Imaging Markers of Progression in Parkinson's Disease

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Abstract:

BACKGROUND: Parkinson’s disease is the second most common neurodegenerative disorder after Alzheimer disease, however to date, there is no approved treatment that stops or slows down disease progression. Over the past decades, neuroimaging studies including molecular and magnetic resonance imaging are trying to provide insights into the mechanisms underlying Parkinson disease.

METHODS AND FINDINGS: Literature review. It is now becoming clear that these imaging modalities can provide biomarkers that can objectively detect brain changes related to Parkinson’s disease and monitor these changes as the disease progresses, and these biomarkers are required to establish a breakthrough in neuroprotective or disease-modifying therapeutics.

CONCLUSIONS: Here, we provide a review of recent observations deriving from positron emission tomography, single positron emission tomography, magnetic resonance imaging studies exploring Parkinson’s disease and other parkinsonian disorders.

Key words: Parkinson’s disease, molecular imaging, magnetic resonance imaging, imaging biomarker.
1. Introduction

To date, it is difficult to predict the progression of Parkinson’s disease (PD) and ensuing treatment complications that may arise. Currently, there is no treatment that stops or slows down disease progression in individuals with PD. It is believed that progress towards neuroprotective or disease-modifying therapeutics has been affected, in part, by the lack of valid, reliable, and clinical-trial ready biomarkers that can objectively detect brain changes related to PD and monitor these changes as the disease progresses\textsuperscript{1,2}.

Modern functional neuroimaging techniques have provided important insights into neural correlates underpinning PD pathophysiology, potential compensatory mechanisms and treatment-related changes from early to advanced stages of the disease. Recent developments in magnetic resonance (MR) as well as molecular imaging including positron emission tomography (PET) and single-photon emission tomography (SPECT) has allowed researchers to gain new insights into the pathophysiology of motor, cognitive and behavioural symptoms in PD and other parkinsonian disorders. In particular, PET and SPECT studies have enabled the investigation of underlying pathological processes such as neurotransmitter dysfunction, changes in blood flow and metabolism, neuroinflammation as well as abnormal protein aggregation. MR imaging techniques including diffusion MRI (dMRI) and functional MRI (fMRI) allow for the exploration of the reorganization of neural pathways and functional disruptions that may be proposed as early biomarkers of PD development and progression. This review will discuss recent findings of molecular and MR imaging studies aiming to better understand symptom development in PD and other parkinsonian disorders as well as attempts to identify biomarkers of disease progression and treatment complications.

2. Molecular Imaging of PD
2.1. The Role of Receptor imaging

2.1.1. Dopamine

The profound dopaminergic dysfunction within the striatum is a critical neurotransmitter change in the development of PD. There is ample evidence showing that this process is widespread and might begin in the lower brainstem and also involving other structures, such as the hypothalamus³.

The level of the membrane dopamine transporter (DAT) seems to be downregulated early in PD⁴, and DAT imaging studies have revealed correlations with the severity of PD motor symptoms⁵-⁷.

Recent investigations⁸ with ¹¹C-dihydrotetrabenazine (DTBZ), a biomarker of dopamine neuron integrity have also assessed the striatal dopamine nerve terminal degeneration found in the associative striatum, which is generally associated with executive processing. Results showed that patients with mild cognitive impairment had severe striatal dopamine depletion in the associative (i.e. cognitive) subdivision. Further, the evaluation of cortical D2 receptor availability (with ¹¹C-FLB 457) in these patients showed also a reduced D2 receptor binding in the bilateral insula compared to cognitively normal patients and controls. Although the prefrontal cortex in general has generally been a critical region in studies involving dopamine and cognition, these findings suggested that striatal dopamine denervation combined with insular D2 receptor loss may underlie mild cognitive impairment in PD, particularly a decline in executive function⁸.

Treatment of the dopaminergic system is of course, not only associated with the amelioration of motor symptoms but also with the development of motor and non-motor complications. Impulse control behaviors (ICBs) represent one of the main non-motor complications of PD and the use of dopamine agonists (DAs) is associated with an
increased risk of their occurrence. It has been recently demonstrated that chronic exposure to treatment with DAs but not levodopa, suppresses the D2R striatal dopamine receptor availability, which may impact output signaling to frontal lobes\textsuperscript{9}. By using a [\textsuperscript{11}C]-raclopride PET challenge, it has been observed that the amount of dopamine release after a reward-related cue exposure is higher in those PD patients experiencing ICBs\textsuperscript{10,11}. In response to reward cues, PD patients with single or multiple ICBs have similar increased ventral striatal dopamine release compared to PD patients without ICBs, but the patients with multiple ICBs are more depressed, and have higher rates of impulsive sensation-seeking compared to participants with single ICBs and without ICBs\textsuperscript{12}. This suggests the implication of both striatal dopamine and possibly non-dopaminergic neurotransmission.

