-Jones, 2012). In late stage PD, dementia can occur with a prevalence of 24–31% (Aarsland et al., 2005). Although dementia in PD is related to a substantial reduction in cortical cholinergic markers, there is also evidence for a more severe loss of noradrenergic input from the LC to cortical areas (Chan-Palay and Asan, 1989). The severity of dementia has been linked to the loss of LC neurons in some studies (Zweig et al., 1993; Del Tredici and Braak, 2013; Li et al., 2019).

NORADRENALINE, NEUROINFLAMMATION, AND NEUROPROTECTION

It is important to mention that NA might protect DA neurons from damage and therefore, integrity of the noradrenergic system may condition the progression of the disease. In this sense, preclinical data from MPTP mice and marmosets suggest that damaging the LC leads to a loss of DA neurons in the SNc followed by more pronounced motor deficits (Mavridis et al., 1991; Marien et al., 1993; Bing et al., 1994; Fornai et al., 1995; Fornai et al., 1997; Yao et al., 2015; Li Y. et al., 2018). Conversely, the damage produced by MPTP is reduced when the synthesis of NA is boosted (Kilbourn et al., 1998; Archer, 2016) or the NET is knocked out (Rommelfanger et al., 2004). A recent publication using mutant mice characterized by the progressive degeneration of dopaminergic neurons demonstrated that chronic pharmacological NET blockade ameliorates such degeneration and the subsequent motor impairment (Kreiner et al., 2019). Peripheral administration of the NET blocker atomoxetine also reduced DA damage in a lipopolysaccharide inflammatory rat model of PD (Yssel et al., 2018). Direct noradrenergic damage by the administration of the neurotoxin N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (or DSP-4) also produces motor deficits and DA cell loss in control rats (Af Bjerkén et al., 2019). In another recent publication, DSP-4 developed motor and non-motor symptoms in control mice and exacerbated motor disability in mice rendered parkinsonian by the injection of lipopolysaccharide (Song et al., 2019). Some studies performed

in 6-OHDA lesioned rodents also suggest that noradrenergic lesions in parkinsonian rats augment dopaminergic neuron vulnerability (Ostock et al., 2014) leading to lower DA levels (Srinivasan and Schmidt, 2003) and worsening motor performance (Srinivasan and Schmidt, 2003; Srinivasan and Schmidt, 2004; Wang et al., 2010; Shin et al., 2014; Ostock et al., 2018). Other studies using the same 6-OHDA model failed, however, to reproduce the latter findings (Delaville et al., 2012a; Guimarães et al., 2013; Ostock et al., 2014; Shin et al., 2014).

The mechanism underlying the neuroprotective effect of NA on DA degeneration is not well understood, although several lines of evidence point at the anti-inflammatory properties of NA as one factor responsible of such an effect. Both PD patients and animal models of the disease show reactive astrogliosis, astrocytic dysfunction, and microglial activation, which has been proposed as the origin of neuroinflammation (Ouchi et al., 2005; Gerhard et al., 2006; Glass et al., 2010; Terada et al., 2016; Liu et al., 2017; Tsutsumi et al., 2019). Importantly, by controlling microglial activation NA is able to halt the damage of dopaminergic neurons. Studies performed in cell cultures and animal models suggest that NA suppresses neuroinflammation by acting, at least in part, on b_2-adrenergic receptors, which are highly expressed in glial cells (Mori et al., 2002; Tanaka et al., 2002; Yao et al., 2015). Low concentrations of NA or long acting β_2-agonists are able to inhibit the microglial production and release of chemokines, interleucines, tumor necrosis factor (TNF-α), superoxide or nitric oxide, among others, (Mori et al., 2002; McNamee et al., 2010a; Qian et al., 2011) or to stimulate the synthesis of interleukin-1 receptor antagonists (McNamee et al., 2010b). Pharmaceutical strategies for increasing NA levels also attenuate nigral microglial activation and ameliorate the behavioral deficits in parkinsonian rats (Yssel et al., 2018). Other authors have also proposed that in addition to the β_2-mediated mechanisms, NA can impact inflammation by inhibiting NADPH oxidase-generated superoxide (Jiang et al., 2015). Additionally, β_2-agonists and NET inhibitors can also induce release of neurotrophic factors from astrocytes promoting neuroprotection and regeneration (Yssel et al., 2018).

By contrast, NA deficit accelerates dopaminergic neurodegeneration by promoting inflammation, diminishing neurotrophic factors and promoting oxidation in the SN (Yao et al., 2015; Af Bjerkén et al., 2019). In this sense, a recent publication observed that additional lesion of the LC in animal models of PD promotes enhanced release of interleukins and cytokines, likely due to an incorrect microglial function, and aggravates DA neuron degeneration (Yao et al., 2015) although some authors fails to confirm this cell loss (Iravani et al., 2014). As commented before, combined dual NA/DA lesions lead to more severe phenotype including motor and non-motor symptomatology, probably due to the higher inflammatory response and faster cell loss (Bharani et al., 2017; Song et al., 2019). The implication of NA in neuroinflamation and neuroprotection could have an important translational impact in the clinic. In this sense, a long-term prospective observatory study has reported that those patients with chronic respiratory diseases, that are chronically treated with β_2-adrenergic agonists may have a lower probability of developing PD (Mittal et al., 2017). One of the reasons for this neuroprotective effect may not only rely on microglial activation, but also on the ability of b_2-adrenoceptors to down regulate expression of human α-synuclein genes and moderate protein expression. When looking for new neuroprotective therapies, it may be relevant to assure a good NA tone for minimizing the contribution of neuroinflammation to the neuropathology.

L-DOPA AND THE NORADRENERGIC SYSTEM

L-DOPA is still considered the most efficient anti-parkinsonian drug. It is a metabolic precursor of NA through its decarboxylation into DA by the aromatic amino acid decarboxylase and the β-hydroxylation of DA by the DA beta-hydroxylase. Unfortunately, L-DOPA is poorly effective against non-motor symptoms, does not control some later-onset motor problems like freezing or "wearing-off" fluctuations, and its long-term use is associated with dyskinesia and hallucinations (Olanow et al., 2009; Hirao et al., 2015). The contribution of the noradrenergic system to LID has been investigated in animal models using NA/DA neurotoxic lesions. Although some studies report no implication of the noradrenergic system (Pérez et al., 2009; Ostock et al., 2014; Ostock et al., 2018), others have demonstrated that additional noradrenergic lesion worsens dyskinetic movements in parkinsonian rodents chronically treated with L-DOPA (Fulceri et al., 2007; Miguelez et al., 2011b; Shin et al., 2014). Interestingly, in one study, the authors induced the noradrenergic lesion to already dyskinetic animals, showing an increase in the duration of the dyskinetic effect of L-DOPA probably due to impaired striatal DA clearance (Miguelez et al., 2011b).

Some noradrenergic drugs have proven antidyskinetic properties in experimental animal models of LID. The α_{2A}-adrenoceptor antagonist idazoxan, reduced LID, and delayed their onset without compromising the motor score in MPTP-treated monkeys. Idazoxan prevented LID appearance while increasing the locomotor response to L-DOPA (Henry et al., 1999; Grondin et al., 2000; Fox et al., 2001). Additional α_{2A}-adrenoceptor antagonists have proven similar antidyskinetic properties (Gomez-Mancilla and Bédard, 1993; Henry et al., 1999; Grondin et al., 2000; Savola et al., 2003; Fox and Brotchie, 2010). Other noradrenergic drugs, as the β-adrenergic receptor antagonist propranolol and the α_2-receptor agonist clonidine also showed antidyskinetic effects in MPTP-treated monkeys, at the cost of reducing the antiparkinsonian efficacy (Gomez-Mancilla and Bédard, 1993). Experiments performed in 6-OHDA lesioned rats support the prodyskinetic action of the α_2-antagonist atipamezole and the antidyskinetic effect of propranonol, clonidine, or idazoxan, which did not worsen motor performance (Dekundy et al., 2007; Gerlach et al., 2013; Bhide et al., 2015; Ostock et al., 2015). These findings

suggest that the limited though beneficial effect of clonidine on LID is probably indirect due to the stimulation of somatodendritic α_2-receptors, which inhibit noradrenergic neuronal activity and NA release. Some authors also suggest that the antidyskinetic effect of clonidine may be related to its sedative properties (Gerlach et al., 2013). Conversely, the antidyskinetic action of idazoxan, even if it enhances brain NA release by antagonizing α_2 autoreceptors, could be related to the blockade of α_2-receptors expressed by NA receptive cells at terminal levels. In any case, the overall picture is complex due to the involvement of the other adrenergic receptor subtypes in LID. The resulting effect of an overall increase in NA extracellular on LID is thus uncertain. In contrast to the beneficial effect of reboxetine (Shin et al., 2014), other NET inhibitors including desipramine have been reported to aggravate LID (Arai et al., 2008; Chotibut et al., 2014; Conti et al., 2016).

As mentioned above, noradrenergic mechanisms are involved in non-motor symptoms and shape the responses to other medications such as antidepressant drugs (Eskow Jaunarajs et al., 2010; Eskow Jaunarajs et al., 2011; Miguelez et al., 2011b; Miguelez et al., 2013). Noradrenergic neurons may also be involved in the effects of L-DOPA, but the preclinical data are not clear. For instance, participation of noradrenergic neurons in the ability of L-DOPA to enhance DA extracellular levels is likely an indirect effect that requires the dorsal raphe nucleus (Tanaka et al., 1999; Navailles et al., 2010; Navailles and De Deurwaerdère, 2012; De Deurwaerdère et al., 2017; Miguelez et al., 2017). In dyskinetic rats, lesion of noradrenergic terminals or neurons did not reduce L-DOPA-stimulated extracellular levels of DA in the striatum (Navailles et al., 2014; Ostock et al., 2018). However, the noradrenergic lesion enhanced the effect of L-DOPA in extrastriatal regions in part due to the loss of clearance of extracellular DA by noradrenergic fibers bearing NET (Navailles et al., 2014). Another study found that the noradrenergic lesion with DSP-4 potentiates L-DOPA-induced rotations, although this behavioral effect is not directly related to DA extracellular levels (Pérez et al., 2007). Evidence suggests that the noradrenergic system participates in the effect of L-DOPA, but preclinical data are difficult to extrapolate to PD patients because the noradrenergic fibers, at least from the LC, are damaged (see above). It is also important to bear in mind that extracellular striatal DA levels induced by L-DOPA in the striatum neither parallel motor effects nor abnormal motor effects (De Deurwaerdère et al., 2017).

Although behavioral data support that noradrenergic drugs can modulate the effect of L-DOPA, the potential effect of L-DOPA on NA content or neuron activity is less clear. In the LC, low doses of L-DOPA did not alter the electrical tonic activity of noradrenergic neurons in control rats (Miguelez et al., 2013). *Post mortem* data vary, reporting no effect, or a decrease in NA tissue concentration in response to L-DOPA administration depending on the dose regimen and the brain region studied (for review see De Deurwaerdère et al., 2017). Acute or chronic L-DOPA increased NA tissue level in the prefrontal cortex of normal macaques, but the same regimen decreased NA tissue level in the prefrontal cortex and the amygdala of MPTP-treated monkeys whether they were dyskinetic or not (Engeln et al., 2015). The only difference regarding NA tissue levels between non-dyskinetic and dyskinetic monkeys was found at the level of the motor cortex. Studies on L-DOPA-evoked extracellular levels of NA also show inconclusive results. In this regard, the latest publications have reported a substantial increase in striatal NA release after L-DOPA administration (Wang et al., 2014; Ostock et al., 2018). However, in one study NA levels were still excessive after noradrenergic neurons were destroyed (Ostock et al., 2018). It is likely that other electrochemically active compounds were confounding the chromatograms (Chagraoui et al., 2019). Other data indicate that L-DOPA either inhibits or does not alter NA release in the cortex (Dayan and Finberg, 2003; Pascucci et al., 2012).

CONCLUSION

The identification of noradrenergic mechanisms in PD is crucial for understanding autonomic functions and non-motor symptomatology, and drugs that target this system may have a beneficial impact in the quality of life of the patients. One major difficulty is to extrapolate the results from animal models to patients where those alterations are variable and depend on the stage of the disease. Meanwhile, the involvement of the noradrenergic system in L-DOPA induced therapeutic effects is controversial, and noradrenergic strategies to limit the side effects accompanying anti-parkinsonian drugs are still not firmly established. In summary, and taking into account that noradrenergic system pathophysiology is a common feature of PD with other neurodegenerative diseases, such as Alzheimer's disease or atypical neurodegenerative dementias, maintenance of this system integrity may provide a common viable therapeutic option as neurodegenerative diseases-modifying strategy.

AUTHOR CONTRIBUTIONS

All authors contributed to writing the manuscript and approved the final version.

FUNDING

This study was supported by grants from the Basque Government (PIBA 2019-38, IT1345-19), UPV/EHU (PPGA19/15), and Spanish Government (SAF2016-77758-R [AEI/FEDER, UE]). EP-R has a fellowship from the Basque Country and SV-S from the UPV/EHU.

REFERENCES

Aarsland, D., Zaccai, J., and Brayne, C. (2005). A systematic review of prevalence studies of dementia in Parkinson's disease. *Mov. Disord. Off. J. Mov. Disord. Soc* 20, 1255–1263. doi: 10.1002/mds.20527

Af Bjerkén, S., Stenmark Persson, R., Barkander, A., Karalija, N., Pelegrina-Hidalgo, N., Gerhardt, G. A., et al. (2019). Noradrenaline is crucial for the substantia nigra dopaminergic cell maintenance. *Neurochem. Int.* 131, 104551. doi: 10.1016/j.neuint.2019.104551

Ahlskog, J. E., Uitti, R. J., Tyce, G. M., O'Brien, J. F., Petersen, R. C., and Kokmen, E. (1996). Plasma catechols and monoamine oxidase metabolites in untreated Parkinson's and Alzheimer's diseases. *J. Neurol. Sci.* 136, 162–168. doi: 10.1016/0022-510x(95)00318-v

Amaral, D. G., and Sinnamon, H. M. (1977). The locus coeruleus: neurobiology of a central noradrenergic nucleus. *Prog. Neurobiol.* 9, 147–196. doi: 10.1016/0301-0082(77)90016-8

Ando, R., Choudhury, M. E., Yamanishi, Y., Kyaw, W. T., Kubo, M., Kannou, M., et al. (2018). Modafinil alleviates levodopa-induced excessive nighttime sleepiness and restores monoaminergic systems in a nocturnal animal model of Parkinson's disease. *J. Pharmacol. Sci.* 136, 266–271. doi: 10.1016/j.jphs.2018.03.005

Arai, A., Tomiyama, M., Kannari, K., Kimura, T., Suzuki, C., Watanabe, M., et al. (2008). Reuptake of L-DOPA-derived extracellular DA in the striatum of a rodent model of Parkinson's disease via norepinephrine transporter. *Synap. N. Y. N* 62, 632–635. doi: 10.1002/syn.20535

Archer, T. (2016). Noradrenergic-Dopaminergic Interactions Due to DSP-4-MPTP Neurotoxin Treatments: Iron Connection. *Curr. Top. Behav. Neurosci.* 29, 73–86. doi: 10.1007/7854_2015_411

Aston-Jones, G., and Bloom, F. E. (1981). Activity of norepinephrine-containing locus coeruleus neurons in behaving rats anticipates fluctuations in the sleep-waking cycle. *J. Neurosci. Off. J. Soc Neurosci.* 1, 876–886. doi: 10.1523/JNEUROSCI.01-08-00876.1981

Aston-Jones, G., Ennis, M., Pieribone, V. A., Nickell, W. T., and Shipley, M. T. (1986). The brain nucleus locus coeruleus: restricted afferent control of a broad efferent network. *Science* 234, 734–737. doi: 10.1126/science.3775363

Aston-Jones, G., Zhu, Y., and Card, J. P. (2004). Numerous GABAergic afferents to locus ceruleus in the pericerulear dendritic zone: possible interneuronal pool. *J. Neurosci. Off. J. Soc Neurosci.* 24, 2313–2321. doi: 10.1523/JNEUROSCI.5339-03.2004

Baldo, B. A., Daniel, R. A., Berridge, C. W., and Kelley, A. E. (2003). Overlapping distributions of orexin/hypocretin- and dopamine-beta-hydroxylase immunoreactive fibers in rat brain regions mediating arousal, motivation, and stress. *J. Comp. Neurol.* 464, 220–237. doi: 10.1002/cne.10783

Beas, B. S., Wright, B. J., Skirzewski, M., Leng, Y., Hyun, J. H., Koita, O., et al. (2018). The locus coeruleus drives disinhibition in the midline thalamus via a dopaminergic mechanism. *Nat. Neurosci.* 21, 963–973. doi: 10.1038/s41593-018-0167-4

Benarroch, E. E. (2017). Locus coeruleus. *Cell Tissue Res.* 373 (1), 221–232. doi: 10.1007/s00441-017-2649-1

Berghauzen-Maciejewska, K., Kuter, K., Kolasiewicz, W., Głowacka, U., Dziubina, A., Ossowska, K., et al. (2014). Pramipexole but not imipramine or fluoxetine reverses the "depressive-like" behaviour in a rat model of preclinical stages of Parkinson's disease. *Behav. Brain Res.* 271, 343–353. doi: 10.1016/j.bbr.2014.06.029

Berridge, C. W., and Waterhouse, B. D. (2003). The locus coeruleus–noradrenergic system: modulation of behavioral state and state-dependent cognitive processes. *Brain Res. Rev.* 42, 33–84. doi: 10.1016/S0165-0173(03)00143-7

Berridge, C. W., Stratford, T. L., Foote, S. L., and Kelley, A. E. (1997). Distribution of dopamine beta-hydroxylase-like immunoreactive fibers within the shell subregion of the nucleus accumbens. *Synap. N. Y. N* 27, 230–241. doi: 10.1002/(SICI)1098-2396(199711)27:3<230::AID-SYN8>3.0.CO;2-E

Bertrand, E., Lechowicz, W., Szpak, G. M., and Dymecki, J. (1997). Qualitative and quantitative analysis of locus coeruleus neurons in Parkinson's disease. *Folia Neuropathol.* 35, 80–86.

Betts, M. J., Kirilina, E., Otaduy, M. C. G., Ivanov, D., Acosta-Cabronero, J., Callaghan, M. F., et al. (2019). Locus coeruleus imaging as a biomarker for noradrenergic dysfunction in neurodegenerative diseases. *Brain* 142, 2558–2571. doi: 10.1093/brain/awz193

Bharani, K. L., Derex, R., Granholm, A.-C., and Ledreux, A. (2017). A noradrenergic lesion aggravates the effects of systemic inflammation on the hippocampus of aged rats. *PloS One* 12, e0189821. doi: 10.1371/journal.pone.0189821

Bhide, N., Lindenbach, D., Barnum, C. J., George, J. A., Surrena, M. A., and Bishop, C. (2015). Effects of the beta-adrenergic receptor antagonist Propranolol on dyskinesia and L-DOPA-induced striatal DA efflux in the hemi-parkinsonian rat. *J. Neurochem.* 134, 222–232. doi: 10.1111/jnc.13125

Bing, G., Zhang, Y., Watanabe, Y., McEwen, B. S., and Stone, E. A. (1994). Locus coeruleus lesions potentiate neurotoxic effects of MPTP in dopaminergic neurons of the substantia nigra. *Brain Res.* 668, 261–265. doi: 10.1016/0006-8993(94)90534-7

Bonito-Oliva, A., Masini, D., and Fisone, G. (2014). A mouse model of non-motor symptoms in Parkinson's disease: focus on pharmacological interventions targeting affective dysfunctions. *Front. Behav. Neurosci.* 8, 290. doi: 10.3389/fnbeh.2014.00290

Braak, H., and Del Tredici, K. (2016). Potential Pathways of Abnormal Tau and α-Synuclein Dissemination in Sporadic Alzheimer's and Parkinson's Diseases. *Cold Spring Harb. Perspect. Biol.* 8, a023630. doi: 10.1101/cshperspect.a023630

Braak, H., Del Tredici, K., Rüb, U., de Vos, R. A. I., Jansen Steur, E. N. H., and Braak, E. (2003). Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiol. Aging* 24, 197–211. doi: 10.1016/S0197-4580(02)00065-9

Braak, H., Ghebremedhin, E., Rüb, U., Bratzke, H., and Del Tredici, K. (2004). Stages in the development of Parkinson's disease-related pathology. *Cell Tissue Res.* 318, 121–134. doi: 10.1007/s00441-004-0956-9

Breton-Provencher, V., and Sur, M. (2019). Active control of arousal by a locus coeruleus GABAergic circuit. *Nat. Neurosci.* 22, 218–228. doi: 10.1038/s41593-018-0305-z

Campos, A. C. P., Berzuino, M. B., Hernandes, M. S., Fonoff, E. T., and Pagano, R. L. (2019). Monoaminergic regulation of nociceptive circuitry in a Parkinson's disease rat model. *Exp. Neurol.* 318, 12–21. doi: 10.1016/j.expneurol.2019.04.015

Carter, M. E., Yizhar, O., Chikahisa, S., Nguyen, H., Adamantidis, A., Nishino, S., et al. (2010). Tuning arousal with optogenetic modulation of locus coeruleus neurons. *Nat. Neurosci.* 13, 1526–1533. doi: 10.1038/nn.2682

Cash, R., Ruberg, M., Raisman, R., and Agid, Y. (1984). Adrenergic receptors in Parkinson's disease. *Brain Res.* 322, 269–275. doi: 10.1016/0006-8993(84)90117-3

Cash, R., Dennis, T., L'Heureux, R., Raisman, R., Javoy-Agid, F., and Scatton, B. (1987). Parkinson's disease and dementia: norepinephrine and dopamine in locus ceruleus. *Neurology* 37, 42–46. doi: 10.1212/wnl.37.1.42

Castellanos, G., Fernández-Seara, M. A., Lorenzo-Betancor, O., Ortega-Cubero, S., Puigvert, M., Uranga, J., et al. (2015). Automated neuromelanin imaging as a diagnostic biomarker for Parkinson's disease. *Mov. Disord. Off. J. Mov. Disord. Soc* 30, 945–952. doi: 10.1002/mds.26201

Cersosimo, M. G., and Benarroch, E. E. (2008). Neural control of the gastrointestinal tract: implications for Parkinson disease. *Mov. Disord. Off. J. Mov. Disord. Soc* 23, 1065–1075. doi: 10.1002/mds.22051

Chagraoui, A., Boulain, M., Juvin, L., Anouar, Y., Barrière, G., and Deurwaerdère, P. D. (2019). L-DOPA in Parkinson's Disease: Looking at the "False" Neurotransmitters and Their Meaning. *Int. J. Mol.* 21. doi: 10.3390/ijms21010294

