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Review

Imaging neurodegeneration in Parkinson's disease

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ABSTRACT

Neuroimaging techniques have evolved over the past several years giving us unprecedented information about the degenerative process in Parkinson's disease (PD) and other movement disorders. Functional imaging approaches such as positron emission tomography (PET) and single photon emission computerised tomography (SPECT) have been successfully employed to detect dopaminergic dysfunction in PD, even while at a preclinical stage, and to demonstrate the effects of therapies on function of intact dopaminergic neurons within the affected striatum. PET and SPECT can also monitor PD progression as reflected by changes in brain levodopa and glucose metabolism and dopamine transporter binding. Structural imaging approaches include magnetic resonance imaging (MRI) and transcranial sonography (TCS). Recent advances in voxel-based morphometry and diffusion-weighted MRI have provided exciting potential applications for the differential diagnosis of parkinsonian syndromes. Substantia nigra hyperechogenicity, detected with TCS, may provide a marker of susceptibility to PD, probably reflecting disturbances of iron metabolism, but does not appear to correlate well with disease severity or change with disease progression. In the future novel radiotracers may help us assess the involvement of non-dopaminergic brain pathways in the pathology of both motor and non-motor complications in PD.

1. Introduction

Parkinson's disease (PD) is a progressive degenerative neurological disorder, characterized by asymmetric onset of resting tremor, rigidity, and bradykinesia in the limbs followed by postural instability. The disease is uncommon before the sixth decade and the prevalence rates increase with age. Results from seven population-based studies performed in European countries suggest that the overall prevalence of PD in people aged over 65 is 1.8%, with an increase from 0.6% for persons aged 65 to 69 years to 2.6% for people aged 85 to 89 years [1]. Progression of symptoms in PD may occur over 10–30 years but can be accelerated in some individuals [2].

The cardinal pathological feature of the disease consists of the formation of proteinaceous intraneuronal Lewy body inclusions and Lewy neurites and progressive neuronal loss particularly targeting the substantia nigra. Using synuclein immunocytochemistry, it has been observed that intraneuronal Lewy body inclusions can be detected in the lower brainstem ahead of midbrain and nigral involvement and later spread to limbic and association cortical areas in a predictable manner [3]. Based on this observation Braak et al. have proposed a six-point staging procedure for the pathological process in PD. They propose that Lewy body pathology begins in the medulla oblongata and olfactory structures in stage 1 and spreads to the pons by stage 2. The substantia nigra (SN) and midbrain brain nuclei are affected during stage 3 and limbic areas in stage 4. Eventually, in stages 5 to 6, the inclusions appear in the neocortex. Despite these findings of widespread Lewy body disease, the hallmark of PD pathology is the loss of the dopaminergic neurons in the SN pars compacta (SNc) which results in striatal dopamine deficiency. This in turn leads to increased inhibitory output activity from the basal ganglia to the ventral thalamus and frontal cortex and subsequent development of parkinsonism. It has been estimated that classical PD symptoms appear when 80% of striatal dopamine and 50% of the nigra compacta cells have been lost [4].

Over the past two decades, neuroimaging techniques such as positron emission tomography (PET), single-photon emission computed tomography (SPECT), magnetic resonance imaging (MRI) and transcranial sonography have increasingly been employed to detect PD, to elucidate the neuropathological mechanisms and compensatory responses underlying symptoms and treatment associated complications, and to monitor disease progression *in vivo*. This paper reviews the different contributions of neuroimaging to the field, with a focus on the assessment of nigrostriatal degeneration. The involvement of other brain structures and neurotransmitter systems, which may be responsible for the onset of non-motor symptoms in PD patients, will also be discussed.

2. PET and SPECT

PET and SPECT have been extensively employed to elucidate the functional changes associated with PD and other neurodegenerative

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Table 1
Summary of functional imaging strategies for the assessment of presynaptic nigrostriatal terminals

Biological marker	Neuroimaging technique	Tracer
Metabolism of levodopa (a) uptake into dopamine neurons (b) metabolism by AADC[a] (c) vesicular storage of ^{18}F-dopamine	PET	^{18}F-dopa
Presynaptic dopamine transporter (DAT)	PET	^{11}C-CFT ^{18}F-CFT ^{11}C-RTI-32 ^{18}F-FP-CIT ^{11}C-methylphenidate
	SPECT	^{123}I-β-CIT ^{123}I-FP-CIT ^{123}I-altropane ^{99m}Tc-TRODAT-1
Type-2 vesicular monoamine transporter (VMAT2)	PET	^{11}C-dihydrotetrabenazine

AADC = aromatic amino acid decarboxylase.
[a]^{18}F-dopa uptake mostly reflects the activity of AADC.

parkinsonian disorders. Both modalities provide a means of assessing: (1) disease severity as reflected by presynaptic dopamine terminal dysfunction, (2) subclinical dysfunction in subjects who are at risk for PD, (3) disease progression and the effects on this of putative neuroprotective agents, and (4) changes in non-dopaminergic neurotransmission.