[\textsuperscript{123}I]-FP-CIT is a radioligand which binds reversibly to striatal presynaptic DAT and is used for SPECT imaging. Pavese and colleagues, recently explored the clinical phenotype of PD and the degree of nigrostriatal dysfunction - as measured by \textsuperscript{123}I-FP-CIT SPECT - at different ages of onset in recently diagnosed patients with untreated PD. The authors found that \textsuperscript{123}I-FP-CIT binding in the most affected putamen was similar across the age subgroups. Conversely, binding in the most affected caudate, the least affected putamen, and the least affected caudate was lower in older age subgroups compared with younger ones, despite similar disease duration. These findings suggest the presence of a more widespread involvement of striatal structures in older patients, which could reflect the contribution of the aging process to the neurodegenerative process of PD and/or a decrease of compensatory mechanisms in older patients\textsuperscript{13}. This age-related variability should be taken into account when using imaging as a surrogate biomarker of disease progression in clinical trials of disease-modifying agent, as a floor effect in older patients could affect the interpretation of the results.

2.1.2. SEROTONIN
The serotonergic system is profoundly affected in PD\textsuperscript{14-18} and PET ligands for the pre-synaptic serotonergic transporter (SERT), such as $[^{11}\text{C}]$-DASB, are reliable tools to assess \textit{in vivo} serotonergic terminals\textsuperscript{19}. Previous work has demonstrated that the administration of levodopa induces markedly higher striatal synaptic dopamine release in PD patients with levodopa-induced dyskinesias (LIDs), which can be ameliorated by the oral administration of the serotonin receptor type 1A agonist buspirone, a pre-synaptic modulator of synaptic release in the serotonergic system, prior to levodopa administration\textsuperscript{15}. This might be due to a relative preservation of serotonergic terminals in the putamen and globus pallidus of PD patients experiencing LIDs\textsuperscript{18}. A further confirmation of this hypothesis comes from a recent study showing that the SERT to DAT ratio increases as PD progresses whereupon patients experience LIDs\textsuperscript{17}. Overall these findings suggest that as the dopaminergic innervation in the striatum becomes critically low, the serotonergic system plays a vital role in the development of LIDs by handling synaptic dopamine levels in an unregulated manner. Serotonergic mechanisms such as excessive striatal innervation and high serotonin to dopamine striatal terminal ratio have also been associated with the development of graft-induced dyskinesias in PD patients who underwent striatal transplantation with fetal ventral mesencephalic tissue\textsuperscript{20-22}. These findings support the role of serotonergic terminals in the aberrant release of striatal dopamine and in promoting the development of dyskinesias in PD patients.

$^{123}\text{I}$-FP-CIT SPECT has also been used to assess clinical correlates of brainstem raphe serotonergic dysfunction in early stages of PD. Results showed that the serotonergic raphe nuclei complex is already dysfunctional in a subgroup of patients with less than 2 years of disease. Additionally, lower $^{123}\text{I}$-FP-CIT binding values in the raphe region, which reflects reduced serotonin transporter availability, was associated with more severe scores of persistent resting tremor. In these early stages, however, levels of raphe serotonergic function did not appear to be related to the non-motor
symptoms of fatigue, depression and sleep disturbance\textsuperscript{23}. In a two-year follow-up study, patients with isolated resting tremor had a significant inverse correlation between raphe \textsuperscript{123}I-FP-CIT binding and resting tremor amplitude scores. However, in the entire cohort, more severe tremor scores were still correlated with lower raphe/putamen binding ratios, indicative of more severe raphe dysfunction compared with nigrostriatal dysfunction. Interestingly, resting tremor in patients with lower raphe/putamen binding ratios responded poorly to dopamine replacement therapy. Taken together, these findings indicate that the occurrence of raphe serotonergic dysfunction is associated with more severe resting tremor and poorer response to dopaminergic drugs\textsuperscript{24}. However, given the non-selective nature of the ligand (\textsuperscript{123}I-FP-CIT), it is not possible to rely entirely on this method to assess serotonergic terminal field integrity in brain structures where other monoaminergic neuronal populations predominate\textsuperscript{23}.