Chandler, D. J., Gao, W.-J., and Waterhouse, B. D. (2014). Heterogeneous organization of the locus coeruleus projections to prefrontal and motor cortices. *Proc. Natl. Acad. Sci. U. S. A.* 111, 6816–6821. doi: 10.1073/pnas.1320827111

Chan-Palay, V., and Asan, E. (1989). Alterations in catecholamine neurons of the locus coeruleus in senile dementia of the Alzheimer type and in Parkinson's disease with and without dementia and depression. *J. Comp. Neurol.* 287, 373–392. doi: 10.1002/cne.902870308

Charles, K.-A., Naudet, F., Bouali-Benazzouz, R., Landry, M., De Deurwaerdère, P., Fossat, P., et al. (2018). Alteration of nociceptive integration in the spinal cord of a rat model of Parkinson's disease. *Mov. Disord. Off. J. Mov. Disord. Soc* 33, 1010–1015. doi: 10.1002/mds.27377

Chaudhuri, K. R., and Schapira, A. H. V. (2009). Non-motor symptoms of Parkinson's disease: dopaminergic pathophysiology and treatment. *Lancet Neurol.* 8, 464–474. doi: 10.1016/S1474-4422(09)70068-7

Chotibut, T., Apple, D. M., Jefferis, R., and Salvatore, M. F. (2012). Dopamine Transporter Loss in 6-OHDA Parkinson's Model Is Unmet by Parallel Reduction in Dopamine Uptake. *PloS One* 7 (12), e52322. doi: 10.1371/journal.pone.0052322

Chotibut, T., Fields, V., and Salvatore, M. F. (2014). Norepinephrine transporter inhibition with desipramine exacerbates L-DOPA-induced dyskinesia: role for

synaptic dopamine regulation in denervated nigrostriatal terminals. *Mol. Pharmacol.* 86, 675–685. doi: 10.1124/mol.114.093302

Conti, M. M., Goldenberg, A. A. A., Kuberka, A., Mohamed, M., Eissa, S., Lindenbach, D., et al. (2016). Effect of tricyclic antidepressants on L-DOPA-induced dyskinesia and motor improvement in hemi-parkinsonian rats. *Pharmacol. Biochem. Behav.* 142, 64–71. doi: 10.1016/j.pbb.2016.01.004

Cullen, K. P., Grant, L. M., Kelm-Nelson, C. A., Brauer, A. F. L., Bickelhaupt, L. B., Russell, J. A., et al. (2018). Pink1 –/– Rats Show Early-Onset Swallowing Deficits and Correlative Brainstem Pathology. *Dysphagia* 33, 749–758. doi: 10.1007/s00455-018-9896-5

Cummings, J., Ritter, A., and Rothenberg, K. (2019). Advances in Management of Neuropsychiatric Syndromes in Neurodegenerative Diseases. *Curr. Psychiatry Rep.* 21, 79. doi: 10.1007/s11920-019-1058-4

Cummings, J. L. (1992). Depression and Parkinson's disease: a review. *Am. J. Psychiatry* 149, 443–454. doi: 10.1176/ajp.149.4.443

Curtis, A. L., Leiser, S. C., Snyder, K., and Valentino, R. J. (2012). Predator stress engages corticotropin-releasing factor and opioid systems to alter the operating mode of locus coeruleus norepinephrine neurons. *Neuropharmacology* 62, 1737–1745. doi: 10.1016/j.neuropharm.2011.11.020

Dayan, L., and Finberg, J. P. M. (2003). L-DOPA increases noradrenaline turnover in central and peripheral nervous systems. *Neuropharmacology* 45, 524–533. doi: 10.1016/s0028-3908(03)00190-4

De Deurwaerdère, P., Di Giovanni, G., and Millan, M. J. (2017). Expanding the repertoire of L-DOPA's actions: A comprehensive review of its functional neurochemistry. *Prog. Neurobiol.* 151, 57–100. doi: 10.1016/j.pneurobio.2016.07.002

Dekundy, A., Lundblad, M., Danysz, W., and Cenci, M. A. (2007). Modulation of L-DOPA-induced abnormal involuntary movements by clinically tested compounds: further validation of the rat dyskinesia model. *Behav. Brain Res.* 179, 76–89. doi: 10.1016/j.bbr.2007.01.013

Del Tredici, K., and Braak, H. (2013). Dysfunction of the locus coeruleus-norepinephrine system and related circuitry in Parkinson's disease-related dementia. *J. Neurol. Neurosurg. Psychiatry* 84, 774–783. doi: 10.1136/jnnp-2011-301817

Del Tredici, K., Rüb, U., De Vos, R. A. I., Bohl, J. R. E., and Braak, H. (2002). Where does parkinson disease pathology begin in the brain? *J. Neuropathol. Exp. Neurol.* 61, 413–426. doi: 10.1093/jnen/61.5.413

Delaville, C., Deurwaerdère, P. D., and Benazzouz, A. (2011). Noradrenaline and Parkinson's disease. *Front. Syst. Neurosci.* 5, 31. doi: 10.3389/fnsys.2011.00031

Delaville, C., Chetrit, J., Abdallah, K., Morin, S., Cardoit, L., De Deurwaerdère, P., et al. (2012a). Emerging dysfunctions consequent to combined monoaminergic depletions in Parkinsonism. *Neurobiol. Dis.* 45, 763–773. doi: 10.1016/j.nbd.2011.10.023

Delaville, C., Navailles, S., and Benazzouz, A. (2012b). Effects of noradrenaline and serotonin depletions on the neuronal activity of globus pallidus and substantia nigra pars reticulata in experimental parkinsonism. *Neuroscience* 202, 424–433. doi: 10.1016/j.neuroscience.2011.11.024

Delfs, J. M., Zhu, Y., Druhan, J. P., and Aston-Jones, G. S. (1998). Origin of noradrenergic afferents to the shell subregion of the nucleus accumbens: anterograde and retrograde tract-tracing studies in the rat. *Brain Res.* 806, 127–140. doi: 10.1016/s0006-8993(98)00672-6

Devoto, P., Flore, G., Pani, L., and Gessa, G. L. (2001). Evidence for co-release of noradrenaline and dopamine from noradrenergic neurons in the cerebral cortex. *Mol. Psychiatry* 6, 657–664. doi: 10.1038/sj.mp.4000904

Devoto, P., Flore, G., Longu, G., Pira, L., and Gessa, G. L. (2003). Origin of extracellular dopamine from dopamine and noradrenaline neurons in the medial prefrontal and occipital cortex. *Synap. N. Y. N* 50, 200–205. doi: 10.1002/syn.10264

Devoto, P., Flore, G., Pira, L., Longu, G., and Gessa, G. L. (2004). Alpha2-adrenoceptor mediated co-release of dopamine and noradrenaline from noradrenergic neurons in the cerebral cortex. *J. Neurochem.* 88, 1003–1009. doi: 10.1046/j.1471-4159.2003.02239.x

Devoto, P., Flore, G., Saba, P., Fà, M., and Gessa, G. L. (2005a). Co-release of noradrenaline and dopamine in the cerebral cortex elicited by single train and repeated train stimulation of the locus coeruleus. *BMC Neurosci.* 6, 31. doi: 10.1186/1471-2202-6-31

Devoto, P., Flore, G., Saba, P., Fà, M., and Gessa, G. L. (2005b). Stimulation of the locus coeruleus elicits noradrenaline and dopamine release in the medial prefrontal and parietal cortex. *J. Neurochem.* 92, 368–374. doi: 10.1111/j.1471-4159.2004.02866.x

Domenici, R. A., Campos, A. C. P., Maciel, S. T., Berzuino, M. B., Hernandes, M. S., Fonoff, E. T., et al. (2019). Parkinson's disease and pain: Modulation of nociceptive circuitry in a rat model of nigrostriatal lesion. *Exp. Neurol.* 315, 72–81. doi: 10.1016/j.expneurol.2019.02.007

Engeln, M., De Deurwaerdère, P., Li, Q., Bezard, E., and Fernagut, P.-O. (2015). Widespread Monoaminergic Dysregulation of Both Motor and Non-Motor Circuits in Parkinsonism and Dyskinesia. *Cereb. Cortex* 25, 2783–2792. doi: 10.1093/cercor/bhu076

Eskow Jaunarajs, K. L., Dupre, K. B., Ostock, C. Y., Button, T., Deak, T., and Bishop, C. (2010). Behavioral and neurochemical effects of chronic L-DOPA treatment on nonmotor sequelae in the hemiparkinsonian rat. *Behav. Pharmacol.* 21, 627–637. doi: 10.1097/FBP.0b013e32833e7e80

Eskow Jaunarajs, K. L., Angoa-Perez, M., Kuhn, D. M., and Bishop, C. (2011). Potential mechanisms underlying anxiety and depression in Parkinson's disease: consequences of l-DOPA treatment. *Neurosci. Biobehav. Rev.* 35, 556–564. doi: 10.1016/j.neubiorev.2010.06.007

Espay, A. J., LeWitt, P. A., and Kaufmann, H. (2014). Norepinephrine deficiency in Parkinson's disease: the case for noradrenergic enhancement. *Mov. Disord. Off. J. Mov. Disord. Soc* 29, 1710–1719. doi: 10.1002/mds.26048

Fallon, J. H., Koziell, D. A., and Moore, R. Y. (1978). Catecholamine innervation of the basal forebrain. II. Amygdala, suprarhinal cortex and entorhinal cortex. *J. Comp. Neurol.* 180, 509–532. doi: 10.1002/cne.901800308

Ferro, M. M., Bellissimo, M. I., Anselmo-Franci, J. A., Angellucci, M. E. M., Canteras, N. S., and Da Cunha, C. (2005). Comparison of bilaterally 6-OHDA- and MPTP-lesioned rats as models of the early phase of Parkinson's disease: histological, neurochemical, motor and memory alterations. *J. Neurosci. Methods* 148, 78–87. doi: 10.1016/j.jneumeth.2005.04.005

Fitoussi, A., Dellu-Hagedorn, F., and De Deurwaerdère, P. (2013). Monoamines tissue content analysis reveals restricted and site-specific correlations in brain regions involved in cognition. *Neuroscience* 255, 233–245. doi: 10.1016/j.neuroscience.2013.09.059

Fornai, F., Bassi, L., Torracca, M. T., Scalori, V., and Corsini, G. U. (1995). Norepinephrine loss exacerbates methamphetamine-induced striatal dopamine depletion in mice. *Eur. J. Pharmacol.* 283, 99–102. doi: 10.1016/0014-2999(95)00313-A

Fornai, F., Alessandrì, M. G., Torracca, M. T., Bassi, L., and Corsini, G. U. (1997). Effects of noradrenergic lesions on MPTP/MPP+ kinetics and MPTP-induced nigrostriatal dopamine depletions. *J. Pharmacol. Exp. Ther.* 283, 100–107.

Fox, S. H., and Brotchie, J. M. (2010). The MPTP-lesioned non-human primate models of Parkinson's disease. Past, present, and future. *Prog. Brain Res.* 184, 133–157. doi: 10.1016/S0079-6123(10)84007-5

Fox, S. H., Henry, B., Hill, M. P., Peggs, D., Crossman, A. R., and Brotchie, J. M. (2001). Neural mechanisms underlying peak-dose dyskinesia induced by levodopa and apomorphine are distinct: evidence from the effects of the alpha(2) adrenoceptor antagonist idazoxan. *Mov. Disord. Off. J. Mov. Disord. Soc* 16, 642–650. doi: 10.1002/mds.1148

Frisina, P. G., Haroutunian, V., and Libow, L. S. (2009). The neuropathological basis for depression in Parkinson's disease. *Parkinsonism Relat. Disord.* 15, 144–148. doi: 10.1016/j.parkreldis.2008.04.038

Fulceri, F., Biagioni, F., Ferrucci, M., Lazzeri, G., Bartalucci, A., Galli, V., et al. (2007). Abnormal involuntary movements (AIMs) following pulsatile dopaminergic stimulation: severe deterioration and morphological correlates following the loss of locus coeruleus neurons. *Brain Res.* 1135, 219–229. doi: 10.1016/j.brainres.2006.12.030

Gagnon, J.-F., Postuma, R. B., and Montplaisir, J. (2006). Update on the pharmacology of REM sleep behavior disorder. *Neurology* 67, 742–747. doi: 10.1212/01.wnl.0000233926.47469.73

Gaspar, P., and Gray, F. (1984). Dementia in idiopathic Parkinson's disease. A neuropathological study of 32 cases. *Acta Neuropathol. (Berl.)* 64, 43–52. doi: 10.1007/bf00695605

Gerhard, A., Pavese, N., Hotton, G., Turkheimer, F., Es, M., Hammers, A., et al. (2006). In vivo imaging of microglial activation with [11C](R)-PK11195 PET in idiopathic Parkinson's disease. *Neurobiol. Dis.* 21, 404–412. doi: 10.1016/j.nbd.2005.08.002

Gerlach, M., Halley, P., Riederer, P., and van den Buuse, M. (2013). The effect of piribedil on L-DOPA-induced dyskinesias in a rat model of Parkinson's

disease: differential role of α(2) adrenergic mechanisms. *J. Neural Transm. Vienna Austria* 1996 120, 31–36. doi: 10.1007/s00702-012-0818-7

German, D. C., Manaye, K. F., White, C. L., Woodward, D. J., McIntire, D. D., Smith, W. K., et al. (1992). Disease-specific patterns of locus coeruleus cell loss. *Ann. Neurol.* 32, 667–676. doi: 10.1002/ana.410320510

Giaime, E., Tong, Y., Wagner, L. K., Yuan, Y., Huang, G., and Shen, J. (2017). Age-Dependent Dopaminergic Neurodegeneration and Impairment of the Autophagy-Lysosomal Pathway in LRRK-Deficient Mice. *Neuron* 96, 796–807.e6. doi: 10.1016/j.neuron.2017.09.036

Giasson, B. I., Duda, J. E., Quinn, S. M., Zhang, B., Trojanowski, J. Q., and Lee, V. M.-Y. (2002). Neuronal α-Synucleinopathy with Severe Movement Disorder in Mice Expressing A53T Human α-Synuclein. *Neuron* 34, 521–533. doi: 10.1016/S0896-6273(02)00682-7

Glass, C. K., Saijo, K., Winner, B., Marchetto, M. C., and Gage, F. H. (2010). Mechanisms underlying inflammation in neurodegeneration. *Cell* 140, 918–934. doi: 10.1016/j.cell.2010.02.016

Goldstein, D. S., Holmes, C., and Sharabi, Y. (2012). Cerebrospinal fluid biomarkers of central catecholamine deficiency in Parkinson's disease and other synucleinopathies. *Brain J. Neurol.* 135, 1900–1913. doi: 10.1093/brain/aws055

Goldstein, D. S. (2006). Orthostatic hypotension as an early finding in Parkinson's disease. *Clin. Auton. Res. Off. J. Clin. Auton. Res. Soc* 16, 46–54. doi: 10.1007/s10286-006-0317-8

Gomez-Mancilla, B., and Bédard, P. J. (1993). Effect of nondopaminergic drugs on L-dopa-induced dyskinesias in MPTP-treated monkeys. *Clin. Neuropharmacol.* 16, 418–427. doi: 10.1097/00002826-199310000-00004

Grant, L. M., Kelm-Nelson, C. A., Hilby, B. L., Blue, K. V., Paul Rajamanickam, E. S., Pultorak, J. D., et al. (2015). Evidence for early and progressive ultrasonic vocalization and oromotor deficits in a PINK1 gene knockout rat model of Parkinson's disease. *J. Neurosci. Res.* 93, 1713–1727. doi: 10.1002/jnr.23625

Grondin, R., Hadj Tahar, A., Doan, V. D., Ladure, P., and Bédard, P. J. (2000). Noradrenoceptor antagonism with idazoxan improves L-dopa-induced dyskinesias in MPTP monkeys. *Naunyn. Schmiedebergs Arch. Pharmacol.* 361, 181–186. doi: 10.1007/s002109900167

Guimarães, J., Moura, E., Silva, E., Aguiar, P., Garrett, C., and Vieira-Coelho, M. A. (2013). Locus Coeruleus Is Involved in Weight Loss in a Rat Model of Parkinson's Disease: An Effect Reversed by Deep Brain Stimulation. *Brain Stimulat.* 6, 845–855. doi: 10.1016/j.brs.2013.06.002

Hakusui, S., Yasuda, T., Yanagi, T., Tohyama, J., Hasegawa, Y., Koike, Y., et al. (1994). A radiological analysis of heart sympathetic functions with meta-[123I] iodobenzylguanidine in neurological patients with autonomic failure. *J. Auton. Nerv. Syst.* 49, 81–84. doi: 10.1016/0165-1838(94)90023-x

Halliday, G., Lees, A., and Stern, M. (2011). Milestones in Parkinson's disease–clinical and pathologic features. *Mov. Disord. Off. J. Mov. Disord. Soc* 26, 1015–1021. doi: 10.1002/mds.23669

Henry, B., Fox, S. H., Peggs, D., Crossman, A. R., and Brotchie, J. M. (1999). The alpha2-adrenergic receptor antagonist idazoxan reduces dyskinesia and enhances anti-parkinsonian actions of L-dopa in the MPTP-lesioned primate model of Parkinson's disease. *Mov. Disord. Off. J. Mov. Disord. Soc* 14, 744–753. doi: 10.1002/1531-8257(199909)14:5<744::AID-MDS1006>3.0.CO;2-7

Hirao, K., Pontone, G. M., and Smith, G. S. (2015). Molecular imaging of neuropsychiatric symptoms in Alzheimer's and Parkinson's disease. *Neurosci. Biobehav. Rev.* 49, 157–170. doi: 10.1016/j.neubiorev.2014.11.010

Hirschberg, S., Li, Y., Randall, A., Kremer, E. J., and Pickering, A. E. (2017). Functional dichotomy in spinal- vs prefrontal-projecting locus coeruleus modules splits descending noradrenergic analgesia from ascending aversion and anxiety in rats. *eLife* 6, e29808. doi: 10.7554/eLife.29808

Hurst, J. H., LeWitt, P. A., Burns, R. S., Foster, N. L., and Lovenberg, W. (1985). CSF dopamine-beta-hydroxylase activity in Parkinson's disease. *Neurology* 35, 565–568. doi: 10.1212/wnl.35.4.565

Iravani, M. M., Sadeghian, M., Rose, S., and Jenner, P. (2014). Loss of locus coeruleus noradrenergic neurons alters the inflammatory response to LPS in substantia nigra but does not affect nigral cell loss. *J. Neural Transm. Vienna Austria* 1996 121, 1493–1505. doi: 10.1007/s00702-014-1223-1

Jiang, L., Chen, S.-H., Chu, C.-H., Wang, S.-J., Oyarzabal, E., Wilson, B., et al. (2015). A novel role of microglial NADPH oxidase in mediating extra-synaptic function of norepinephrine in regulating brain immune homeostasis. *Glia* 63, 1057–1072. doi: 10.1002/glia.22801

Jin, X., Li, S., Bondy, B., Zhong, W., Oginsky, M. F., Wu, Y., et al. (2016). Identification of a Group of GABAergic Neurons in the Dorsomedial Area of the Locus Coeruleus. *PloS One* 11, e0146470. doi: 10.1371/journal.pone.0146470

Kadar, H., Le Douaron, G., Amar, M., Ferrié, L., Figadère, B., Touboul, D., et al. (2014). MALDI mass spectrometry imaging of 1-methyl-4-phenylpyridinium (MPP+) in mouse brain. *Neurotox. Res.* 25, 135–145. doi: 10.1007/s12640-013-9449-5

Kamińska, K., Lenda, T., Konieczny, J., Czarnecka, A., and Lorenc-Koci, E. (2017). Depressive-like neurochemical and behavioral markers of Parkinson's disease after 6-OHDA administered unilaterally to the rat medial forebrain bundle. *Pharmacol. Rep. PR* 69, 985–994. doi: 10.1016/j.pharep.2017.05.016

Kempadoo, K. A., Mosharov, E. V., Choi, S. J., Sulzer, D., and Kandel, E. R. (2016). Dopamine release from the locus coeruleus to the dorsal hippocampus promotes spatial learning and memory. *Proc. Natl. Acad. Sci. U. S. A.* 113, 14835–14840. doi: 10.1073/pnas.1616515114

Kilbourn, M. R., Sherman, P., and Abbott, L. C. (1998). Reduced MPTP neurotoxicity in striatum of the mutant mouse tottering. *Synap. N. Y. N* 30, 205–210. doi: 10.1002/(SICI)1098-2396(199810)30:2<205::AID-SYN10>3.0.CO;2-0

Kim, M.-A., Lee, H. S., Lee, B. Y., and Waterhouse, B. D. (2004). Reciprocal connections between subdivisions of the dorsal raphe and the nuclear core of the locus coeruleus in the rat. *Brain Res.* 1026, 56–67. doi: 10.1016/j.brainres.2004.08.022

Kim, S., Kwon, S.-H., Kam, T.-I., Panicker, N., Karuppagounder, S. S., Lee, S., et al. (2019). Transneuronal Propagation of Pathologic α-Synuclein from the Gut to the Brain Models Parkinson's Disease. *Neuron* 103, 627–641.e7. doi: 10.1016/j.neuron.2019.05.035

Kish, S. J., Shannak, K. S., Rajput, A. H., Gilbert, J. J., and Hornykiewicz, O. (1984). Cerebellar norepinephrine in patients with Parkinson's disease and control subjects. *Arch. Neurol.* 41, 612–614. doi: 10.1001/archneur.1984.04210080020007

Kreiner, G., Rafa-Zabłocka, K., Barut, J., Chmielarz, P., Kot, M., Bagińska, M., et al. (2019). Stimulation of noradrenergic transmission by reboxetine is beneficial for a mouse model of progressive parkinsonism. *Sci. Rep.* 9, 5262. doi: 10.1038/s41598-019-41756-3

Leclair-Visonneau, L., Magy, L., Volteau, C., Clairembault, T., Le Dily, S., Préterre, C., et al. (2018). Heterogeneous pattern of autonomic dysfunction in Parkinson's disease. *J. Neurol.* 265, 933–941. doi: 10.1007/s00415-018-8789-8

Li, L., Feng, X., Zhou, Z., Zhang, H., Shi, Q., Lei, Z., et al. (2018). Stress Accelerates Defensive Responses to Looming in Mice and Involves a Locus oeruleus-Superior Colliculus Projection. *Curr. Biol. CB* 28, 859–871.e5. doi: 10.1016/j.cub.2018.02.005