Additionally, PET studies with the peripheral benzodiazepine ligand ^{11}C-(R)-PK11195, a selective marker of activated microglia [5], have been used for imaging brain inflammation *in vivo* in PD patients and to help clarify the role of activated microglia in the ongoing degenerative process.

2.1. Presynaptic dopaminergic function

Assessment of the functional integrity of presynaptic nigrostriatal projections is a major goal for the functional imaging techniques used in the evaluation of patients with parkinsonian features. At present, three different markers of presynaptic dopaminergic terminals are commonly studied; these are discussed below and summarized in Table 1.

2.1.1. Measurement of striatal aromatic amino acid decarboxylase activity

^{18}F-dopa PET was the first neuroimaging technique validated for the assessment of presynaptic dopaminergic integrity. The uptake of ^{18}F-dopa in the striatal nuclei over 90 min, as measured by an influx constant Ki, reflects both the density of the axonal terminal plexus and the activity of the striatal aromatic amino acid decarboxylase (AADC), the enzyme responsible for the conversion of ^{18}F-dopa to ^{18}F-dopamine. Measurements of ^{18}F-dopa uptake in the striatum of patients with PD will, therefore, be influenced by the number of remaining dopaminergic cells. This is supported by pathological studies which have demonstrated that levels of striatal ^{18}F-dopa uptake correlated well with nigral cell counts in both human cases and in non-human primates where parkinsonism was induced by the nigral toxin MPTP [6,7]. However, particularly in early stages of disease, ^{18}F-dopa PET may underestimate the degenerative process due to the presence of compensatory upregulation of AADC in remaining terminals [8].

Putamen uptake of ^{18}F-dopa in PD has been shown to correlate with the clinical severity of locomotor disability as measured by the Unified Parkinson's Disease Rating scale (UPDRS) [9–11]. Interestingly, while putamen ^{18}F-dopa reductions correlate well with the degree of rigidity and bradykinesia in PD this is not true of tremor severity suggesting that either non-nigrostriatal and/or non-dopaminergic pathways are implicated in the pathogenesis of this symptom.

Typically, PD patients show a gradient of reduced striatal ^{18}F-dopa uptake along a rostro-caudal axis. Patients with hemiparkinsonism have their greatest ^{18}F-dopa uptake reduction in the dorsal posterior putamen contralateral to the side of clinical symptoms [12]. As the disease progresses to become bilateral, additional reductions are seen within the ventral and anterior putamen and dorsal caudate. In the most advanced stages, uptake within the ventral head of caudate also falls. These ^{18}F-dopa PET findings are in line with post-mortem data that have reported an uneven pattern of dopamine loss in the striatum in PD, posterior dorsal putamen being targeted. Nigral cell counts are lowest in ventrolateral subregions which send dopaminergic projections to the dorsal putamen. [4,13]

Reductions of ^{18}F-dopa uptake in PD can be localised at a voxel level across the whole brain by interrogating PET images with statistical parametric mapping (SPM). This analytical approach has made it possible to detect changes in ^{18}F-dopa uptake in extrastriatal as well as striatal regions and to explore compensatory responses to the neurodegenerative process at different stages of the disease. With SPM, several authors have reported increases in ^{18}F-dopa uptake in dorsolateral prefrontal cortex, anterior cingulate, and globus pallidus interna of patients with early PD compared to both normal controls and patients with more advanced disease [14–16]. It is likely that these increases in extrastriatal ^{18}Fdopa uptake in early PD reflect compensatory upregulation of AADC though some uptake of ^{18}F-dopa into serotonergic terminals may also be a contributor. Whone et al. observed a 40% increase in ^{18}F-dopa uptake in the globus pallidus interna in early PD which was then lost in advanced disease as motor fluctuations developed [16]. It may well be that raised pallidal dopamine storage in early PD is important for normalising basal ganglia output to the ventral thalamus and motor cortex and, when pallidal as well as putamen dopamine storage fails, motor responses to levodopa therapy become fluctuating and unpredictable.

2.1.2. Measurement of presynaptic dopamine transporter binding

The presynaptic dopamine transporter (DAT) is the plasma membrane transporter responsible for the high-affinity uptake of dopamine. It is found exclusively in dendrites and axons of dopaminergic neurons and is therefore a potential marker of integrity of nigrostriatal projections. Several PET ligands (^{11}C-CFT, ^{18}F-CFT, ^{18}F-FP-CIT, and ^{11}C-RTI-32) and SPECT tracers (such as ^{123}I-β-CIT, ^{123}I-FP-CIT, ^{123}I-altropane, ^{11}C-methylphenidate, and ^{99m}Tc-TRODAT-1) are now available to measure DAT availability.