2.1.3. Cholinergic dysfunction

Cognitive changes in PD may result from multifactorial processes that include cellular or network dysfunctions due to regional deposition of proteinopathies, neurotransmitter changes due to the involvement of important neuromodulator projection systems, neuroinflammation or effects of accelerated aging due to the interaction of disease-specific changes and cerebral effects of medical comorbidities\textsuperscript{25,26}. A dual-syndrome hypothesis of cognitive impairment in PD has been proposed where the concept of mild cognitive impairment and fronto-executive dysfunction in particular is mainly driven by dopaminergic dysfunction and manifesting as deficits in flexibility, planning, working memory, and reinforcement learning. In contrast, conversion to dementia in PD might depend on non-dopaminergic, cholinergic and more posterior cortical dysfunctions\textsuperscript{27}. More recent studies emphasize the role of disruption in global brain networks underlying the development of cognitive impairment.
in PD\textsuperscript{28}. Interestingly, brain imaging studies have found early changes in the posterior temporal-occipital regions which correlated with visual cognitive changes in PD\textsuperscript{28}.

The cholinergic system is believed to be one of the major neural mechanisms underlying progressive cognitive decline in PD. Therefore, failure of the cholinergic system may worsen cognitive deficits and make the severity of the dementia syndrome worse. A previous acetylcholinesterase PET study found early regional cortical vulnerability of occipital association cortices in PD, especially in Brodmann area 18\textsuperscript{29}. These are areas that overlap with the ventral and dorsal visual stream of visual cortical projections\textsuperscript{30}, which are important for the processing of shape functions and may provide a link to the susceptibility of PD to develop visual illusions and ultimately visual hallucinations when dementia emerges. Interestingly, a glucose metabolic PET study found that patients with PD with mild cognitive impairment who also had visual hallucinations had more severe posterior cortical hypometabolism and higher rate of conversion to dementia compared to patients with similar mild cognitive impairment but no hallucinations\textsuperscript{31}.

The cholinergic system plays an important role in widespread and diffuse brain networks that sub-serve cognitive functioning\textsuperscript{32}. For example, a muscarinic cholinergic receptor [\textsuperscript{123}I]-QNB brain SPECT study in patients with PD dementia (PDD) found co-occurring receptor reductions in anterior cingulate, basal forebrain, insula, temporal, and striatal regions. Higher or stable binding, however, was seen in parieto-occipital and frontal regions compared to controls\textsuperscript{33}. Interestingly, the topography of receptor binding changes that reflected a beneficial response to cholinesterase inhibitor treatment had regional overlap with default mode and frontoparietal brain networks. These observations may imply a cholinergic role for the maintenance of these networks\textsuperscript{33}.

More recent studies illustrate interactive cognitive effects of dopaminergic and cholinergic neurotransmitter changes in PD. The so-called 'compensatory' hypothesis posits that fronto-parietal cortical cholinergic functions associated with top-down control
may be recruited (increased cholinergic activity in cortical circuits) to compensate for executive dysfunctions associated with striatal dopaminergic declines in early stage disease\textsuperscript{34}. Conversely, combined dopaminergic and cholinergic neurotransmitter system losses may aggravate cognitive, especially executive function, deficits and jointly increase the risk of conversion to PDD\textsuperscript{34}. For example, Kim et al. showed that cortical cholinergic integrity was a stronger predictor of conflict processing in PD patients with relatively low caudate dopaminergic function\textsuperscript{35}. These findings imply also a more nuanced perspective that diverges from the dual-syndrome hypothesis as the cholinergic system may make an important contribution to executive dysfunction in PD.

Acetylcholine can dynamically switch between different cognitive networks processing extrinsic versus intrinsic signals\textsuperscript{36,37}, thereby optimizing cognitive flexibility and performance. Recent studies in PD support a cholinergic role in switching function between large-scale brain network functions where the cortical cholinergic system is involved in top-down cognitive control function\textsuperscript{38}, whereas thalamic cholinergic nerve terminals may have an important role in bottom-up saliency processing\textsuperscript{39}. Consequently, breakdown of these diverse cholinergic systems may result in attentional and executive function deficits and behavioral inflexibility in PD. Cognitive control plays an important role in mobility\textsuperscript{40,41} and the cholinergic system has also been implicated in postural instability and gait difficulties, in particular slow gait speed, falls and freezing of gait, in PD\textsuperscript{42-45}. A post-mortem study confirmed more severe loss of penduculopontine nucleus cholinergic neurons in PD fallers compared to non-fallers\textsuperscript{46}. Pharmacological studies of fall reducing effects cholinesterase inhibitors provide supplemental evidence of the cholinergic system and mobility in PD\textsuperscript{47,48}.