Li, Y., Jiao, Q., Du, X., Bi, M., Han, S., Jiao, L., et al. (2018). Investigation of Behavioral Dysfunctions Induced by Monoamine Depletions in a Mouse Model of Parkinson's Disease. *Front. Cell. Neurosci.* 12, 241. doi: 10.3389/fncel.2018.00241

Li, Y., Wang, C., Wang, J., Zhou, Y., Ye, F., Zhang, Y., et al. (2019). Mild cognitive impairment in de novo Parkinson's disease: A neuromelanin MRI study in locus coeruleus. *Mov. Disord. Off. J. Mov. Disord. Soc* 34, 884–892. doi: 10.1002/mds.27682

Liu, B., Teschemacher, A. G., and Kasparov, S. (2017). Astroglia as a cellular target for neuroprotection and treatment of neuro-psychiatric disorders. *Glia* 65, 1205–1226. doi: 10.1002/glia.23136

Llorca-Torralba, M., Suárez-Pereira, I., Bravo, L., Camarena-Delgado, C., Garcia-Partida, J. A., Mico, J. A., et al. (2019). Chemogenetic Silencing of the Locus Coeruleus-Basolateral Amygdala Pathway Abolishes Pain-Induced Anxiety and Enhanced Aversive Learning in Rats. *Biol. Psychiatry* 85, 1021–1035. doi: 10.1016/j.biopsych.2019.02.018

Loughlin, S. E., Foote, S. L., and Bloom, F. E. (1986). Efferent projections of nucleus locus coeruleus: topographic organization of cells of origin demonstrated by three-dimensional reconstruction. *Neuroscience* 18, 291–306. doi: 10.1016/0306-4522(86)90155-7

Lu, Y., Simpson, K. L., Weaver, K. J., and Lin, R. C. S. (2012). Differential distribution patterns from medial prefrontal cortex and dorsal raphe to the locus coeruleus in rats. *Anat. Rec. Hoboken NJ* 2007 295, 1192–1201. doi: 10.1002/ar.22505

Luchtman, D. W., Shao, D., and Song, C. (2009). Behavior, neurotransmitters and inflammation in three regimens of the MPTP mouse model of Parkinson's disease. *Physiol. Behav.* 98, 130–138. doi: 10.1016/j.physbeh.2009.04.021

Marien, M., Briley, M., and Colpaert, F. (1993). Noradrenaline depletion exacerbates MPTP-induced striatal dopamine loss in mice. *Eur. J. Pharmacol.* 236, 487–489. doi: 10.1016/0014-2999(93)90489-5

Martin-Bastida, A., Pietracupa, S., and Piccini, P. (2017). Neuromelanin in parkinsonian disorders: an update. *Int. J. Neurosci.* 127, 1116–1123. doi: 10.1080/00207454.2017.1325883

Martinez-Martin, P., Chaudhuri, K. R., Rojo-Abuin, J. M., Rodriguez-Blazquez, C., Alvarez-Sanchez, M., Arakaki, T., et al. (2015). Assessing the non-motor symptoms of Parkinson's disease: MDS-UPDRS and NMS Scale. *Eur. J. Neurol.* 22, 37–43. doi: 10.1111/ene.12165

Masilamoni, G. J., Groover, O., and Smith, Y. (2017). Reduced noradrenergic innervation of ventral midbrain dopaminergic cell groups and the subthalamic nucleus in MPTP-treated parkinsonian monkeys. *Neurobiol. Dis.* 100, 9–18. doi: 10.1016/j.nbd.2016.12.025

Mason, S. T., and Fibiger, H. C. (1979). Regional topography within noradrenergic locus coeruleus as revealed by retrograde transport of horseradish peroxidase. *J. Comp. Neurol.* 187, 703–724. doi: 10.1002/cne.901870405

Mavridis, M., Degryse, A. D., Lategan, A. J., Marien, M. R., and Colpaert, F. C. (1991). Effects of locus coeruleus lesions on parkinsonian signs, striatal dopamine and substantia nigra cell loss after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in monkeys: a possible role for the locus coeruleus in the progression of Parkinson's disease. *Neuroscience* 41, 507–523. doi: 10.1016/0306-4522(91)90345-o

McCall, J. G., Al-Hasani, R., Siuda, E. R., Hong, D. Y., Norris, A. J., Ford, C. P., et al. (2015). CRH Engagement of the Locus Coeruleus Noradrenergic System Mediates Stress-Induced Anxiety. *Neuron* 87, 605–620. doi: 10.1016/j.neuron.2015.07.002

McCall, J. G., Siuda, E. R., Bhatti, D. L., Lawson, L. A., McElligott, Z. A., Stuber, G. D., et al. (2017). Locus coeruleus to basolateral amygdala noradrenergic projections promote anxiety-like behavior. *eLife* 6, e18247. doi: 10.7554/eLife.18247

McMillan, P. J., White, S. S., Franklin, A., Greenup, J. L., Leverenz, J. B., Raskind, M. A., et al. (2011). Differential response of the central noradrenergic nervous system to the loss of locus coeruleus neurons in Parkinson's disease and Alzheimer's disease. *Brain Res.* 1373, 240–252. doi: 10.1016/j.brainres.2010.12.015

McNamee, E. N., Griffin, E. W., Ryan, K. M., Ryan, K. J., Heffernan, S., Harkin, A., et al. (2010a). Noradrenaline acting at beta-adrenoceptors induces expression of IL-1beta and its negative regulators IL-1ra and IL-1RII, and drives an overall anti-inflammatory phenotype in rat cortex. *Neuropharmacology* 59, 37–48. doi: 10.1016/j.neuropharm.2010.03.014

McNamee, E. N., Ryan, K. M., Kilroy, D., and Connor, T. J. (2010b). Noradrenaline induces IL-1ra and IL-1 type II receptor expression in primary glial cells and protects against IL-1beta-induced neurotoxicity. *Eur. J. Pharmacol.* 626, 219–228. doi: 10.1016/j.ejphar.2009.09.054

Mejías-Aponte, C. A., Drouin, C., and Aston-Jones, G. (2009). Adrenergic and Noradrenergic Innervation of the Midbrain Ventral Tegmental Area and Retrorubral Field: Prominent Inputs from Medullary Homeostatic Centers. *J. Neurosci.* 29, 3613–3626. doi: 10.1523/JNEUROSCI.4632-08.2009

Micieli, G., Tosi, P., Marcheselli, S., and Cavallini, A. (2003). Autonomic dysfunction in Parkinson's disease. *Neurol. Sci. Off. J. Ital. Neurol. Soc Ital. Soc Clin. Neurophysiol.* 24 (Suppl 1), S32–S34. doi: 10.1007/s100720300035

Miguelez, C., Aristieta, A., Cenci, M. A., and Ugedo, L. (2011a). The locus coeruleus is directly implicated in L-DOPA-induced dyskinesia in parkinsonian rats: an electrophysiological and behavioural study. *PloS One* 6, e24679. doi: 10.1371/journal.pone.0024679

Miguelez, C., Grandoso, L., and Ugedo, L. (2011b). Locus coeruleus and dorsal raphe neuron activity and response to acute antidepressant administration in a rat model of Parkinson's disease. *Int. J. Neuropsychopharmacol.* 14, 187–200. doi: 10.1017/S146114571000043X

Miguelez, C., Berrocoso, E., Mico, J. A., and Ugedo, L. (2013). L-DOPA modifies the antidepressant-like effects of reboxetine and fluoxetine in rats. *Neuropharmacology* 67, 349–358. doi: 10.1016/j.neuropharm.2012.11.016

Miguelez, C., Benazzouz, A., Ugedo, L., and De Deurwaerdère, P. (2017). Impairment of Serotonergic Transmission by the Antiparkinsonian Drug L-DOPA: Mechanisms and Clinical Implications. *Front. Cell. Neurosci.* 11, 274. doi: 10.3389/fncel.2017.00274

Mittal, S., Bjørnevik, K., Im, D. S., Flierl, A., Dong, X., Locascio, J. J., et al. (2017). β2-Adrenoreceptor is a regulator of the α-synuclein gene driving risk of Parkinson's disease. *Science* 357, 891–898. doi: 10.1126/science.aaf3934

Monaca, C., Laloux, C., Jacquesson, J.-M., Gelé, P., Maréchal, X., Bordet, R., et al. (2004). Vigilance states in a parkinsonian model, the MPTP mouse. *Eur. J. Neurosci.* 20, 2474–2478. doi: 10.1111/j.1460-9568.2004.03694.x

Morón, J. A., Brockington, A., Wise, R. A., Rocha, B. A., and Hope, B. T. (2002). Dopamine uptake through the norepinephrine transporter in brain regions with low levels of the dopamine transporter: evidence from knock-out mouse lines. *J. Neurosci. Off. J. Soc Neurosci.* 22, 389–395. doi: 10.1523/JNEUROSCI.22-02-00389.2002

Mori, K., Ozaki, E., Zhang, B., Yang, L., Yokoyama, A., Takeda, I., et al. (2002). Effects of norepinephrine on rat cultured microglial cells that express alpha1, alpha2, beta1 and beta2 adrenergic receptors. *Neuropharmacology* 43, 1026–1034. doi: 10.1016/s0028-3908(02)00211-3

Navailles, S., and De Deurwaerdère, P. (2012). Imbalanced Dopaminergic Transmission Mediated by Serotonergic Neurons in L-DOPA-Induced Dyskinesia. *Park. Dis.* 2012, 323686. doi: 10.1155/2012/323686

Navailles, S., Bioulac, B., Gross, C., and De Deurwaerdère, P. (2010). Serotonergic neurons mediate ectopic release of dopamine induced by L-DOPA in a rat model of Parkinson's disease. *Neurobiol. Dis.* 38, 136–143. doi: 10.1016/j.nbd.2010.01.012

Navailles, S., Milan, L., Khalki, H., Di Giovanni, G., Lagière, M., and De Deurwaerdère, P. (2014). Noradrenergic terminals regulate L-DOPA-derived dopamine extracellular levels in a region-dependent manner in Parkinsonian rats. *CNS Neurosci. Ther.* 20, 671–678. doi: 10.1111/cns.12275

Nayyar, T., Bubser, M., Ferguson, M. C., Neely, M. D., Goodwin, J. S., Montine, T. J., et al. (2009). Cortical serotonin and norepinephrine denervation in parkinsonism: preferential loss of the beaded serotonin innervation. *Eur. J. Neurosci.* 30, 207–216. doi: 10.1111/j.1460-9568.2009.06806.x

O'Connor, D. T., Cervenka, J. H., Stone, R. A., Levine, G. L., Parmer, R. J., Franco-Bourland, R. E., et al. (1994). Dopamine beta-hydroxylase immunoreactivity in human cerebrospinal fluid: properties, relationship to central noradrenergic neuronal activity and variation in Parkinson's disease and congenital dopamine beta-hydroxylase deficiency. *Clin. Sci. Lond. Engl.* 1979 86, 149–158. doi: 10.1042/cs0860149

Olanow, C. W., Stern, M. B., and Sethi, K. (2009). The scientific and clinical basis for the treatment of Parkinson disease, (2009). *Neurology* 72, S1–136. doi: 10.1212/WNL.0b013e3181a1d44c

Ostock, C. Y., Lindenbach, D., Goldenberg, A. A., Kampton, E., and Bishop, C. (2014). Effects of noradrenergic denervation by anti-DBH-saporin on behavioral responsivity to L-DOPA in the hemi-parkinsonian rat. *Behav. Brain Res.* 270, 75–85. doi: 10.1016/j.bbr.2014.05.009

Ostock, C. Y., Hallmark, J., Palumbo, N., Bhide, N., Conti, M., George, J. A., et al. (2015). Modulation of L-DOPA's antiparkinsonian and dyskinetic effects by α2-noradrenergic receptors within the locus coeruleus. *Neuropharmacology* 95, 215–225. doi: 10.1016/j.neuropharm.2015.03.008

Ostock, C. Y., Bhide, N., Goldenberg, A. A., George, J. A., and Bishop, C. (2018). Striatal norepinephrine efflux in l-DOPA-induced dyskinesia. *Neurochem. Int.* 114, 85–98. doi: 10.1016/j.neuint.2018.01.010

Ouchi, Y., Yoshikawa, E., Sekine, Y., Futatsubashi, M., Kanno, T., Ogusu, T., et al. (2005). Microglial activation and dopamine terminal loss in early Parkinson's disease. *Ann. Neurol.* 57, 168–175. doi: 10.1002/ana.20338

Pérez, V., Sosti, V., Rubio, A., Barbanoj, M., Rodríguez-Alvarez, J., and Kulisevsky, J. (2007). Modulation of the motor response to dopaminergic drugs in a parkinsonian model of combined dopaminergic and noradrenergic degeneration. *Eur. J. Pharmacol.* 576, 83–90. doi: 10.1016/j.ejphar.2007.08.024

Pérez, V., Marin, C., Rubio, A., Aguilar, E., Barbanoj, M., and Kulisevsky, J. (2009). Effect of the additional noradrenergic neurodegeneration to 6-OHDA-lesioned rats in levodopa-induced dyskinesias and in cognitive disturbances. *J. Neural Transm. Vienna Austria* 1996 116, 1257–1266. doi: 10.1007/s00702-009-0291-0

Park, A., and Stacy, M. (2009). Non-motor symptoms in Parkinson's disease. *J. Neurol.* 256 Suppl 3, 293–298. doi: 10.1007/s00415-009-5240-1

Pascucci, T., Giacovazzo, G., Andolina, D., Conversi, D., Cruciani, F., Cabib, S., et al. (2012). In vivo catecholaminergic metabolism in the medial prefrontal cortex of ENU2 mice: an investigation of the cortical dopamine deficit in phenylketonuria. *J. Inherit. Metab. Dis.* 35, 1001–1009. doi: 10.1007/s10545-012-9473-2

Patt, S., and Gerhard, L. (1993). A Golgi study of human locus coeruleus in normal brains and in Parkinson's disease. *Neuropathol. Appl. Neurobiol.* 19, 519–523. doi: 10.1111/j.1365-2990.1993.tb00480.x

Pavese, N., Rivero-Bosch, M., Lewis, S. J., Whone, A. L., and Brooks, D. J. (2011). Progression of monoaminergic dysfunction in Parkinson's disease: a longitudinal 18F-dopa PET study. *NeuroImage* 56, 1463–1468. doi: 10.1016/j.neuroimage.2011.03.012

Pifl, Ch., Schingnitz, G., and Hornykiewicz, O. (1991). Effect of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine on the regional distribution of brain monoamines in the rhesus monkey. *Neuroscience* 44, 591–605. doi: 10.1016/0306-4522(91)90080-8

Pifl, C., Kish, S. J., and Hornykiewicz, O. (2012). Thalamic noradrenaline in Parkinson's disease: deficits suggest role in motor and non-motor symptoms. *Mov. Disord. Off. J.Mov. Disord. Soc* 27, 1618–1624. doi: 10.1002/mds.25109

Qian, L., Wu, H., Chen, S.-H., Zhang, D., Ali, S. F., Peterson, L., et al. (2011). β2-adrenergic receptor activation prevents rodent dopaminergic neurotoxicity by inhibiting microglia via a novel signaling pathway. *J. Immunol. Baltim. Md* 1950 186, 4443–4454. doi: 10.4049/jimmunol.1002449

Remy, P., Doder, M., Lees, A., Turjanski, N., and Brooks, D. (2005). Depression in Parkinson's disease: loss of dopamine and noradrenaline innervation in the limbic system. *Brain J. Neurol.* 128, 1314–1322. doi: 10.1093/brain/awh445

Rey, N. L., Petit, G. H., Bousset, L., Melki, R., and Brundin, P. (2013). Transfer of human α-synuclein from the olfactory bulb to interconnected brain regions in mice. *ActaNeuropathol. (Berl.)* 126, 555–573. doi: 10.1007/s00401-013-1160-3

Rommelfanger, K. S., and Weinshenker, D. (2007). Norepinephrine: The redheaded stepchild of Parkinson's disease. *Biochem. Pharmacol.* 74, 177–190. doi: 10.1016/j.bcp.2007.01.036

Rommelfanger, K. S., Weinshenker, D., and Miller, G. W. (2004). Reduced MPTP toxicity in noradrenaline transporter knockout mice. *J. Neurochem.* 91, 1116–1124. doi: 10.1111/j.1471-4159.2004.02785.x

Rusconi, R., Ulusoy, A., Aboutalebi, H., and Di Monte, D. A. (2018). Long-lasting pathological consequences of overexpression-induced α-synuclein spreading in the rat brain. *Aging Cell* 17 (2). doi: 10.1111/acel.12727

Ryan, M., Eatmon, C. V., and Slevin, J. T. (2019). Drug treatment strategies for depression in Parkinson disease. *Expert Opin.Pharmacother.* 20, 1351–1363. doi: 10.1080/14656566.2019.1612877

Santiago, R. M., Barbieiro, J., Lima, M. M. S., Dombrowski, P. A., Andreatini, R., and Vital, M. A. B. F. (2010). Depressive-like behaviors alterations induced by intranigral MPTP, 6-OHDA, LPS and rotenone models of Parkinson's disease are predominantly associated with serotonin and dopamine. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 34, 1104–1114. doi: 10.1016/j.pnpbp.2010.06.004

Sara, S. J., and Bouret, S. (2012). Orienting and reorienting: the locus coeruleus mediates cognition through arousal. *Neuron* 76, 130–141. doi: 10.1016/j.neuron.2012.09.011

Sasaki, M., Shibata, E., Tohyama, K., Takahashi, J., Otsuka, K., Tsuchiya, K., et al. (2006). Neuromelanin magnetic resonance imaging of locus ceruleus and substantia nigra in Parkinson's disease. *Neuroreport* 17, 1215–1218. doi: 10.1097/01.wnr.0000227984.84927.a7

Savola, J.-M., Hill, M., Engstrom, M., Merivuori, H., Wurster, S., McGuire, S. G., et al. (2003). Fipamezole (JP-1730) is a potent alpha2 adrenergic receptor antagonist that reduces levodopa-induced dyskinesia in the MPTP-lesioned primate model of Parkinson's disease. *Mov. Disord. Off. J.Mov. Disord. Soc* 18, 872–883. doi: 10.1002/mds.10464

Schapira, A. H. V., Chaudhuri, K. R., and Jenner, P. (2017). Non-motor features of Parkinson disease. *Nat. Rev. Neurosci.* 18, 435–450. doi: 10.1038/nrn.2017.62

Schwarz, L. A., and Luo, L. (2015). Organization of the locus coeruleus-norepinephrine system. *Curr. Biol. CB* 25, R1051–R1056. doi: 10.1016/j.cub.2015.09.039

Schwarz, L. A., Miyamichi, K., Gao, X. J., Beier, K. T., Weissbourd, B., DeLoach, K. E., et al. (2015). Viral-genetic tracing of the input-output organization of a central noradrenaline circuit. *Nature* 524, 88–92. doi: 10.1038/nature14600

Schwarz, S. T., Xing, Y., Tomar, P., Bajaj, N., and Auer, D. P. (2017). In Vivo Assessment of Brainstem Depigmentation in Parkinson Disease: Potential as a Severity Marker for Multicenter Studies. *Radiology* 283, 789–798. doi: 10.1148/radiol.2016160662

Senard, J. M., Valet, P., Durrieu, G., Berlan, M., Tran, M. A., Montastruc, J. L., et al. (1990). Adrenergic supersensitivity in parkinsonians with orthostatic hypotension. *Eur. J. Clin. Invest.* 20, 613–619. doi: 10.1111/j.1365-2362.1990.tb01909.x

Shannak, K., Rajput, A., Rozdilsky, B., Kish, S., Gilbert, J., and Hornykiewicz, O. (1994). Noradrenaline, dopamine and serotonin levels and metabolism in the human hypothalamus: observations in Parkinson's disease and normal subjects. *Brain Res.* 639, 33–41. doi: 10.1016/0006-8993(94)91761-2

Sharabi, Y., Imrich, R., Holmes, C., Pechnik, S., and Goldstein, D. S. (2008). Generalized and neurotransmitter-selective noradrenergic denervation in Parkinson's disease with orthostatic hypotension. *Mov. Disord. Off. J. Mov. Disord. Soc* 23, 1725–1732. doi: 10.1002/mds.22226

Shiba, M., Bower, J. H., Maraganore, D. M., McDonnell, S. K., Peterson, B. J., Ahlskog, J. E., et al. (2000). Anxiety disorders and depressive disorders preceding Parkinson's disease: a case-control study. *Mov. Disord. Off. J. Mov. Disord. Soc* 15, 669–677. doi: 10.1002/1531-8257(200007)15:4<669::AID-MDS1011>3.0.CO;2-5

Shin, E., Rogers, J. T., Devoto, P., Björklund, A., and Carta, M. (2014). Noradrenaline neuron degeneration contributes to motor impairments and development of L-DOPA-induced dyskinesia in a rat model of Parkinson's disease. *Exp. Neurol.* 257, 25–38. doi: 10.1016/j.expneurol.2014.04.011

Shipley, M. T., Halloran, F. J., and de la Torre, J. (1985). Surprisingly rich projection from locus coeruleus to the olfactory bulb in the rat. *Brain Res.* 329, 294–299. doi: 10.1016/0006-8993(85)90537-2

Slaets, S., Van Acker, F., Versijpt, J., Hauth, L., Goeman, J., Martin, J.-J., et al. (2015). Diagnostic value of MIBG cardiac scintigraphy for differential dementia diagnosis. *Int. J. Geriatr. Psychiatry* 30, 864–869. doi: 10.1002/gps.4229

Smith, C. C., and Greene, R. W. (2012). CNS dopamine transmission mediated by noradrenergic innervation. *J. Neurosci. Off. J. Soc Neurosci.* 32, 6072–6080. doi: 10.1523/JNEUROSCI.6486-11.2012

Sommerauer, M., Fedorova, T. D., Hansen, A. K., Knudsen, K., Otto, M., Jeppesen, J., et al. (2018a). Evaluation of the noradrenergic system in Parkinson's disease: an 11C-MeNER PET and neuromelanin MRI study. *Brain J. Neurol.* 141, 496–504. doi: 10.1093/brain/awx348

Sommerauer, M., Hansen, A. K., Parbo, P., Fedorova, T. D., Knudsen, K., Frederiksen, Y., et al. (2018b). Decreased noradrenaline transporter density in the motor cortex of Parkinson's disease patients. *Mov. Disord. Off. J. Mov. Disord. Soc* 33, 1006–1010. doi: 10.1002/mds.27411

Song, S., Wang, Q., Jiang, L., Oyarzabal, E., Riddick, N. V., Wilson, B., et al. (2019). Noradrenergic dysfunction accelerates LPS-elicited inflammation-related ascending sequential neurodegeneration and deficits in non-motor/motor functions. *Brain. Behav. Immun.* 81, 374–387. doi: 10.1016/j.bbi.2019.06.034

Sotiriou, E., Vassilatis, D. K., Vila, M., and Stefanis, L. (2010). Selective noradrenergic vulnerability in alpha-synuclein transgenic mice. *Neurobiol. Aging* 31, 2103–2114. doi: 10.1016/j.neurobiolaging.2008.11.010

Srinivasan, J., and Schmidt, W. J. (2003). Potentiation of parkinsonian symptoms by depletion of locus coeruleus noradrenaline in 6-hydroxydopamine-induced partial degeneration of substantia nigra in rats. *Eur. J. Neurosci.* 17, 2586–2592. doi: 10.1046/j.1460-9568.2003.02684.x

Srinivasan, J., and Schmidt, W. J. (2004). Behavioral and neurochemical effects of noradrenergic depletions with N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine in 6-hydroxydopamine-induced rat model of Parkinson's disease. *Behav. Brain Res.* 151, 191–199. doi: 10.1016/j.bbr.2003.08.016

Stacy, M. (2002). Sleep disorders in Parkinson's disease: epidemiology and management. *Drugs Aging* 19, 733–739. doi: 10.2165/00002512-200219100-00002

Szot, P., Franklin, A., Miguelez, C., Wang, Y., Vidaurrazaga, I., Ugedo, L., et al. (2016). Depressive-like behavior observed with a minimal loss of locus coeruleus (LC) neurons following administration of 6-hydroxydopamine is associated with electrophysiological changes and reversed with precursors of norepinephrine. *Neuropharmacology* 101, 76–86. doi: 10.1016/j.neuropharm.2015.09.003

Tadaiesky, M. T., Dombrowski, P. A., Figueiredo, C. P., Cargnin-Ferreira, E., Da Cunha, C., and Takahashi, R. N. (2008). Emotional, cognitive and neurochemical alterations in a premotor stage model of Parkinson's disease. *Neuroscience* 156, 830–840. doi: 10.1016/j.neuroscience.2008.08.035

Takatsu, H., Nishida, H., Matsuo, H., Watanabe, S., Nagashima, K., Wada, H., et al. (2000). Cardiac sympathetic denervation from the early stage of Parkinson's disease: clinical and experimental studies with radiolabeled MIBG. *J. Nucl. Med. Off. Publ. Soc Nucl. Med.* 41, 71–77.