^{123}I-β-CIT, a tropane derivative, binds with equal nanomolar affinity to DAT, noradrenergic (NART), and serotonergic (SERT) transporters. Striatal uptake at 24 h post-injection primarily reflects DAT binding whereas brainstem uptake at 1 h post-injection reflects SERT binding. Similarly to ^{18}F-dopa, striatal ^{123}I-β-CIT uptake correlates well with stage of disease and symptom severity in PD, particularly with bradykinesia but not rest tremor [17–19]. A disadvantage of ^{123}I-β-CIT is its slow striatal uptake kinetics. It takes 24 h to equilibrate in this brain region following its administration so SPECT must be delayed to the following day. More recently developed SPECT tracers such as ^{123}I-FP-CIT, ^{123}I-altropane, and ^{123}I-PE21, have faster uptake kinetics though they give higher non-specific signals. In practice diagnostic scans can be performed within 2 to 3 h of tracer administration. ^{99m}Tc-TRODAT-1 has the advantage of being technetium based and so available in kit form. Its specific signal, however, is lower than the ^{123}I based SPECT tracers.

In general, all these DAT markers show similar findings in PD to those seen with ^{18}F-dopa PET and are able to differentiate early PD from normal subjects with a sensitivity of around 90%. In contrast to ^{18}F-dopa, striatal uptake of DAT ligands in early PD may overestimate the reduction in terminal density due to the relative downregulation of DAT in remaining neurons as a response to nigral neuron loss in order to maintain synaptic dopamine levels. While striatal ^{18}F-dopa

does not appear to be age dependent in healthy subjects, DAT binding falls with age [20–22].

2.1.3. Measurement of the vesicular monoamine transporter 2

The type-2 vesicular monoamine transporter (VMAT2) is exclusively expressed in the brain and is responsible for the uptake of monoamines from the cytoplasm into the secretory vesicles in dopamine neurones [23]. ^{11}C-dihydrotetrabenazine (DTBZ) is a PET tracer that binds to VMAT2. Lee et al. compared striatal uptake of ^{11}C-dihydrotetrabenazine, ^{18}F-dopa, and the DAT ligand ^{11}C-methylphenidate in PD. They found that ^{18}F-dopa Ki was reduced relatively less than the ^{11}C-dihydrotetrabenazine binding potential in the parkinsonian striatum, while ^{11}C-dihydrotetrabenazine binding was reduced less than ^{11}C-methylphenidate binding [24]. This finding is in line with the presence of relative AADC upregulation and DAT downregulation in the striatum of parkinsonian patients in order to increase dopamine turnover and diminish its re-uptake. The authors propose that ^{11}C-dihydrotetrabenazine PET gives the most reliable measurement of the density of dopaminergic terminals. This suggestion, however, remains to be validated by comparing ^{11}C-dihydrotetrabenazine striatal binding with post-mortem nigral cell counts and demonstrating that dopaminergic drugs have no effect on tracer uptake.

Fig. 1 shows striatal uptake of different PET and SPECT tracers in healthy controls and early Parkinson's disease.

2.2. Detection of subclinical disease

Measures of dopaminergic presynaptic integrity with both ^{18}F-dopa PET and ^{123}I-β-CIT SPECT have allowed detection of subclinical dysfunction in subjects who are at risk for PD. Reductions in ^{18}F-dopa putaminal uptake has been reported in 18% of asymptomatic dizygotic co-twins and in 55% of asymptomatic monozygotic co-twins of patients with idiopathic PD. Over the 4-year follow-up of this study, two of ten asymptomatic monozygotic co-twins subsequently developed clinical parkinsonism and all 10 subjects showed a further decrease in putaminal ^{18}F-dopa uptake [25]. A significant reduction of putaminal ^{18}F-dopa uptake has also been reported in around 25% of asymptomatic siblings in kindreds with familial PD, and about one-third of those with abnormal imaging developed clinical parkinsonism over a five-year follow-up [26]. These findings support a role of inheritance in PD, but do not fully rule out the effect of possible concomitant environmental factors.

PARK2 is a recessive form of Parkinson's disease caused by parkin gene mutations. Recently, two separate studies [27,28] have reported reductions of striatal ^{18}F-dopa uptake in asymptomatic carriers of a single *parkin* mutation compared to normal subjects. Longitudinal studies are now required to establish whether these subjects will convert to clinical PD later on in life.