Other research groups aim to explore the evidence that alpha-synuclein pathology in PD could start in peripheral organs including the gastrointestinal tract (GIT). Pavese and colleagues used $^{11}$C-donepezil PET, a marker of acetylcholinesterase density in the brain and peripheral organs, to assess
parasympathetic innervation in the GIT of patients with early PD. They found that compared with controls, PD patients had significantly reduced $^{11}$C-donepezil uptake in the small intestine, colon, and kidneys, providing further support that parasympathetic denervation in these organs is present early in the natural history of PD$^{49}$.

2.1.4 Phosphodiesterase 10A, Cannabinoid and Noradrenergic system

Lesions in nigrostriatal dopaminergic projections in animal models of PD lead to increased levels of cyclic adenosine monophosphate (cAMP). Furthermore, treatment with levodopa reduces the high cAMP levels observed in the denervated striatum$^{50}$. In striatal neurons, cAMP catabolism is mediated by phosphodiesterases (PDE) such as PDE10A. PDE10A is a regulator of cAMP signaling within the striatum and has been studied in animal models of PD. Pharmacological modulations with PDE10A inhibitors have been shown to ameliorate the severity of levodopa-induced dyskinesias (LIDs), restoring physiological cAMP levels in the cortico-striatal-pallidal pathways$^{51}$. Recent findings have demonstrated that PDE10A levels are reduced in the striatum and globus pallidus of PD patients and that these measures can be associated with the severity of motor symptoms and LIDs$^{52}$. In particular, using a selective PDE10A radioligand, i.e. $^{11}$C-IMA107, a reduced binding was observed in the caudate, putamen and globus pallidus which correlated with severity of Unified Parkinson’s Disease Rating Scale part-III motor scores and Unified Dyskinesia Rating Scale scores. These findings suggest that nigrostriatal degeneration affects the expression of PDE10A$^{52}$. Type 1 cannabinoid receptor (CB1) is a modulator of synaptic transmission and potential therapeutic target for LIDs$^{53}$. Using, a CB1-selective radioligand, i.e. $^{[18]}$F] MK-9470, PD patients showed an increased of CB1 availability in nigrostriatal, mesolimbic, and mesocortical dopaminergic projection areas. However, CB1 availability did not differ significantly in patients with and without LIDs, and there was no correlation with LID severity. Thus, these observations demonstrated some regional changes in CB1 availability in PD, but
did not reveal a role of CB1 in the pathogenesis of LIDs\(^5\). Noradrenergic impairment may also play an important role in PD complications\(^5\). Using a noradrenaline transporter, \(^{11}\)C-MeNER, PD patients had a reduced binding which correlated with amount of REM sleep behavioral disorders (RBDs), cognitive performance, and orthostatic hypotension. Thus, impaired noradrenergic function in PD may contribute to a number of non-motor symptoms\(^5\).

2.2. The Contribution of neuro-inflammation and proteinopathies

The development of radiotracers specifically targeting pathological protein aggregates such as tau and \(\beta\)-amyloid, as well as markers of neuroinflammation has been a particular focus within the last years.

The radiotracer \(^{18}\)F-AV-1451, a ligand that binds to paired helical filaments of tau in Alzheimer's disease, has recently been used to explore its potential as a biomarker for the diagnosis and disease progression monitoring in progressive supranuclear palsy (PSP). Previous findings did not show significant tracer retention in PSP patients compared to PD and controls\(^5\), however further investigations with the same tracer showed an off-target binding to neuromelanin-containing neurons in the midbrain\(^5\). These observations demonstrated that \(^{18}\)F-AV-1451 might be the first PET radiotracer capable of imaging neurodegeneration of the substantia nigra in parkinsonism. Other tau PET imaging with \(^{11}\)C-PBB3, \(^{18}\)FTHK-5317, \(^{18}\)FTHK-5351, showed specific patterns of tau tracer retention in atypical parkinsonism\(^5\). These observations provide some evidence for the underlying neuropathology, which may in future allow tauopathies (e.g. corticobasal degeneration or PSP) to be distinguished from non-tauopathies (e.g. multiple-system atrophy [MSA])\(^6\). However, the primary concern with all these tracers is often the lack of specificity for tau with off target binding.
(e.g. neuromelanin, monoamine oxidase A/B). Neuroimaging\textsuperscript{65-69} studies have demonstrated as well a relationship between elevated striatal and cortical $\beta$-amyloid deposits and cognitive impairment in PD\textsuperscript{65,66}. However, in PD with dementia (PDD), the distribution of $\beta$-amyloid measured with $[^{11}\text{C}]$ PIB had a different pattern than those with Alzheimer disease\textsuperscript{69}. The presence of an abnormal $[^{11}\text{C}]$ PIB binding in PDD underestimated at autopsy the risk of $\beta$-amyloid-deposition in these people\textsuperscript{67,68}.