Takeuchi, T., Duszkiewicz, A. J., Sonneborn, A., Spooner, P. A., Yamasaki, M., Watanabe, M., et al. (2016). Locus coeruleus and dopaminergic consolidation of everyday memory. *Nature* 537, 357–362. doi: 10.1038/nature19325

Tanaka, H., Kannari, K., Maeda, T., Tomiyama, M., Suda, T., and Matsunaga, M. (1999). Role of serotonergic neurons in L-DOPA-derived extracellular dopamine in the striatum of 6-OHDA-lesioned rats. *Neuroreport* 10, 631–634. doi: 10.1097/00001756-199902250-00034

Tanaka, K. F., Kashima, H., Suzuki, H., Ono, K., and Sawada, M. (2002). Existence of functional beta1- and beta2-adrenergic receptors on microglia. *J. Neurosci. Res.* 70, 232–237. doi: 10.1002/jnr.10399

Terada, T., Yokokura, M., Yoshikawa, E., Futatsubashi, M., Kono, S., Konishi, T., et al. (2016). Extrastriatal spreading of microglial activation in Parkinson's disease: a positron emission tomography study. *Ann. Nucl. Med.* 30, 579–587. doi: 10.1007/s12149-016-1099-2

Titova, N., Schapira, A. H. V., Chaudhuri, K. R., Qamar, M. A., Katunina, E., and Jenner, P. (2017). Nonmotor Symptoms in Experimental Models of Parkinson's Disease. *Int. Rev. Neurobiol.* 133, 63–89. doi: 10.1016/bs.irn.2017.05.018

Tsutsumi, R., Hori, Y., Seki, T., Kurauchi, Y., Sato, M., Oshima, M., et al. (2019). Involvement of exosomes in dopaminergic neurodegeneration by microglial activation in midbrain slice cultures. *Biochem. Biophys. Res. Commun.* 511, 427–433. doi: 10.1016/j.bbrc.2019.02.076

Uchiyama, M., Isse, K., Tanaka, K., Yokota, N., Hamamoto, M., Aida, S., et al. (1995). Incidental Lewy body disease in a patient with REM sleep behavior disorder. *Neurology* 45, 709–712. doi: 10.1212/wnl.45.4.709

Ulusoy, A., Rusconi, R., Pérez-Revuelta, B. I., Musgrove, R. E., Helwig, M., Winzen-Reichert, B., et al. (2013). Caudo-rostral brain spreading of α-synuclein through vagal connections. *EMBO Mol. Med.* 5, 1119–1127. doi: 10.1002/emmm.201302475

Ulusoy, A., Phillips, R. J., Helwig, M., Klinkenberg, M., Powley, T. L., and Di Monte, D. A. (2017). Brain-to-stomach transfer of α-synuclein via vagal preganglionic projections. *Acta Neuropathol. (Berl.)* 133, 381–393. doi: 10.1007/s00401-016-1661-y

Vanle, B., Olcott, W., Jimenez, J., Bashmi, L., Danovitch, I., and IsHak, W. W. (2018). NMDA antagonists for treating the non-motor symptoms in Parkinson's disease. *Transl. Psychiatry* 8, 117. doi: 10.1038/s41398-018-0162-2

Vazey, E. M., and Aston-Jones, G. (2012). The emerging role of norepinephrine in cognitive dysfunctions of Parkinson's disease. *Front. Behav. Neurosci.* 6, 48. doi: 10.3389/fnbeh.2012.00048

Vieira, J. C. F., Bassani, T. B., Santiago, R. M., de O. Guaita, G., Zanoveli, J. M., da Cunha, C., et al. (2019). Anxiety-like behavior induced by 6-OHDA animal model of Parkinson's disease may be related to a dysregulation of neurotransmitter systems in brain areas related to anxiety. *Behav. Brain Res.* 371, 111981. doi: 10.1016/j.bbr.2019.111981

Vila, M. (2019). Neuromelanin, aging, and neuronal vulnerability in Parkinson's disease. *Mov. Disord.* 34 (10), 1440–1451. doi: 10.1002/mds.27776

Vo, Q., Gilmour, T. P., Venkiteswaran, K., Fang, J., and Subramanian, T. (2014). Polysomnographic Features of Sleep Disturbances and REM Sleep Behavior Disorder in the Unilateral 6-OHDA Lesioned Hemiparkinsonian Rat. *Park. Dis.* 2014, 852965. doi: 10.1155/2014/852965

Von Coelln, R., Thomas, B., Savitt, J. M., Lim, K. L., Sasaki, M., Hess, E. J., et al. (2004). Loss of locus coeruleus neurons and reduced startle in parkin null mice. *Proc. Natl. Acad. Sci. U. S. A.* 101, 10744–10749. doi: 10.1073/pnas.0401297101

Wakabayashi, K., and Takahashi, H. (1997). Neuropathology of autonomic nervous system in Parkinson's disease. *Eur. Neurol.* 38 Suppl 2, 2–7. doi: 10.1159/000113469

Wang, T., Zhang, Q.-J., Liu, J., Wu, Z.-H., and Wang, S. (2009). Firing activity of locus coeruleus noradrenergic neurons increases in a rodent model of Parkinsonism. *Neurosci. Bull.* 25, 15–20. doi: 10.1007/s12264-009-1023-z

Wang, Y., Zhang, Q. J., Liu, J., Ali, U., Gui, Z. H., Hui, Y. P., et al. (2010). Noradrenergic lesion of the locus coeruleus increases apomorphine-induced circling behavior and the firing activity of substantia nigra pars reticulata neurons in a rat model of Parkinson's disease. *Brain Res.* 1310, 189–199. doi: 10.1016/j.brainres.2009.10.070

Wang, Y., Wang, H. S., Wang, T., Huang, C., and Liu, J. (2014). L-DOPA-induced dyskinesia in a rat model of Parkinson's disease is associated with the fluctuational release of norepinephrine in the sensorimotor striatum. *J. Neurosci. Res.* 92, 1733–1745. doi: 10.1002/jnr.23439

Wang, J., Li, Y., Huang, Z., Wan, W., Zhang, Y., Wang, C., et al. (2018). Neuromelanin-sensitive magnetic resonance imaging features of the substantia nigra and locus coeruleus in de novo Parkinson's disease and its phenotypes. *Eur. J. Neurol.* 25, 949–e73. doi: 10.1111/ene.13628

Westlund, K. N., Bowker, R. M., Ziegler, M. G., and Coulter, J. D. (1983). Noradrenergic projections to the spinal cord of the rat. *Brain Res.* 263, 15–31. doi: 10.1016/0006-8993(83)91196-4

Yamasaki, M., and Takeuchi, T. (2017). Locus Coeruleus and Dopamine-Dependent Memory Consolidation. *Neural Plast.* 2017, 8602690. doi: 10.1155/2017/8602690

Yao, N., Wu, Y., Zhou, Y., Ju, L., Liu, Y., Ju, R., et al. (2015). Lesion of the locus coeruleus aggravates dopaminergic neuron degeneration by modulating microglial function in mouse models of Parkinson's disease. *Brain Res.* 1625, 255–274. doi: 10.1016/j.brainres.2015.08.032

Yssel, J. D., O'Neill, E., Nolan, Y. M., Connor, T. J., and Harkin, A. (2018). Treatment with the noradrenaline re-uptake inhibitor atomoxetine alone and in combination with the α2-adrenoceptor antagonist idazoxan attenuates loss of dopamine and associated motor deficits in the LPS inflammatory rat model of Parkinson's disease. *Brain. Behav. Immun.* 69, 456–469. doi: 10.1016/j.bbi.2018.01.004

Zarow, C., Lyness, S. A., Mortimer, J. A., and Chui, H. C. (2003). Neuronal loss is greater in the locus coeruleus than nucleus basalis and substantia nigra in Alzheimer and Parkinson diseases. *Arch. Neurol.* 60, 337–341. doi: 10.1001/archneur.60.3.337

Zweig, R. M., Cardillo, J. E., Cohen, M., Giere, S., and Hedreen, J. C. (1993). The locus ceruleus and dementia in Parkinson's disease. *Neurology* 43, 986–991. doi: 10.1212/wnl.43.5.986

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pharmacology

REVIEW
published: 23 April 2020
doi: 10.3389/fphar.2020.00356

Modeling Parkinson's Disease With the Alpha-Synuclein Protein

Mónica Gómez-Benito[1,2†], *Noelia Granado*[1,2†], *Patricia García-Sanz*[1,2], *Anne Michel*[3], *Mireille Dumoulin*[4] *and Rosario Moratalla*[1,2*]

[1] *Cajal Institute, Consejo Superior de Investigaciones Científicas (CSIC), Madrid, Spain,* [2] *CIBERNED, Instituto de Salud Carlos III, Madrid, Spain,* [3] *UCB Biopharma, Neuroscience TA, Braine L'Alleud, Belgium,* [4] *Centre of Protein Engineering, InBios, University of Liege, Liège, Belgium*

Alpha-synuclein (α-Syn) is a key protein involved in Parkinson's disease (PD) pathology. PD is characterized by the loss of dopaminergic neuronal cells in the substantia nigra pars compacta and the abnormal accumulation and aggregation of α-Syn in the form of Lewy bodies and Lewy neurites. More precisely, the aggregation of α-Syn is associated with the dysfunctionality and degeneration of neurons in PD. Moreover, mutations in the *SNCA* gene, which encodes α-Syn, cause familial forms of PD and are the basis of sporadic PD risk. Given the role of the α-Syn protein in the pathology of PD, animal models that reflect the dopaminergic neuronal loss and the widespread and progressive formation of α-Syn aggregates in different areas of the brain constitute a valuable tool. Indeed, animal models of PD are important for understanding the molecular mechanisms of the disease and might contribute to the development and validation of new therapies. In the absence of animal models that faithfully reproduce human PD, in recent years, numerous animal models of PD based on α-Syn have been generated. In this review, we summarize the main features of the α-Syn pre-formed fibrils (PFFs) model and recombinant adeno-associated virus vector (rAAV) mediated α-Syn overexpression models, providing a detailed comparative analysis of both models. Here, we discuss how each model has contributed to our understanding of PD pathology and the advantages and weakness of each of them.

Significance: Here, we show that injection of α-Syn PFFs and overexpression of α-Syn mediated by rAAV lead to a different pattern of PD pathology in rodents. First, α-Syn PFFs models trigger the Lewy body-like inclusions formation in brain regions directly interconnected with the injection site, suggesting that there is an inter-neuronal transmission of the α-Syn pathology. In contrast, rAAV-mediated α-Syn overexpression in the brain limits the α-Syn aggregates within the transduced neurons. Second, phosphorylated α-Syn inclusions obtained with rAAV are predominantly nuclear with a punctate appearance that becomes diffuse along the neuronal fibers, whereas α-Syn PFFs models lead to the formation of cytoplasmic aggregates of phosphorylated α-Syn reminiscent of Lewy bodies and Lewy neurites.

Keywords: Lewy body pathology, α-Syn pre-formed fibrils, α-Syn AVV-viral particles, prion-like propagation, Braak hypothesis, α-Syn aggregation

OPEN ACCESS

Edited by:
Cristina Miguelez, University of the Basque Country, Spain

Reviewed by:
Ledia F. Hernandez, Centro Integral en Neurociencias A.C. HM CINAC, Spain
Chris Van Den Haute, KU Leuven, Belgium

***Correspondence:**
Rosario Moratalla
moratalla@cajal.csic.es

[†]*These authors have contributed equally to this work*

Specialty section:
This article was submitted to Neuropharmacology, a section of the journal Frontiers in Pharmacology

Received: *03 January 2020*
Accepted: *10 March 2020*
Published: *23 April 2020*

Citation:
Gómez-Benito M, Granado N, García-Sanz P, Michel A, Dumoulin M and Moratalla R (2020) Modeling Parkinson's Disease With the Alpha-Synuclein Protein. Front. Pharmacol. 11:356. doi: 10.3389/fphar.2020.00356

PARKINSON'S DISEASE

Parkinson's Disease (PD) is the second most common neurodegenerative disorder after Alzheimer's disease. Currently, PD affects 1%–2% of people over the age of 60 years, rising to 4% at age 80 years (Rizek et al., 2016). PD is mainly characterized by the progressive loss of dopaminergic neurons of the substantia nigra that project to the striatum (Xu and Pu, 2016). The deficiency of dopamine in the striatum leads to the development of the classic motor symptoms of PD, including bradykinesia, resting tremor, muscular rigidity, and postural instability. In addition to motor symptomatology, non-motor manifestations, such as autonomic dysfunction, olfactory impairment, mood disorders (i.e., depression and anxiety), cognitive deficits, or sleep disturbances are also frequently present in PD. Most of these symptoms appear even before the motor symptoms and have a serious impact on the quality of life of patients (Kalia and Lang, 2015; Schapira et al., 2017). Because the PD motor symptoms emerge when the striatal dopamine levels have decreased by 60%–80% (Dauer and Przedborski, 2003), the study of PD non-motor symptoms is important to identify early biomarkers as well as targets to develop disease-modifying therapies that slow or prevent the progression of neurodegeneration.

The neuropathological mechanisms underlying non-motor symptoms of PD are still poorly understood, but growing evidence suggests that the evolution of these symptoms may arise from the disruption of both dopaminergic and non-dopaminergic systems, and the involvement of diverse structures outside the nigrostriatal system (Jellinger, 2017; Schapira et al., 2017). Besides the dopamine, the further key neurotransmission systems that have been described to be involved in the pathogenesis of PD are the noradrenergic system of locus coeruleus, the serotonergic system of the dorsal raphe nuclei, and the cholinergic system of the nucleus basalis of Meynert and the pedunculopontine nucleus (Qamar et al., 2017). Since the relative contribution of each of these pathways to motor and non-motor symptoms has only been partially explored, additional research is needed to fully understand their involvement in the clinical and pathological features of the disease.

The neuropathological hallmark of PD is the abnormal accumulation and aggregation of alpha synuclein protein (α-Syn) in form of Lewy bodies and Lewy neurites (Xu and Pu, 2016). It is well established that pathological aggregation of α-Syn is a common feature of several neurodegenerative diseases including PD, dementia with Lewy bodies (DLB) and multiple system atrophy (MSA), that are collectively referred as synucleinopathies (Goedert et al., 2017). α-Syn is a protein with remarkable conformational plasticity since it can adopt a wide range of structural conformations (oligomers, protofibrils and fibrils; Deleersnijder et al., 2013; Mehra et al., 2019). Each α-Syn conformation displays distinct properties in terms of neurotoxicity, stability and seeding and propagation ability. It has been proposed that the existence of structurally distinct α-Syn assemblies or α-Syn "strains" may contribute to explain the clinical-pathological heterogeneity among synucleinopathies and help to develop strain-specific medications (Melki, 2015; Peelaerts et al., 2015; Candelise et al., 2019).

Parallel to deficits in both dopaminergic and non-dopaminergic neurotransmission systems, the appearance of PD non-motor symptoms is also attributed to the sequential development of Lewy bodies in different brain regions, including the olfactory bulb, the dorsal motor nucleus of the vagal nerve, locus coeruleus, raphe nucleus, basal nucleus of Meynert and pedunculopontine nucleus (Braak et al., 2003; Braak et al., 2004; Schindlbeck and Eidelberg, 2019). Both hypotheses are not mutually exclusive, since sequential distribution of Lewy bodies through the brain may alter various neurotransmission pathways that may be the basis of non-motor manifestations. To date, the etiology of PD remains unknown, but cumulative evidence suggests that the presence of intraneuronal inclusions of α-Syn affects the functional integrity of neurons, ultimately causing their death. It has been demonstrated that α-Syn aggregates can induce neuronal toxicity leading to neuronal death by multiple mechanisms, including mitochondrial dysfunction, lysosomal impairment, membrane disturbance, endoplasmic reticulum stress, and synaptic dysfunction (reviewed in Roberts and Brown, 2015; Xu and Pu, 2016; Zhang et al., 2018; Zhang et al., 2019). However, although the Lewy pathology is commonly observed in PD, there remains much debate over whether α-Syn aggregation is a key feature for the development and progression of the disease. Two observations contribute to this debate: first, there is solid evidence that not all cases of parkinsonism are characterized by the presence of α-Syn inclusions—several studies have reported that PD patients carrying familial mutations in *Parkin* gene, and some of those with the *LRRK2* G2019S mutation, show neuronal degeneration but do not develop Lewy bodies (Gaig et al., 2009; Johansen et al., 2018) and, second, postmortem analysis reflect that Lewy bodies and Lewy neurites may be present in the absence of clinical PD symptoms (Parkkinen et al., 2005). Strengths and limitations of the evidence that correlate the aggregation of α-Syn with the progression of PD pathology will be addressed throughout the review.

THE ROLE OF ALPHA-SYNUCLEIN IN PD PATHOLOGY

α-Syn is a small protein encoded by the *SNCA* gene that is abundantly expressed in the presynaptic terminals of the central nervous system. The exact function of α-Syn remains largely unknown, although mounting evidence supports the notion that α-Syn is involved in synaptic plasticity and neurotransmitter release (Burré et al., 2010; Venda et al., 2010). Likewise, recent studies have shown that neuronal/synaptic activity regulates dynamically the physiological release of endogenous α-Syn, so an elevated neuronal activity increases the release of α-Syn (Yamada and Iwatsubo, 2018). Several lines of evidence demonstrate the pathogenic role of α-Syn in PD: 1) point mutations (A30P, E46K, H50Q, G51D, A53T, and A53E) and duplication or triplication of the *SNCA* gene cause autosomal

dominant forms of PD (Polymeropoulos et al., 1997; Zarranz et al., 2004); 2) polymorphic variants of the *SNCA* gene constitute an important risk factor for developing idiopathic PD (Nalls et al., 2014); and 3) α-Syn is the major component of Lewy bodies (Spillantini et al., 1997; Wakabayashi et al., 2013).

Under normal conditions, native α-Syn exists in a dynamic equilibrium between unfolded monomers and α-helically folded tetramers with a low propensity to aggregation (Lashuel et al., 2013). The decline of the tetramer:monomer ratio and the consequent increase in the level of α-Syn unfolded monomers favor its aggregation (Nuber et al., 2018). The aggregation process of α-Syn involves a conformational change whereby it adopts a β-sheet-rich structure that facilitates its aggregation into oligomers, protofibrils, and insoluble fibrils that finally accumulate in Lewy bodies. There is an intense debate about what α-Syn species are cytotoxic. Although both oligomeric and fibrillar species of α-Syn have been shown to be toxic, recent studies suggest that oligomers and protofibrils, forming during the initial stages of the aggregation process, are the potent neurotoxic species causing cell death in PD. Conversely, α-Syn fibrils appear to be the most efficient species at propagating, thus contributing to the spread and progression of the disease (Alam et al., 2019; Mehra et al., 2019). Most of studies that confirm the pathogenic effects of different α-Syn assemblies have used in vitro formed species; so the extent to which these oligomers recapitulate the structure and properties of those found in brain tissue from PD patients remains unclear (Bengoa-Vergniory et al., 2017). Mutations, post-translational modifications, an imbalance between synthesis and degradation of α-Syn, and environmental factors influence the aggregation propensity of α-Syn. The A53T mutation was the first to be documented, and it is associated with an early-onset PD (Polymeropoulos et al., 1997). The E46K mutation predisposes to the development of severe parkinsonism with dementia and a large number of Lewy bodies that are widely distributed (Zarranz et al., 2004). Both mutations alter the α-Syn protein structure, which facilitates its aggregation (Li et al, 2001; Greenbaum et al., 2005; Tosatto et al., 2015).

α-Syn undergoes various post-translational modifications, such as phosphorylation, truncation, ubiquitination, and nitration. Phosphorylation of α-Syn at the serine 129 residue is one of the major pathological markers of PD; 90% of α-Syn is phosphorylated in the brain of patients with PD while only 4% of α-Syn is phosphorylated in healthy brains (Oueslati, 2016; Ghosh et al., 2017). However, there is a great controversy over whether phosphorylation has an active role in the α-Syn aggregation or if it is a response mechanism of cells to try to label and eliminate toxic species of α-Syn (Oueslati, 2016). Smith et al. (2005) proposed that phosphorylation of α-Syn promotes the formation of cytoplasmic inclusions in some cell culture models (Smith et al., 2005). Nevertheless, Oueslati et al. (2013) show that phosphorylation of α-Syn induced by polo-like kinase 2 has no effect on the aggregation and regulates α-Syn clearance via the lysosomal autophagy pathway (Oueslati et al., 2013). Additional lines of evidence show crosstalk between phosphorylation and α-Syn degradation. The inhibition of the ubiquitin-proteasome system (Chau et al., 2009) or the autophagy-lysosomal pathway (Machiya et al., 2010) induced a significant increase in phosphorylated α-Syn in human neuroblastoma, suggesting that phosphorylation regulates the α-Syn degradation.