^{123}I-β-CIT SPECT has been used to evaluate dopamine terminal integrity in relatives of PD patients with no parkinsonian symptoms but with a complaint of idiopathic hyposmia, a known risk factor for PD. 11% of the relatives exhibited hyposmia on UPSIT testing and 17.5% (7 out of 40) of these siblings with hyposmia had reduced striatal ^{123}I-β-CIT binding. 57% (4 out of 7) of those hyposmic relatives with subclinically reduced DAT binding converted to clinical PD over a 2-year follow-up [29]. While ^{123}I-β-CIT SPECT can detect subclinical dysfunction in PD relatives only 2% in total, however, exhibit both hyposmia and dopaminergic loss.

2.3. Disease progression and the effects of putative neuroprotective agents

Both PET and SPECT have been used to monitor the progression of nigrostriatal degeneration in PD. Several series have now demonstrated that the loss of striatal ^{18}F-dopa uptake occurs more rapidly in PD patients than in age-matched controls [30–33]. In these studies the mean annual rate of ^{18}F-dopa uptake decline in PD patients has been reported to range from 8% to 12% in the putamen and 4% to 6% in the caudate, whereas the annual decline in normal volunteers is lower than 1% (0.5% and 0.7% in the putamen and in the caudate respectively) [33]. It has been suggested that the absolute rate of decline does not vary between different regions of the striatum. In a 5-year longitudinal study with ^{18}F-dopa PET, Nurmi et al. evaluated the annual rate of decline of tracer uptake in different striatal subregions [33]. They found a 10.3% annual reduction in ^{18}F-dopa uptake in the posterior putamen and an 8.3% reduction in the anterior putamen. Caudate nucleus showed a 5.9% annual reduction. The absolute rate of decline however was similar in all striatal subregions. An analogous study was performed in 31 untreated patients with early PD [34]. Patients were studied with ^{18}F-dopa PET twice, at the time of the diagnosis and 2 years later. Results from this study also indicate a similar rate of progression between the subregions of the striatum.

Based on this evidence ^{18}F-dopa PET provides a reliable biological marker of the progression of PD. It has been argued, however, that

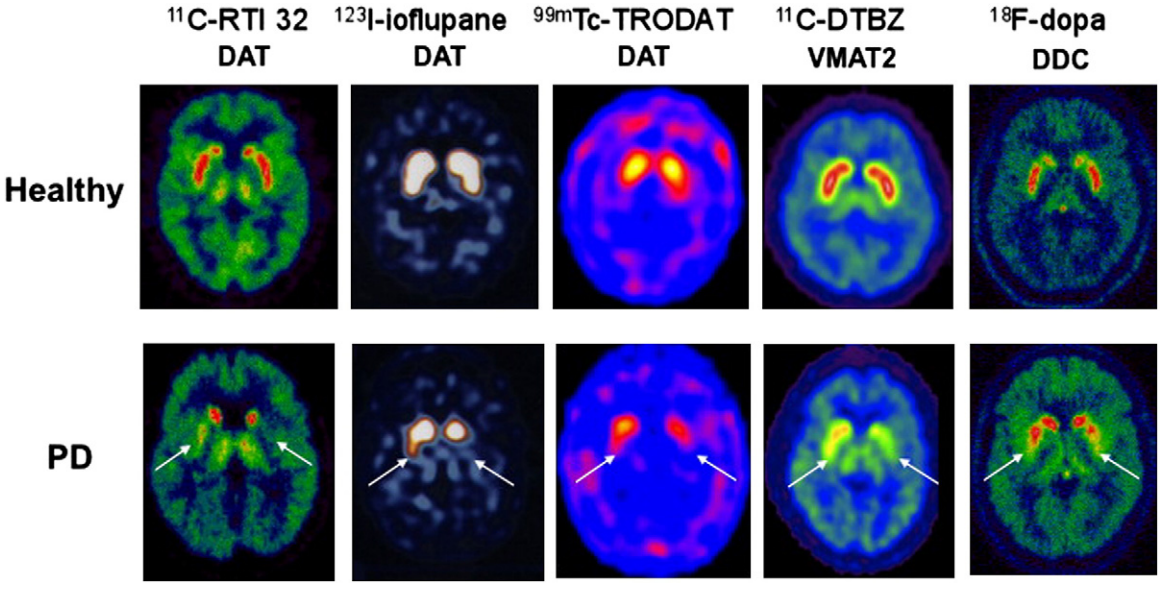

Fig. 1. Imaging dopamine terminal function in healthy controls and early Parkinson's disease.