Another area of current interest is the potential usage of the mitochondrial translocator protein 18kDa (TSPO) as an \textit{in vivo} biomarker of neuroinflammation. The recent development of second-generation radiotracers such as $[^{18}\text{F}]$-FEPPA enables researchers to explore the role of TSPO expression in PD by controlling for genotyping expression. While Strafella and colleagues could not observe increased tracer uptake in the striatum\textsuperscript{70} or in the cortical and subcortical brain regions\textsuperscript{71} in patients with PD compared to controls, the combination of the radioligands $[^{18}\text{F}]$ FEPPA and $[^{11}\text{C}]$ PIB, a measure of $\beta$-amyloid load, revealed interesting findings. The dual-tracer PET study detected significantly higher $[^{18}\text{F}]$ FEPPA binding in PD patients with cognitive impairment in the frontal and temporal lobe, striatum, precuneus and dorsolateral prefrontal cortex, when $\beta$-amyloid was present in these brain regions\textsuperscript{72}. Other areas of research focus on patients with idiopathic Rapid Eye Movement Sleep Behavior Disorder (iRBD) who could be in the pre-motor phase of alpha-synucleinopathies such as PD, dementia with Lewy bodies (DLB), and MSA. Pavese and colleagues used PET imaging to explore the extent and distribution of early inflammation (microglia activation) in the brain of iRBD to clarify whether it could contribute to the neurodegenerative process of developing alpha-synucleinopathies. The relationship between neuroinflammation and striatal and extrastriatal dopamine dysfunction was also investigated. They found that $^{11}\text{C}$-PK11195 binding, a marker of activated microglia was significantly increased in the substantia nigra of iRBD patients compared to
controls. Increased binding was also observed at a lesser extent in the putamen and caudate nuclei bilaterally. Individual analysis showed that 26% of the iRBD patients had significant increases in $^{11}$C-PK11195 binding in the putamen and/or caudate (> 2 standard deviations above the mean of the controls), suggesting that $^{11}$C-PK11195 PET can be used to identify individual iRBD patients who have high levels of neuroinflammation. Activated microglia were also found in the visual associative cortex of the occipital lobe of these patients, which might potentially indicate those at greater risk of developing DLB. However, $^{11}$C-PK11195 is known for its technical limitations such as low signal-to-noise ratio, high nonspecific binding, low brain penetration, and high plasma protein binding. Analysis of nigrostriatal function with $^{18}$F-dopa PET in individual iRBD patients showed that a subgroup of these iRBD patients had a homogeneous and more widespread decrease of tracer uptake in the striatal sub-regions including the caudate, possibly suggesting that these patients are in an early stage of DLB or MSA rather than idiopathic PD.

2.3. Blood flow and metabolism

The valuable role of $^{18}$F-FDG PET in the detection of spatial patterns of metabolic dysfunction allowing differentiation between different parkinsonian disorders has repeatedly been demonstrated. Moreover, it has been shown that patterns of metabolism detected with FDG-PET may correlate more with the clinical phenotype (e.g. motor vs. cognitive impairments) than with a specific underlying pathology.

When investigating fatigue in PD by using $^{18}$F-FDG-PET, results revealed that PD patients with higher levels of fatigue showed anti-correlated metabolic changes in cortical regions associated with the salience (i.e., right insular region) and default (i.e., bilateral posterior cingulate cortex) networks. These observations propose that fatigue in PD might be the expression of metabolic abnormalities and impaired functional interactions between brain regions linked to the salience and other neural networks.
As mentioned earlier, increased impulsivity and hypomania are non-motor symptoms associated with behavioral addictions (e.g. pathological gambling) that may occur with dopamine replacement therapy. Recent studies have shown that metabolic patterns associated with impulsivity and hypomania in PD are mostly found within the fronto-insular network\textsuperscript{79-81}. This is in line with the view that the insula plays an important role in various non-motor disturbances in PD\textsuperscript{82}.

Metabolic activity has also demonstrated spatially distributed networks of brain function. In particular, \textsuperscript{18}F-FDG-PET has provided a means of quantifying highly specific spatial covariance patterns associated with PD motor and cognitive functions\textsuperscript{83-85}.

3. Magnetic resonance imaging in PD

3.1. Magnetic Resonance Imaging Techniques

Over the past years, researchers are using as well different MR imaging techniques such as dMRI and fMRI to study functional disruptions and the reorganization of neural pathways in PD and other movement disorders.