PROPAGATION OF ALPHA-SYNUCLEIN: EVIDENCE AND CONSIDERATIONS

Although dopaminergic neurons of substantia nigra seem to be particularly vulnerable in PD, the examination of PD progression indicates that α-Syn pathology is not restricted exclusively to this region. In 2003, Braak et al. postulated the hypothesis that the progression of α-Syn pathology follows a specific caudo-rostral pattern through the central nervous system (Braak et al., 2003; Braak et al., 2004). These authors proposed that the two starting points of PD pathology were the olfactory bulb and the enteric nerves, and from them, the damage extends via the olfactory tract or the vagus nerve, respectively, to other brain regions. According to this theory, PD can be divided into six stages, and each of them is characterized by the development of α-Syn inclusions in specific brain areas, including dorsal motor nucleus of vagus nerve, raphe nuclei, magnocellular portions of reticular formation, locus coeruleus, substantia nigra, and cortex (Killinger and Kordower, 2019). The presence of these inclusion bodies causes a dysfunctionality of the cells, which is ultimately responsible for the development of the clinical symptoms associated with PD (Braak et al., 2003; Braak et al., 2004). Given that α-Syn is involved in neuronal plasticity, the functional consequences of its aggregation have been explored at both the presynaptic and postsynaptic level. A recent study has shown that neurons derived from human induced pluripotent stem cells (iPSC) of PD patients that express oligomer-forming α-Syn mutants (E46K and E57K) display a reduction in presynaptic protein levels, an impaired anterograde axonal transport, and structural abnormalities of the axonal and synaptic compartments (Prots et al., 2018). Moreover, other studies have shown that α-Syn plays a role in N-Methyl-D-aspartic acid (NMDA) receptor trafficking, suggesting that α-Syn has postsynaptic effects. In a transgenic mouse model expressing C-terminally truncated α-Syn (aa 1-120), an impaired hippocampal long-term potentiation due to alterations in dopaminergic transmission and plastic changes in the composition of NMDA receptors has been reported (Tofaris et al., 2006; Costa et al., 2012). Electrophysiological recordings after treating mouse brain slices with α-Syn oligomers show impaired synaptic transmission and long-term potentiation in the hippocampus (Martin et al., 2012; Diógenes et al., 2012). *In vivo* amperometry recordings in rodents injected with α-Syn PFFs in combination with AAV-mediated overexpression of α-Syn reveal a reduction in dopamine release and reuptake rates in the striatum (Thakur et al., 2017). Recently, an electrophysiological analysis from slices from α-Syn PFFs-injected mice has shown that α-Syn reduces NMDA receptor-mediated synaptic currents and impairs long-term potentiation in the striatal medium spiny neurons (Durante et al., 2019).

Moreover, Kordower et al. found Lewy body-like inclusions in embryonic grafted neurons in PD patients, suggesting that α-Syn can spread from the host to the graft neurons (Kordower et al., 2008). The α-Syn protein has been detected in cerebrospinal fluid, and blood plasma of both PD and healthy subjects (El-Agnaf et al., 2003) and, in addition, numerous *in vitro* studies have demonstrated that cultured neurons can secrete α-Syn and take it up from the extracellular space (Luk et al., 2009; Volpicelli-Daley et al., 2011; Reyes et al., 2015). All these findings suggest that pathological α-Syn acts as a prion-like protein that can propagate throughout the brain through cell-to-cell transmission mechanisms. Moreover, the treatment of cultured cells with recombinant α-Syn fibrils induces the aggregation of endogenous α-Syn in insoluble inclusions that resemble Lewy bodies. These data suggest that PFFs can seed the recruitment of endogenous α-Syn and induce its pathological conversion (Luk et al., 2009; Volpicelli-Daley et al., 2011). In addition to *in vitro* studies, in the last decade, several animal models of PD have been developed to study (*in vivo*) the ability of propagation of α-Syn protein. These models can be classified as (a) neural stem cell transplantation into transgenic mice expressing human α-Syn (Desplats et al., 2009), (b) administration of brain extracts derived from PD patients (Recasens et al., 2014) or α-Syn pre-formed fibrils (PFFs; Chung et al., 2019), and (c) AAV-α-Syn viral particles (Ulusoy et al., 2013; Ip et al., 2017). Among these, the PFFs and the AAV-α-Syn viral particle models, which are the subject of this review, are the most extensively used models to study the α-Syn pathology and propagation.

Although many studies support the prion-like nature of α-Syn and show that its propagation determines the temporal course of the disease, some observations have recently challenged this theory. The Braak staging is neither a proof nor an argument of the spreading hypothesis, it might be rather that certain subsets of neurons are affected by Lewy bodies much earlier than others and that, therefore, the intraneuronal lesions evolve sequentially (Walsh and Selkoe, 2016). Another clinical observation that has questioned the pathogenic spread hypothesis is that only a minority of grafted neurons in patients with advanced PD exhibited Lewy body-like inclusions, and their presence appeared to have little functional consequences for neurons that survived for long periods of time (Kordower et al., 2008; Cooper et al., 2009; Hallett et al., 2014). In this context, a new possibility raised is that a selective vulnerability of specific neuronal populations to certain adverse stimuli, such as neuroinflammation, contributes to the propagation of α-Syn (Walsh and Selkoe, 2016). Misfolded α-Syn can trigger the activation of microglia. Likewise, activated microglia can enhance the aggregation and spreading of α-Syn, creating a positive feedback loop between inflammation and α-Syn aggregation. Both phenomena are interconnected, and this interaction plays a key role in the pathogenesis of PD. Therefore, it remains unclear whether α-Syn aggregation is a cause or a consequence of inflammation. A recent study suggests that activation of microglia with toxic cytokine release, such as caspase-1 and calpains, plays a critical role in promoting the misfolding of native α-Syn and in the spread of misfolded α-Syn in PD (Olanow et al., 2019). The two proposed possibilities, the prion-like hypothesis and the selective vulnerability hypothesis, are not mutually exclusive, and a combination of both hypotheses might occur. It is noteworthy that in the PFFs models, microglial activation might appear as resulting from an immune reaction due to the inoculation of foreign α-Syn fibrils. Indeed, it has been shown that striatal injection of α-Syn PFFs but not monomers in WT mice can trigger neuroinflammation by increasing peripheral immune cells infiltration in the CNS (Earls et al., 2019). Apparently, this immune reaction would precede the dopaminergic neurodegeneration (Harms et al., 2017) but, it cannot be excluded that the magnitude and the efficacy of the immune reaction can also modulate the spread of the pathology observed as it was recently suggested by (Earls et al., 2020).

Another crucial question that is not clear is whether the spreading of α-Syn is a driving factor for neuronal degeneration and progression of PD, or if it is an epiphenomenon that appears as a result of other alterations, such as lysosomal dysfunction (Killinger and Kordower, 2019). Despite the unequivocal evidence of the presence of Lewy body-like inclusions and the ability of α-Syn to propagate in animal models, why dopaminergic neurons of SNc should be particularly vulnerable to propagated aggregates of α-Syn remains uncertain. The lack of α-Syn deposits in some PD, patients (Gaig et al., 2009; Johansen et al., 2018) has led some authors to the belief that the presence and spread of Lewy-type aggregates are not sufficient to explain the dysfunction and loss of neurons and the development of parkinsonian symptoms.

Lysosomal dysfunction impairs the ability to remove toxic aggregates, which increases the probability of α-Syn aggregation and spreading (Klein and Mazzulli, 2018). α-Syn is degraded through the lysosome in physiological conditions, so some perturbations in lysosomal functions can affect the α-Syn levels (Martinez-Vicente et al., 2008). Likewise, α-Syn aggregates might impair the autophagic-lysosomal pathway function (Xilouri et al., 2013a), establishing a reciprocal relationship. The accumulation of α-Syn reduces lysosomal degradation capacity by disrupting hydrolases trafficking, such as of glucocerebrosidase 1 (GCase1), from the endoplasmic reticulum to the lysosome (Mazzulli et al., 2011; García-Sanz et al., 2017; García-Sanz et al., 2018). Currently, mutations in the *GBA1* gene are the main genetic risk factor for PD. The *GBA1* gene encodes for GCase1, a lysosomal enzyme responsible for degrading the lipid glucosylceramide into ceramide and glucose (Do et al., 2019). *GBA1* mutations result in reduced enzymatic activity that leads to the accumulation of glucosylceramide and of cholesterol and its esters in lysosomes, which can compromise lysosomal function and promote α-Syn aggregation, creating a bidirectional loop (Schapira, 2015). Previous studies from our laboratory have shown that fibroblasts derived from PD patients with the *GBA1* mutation accumulate cholesterol in lysosomes and present multilamellar bodies (García-Sanz et al., 2017; García-Sanz et al., 2018). Also, membrane structures resembling lysosomes and autophagosomes have been found in

the inner architecture of Lewy bodies (Shahmoradian et al., 2019), suggesting that the alteration of these organelles might contribute to the formation of Lewy bodies.

In addition to *GBA1* mutations, numerous PD-related genetic variants have been identified in several genes involved in the autophagic-lysosomal pathway including *Parkin*, *PINK1*, *DJ-1*, *LRRK2*, *ATP13A2*, or *VPS35* (Klein and Westenberger, 2012). Some of these genes encode lysosomal enzymes/proteins (e.g. *ATP13A2*), whereas others correspond to proteins that are involved in the transport to the lysosome (e.g. *VPS35*), mitophagy (e.g. *Parkin*, *PINK1*, and *DJ-1*), or other autophagic-related functions (e.g. *LRRK2*; Gan-Or et al., 2015). Mutations in these genes are directly related to autosomal -dominant or recessive- PD forms or may contribute to increase PD susceptibility.

Propagation and aggregation of α-Syn is an important molecular mechanism that contributes to PD progression. However, this event might require other factors to promote the pathological development of the disease. Mitochondrial dysfunction, oxidative stress, failure of the lysosomal autophagy and ubiquitin-proteasome systems, and neuroinflammation have been recognized as potential triggers of the misfolding of α-Syn, the spread of pathology, and the progression of PD. Precisely, Lewy bodies are composed by fragmented organelles including mitochondria, lipid membranes, lysosomal structures as well as other proteins involved in the degradation systems such as ubiquitin and p62/SQSTM1, suggesting a potential role of damaged and disrupted organelles in the formation of α-Syn inclusions (Shahmoradian et al., 2019). All these factors depend on the age, genetic background, and environment to which each individual is exposed. Likewise, the misfolding of α-Syn and its consequent aggregation might cause several alterations, such as mitochondrial dysfunction, endoplasmic reticulum stress, impairment of protein clearance pathways, disruption of biological membranes, and synaptic dysfunction (reviewed in Roberts and Brown, 2015). Future research should focus on determining which events lead first to the development of pathology and understand how the different mechanisms involved interact with each other.

ANIMAL MODELS OF PD BASED ON ALPHA-SYNUCLEIN

Given the close relationship between α-Syn aggregation and PD pathology, a wide variety of PD animal models has been generated in recent years. This review includes a detailed analysis of models based on the inoculation of α-Syn PFFs and models based on overexpression of α-Syn by recombinant adeno-associated viral vectors (rAAV), giving special interest to the differences observed between these two models. These observations are based on results obtained by our group and which are in line with the findings of previous studies.

Injection of α-Syn Pre-Formed Fibrils or Pathological Extracts

One of the approaches that have been developed to study the propagation of α-Syn is the intracerebral or systemic administration of either α-Syn pre-formed fibrils (PFFs) or brain extracts containing Lewy bodies and α-Syn derived from PD patients or transgenic mice exhibiting α-Syn pathology. PFFs are generated *in vitro* from recombinant α-Syn monomers. Subsequently, the aggregation of monomers into fibrils is induced and the fibrils are sonicated to generate short fibrils which after injection, trigger the aggregation, hyperphosphorylation and ubiquitination of endogenous α-Syn (Patterson et al., 2019). Intracerebral injection of α-Syn PFFs has been extended to rodents, both mice (Luk et al., 2012a; Masuda-Suzukake et al., 2013; Karampetsou et al., 2017; Milanese et al., 2018; Okuzumi et al., 2018; Patterson et al., 2019) and rats (Paumier et al., 2015; Harms et al., 2017; Thakur et al., 2017; Duffy et al., 2018b; Durante et al., 2019) and non-human primates (Shimozawa et al., 2017; Chu et al., 2019). PFFs have been mainly injected in the striatum (Luk et al., 2012a; Paumier et al., 2015; Karampetsou et al., 2017; Shimozawa et al., 2017; Duffy et al., 2018b; Milanese et al., 2018; Okuzumi et al., 2018; Chu et al., 2019; Durante et al., 2019; Patterson et al., 2019), the substantia nigra (Masuda-Suzukake et al., 2013; Harms et al., 2017) and the cortex (Luk et al., 2012b). Similarly, the inoculation of purified brain extracts from PD patients or transgenic mice containing pathological α-Syn has been performed in rodents and non-human primates (Luk et al., 2012b; Recasens et al., 2014). Most of these models have succeeded in producing the accumulation and aggregation of phosphorylated α-Syn (pα-Syn), the progressive degeneration of dopaminergic neurons, a significant reduction in striatal dopamine levels, neuroinflammation, and the development of motor deficits (Recasens et al., 2014; Paumier et al., 2015; Karampetsou et al., 2017; Shimozawa et al., 2017; Patterson et al., 2019). It is well described that intracerebral inoculation of PFFs leads to the formation of pα-Syn-immunoreactive aggregates that are distributed both in the soma and neuronal processes. α-Syn inclusions exhibit different morphological features that resemble human Lewy bodies: from granules with cytoplasmic staining to compact and rounded structures with dark staining that fills entirely cells (Luk et al., 2012a; Paumier et al., 2015; Chu et al., 2019). Some studies have shown that these aggregates commonly colocalize with key markers of Lewy bodies, including ubiquitin and p62/proteasome 1 (Wakabayashi et al., 2013), and they are thioflavin S-positive and proteinase K-resistant, indicating that they share common properties with human Lewy bodies (Paumier et al., 2015; Chu et al., 2019). Our group has observed the presence of pα-Syn-immunoreactive structures with similar morphological characteristics at 12 weeks after intrastriatal inoculation of human wild-type αSyn PFFs in the SNCA-OVX transgenic mouse model (Janezic et al., 2013; **Figure 1**).

Also, these models have shown that pathological α-Syn can spread from the injection site to other anatomically

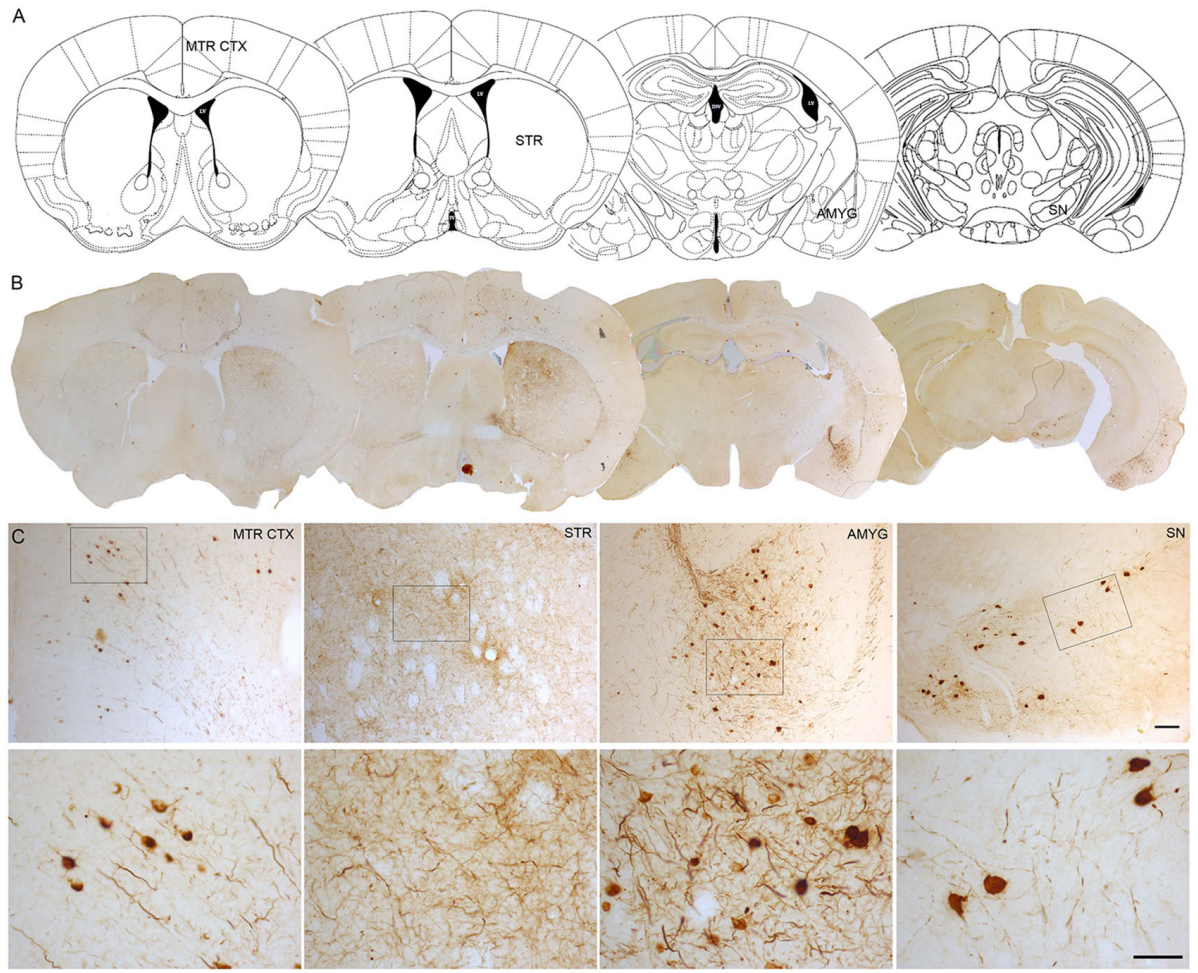


FIGURE 1 | α-Syn aggregation 3 months after intrastriatal α-Syn PFFs injection. **(A, B)**. Representative photomicrographs of pα-Syn-stained sections of the motor cortex (Mrt Ctx), striatum (STR), amygdala (Amyg), and substantia nigra (SN). **(C)**. High magnification images show the presence of pα-Syn. Scale bar: 100 μm (**C**, upper panel) and 50 μm (**C**,lower panel). PFFs, pre-formed fibrils.

interconnected brain regions following a fibrillary retrograde transport (**Figure 4**). The fibrils are picked up by the nerve terminals of dopaminergic neurons, travelling from them to the cell body located in the substantia nigra pars compacta. At early stages after the injection of PFFs, α-Syn inclusions are visible in regions close to the injection site, but over time the inclusions can be detected in other areas, including locus coeruleus, SNc, thalamus, hypothalamus, amygdala, and neocortex, confirming that there is a time-dependent propagation of α-Syn pathology (Luk et al., 2012a; Luk et al., 2012b; Paumier et al., 2015; Duffy et al., 2018b; Patterson et al., 2019). Some studies have reported that unilateral injection of PFFs leads to the accumulation of pα-Syn in the hemisphere contralateral to the injection (Luk et al., 2012b; Polinski et al., 2018). Indeed, we also observed small and faint pα-Syn-positive fibers into the contralateral hemisphere. These fibers are mainly found into the striatum, but also into motor cortex, amygdala and substantia nigra (**Figure 2**). The presence of α-Syn-immunoreactive inclusions in the fibers of the corpus callosum and anterior commissure, which extend bilaterally, might explain the interhemispheric transmission of α-Syn pathology.

Notably, the injection of α-Syn PFFs in transgenic null mice for murine *Snca* (i.e. α-Syn KO mice) does not lead to the formation of α-Syn inclusions or degeneration; an observation which supports that endogenous α-Syn is required for the development of the pathology (Luk et al., 2012b; Kim et al., 2019). Recasens et al. (2014) have shown that the cytoplasmic accumulation of α-Syn detected in the SNc of mice injected with Lewy body extracts at 4 months post-injection could be exclusively attributed to endogenous α-Syn, since at this time, the exogenous α-Syn could not be detected. These findings suggest that fibrils of α-Syn act as a template to convert the endogenously expressed α-Syn into pathological aggregates (Recasens et al., 2014).

Nevertheless, it is important to highlight the great variability that exists in these models because the injection site, the amount and

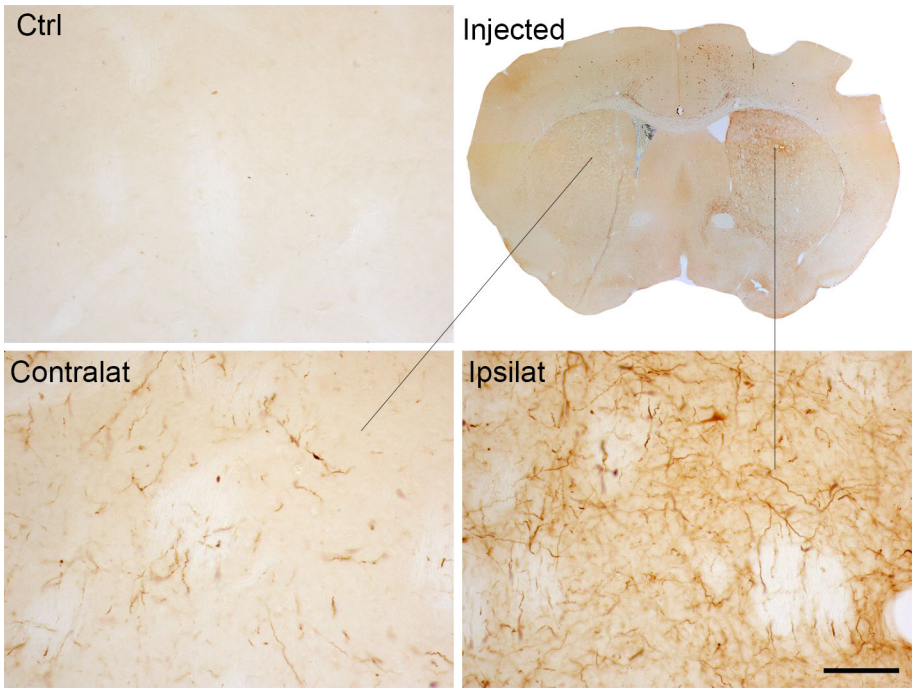


FIGURE 2 | α-Syn expression in the contralateral side to the injection of α-Syn PFFs. Representative photomicrographs of pα-Syn-stained sections of the striatum of control mice or mice injected with PFFs. Scale bar: 50 μm. PFFs, pre-formed fibrils.

type of PFFs injected, and the animal species/strains used might influence the development of neuropathology. Regarding the injection site, in most of the studies the α-Syn PFFs have been injected into the striatum because it is a large area with easy access. The precision required to inject PFFs is critical given that the fibrils do not spread efficiently through tissues, so adequate inoculation at the site of interest is important. Interestingly, it has been demonstrated that the injection of α-Syn PFFs into the substantia nigra of mice leads to a restriction of the pathological α-Syn expression around this brain area (Masuda-Suzukake et al., 2014) and does not induce a dopaminergic degeneration even 15 months after PFFs inoculation (Masuda-Suzukake et al., 2013). In addition, the genetic background of rodents can also influence the severity and the extent of the spreading of Lewy body-like pathology in these models. Intrastriatal injection of mouse α-Syn PFFs into different mice strains leads to differences in the severity of the α-Syn-induced pathology (Luk et al., 2012a).