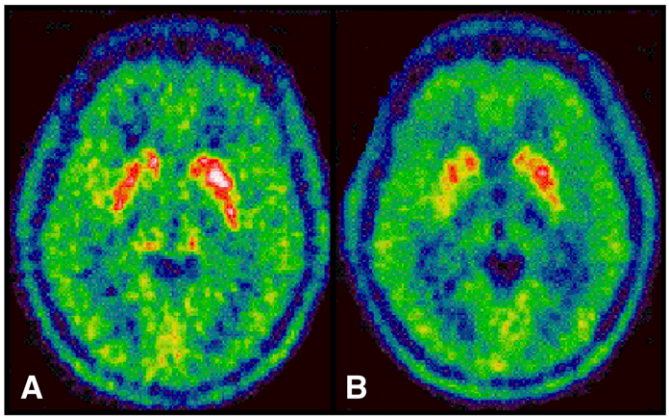

Fig. 2. Images of ^{18}F-dopa uptake in a patient with Parkinson's disease at baseline (A) and after 2 years follow-up (B).

decreases in striatal ^{18}F-dopa uptake over time may not provide an accurate measure of the neurodegenerative process as they reflect both neuronal loss and failure of compensatory mechanisms (AADC upregulation). Fig. 2 shows images of ^{18}F-dopa uptake in a PD patient at baseline and after 2 years follow-up.

^{123}I-β-CIT, ^{123}I-FP-CIT, and ^{123}I-IPT SPECT and ^{18}F-CFT PET have all been used to monitor the rate of the loss of DAT binding in PD. Several ^{123}I-β-CIT SPECT studies have evaluated the rate of PD progression and reported similar rates of the loss of putamen dopamine transporters with a mean 8% annual decline [19,35–37]. A confounding factor when assessing disease progression with ^{123}I-β-CIT SPECT is that tracer uptake decreases with age (3.3% to 10% per decade) in healthy subjects [20–22].

Nurmi et al. [38] have used ^{18}F-CFT PET to investigate DAT loss in striatal subregions in patients with early PD. At variance with previous ^{18}F-dopa PET findings, they found that the decline in tracer uptake was significantly different in anterior and posterior putamen. When the rates of progression were calculated compared to the normal control mean, the caudate had the highest rate of progression (5.6%), followed by the anterior putamen (5.3%) and then the posterior putamen (3.3%). Additionally, the absolute decline in ^{18}F-CFT PET uptake was greater in the less affected putamen. If confirmed in larger longitudinal studies, this finding would suggest that progression is non-linear – possibly exponential – and slower in the posterior putamen where the disease is more advanced at baseline.

At present, ^{11}C-DTBZ PET is only available in few centres and not many longitudinal studies have been performed with this technique. The Vancouver group has reported two separate longitudinal studies in PD patients over a 4-year follow-up [39,40]. The annual rate of decline in putamen DTBZ was around 5% of baseline. A faster rate of progression was observed in patients with a milder disease.

Due to their capacity to monitor the loss of dopaminergic function in PD objectively and to their relatively wide availability worldwide, ^{18}F-dopa PET and ^{123}I-β-CIT SPECT have been used as biomarkers of disease progression when assessing the efficacy of putative neuroprotective agents. Over the past years, clinical trials have been performed to evaluate the effects of dopamine agonists, levodopa, and possible neuroprotective agents such as riluzole and CEP1347 on the natural course of the disease in PD patients [41–47] (Table 2). In all these studies, drug efficacy was evaluated using both clinical and imaging outcome measures. The clinical endpoints in studies comparing dopamine agonists and levodopa have generally been the incidence or prevalence of dyskinesias after a given time interval. In studies of other possible neuroprotective agents the time to requiring levodopa treatment in de novo patients is a frequent measure of efficacy. Imaging outcomes were typically the change in dopamine terminal function between baseline and end-of-study evaluation as assessed by ^{18}F-dopa PET or ^{123}I-β-CIT SPECT.

To date, functional imaging studies in these trials have failed to demonstrate a clear-cut neuroprotective effect on nigrostriatal degeneration of agonists or other agents. In addition, discordance between clinical and imaging outcomes has been reported in dopamine agonist vs levodopa studies. Whereas dopamine terminal function declined more slowly when early PD patients were treated with dopamine agonists, as judged by imaging, the response of their parkinsonism was poorer. The ELLDOPA study was a randomized, double-blind, placebo-controlled trial designed to assess the effect of levodopa itself on the rate of progression of PD. Levodopa improved the clinical status of de novo PD cases over 9 months, even after a 2 week withdrawal period, but the mean percent decline in the ^{123}I-β-CIT uptake over this period was significantly greater with levodopa than placebo [46].

The poor correlation between clinical changes over time and decline in ^{18}F-dopa or ^{123}I-β-CIT uptake in studies comparing levodopa with placebo or agonists raises the issue of differential direct effects of these dopaminergic medications on imaging parameters acting as a confounding factor. Levodopa may act to depress DAT binding and AADC activity relative to dopamine agonists. The findings certainly prevent the use of these imaging techniques as surrogate biomarkers of disease progression. Additionally, if dopamine agonists are indeed neuroprotective or levodopa neurotoxic these effects may be masked by the greater clinical efficacy of the latter, particularly when short wash-out periods are employed. Finally, clinical rating scales are vulnerable to both patient and evaluator subjectivity. Further trials, possibly in untreated patients and with better study designs and longer wash-out periods are, therefore, needed in order to determine the contribution of possible confounding factors and to better validate imaging outcomes as biomarkers of disease progression. Despite the above criticisms, however, functional imaging still offers an objective method of assessing disease progression in PD. In trials of direct infusion of the growth factor GDNF [48] and implantation of fetal dopamine cells [49] into putamen ^{18}F-dopa PET provided proof of mechanism by detecting increased dopamine storage capacity after these treatments.

Table 2
Summary of clinical trials that have used imaging outcome measures to evaluate efficacy of putative neuroprotective agents

Study name	Study medication	Comparator	Imaging technique	Patient numbers	Duration of follow-up	Striatal decline[a]	
						Treated group	Comparison group
PELMOPET[42]	Pergolide	Levodopa	^{18}F-dopa PET	88	36 months	−7.9%	−14.5
056[43]	Ropinirole	Levodopa	^{18}F-dopa PET	45	24 months	−13	−18%
CALM-PD[44]	Pramipexole	Levodopa	^{123}I-β-CIT SPECT	82	46 months	−16%	−25.5%[c]
REAL-PET[45]	Ropinirole	Levodopa	^{18}F-dopa PET	186	24 months	−13.4%	−20.3%[b]
Riluzole[47]	Riluzole	Placebo	^{18}F-dopa PET	97	24 months		−15%
	50 mg					−21%	
	100 mg					−18%	

[a] Percentage of change from baseline as measured by ^{18}F-dopa PET or ^{123}I-β-CIT SPECT.
[b] $p<0.05$.
[c] $p=0.01$.

2.4. Involvement of non-dopaminergic pathways in PD

Although the nigrostriatal pathway is the neuronal system most severely affected in PD, it is now well established that the degenerative process also targets serotonergic, noradrenergic and cholinergic neurons. Post-mortem studies have shown involvement of the nucleus basalis of Meynert, the raphe nuclei and the locus ceruleus [50]. It is, therefore, likely that Lewy body pathology in the noradrenergic, serotonergic and cholinergic systems is responsible for the onset of some non-motor symptoms in PD. Non-motor symptoms, which can appear even before the onset of the classical motor symptoms, include hyposomia, autonomic dysfunction, sleep disorders, mood disorders, psychosis, impaired cognition and dementia.

^{18}F-dopa PET has been employed to evaluate the distribution and the function of serotonergic and noradrenergic systems in the brain. As ^{18}F-dopa uptake reflects the activity of AADC, which is present in the terminals of all monoaminergic neurones, measurements of its uptake into extrastriatal areas provide an index of the density of the serotonergic and noradrenergic along with dopaminergic terminals. Our group has recently used ^{18}F-dopa PET in a cross-sectional study to characterize extrastriatal monoamine neuronal dysfunction in PD. Interestingly, the midbrain raphe showed raised and the locus coeruleus normal ^{18}F-dopa uptake in early PD and then levels fell below normal in advanced patients. Uptake into the red nucleus, subthalamus, ventral thalamus and pineal gland was also targeted in more advanced patients, whereas limbic areas, except for hypothalamus, were spared even in late disease [51].

The serotonergic system has also been directly investigated in PD with several specific PET and SPECT ligands. Most of these studies were directed towards evaluating the role of serotonergic function in PD depression. The DAT ligand ^{123}I-β-CIT binds with lower affinity to serotonin transporters in the brainstem [52]. Using ^{123}I-β-CIT SPECT, Kim et al. [53] found no difference in levels of reduction of ^{123}I-β-CIT binding in the brainstem of PD patients with and without depression. Additionally, there were no correlations between radiotracer binding in this region and Hamilton Depression Rating Scale scores.

^{11}C-WAY-100635 PET is a marker of serotonin 5-HT$_{1A}$ receptors which act as autoreceptors on the soma of serotonergic neurones in the median raphe. A 25% reduction in binding of ^{11}C-WAY-100635 has been observed in the midbrain raphe of PD patients compared to healthy controls but the magnitude of reduction was similar whether depression was present or absent [54]. ^{11}C-DASB PET is a marker of brain serotonin transporter binding. In advanced, non-depressed PD patients, Guttman et al. have reported 20–30% reduced binding of ^{11}C-DASB in all examined brain areas compatible with a modest, widespread loss of brain serotonergic innervation [55]. The results from these studies, therefore, do not support a major role for serotonergic loss in PD depression. Interestingly, the severity of resting tremor in PD has been found to correlate with a decrease in median raphe 5-HT$_{1A}$ receptor binding, as measured by ^{11}C-WAY 100635 PET [56]. This observation suggests that midbrain tegmental rather than nigrostriatal pathology may be more relevant to the pathogenesis of parkinsonian tremor.

^{11}C-RT132 is a PET tracer that binds with similar nanomolar affinity to both dopamine and noradrenaline membrane transporters. This tracer has recently been used to assess noradrenergic and dopaminergic neurotransmission in PD patients with depression. Depressed PD patients showed lower ^{11}C-RT132 binding in locus coeruleus and areas of the limbic system than non-depressed PD patients. In areas with low dopaminergic innervation such as locus coeruleus, ^{11}C-RT132 uptake mainly reflects the density of noradrenergic neurons. In limbic areas, such as the ventral striatum and amygdala, ^{11}C-RT132 uptake reflects function of both dopaminergic and noradrenergic pathways [57]. This finding, therefore, suggests an important role for noradrenaline and limbic dopaminergic rather than serotonergic dysfunction in the pathogenesis of depression in patients with PD.

PET and SPECT have also been employed to assess the role of cholinergic deficiency in PD patients with dementia. The SPECT tracer ^{123}I-BMV is an *in vivo* marker of the vesicular acetylcholine transporter. Kuhl et al. have reported that PD patients without dementia showed reduced ^{123}I-BMV uptake in the parietal and occipital cortex, whereas PD with dementia had a more severe global reduction of cortical binding similar to that seen in Alzheimer's disease patients [58]. Hilker et al. have assayed cortical acethylcholinesterase activity in PD with ^{11}C-MP4A PET. They found that cholinergic function was lost throughout the cortex in parallel with the loss of striatal dopaminergic function in PD, demented cases showing the most severe reductions [59]. Cortical acethylcholinesterase activity in PD with and without dementia has also been investigated with ^{11}C-PMP PET. The greatest reduction in cortical acetylcholinesterase activity was again found in PD patients with dementia (PDD). Interestingly, patients with Alzheimer's disease, despite being matched to PD patients for dementia severity, showed a lesser reduction in cholinergic innervation to PDD, the lateral temporal cortex being selectively targeted [60]. This result supports the usage of acethylcholinesterase inhibitors in PD dementia as well as Alzheimer's disease.

Finally, PET ligands have recently been developed for imaging β-amyloid plaques in Alzheimer's disease and other dementias. We have used ^{11}C-PIB PET, a marker for fibrillar β-amyloid plaques, and ^{18}F-FDG PET to correlate *in vivo* regional brain β-amyloid load with glucose metabolism in seven PD patients with later onset dementia [61]. None of these seven PDD patients showed any increase in ^{11}C-PIB uptake despite the presence of significantly reduced glucose metabolism in frontal, temporal, parietal and occipital association areas. These findings suggest that β-amyloid deposition does not contribute significantly to the pathogenesis of later onset dementia in PD.

2.5. Neuroinflammation in PD

^{11}C-(R)-PK11195 PET is a marker of microglial activation, the natural immune defence to brain injury. Two recent papers have reported significantly increases in ^{11}C-(R)-PK11195 binding in both striatal and extrastriatal regions in PD patients compared to normal controls, suggesting increased levels of activated microglia in this condition [62,63]. Ouchi et al. reported a correlation between midbrain tracer uptake and reduced striatal DAT binding supporting the hypothesis that neuroinflammatory responses by activated microglia may contribute to dopamine neurone loss in PD [62].

3. Magnetic resonance imaging

MRI is far more widely available than PET and SPECT and is most commonly used in clinical practice to differentiate idiopathic PD from secondary causes of parkinsonism, such as vascular disease and other structural lesions. MRI findings may also help differentiate PD from multiple system atrophy by showing a reduced T2-weighted Putman signal, and progressive supranuclear palsy and cortical-basal degeneration by revealing midbrain and cortical atrophy [64]. There is an abundance of papers published on this topic, but they are beyond the scope of the current review and will not be discussed here further.

Conventional MRI is normal in patients with idiopathic PD without dementia, as standard MRI sequences have proved unable to detect definitive abnormalities in the basal ganglia structures. Several researchers have used MRI sequences designed to reveal changes in midbrain iron content as post-mortem studies in PD have shown an increase in iron concentration in the SN [65]. Early MRI studies failed to show significant differences in SN iron levels between PD patients and controls [66,67]. New MRI methodologies, however, appear to be more sensitive to iron increases in PD patients [68–71]. Using inversion recovery white and grey matter signal-suppression sequences, structural changes have been found in the SN of PD

patients, even at very early stages, with significant differences between patients and control group [68,69]. In one of these studies, MRI changes within the SN of PD patients correlated with a striatal dopaminergic function measured by ^{18}F-dopa PET. ^{18}F-dopa PET, however, was more reliable than inversion recovery MRI in discriminating patients with moderately severe PD from normal subjects [69]. Recently, Martin et al. [71] have assessed 26 untreated PD patients with a 3 T MRI and a multiple gradient echo sequence designed for rapid single-scan mapping of the proton transverse relaxation rate (R_2*). They found that PD patients had significantly higher R_2* values in the lateral SNc compared to controls. Interestingly, there was an association between the lateralized motor score from the clinically most affected side and R_2* values obtained from the opposite lateral SNc. Longitudinal studies are currently underway to assess the validity of nigral R_2* measurements as a marker of disease duration.

Voxel based morphometry (VBM) [72] is an MRI technique that localises significant changes in grey matter density related to disease. MR images are spatially normalized into standard stereotaxic space, segmented into gray and white matter and cerebrospinal fluid, smoothed and submitted to statistical parametric mapping at a voxel level. VBM has been used in PD to evaluate patterns of brain atrophy in patients with and without dementia [73–77]. These studies have revealed significant cortical atrophy in PD patients with dementia which progresses over time. Feldmann et al. [78] have recently reported VBM findings in a group of PD patients with and without depression. They found gray matter decrease in the bilateral orbitrofrontal cortex, the right superior temporal pole, and the limbic system of depressed PD patients.

Diffusion tensor imaging (DTI) and diffusion tensor tractography (DTT) are promising new MRI techniques which evaluate the integrity of tracts in white matter and, indirectly, neuronal connectivity in the brain. Normally water diffusion is constrained along nerve fibres in brain tissue and so is anisotropic. Degeneration of tracts leads to the loss of this directionality of diffusion or anisotropy. DTI measures the direction and magnitude of diffusivity of water molecules in tissues and can be used as an index of damage to neuronal tracts. There are two main quantities of interest: the mean apparent diffusion coefficient (ADC), which measures total molecular motion averaged over all directions, and fractional anisotropy (FA), which is a measure of the directional diffusivity of water. DTT is a computational procedure that reconstructs major fiber bundles in the brain based on the anisotropy of water movement in myelinated white matter. Yoshikawa et al. [79] and Chan et al. [80] have reported lower values of FA in the SN of PD patients compared to controls. FA values were inversely correlated with disease severity [80]. Another study has shown evidence of olfactory tract degeneration in early PD patients [81]. This finding is in line with the 50% prevalence of hyposmia in PD on UPSIT testing and gives support to the staging procedure proposed by Braak. Finally, DTI has been used to investigate non-motor symptoms of PD. Matsui et al. scanned a group of PD patients with and without depression and found bilateral abnormalities in the anterior cingulate in depressed patients [82]. The same group also compared PD patients with and without dementia and found abnormalities in the posterior cingulate in the latter [83]. These interesting findings remain to be confirmed by larger studies.

4. Transcranial sonography

Transcranial brain sonography (TCS) is a neuroimaging technique which measures brain tissue echogenicity through the intact skull. TCS is usually performed using a phase-array ultrasound system with a 2.5 Mhz transducer. The butterfly-shaped mesencephalon can be identified through a preauricular acoustic bone window in most subjects.

The typical TCS finding in PD patients is an increased echogenicity from the lateral midbrain, probably arising from the SN and reflecting increased amounts of iron deposition. The SN, which in normal subjects appear as a small patchy area of slightly increased echogenicity, become more demarcated and identifiable in PD patients. SN hyperechogenicity has been reported in up to 90% of clinically probable PD patients assessed with this technique [84–86]. A similar finding, however, has also been reported in 17% of patients with essential tremor [87], 40% of depressed patients without signs of PD [88], and in 10% of healthy age-matched volunteers where it could possibly reflect subclinical involvement of the nigrostriatal system [89,90]. Conversely, normal SN echogenicity is observed in atypical parkinsonian syndromes despite involvement of the dopaminergic system and this may help in the differential diagnosis with PD [91]. Levels of nigral echogenicity in PD do not correlate with striatal dopamine transporter binding measured with ^{123}I-FP-CIT SPECT [92] and do not appear to change over a five-year follow-up period despite clinical progression [93]. This suggests that SN hyperechogenicity is a trait rather than state marker of PD, probably reflecting disturbances of iron metabolism rather than neuronal degeneration.

5. Future perspectives

Neuroprotection remains an open challenge in PD research and it is expected that in the near future novel putative neuroprotective agents will become available which will need to be tested. Current imaging biomarkers have generally proved to be a valuable adjunct to clinical data when assessing both mechanism and efficacy of neuroprotective agents but they have also shown on occasion a discordance with clinical outcome. Further research should aim to determine direct drug effects on the imaging biomarkers currently available but also to develop new imaging techniques to longitudinally monitor progression and treatment of PD. Development of new radiotracers to target non-dopaminergic brain pathways and the glial reaction to disease is also needed. A better understanding of the involvement of non-dopaminergic structures in PD could potentially provide insight into non-motor symptoms experienced by subgroups of patients and hopefully rationalise the therapeutic options for the management of these disabling complications.

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