Using dMRI and a computational bi-tensor model instead of a single-tensor model, several studies have uncovered a consistent pattern of elevated free water in the substantia nigra of patients with PD that progresses over time. For instance, it was shown that free water levels in the posterior substantia nigra were elevated in PD relative to healthy controls across separate single- and multi-site cohorts\textsuperscript{86}, and this region is consistent with the ventrolateral tier of the substantia nigra where dopaminergic cell loss is greatest in PD\textsuperscript{87,88}. Furthermore, free water in posterior substantia nigra was also elevated in atypical parkinsonian syndromes, consistent with more severe pathology in these disorders as compared to PD\textsuperscript{89}. Most importantly, the results of a prospective, single-site longitudinal study revealed that free water in
posterior substantia nigra increased over the course of 1 year in PD but not in controls, and baseline free water predicted changes in bradykinesia and cognitive scores\textsuperscript{90}. In a recent validation study using the Parkinson Progression Marker Initiative Cohort\textsuperscript{91}, it was found that (i) free water level in the posterior substantia nigra increased over 1 year in de novo PD but not in controls; (ii) free water kept increasing over 4 years in PD; (iii) sex and baseline free water predicted 4-year changes in free water; (iv) free water increases over 1 and 2 years were related to worsening on the Hoehn and Yahr scale over 4 years; and (v) the 4-year increase in free water was associated with the 4-year decrease in striatal binding ratio in the putamen. Importantly, all longitudinal results were consistent across sites.

In addition to studies of dMRI, task-based fMRI studies have also revealed progression changes in the putamen and motor cortex in parkinsonism. To explore longitudinal changes in brain activity in patients with PD, MSA, and PSP a robust task-based fMRI protocol was used that has consistently shown cross-sectional changes in PD, MSA, and PSP\textsuperscript{92-94}. A total of 112 individuals were scanned 1 year apart while performing a unimanual grip force task: 46 PD, 13 MSA, 19 PSP, and 34 controls\textsuperscript{95}. Compared with the control group, patients with PD showed a decline in functional activity over the course of 1 year in the putamen and motor cortex compared to controls. Changes after 1 year in MSA were exclusively extrastriatal, and included a reduction in functional activity in the primary motor cortex (M1), supplementary motor area (SMA), and superior cerebellum. In PSP, all regions of interest across putamen, cerebellum, and motor cortex were less active at 1 year compared to baseline. A key finding was that the functional activity of these regions did not change in the control group.

Latest investigations from Strafella and colleagues\textsuperscript{96} using fMRI, explored the dynamic functional connectivity in PD, focusing on temporal properties of functional connectivity states, and the variability of network topological organization. Results revealed changes within- as well as between network states in PD versus controls,
confirming the vulnerability of functional connectivity networks in this movement disorder.

Recent fMRI work from Lewis and colleagues focuses on the investigation of the common clinical phenomena of freezing of gait (FOG) and hallucinations in PD.

Freezing of gait is a complex, heterogeneous, and highly variable phenomenon whose pathophysiology and neural signature remains enigmatic. Evidence suggests that freezing is associated with impairments across cognitive, motor and affective domains; however, most research to date has focused on investigating one axis of freezing of gait in isolation. By contrast, recent work using an established virtual reality gait paradigm to elicit freezing behaviour has examined individual differences in the differential component processes that underlie freezing of gait (i.e. cognitive, motor and affective function)\(^9^7\). Simultaneously probing three freezing triggers: set-shifting ability for cognition, step time variability for motor function, and self-reported anxiety measures in a principal components analysis, has allowed a multivariate approach to interrogate the pattern of task-based functional connectivity associated with the freezing phenomena. Specifically, the investigators used the first principal component from their behavioral analysis to classify patterns of functional connectivity into those that were associated with: (i) increased severity; (ii) increased compensation; or (iii) those that were independent of freezing severity. Coupling between the cognitive and limbic networks was associated with ‘worse freezing severity’, whereas anti-coupling between the putamen and the cognitive and limbic networks was related to ‘increased compensation’. Additionally, anti-coupling between cognitive cortical regions and the caudate nucleus were ‘independent of freezing severity’ and thus may represent common neural underpinnings of freezing that are unaffected by heterogenous factors. Subsequently, these investigators related these connectivity patterns to each of the individual components of freezing in turn (i.e. cognitive, motor, affective), thus exposing latent heterogeneity in the freezing phenotype whilst also identifying critical functional
network signatures that may represent potential targets for novel therapeutic intervention. In conclusion, the findings from this study provided confirmatory evidence for systems-level impairments in the pathophysiology of freezing of gait and further advances our understanding of the whole-brain deficits that mediate symptom expression in PD.

Visual hallucinations are a common and troubling neuropsychiatric feature in more advanced PD but the ability to capture such activity using fMRI has been severely restricted. However, the recent development of a neuropsychological paradigm that can reliably induce visual misperceptions in PD patients reporting visual hallucinations has allowed for its combination with fMRI to provide greater insights into the pathophysiology of this phenomenon. This work has demonstrated that visual hallucinations seem to arise from an increased engagement of the Default Mode Network (DMN) (operating across the hippocampal formations, posterior cingulate and intraparietal sulcus) with the primary visual system. This is the result of a disengaged Dorsal Attention Network (representing regions of the frontal eye fields and superior parietal lobule), which would normally maintain directed attention and prevent hallucinations arising. These fresh clues may provide the basis of future treatments targeting pathological network activity.

Resting-state functional magnetic resonance imaging (RS-fMRI) is a relatively new, non-invasive tool to assess functional abnormalities observed in PD without the effects of motor or cognitive tasks. A recent meta-analysis of RS-fMRI studies in PD found evidence for an intrinsic functional disturbance of the inferior parietal lobule (IPL) and the supra-marginal gyrus potentially linked to functional impairment of perception and executive processes. However, when interpreting RS-fMRI results, it is important to consider the impact of dopamine replacement therapy.

Tessitore and colleagues applied RS-fMRI to study functional disruption and reorganization of neural pathways that may be proposed as early biomarkers of PD.
development and progression. Among the most reported RS networks, alterations within the sensori-motor network (SMN) have been consistently reported across PD stages by means of different analytic approaches. In a cohort of drug-naïve early PD patients, the authors have demonstrated the presence of functional connectivity disruption in the SMA compared to controls, partially restored by the first levodopa administration compared to placebo. Moreover, a region of interest analysis of the SMN functional connectivity within the basal ganglia revealed that levodopa significantly increased the participation of these subcortical regions to the SMN activity and may selectively induce low-frequency rhythm changes. Interestingly, SMN connectivity abnormalities were also detected in asymptomatic LRRK2 (G2019S) mutation carriers, suggesting that functional changes may also occur earlier during the preclinical phase of the disease.

As SMN functional connectivity has consistently shown a levodopa modulation and symptoms severity correlation, the authors could speculate that: 1) SMN functional connectivity disruption may be considered as a neural correlate of the cortico-striatal disruption which underlies PD pathophysiology, even before symptoms emerge, 2) SMN connectivity may be potentially used as a biomarker of both symptom development and treatment response throughout the disease course.

In recent years, an intrinsic aberrant functional connectivity within the DMN has been implicated in cognitive processing in several neurodegenerative disorders, including PD, with and without cognitive impairment. In a cohort of early-stage cognitively unimpaired patients with PD, researchers demonstrated the presence of decreased medial temporal and inferior parietal connectivity within the DMN, which correlated with cognitive performance. This finding suggests that functional disconnection of posterior brain regions can precede clinically measurable cognitive impairment in PD and may be proposed to develop a sensitive and specific biomarker of dementia in PD for prognostic and disease-monitoring purposes.
Together with the DMN, other so-called neurocognitive networks, such as the salience (SN) and the central-executive (CEN) networks, have been implicated into PD progression. Typically, the SN and CEN show increased activation in response to external stimuli\textsuperscript{119}, whereas DMN activity is suppressed, resulting in anti-correlated coupling between the CEN and DMN\textsuperscript{120,121}. The same pattern of interaction among the three neurocognitive networks has been also shown at rest\textsuperscript{122,123}. This dynamic balance may allow an individual to remain prepared for unexpected environmental events\textsuperscript{120} and seems to be critical in generating and maintaining an efficient behavioral and cognitive performance\textsuperscript{122}. Interestingly, using RS-fMRI Tessitore and colleagues highlighted the presence of a disrupted connectivity within these three networks in treated PD patients with ICBs compared to those without\textsuperscript{124}. These behavioral symptoms may be triggered by dopamine replacement treatment in a specific subset of patients, showing clinical risk factors which are not able, to date, to predict their development. To fill this gap, the researchers investigated the intrinsic brain networks connectivity at baseline in a cohort of drug-naïve PD patients that subsequently developed ICB (ICB+) over a 36-month follow-up period compared with patients who did not (ICB-).\textsuperscript{125} The imaging data demonstrated the presence of a specific decreased connectivity within and between the DMN and CEN networks at baseline as well as an increased connectivity within the SN in ICB+ compared with ICB- patients. Specifically, the authors found that the physiological anti-correlation between DMN/CEN is lost at the time of diagnosis in PD patients who are more prone to develop ICB and this inverse pattern seems to predict ICB emergence over time. Moreover, this altered DMN/CEN coupling showed a positive correlation with the time to ICB onset (i.e. the less the anti-correlation between DMN and CEN the earlier is the emergence of ICB). These connectivity changes are independent from motor, behavioral and cognitive features and do not result from chronic dopaminergic treatment, suggesting that it may represent a potential biomarker for the emergence of ICB symptoms.
Further investigations have aimed to explore functional changes in sensorimotor and cognitive networks in PD, focusing on inter- and intra-connectivity organization in the disease-associated nodal and hub regions using graph theoretical analyses. To date, findings have highlighted diffuse alterations in nodal organization and regional hub disruption that result in a number of distributed abnormalities across brain networks that may be related to the specific clinical manifestations of PD\textsuperscript{126}.

### 3.2. The role of Ultra-High Field (UHF) Imaging

Despite several limitations, MR imaging has certainly enhanced the diagnostic accuracy in the differential diagnosis of neurodegenerative parkinsonism over the last years.

Technological advances and the introduction of high-field 3 Tesla (3T) and ultra-high-field 7 Tesla (7T) MRI have led to improved spatial resolution and contrast thus enabling better visualization of brain structures affected in neurodegenerative disorders. Consequently, a new MRI finding has been described in the substantia nigra on iron-sensitive MRI sequences capable of differentiating between PD patients and controls\textsuperscript{127}. In controls, a hyperintense ovoid area within the dorsolateral border of the otherwise hypointense substantia nigra pars compacta (SNpc), referred to as dorsolateral nigral hyperintensity (DNH), has been recognized. DNH seems to correspond histopathologically to nigrosome-1, a calbindin-negative subregion in the SNpc\textsuperscript{127}. A recent meta-analysis\textsuperscript{128} evaluated DNH as an imaging marker for PD, concluding that visual assessment of DNH could provide excellent diagnostic accuracy for PD compared to controls. The loss of DNH may enable the discrimination between PD and other movement disorders including drug-induced parkinsonism, essential tremor, and dystonic tremor\textsuperscript{128}. Studies in iRBD cohorts\textsuperscript{129} have shown that at least two third of these patients had a loss of DNH as seen in PD. Therefore, loss of DNH could represent a potential marker for prediagnostic stages of PD\textsuperscript{128}. 
4. Future Directions & Conclusions

Overall, previous and current work has provided some insights on PD and parkinsonian disorders and their underlying pathophysiological abnormalities. These observations have offered researchers and clinicians a better understanding of the neural correlates of the symptoms experienced in PD such as cognitive dysfunction, FOG and hallucinations as well as subsequent treatment complications including ICBs. Nonetheless, the visualization of neuroanatomical and functional hallmarks of these pathological conditions remains an active and challenging area, while PET and MR imaging have raised their value as powerful tools to detect brain changes. These neuroimaging techniques have also proven to be helpful in the clinical setting e.g. metabolic PET and structural or dMRI can accurately distinguish PD from atypical parkinsonism. Further, dopaminergic and serotonergic PET and SPECT imaging can follow the development of motor and nonmotor symptoms and complications as the disease progresses whereas metabolic, cholinergic and β-amyloid PET methods are useful for investigating cognitive decline in PD.

There are several factors delaying the transfer of neuroimaging techniques from the research setting into clinical practice. For instance, many research achievements have derived from PET imaging, which is a powerful tool, however also expensive and not widely available. Most biomarkers are unsuitable for individual level type of analysis, and allow only group-wise comparison. Moreover, several PET and MRI techniques depend on sophisticated quantification, which necessitates specialized software and skilled technicians. Another prerequisite for clinical application is standardized data processing and the availability of well-defined imaging criteria. The combination of PET and MR imaging may be helpful in determining diagnostic criteria based on these innovative MRI techniques. Also, data from different investigational modalities need to
be united to recognize mechanisms that are meaningful drug targets. Lastly, physicians require training and experience with these techniques to obtain optimization\textsuperscript{130}.

PD remains a complex neurodegenerative disease, and several novel neuroimaging techniques require further research and longitudinal assessments to evaluate their stability and validity as PD biomarkers before being recommended for routine clinical practice\textsuperscript{130}.

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3) We confirm that we have read the Journal’s position on issues involved in ethical publication and affirm that this work is consistent with those guidelines.

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