Another important consideration of this model is the concentration and type of PFFs injected. Given that the *in vitro* generation of PFFs occurs under different conditions (i.e. pH, temperature, ionic strength), the sonicated α-Syn PFFs are heterogenous in nature and have different conformational features and biological effects (i.e. different aggregation pattern, propensity to propagate, seeding ability and/or toxicity). Rodents injected with different PFFs strains manifest different pathological severity and behavioral phenotypes, reflecting the great variability of the results, especially among different research studies (Peelaerts et al., 2015; Polinski et al., 2018; Chung et al., 2019). A recent study in which α-Syn PFFs have been injected into the mouse gastric wall shows that mice developed α-Syn-immunoreactive aggregates in the dorsal motor nucleus at 45 days post-inoculation, but no propagation of pα-Syn aggregates beyond of the dorsal motor nucleus of the vagus nerve was observed 12 months after inoculation (Uemura et al., 2018). These findings support that each α-Syn strain has a different potency for inducing the propagation. In addition, the animal species from which the α-Syn is derived seems to be critical. Although human and mouse α-Syn share 95% sequence identity, their ability to induce the formation of the inclusions seems to differ. Injection of mouse PFFs into the duodenal and pyloric muscularis layers of mice leads to the pathological accumulation of α-Syn in the SNc at 3 months post-injection, while the injection of human PFFs does not cause α-Syn accumulation in the SNc at the same time point (Kim et al., 2019). Moreover, previous studies have shown that human PFFs injection leads to slower propagation of α-Syn pathology compared to the injection of mouse PFFs (Rey et al., 2016). According to this finding, the formation of aggregates is more efficient if the PFFs and endogenous α-Syn are from the same species (Fares et al., 2016). Moreover, some studies have used modified forms of α-Syn PFFs, for example phosphorylated S129 fibrils or N- and C-terminally truncated forms. Mice injected with phosphorylated PFFs exhibited more α-Syn inclusions in the SNc than mice injected with wild type α-Syn PFFs (Karampetsou et al., 2017).

According to the Braak hypothesis, the PD pathology is initiated in two independent sites: the gastrointestinal tract and

the olfactory bulb (Braak et al., 2003). To address the need to understand the mechanisms by which the pathologic α-Syn spread through the brain from these two starting sites, α-Syn PFFs have been administered through peripheral routes (i.e., intramuscularly and intravenously), and in the olfactory bulb. Recent studies demonstrate that the injection of α-Syn PFFs in the muscular layers of pylorus and duodenum produces α-Syn aggregation and a significant progressive degeneration of dopaminergic neurons. The accumulation of pα-Syn in the SN at 7 months post-injection coincides with a reduction of TH- and Nissl-positive cells in this region. In contrast, at 1 and 3 months after injection, there is no significant loss of TH- or Nissl-positive cells. Accompanying the loss of dopaminergic neurons, a reduction of tyrosine hydroxylase immunoreactivity in the striatum is observed at 7 months and, much more at 10 months after PFFs injection, coinciding with the appearance of pα-Syn aggregates in this area. Also, high-performance liquid chromatography analysis to measure dopamine concentration indicates that striatal dopamine levels are significantly reduced at 3 months post-injection (Kim et al., 2019). These mice develop motor, and non-motor PD symptoms, including psychiatric behavioral and olfactory dysfunction (Kim et al., 2019) and gastrointestinal dysfunction (Challis et al., 2020). In addition, these studies demonstrated that the vagus nerve and endogenous α-Syn are required for the gut-to-brain transmission of pathologic α-Syn because truncal vagotomy was performed prior to inoculation of PFFs (Uemura et al., 2018; Kim et al., 2019) and the PFFs injection in the *Snca* null mice (Kim et al., 2019) prevents the spread of pathologic α-Syn to the brain. Similarly, another recent study has shown that intrastriatal injection of α-Syn PFFs induces the Lewy body-like pathology within the enteric nervous system of wild type mice (Earls et al., 2019). Likewise, other studies have shown that nigral overexpression of rAAV-α-Syn in rats produces concomitant alterations on the enteric nervous system—accumulation of α-Syn deposits (Ulusoy et al., 2017) and a loss of enteric neurons and changes in the gut microbiome (O'Donovan et al., 2020), which reflects that α-Syn pathology in the brain may impact on the gastrointestinal system and that the gut-to-brain communication via the vagus nerve may underlie the path of pathology progression. In other studies, α-Syn PFFs have been injected into the olfactory bulb, which produces an accumulation of pα-Syn in different areas distant to the injection site, which shows that there is a spatial and multi-synaptic progression of α-Syn pathology over time. Moreover, those studies showed degeneration and olfactory dysfunction (Rey et al., 2016; Rey et al., 2018), so these models can be used to reproduce prodromal PD and test therapies designed to slow or halt the development of PD.

Given that idiopathic PD is not strictly associated with an increase in the α-Syn levels, as opposed to PD associated with duplications and triplications of the *SNCA* gene, an environment in which the levels of endogenous α-Syn are physiological would more faithfully recapitulate the non-genetic forms of PD (Duffy et al., 2018a). The α-Syn PFFs models, in contrast to those based on overexpression of α-Syn (viral vector-based and transgenic models), represent an approach in which the pathology is induced in a context of physiological levels of endogenous α-Syn (Chung et al., 2019). Thus, these models are especially useful for assessing the effect of pathological α-Syn injected exogenously on the aggregation of endogenous α-Syn, and neuronal function, and to evaluate the resultant parkinsonian phenotype. Moreover, PFFs models are considered as valuable tools to develop and test therapies based on preventing the formation of α-Syn inclusions and their spreading at the early stages of the disease. While common features are well reproducible among the different groups working on this model, a certain degree of variability still exists between the results. Establishing standard protocols for the preparation of PFFs can improve the reproducibility of the results obtained. Additional experiments would be helpful to understand the best conditions to achieve the most efficient and robust phenotype (e.g. concentrations of PFFs, site of injection, and comparison across animal species and strains). Another important consideration when using the α-Syn PFFs model is to established whether or not recombinant α-Syn PFFs are identical to the species of α-Syn present in the pathology of human PD patients (Polinski et al., 2018).

Overexpression of α-Syn Mediated by rAAV

Another alternative to model PD is the α-Syn overexpression by recombinant adeno-associated virus vectors (rAAV). rAAV are an efficient vehicle for gene delivery in the brain area of interest and offer some characteristics that favor their use in modeling PD. rAAV efficiently transduce various cell types, confer long-lasting transgene expression, and can transduce dividing and non-dividing cells in the absence of an immune reaction (Pignataro et al., 2018). Given that neurons are post-mitotic cells, the capacity of rAAV to transduce non-dividing cells is crucial in the context of neurodegenerative disease. rAAV are smaller particles than lentivirus, which gives them the advantage of spreading efficiently within tissues, being a good choice for tissue infection. Another advantage of their small size is that many more rAAV-viral particles can be injected in the same volume compared to lentiviral particles, resulting in a much greater functional titer per injected volume. In addition, rAAV rarely integrate into the host genome, which reduces the occurrence of mutagenesis. This is crucial since random integration of the vector into the host DNA can lead to both loss- and gain-of-function mutations that might alter cell functionality and homeostasis (Albert et al., 2017).

Overexpression of wild type α-Syn or PD-associated mutants (A53T or A30P α-Syn) utilizing rAAV leads to a progressive loss of dopaminergic neurons in the SNc, a loss of dopamine terminals in the striatum (Koprich et al., 2010; Koprich et al., 2011; Oliveras-Salvá et al., 2013; Bourdenx et al., 2015; Caudal et al., 2015; Lu et al., 2015; Ip et al., 2017), and a reduction of striatal dopamine content (Koprich et al., 2011; Ip et al., 2017). However, the extent of neurodegeneration achieved with the rAAV model is variable among the different studies. Several serotypes, promoters, α-Syn species, doses, and time-course after

injection have been tested, and all these factors influence the parkinsonian phenotype achieved. rAAV-α-Syn expression leads to the accumulation of pα-Syn. Unlike models based on the administration of α-Syn PFFs, in these models, the α-Syn-immunoreactive structures are commonly nuclear with a small and punctate appearance. Some studies have demonstrated that these structures are proteinase-K resistant (Koprich et al., 2010; Taschenberger et al., 2012; Lu et al., 2015; Ip et al., 2017) or urea-resistant (Oliveras-Salvá et al., 2013), but they do not reproduce the morphological features of human Lewy bodies. Our group have found that the overexpression of E46K human α-Syn mediated by rAAV2/9 in the striatum leads to the accumulation of multiple pα-Syn-immunoreactive structures in striatal cells, most likely in medium spiny projection neurons because we observe a diffuse staining of pα-Syn in the terminals in the projection fields (the globus pallidus and substantia nigra reticulata) at 12 weeks after rAAV injection (**Figure 3**). According to previous studies (Koprich et al., 2010; Ip et al., 2017), the pα-Syn-immunopositive structures are small, with a rounded appearance. Although several transgenic mice lines expressing E46K α-Syn have been generated (Emmer et al., 2011; Nuber et al., 2018), it is the first time that E46K human α-Syn form is overexpressed by viral vectors in mice. In the cell body, α-Syn aggregates are located in the nucleus while in the projection fields are in the axon terminals. We find that pα-Syn expression is maintained within the striatal medium spiny neurons, travelling from the cell body to the terminals, with an anterograde transport, but we do not observe a transsynaptic transmission between neurons (**Figure 4**). These observations suggest that rAAV-α-Syn expression does not constitute a propagation model of α-Syn as it is observed with the PFFs model, so further investigations are needed to understand the pathological mechanism of excessive amount of α-Syn coming from external source and those that are endogenously produced.

In the rAAV-α-Syn model, the presence of pα-Syn inclusions in the nigrostriatal system is concomitant with a significant loss of nigral dopaminergic neurons and the reduction in tyrosine hydroxylase immunoreactivity in the striatum. Overexpression of wild type or A53T human α-Syn induces a progressive loss of dopaminergic neurons in the SN over time (Oliveras-Salvá et al., 2013). At 5 days post-injection, no degeneration was observed. The dopaminergic cell loss in the SN increased from 57% at 4 weeks after injection to 82% at 8 weeks for wild type α-Syn; and from 51% at 4 weeks after injection to 59% at 8 weeks for A53T α-Syn. Similar to the observation for the SN, immunohistochemical staining for TH in the striatum revealed a gradual reduction of TH expression over time (Oliveras-Salvá et al., 2013). The maximum of pα-Syn-positive cells in the SN is reached at 4 weeks post-injection when neurodegeneration begins to be evident (Oliveras-Salvá et al., 2013). However, in absence of pα-Syn aggregates, i.e. when animals are injected with empty viral particles (Koprich et al., 2010; Ip et al., 2017) or in the contralateral injection side (Oliveras-Salvá et al., 2013), neurodegeneration is not observed.

Some studies show that rAAV-α-Syn expression causes the development of motor alterations, such as an increased apomorphine or amphetamine-induced rotation, defects in the stepping test or increased forepaw asymmetry in the cylinder test (Kirik et al., 2002; Decressac et al., 2011; Koprich et al., 2011; Decressac et al., 2012; Gaugler et al., 2012; Gombash et al., 2013; Oliveras-Salvá et al., 2013; Bourdenx et al., 2015; Caudal et al., 2015; Ip et al., 2017). These motor deficits appear several weeks after injection in animals with a significant loss of dopaminergic neurons.

In the rAAV-α-Syn models, the transgene expression is dependent on the serotype, the promoter, the injection site, and the titer of rAAV. The serotype rAAV2 is the most extensively used to date, probably because its production and purification methods are well-established (Van der Perren et al., 2014). However, there are some limitations associated with the rAAV2 serotype. First, rAAV2 efficiently transduces neurons but requires high doses; second, rAAV2 can induce a weak immune response in human hepatocytes (Mingozzi and High, 2013). A novel generation of viral particles has recently been produced;

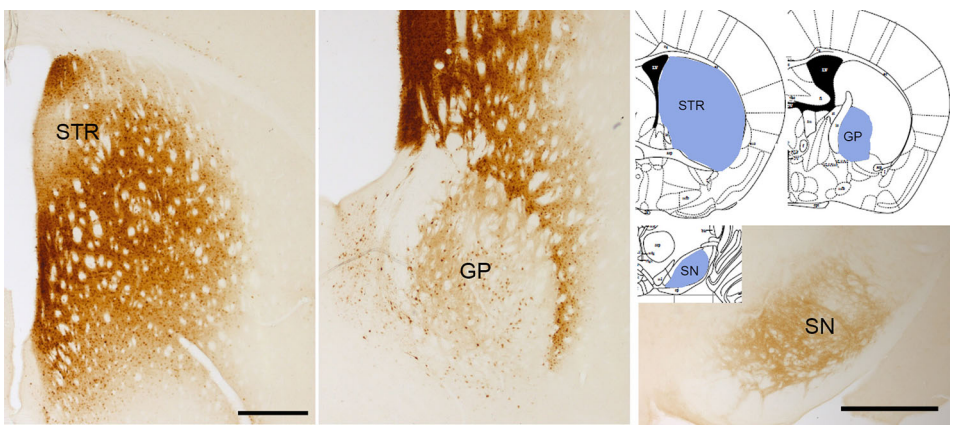


FIGURE 3 | α-Syn aggregation 3 months after rAAV-E46K viral particle injection. Representative photomicrographs illustrating the expression of pα-Syn in the striatum (STR), globus pallidus (GP), and substantia nigra (SN). Scale bar: 500 μm.

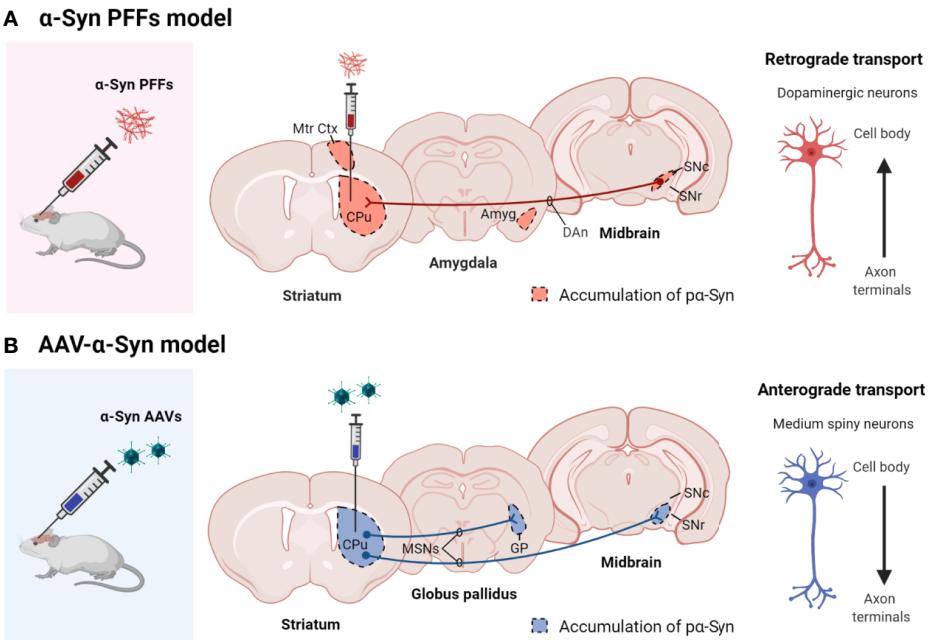


FIGURE 4 | Scheme showing the propagation pattern of α-Syn in PFFs and AAV models. The colored areas represent the brain areas where we find pα-Syn expression after **(A)** α-Syn PFFs inoculation or **(B)** AAV-α-Syn administration. Created with: BioRender.com

these particles show higher efficiency and no immunity associated with the rAAV2 capsid. Indeed, rAAV2/1, rAAV2/5, rAAV2/6, rAAV2/7, and rAAV2/8 exhibit a higher transduction efficiency than the rAAV2 in the nigrostriatal pathway (Taymans et al., 2007; McFarland et al., 2009b; Ulusoy et al., 2012; Oliveras-Salvá et al., 2013).

The efficiency of transgene expression is also determined by the design of the vector construct, and the promoter used to control the transgene expression. The most common promoters used are hybrid cytomegalovirus (CMV), chicken β-actin (CBA), phosphoglycerate kinase (PGK), and human synapsin I (Syn-1). These promoters provide high levels of transgene expression. In addition, some studies use post-transcriptional regulatory elements to improve the transgene expression, such as woodchuck hepatitis virus posttranscriptional regulatory element or polyadenylation sequence (McFarland et al., 2009a; Koprich et al., 2010; Koprich et al., 2011; Gaugler et al., 2012; Gombash et al., 2013; Oliveras-Salvá et al., 2013; Lu et al., 2015).

Determination of viral titer after its production and purification is a critical factor in defining the transduction efficiency. In contrast to α-Syn PFFs models, the levels of α-Syn achieved after transduction with rAAV-α-Syn typically exceed those found in idiopathic PD or even in PD associated with *SNCA* multiplications (Duffy et al., 2018a), despite that the phenotype severity depends on the viral dose injected (Oliveras-Salvá et al., 2013). However, although high levels of α-Syn lead to a more robust phenotype, this does not adequately reflect what happens in parkinsonian patients. Additional studies are required to determine the optimal conditions (serotype, promoter, and viral titer) to improve the ability of this model to recapitulate the human PD. However, although the modification of these factors may help to potentiate the PD phenotype, considering other factors, such as the expression of endogenous α-Syn or the nature of injected α-Syn, are necessary to reproduce the complexity of human disease in rodents.

Several parameters, including the species, the strain, and the age of animals used, influence the development of pathology. Although most studies have used rats (Kirik et al., 2002; Koprich et al., 2010; Decressac et al., 2011; Koprich et al., 2011; Gaugler et al., 2012; Gombash et al., 2013; Caudal et al., 2015; Rocha et al., 2015; Thakur et al., 2017; O'Donovan et al., 2020), the model has also been adapted to mice. This opens a wide range of possibilities due to the greater availability of knockout and transgenic mice, which could be used to assess whether certain genes protect or enhance neurodegeneration. Viral α-Syn overexpression in L444P *GBA1* mice produces a greater loss of dopaminergic neurons in *GBA1* than wild type mice, suggesting that *GBA1* mutations enhance the vulnerability of dopaminergic neurons induced by α-Syn (Migdalska-Richards et al., 2017). In *PINK1* knock out mice, overexpression of α-Syn in the substantia nigra resulted in enhanced dopaminergic degeneration as well as high levels of phosphorylated α-Syn, suggesting that the loss of *PINK1* leads to an increased sensitivity to α-Syn-induced neuropathology (Oliveras-Salvá et al., 2014). The injection of rAAV-mediating human α-Syn overexpression in knock out mice for synapsin III (Syn III) shows that silencing of Syn III could prevent the α-Syn aggregation (Faustini et al., 2018). Moreover, the genetic background of animals is important in response to α-Syn overexpression. Human α-Syn overexpression produced a larger decrease of dopaminergic

neurons in C57BL/6 than in other strains. Likewise, different rat strains (Sprague-Dawley and Wistar) displayed different susceptibility to hα-Syn overexpression (Bourdenx et al., 2015). A recent study shows that deficiency of CX3CR1 receptor in mice ($Cx3cr1^{-/-}$) exacerbates the neurodegeneration and neuroinflammation induced by overexpression of A53T human α-Syn, reflecting the importance of genetic background (Castro-Sánchez et al., 2018). The age of the animals also affects the vulnerability of dopaminergic neurons to α-Syn. Overexpression of human α-Syn into old rats produced a more robust loss of dopaminergic neurons than into young rats (Salganik et al., 2015). Also, α-Syn levels increase with age in monkeys and humans (Chu and Kordower, 2007), so the parkinsonian phenotype might be aggravated in aged mice.

In general, the rAAV-α-Syn are directly injected into the SNc to transduce dopaminergic neurons of the nigrostriatal system. Taking into consideration the special characteristics of dopaminergic neurons of the substantia nigra, that are particularly vulnerable to stress (Bolam and Pissadaki, 2012), it is important to include appropriate controls to specifically determine the neuronal damage and dysfunction caused by α-Syn overexpression. It is also crucial to analyze whether the control proteins (e.g., GFP) or empty vectors are toxic to the dopaminergic neurons (Albert et al., 2017). Although the immune response produced by rAAV in the brain is minimal, the extent of the defense response after rAAV injection must be evaluated. Some studies have demonstrated that the injection of rAAV carrying GFP results in a significant loss of dopaminergic neurons (Klein et al., 2006; Koprich et al., 2011; Landeck et al., 2017). These findings demonstrate the need to use proper controls to ensure the α-Syn specificity on the degeneration of dopaminergic neurons.

One important consideration of these models is that the α-Syn pathology is exclusively restricted to neurons transduced by rAAV to express α-Syn. There is no evidence of the transmission of the pathology to other non-transduced neurons despite the expression of endogenous α-Syn. Another significant drawback is that individual stereotaxic injection of rAAV might cause a wide variety in the expression pattern of α-Syn between animals, which makes it difficult to obtain robust and reproducible results. Morever, the injections into the SNc are technically difficult, especially in the mouse brain due to its small size, so high precision in the stereotaxic procedure is required to minimize the inter-animal variability (Volpicelli-Daley et al., 2016).

Overexpression of α-Syn mediated by rAAV represents a valuable tool to induce progressive degeneration of dopaminergic neurons, accompanied by the development of α-Syn inclusions in these neurons. Although the progression of pathological changes observed in rAAV-α-Syn models is faster than in PD patients, the time-course of these changes is sufficient to observe the different stages defined in PD patients (pre-symptomatic, early symptomatic, and advantage stage), which facilitates the identification of new therapeutic targets (Decressac et al., 2012). These models are especially useful to study how α-Syn accumulation and aggregation contributes to neuronal degeneration and its consequences, such as motor or cognitive impairment. The use of these models has also been extended to develop and evaluate potential therapies aimed at reducing the aggregation of α-Syn and prevent against neurodegeneration induced by α-Syn (Decressac et al., 2013; Xilouri et al., 2013b; Rocha et al., 2015).

CONCLUDING REMARKS

More than two decades ago, α-Syn was identified as the main component of Lewy bodies. Since then, this protein has become established as a possible diagnostic biomarker in PD and therapeutic target. Also, numerous animal models have used this protein in attempts to reproduce PD. Each animal model offers specific aspects of the pathology of human PD, although none of them reproduce all the defining pathological and clinical features of the disease. The lack of comparable phenotypes between rodents overexpressing α-Syn or rodents injected with toxic α-Syn species reflects the difficulty of reproducing PD in animal models. Therefore, a thorough knowledge of the key features of these models is essential to choose the model that best suits the scientific questions that we want to solve. Here, we show that α-Syn PFFs models are suitable for studying the prion-like behavior of α-Syn and its propagation through the brain. While viral α-Syn overexpression models are especially useful to determine the mechanisms of α-Syn-induced toxicity but do not allow the study of their prion-like behavior. Despite these differences, both models are valuable tools for identifying novel therapeutic targets and the design and evaluation of potential therapies aimed at reducing the aggregation of α-Syn to alter disease progression.

ETHICS STATEMENT

All experimental procedures were approved by Cajal Institute´s Bioethics Committee in accordance with the guidelines of the European Union Council Directive (DC86/609/CEE).

AUTHOR CONTRIBUTIONS

RM conceptualized, arranged the review, and provided funding. MG-B generated the first draft, performed the experiments, and provided the figures. NG performed the experiments and supervised and approved the figures. PG-S contributed with the experiments and provided critical feedback. AM synthetized the α-Syn PFFs and provided mice injected with PFFs, and MD provided antibodies and tools for the experiments.

FUNDING

This work was supported by grants from the Spanish Ministries of Science and Innovation (SAF2016-78207-R) and of Health,

Consumption and Social Welfare, Instituto de Salud Carlos III (ISCIII), Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas [CIBERNED] (CB06/05/00559 and PNSD2016I033) and from Ramón Areces Foundation (172275) and from Europan Union´s Horizon 2020, research and innovation program, AND-PD project, grant agreement n° 848002 to RM and from the ERA-Net Euronanomed II JTC2015-DiaSyn project through the Spanish Ministries of Innovation, Science and Universities (PCIN-2015-98) to RM and the Walloon Region (SPW, DG06, Belgium) to AM and MD (project N° n°1510352). MG-B was supported by a fellowship JAE-Intro 2019 from Consejo Superior de Investigaciones Científicas. The authors acknowledge support of the publication fee by the CSIC Open Access Publication Support Initiative through its Unit of Information Resources for Research (URICI).

REFERENCES

Alam, P., Bousset, L., Melki, R., and Otzen, D. E. (2019). α-synuclein oligomers and fibrils: a spectrum of species, a spectrum of toxicities. *J. Neurochem*. 150 (5), 522–534. doi: 10.1111/jnc.14808

Albert, K., Voutilainen, M. H., Domanskyi, A., and Airavaara, M. (2017). AAV vector-mediated gene delivery to substantia nigra dopamine neurons: Implications for gene therapy and disease models. *Genes* 8 (2), 63. doi: 10.3390/genes8020063

Bengoa-Vergniory, N., Roberts, R. F., Wade-Martins, R., and Alegre-Abarrategui, J. (2017). Alpha-synuclein oligomers: a new hope. *Acta Neuropathol*. 134 (6), 819–838. doi: 10.1007/s00401-017-1755-1

Bolam, J. P., and Pissadaki, E. K. (2012). Living on the edge with too many mouths to feed: Why dopamine neurons die. *Mov. Disord*. 27 (12), 1478–1483. doi: 10.1002/mds.25135

Bourdenx, M., Dovero, S., Engeln, M., Bido, S., Bastide, M. F., Dutheil, N., et al. (2015). Lack of additive role of ageing in nigrostriatal neurodegeneration triggered by α-synuclein overexpression. *Acta Neuropathol. Commun*. 3, 46. doi: 10.1186/s40478-015-0222-2

Braak, H., Del Tredici, K., Rüb, U., De Vos, R. A. I., Jansen Steur, E. N. H., and Braak, E. (2003). Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiol. Aging* 24 (2), 197–211. doi: 10.1016/S0197-4580(02)00065-9

Braak, H., Ghebremedhin, E., Rüb, U., Bratzke, H., and Del Tredici, K. (2004). Stages in the development of Parkinson's disease-related pathology. *Cell Tissue Res*. 18 (1), 121–134..doi: 10.1007/s00441-004-0956-9

Burré, J., Sharma, M., Tsetsenis, T., Buchman, V., Etherton, M. R., and Südhof, T. C. (2010). α-Synuclein promotes SNARE-complex assembly in vivo and in vitro. *Science*. 329 (5999), 1663–1667. doi: 10.1126/science.1195227

Candelise, N., Schmitz, M., Llorens, F., Villar-Piqué, A., Cramm, M., Thom, T., et al. (2019). Seeding variability of different alpha synuclein strains in synucleinopathies. *Ann. Neurol*. 85, 691–703. doi: 10.1002/ana.25446

Castro-Sánchez, S., García-Yagüe, Á.J., López-Royo, T., Casarejos, M., Lanciego, J. L., and Lastres-Becker, I. (2018). Cx3cr1-deficiency exacerbates alpha-synuclein-A53T induced neuroinflammation and neurodegeneration in a mouse model of Parkinson's disease. *GLIA*. 66 (8), 1752–1762. doi: 10.1002/glia.23338

Caudal, D., Alvarsson, A., Björklund, A., and Svenningsson, P. (2015). Depressive-like phenotype induced by AAV-mediated overexpression of human α-synuclein in midbrain dopaminergic neurons. *Exp. Neurol*. 273, 243–252. doi: 10.1016/j.expneurol.2015.09.002

Challis, C., Hori, A., Sampson, T. R., Yoo, B. B., Challis, R. C., Hamilton, A. M., et al. (2020). Gut-seeded α-synuclein fibrils promote gut dysfunction and brain pathology specifically in aged mice. *Nat. Neurosci*. 23 (3), 327–336. doi: 10.1038/s41593-020-0589-7

Chau, K. Y., Ching, H. L., Schapira, A. H. V., and Cooper, J. M. (2009). Relationship between alpha synuclein phosphorylation, proteasomal inhibition and cell death: Relevance to Parkinson's disease pathogenesis. *J. Neurochem*. 110 (3), 1005–1013. doi: 10.1111/j.1471-4159.2009.06191.x

Chu, Y., and Kordower, J. H. (2007). Age-associated increases of α-synuclein in monkeys and humans are associated with nigrostriatal dopamine depletion: Is this the target for Parkinson's disease? *Neurobiol. Dis*. 25 (1), 134–149. doi: 10.1016/j.nbd.2006.08.021

Chu, Y., Muller, S., Tavares, A., Barret, O., Alagille, D., Seibyl, J., et al. (2019). Intrastriatal alpha-synuclein fibrils in monkeys: spreading, imaging and neuropathological changes. *Brain* 142 (11), 3565–3579. doi: 10.1093/brain/awz296

Chung, H. K., Ho, H.-A., Pérez-Acuña, D., and Lee, S.-J. (2019). Modeling α-Synuclein Propagation with Preformed Fibril Injections. *J. Mov. Disord*. 12 (3), 139–151. doi: 10.14802/jmd.19046

Cooper, O., Astradsson, A., Hallett, P., Robertson, H., Mendez, I., and Isacson, O. (2009). Lack of functional relevance of isolated cell damage in transplants of Parkinson's disease patients. *J. Neurol*. 256 (Suppl 3), 310–316. doi: 10.1007/s00415-009-5242-z

Costa, C., Sgobio, C., Siliquini, S., Tozzi, A., Tantucci, M., Ghiglieri, V., et al. (2012). Mechanisms underlying the impairment of hippocampal long-term potentiation and memory in experimental Parkinson's disease. *Brain*. 135 (6), 1884–1899. doi: 10.1093/brain/aws101

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

Decressac, M., Ulusoy, A., Mattsson, B., Georgievska, B., Romero-Ramos, M., Kirik, D., et al. (2011). GDNF fails to exert neuroprotection in a rat α-synuclein model of Parkinson's disease. *Brain* 134 (8), 2302–2311. doi: 10.1093/brain/awr149

Decressac, M., Mattsson, B., Lundblad, M., Weikop, P., and Björklund, A. (2012). Progressive neurodegenerative and behavioural changes induced by AAV-mediated overexpression of α-synuclein in midbrain dopamine neurons. *Neurobiol. Dis*. 45 (3), 939–953. doi: 10.1016/j.nbd.2011.12.013

Decressac, M., Mattsson, B., Weikop, P., Lundblad, M., Jakobsson, J., and Björklund, A. (2013). TFEB-mediated autophagy rescues midbrain dopamine neurons from α-synuclein toxicity. *Proc. Natl. Acad. Sci. U. S. A*. 110(19, E1817–E1826. doi: 10.1073/pnas.1305623110

Deleersnijder, A., Gerard, M., Debyser, Z., and Baekelandt, V. (2013). The remarkable conformational plasticity of alpha-synuclein: Blessing or curse? *Trends Mol. Med*. 19 (6), 368–377. doi: 10.1016/j.molmed.2013.04.002

Desplats, P., Lee, H. J., Bae, E. J., Patrick, C., Rockenstein, E., Crews, L., et al. (2009). Inclusion formation and neuronal cell death through neuron-to-neuron transmission of α-synuclein. *Proc. Natl. Acad. Sci. U. States A*. 106 (31), 13010–13015. doi: 10.1073/pnas.0903691106

Diógenes, M. J., Dias, R. B., Rombo, D. M., Vicente Miranda, H., Maiolino, F., Guerreiro, P., et al. (2012). Extracellular alpha-synuclein oligomers modulate synaptic transmission and impair LTP via NMDA-receptor activation. *J. Neurosci*. 32 (34), 11750–11762.. doi: 10.1523/JNEUROSCI.0234-12.2012

Do, J., McKinney, C., Sharma, P., and Sidransky, E. (2019). Glucocerebrosidase and its relevance to Parkinson disease. *Mol. Neurodegener*. 14 (1), 36.. doi: 10.1186/s13024-019-0336-2

Duffy, M. F., Collier, T. J., Patterson, J. R., Kemp, C. J., Fischer, D. L., Stoll, A. C., et al. (2018a). Quality over quantity: Advantages of using alpha-synuclein preformed fibril triggered synucleinopathy to model idiopathic Parkinson's disease. *Front. Neurosci*. 12, 621. doi: 10.3389/fnins.2018.00621

Duffy, M. F., Collier, T. J., Patterson, J. R., Kemp, C. J., Luk, K. C., Tansey, M. G., et al. (2018b). Lewy body-like alpha-synuclein inclusions trigger reactive microgliosis prior to nigral degeneration. *J. Neuroinflammation*. 15 (1), 129. doi: 10.1186/s12974-018-1171-z

Durante, V., de Iure, A., Loffredo, V., Vaikath, N., De Risi, M., Paciotti, S., et al. (2019). Alpha-synuclein targets GluN2A NMDA receptor subunit causing striatal synaptic dysfunction and visuospatial memory alteration. *Brain*. 142 (5), 1365–1385. doi: 10.1093/brain/awz065

Earls, R. H., Menees, K. B., Chung, J., Barber, J., Gutekunst, C. A., Hazim, M. G., et al. (2019). Intrastriatal injection of preformed alpha-synuclein fibrils alters central and peripheral immune cell profiles in non-transgenic mice. *J. Neuroinflammation*. 16 (1), 250. doi: 10.1186/s12974-019-1636-8

Earls, R. H., Menees, K. B., Chung, J., Gutekunst, C. A., Lee, H. J., Hazim, M. G., et al. (2020). NK cells clear α-synuclein and the depletion of NK cells exacerbates synuclein pathology in a mouse model of α-synucleinopathy. *Proc. Natl. Acad. Sci. U. S. A.* 117 (3), 1762–1771. doi: 10.1073/pnas.1909110117

El-Agnaf, O. M. A., Salem, S. A., Paleologou, K. E., Cooper, L. J., Fullwood, N. J., Gibson, M. J., et al. (2003). α-Synuclein implicated in Parkinson's disease is present in extracellular biological fluids, including human plasma. *FASEB J.* 17 (13), 1945–1947. doi: 10.1096/fj.03-0098fje

Emmer, K. L., Waxman, E. A., Covy, J. P., and Giasson, B. I. (2011). E46K human α-synuclein transgenic mice develop lewy-like and tau pathology associated with age-dependent, detrimental motor impairment. *J. Biol. Chem.* 286 (40), 35104–35118. doi: 10.1074/jbc.M111.247965

Fares, M. B., Maco, B., Oueslati, A., Rockenstein, E., Ninkina, N., Buchman, V. L., et al. (2016). Induction of de novo a-synuclein fibrillization in a neuronal model for Parkinson's disease. *Proc. Natl. Acad. Sci. U. S. A.* 113 (7), E912–E921. doi: 10.1073/pnas.1512876113

Faustini, G., Longhena, F., Varanita, T., Bubacco, L., Pizzi, M., Missale, C., et al. (2018). Synapsin III deficiency hampers α-synuclein aggregation, striatal synaptic damage and nigral cell loss in an AAV-based mouse model of Parkinson's disease. *Acta Neuropathol.* 136 (4), 621–639. doi: 10.1007/s00401-018-1892-1

Gaig, C., Martí, M. J., Ezquerra, M., Cardozo, A., Rey, M. J., and Tolosa, E. (2009). G2019S LRRK2 mutation causing Parkinson's disease without Lewy bodies. *BMJ Case Rep.* 2009, bcr08.2008.0632 doi: 10.1136/bcr.08.2008.0632

Gan-Or, Z., Dion, P. A., and Rouleau, G. A. (2015). Genetic perspective on the role of the autophagy-lysosome pathway in Parkinson disease. *Autophagy*. 11 (9), 1443–1457. doi: 10.1080/15548627.2015.1067364

García-Sanz, P., Orgaz, L., Bueno-Gil, G., Espadas, I., Rodríguez-Traver, E., Kulisevsky, J., et al. (2017). N370S-GBA1 mutation causes lysosomal cholesterol accumulation in Parkinson's disease. *Mov. Disord.* 32 (10), 1409–1422. doi: 10.1002/mds.27119

García-Sanz, P., Orgaz, L., Fuentes, J. M., Vicario, C., and Moratalla, R. (2018). Cholesterol and multilamellar bodies: Lysosomal dysfunction in GBA-Parkinson disease. *Autophagy* 14 (4), 717–718. doi: 10.1080/15548627.2018.1427396

Gaugler, M. N., Genc, O., Bobela, W., Mohanna, S., Ardah, M. T., El-Agnaf, O. M., et al. (2012). Nigrostriatal overabundance of α-synuclein leads to decreased vesicle density and deficits in dopamine release that correlate with reduced motor activity. *Acta Neuropathol.* 123 (5), 653–669. doi: 10.1007/s00401-012-0963-y

Ghosh, D., Mehra, S., Sahay, S., Singh, P. K., and Maji, S. K. (2017). α-synuclein aggregation and its modulation. *Int. J. Biol. Macromol.* 100, 37–54. doi: 10.1016/j.ijbiomac.2016.10.021

Goedert, M., Jakes, R., and Spillantini, M. G. (2017). The Synucleinopathies: Twenty Years on. *J. Parkinson's Dis.* 7 (s1), S51–S69. doi: 10.3233/JPD-179005

Gombash, S. E., Manfredsson, F. P., Kemp, C. J., Kuhn, N. C., Fleming, S. M., Egan, A. E., et al. (2013). Morphological and behavioral impact of AAV2/5-mediated overexpression of human wildtype alpha-synuclein in the rat nigrostriatal system. *PloS One* 8 (11), e81426. doi: 10.1371/journal.pone.0081426

Greenbaum, E. A., Graves, C. L., Mishizen-Eberz, A. J., Lupoli, M. A., Lynch, D. R., Englander, S. W., et al. (2005). The E46K mutation in α-synuclein increases amyloid fibril formation. *J. Biol. Chem.* 280 (9), 7800–7807. doi: 10.1074/jbc.M411638200

Hallett, P. J., Cooper, O., Sadi, D., Robertson, H., Mendez, I., and Isacson, O. (2014). Long-Term Health of Dopaminergic Neuron Transplants in Parkinson's Disease Patients. *Cell Rep.* 7 (6), 1755–1761. doi: 10.1016/j.celrep.2014.05.027

Harms, A. S., Delic, V., Thome, A. D., Bryant, N., Liu, Z., Chandra, S., et al. (2017). α-Synuclein fibrils recruit peripheral immune cells in the rat brain prior to neurodegeneration. *Acta Neuropathol. Commun.* 5 (1), 85. doi: 10.1186/s40478-017-0494-9

Ip, C. W., Klaus, L. C., Karikari, A. A., Visanji, N. P., Brotchie, J. M., Lang, A. E., et al. (2017). AAV1/2-induced overexpression of A53T-α-synuclein in the substantia nigra results in degeneration of the nigrostriatal system with Lewy-like pathology and motor impairment: a new mouse model for Parkinson's disease. *Acta Neuropathol. Commun.* 5 (1), 11. doi: 10.1186/s40478-017-0416-x

Janezic, S., Threlfell, S., Dodson, P. D., Dowie, M. J., Taylor, T. N., Potgieter, D., et al. (2013). Deficits in dopaminergic transmission precede neuron loss and dysfunction in a new Parkinson model. *Proc. Natl. Acad. Sci. U. S. A.* 110 (42), E4016–E4025. doi: 10.1073/pnas.1309143110

Jellinger, K. A. (2017). Neuropathology of Nonmotor Symptoms of Parkinson's Disease. *Int. Rev. Neurobiol.* 133, 13–62. doi: 10.1016/bs.irn.2017.05.005

Johansen, K. K., Torp, S. H., Farrer, M. J., Gustavsson, E. K., and Aasly, J. O. (2018). A Case of Parkinson's Disease with No Lewy Body Pathology due to a Homozygous Exon Deletion in Parkin. *Case Rep. Neurol. Med.* 2018, 6838965. doi: 10.1155/2018/6838965

Kalia, L. V., and Lang, A. E. (2015). Parkinson's disease. *Lancet* 386 (9996), 896–912.. doi: 10.1016/S0140-6736(14)61393-3

Karampetsou, M., Ardah, M. T., Semitekolou, M., Polissidis, A., Samiotaki, M., Kalomoiri, M., et al. (2017). Phosphorylated exogenous alpha-synuclein fibrils exacerbate pathology and induce neuronal dysfunction in mice. *Sci. Rep.* 7 (1), 16533. doi: 10.1038/s41598-017-15813-8

Killinger, B. A., and Kordower, J. H. (2019). Spreading of alpha-synuclein – relevant or epiphenomenon? *J. Neurochem.* 9 (Suppl 2), S345–S358. doi: 10.1111/jnc.14779

Kim, S., Kwon, S.-H., Kam, T.-I., Panicker, N., Karuppagounder, S. S., Lee, S., et al. (2019). Transneuronal Propagation of Pathologic α-Synuclein from the Gut to the Brain Models Parkinson's Disease. *Neuron*. 103 (4), 627–641. doi: 10.1016/j.neuron.2019.05.035

Kirik, D., Rosenblad, C., Burger, C., Lundberg, C., Johansen, T. E., Muzyczka, N., et al. (2002). Parkinson-Like Neurodegeneration Induced by Targeted Overexpression of α-Synuclein in the Nigrostriatal System. *J. Neurosci.* 22 (7), 2780–2791. doi: 10.1523/jneurosci.22-07-02780.2002

Klein, A. D., and Mazzulli, J. R. (2018). Is Parkinson's disease a lysosomal disorder? *Brain*. 141 (8), 2255–2262. doi: 10.1093/brain/awy147

Klein, C., and Westenberger, A. (2012). Genetics of Parkinson's disease. *Cold Spring Harbor Perspect. Med.* 2 (1), a008888. doi: 10.1101/cshperspect.a008888

Klein, R. L., Dayton, R. D., Leidenheimer, N. J., Jansen, K., Golde, T. E., and Zweig, R. M. (2006). Efficient neuronal gene transfer with AAV8 leads to neurotoxic levels of tau or green fluorescent proteins. *Mol. Ther.* 13 (3), 517–527. doi: 10.1016/j.ymthe.2005.10.008

Koprich, J. B., Johnston, T. H., Reyes, M. G., Sun, X., and Brotchie, J. M. (2010). Expression of human A53T alpha-synuclein in the rat substantia nigra using a novel AAV1/2 vector produces a rapidly evolving pathology with protein aggregation, dystrophic neurite architecture and nigrostriatal degeneration with potential to model the pat. *Mol. Neurodegener.* 5, 43. doi: 10.1186/1750-1326-5-43

Koprich, J. B., Johnston, T. H., Huot, P., Reyes, M. G., Espinosa, M., and Brotchie, J. M. (2011). Progressive neurodegeneration or endogenous compensation in an animal model of Parkinson's disease produced by decreasing doses of alpha-synuclein. *PloS One* 6 (3), e17698. doi: 10.1371/journal.pone.0017698

Kordower, J. H., Chu, Y., Hauser, R. A., Freeman, T. B., and Olanow, C. W. (2008). Lewy body-like pathology in long-term embryonic nigral transplants in Parkinson's disease. *Nat. Med.* 14 (5), 504–506. doi: 10.1038/nm1747

Landeck, N., Buck, K., and Kirik, D. (2017). Toxic effects of human and rodent variants of alpha-synuclein in vivo. *Eur. J. Neurosci.* 45 (4), 536–547. doi: 10.1111/ejn.13493

Lashuel, H. A., Overk, C. R., Oueslati, A., and Masliah, E. (2013). The many faces of α-synuclein: From structure and toxicity to therapeutic target. *Nat. Rev. Neurosci.* 14 (1), 38–48. doi: 10.1038/nrn3406

Li, J., Uversky, V. N., and Fink, A. L. (2001). Effect of familial Parkinson's disease point mutations A30P and A53T on the structural properties, aggregation, and fibrillation of human α-synuclein. *Biochemistry* 40 (38), 11604–11613. doi: 10.1021/bi010616g

Lu, J., Sun, F., Ma, H., Qing, H., and Deng, Y. (2015). Comparison between α-synuclein wild-type and A53T mutation in a progressive Parkinson's disease model. *Biochem. Biophys. Res. Commun.* 464 (4), 988–993. doi: 10.1016/j.bbrc.2015.07.007

Luk, K. C., Song, C., O'Brien, P., Stieber, A., Branch, J. R., Brunden, K. R., et al. (2009). Exogenous α-synuclein fibrils seed the formation of Lewy body-like intracellular inclusions in cultured cells. *Proc. Natl. Acad. Sci. U. S. A.* 106 (47), 20051–20056. doi: 10.1073/pnas.0908005106

Luk, K. C., Kehm, V., Carroll, J., Zhang, B., O'Brien, P., Trojanowski, J. Q., et al. (2012a). Pathological α-synuclein transmission initiates Parkinson-like neurodegeneration in nontransgenic mice. *Science* 338 (6109), 949–953. doi: 10.1126/science.1227157

Luk, K. C., Kehm, V. M., Zhang, B., O'Brien, P., Trojanowski, J. Q., and Lee, V. M. Y. (2012b). Intracerebral inoculation of pathological α-synuclein initiates a rapidly progressive neurodegenerative α-synucleinopathy in mice. *J. Exp. Med.* 209 (5), 975–986. doi: 10.1084/jem.20112457

Machiya, Y., Hara, S., Arawaka, S., Fukushima, S., Sato, H., Sakamoto, M., et al. (2010). Phosphorylated α-Synuclein at Ser-129 is targeted to the proteasome pathway in a ubiquitin-independent manner. *J. Biol. Chem.* 285 (52), 40732–40744. doi: 10.1074/jbc.M110.141952

Martin, Z. S., Neugebauer, V., Dineley, K. T., Kayed, R., Zhang, W., Reese, L. C., et al. (2012). α-Synuclein oligomers oppose long-term potentiation and impair memory through a calcineurin-dependent mechanism: Relevance to human synucleopathic diseases. *J. Neurochem.* 120 (3), 440–452. doi: 10.1111/j.1471-4159.2011.07576.x

Martinez-Vicente, M., Talloczy, Z., Kaushik, S., Massey, A. C., Mazzulli, J., Mosharov, E. V., et al. (2008). Dopamine-modified α-synuclein blocks chaperone-mediated autophagy. *J. Clin. Invest.* 118 (2), 777–788. doi: 10.1172/JCI32806

Masuda-Suzukake, M., Nonaka, T., Hosokawa, M., Oikawa, T., Arai, T., Akiyama, H., et al. (2013). Prion-like spreading of pathological α-synuclein in brain. *Brain* 136 (Pt 4), 1128–1138. doi: 10.1093/brain/awt037

Masuda-Suzukake, M., Nonaka, T., Hosokawa, M., Kubo, M., Shimozawa, A., Akiyama, H., et al. (2014). Pathological alpha-synuclein propagates through neural networks. *Acta Neuropathol. Commun.* 2, 88. doi: 10.1186/s40478-014-0088-8

Mazzulli, J. R., Xu, Y. H., Sun, Y., Knight, A. L., McLean, P. J., Caldwell, G. A., et al. (2011). Gaucher disease glucocerebrosidase and α-synuclein form a bidirectional pathogenic loop in synucleinopathies. *Cell* 146 (1), 37–52. doi: 10.1016/j.cell.2011.06.001

McFarland, N. R., Fan, Z., Xu, K., Schwarzschild, M. A., Feany, M. B., Hyman, B. T., et al. (2009a). α-Synuclein S129 phosphorylation mutants do not alter nigrostriatal toxicity in a rat model of parkinson disease. *J. Neuropathol. Exp. Neurol.* 68 (5), 515–524. doi: 10.1097/NEN.0b013e3181a24b53

McFarland, N. R., Lee, J. S., Hyman, B. T., and McLean, P. J. (2009b). Comparison of transduction efficiency of recombinant AAV serotypes 1, 2, 5, and 8 in the rat nigrostriatal system. *J. Neurochem.* 109 (3), 838–845. doi: 10.1111/j.1471-4159.2009.06010.x

Mehra, S., Sahay, S., and Maji, S. K. (2019). α-Synuclein misfolding and aggregation: Implications in Parkinson's disease pathogenesis. *Biochim. Biophys. Acta - Proteins Proteomics.* 1867 (10), 890–908. doi: 10.1016/j.bbapap.2019.03.001

Melki, R. (2015). Role of different alpha-synuclein strains in synucleinopathies, similarities with other neurodegenerative diseases. *J. Parkinson's Dis.* 5 (2), 217–227. doi: 10.3233/JPD-150543

Migdalska-Richards, A., Wegrzynowicz, M., Rusconi, R., Deangeli, G., Di Monte, D. A., Spillantini, M. G., et al. (2017). The L444P Gba1 mutation enhances alpha-synuclein induced loss of nigral dopaminergic neurons in mice. *Brain* 140 (10), 2706–2721. doi: 10.1093/brain/awx221

Milanese, C., Cerri, S., Ulusoy, A., Gornati, S. V., Plat, A., Gabriels, S., et al. (2018). Activation of the DNA damage response in vivo in synucleinopathy models of Parkinson's disease. *Cell Death Dis.* 9, 818. doi: 10.1038/s41419-018-0848-7

Mingozzi, F., and High, K. A. (2013). Immune responses to AAV vectors: Overcoming barriers to successful gene therapy. *Blood* 122 (1), 23–36. doi: 10.1182/blood-2013-01-306647

Nalls, M. A., Pankratz, N., Lill, C. M., Do, C. B., Hernandez, D. G., Saad, M., et al. (2014). Large-scale meta-analysis of genome-wide association data identifies six new risk loci for Parkinson's disease. *Nat. Genet.* 46 (9), 989–993. doi: 10.1038/ng.3043

Nuber, S., Rajsombath, M., Minakaki, G., Winkler, J., Müller, C. P., Ericsson, M., et al. (2018). Abrogating Native α-Synuclein Tetramers in Mice Causes a L-DOPA-Responsive Motor Syndrome Closely Resembling Parkinson's Disease. *Neuron* 100 (1), 75–90.e5. doi: 10.1016/j.neuron.2018.09.014

O'Donovan, S. M., Crowley, E. K., Brown, J. R. M., O'Sullivan, O., O'Leary, O. F., Timmons, S., et al. (2020). Nigral overexpression of α-synuclein in a rat Parkinson's disease model indicates alterations in the enteric nervous system and the gut microbiome. *Neurogastroenterol. Motil.* 32 (1), e13726. doi: 10.1111/nmo.13726

Okuzumi, A., Kurosawa, M., Hatano, T., Takanashi, M., Nojiri, S., Fukuhara, T., et al. (2018). Rapid dissemination of alpha-synuclein seeds through neural circuits in an in-vivo prion-like seeding experiment. *Acta Neuropathol. Commun.* 6 (1), 96. doi: 10.1186/s40478-018-0587-0

Olanow, C. W., Savolainen, M., Chu, Y., Halliday, G. M., and Kordower, J. H. (2019). Temporal evolution of microglia and α-synuclein accumulation following foetal grafting in Parkinson's disease. *Brain: A J. Neurol.* 142 (6), 1690–1700. doi: 10.1093/brain/awz104

Oliveras-Salvá, M., Van Der Perren, A., Casadei, N., Stroobants, S., Nuber, S., D'Hooge, R., et al. (2013). RAAV2/7 vector-mediated overexpression of alpha-synuclein in mouse substantia nigra induces protein aggregation and progressive dose-dependent neurodegeneration. *Mol. Neurodegener.* 8, 44. doi: 10.1186/1750-1326-8-44

Oliveras-Salvá, M., Macchi, F., Coessens, V., Deleersnijder, A., Gérard, M., Van der Perren, A., et al. (2014). Alpha-synuclein-induced neurodegeneration is exacerbated in PINK1 knockout mice. *Neurobiol. Aging* 35 (11), 2625–2636. doi: 10.1016/j.neurobiolaging.2014.04.032

Oueslati, A., Schneider, B. L., Aebischer, P., and Lashuel, H. A. (2013). Polo-like kinase 2 regulates selective autophagic synuclein clearance and suppresses its toxicity in vivo. *Proc. Natl. Acad. Sci. U. S. A.* 110 (41), E3945–E3954. doi: 10.1073/pnas.1309991110

Oueslati, A. (2016). Implication of Alpha-Synuclein Phosphorylation at S129 in Synucleinopathies: What Have We Learned in the Last Decade? *J. Parkinson's Dis.* 6 (1), 39–51. doi: 10.3233/JPD-160779

Parkkinen, L., Pirttilä, T., Tervahauta, M., and Alafuzoff, I. (2005). Widespread and abundant α-synuclein pathology in a neurologically unimpaired subject. *Neuropathology* 25 (4), 304–314. doi: 10.1111/j.1440-1789.2005.00644.x

Patterson, J. R., Polinski, N. K., Duffy, M. F., Kemp, C. J., Luk, K. C., Volpicelli-Daley, L. A., et al. (2019). Generation of Alpha-Synuclein Preformed Fibrils from Monomers and Use In Vivo. *J. Vis. Exp.* 148. doi: 10.3791/59758

Paumier, K. L., Luk, K. C., Manfredsson, F. P., Kanaan, N. M., Lipton, J. W., Collier, T. J., et al. (2015). Intrastriatal injection of pre-formed mouse α-synuclein fibrils into rats triggers α-synuclein pathology and bilateral nigrostriatal degeneration. *Neurobiol. Dis.* 82, 185–199. doi: 10.1016/j.nbd.2015.06.003

Peelaerts, W., Bousset, L., Van Der Perren, A., Moskalyuk, A., Pulizzi, R., Giugliano, M., et al. (2015). α-Synuclein strains cause distinct synucleinopathies after local and systemic administration. *Nature* 522 (7556), 340–344. doi: 10.1038/nature14547

Pignataro, D., Sucunza, D., Rico, A. J., Dopeso-Reyes, I. G., Roda, E., Rodríguez-Perez, A. I., et al. (2018). Gene therapy approaches in the non-human primate model of Parkinson's disease. *J. Neural Transm.* 125 (3), 575–589. doi: 10.1007/s00702-017-1681-3

Polinski, N. K., Volpicelli-Daley, L. A., Sortwell, C. E., Luk, K. C., Cremades, N., Gottler, L. M., et al. (2018). Best practices for generating and using alpha-synuclein pre-formed fibrils to model Parkinson's disease in rodents. *J. Parkinson's Dis.* 8 (2), 303–322. doi: 10.3233/JPD-171248

Polymeropoulos, M. H., Lavedan, C., Leroy, E., Ide, S. E., Dehejia, A., Dutra, A., et al. (1997). Mutation in the α-synuclein gene identified in families with Parkinson's disease. *Science* 276 (5321), 2045–2047. doi: 10.1126/science.276.5321.2045

Prots, I., Grosch, J., Brazdis, R.-M., Simmnacher, K., Veber, V., Havlicek, S., et al. (2018). α-Synuclein oligomers induce early axonal dysfunction in human iPSC-based models of synucleinopathies. *Proc. Natl. Acad. Sci.* 115 (30), 7813–7818. doi: 10.1073/pnas.1713129115

Qamar, M. A., Sauerbier, A., Politis, M., Carr, H., Loehrer, P., and Chaudhuri, K. R. (2017). Presynaptic dopaminergic terminal imaging & non-motor symptoms assessment of Parkinson's disease: Evidence for dopaminergic basis? *Parkinson's Dis.* 3, 5. doi: 10.1038/s41531-016-0006-9

Recasens, A., Dehay, B., Bové, J., Carballo-Carbajal, I., Dovero, S., Pérez-Villalba, A., et al. (2014). Lewy body extracts from Parkinson disease brains trigger α-synuclein pathology and neurodegeneration in mice and monkeys. *Ann. Neurol.* 75 (3), 351–362. doi: 10.1002/ana.24066

Rey, N. L., Steiner, J. A., Maroof, N., Luk, K. C., Madaj, Z., Trojanowski, J. Q., et al. (2016). Widespread transneuronal propagation of α-synucleinopathy triggered in olfactory bulb mimics prodromal Parkinson's disease. *J. Exp. Med.* 213 (9), 1759–1778. doi: 10.1084/jem.20160368

Rey, N. L., George, S., Steiner, J. A., Madaj, Z., Luk, K. C., Trojanowski, J. Q., et al. (2018). Spread of aggregates after olfactory bulb injection of α-synuclein fibrils

is associated with early neuronal loss and is reduced long term. *Acta Neuropathol.* 135 (1), 65–83. doi: 10.1007/s00401-017-1792-9

Reyes, J. F., Olsson, T. T., Lamberts, J. T., Devine, M. J., Kunath, T., and Brundin, P. (2015). A cell culture model for monitoring α-synuclein cell-to-cell transfer. *Neurobiol. Dis.* 77, 266–275. doi: 10.1016/j.nbd.2014.07.003

Rizek, P., Kumar, N., and Jog, M. S. (2016). An update on the diagnosis and treatment of Parkinson disease. *CMAJ*. 188 (16), 1157–1165. doi: 10.1503/cmaj.151179

Roberts, H. L., and Brown, D. R. (2015). Seeking a mechanism for the toxicity of oligomeric α-synuclein. *Biomolecules*. 5(2), 282–305. doi: 10.3390/biom5020282

Rocha, E. M., Smith, G. A., Park, E., Cao, H., Brown, E., Hayes, M. A., et al. (2015). Glucocerebrosidase gene therapy prevents α-synucleinopathy of midbrain dopamine neurons. *Neurobiol. Dis.* 82, 495–503. doi: 10.1016/j.nbd.2015.09.009

Salganik, M., Sergeyev, V. G., Shinde, V., Meyers, C. A., Gorbatyuk, M. S., Lin, J. H., et al. (2015). The loss of glucose-regulated protein 78 (GRP78) during normal aging or from siRNA knockdown augments human alpha-synuclein (α-syn) toxicity to rat nigral neurons. *Neurobiol. Aging* 36 (6), 2213–2223. doi: 10.1016/j.neurobiolaging.2015.02.018

Schapira, A. H. V., Chaudhuri, K. R., and Jenner, P. (2017). Non-motor features of Parkinson disease. *Nat. Rev. Neurosci.* 18 (7), 435–450. doi: 10.1038/nrn.2017.62

Schapira, A. H. V. (2015). Glucocerebrosidase and Parkinson disease: Recent advances. *Mol. Cell. Neurosci.* 66 (Pt A), 37–42. doi: 10.1016/j.mcn.2015.03.013

Schindlbeck, K. A., and Eidelberg, D. (2019). Serotonergic pathology and Braak's staging hypothesis in Parkinson's disease. *Lancet Neurol.* 18 (8), 713–714. doi: 10.1016/S1474-4422(19)30242-X

Shahmoradian, S. H., Lewis, A. J., Genoud, C., Hench, J., Moors, T. E., Navarro, P. P., et al. (2019). Lewy pathology in Parkinson's disease consists of crowded organelles and lipid membranes. *Nat. Neurosci.* 22 (7), 1099–1109. doi: 10.1038/s41593-019-0423-2

Shimozawa, A., Ono, M., Takahara, D., Tarutani, A., Imura, S., Masuda-Suzukake, M., et al. (2017). Propagation of pathological α-synuclein in marmoset brain. *Acta Neuropathol. Commun.* 5 (1), 12. doi: 10.1186/s40478-017-0413-0

Smith, W. W., Margolis, R. L., Li, X., Troncoso, J. C., Lee, M. K., Dawson, V. L., et al. (2005). α-synuclein phosphorylation enhances eosinophilic cytoplasmic inclusion formation in SH-SY5Y cells. *J. Neurosci.* 25 (23), 5544–5552. doi: 10.1523/JNEUROSCI.0482-05.2005

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

Taschenberger, G., Garrido, M., Tereshchenko, Y., Bähr, M., Zweckstetter, M., and Kügler, S. (2012). Aggregation of αsynuclein promotes progressive in vivo neurotoxicity in adult rat dopaminergic neurons. *Acta Neuropathol.* 123 (5), 671–683. doi: 10.1007/s00401-011-0926-8

Taymans, J. M., Vandenberghe, L. H., Van Den Haute, C., Thiry, I., Deroose, C. M., Mortelmans, L., et al. (2007). Comparative analysis of adeno-associated viral vector serotypes 1, 2, 5, 7, and 8 in mouse brain. *Hum. Gene Ther.* 18 (3), 195–206. doi: 10.1089/hum.2006.178

Thakur, P., Breger, L. S., Lundblad, M., Wan, O. W., Mattsson, B., Luk, K. C., et al. (2017). Modeling Parkinson's disease pathology by combination of fibril seeds and α-synuclein overexpression in the rat brain. *Proc. Natl. Acad. Sci. U. S. A.* 114 (39), E8284–E8293. doi: 10.1073/pnas.1710442114

Tofaris, G. K., Garcia Reitböck, P., Humby, T., Lambourne, S. L., O´Connell, M., Ghetti, B., et al. (2006). Pathological Changes in Dopaminergic Nerve Cells of the Substantia Nigra and Olfactory Bulb in Mice Transgenic for Truncated Human α-Synuclein(1-120): Implications for Lewy Body Disorders. *J. Neurosci.* 26 (15), 3942– 3950.. doi: 10.1523/JNEUROSCI.4965-05.2006

Tosatto, L., Horrocks, M. H., Dear, A. J., Knowles, T. P. J., Dalla Serra, M., Cremades, N., et al. (2015). Single-molecule FRET studies on alpha-synuclein oligomerization of Parkinson's disease genetically related mutants. *Sci. Rep.* 5, 16696. doi: 10.1038/srep16696

Uemura, N., Yagi, H., Uemura, M. T., Hatanaka, Y., Yamakado, H., and Takahashi, R. (2018). Inoculation of α-synuclein preformed fibrils into the mouse gastrointestinal tract induces Lewy body-like aggregates in the brainstem via the vagus nerve. *Mol. Neurodegener.* 13 (1), 21. doi: 10.1186/s13024-018-0257-5

Ulusoy, A., Björklund, T., Buck, K., and Kirik, D. (2012). Dysregulated dopamine storage increases the vulnerability to α-synuclein in nigral neurons. *Neurobiol. Dis.* 47 (3), 367–377. doi: 10.1016/j.nbd.2012.05.012

Ulusoy, A., Rusconi, R., Pérez-Revuelta, B. I., Musgrove, R. E., Helwig, M., Winzen-Reichert, B., et al. (2013). Caudo-rostral brain spreading of α-synuclein through vagal connections. *EMBO Mol. Med.* 5 (7), 1119–1127. doi: 10.1002/emmm.201302475

Ulusoy, A., Phillips, R. J., Helwig, M., Klinkenberg, M., Powley, T. L., and Di Monte, D. A. (2017). Brain-to-stomach transfer of α-synuclein via vagal preganglionic projections. *Acta Neuropathol.* 133 (3), 381–393. doi: 10.1007/s00401-016-1661-y

Van der Perren, A., Van den Haute, C., and Baekelandt, V. (2014). Viral Vector-based models of Parkinson's disease. *Curr. Top. Behav. Neurosci.* 22, 271–301. doi: 10.1007/7854_2014_310

Venda, L. L., Cragg, S. J., Buchman, V. L., and Wade-Martins, R. (2010). α-Synuclein and dopamine at the crossroads of Parkinson's disease. *Trends Neurosci.* 33 (12), 559–568. doi: 10.1016/j.tins.2010.09.004

Volpicelli-Daley, L. A., Luk, K. C., Patel, T. P., Tanik, S. A., Riddle, D. M., Stieber, A., et al. (2011). Exogenous α-Synuclein Fibrils Induce Lewy Body Pathology Leading to Synaptic Dysfunction and Neuron Death. *Neuron*. 72 (1), 57–71. doi: 10.1016/j.neuron.2011.08.033

Volpicelli-Daley, L. A., Kirik, D., Stoyka, L. E., Standaert, D. G., and Harms, A. S. (2016). How can rAAV-α-synuclein and the fibril α-synuclein models advance our understanding of Parkinson's disease? *J. Neurochem.* 139 (Suppl 1), 131–155. doi: 10.1111/jnc.13627

Wakabayashi, K., Tanji, K., Odagiri, S., Miki, Y., Mori, F., and Takahashi, H. (2013). The Lewy body in Parkinson's disease and related neurodegenerative disorders. *Mol. Neurobiol.* 47 (2), 495–508. doi: 10.1007/s12035-012-8280-y

Walsh, D. M., and Selkoe, D. J. (2016). A critical appraisal of the pathogenic protein spread hypothesis of neurodegeneration. *Nat. Rev. Neurosci.* 17 (4), 251–260. doi: 10.1038/nrn.2016.13

Xilouri, M., Brekk, O. R., and Stefanis, L. (2013a). α-Synuclein and protein degradation systems: a reciprocal relationship. *Mol. Neurobiol.* 47 (2), 537–551. doi: 10.1007/s12035-012-8341-2

Xilouri, M., Brekk, O. R., Kirik, D., and Stefanis, L. (2013b). LAMP2A as a therapeutic target in Parkinson disease. *Autophagy* 9 (12), 2166–2168. doi: 10.4161/auto.26451

Xu, L., and Pu, J. (2016). Alpha-Synuclein in Parkinson's Disease: From Pathogenetic Dysfunction to Potential Clinical Application. *Parkinson's Dis.* 1720621. doi: 10.1155/2016/1720621

Yamada, K., and Iwatsubo, T. (2018). Extracellular α-synuclein levels are regulated by neuronal activity. *Mol. Neurodegener.* 13 (1), 9. doi: 10.1186/s13024-018-0241-0

Zarranz, J. J., Alegre, J., Gómez-Esteban, J. C., Lezcano, E., Ros, R., Ampuero, I., et al. (2004). The New Mutation, E46K, of α-Synuclein Causes Parkinson and Lewy Body Dementia. *Ann. Neurol.* 55 (2), 164–173. doi: 10.1002/ana.10795

Zhang, G., Xia, Y., Wan, F., Ma, K., Guo, X., Kou, L., et al. (2018). New Perspectives on Roles of Alpha-Synuclein in Parkinson's Disease. *Front. Aging Neurosci.* 10, 370. doi: 10.3389/fnagi.2018.00370

Zhang, J., Li, X., Li, J., and Da, (2019). The Roles of Post-translational Modifications on α-Synuclein in the Pathogenesis of Parkinson's Diseases. *Front. Neurosci.* 13, 381. doi: 10.3389/fnins.2019.00381

Conflict of Interest: AM is working as UCB Biopharma employee.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: info@frontiersin.org | +41 21 510 17 00

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership