

***In vivo* assessment of non-dopaminergic
systems in Parkinson's disease with
Positron Emission Tomography**

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Abstract

Parkinson's disease (PD) is characterized by a progressive loss of nigrostriatal dopaminergic neurons. Non-dopaminergic neurotransmission is also impaired. Intraneuronal Lewy bodies, the pathological hallmark of PD, have been observed in serotonergic, noradrenergic, and cholinergic neurons. Dysfunction of these systems could play a role in the occurrence of non-motor symptoms including fatigue. However, the extent of non-dopaminergic degeneration in PD, rates of its progression, and its contribution to the development of non-motor symptoms is unclear.

First, I used ^{18}F -dopa Positron Emission Tomography (PET), a marker of monoaminergic terminal function, to assess the involvement of dopaminergic, noradrenergic, and serotonergic pathways in PD and in *parkin*-linked parkinsonism, a genetic form of PD. I found that *parkin* patients and PD patients have distinct patterns of monoaminergic involvement, with more widespread dysfunction in PD.

In a second study, I used serial ^{18}F -dopa PET to assess longitudinal changes in tracer uptake in brain monoaminergic structures over a 3-year period in a group of PD patients. I also assessed the relationship between striatal function decline and dysfunction in extra-striatal areas in the same patients. I found that the degeneration in extrastriatal monoaminergic structures in PD occurs independently from nigrostriatal degeneration and at a slower rate. Brain compensatory mechanisms disappear within the first years of disease.

I then used ^{18}F -dopa and ^{11}C -DASB PET to investigate whether fatigue in PD is associated with dysfunction of dopaminergic/serotonergic innervation. I found that PD patients with fatigue show severe loss of serotonergic innervation in basal ganglia and limbic areas.

Finally, I assessed the relationship between ^{18}F -dopa uptake and measurements of serotonin transporter availability by ^{11}C -DASB PET within brain serotonergic structures and I provided evidence for the hypothesis that ^{18}F -dopa PET can be used to evaluate the distribution and the function of serotonergic systems in the brain of PD patients.

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List of Abbreviations

^{11}C MP4A = ^{11}C -methylpiperidin-4-yl acetate

^{11}C -DASB = *N,N*-dimethyl-2-(2-amino-4-cyanophenylthio) benzylamine

^{11}C -PMP = ^{11}C - methyl-4-piperidinyl propionate

^{18}F - DOPAC = dihydroxyphenylacetic acid

^{18}F -dopa = ^{18}F -Fluorodopa

^{18}F -HVA = homovanillic acid

6-OHDA = 6-hydroxydopamine

A2A = adenosine A2A (receptor)

AADC= aromatic amino acid decarboxylase

AChE = acetylcholinesterase

AMPA = α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (receptor)

ARSAC = Administration of Radioactive Substances Advisory Committee

ATP = adenosine triphosphate

BA = Brodmann area

BP = binding potential

BP_{ND} = binding potential (non displaceable)

CALM-PD = comparison of the agonist pramipexole with levodopa on motor complications of parkinson's disease

CBD = corticobasal degeneration

CFS = chronic fatigue syndrome

D1 = dopamine D1 (receptor)

D2 = dopamine D2 (receptor)

D3 = dopamine D3 (receptor)

DAT = dopamine transporter

DBS = deep brain stimulation

DDS = dopamine dysregulation syndrome

DLPFC = dorsolateral prefrontal cortex

EDS = excessive daytime sleepiness

ELLDOPA = earlier vs. later levodopa

FDG = deoxyglucose
 FWHM = full width at half maximum
 GABA = γ -aminobutyric acid
 GDNF = glial cell line derived neurotrophic factor
 GLT1= glial glutamate transporter 1
 GPe = globus pallidus externa;
 GPi = globus pallidus interna;
 IBZM = ^{123}I -iodobenzamide
 Kc= uptake rate constants
 Ki = influx rate constant
 L = left
 LC =locus coeruleus
 LEU = levodopa equivalent units
 LOR = line of response
 LRRK2 = leucine-rich repeat kinase 2
 MNI = Montreal Neurological Institute
 MPP+ = 1-methyl-4-phenylpyridinium
 MPPP = 1-methyl-4-phenyl-4-propionoxypiperidine
 MPTP = 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
 MSA = multiple system atrophy,
 NMDA = N-methyl-D-aspartic acid (receptor)
 PD = Parkinson's disease
 PDD = Parkinson's disease with dementia
 PD-F = Parkinson's disease with fatigue
 PD-NF = Parkinson's disease patients without fatigue
 PELMOPET = pergolide-versus-L-dopa-monotherapy-and-positron-emission-tomography trial
 PET = positron emission tomography
 PFS-16 Parkinson Fatigue Scale
 PINK1 = phosphatase and tensin homolog-induced kinase 1
 PPN = pedunculopontine nucleus
 PPNc = pedunculopontine nucleus pars compacta

PPNd = pedunculopontine nucleus pars dissipatus
PRIAMO = Parkinson and non motor symptoms
PSP = progressive supranuclear palsy
R = right
rCBF = regional cerebral blood flow
rCMRglc = regional cerebral glucose metabolic rate
REAL-PET = Requip as early therapy versus L-dopa and PET
REM = rapid eye movement
RN = red nucleus
ROI = region of interest
SERT = serotonin transporter
SPECT = single photon emission computed tomography
SPM = statistical parametric mapping
SRTCM= simplified reference tissue compartmental model
SSRIs = selective serotonin reuptake inhibitors
STN = subthalamic nucleus
TSPO = translocator protein
UCHL1 = ubiquitin carboxy-terminal hydrolase L1
UPDRS = unified Parkinson's disease rating scale
UPS = ubiquitin-proteasome system
VAT = ventral anterior thalamus
VMAT2 = vesicular monoamine transporter

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Publications arising from this thesis

1. **Pavese, N.** & Brooks DJ. (2009). Imaging neurodegeneration in Parkinson's disease. *Biochimica et Biophysica Acta*, 1792(7), 722-729.
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7. **Pavese, N.**, Simpson, B.S, Metta, V., Ramlackhansingh A., Chaudhuri, K.R. & Brooks, D.J. (2010). ^{18}F -dopa uptake in the raphe nuclei complex reflects serotonin transporter availability. A combined ^{18}F -dopa and ^{11}C -DASB PET study in Parkinson's disease. *Neuroimage*, under peer review.

Acknowledgments and Declaration of originality

I declare that the work contained in this thesis is the work of my own.

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General introduction and Background

1

1.1 Idiopathic Parkinson's disease

Parkinson's disease (PD) is a chronic and progressive degenerative neurological disorder characterized by the degeneration of dopamine neurons in the substantia nigra, an important relay station in the motor system. The disease, which causes a prominent movement disorder, was first described by James Parkinson in 1817 and named paralysis agitans by Marshall Hall in 1841. Since then, remarkable advances have been made in understanding the pathophysiology of the disease and in developing effective symptomatic treatments. The introduction of levodopa, the amino-acid precursor of dopamine, in 1967 represented a milestone in the treatment of PD. The drug, which remains the mainstay approach for controlling PD symptoms, has significantly affected the natural history of the disease. Before the introduction of levodopa, PD caused severe disability in 16% of patients within 5 years of onset, in 37% in the following 5 years, and in 42% of those surviving for 15 years. The mortality rate was three times higher than that of the general population matched for age, sex and racial origin (Fahn, 1995). Treatment with levodopa and, more recently, with other dopamine replacement therapies have near normalized life expectancy; death rates in patients with PD and non-parkinsonian individuals are now approximately equal (Hohen, 1992). However, a longer disease duration will also result in the development of a more advanced and widespread neurodegenerative process, which in turn leads to the occurrence of more complex symptoms and complications. Additionally, complications of long-term exposure to levodopa must

now be considered as part of the natural history of the disease. For these reasons, PD is now considered the clinical manifestation of a multisystem degenerative process, affecting not only the dopaminergic system, as initially thought, but also serotonergic, cholinergic, noradrenergic, and other central neurotransmitter systems.

1.2 Epidemiology of Parkinson's disease

PD affects at least 4 million people worldwide. Although the disease targets all ethnic groups, some race/ethnicity groups seem to have a lower incidence, including the black populations of South Africa and Nigeria and African-Americans (Martilla and Rinne, 1989; Richards & Chaudhuri, 1996; Van Den Eeden et al., 2003). However, this may represent ascertainment bias as black persons living in Mississippi appeared to be affected to a similar degree as the white population (Schoenberg et al., 1985). Lower incidence has also been reported in Asian populations (Muangpaisan et al., 2009). Men have a higher prevalence rate than women. In general, it is reported that men are about 1.5 times more likely to develop PD than women. However, this difference is not the same across different studies, and is more pronounced in people with an older age of onset and in Western populations (Taylor et al., 2007). The disease onset 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 (de Rijk et al., 2000).

1.1 Aetiology and pathogenesis of Parkinson's disease

The aetiology of PD is unknown and a number of possible factors must be considered. In fact, it is likely that the cause of PD is multifactorial, with contributions from aging, genetic predisposition, exposure to toxins, and impaired oxidative mechanisms (Jankovic, 1993). These factors are briefly summarized in the following paragraphs.

1.3.1 Aging

Attrition of nigral neurons occurs as part of the normal aging process (Hirai, 1968; Mann, 1988). However, this cellular loss is most evident in the dorsal tier and pars lateralis of the substantia nigra pars compacta, whereas the pathological process in PD targets the ventrointermediate and ventrolateral regions of this structure - see paragraph 1.5.3. The nigral neuronal loss in PD patients has been estimated to occur ten times more rapidly than in normal aging, and motor symptoms begin when less than 60% of neurones remain (Gibb and Lees, 1994), suggesting that age is not the primary cause of PD. Nevertheless, advanced age seems to be a contributing factor for specific clinical features. Diederich and colleagues (2003) reported that PD patients with old age onset (>78 years) had more severe rigidity, bradykinesia, and axial impairment than patients with middle-age onset (between 43 and 66 years) with similar disease duration, even when adjusting for comorbidities. Severity of tremor, however, was not different between the two groups.

1.3.2 Environmental factors

In 1979, Davis and colleagues reported a case of chronic parkinsonism in a chemist after intravenous injection of a mixture of 1-methyl-4-phenyl-4-propionoxypiperidine (MPPP), a potent meperidine analogue, and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Shortly after this first observation, Ballard and colleagues (1985) reported 7 additional cases of parkinsonism induced by MPTP. Autopsy of these cases revealed selective destruction of the dopaminergic neurons of the pars compacta of the substantia nigra. MPTP is oxidized to MPP⁺ (1-methyl-4-phenylpyridinium) by the enzyme monoamine oxidase type B, one of the isoenzymes that catabolise dopamine. MPP⁺ is a highly toxic molecule that is taken up by the dopamine transporter, accumulates in mitochondria and interferes with the function of Complex I of the respiratory chain, causing depletion of ATP and cell death. MPTP is currently used to produce animal lesion models of PD in various animal species, including rodents and non-human primates. Other exogenous toxins which are reported to induce parkinsonism include manganese (Calne et al., 1994), carbon monoxide (Pavese et al., 1999), and tetrahydroisoquinolone (Yoshida et al., 1993).

Rural living, farming experience, herbicide or pesticide exposure, and industrial chemical exposure have been suggested to be associated with an increased risk of developing PD and are possibly related to exposure to unknown toxins (Mizuno et al., 2004). Rotenone and paraquat, two common pesticides, cause mitochondrial dysfunction and promote alpha-synuclein aggregation in rodents (Uversky et al., 2002).

A number of infections have been associated with parkinsonism. One of the most highlighted epidemics of viral parkinsonism is that following the outbreak of encephalitis lethargica (von Economo's encephalopathy) from 1917 to 1926. The main interest in this disease was the long latent period - in some cases years or decades - from acute infection to the appearance of parkinsonism, suggesting that the infection might have triggered premature death of nigral neurons. However, in postencephalitic parkinsonism the protein that aggregates abnormally is tau and not alpha-synuclein, in contrast to PD. Other viruses known to induce parkinsonism include Coxsackie, Japanese encephalitis B, St. Louis, West Nile and HIV viruses (Jang et al., 2009). However, virologic studies performed on brains of PD patients using electron microscopy and immunofluorescent studies have failed to detect specific viral particles or antibodies (Schwartz and Elizan, 1979).

A history of minor head trauma is also more common in PD (Semchuk et al., 1993).

1.3.3 Genetic factors

Although PD was initially considered to be a sporadic degenerative disorder, it is now well established that genetic factors are important in PD and that the disease can arise as a result of a variety of genetic mutations. Several studies have indicated that PD is more common among relatives of index cases. Over the last decade, possible causative genes have been investigated in several kindreds with family history of PD. So far 9 genetic defects have been identified in these families and linkages have been reported for at least four more (Farrer, 2006; Schapira, 2006; Lesage and Brice, 2009).

Recessive forms of young onset PD are known to be caused by mutations in *parkin*

(PARK2), *PINK1* (PARK6), *DJ-1* (PARK7) and *ATP13A2* (PARK9) genes.

Autosomal dominant forms of parkinsonism are caused by missense mutations and duplication of *alpha-synuclein* (PARK1/4), by mutations in the gene for *leucine-rich repeat kinase 2* (LRRK2) (PARK8) and *ubiquitin carboxy-terminal hydrolase L1* (UCHL1) (PARK5). Susceptibility genes such as mutations in the glucocerebrosidase A (GBA) gene have also been reported. From a clinical perspective, familial parkinsonisms often present with clinical features resembling idiopathic PD. However, neuropathological (Mori et al., 1998; Hayashi et al., 2000; van de Warrenburg et al., 2001; Farrer et al., 2001; Morales et al., 2002; Farrer et al., 2004; Sasaki et al., 2004; Zimprich et al., 2004; Khan et al., 2005a) and Positron Emission Tomography (PET) (Khan, 2002a; Khan, 2002b; Hering, 2004; Scherfler, 2004; Khan et al., 2005a; Adams et al., 2005; Hernandez et al., 2005) findings in these patients suggest that their pathology is distinct from PD. One of the aims of this thesis is to further clarify the pathological process in genetic forms of parkinsonism.

1.3.4 Abnormality in oxidative mechanisms

Aerobic cells use molecular oxygen as the terminal electron acceptor in oxidative phosphorylation, the mitochondrial metabolic pathway that leads to the generation of energy-storing adenosine triphosphate (ATP). On the other hand, oxidation reactions lead to the formation of reactive oxygen derivatives including superoxide anion radical, hydrogen peroxide, and hydroxy radicals. These highly reactive and unstable molecules with an unpaired electron can react with membrane lipids, inducing lipid peroxidation and cell damage. However, under physiological conditions, reactive oxygen derivatives are cleared by glutathione and oxyradical scavenger enzymes such as superoxide dismutases, catalases, glutathione peroxidase, and peroxiredoxins.

Oxidative stress is associated with increased production of oxidizing species or a significant decrease in the capability of antioxidant defences. Additionally, oxidation reactions are facilitated by accumulation of transition metals including iron, copper, and manganese.

The production of free radicals is accelerated in the dopaminergic neurons of the substantia nigra due to the oxidative metabolism of dopamine and the high

concentration of iron and neuromelanin, which has a high affinity for iron (Youdim et al., 1994).

PD patients have been reported to have increased iron levels and a 40 % decrease in levels of glutathione in the substantia nigra pars compacta both of which could promote a marked increase in oxidative reactions (Gerlach et al., 1994; Sian et al., 1994). Oxidative metabolism of exogenously administered levodopa could also potentially generate toxic free radicals and induce further oxidative stress. However, the effect of levodopa on dopamineergic neurons in vivo is controversial; in vitro cell culture studies have suggested it is toxic but in vivo studies have reported protective effects at therapeutic doses (Fahn, 1996; Murer et al., 1998; Conway et al., 2001). A recently performed randomized, double-blind, placebo-controlled trial to assess the effect of levodopa on the rate of progression of PD (ELLDOPA study) failed to give a definitive answer. The results of the ELLDOPA study will be further discussed in paragraph 1.6.2.1.3.

1.3.5 Pathogenesis

Despite the well-characterized pathological process, the molecular mechanisms, which lead to progressive neuronal degeneration in PD, remain elusive. Several factors and processes including oxidative stress and free radical generation, failure of the ubiquitin-proteasome system (UPS), mitochondrial dysfunction, glutamate receptor mediated excitotoxicity, apoptosis, and inflammation all seem to be involved in PD pathology.

The effects of oxidative stress have been described in the previous paragraph.

The UPS is a multicomponent pathway that identifies abnormal proteins and tags them with ubiquitin for degradation by the 26/20S proteasome. In addition to degrading misfolded and damaged proteins, the UPS system is also essential in regulating cellular signaling pathways involved in proliferation, adaptation to stress, regulation of cell size, and cell death.

In recent years, evidence for UPS dysfunction in PD has arisen from molecular studies in cell cultures, animal lesion models of PD, as well as human studies on postmortem brains from PD patients (McNaught et al., 2002; Petrucelli and Dawson, 2004;

Bukhatwa et al., 2010; Xie et al., 2010). These studies indicate that impaired capacity of the UPS to clear aberrant, misfolded proteins promotes their aggregation and degeneration of dopaminergic neurons.

Mitochondrial dysfunction results in oxidative stress, as respiratory failure leads to uncoupling of ATP production and excessive oxygen free-radical formation with consumption of glutathione. However, respiratory failure also results in cellular death via excitotoxic release of glutamate and influx of calcium into cells (Beal, 1992; Celsi et al., 2009). Several laboratories have reported deficiencies of mitochondrial electron transport chain complex I in the substantia nigra of PD patients (Mizuno et al., 1989; Schapira et al., 1990; Shapira et al., 1998). Interestingly, similar reductions in complex I have been reported in platelets, fibroblasts, and muscle of PD patients, although these findings are less consistent (Krige et al., 1992; Benecke et al., 1993; Anderson et al., 1993; Haas et al., 1995; Wiedemann, et al., 1999; Hanagasi et al., 2005).

An increasing body of evidence suggests that modifications in the mechanisms that control the apoptotic cascade, known as programmed cell death, may play a central role in the neurodegenerative process characterizing PD (Anglade et al., 1997; Tatton, 2000; Hartmann et al., 2001; Singh and Dikshit, 2007). Enhanced expression of several pro-inflammatory cytokines and apoptosis-related proteins including IL-2, IL-4, IL-6, interferon-gamma, NF-kappaB, CD95/Fas, and the tumor suppressor protein p53 has been reported in the parkinsonian brain (Mogi et al., 1994; Mogi et al., 1995; de la Monte et al., 1998; Mogi et al., 2007). A significant increase in caspase activation has also been observed in dopaminergic neurons of PD patients (Mogi et al., 2000; Hartmann et al., 2001; Anderson et al., 2001).

Excitotoxicity mediated by overactivity of glutamatergic output from the subthalamic nucleus is another proposed pathogenic mechanism for neuronal degeneration in PD (Choi, 1988; Beal, 1992). Excess glutamate release could induce excitotoxic damages in targets of the subthalamic nucleus including the nigra and pallidum (Rodriguez et al., 1998).

Activation of microglia in response to acute and chronic brain insults is thought to represent a reparative measure in the affected areas. These cells are able to promote

neuronal survival by releasing growth factors, walling off necrotic tissue, and stripping and remodeling synapses (Kim and de Vellis, 2005). However, there is cumulative evidence suggesting that activated microglia may also cause neuronal death through the release of a variety of cytokines and other neurotoxic factors (Wilms et al., 2003). It is possible that in conditions characterized by extensive chronic microglial activation, these harmful effects overcome the beneficial ones leading to a self-propagating cycle, where activated microglia promote further neurodegeneration and subsequent further induction of microglia activation with production of neurotoxic factors.

1.4 Clinical features of Parkinson's disease

1.4.1 Motor symptoms

PD is clinically diagnosed when asymmetrical limb bradykinesia along with one or more of the following resting tremor, rigidity or postural instability.

Bradykinesia is the most characteristic symptom of PD. The term refers to slowness in the initiation and execution of movements. Bradykinesia results in loss of facial expression, impairment of fine motor movements, and loss of spontaneous movements such as decreased arm swing and reduced blinking and swallowing. It also leads to hypophonic dysarthria with slowing of lip, tongue and pharyngeal muscles movements during talking, difficulty in rising from a chair and initiating gait, and shuffling gait with shorter steps.

Resting tremor is one of the most easily recognizable and noticeable features of PD and is the initial symptom in approximately 50% of patients (Jankovic, 2003). The tremor, characteristically in the 3-6Hz range, may appear unilaterally in the fingers and thumb or in a leg but eventually spreads to involve both the arm and leg and then the opposite limbs. In severe cases, the tremor involves all four limbs, the head, the lips and eyelids. Only 15% of the patients never have a tremor throughout their disease course (Jankovic, 2003).

Rigidity is increased muscle tone, which in combination with a resting tremor may produce a characteristic cogwheel phenomenon when the limb is passively moved. Rigidity usually appears unilaterally and proximally in an upper limb, spreading later to involve all the muscles of that limb and eventually the opposite side and muscles of the neck and trunk.

As PD becomes more advanced, many patients develop gait and balance problems and these can lead to recurrent falls. Unfortunately, neither postural instability nor tendency to fall are improved by dopaminergic therapy, implying that dopaminergic pathways may not be involved in the pathogenesis of these symptoms.

Recent observations suggest that dysfunction of the pedunculopontine nucleus (PPN) is an important causative factor for postural instability and gait disturbances. In animal models, lesioning of the PPN causes akinesia (Aziz et al., 1998; Munro-Davies et al., 1999). Conversely, akinesia in MPTP lesioned primate model of PD is improved by stimulating the PPN at low frequencies (Nandi et al., 2004). Further evidence pointing to the role of the PPN in modulating gait in humans comes from post-mortem studies of patients with advanced PD disease, progressive supranuclear palsy, and multisystem atrophy, as the extent of PPN degeneration in the brains of these patients correlates with their levels of pre-mortem gait dysfunction (Jellinger et al., 1988). Low frequency deep brain stimulation of the PPN is emerging as a treatment for postural and gait disorders non-levodopa responsive in PD patients.

Other motor features of PD include eyelid apraxia, blepharospasm, micrographia, and freezing of gait, a sudden and transient inability to move as if the feet were glued to the ground.

1.4.2. Non-motor symptoms spectrum

Non-motor symptoms are very common in PD patients but are generally under-appreciated by clinicians and healthcare professionals. The spectrum of non-motor symptoms in PD is wide and includes hyposmia, autonomic dysfunction, chronic

fatigue, sleep disorders, mood disorders, psychosis, impaired cognition and dementia (Chaudhuri et al., 2006). Some of these symptoms may be present long before the development of the classical motor syndrome (“pre-motor features”), while others complicate the advanced stages of the disease with a negative impact on patients’ quality of life.

Olfactory dysfunction (hyposmia) is a frequent non-motor symptom in PD patients, affecting up to 60% of patients. It generally is an early feature of PD, often preceding the motor symptoms so providing a potential marker of subclinical disease.

Autonomic dysfunction is commonly seen in the middle to late stages of PD. An early onset of autonomic features generally suggests atypical parkinsonian syndromes and particularly multiple system atrophy. Autonomic symptoms seen in PD patients include orthostatic hypotension, constipation, seborrhoea and excessive sweating, drooling, dysphagia, urinary disturbances, erectile difficulties and ejaculatory disturbances in the male and vaginismus and anorgasmia in the female.

Disabling fatigue is estimated to occur in about one-third of PD patients and manifests as a feeling of constant exhaustion or tiredness, either mental, physical, or both. (Brown et al., 2005). The reason for the high prevalence of fatigue in PD remains poorly understood. One of the aims of this thesis will be to elucidate possible pathophysiological mechanisms underlying chronic fatigue in patients with PD who experience this symptom.

Sleep disorders in PD can be categorised as insomnia, parasomnias (dreams / nightmares / somnambulism / sleep behavior disorder), and daytime somnolence (Garcia-Borreguero et al., 2003). Although sedative dopaminergic drugs and systemic problems can play a part in the pathogenesis of these disorders, it has been suggested that degeneration of the central nervous system neurons in PD may affect central sleep regulatory centres, which modulate wakefulness and REM sleep (Rye and Jankovic, 2002). These centres include the noradrenergic locus ceruleus, serotonergic dorsal

raphe, histaminergic tuberomammillary nucleus, dopaminergic ventral tegmental area, and the hypothalamus.

Depression is also common in PD having a reported prevalence that varies from 10 to 45%, depending on the criteria used for diagnosis (Burn, 2002). Symptoms of depression can precede the onset of motor symptoms in PD patients but the precise pathophysiological substrates of depression in PD remain obscure. Although a pure reactive basis cannot be excluded and may certainly play a significant role, it is also conceivable that depression in PD could be the result of damage to serotonergic and noradrenergic pathways along with loss of limbic dopaminergic neurotransmission.

Finally, the incidence of dementia in PD patients is about six-times higher than that in age-matched healthy people and increases exponentially with age. Reported prevalences vary from 0-80% across different series but averages at around 40% (Emre, 2003). Dementia in PD patients is characterized by a dysexecutive syndrome along with impairment of visuospatial capacities, attentional control, and short-term memory. Compared with Alzheimer's disease, language is relatively preserved. Many of these cognitive deficits are thought to be associated with degeneration of the medial substantia nigra and ventral tegmental area and subsequent loss of mesolimbic and mesocortical dopaminergic projections. However, reported pathological changes in PD patients with dementia also include: a) direct involvement of the cortex by Lewy body pathology, the pathological hallmark of PD, which targets the cingulate gyrus and association areas; b) cholinergic cell loss in the nucleus basalis of Meynert; c) incidental Alzheimer's disease; d) vascular pathology.

In summary, non-motor symptoms are associated with significant morbidity since they have functional effects on the patients' working and social life (Chaudhuri et al., 2006). However, many patients do not develop disabling non-motor symptom and continue to function relatively well, especially in early disease stages. The variability in the expression of non-motor symptoms reflects the heterogenous extent and progression of the degenerative process at extrastriatal sites.

1.5 The pathological characterization of Parkinson's disease

1.5.1 Pathological hallmarks of Parkinson's disease

The cardinal pathological feature of the PD consists of the formation of proteinaceous intraneuronal inclusions (Lewy bodies and Lewy neuritis) and progressive neuronal loss particularly targeting the substantia nigra pars compacta.

1.5.2 Lewy bodies and Lewy neurites

The presence of Lewy bodies and Lewy neurites is a prerequisite for the post mortem diagnosis of PD. The major component of Lewy bodies and Lewy neuritis is an aggregated form of alpha-synuclein, a presynaptic protein that is probably involved in controlling synaptic plasticity (Mezey et al., 1998). The mechanisms leading to the development of Lewy neurites and Lewy body remain unknown. Oxidative stress and nitrosylation could cause a change in the conformation of normal alpha-synuclein molecules leading to aggregation and formation of insoluble intraneuronal inclusions (Mezey et al., 1998).

1.5.3 Nigrostriatal dysfunction

The substantia nigra, the largest nucleus in the midbrain, is an anatomically heterogeneous structure that is composed of two zones: the pars reticulata, a pale ventral area with a relatively small number of cells, and the pars compacta, a darkly pigmented dorsal area packed with dopaminergic perikarya that contain neuromelanin, a polymer of dopamine or its metabolite (Hassler, 1937). The pars compacta is divided into two tiers: ventral and dorsal. The ventral tier has two further divisions (ventro-medial and ventro-lateral), whereas the dorsal tier has three further divisions: dorso-medial, dorso-lateral, and pars lateralis.

The initial neuronal loss in the substantia nigra in PD involves the caudal pars compacta in the lateral ventral tier, which projects specifically to dorso-lateral putamen (Fearnley and Lees, 1991; Damier et al., 1999). As PD progresses, nigral pathology extends medially and dorsally into the dorsal tier and the contiguous ventral

tegmental area (Damier et al., 1999; Fearnley and Lees, 1991), with consequent loss of nigral projections to the rostral putamen, caudate nucleus, nucleus accumbens, and globus pallidus (Kish et al., 1988; Jan et al., 2000).

Loss of nigral cells results in profound dopamine depletion in the nigrostriatal projections, which, in turn, leads to increased inhibitory output activity from the basal ganglia to the ventral thalamus and the frontal cortex implicated in the control of motor performance. When 80% of striatal dopamine and 50% of the nigra compacta cells have been lost, classical motor signs of PD become manifested (Fearnley and Lees, 1991). The classical model of basal ganglia function proposes that signals from the cerebral cortex loop through the basal ganglia and are then returned after focussing and filtering back to the cortex (Obeso et al., 2000). Afferent inputs from the cerebral cortex and the substantia nigra pars compacta converge on the striatum, from which two pathways with opposing effects project to the internal pallidum, the main output nucleus of the basal ganglia. The direct pathway involves monosynaptic outputs from the striatum that inhibit the globus pallidus interna and the substantia nigra pars reticulata. The indirect pathway involves polysynaptic outputs from the striatum to globus pallidus interna via inhibition of the globus pallidus externa, which in turn inhibits the subthalamic nucleus. The subthalamic nucleus sends excitatory output to the globus pallidus interna and the substantia nigra pars reticulata. Therefore, the direct and indirect pathways have opposing actions on the globus pallidus interna. Activity of the direct pathway inhibits internal pallidum output, while activity in the indirect pathway facilitates internal pallidum output. Projections from the globus pallidus interna and the substantia nigra pars reticulata inhibit the ventral lateral nucleus of the thalamus which send excitatory efferents to the cerebral cortex. The net effect is that the direct pathway acts to facilitate thalamocortical activity, whereas activity in the indirect pathway inhibits this. Dopamine activates the direct and inhibits the indirect pathway. Therefore, reduction of dopamine levels in PD induces increased inhibitory output from the globus pallidus interna/substantia nigra par reticulata with subsequent increased inhibition of the thalamocortical pathway leading to suppression of movement.

1.5.4 Involvement of non-dopaminergic pathways

Although nigrostriatal dopaminergic denervation remains the pathological hallmark of PD, neuropathological studies and biochemical and neuroimaging findings in PD patients have documented the occurrence of concomitant degeneration in non-dopaminergic pathways, including serotonin, noradrenaline, and acetylcholine neuronal systems. Dysfunction of these systems could contribute to the development of both motor and non-motor features and may provide targets for pharmacological interventions to treat these symptoms.

The pathological evidence of the involvement of non-dopaminergic pathways in PD is briefly reviewed in the following paragraphs. Neuroimaging findings will be discussed elsewhere (paragraph 1.6.3).

1.5.4.1 Involvement of serotonergic pathways

In the normal brain, serotonergic neurons are located predominantly in the raphe nuclei complex in the midline of the brainstem (Dahlström and Fuxe, 1964). The more rostral cell groups in the pontine and midbrain raphe including the dorsal raphe nuclei send extensive projections to the basal ganglia, frontal cortex, limbic system, and diencephalon, whereas the caudal raphe nuclei in the medulla project towards the spinal cord and brain stem.

A reduction of serotonin and its metabolite has been reported in brains of patients with PD, although not to the same degree as dopamine loss (Scatton et al., 1983; Shannak et al., 1994; Kish et al., 2008). In early post-mortem studies depletion of serotonin was observed in hypothalamus (Shannak et al., 1994) as well as in the caudate nucleus, hippocampus and frontal cortex (Scatton et al., 1983) of PD patients. The 5-hydroxyindoleacetic acid to serotonin ratio, a catabolic index to identify changes in the ratio of catabolic processes and transmitter synthesis, appears to be increased in the caudate nucleus and frontal cortex but not in the other cortical areas (Scatton et al., 1983). A recent pathological study has extended some of these early findings, showing preferential loss of serotonin in the caudate compared with the putamen (Kish et al., 2008). However, some patients had normal levels in both structures. Other serotonin

markers including serotonin transporter, 5-hydroxyindoleacetic acid, and tryptophan hydroxylase were also reduced, with the caudate more affected than putamen. The loss of striatal serotonin in PD may be secondary to neurodegeneration within the raphe nuclei. Indeed, Lewy bodies are seen in the raphe nuclei in association with neuronal loss (D'Amato et al., 1987; Halliday et al., 1990 Braak et al., 2003). However, none of these studies reported any correlation with motor disability, dyskinesia, mood or psychiatric comorbidities. Conversely, Paulus and Jellinger (1991) reported a lower density of neurons in the dorsal raphe nuclei in PD patients with depression compared to non-depressed PD patients. Depressed PD patients also show reduced cerebrospinal fluid levels of 5-hydroxyindoleacetic acid (Mayeux et al., 1984).

1.5.4.2 Involvement of cholinergic pathways

The nucleus basalis of Meynert and the PPN in the brainstem, the two major sources of cholinergic projections in the brain, are affected in PD. The nucleus basalis together with the medial septum, and the vertical and horizontal limbs of the diagonal band of Broca constitute the basal forebrain cholinergic complex which extensively innervate the cerebral cortex (Mesulam and Geula, 1988), whereas brainstem cholinergic neurons primarily innervate thalamus, cerebellum, several brainstem nuclei, the striatum, and the spinal cord (Pahapill and Lozano, 2000; Hamani et al., 2007). Several post-mortem studies have shown that significant loss of cholinergic neurons in the nucleus basalis is an early phenomenon in PD (Candy et al., 1983, Arendt et al., 1983; Nakano and Hirano, 1984; Tagliavini et al., 1984). Tagliavini and colleagues (1984) reported a mean 45.8% reduction in nucleus basalis neurons of PD patients compared to age-matched controls. Additionally, numerous surviving cells contained Lewy inclusion bodies. The percentage loss of cells in these patients (range from 29.9% to 68.3%) did not correlate with the duration of illness, age of the patients, their mental state or drug regime. There is evidence that the neuronal loss from the nucleus of Meynert may be greater in PD than in Alzheimer's disease (Arendt et al., 1983). Cortical acetylcholinesterase activity, a presynaptic cholinergic marker, is also decreased significantly in the frontal cortex of both demented and non-demented

parkinsonian subjects compared to controls, probably as a consequence of the dysfunction of the basal forebrain cholinergic complex (Ruberg et al., 1986).

The PPN comprises two histochemically different structures, the pars compacta (PPNc) and the pars dissipatus (PPNd), which are thought to have different physiological roles. The PPNc has predominantly cholinergic neurons and is part of the ascending reticular activating system. It is involved in frontal cortical activation, including focused attention during behavioural tasks and the sleep wake cycle (Brudzynski et al., 1986; Rye, 1997; Pahapill and Lozano, 2000). The PPNd contains glutamatergic projections and is involved in the generation of axial and proximal gross limb movements (Brudzynski et al., 1986; Nandi et al., 2002; Winn, 2006).

Human post-mortem studies in Parkinsonian patients demonstrate loss of cholinergic neurones in PPNc, the degree of which clinically correlates with the patients' pre-mortal gait dysfunction and postural instability (Hirsch et al., 1987; Jellinger, 1988; Zweig et al., 1989).

The PPN is a novel target for deep brain stimulation (DBS), and low frequency modulation alleviates postural instability and gait dysfunction.

1.5.4.3 Involvement of noradrenergic pathways

Compared with dopamine deficiency, the degree of noradrenaline decrease in PD is moderate (Ehringer and Hornykiewicz, 1998). In the normal brain there are two main noradrenergic pathways. The caudal complex is composed of groups of neurons mainly located in the medullary ventrolateral reticular formation, the dorsal vagal complex, and the nucleus of the solitary tract. These noradrenergic neurons project to the hypothalamus, the locus coeruleus, and the spinal cord (Moore and Bloom, 1979). The rostral complex is located in the pons and mainly comprises the locus ceruleus and the subceruleus. The locus coeruleus is the principal noradrenergic cell group in brain. Through a widespread efferent projection system, this nucleus supplies noradrenaline throughout the entire brain (Moore and Bloom, 1979). The involvement of locus coeruleus in PD has been well documented by various neuropathological series (Mann, 1983; German et al., 1992; Forno, 1996). Intracellular Lewy bodies

(Forno, 1996), neurofibrillary tangles, neuronal loss, and reactive fibrosis have all been observed (Bertrand et al., 1997). Bertrand and colleagues (1997) have reported a 70 % reduction of coeruleus neurons in PD patients compared with age-matched controls. Degeneration was more pronounced in the middle and the caudate segment of the locus coeruleus, although the entire nucleus was affected. The authors suggested that the neuronal loss might begin in the middle segment of the nucleus and subsequently spread towards its caudal part. However, other authors have reported comparable cell loss throughout the rostral-caudal extent of the nucleus in PD patients (German et al., 1992). Similar magnitudes of reduced noradrenergic innervation of the motor and prefrontal regions of the cortex have been reported in PD patients (Gaspar et al., 1991).

1.5.4.4 Involvement of other neurotransmitters/modulators

The concentrations of GABA in the striatum of PD patients can be increased compared to age-matched healthy volunteers (Perry et al., 1983; Kish et al., 1986). The GABA elevation appears to be most pronounced in the caudal subdivision of the putamen (Kish et al., 1986). Milder non-significant elevations of GABA levels were observed in the intermediate and rostral putamen, in the head of the caudate nucleus, and in the external and internal globus pallidus, whereas levels in other extrastriatal brain areas including thalamus, subthalamic nucleus and substantia nigra were normal. A significant negative correlation between increased GABA levels and reduced dopamine levels was observed in the caudal putamen (Kish et al., 1986).

Glutamate concentrations can also be elevated in the PD striatum compared to age-matched controls, with a statistically significant increase in the putamen and a trend towards increased concentrations in the subthalamic nucleus (Kish et al., 1986). Abnormalities in glutamate transmission have been associated with the presence of levodopa-induced dyskinesias in animal models of PD (Oh et al., 1998; Chase et al., 2000; Nash et al., 2002; Calon et al., 2002; Robelet et al., 2004). High levels of extracellular glutamate and increased expression of the glial glutamate transporter GLT1 have been observed in the striatum and substantia nigra of dyskinetic 6-OHDA lesioned rats after chronic levodopa treatment (Robelet et al., 2004). When nigral

lesioned rats develop dyskinesias after chronic levodopa exposure increased phosphorylation of striatal NMDA receptor subunits is found (Chase et al., 2000) while NMDA and AMPA receptor upregulation is seen in MPTP-lesioned monkeys (Calon et al., 2002).

In the human brain, the striatum contains the highest concentrations of adenosine A2A receptors (Svenningsson et al., 1997; Svenningsson et al., 1999; Jenner et al., 2009). A2A sites are found mainly on the spinous processes of the medium spiny neurones of the indirect pathway (Jenner et al., 2009) and are G protein coupled. Their activation leads to inhibition of D2 receptors and an overall suppression of indirect pathway activity (Svenningsson et al., 1999). A2A receptor mRNA expression has been reported to be elevated in dyskinetic patients (Calon et al., 2004) and in dyskinetic animal models of PD (Kaelin-Lang et al., 2000).

Finally, enkephalin and dynorphin are transmitted by functionally distinct indirect and direct striatal GABAergic pathways to the external and internal globus pallidus and are thought to play a neuromodulatory role in motor function (Austin and Kalivas, 1990). High levels of enkephalin are observed in the external pallidum and striatum of 6-OHDA-lesioned rats after levodopa replacement therapy (Engber et al., 1991; Taylor et al., 1992), and increases in enkephalin gene expression have been reported in the putamen of MPTP-exposed nonhuman primates with levodopa-induced dyskinesias (Jolkkonen et al., 1995). In PD patients with levodopa-induced dyskinesias, Nisbet and colleagues (1995) have reported statistically significant increases in preproenkephalin messenger RNA expression in the body of the caudate and in the intermediolateral putamen.

Since the intrinsic neuronal population of the basal ganglia is preserved in PD the observed changes in GABA, glutamate, adenosine and neuropeptide levels are likely to reflect changes in basal ganglia function secondary to loss of dopaminergic input and non-physiological exposure to exogenous levodopa.

1.5.5 The Braak hypothesis

The recognition of the importance of alpha-synuclein, the main component of Lewy bodies, in the pathophysiology of familial and sporadic PD (Trojanowski and Lee, 2003) has led to a new concept of the neuropathology of PD as a synucleinopathy. Using synuclein immuno-cytochemistry, Braak and colleagues (2004) have reported that the intraneuronal lesions in PD evolve sequentially, beginning in the dorsal nucleus of the vagus and ascending in a predictable manner throughout vulnerable regions of the gray matter. Based on this observation Braak and colleagues (2004) 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 and midbrain brain nuclei are only affected by stage 3, when motor symptoms appear. In stage 4 limbic areas become affected and, in stages 5 to 6, inclusions appear in the association and primary neocortex. Figure 1-1 shows the Braak stages in the development of PD.

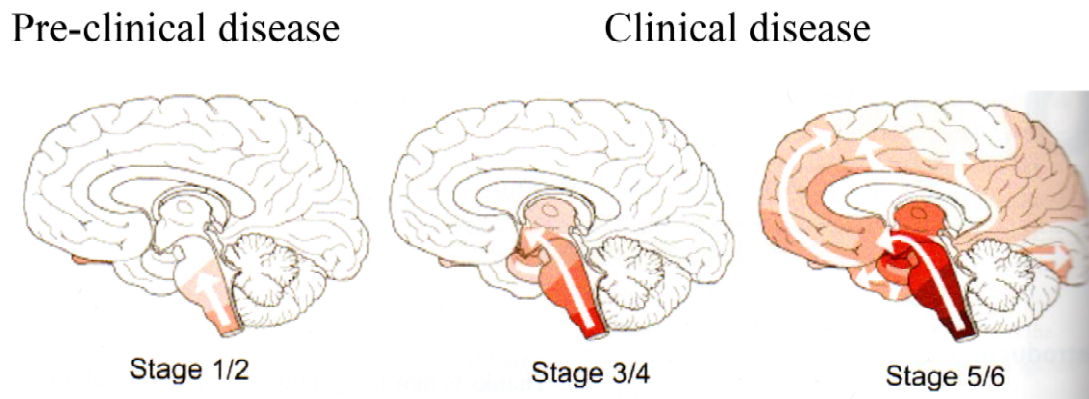


Figure 1-1 Braak stages in the development of Parkinson's disease. Lewy body pathology begins in the medulla oblongata, olfactory structures, and pons (stages 1 and 2). In stages 3 and 4, pathology progressively spreads to the substantia nigra, other midbrain brain nuclei, and the limbic areas. In stages 5 to 6, inclusions appear in the association and primary neocortex. First signs of motor parkinsonism start in stage 4 and 5

1.5.6 Controversies

In other neuropathologic studies only one third of incidental Lewy body cases conform to Braak staging (Dickson, 2010) and brains with prominent neurodegeneration and alpha-synuclein pathology in the substantia nigra pars compacta and higher brainstem structures in the absence of medullary involvement are recorded (Jellinger, 2003; Parkkinen et al., 2003; Kalaitzakis et al., 2008). Additionally, Braak staging is based exclusively on the presence of immunoreactive Lewy neurites and Lewy bodies, and it is unclear whether these alpha-synuclein-related pathologic changes correlate with neuronal dysfunction or loss.

1.6 Functional imaging with Positron Emission Tomography in Parkinson's disease

1.6.1 Introduction

Over the last two decades, functional neuroimaging with positron emission tomography (PET) has increasingly been employed to understand the complex pattern of basal ganglia functional neuroanatomy in humans and to elucidate pathophysiological mechanisms underlying PD and other common movement disorders. To date, PET has been mainly employed in PD to evaluate: (1) presynaptic dopaminergic terminal function, (2) postsynaptic dopaminergic receptor status, (3) the changes in levels of dopamine in the synaptic cleft, (4) changes in cerebral metabolism, (5) the consequences of striatal dysfunction on cortical activation, (6) the contribution of neuroinflammation to the pathogenesis of PD, (7) changes in non-dopaminergic transmitters.

Figure 1–2 is a schematic representation of a striatal presynaptic dopamine terminal showing neurobiological substrates for imaging techniques.

1.6.2 Assessment of dopaminergic dysfunction

1.6.2.1 Presynaptic dopamine function

PET has extensively been employed to investigate presynaptic dopamine function in PD and atypical parkinsonisms. The technique detects the functional activity of intact

dopaminergic neurons within the affected striatum. This can be achieved by measuring: (1) striatal aromatic amino acid decarboxylase (AADC) activity with ^{18}F -dopa PET; (2) availability of presynaptic dopamine transporter (DAT), which is the plasma membrane transporter responsible for the high-affinity uptake of dopamine, with PET tracers such as ^{11}C -CFT, ^{18}F -CFT, and ^{11}C -RTI-32; (3) the density of vesicular monoamine transporter, which is the vesicular transporter responsible for the transport of dopamine from the cytoplasm into secretory vesicles, with $^{11}\text{C}/^{18}\text{F}$ -dihydro-tetrabenazine PET. Table 1-1 summarizes functional imaging strategies for the assessment of presynaptic nigrostriatal terminals.

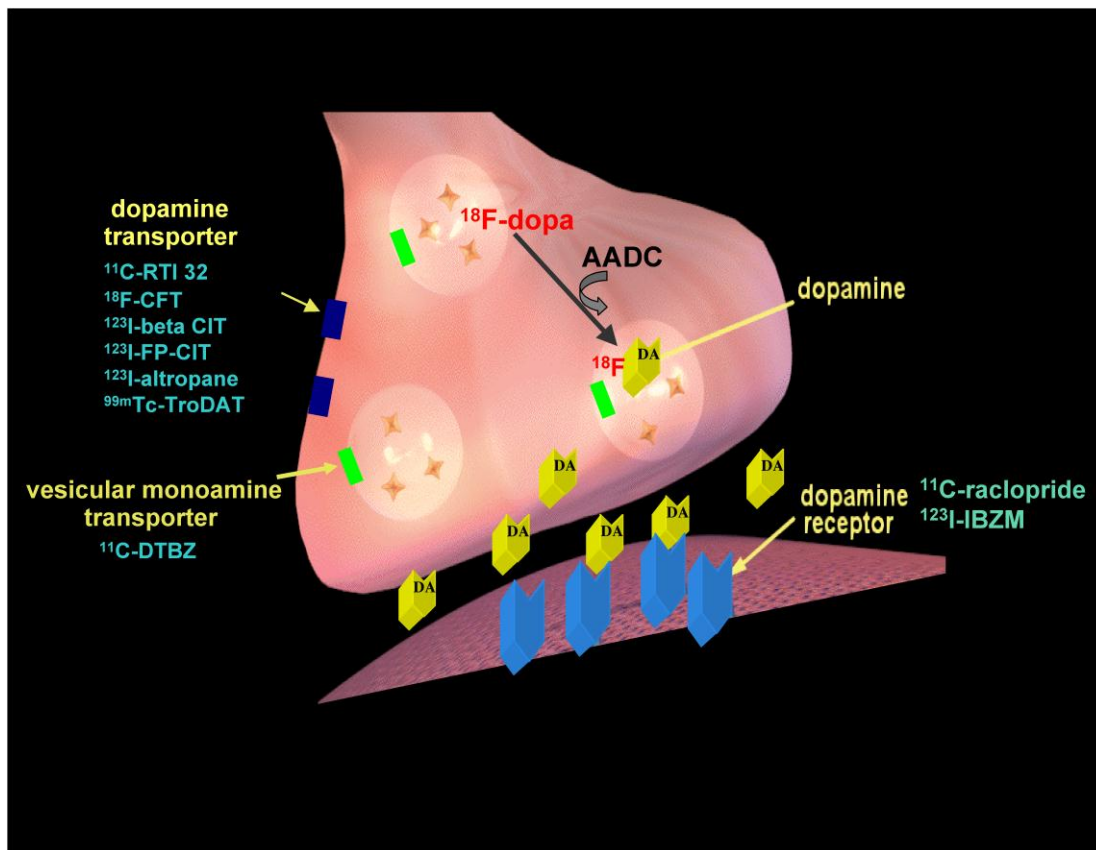


Figure 1–2. Schematic representation of a striatal presynaptic dopamine terminal showing neurobiological substrates of PET tracers. SPECT tracers for dopamine receptors (^{123}I -IBZM) and dopamine transporter (^{123}I -FP-CIT, ^{123}I -altropane, $^{99\text{m}}\text{TC}$ -TroDAT) are also represented

^{18}F -dopa PET was the first neuroimaging technique used in research. The metabolism of intravenously injected ^{18}F -dopa will be described in detail in chapter 3. Briefly, striatal uptake of ^{18}F -dopa depends upon five processes: (a) transport across the blood-brain barrier, (b) uptake into dopaminergic neurons, (c) metabolism by AADC, (d) vesicular storage of ^{18}F -dopamine, (e) degradation of ^{18}F -dopamine. Among these processes, AADC activity and density of the axonal terminal plexus are the principal determinants of ^{18}F -dopa uptake as measured by the influx constant K_i over 90 minutes. Therefore, measurements of ^{18}F -dopa uptake in the striatum in PD reflect the number of remaining dopaminergic cells. This is supported by human and MPTP-lesioned monkey autopsy studies which have demonstrated that reduced striatal ^{18}F -dopa uptake correlated well with nigral neuronal counts (Snow et al., 1993; Pate et al., 1993). However, in early stages of disease, ^{18}F -dopa PET may underestimate the extent of the degenerative process due to compensatory upregulation of AADC in functioning terminals (Ribeiro et al., 2002).

Putamen uptake of ^{18}F -dopa in PD correlates with motor impairment. Several authors have described a significant inverse correlation between ^{18}F -dopa uptake and the degree of motor disability as measured by the Hoehn and Yahr and Unified Parkinson's Disease Rating (UPDRS) scales (Brooks et al., 1990a; Broussolle et al., 1999; Vingerhoets et al., 1997).

Interestingly, putamen levels of ^{18}F -dopa uptake correlate inversely with the degree of rigidity and bradykinesia but not with severity of tremor suggesting that pathways other than the nigrostriatal pathway might be implicated in the pathogenesis of this last symptom (Otsuka et al., 1996).

Table 1.1 Summary of functional imaging strategies for the assessment of presynaptic nigrostriatal terminals		
Biological Marker	Neuroimaging Technique	Tracer
Metabolism of levodopa: (a) aminoacid transport into dopamine neurons (b) metabolism by AADC * (c) vesicular storage of ^{18}F -dopamine (d) metabolism of ^{18}F -dopamine to HVA and DOPAC	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
Type-2 vesicular monoamine transporter (VMAT2)	PET	$^{11}\text{C}/^{18}\text{F}$ -dihydrotetrabenazine

AADC= aromatic amino acid decarboxylase, * ^{18}F -dopa uptake primarily reflects AADC activity

As discussed in paragraph 1.5.3, the neurodegenerative process in PD is not uniform. The ventrolateral nigral dopaminergic projections to the dorsal putamen are more affected than the dorsomedial projections to the head of caudate (Fearnley and Less, 1991). Similarly, reduction in ^{18}F -dopa uptake is maximal in the putamen when compared to the caudate. Patients with hemiparkinsonism have more pronounced reduction in the dorsal posterior putamen contralateral to the side of clinical symptoms (Morrish et al., 1995), whereas patients with bilateral disease present with reductions extending into the ventral and anterior putamen and dorsal caudate. Finally, when PD is in more advanced stages, the uptake within the ventral head of caudate also falls.

^{18}F -dopa has also been used to assess disease progression *in vivo*. Several series have now demonstrated that loss of striatal ^{18}F -dopa uptake occurs more rapidly in PD patients than in age-matched controls (Vingerrhoets et al., 1994, Morrish et al., 1996, 1998, Nurmi et al., 2001, 2003). In early PD patients, putaminal ^{18}F -dopa uptake has been reported to decline annually by around 10% of baseline (5% of the normal mean), whereas when disease duration is longer, the annual loss is reduced (Morrish et al., 1996). Caudate uptake decreases at a slower rate than putamen. It must be acknowledged that decreases in striatal ^{18}F -dopa uptake over the time do not provide an accurate measure of the neurodegenerative process as they reflect both a failure of compensatory mechanisms (AADC upregulation) and neuron loss. Nonetheless, this approach potentially provides an objective biological marker for objectively monitoring disease progression *in vivo* in PD.

In more recent years, the possibility of analyzing changes in ^{18}F -dopa uptake across the whole brain at a voxel level with statistical parametric mapping (SPM) has enabled several groups to explore compensatory responses in extrastriatal pathways at different stages of the disease. Increased ^{18}F -dopa uptake, which probably reflects both a compensatory hyperactivity of AADC and uptake of ^{18}F -dopa into serotonergic terminals, in dorsolateral prefrontal cortex, anterior cingulate, and globus pallidus interna compared to normal controls was found in early PD patients but not in more advanced cases (Rakshi et al., 1999; Kaasinen et al., 2000; Whone et al., 2003).

Interestingly, globus pallidus interna ^{18}F -dopa uptake is increased by 30–40% at the onset of PD symptoms - whereas putamen ^{18}F -dopa uptake is reduced by 30–40% - but it subsequently falls as the disease advances and symptoms become more severe (Whone et al., 2003). Enhanced function of the dopamine projections to the globus pallidus interna is possibly important in maintaining a normal pattern of pallidal output to the ventral thalamus and motor cortex in early PD, whereas the loss of this compensatory pathway in advanced disease may be a pivotal step in the progression of the disease.

^{11}C -dihydrotetrabenazine is a PET tracer that binds to the vesicular monoamine transporter (VMAT2). Lee and colleagues (2000) compared striatal PET measurements obtained with ^{11}C -dihydrotetrabenazine, ^{18}F -dopa, and ^{11}C -methylphenidate, a DAT ligand. They found that ^{18}F -dopa K_i was reduced less than ^{11}C -dihydrotetrabenazine binding potential in the parkinsonian striatum, whereas the ^{11}C -methylphenidate binding potential was reduced more than the ^{11}C -dihydrotetrabenazine binding potential. This finding suggests the presence of relative AADC upregulation and DAT downregulation in the striatum of parkinsonian patients. It also indicates that ^{11}C -dihydrotetrabenazine binding probably gives the most reliable measurement of the density of dopaminergic terminals.

Presynaptic dopaminergic terminal function in PD can also be assessed with single photon emission computed tomography (SPECT). Several SPECT ligands (such as ^{123}I - β -CIT, ^{123}I -FP-CIT, ^{123}I -altropine, and $^{99\text{m}}\text{Tc}$ -TRODAT-1) are available for imaging DAT. Most of these ligands bind non-selectively to dopamine, noradrenaline, and serotonin transporters. However, since the majority of transporters within the striatum are dopaminergic, reductions in striatal uptake of these tracers provide a useful marker of DAT functional loss in that region. It must be taken into account that DAT binding may underestimate true terminal density due to the relative downregulation of DAT in remaining neurons as a response to reductions in synaptic dopamine. Additionally, there is evidence that DAT binding decreases with age (3.3 % to 10% per decade) in healthy subjects (vanDyck et al., 1995; Tissingh et al., 1997; Pirker et al., 2000).

The assessment of presynaptic dopaminergic dysfunction with PET has some important clinical applications which will be discussed below.

1.6.2.1.1 Detection of preclinical disease

In several studies, evaluation of the dopaminergic pre-synaptic system has allowed detection of preclinical dysfunction. In kindreds with familial PD, about 25% of asymptomatic family members showed abnormal reduction of putaminal ^{18}F -dopa uptake, and around one-third of those with abnormal imaging developed clinical parkinsonism over a five-year follow-up (Piccini et al., 1997a). Another study by the same authors has reported abnormal putaminal ^{18}F -dopa uptake in 18% of asymptomatic dizygotic co-twins and in 55% of asymptomatic monozygotic co-twins of patients with idiopathic PD. Subsequently, two of the ten asymptomatic monozygotic co-twins who had reduced ^{18}F -dopa uptake developed clinical parkinsonism over four years while all ten co-twins showed a decrease in putaminal ^{18}F -dopa uptake (Piccini et al., 1999a). Taken together, these findings suggest an important role for inheritance even in apparently sporadic PD. Khan and colleagues (2005b) have reported reductions of ^{18}F -dopa uptake in caudate putamen, ventral and dorsal midbrain in asymptomatic carriers of a single *parkin* mutation compared to normal subjects. However, the rate of nigrostriatal dysfunction progression seems to be slow in these subjects suggesting that heterozygous *parkin* gene mutations are unlikely to represent a major risk factor for late onset PD (Pavese et al., 2009). In summary, ^{18}F -dopa PET has been capable of detecting preclinical dopaminergic dysfunction when present in subjects who are at risk for PD. It is easy to predict that this ability will be of importance if neuroprotective agents become available for PD.

1.6.2.1.2 Differential diagnosis between Parkinson's disease, essential tremor, and other parkinsonian syndromes

All the above-mentioned PET tracers differentiate clinically probable PD from normal subjects or patients with essential tremor with a sensitivity of around 90%. Post-neuroleptic parkinsonism can be separated from PD as ^{18}F -dopa PET and DAT imaging show an intact pre-synaptic dopaminergic system in the former (Burn and

Brooks, 1993; Eerola et al., 2005). Unfortunately, the ability of ^{18}F -dopa PET and markers of DAT binding to differentiate between the different dopamine deficient parkinsonian syndromes is poor due to the overlap of individual ranges of putamen and caudate tracer uptake in PD, Multiple System Atrophy, and Progressive Supranuclear Palsy patients (Burn et al., 1994). Table 1-2 gives an overview of neuroimaging findings in parkinsonian syndromes.

1.6.2.1.3 Evaluation of disease modifying agents in Parkinson's disease

To date no treatment is available to slow down the progression of PD and agents with neuroprotective effects are greatly needed. Due to its capacity to objectively follow loss of dopamine terminal function in PD, ^{18}F -dopa PET provides a potential tool for monitoring the efficacy of such agents.

Over the past years, an increasing body of basic science evidences suggest that dopamine agonists may have neuroprotective effects by slowing down the progression of PD. Based on that, several clinical trials have been performed to directly compare the effects of dopamine agonists and LD on the natural course of PD in de novo patients (Table 1-3). In all these studies, the effect of the drugs on disease progression was evaluated using clinical and imaging outcome measures with ^{18}F -dopa PET or ^{123}I - β -CIT SPECT.

The first ^{18}F -dopa PET study to be reported was a 5-year double-blind trial comparing ropinirole with levodopa in 268 de novo patients (056 study) (Rascol et al., 2000). A small subgroup of these patients (N=45) had ^{18}F -dopa PET, which showed a trend towards slower loss of tracer uptake in the ropinirole group (Rakshi et al., 2002).

A more extensive ^{18}F -dopa PET study (REAL-PET) was conducted over two years in 186 patients with early PD randomized to ropinirole or levodopa (Whone et al., 2003). Region of interest analysis showed a significantly slower reduction in putaminal ^{18}F -dopa uptake over two years with ropinirole compared with levodopa (13.4% vs 20.3%).

Table 1.2 Summary of striatal functional imaging in Parkinson's disease and other parkinsonisms

	¹⁸ F-dopa uptake	DAT binding	Dopamine D2 receptor binding	Glucose metabolism
Parkinson's disease	reduced with a typical rostro-caudal gradient	reduced	increased in untreated patients/normal in treated patients	normal or increased in the striatum
MSA	reduced	reduced	reduced	reduced
PSP	reduced	reduced	reduced	reduced
CBD	reduced	reduced	reduced	reduced
Essential Tremor	normal	normal	normal	hyperactivity of cerebellar-thalamo-cortical pathways
Vascular parkinsonism	normal/reduced	normal/reduced	normal / reduced	reduced
Drug-induced parkinsonism	normal	normal	reduced	normal

DAT=Dopamine transporter, MSA=Multiple System Atrophy, PSP= Progressive Supranuclear Palsy, CBD=Corticobasal Degeneration

The PELMOPET study evaluated pergolide versus levodopa monotherapy in 294 PD patients over three years. Eighty-eight patients in this study underwent ^{18}F -dopa PET. Over three years, a similar decrease in mean putaminal Ki/Occ was found in the two groups, although a weak trend was observed in favour of pergolide (7.9%, vs 14.5% in the levodopa group) (Oertel et al., 2000).

An ^{18}F -dopa PET was performed to evaluate the neuroprotective effect of riluzole (daily doses of 50mg and 100 mg) versus placebo in early PD. The trial was interrupted after the first interim analysis (when half of the patients had completed the two year trial) as no clinical differences were seen between the three groups. Concordantly, in the subgroup of patients who had ^{18}F -dopa PET similar decreases in mean putaminal Ki values were seen in the patients whether treated with riluzole or placebo (Pavese et al., 2005).

In the CALM-PD trial, patients were randomized to double-blind therapy with levodopa or pramipexole. Eighty-two patients in this study had ^{123}I - β -CIT SPECT to assess striatal DAT binding (Parkinson Study Group, 2002). Patients initially treated with pramipexole demonstrated a significantly attenuated loss of striatal ^{123}I - β -CIT uptake from baseline compared with those in the levodopa group (16% vs 25.5%) at 46 months.

Finally, a randomized, double-blind, placebo-controlled trial has also been performed to assess the toxicity of levodopa by determining its effect on the rate of progression of PD as judged clinically and by changes in DAT availability (ELLDOPA study). This study failed to give a definitive answer, as clinical and imaging findings were discordant. Levodopa slowed the progression of PD in a dose dependent manner based on clinical ratings but the mean percent decline in the ^{123}I - β -CIT uptake was significantly greater with levodopa than with placebo (Fahn et al., 2004).

In conclusion, functional imaging studies in these trials have provided a valuable adjunct to clinical data but they have all failed to demonstrate an unequivocal neuroprotective effect of any of the agents trialled. It has been argued that several possible factors may have influenced and confounded the neuroimaging findings

including direct effects of the drug itself on tracer binding, differential compensatory up- or down-regulation of dopamine terminal function, and differential loss of cases from treatment arms. Despite these criticisms, functional imaging can offer an objective method of assessing disease progression in PD and remains an important potential adjunct for monitoring neuroprotection in this disease (Brooks, 2003). However, in designing future studies, which are greatly needed, all the above mentioned confounding factors must be addressed in order to better validate the imaging outcome.

Table 1-3. Clinical Trials of putative neuroprotective agents in Parkinson's disease

Study medication	Study Name	Imaging technique	Follow-up duration	Striatal decline in the treated group	Striatal decline in the comparison group
Ropinirole ¹	056	¹⁸ F-dopa PET	24 months	13%	18%
Pramipexole ¹	CALM-PD	¹²³ I-β-CIT SPECT	46 months	16%	25.5%
Ropinirole ¹	REAL-PET	¹⁸ F-dopa PET	24 months	13.4%	20.3%
Riluzole ²	NA	¹⁸ F-dopa PET	24 months	21% (50mg) 18% (100mg)	15%

1=compared to levodopa, 2=compared to placebo

1.6.2.1.4 Evaluation of restorative approaches in Parkinson's disease

The goal of restorative therapies in PD is to correct the basic chemical defect of the disease by increasing the production of dopamine in the brain. These procedures include transplantation of foetal or engineered stem cells, growth factors and viral gene therapy. At this time, all the restorative therapies are experimental and are not available as treatment. Transplantation of human foetal dopamine neurons in patients' striatum has been the most investigated among these procedures and ^{18}F -dopa PET has been used to demonstrate *in vivo* that graft cells survive and store dopamine. Several small open series showed significant clinical improvement and increases from baseline of ^{18}F -dopa uptake after striatal grafting (Remy et al., 1995; Wenning et al., 1997; Piccini et al., 1999b; Hauser et al., 1999; Brundin et al., 2000; Piccini et al., 2000). Using ^{18}F -dopa, it has been possible to demonstrate *in vivo* that graft cells survive up to 10 years. ^{11}C -raclopride PET provides a marker of fluxes in synaptic dopamine and has been used to show that grafts are able to release dopamine following a methamphetamine challenge (Piccini et al., 1999b). Most importantly, grafts form connections in the host brain which restore activation of motor cortical areas during limb movement (Piccini et al., 2000).

Unfortunately, the first two double blind controlled trials of foetal dopamine cell transplantation into putamen failed to replicate the promising results of the open studies. In fact, despite both post-mortem and ^{18}F -dopa evidence of graft function, neither of these trials demonstrated significant clinical improvements. Nevertheless, there were indications that younger, less affected patients could benefit from transplantation (Freed et al., 2001; Olanow et al., 2003). A serious adverse event seen in these two trials was the occurrence of problematic "off" period dyskinesias affecting up to 56% of the implanted patients. It has been suggested that graft induced dyskinesias may be associated with uncontrolled dopamine release from grafts and greater baseline dopamine loss in the ventral putamen (Ma et al., 2002). A recent review argued against these hypotheses (Hagell et al., 2002; Piccini et al., 2006). Piccini and colleagues have also used SPM to interrogate functional maps of ^{18}F -dopa

uptake of individual PD patients before and one and two years after neural transplant (2006). The patients with the best functional outcome after transplantation exhibited no dopaminergic denervation in areas outside the grafted areas either preoperatively or at one or two years post-operatively. In contrast, patients with no or modest clinical benefit showed reduction of ^{18}F -dopa in ventral striatum prior to or following transplantation. These findings indicate that poor outcome after transplantation is in part associated with progressive dopaminergic denervation in areas outside the grafted area, a process which may have started already before surgery.

Infusion of glial cell line derived neurotrophic factor (GDNF) via a catheter into the putamen is another restorative approach that has been tested in PD. GDNF, which is a potent neurotrophic factor, is known to protect dopamine neurones in rodent and primate lesion models of PD. Gill and colleagues have examined the safety and efficacy of putaminal infusion of GDNF in five patients with PD. A significant improvement in all UPDRS score was seen after 12 months of treatment and PET studies revealed a 28 % increase in putaminal ^{18}F -dopa uptake (Gill et al., 2003). A subsequent randomized placebo-controlled study, however, failed to show a sustained clinical benefit of this procedure (Lang et al., 2006).

1.6.2.2 Postsynaptic dopamine function

Both PET and SPECT ligands have been used to examine post-synaptic dopaminergic receptor availability in PD. ^{11}C -raclopride, a D2/3 receptor ligand, has been used in many PET studies. With one exception (Dentresangle et al., 1999), these studies have generally shown a mild increase in putaminal ^{11}C -raclopride binding in de novo (untreated) PD patients suggesting an increase in D2 receptor availability in early stages of the disease (Rinne et al., 1993; Sawle et al., 1993; Turjanski et al., 1997). In one study, ^{11}C -raclopride binding inversely correlated with presynaptic reduction of ^{18}F -dopa uptake in patients with early disease (Sawle et al., 1993). As disease progresses and dopaminergic therapy is started, ^{11}C -raclopride binding normalizes in the putamen and often decreases in the caudate (Antonini et al., 1994; Brooks et al., 1992; Turjanski et al., 1997; Dentresangle et al., 1999). Whilst increased putaminal

^{11}C -raclopride binding in de novo patients may represent a compensatory response involving receptor upregulation, it could equally reflect reduced competition from low levels of synaptic dopamine. Subsequent reduction of D2 binding in more advanced patients may reflect chronic exposure to dopaminergic treatment. The real significance of these changes in D2 receptor availability, however, remains unclear.

Scherfler and co-workers (2006) investigated D2 receptor availability in patients with *parkin*-linked parkinsonism. Interestingly, never-treated patients, similarly to idiopathic PD patients, showed an increased putaminal ^{11}C -raclopride binding compared to normal subjects, whereas levodopa-treated patients showed significant reductions in ^{11}C -raclopride binding in both caudate and putamen. The authors interpreted these findings as parkin cases having a greater sensitivity to D2 downregulation following exposure to dopaminergic medication compared with idiopathic PD patients.

The availability of post-synaptic D2 receptors can also be evaluated with the SPECT tracer ^{123}I -iodobenzamide (IBZM). Striatal ^{123}I -IBZM binding is normal in patients with early PD and is either normal or mildly reduced in patients with more advanced disease (Playford and Brooks, 1992; Schwarz et al., 1993; Schulz et al., 1994). In contrast, striatal ^{123}I -IBZM binding is more consistently reduced in multiple system atrophy suggesting that ^{123}I -IBZM SPECT may be a useful tool for differentiating the two conditions (Schulz et al., 1994).

Expression of extrastriatal D2 and D3 receptors has also been investigated with the high affinity PET ligand ^{11}C -FLB 457. Advanced but not early PD patients showed reduced ^{11}C -FLB 457 binding in thalamus, anterior cingulate, dorsolateral prefrontal and temporal cortex (Kaasinen et al., 2000, 2003). The physiological significance of loss of extrastriatal dopamine D2 receptor function in PD patients remain to be ascertained but it may contribute to the occurrence of cognitive and emotional deficits. Striatal D1 receptor binding can be assessed with the PET tracer ^{11}C -SCH23390 (Rinne et al., 1990; Sinotoh et al., 1993; Turjanski et al., 1997). Striatal D1 receptor availability, as measured by this ligand, is generally preserved in de novo PD patients but slightly reduced in patients who have been chronically treated with levodopa in good agreement with post-mortem reports.

Dopamine D1 and D2 receptor availability changes associated with levodopa-induced dyskinesias have also been investigated. Two PET studies found no difference in striatal D1 and D2 receptor binding between stable responders and dyskinetic patients matched for disease duration (Turjanski et al., 1997; Kishore et al., 1997). These findings suggest that changes in presynaptic function and/or downstream postsynaptic (cellular) transmission, rather than dopamine receptor density are more likely to be responsible for the development of motor complications in PD.

1.6.2.3 Changes in levels of dopamine in the synaptic cleft

A number of studies have confirmed the ability of ^{11}C -raclopride PET to detect *in vivo* increases in synaptic cleft dopamine levels following pharmacological or behavioural challenges, evidenced as decreases in D2 receptor availability (Laruelle, 2000). It has been estimated that a 10% reduction in the availability of D2 receptors for ^{11}C -raclopride binding reflects a five-fold increase in synaptic dopamine levels (Breier et al., 1997).

Piccini and colleagues (2003) evaluated the endogenous release induced by methamphetamine in striatal and cortical areas in normal subjects and patients with advanced PD. In the PD group, the mean reductions in caudate and putamen ^{11}C -raclopride binding were significantly smaller than in the normal subjects (8% vs 17% in caudate and 7% vs 25% in putamen). In individual patients, the percentage reduction in putamen ^{11}C -raclopride binding following methamphetamine correlated with both putaminal ^{18}F -dopa uptake and UPDRS scores when withdrawn from medication. SPM also detected reduced striatal dopamine release after amphetamine in PD but, interestingly, dopamine release was preserved in cortical areas (dorsal and ventrolateral prefrontal and orbito-frontal areas). These findings indicate that dopamine release in frontal areas after a pharmacological challenge is preserved even in late stage of PD and support the hypothesis that reduced striatal rather than frontal release of endogenous dopamine may be more relevant to the executive disabilities associated with PD.

^{11}C -raclopride PET has also been used to estimate the changes in synaptic levels of dopamine induced by exogenous levodopa in PD patients (Tedroff et al. 1996; de la

Fuente-Fernandez et al., 2001, 2004; Pavese et al., 2006). These studies have shown that striatal reductions in ^{11}C -raclopride binding after administration of levodopa become larger as the disease progresses. The increasing amplitude of the synaptic dopamine response to levodopa with PD severity reflects a failure of terminals to store and so buffer swings in dopamine and so may be responsible for the emergence of peak-dose dyskinesias and fluctuating motor responses (de la Fuente-Fernandez et al., 2004).

The increased synaptic dopamine levels that result from levodopa administration in more advanced PD patients probably reflect the reduced dopamine storage capacity of the severely affected putamen. However, another possible explanation could be reduction of DATs available to clear the transmitter. In line with this theory, Sossi and colleagues (2007) found a significant negative correlation between changes in synaptic dopamine concentration and binding of the DAT marker ^{11}C -methylphenidate. Greater reductions in ^{11}C -raclopride, indicative of lower changes in synaptic dopamine concentration and lower dopamine turnover, were observed to be associated with lower ^{11}C -methylphenidate binding. This finding suggests that DAT might play an important functional role in maintaining synaptic dopamine levels when oral levodopa is administered. Decreases in DAT availability may result in greater oscillations in synaptic dopamine levels concentration, contributing to the development of motor complications as the disease progresses.

The relationship between clinical improvement and *in vivo* synaptic dopamine release after a single oral dose of levodopa (250 mg levodopa/25 mg carbidopa) has been investigated in advanced PD (Pavese et al., 2006). Individual improvements in UPDRS scores induced by levodopa correlated significantly with reductions in putaminal ^{11}C -raclopride binding potential. Additionally, large putaminal ^{11}C -raclopride binding changes were associated with higher dyskinesias scores. When motor UPDRS sub-items were examined, improvements in rigidity and bradykinesia, but not in tremor or axial symptoms, correlated with putamen dopamine release. In summary, these findings indicate that in advanced PD the improvements of rigidity and bradykinesia and the presence of dyskinesias after a single dose of oral levodopa is governed by the levels of dopamine generated at striatal D2 receptors. They also

confirm that relief of parkinsonian tremor and axial symptoms is not related to striatal synaptic dopamine levels and presumably occurs via extra-striatal and/or non-dopaminergic mechanisms

Besides pharmacological challenges, endogenous release of dopamine in normal subjects and PD patients (less frequently) has been demonstrated during repetitive transcranial magnetic stimulation (Strafella et al., 2001, 2003), the performances of behavioural rewarded tasks (Koepp et al 1998; Lawrence and Brooks, 1999) or simple fingers movements (Goerendt et al., 2003) contributing to the understanding of the complex basal ganglia network.

Finally, Evans and colleagues (2006) have reported that PD patients with compulsive use of dopaminergic drugs (Dopamine dysregulation syndrome, DDS) (Giovannoni et al., 2000) exhibited enhanced levodopa-induced dopamine release in ventral striatum compared with PD patients who are not compulsively taking their medication. Ventral striatal dopamine release in DDS patients correlated with self-reported compulsive drug “wanting” but not “liking” providing evidence that links sensitization of ventral striatal circuitry to compulsive drug use.

In conclusion, the ^{11}C -raclopride PET paradigm provides a valid and useful research tool to evaluate the function of the presynaptic terminals in both normal subjects and patients with dopaminergic dysfunction.

1.6.3 Assessment of non-dopaminergic pathways

PET has also been used to explore the dysfunction of non-dopaminergic neurotransmitter systems.

1.6.3.1 Assessment of brain serotonergic function

Function of the serotonergic system has been investigated in PD patients with several PET and SPECT ligands. ^{11}C -DASB PET measures levels of the brain serotonin transporter (SERT), a marker of serotonergic terminal function. In advanced PD patients, ^{11}C -DASB binding levels have been reported to be significantly lower than those in controls in all examined brain areas, including orbitofrontal cortex (-22%), caudate (-30%), putamen (-26%), and midbrain (-29%)

suggesting that a modest, widespread loss of brain serotonergic innervation is a feature of advanced PD. Interestingly, a non-significant 7% reduction was also observed in dorsolateral pre-frontal cortex, an area implicated in major depression (Guttman et al., 2007).

^{11}C -WAY 100635 is a PET ligand which binds to serotonin 5-HT_{1A} receptors. These receptors are both presynaptic somatodendritic autoreceptors localized on 5-HT cell bodies in the midbrain raphe nuclei, where they inhibit serotonin release, and are also distributed postsynaptically on cortical pyramidal neurons and on glia having the highest density in the hippocampus, low density in the striatum and are absent in the cerebellum. Doder and colleagues (2000) reported a 25% reduction of ^{11}C -WAY 100635 binding in midbrain raphe in PD patients compared to healthy controls. Interestingly, the magnitude of ^{11}C -WAY 100635 binding reduction was similar in PD patients with and without a history of depression suggesting that serotonergic loss may not be relevant to the development of depression in PD. However, severity of total tremor and resting tremor in PD correlated with decrease in median raphe 5-HT_{1A} receptor binding and this observation suggests a possible role of serotonergic neurons in the pathogenesis of tremor.

1.6.3.2 Assessment of brain cholinergic function

PET tracers to image the cholinergic system have also been developed and several reports of findings in patients with PD are now available. Cortical acetylcholinesterase (AChE) activity in PD with and without dementia has been investigated with the PET ligands ^{11}C -methylpiperidin-4-yl acetate (^{11}C -MP4A) and ^{11}C -methyl-4-piperidinyl propionate (^{11}C -PMP), acetylcholine analogues that serve as selective substrates for AChE (Bohnen et al., 2003; Hilker 2005). Hilker and colleagues (2005) have assayed cortical AChE activity in PD with ^{11}C -MP4A PET. They found that cholinergic function was lost throughout the cortex in parallel with loss of striatal dopaminergic function in PD, demented cases showing the most severe reductions. Bohnen and colleagues (2003) used ^{11}C -PMP PET to investigate cortical AChE activity in PD with and without dementia and patients with Alzheimer's disease. The greatest reduction in cortical AChE 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 less reduction in cholinergic innervation than PDD, the lateral temporal cortex being selectively targeted. These results provide a rationale for the usage of AChE inhibitors in PD dementia as well as Alzheimer's disease.

Muscarinic acetylcholine receptor availability has also been assessed in PD. Asahina and colleagues (1998) reported that binding of the muscarinic M1 receptor PET ligand ^{11}C -NMPB was significantly higher in the frontal cortex of PD patients. One of the PD patients in this study had dementia and he had the highest binding in frontal and temporal areas. The authors suggested that the increased muscarinic receptor availability in frontal cortex in PD reflected denervation hypersensitivity caused by loss of ascending cholinergic input to the cortex.

Bohnen and colleagues (2009) have recently investigated the role of cholinergic and dopaminergic neurotransmission in the occurrence of recurrent falls in patients with PD. Seventeen PD patients with a previous history of recurrent falls and 27 patients who had no falls were investigated. Patients were divided into fallers and non-fallers based on their answer to item 13 of the UPDRS. Patients with a score of 1 or higher were classified as fallers. Each patient underwent ^{11}C -PMP PET to assess brain AChE activity and ^{11}C -DTBZ PET to measure nigrostriatal dopaminergic function. There was no significant difference in nigrostriatal dopaminergic activity between fallers and non-fallers, providing further evidence that the impairment of nigrostriatal dopaminergic transmission does not play a direct role in the occurrence of tendency to fall in PD. Conversely, the PD fallers had significantly lower thalamic AChE activity than controls and PD non-fallers. Cortical AChE activity was also reduced in the PD fallers compared to non-fallers though this did not reach statistical significance. Loss of thalamic cholinergic function is likely to reflect loss of input from the degenerating PPN nucleus, which is the principal source of cholinergic innervation to the thalamus. However, AChE activity in the PPN itself was not measured in this study due to the small size of this nucleus and resolution limitations of PET. In conclusion, this paper suggests an association between cholinergic dysfunction and fall status in PD,

providing possible new targets for pharmacological interventions in those patients with poor balance and a tendency to fall.

1.6.3.3 Assessment of brain noradrenergic function

^{11}C -RT132 is a PET tracer that binds with similar affinity to both dopamine and noradrenaline membrane transporters. PD patients with depression have been reported to have lower ^{11}C -RT132 binding in locus coeruleus and several areas of the limbic system than non-depressed PD patients (Remy et al., 2005).

In areas with low dopaminergic innervation such as locus coeruleus, ^{11}C -RT132 mainly reflects the density of noradrenergic neurons. In the other areas a combined involvement of both dopaminergic and noradrenergic pathways is more likely. This finding suggests an important role of noradrenaline but also limbic dopamine dysfunction in the pathogenesis of depression in patients with PD.

1.6.3.4 Assessment of other neurotransmitter systems

The striatal and extrastriatal opioid binding in PD has been investigated by Piccini and colleagues (1997b) with ^{11}C -diprenorphine PET. They found significantly reduced striatal and thalamic opioid binding in dyskinetic but not non-dyskinetic patients. SPM showed additional binding reductions in cingulate and prefrontal areas. These findings are compatible with enhanced endogenous opioid transmission, which in turn would dampen inhibitory output from the internal segment of the globus pallidus to thalamus, possibly causing dyskinesias.

1.6.4 Changes in cerebral metabolism and cerebral blood flow

^{18}F -deoxyglucose (FDG) PET is a marker of hexokinase activity which regulates regional cerebral glucose metabolic rate (rCMRglc). In contrast to other neurodegenerative parkinsonism such as multiple system atrophy and progressive supranuclear palsy where resting striatal metabolism is reduced, in PD it is usually normal or slightly elevated (Antonini et al., 1997; Jul et al., 2004).

The distinctive patterns of regional glucose metabolism in each of these conditions can be useful for differentiating PD from other parkinsonisms. Eckert and co-workers

(2005) reported that ^{18}F FDG PET separates PD from other parkinsonian syndromes with 95% sensitivity and 94% specificity when computer assisted methodologies are applied to discriminate patterns of rCMRglc covariance at a voxel level. The relative pallidal and putaminal hypermetabolism observed in PD may represent metabolic compensatory responses to dopamine deficiency (Lozza et al., 2002).

Other metabolic abnormalities observed in PD include thalamic hypermetabolism and regional metabolic decrements in the lateral and mesial premotor cortex, the supplementary motor area, the dorsolateral prefrontal cortex, the temporo-parietal cortex, and the parieto-occipital association regions (Eidelberg et al., 1990; Antonini et al., 1998; Hu et al., 2000; Fukuda et al., 2001).

The presence of cognitive impairment and depression are associated with further reductions in temporo-parietal and frontal metabolism (Hu et al., 2000; Berding et al., 2001; Mentis et al., 2002), whereas treatment with dopaminergic drugs can normalise metabolic patterns in PD. These confounding factors need to be considered when designing ^{18}F FDG PET studies in PD.

Several studies have compared changes in neuronal activity during the execution of simple motor tasks, such as cued or freely chosen finger or hand movements, in normal subjects and PD patients. The striatum receives input from all cortical areas and, via the internal pallidum and the ventral thalamus, in turn sends output principally to frontal lobe areas (premotor, supplementary motor, and prefrontal areas) that are related with motor preparation and planning. The basal ganglia are involved in many neuronal pathways subserving emotional, motivational, associative and cognitive functions. The effects of the striatal dysfunction in PD and the changes induced by dopaminergic treatments on this complex network of neuronal circuits has been extensively studied with PET and more recently with fMRI. A short summary of these studies will be reported in this section.

Using H_2^{15}O PET to detect changes in regional cerebral blood flow (rCBF), Playford and colleagues (1992) found that PD patients showed reduced neuronal activity in the rostral supplementary motor area (SMA) and right dorsolateral prefrontal cortex (DLPFC) compared to normal subjects during the execution of free-choice joystick movements. Similar findings were observed in PD patients during the execution of

fingers movements (Jahanshahi et al., 1995). These findings suggest reduced thalamocortical activation in PD. The administration of the non-selective dopaminergic agonist apomorphine normalized the activation pattern as patients switched to “on” state (Jenkins et al., 1992). PET studies and, more recently, fMRI studies have also shown higher activation in lateral premotor and parietal areas in PD patients compared to healthy subjects during sequential manual movements (Samuel et al., 1997; Catalan et al., 1999; Sabatini et al., 2000; Haslinger et al., 2001). The hyperactivity in these areas may represent compensatory mechanisms to loss of medial frontal activation but this is still debated.

1.6.5 Contribution of neuroinflammation to the pathogenesis of Parkinson’s disease

Microglia constitute 15% of normal white cells and are the natural immune defence of the brain. They are normally in an inactive state but any change in brain milieu due to trauma, stroke, infection, or neurodegeneration can cause them to become activated. The isoquinoline ^{11}C -(R)-PK11195 PET is a selective *in vivo* marker of activated microglia. The 18 kDa translocator protein (TSPO), which was previously known as the peripheral benzodiazepine receptor, is expressed on the mitochondrial membranes of activated but not resting microglia and has a binding site for ^{11}C -(R)-PK11195 (Banati et al., 2000). Two papers have reported significant increases in ^{11}C -(R)-PK11195 binding in both striatal and extrastriatal regions in PD patients compared to normal controls (Ouchi et al., 2005; Gerhard et al., 2006). Increased levels of activated microglia have also been observed in patients with multiple system atrophy (Gerhard et al., 2003). Taken together these findings support the hypothesis that neuroinflammatory responses, as evidenced by activated microglia, contribute to neuronal loss in PD and in other neurodegenerative diseases.

1.7 Functional neuroimaging in genetic forms of Parkinson’s disease

Due to the limited series of post-mortem cases, functional neuroimaging techniques play an important role to better understand the pathological processes of genetic forms of PD and the possible differences from the more common idiopathic PD.

In PARK1 due to mutations in the alpha synuclein gene striatal ^{18}F -dopa uptake reductions resemble those observed in idiopathic PD with a caudal-rostral gradient loss of uptake (Samii et al., 1999). A similar pattern is also observed in another autosomic dominant form PARK8 caused by mutations in the gene for LRRK2 (Adams et al., 2005). Autosomic recessive forms of PD, PARK2 (*parkin*-mutations), PARK6 (Pink1), and PARK7 (DJ1) are associated with more severe, symmetrical, and uniform reduction of ^{18}F -dopa uptake (Broussolle et al., 2000; Hilker et al., 2001; Khan et al., 2002b; Bonifati et al., 2003; Scherfler et al., 2004).

Results from a recent study with clinical and PET longitudinal follow-up in patients with *parkin*-linked confirm clinical impression that disease progression in these patients is slower than that of idiopathic later onset PD patients (Pavese et al., 2009). In the group of patients with *parkin*-linked parkinsonism, the loss of nigrostriatal function, as measured by annual reduction ^{18}F -dopa uptake, occurred at a slow rate (0.5% and 2% annual reduction in putamen and caudate ^{18}F -dopa uptake over five years). This is slower than in idiopathic PD patients (Morris et al., 1998) where putamen ^{18}F -dopa uptake has been reported to decline annually by around 9% of baseline (5% of the normal mean) while caudate uptake decreases at a slower rate (3.5% of baseline; 2.8% of the normal mean). Reduction of midbrain ^{18}F -dopa uptake in the *parkin* patients can also be detected at a rate of 0.59% per annum. The annual rate of decline in the total UPDRS scores was 1.2 %, slightly slower than the 1.58% reported for PD (Jankovic and Kapadia, 2001).

D2 receptor availability, measured with ^{11}C -raclopride PET, has been reported to be normal in PARK8 (Adams et al., 2005) and in treated PD, but is reduced in treated *parkin*-patients (Hilker et al., 2001; Portman et al., 2001; Scherfler et al., 2004, 2006). These findings suggest a greater susceptibility of D2 expression to downregulation following exposure to dopaminergic medication in *parkin*-disease than in idiopathic PD.

Aim and research questions to be addressed

2

2.1 Aim and research questions to be addressed

A better understanding of the pharmacological involvement of systems beyond the nigrostriatal dopaminergic pathway is still required in PD. The heterogeneous nature of the disease, once motor symptoms develop, suggests that the onset and progression of non-dopaminergic involvement is highly variable. Onset and progression of non-dopaminergic involvement will depend on the cause/s of the syndrome.

Approximately, 5% of PD patients have a clear familial aetiology with a recessive or dominant Mendelian mode of inheritance. Serotonergic and noradrenergic functions in patients with genetic parkinsonism have yet to be investigated *in vivo*. A better understanding of the neurodegenerative process in these monogenic variants of PD could potentially shed light on the molecular pathogenesis of non-dopaminergic dysfunction in idiopathic PD.

The neurochemical substrates underlying the numerous non-motor symptoms are unclear and remain to be established.

In the present project I have used PET imaging to:

- 1) assess *in vivo* the involvement of non-dopaminergic pathways in idiopathic PD and in genetic forms of parkinsonism by using ^{18}F -dopa PET as a marker of monoaminergic function in the brain.
- 2) assess with serial ^{18}F -dopa PET scans the rate of progression of the degenerative process in extrastriatal monoaminergic structures, including locus coeruleus, hypothalamus, ventral anterior thalamus, and red nucleus.
- 3) elucidate possible pathophysiological mechanisms underlying chronic fatigue in PD by investigating dopamine and serotonin function in the basal ganglia and in the limbic system of PD patients with and without a complaint of fatigue.
- 4) verify the hypothesis that ^{18}F -dopa PET can be used to evaluate the distribution and the function of serotonergic systems in the brain by assessing correlations between ^{11}C -DASB binding values and ^{18}F -dopa K_i values within serotonergic structures.

Methodology

3

3.1 Introduction

This chapter outlines the methodology employed in the studies reported in the experimental chapters of this thesis. The first section of the chapter provides a background description of basic principles of PET scanning and the characteristics of the PET scanners used for the experiments. The following sections describe the radioligands administered and the standard processing procedures that have been used to analyze the PET images.

The characteristics of the subjects investigated, the clinical assessments performed, and the statistic applied in each study will be described in the methods section of the respective experimental chapters.

3.2 PET (Positron Emission Tomography)

3.2.1 Introduction

PET is a scintigraphic technique that employs extremely short-lived radioisotopes linked to chemical compounds of biological interest. Following intravenous injection, PET radiotracers can be monitored in body organs in real time and, depending upon their metabolic fate, provide functional information such as blood flow, receptor density, metabolic rates in health and disease. Therefore, the technique has been widely used in several medical specialties including neurology, psychiatry, oncology, and cardiology. Within the neurosciences, brain PET has been used in the

investigation of dementia, epilepsy, stroke, PD, Huntington's disease and other neurodegenerative disorders.

After administration, positron-emitting radioisotopes, which have an excess of protons, decay and are detected as pairs of photons (annihilation event). The photons travel in opposite directions and are detected simultaneously as a coincident event by the PET scanner. The tomograph includes multiple rings of bismuth germanate (BGO) crystal detectors coupled with photomultiplier tubes. When two photons are detected in close temporal proximity by two opposed detectors in the ring, they are likely to have originated from a single annihilation event in the body, somewhere along the line between the two detectors. These lines of coincidence are recorded by the PET computer system. By measuring the number and directions of these lines a quantitative 3D image of tracer distribution can then be reconstructed using backprojection. More details about PET production of PET radiotracers and physical properties of radioactive decay by positron emission are provided in the paragraphs below.

3.2.2 Production of positron emitting isotopes

The short-lived radioisotopes (^{11}C , ^{18}F) employed in PET are typically produced in a cyclotron which acts to accelerate subatomic particles such as protons, deuterons, or helium nuclei. A beam of these particles is then directed to bombard stable elements on a target leading to formation of positron emitting unstable isotopes. The cyclotron contains both a magnetic field and an electric field at right angles within a vacuum chamber (Birattari et al., 1987). The core of the cyclotron contains an ion source emitting charged particles, generally protons or deuterons. Following emission, the charged particle is accelerated by the electric field along a path, which is made circular by the presence of the magnetic field. As the charged particle accelerates it gains energy until it is focused by an electrostatic deflecting beam to bombard a target. Once the radioisotope has been produced, the next step in the production of the radiotracer is the incorporation of the isotope into an organic compound of biological interest. This is achieved by replacing one atom of the organic compound with the

radioactive nuclide, without significantly affecting the biochemical properties of the initial compound.

3.2.3 Positron detection

The radiotracer is usually administered by intravenous bolus injection and, after an appropriate uptake period, the concentration of tracer in the tissue, in our case the brain, is measured by the tomograph.

During the imaging period, the unstable nucleus of the short-lived isotope decays into a more stable structure by emitting positrons, subatomic particles having the same mass and energy as the electrons but exhibiting a positive charge. Each emitted positron travels for a short distance from the site of origin (usually 1-3 millimeters) and gradually loses kinetic energy as it passes through the surrounding tissue. When most of its kinetic energy has been lost, the positron combines with an electron in the immediate area. This reaction results in the annihilation of both particles, their mass being conserved as energy in the form of emission of two high-energy 511 keV photons of electromagnetic gamma radiation (gamma rays). The two 511 keV photons are emitted in opposite directions at approximately 180 degrees from each other and travel through the brain and the skull before reaching the scintillation crystals of the PET detector ring at near opposite sites (Figure 3-1). When they absorb gamma rays, scintillation crystals emit low energy photons of visible light. The photomultiplier tube then enhances the light signal and converts the scintillation events into electrical pulses.

When two photons are detected within a very short time interval (on the order of 6 to 12 nanoseconds) by opposed detectors in the ring, they are assumed to have arisen from the same annihilation event. This is referred to as a coincidence event and is indicative that the annihilation occurred at some point along a straight line, called coincidence line or line of response (LOR), between the two detectors. By detecting multiple LORs from the emission site a reasonably precise localization of the origin of the annihilation events can be computed.

During a PET scan several million coincidence events are recorded forming a large number of coincidences lines. These are recorded by the PET computer system and saved as a two-dimensional matrix referred to as a sinogram (Figure 3-2). More

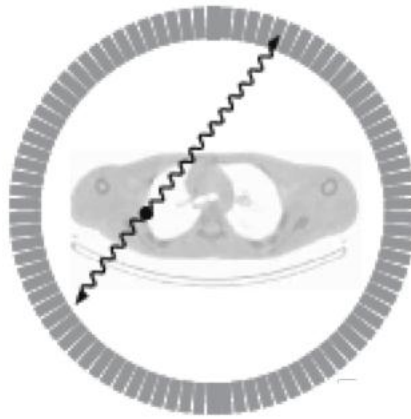


Figure 3-1 Diagram of a PET detector ring showing two photons from a single annihilation event in the body reaching opposite crystals

precisely, a sinogram is a histogram of the response count for a particular pair of detectors.

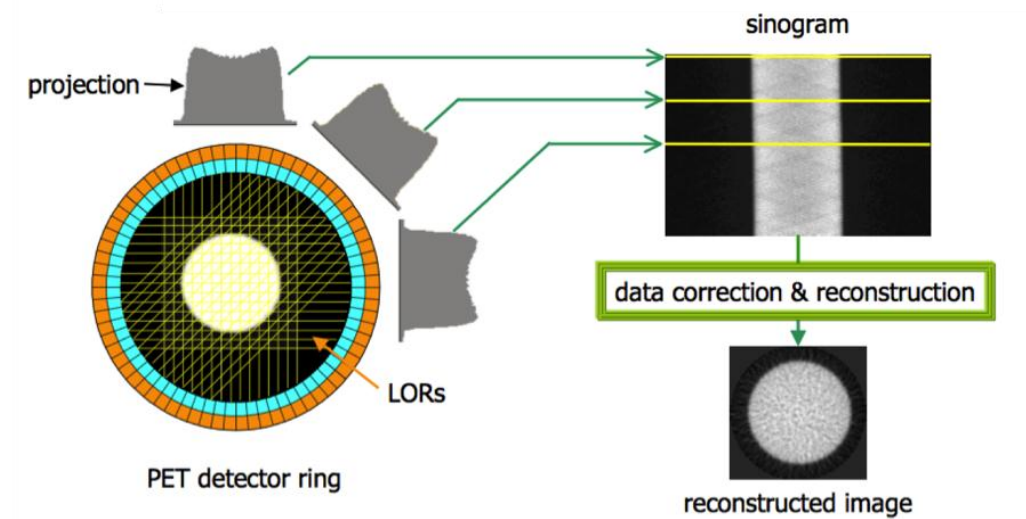


Figure 3-2 Diagram of a PET acquisition and image reconstruction

The size of the crystals in the detection system is a major factor determining the resolution of the PET scanner. The maximal theoretical image resolution possible is 1.1 to 1.2 times greater than the width of the individual detector crystals. Ring diameter, detector decoding systems, and the average distance positrons travel before annihilating with an electron, however, act to lower the spatial resolution of the PET scanner.

Early PET scanners had only a single ring of detectors and the acquisition of data and subsequent reconstruction was restricted to a single transverse plane (2D reconstruction). Modern scanners contain multiple rings, resulting in an effective cylinder of detectors and allow coincidences to be detected between rings as well as within rings and subsequent reconstruction of the entire brain volume (3D reconstruction). 3D reconstruction offers higher sensitivity (because more coincidences are detected and used) and, therefore, less noise but is more sensitive to the effects of scatter and random coincidences described in the next paragraph.

3.2.4 Detection of random and scattered events and attenuation correction

Before a three dimensional image can be reconstructed the sinogram data must be corrected for false positive coincidence and for absorption of photons by the body (attenuation correction).

There are two types of false positive coincidence that can be recorded by the PET computer system known as random and scattered events.

Random coincidence happens when two photons from different individual events are detected within coincidence resolving time and computed as a true event. The most common method of correcting for random coincidences is the real-time subtraction of a delayed coincidence channel (Dyson, 1960; Noll, 1999). Briefly, random events are identified by introducing a significant delay into one side of the coincidence detection circuit. Under these circumstances, coincidences resulting from a single annihilation event are not detected and the number of coincidences found is a good estimate of the

number of random coincidences. The estimated number of random coincidences is then subtracted from the measured data in each LOR.

Scattered coincidences occur when one or both gamma rays have been deflected off line by an interaction with an atomic nucleus in the tissue before being detected. If the remaining energy of the gamma rays is above the energy threshold of the detector window, the coincidence will still be counted. However, the line of assumed response will be between the wrong pair of detectors and this will cause the site of the annihilation event to be falsely localized. Several methods are available in order to correct for scatter during the reconstruction process. In the case of the PET studies reported in this thesis a dual-window scatter correction was applied (Shao et al., 1996; Grooten et al., 1996). Briefly, dual-window scatter correction is applied by adapting the detector properties such that the gamma rays are collected at two different energy windows simultaneously, the data window, set at the energy level of emitted unscattered photons, and the scatter window, set at a lower energy level appropriate for scattered photons (Grooten et al., 1996).

Although the photons of gamma radiation produced by the annihilation event have high 511 keV energy levels, some of them are absorbed by the surrounding tissue through which they have to pass to reach the detectors. This process is known as attenuation and results in a loss of detection of true coincidence events, which can range between 50 to 95%. Loss of counts due to attenuation increases image noise, image artefacts, and image distortion. Therefore, attenuation correction of data is necessary in order to obtain accurate qualitative and quantitative PET measurements (Phelps, 1997).

The tissue attenuation is measured by performing two additional scans, known as blank and transmission emission scans. The blank emission scan is performed with no subject in the camera to detect levels of background radiation. The transmission scan is generally performed prior to the injection of the radiotracer by exposing the subject to be imaged to an external rotating positron source emitting 511 keV gamma radiation. The tissue attenuation of 511 keV photons along all coincidence lines is then

calculated as the ratio of the blank and transmission scan data (measured blank count/measured transmission count) and this factor is applied to each LOR in the emission data (de Kemp and Nahmias, 1994; Xu et al., 1991).

Sinogram data must also be corrected for detector non-uniformity, detector dead-time correction (refractory period after the detection of a photon) and detector-sensitivity correction (for both inherent detector sensitivity and changes in sensitivity due to angle of incidence). Non-uniformity is corrected by scanning a phantom source of radioactivity and calibrating the detectors. Dead time is rarely a problem with radiopharmaceuticals due to the low count rates involved. Specific details regarding correction for angles of incidence are outside the remit of this thesis and will not be further discussed.

3.2.5 PET image reconstruction

After these corrections have been performed the image can be reconstructed using a filtered back-projection algorithm. The algorithm, similar to that used in computed tomography (Kinahan and Rogers, 1989), comprises the projection of sinograms recorded at different angles to produce a 3D image of tissue radioactivity.

After reconstruction, data are presented in the form of a series of three-dimensional images acquired during contiguous periods of time during the scan (3D dynamic images). The scanning period is divided into time intervals, generally referred to as time frames. The length of the time frames is variable and defined according to the characteristics of the radiotracer. The first frames following radiotracer injection reflect blood flow and radioligand delivery and are generally very short (15-60 seconds). Towards the end of the scan, the time frames become longer in order to maintain reasonable signal to noise ratio despite the decrease in detected count rate due to radioactive decay over the scanning period. The total length of the scanning period depends on the characteristics of each specific tracer and the kinetic model required for its analysis.

3.2.6 PET scanners

PET scanning took place at the Cyclotron Building, Hammersmith Hospital, in the facility of Hammersmith Imanet, GE Healthcare, London, UK. I used two different PET scanners for the research studies in this thesis. However, in each study, all patients were scanned with a single camera, as specified in the respective experimental chapters.

The ECAT EXACT HR++ (CTI/Siemens 966) camera covers an axial field of view of 23.4 cm and provides 95 transaxial planes. The tomograph has a spatial resolution of 5.6 mm after image reconstruction (Spinks et al., 2000). A transmission scan, used to correct for attenuation of emitted radiation by skull and soft tissues, was acquired using a single rotating photon point source of 150 MBq of ^{137}Cs . The high resolution and sensitivity of this tomograph permits interrogation of the function of subcortical nuclei.

The ECAT EXACT HR+ (CTI/Siemens 962) camera covers an axial field of view of 15.5 cm and provides 63 transaxial planes. This tomograph has an axial resolution of 5.4 mm full width at half maximum (FWHM) and a transaxial resolution of 5.6 mm FWHM at 10 cm distance from the centre (Brix et al., 1997). Head position was checked using a short two-minute transmission scan using an external rotating ^{68}Ge source. A 10-minute transmission scan was then performed to measure tissue attenuation.

Subjects were positioned in the scanners such that their orbitomeatal plane was parallel to the transaxial plane of the tomograph. Head position was carefully monitored with a video camera and by direct observation throughout.

3.3 ^{18}F -dopa

3.3.1 Introduction

I have used ^{18}F -dopa [^{18}F -Fluorodopa] PET, a marker of neuronal AADC activity, to assess the function of the monoaminergic systems in PD patients (Figure 3-3).

The use of ^{18}F -dopa PET to visualize the nigrostriatal dopaminergic pathway in the living brain was first introduced by Garnett and colleagues in 1983. Following

intravenous administration, the metabolic pathways for ^{18}F -dopa are qualitatively

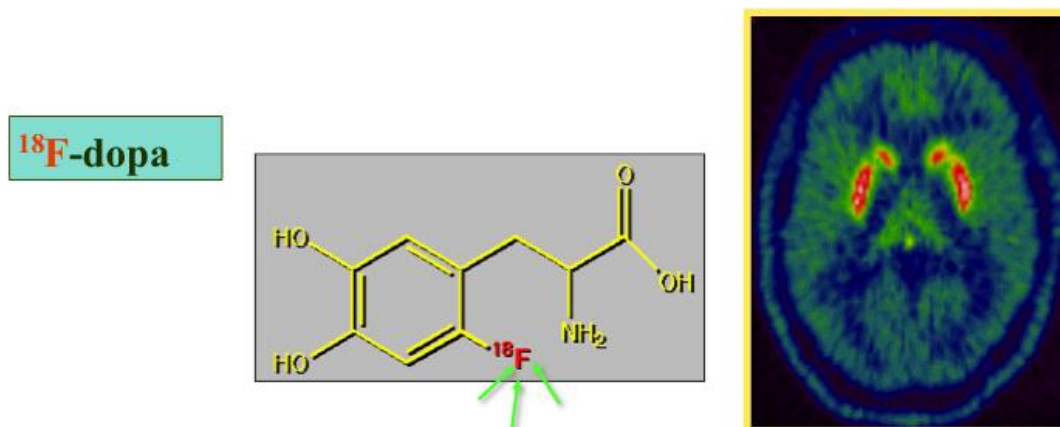


Figure 3-3 Dash formula of ^{18}F -dopa and PET image of ^{18}F -dopa uptake (axial plane at basal ganglia level)

similar to those for exogenous levodopa, although quantitative differences have been observed (Firnau et al., 1988; Melega et al., 1990). Similar to exogenous levodopa, ^{18}F -dopa is transported across the blood-brain barrier by the neutral amino acid carrier (Leenders et al., 1986) and then converted by AADC to ^{18}F -dopamine (Firnau et al., 1987). The rate of AADC activity is the limiting step in ^{18}F -dopamine formation. Striatal ^{18}F -dopamine is then metabolised to ^{18}F -DOPAC (dihydroxyphenylacetic acid) and ^{18}F -HVA (homovanillic acid) by monoamine oxidase B and catechol-O-methyltransferase.

A comprehensive kinetic analysis of striatal ^{18}F -dopa uptake and washout is complex because of its peripheral and central metabolism. However, over the 90 minutes duration of a PET scan it is reasonable to assume that the fluorine radioisotope taken up by the brain remains trapped within the nerve terminals as ^{18}F -dopamine and its metabolites. ^{18}F -dopa uptake in the striatum can, therefore, be regarded as irreversible and analysed as an influx constant using a graphical approach to linearise the time course of the data.

Multiple-time graphical (Patlak) analysis evaluates the unidirectional uptake of tracer from the time course of the total tissue radioactivity. The method is suitable for single or multiple compartment models, as long as net uptake is irreversible, in other words the tracer is trapped in the region and cannot leave during the study. It assumes that the flow of the injected tracer is unidirectional into the compartment, follows first-order kinetics and, if the tracer is metabolised, it occurs only in an irreversible manner so that the metabolites are also irreversibly trapped. The method also requires the identification of: (i) a lower time limit when all tissue free and exchangeable tracer pools have reached equilibrium with the arterial plasma, and (ii) an upper time limit before significant product loss occurs. In conclusion, graphical analysis is based on the assumption that following equilibration between exchangeable tissue pools and plasma, the apparent distribution space of the tracer, plotted against the normalised plasma integral of the tracer, will increase linearly as long as there is no product loss. The slope of the linear portion of the graph is an influx rate constant (K_i) and reflects specific tracer accumulation.

The Patlak plot can also be employed using a non-specific tissue reference input function as a replacement for the plasma input function provided there is a region which represents the exchangeable tissue compartment in areas of specific binding (Patlak and Blasber, 1985). Striatal ^{18}F -dopa K_i values are commonly computed by graphical analysis using the occipital cortex as reference input function, as this region is known to have the lowest dopamine concentration (Brown et al., 1979) and lowest AADC activity (Lloyd and Hornykiewicz, 1972). The cerebellar cortex can also be used as a reference region. However, the degree of cerebellar volume available depends on the PET scanner's field of view.

In summary, the pattern of regional ^{18}F -dopa uptake in the brain depends upon five processes: 1) passage of the ligand through the blood-brain barrier; 2) uptake into presynaptic terminals; 3) conversion to ^{18}F -dopamine by AADC; 4) trapping of ^{18}F -dopamine in neurons (5) degradation of ^{18}F -dopamine. Among these processes, the principal determinants of uptake as shown by the influx constant K_i , are the AADC activity and the density of the axonal plexus.

3.3.2 Extra-striatal uptake of ^{18}F -dopa

In the striatum where the nigrostriatal terminals are the major monoaminergic constituent, ^{18}F -dopa uptake reflects presynaptic dopaminergic function. Indeed, as mentioned in Chapter 1 (paragraph 1.6.2.1), post-mortem and animal studies have shown that striatal ^{18}F -dopa uptake correlates with both nigrostriatal cell counts and levels of dopamine in striatal terminals (Snow et al., 1993; Pate et al., 1993). However, ^{18}F -dopa PET can also be used to evaluate the distribution and the function of the other monoaminergic systems in the brain. ^{18}F -dopa uptake, when expressed as an influx constant, K_i , reflects the activity of AADC contained in the axonal plexus (Snow et al. 1993) and AADC is contained in the terminals of all monoaminergic neurons, including noradrenaline and serotonin systems. Measurements of ^{18}F -dopa uptake into serotonergic and noradrenergic structures, therefore, provide an indication of the functional integrity of these nerve terminals (Brown et al. 1999). Moore and colleagues have demonstrated that ^{18}F -dopa uptake can be reliably quantitated in a variety of extra-striatal regions in normal brain (2003) and in patients with idiopathic PD (2008). Figure 3-4 shows examples of ^{18}F -dopa uptake in the hypothalamus and midbrain raphe of a healthy volunteer.

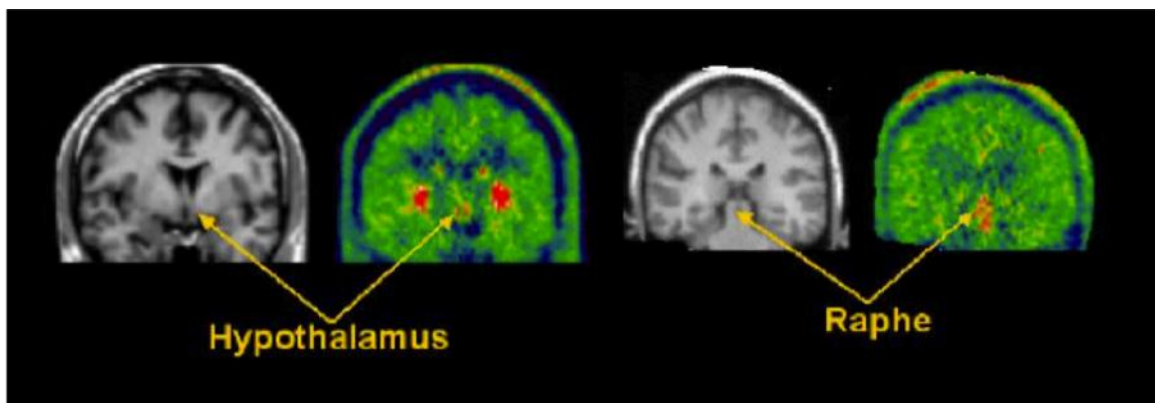


Figure 3-4. ^{18}F -dopa uptake in the hypothalamus and midbrain raphe of a healthy volunteer

3.3.3 Radiopharmaceutical preparation

^{18}F -dopa was supplied by Hammersmith Imanet.

The radiotracer was synthesized as previously described (Namavari et al., 1992) by ^{18}F -fluorine reaction with the DOPA precursor (4, 5-Di-BOC-N-ormyl-2-trimethyl-L-phenylalanine ethyl ester). ^{18}F -fluorine was produced by the $^{18}\text{O}(\text{p},\text{n})\ ^{18}\text{F}$ reaction, bombarding enriched ^{18}O -oxygen with 19MeV protons at 40 μA for 30-45 minutes followed by a second bombardment of 19 MeV protons on 0.2% fluorine in argon during which time the ^{18}F bound to the target walls is isotopically exchanged.

A sample from each synthesis is then taken for quality assurance and analysed using reverse phase high pressure liquid chromatography.

The radiochemical purities of synthesis requested to proceed with injection was 95.0% or higher.

3.3.4 Patient preparation and scan protocol

All patients were asked to stop their dopamine replacement therapy for at least 12h before PET. Cabergoline was stopped 3 days before scanning because of its longer half-life. To improve availability of ^{18}F -dopa to the brain, patients were pretreated with 150 mg of carbidopa, a peripheral inhibitor of AADC, 1 hour prior to scanning (Hoffman et al., 1992).

The scanning protocol involved list mode (event-by-event) 3D acquisition over 95 minutes subsequently divided into 26 time frames following a bolus injection of 110 MBq of ^{18}F -dopa.

3.4 ^{11}C -DASB [*N,N*-dimethyl-2-(2-amino-4-cyanophenylthio) benzylamine]

3.4.1 Introduction

^{11}C -DASB [*N,N*-dimethyl-2-(2-amino-4-cyanophenylthio) benzylamine] PET, is a marker of serotonin transporter (SERT) binding and reflects functional integrity of the serotonergic system in PD patients (Figure 3-5). SERTs facilitate the inactivation of serotonin by uptake from the synaptic cleft into the presynaptic nerve terminals. SERT

availability on the serotonin presynaptic terminals is thought to reflect the degree of serotonin innervation in that region.

^{11}C -DASB has high specificity and sensitivity for SERT (nanomolar affinity) and much lower affinity for the dopamine and norepinephrine transporters (micromolar



Figure 3-5 Dash formula of ^{11}C -DASB and PET image of ^{11}C -DASB binding (axial plane at basal ganglia level)

affinity) (Houle et al., 2000). The main advantage of ^{11}C -DASB over other SERT radiotracers available for human brain imaging, such as ^{11}C (+)McN5652, is its higher ratio of specific binding to non-specific binding in vivo. The binding potential values (an index of available receptor density proportional to the ratio of $B_{\text{max}}:K_d$) found with ^{11}C -DASB PET are approximately two to threefold greater than those found with ^{11}C - (+)McN5652 PET in the same cerebral areas when using the same modelling method. Furthermore, binding potential values found with ^{11}C -DASB PET are reliable for most brain regions under test-retest conditions (Kim et al., 2006). For all these reasons, ^{11}C -DASB PET has represented a major advance in brain imaging of the SERT in living humans.

^{11}C -DASB PET images in humans show high binding in the midbrain, thalamus and striatum, moderate binding in the medial temporal lobe and anterior cingulate, and low binding in neocortex (Frankle et al., 2006). The cerebellum (excluding vermis) is regarded as a region with extremely low or undetectable specific SERT binding

(Cortes et al., 1988). A recent study has reported that SERT density in the cerebellar cortex is less than 3% of the striatal values, as measured with Western blot method (Kish et al., 2005). I have therefore used the cerebellum (excluding vermis) as a reference region for the analysis of ^{11}C -DASB PET images in this research project.

3.4.2 Radiopharmaceutical preparation

^{11}C -DASB was supplied by Hammersmith Imanet.

^{11}C was produced by the $^{14}\text{N}(\text{p}, \text{a})^{11}\text{C}$ reaction using natural nitrogen gas

The radiotracer was synthesized from the precursor (3-amino-4-(2-methylamminoethylphenylsulphonyl)-benzonitrile by alkylating with ^{11}C -methyl iodide. ^{11}C -Methyl iodide was prepared by reduction of $^{11}\text{CO}_2$ with LiAlH_4 to $^{11}\text{CH}_3\text{OH}$ followed by iodination with hydriodic acid. The crude drug substance was then purified by preparative reverse phase high pressure liquid chromatography. The radiochemical purities of synthesis requested to proceed with injection was 95.0% or higher.

3.4.3 Patient preparation and scan protocol

All patients were asked to stop their dopamine replacement therapy for at least 12h before PET. Cabergoline was stopped 3 days before scanning because of their longer half-life. The patients were also requested to refrain from consuming alcohol, coffee or caffeine or xanthine containing products from 12 hours prior to radiotracer administration. Smoking or other use of nicotine-containing products was not allowed during the study.

The scanning protocol for ^{11}C -DASB involved 3D acquisition over 90 minutes and 30 seconds (17 time frames) following a bolus injection of 450 MBq of ^{11}C -DASB.

3.5 Analysis of ^{18}F -dopa and ^{11}C -DASB PET images

3.5.1 Introduction

The following paragraphs and the flowcharts in figure 3-6 and figure 3-7 outline the standard processing procedures used to analyze ^{18}F -dopa and ^{11}C -DASB PET images.

Study specific procedures will be described in details in the respective experimental chapters along with a brief summary of the standard procedures employed.

3.5.2 Generation of parametric images of ^{18}F -dopa

The reconstructed dynamic image series of ^{18}F -dopa uptake were analyzed using Kronos, software developed in-house and written in IDL (Interactive Data Language; Research Systems International). The software, which has been previously validated (Rakshi et al., 1996), creates parametric images of ^{18}F -dopa influx constant K_i 's [$\text{mL}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$] (K_i maps) at a voxel level for the whole brain, using the reference tissue adaptation of the multiple-time graphical approach (Patlak et al., 1985). The resulting parametric images contain all the transaxial planes from the tip of the vertex to the base of the cerebellum.

The occipital cortex was used to provide a reference region input function (Brooks et al., 1990b). A standard size ellipse was used to sample both the right and left occipital hemispheres over 5 transaxial planes. The linear portion of the Patlak plot, the gradient of which provides the K_i value, was taken from the time frame closest to 24 minutes until the time frame closest to 91 minutes.

Additionally, all 26 frames of the dynamic ^{18}F -dopa time series were integrated to form a summated image (ADD image) reflecting both tracer delivery and specific uptake. ADD images are required for spatial normalization purposes, as explained in paragraph 3.5.4.

In order to optimise image quality, the quality of the reference region input function time activity curves, alignment of K_i maps and corresponding ADD images were all audited.

3.5.3 Generation of parametric images of ^{11}C -DASB

Parametric images of specific ^{11}C -DASB binding potentials [BP_{ND} (non-displaceable) in consensus nomenclature (Innis et al., 2007)] were generated on a voxel wise basis

for the whole brain using a basis function implementation of the simplified reference tissue compartmental model with the cerebellum providing the reference tissue input function (Lammertsma and Hume, 1996; Gunn et al., 1997).

The simplified reference tissue compartmental model allows estimation of the parameters R1 (relative rate of radioligands delivery normalized to the cerebellum) and binding potential (BP).

BP is defined as:

$$BP = f_2 B_{\max} / [K_D (1 + F_1 / K_{Di})]$$

Where f_2 is the free fraction of the non specifically bound radioligands in the tissue, $B_{\max}$ is the total concentration of specific binding sites, K_D is the dissociation constant of the radioligands indicating its affinity, and F_1 and K_{Di} are respectively the free concentration and the dissociation constant of competing endogenous ligand.

Integrated ADD images of the data from the 17 time frames were also created for spatial normalization purposes.

In order to optimise image quality, the quality of the reference region input function time activity curves, alignment of parametric images of ^{11}C -DASB BP_{ND} , and corresponding ADD images were all audited.

3.5.4 Spatial normalization

Spatial normalization is an image registration method, which consists in registering individual brains to a standard template in stereotaxic space. The normalization of individual brain images into the same orientation and shape minimizes spatial differences between subjects and deform human brain scans so that one location in one subject's brain scan corresponds to the same location in another subject's brain scan (Figure 3-8).

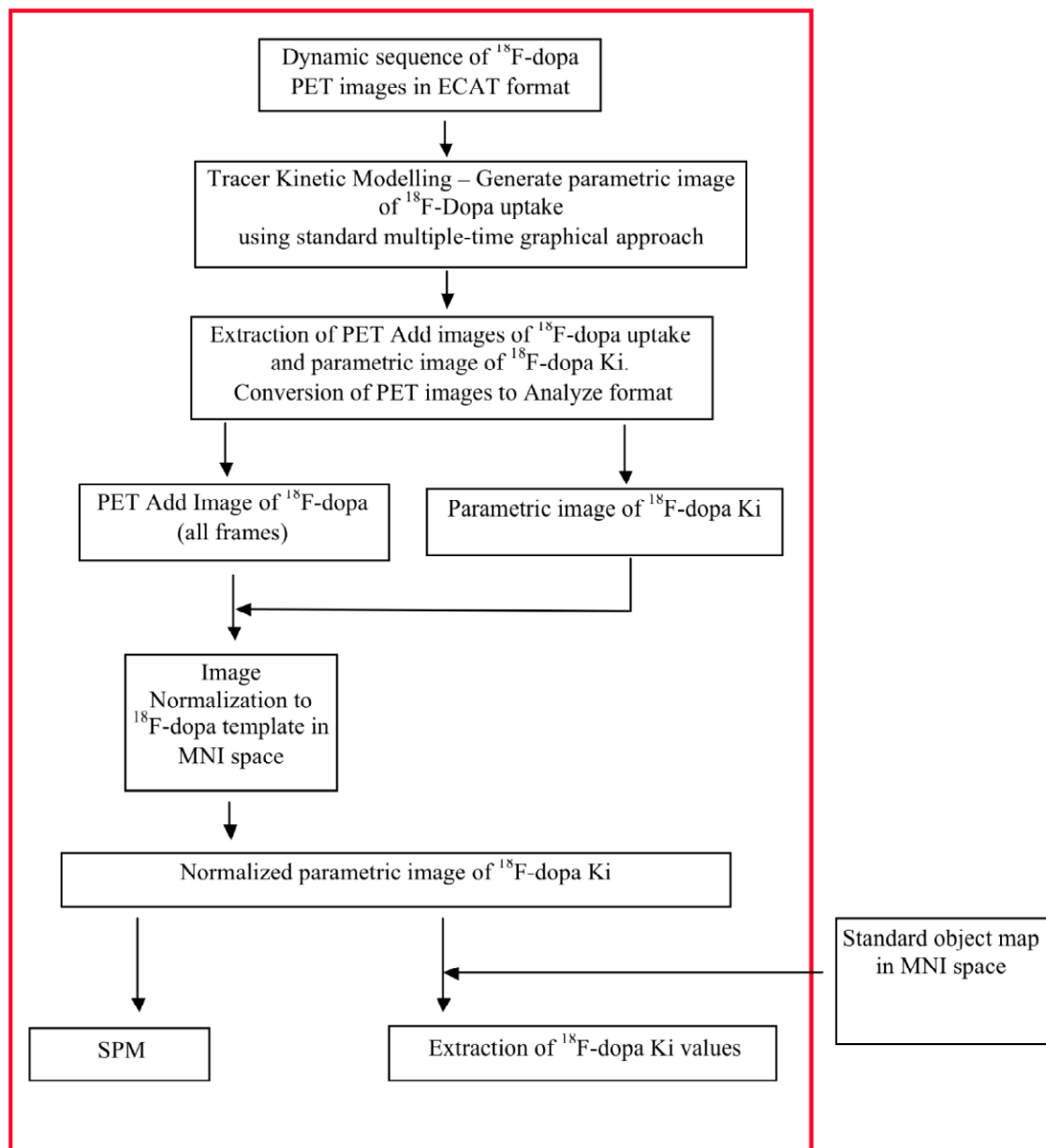


Figure 3-6. Flowchart of ¹⁸F-Dopa PET Analysis

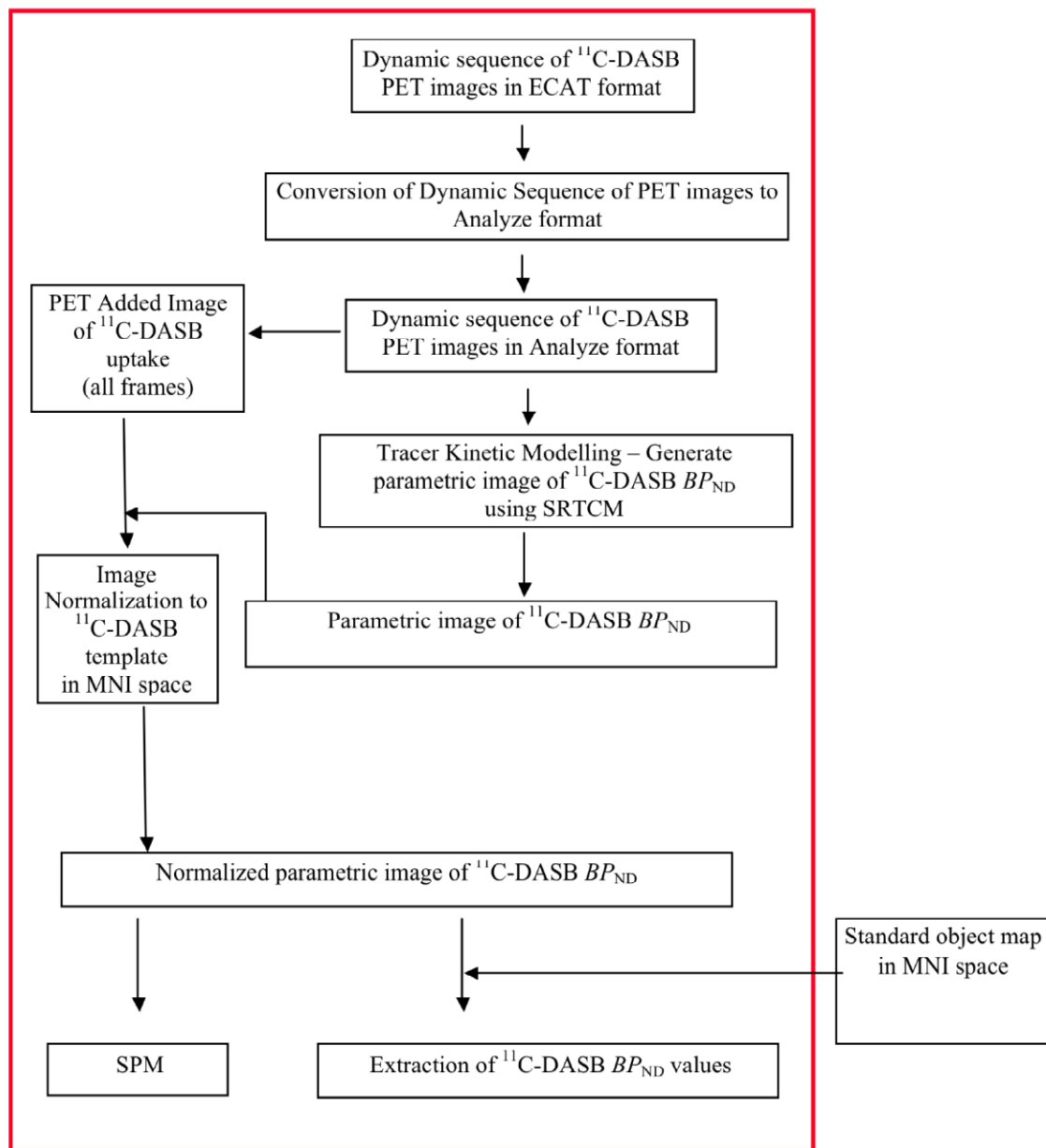


Figure 3-7. Flowchart of ¹¹C-DASB PET Analysis. SRTCM= simplified reference tissue compartmental model

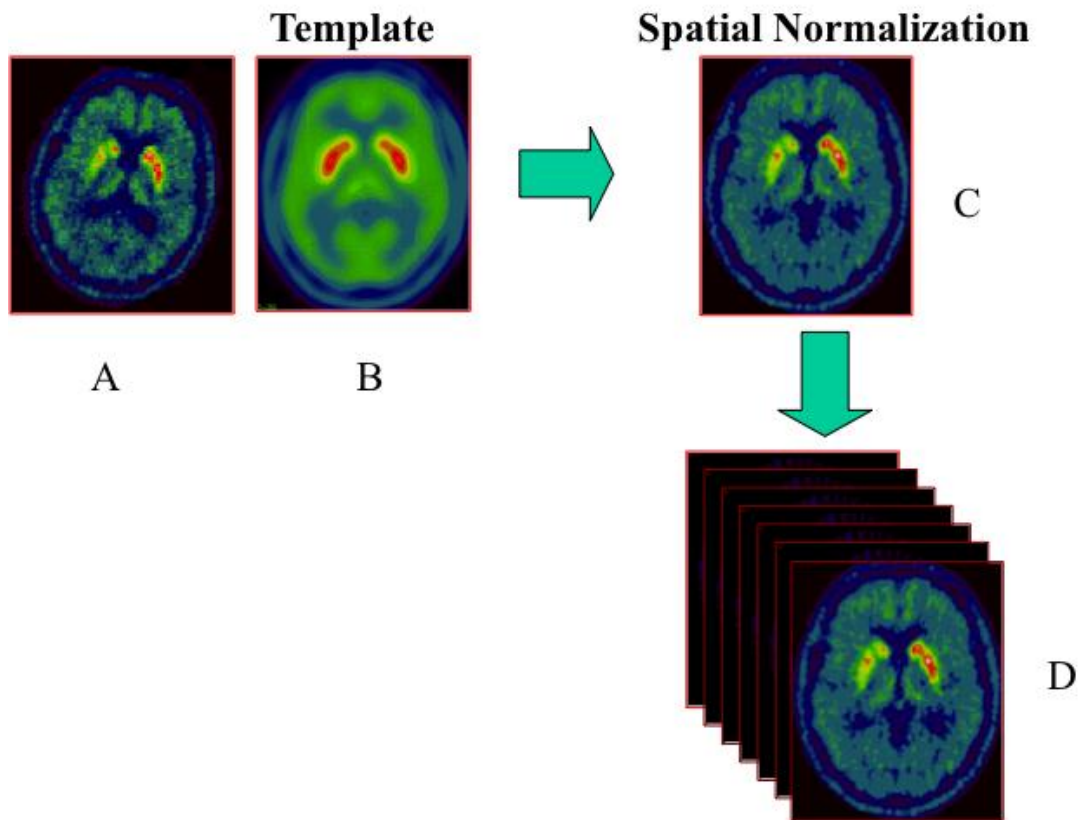


Figure 3-8 Example of spatial normalization. The individual PET image (A) is registered to the template in stereotaxic space (B). The resulting normalized image (C) will have the same standard spatial coordinates as previously normalized images (D)

To effect spatial normalization, the algorithm available in SPM software was used (www.fil.ion.ucl.ac.uk/spm) implemented in Matlab5, which transforms individual brain images into standard Montreal Neurological Institute (MNI) stereotaxic space. ADD images were used to determine spatial transformation parameters, as they contain emission data reflecting both delivery and specific tracer binding and provide greater anatomical detail than parametric Ki images.

First the ADD images of ^{18}F -dopa and ^{11}C -DASB uptake were normalized to a smoothed PET ADD image template in MNI space. The ^{18}F -dopa and ^{11}C -DASB

templates in MNI space used for spatial normalization were created in-house from healthy control subjects. Having normalized the ADD images to MNI space, the parametric images were then spatially normalized using the same parameters created during transformation of the ADD images.

Since the accuracy of the stereotaxic normalization is essential for the subsequent analysis of PET images, the outcome was audited by comparing the normalized ADD image and the normalized parametric image of each patient to the template in all three planes. Cases where stereotaxic normalization was considered non successful were excluded from further analyses.

3.5.5 PET analysis

Parametric images of ^{18}F -dopa K_i and ^{11}C -DASB BP_{ND} were analysed using two different methods: (i) a region of interest (ROI) approach; and (ii) SPM, allowing exploratory group comparisons throughout the entire brain volume at a voxel level without requiring an *a priori* hypothesis. These two methods, which provide complementary information, are described below:

3.5.5.1 Region of interest approach

The region of interest (ROI) approach samples tracer uptake in preselected areas of the brain that have been identified to respond to specific research questions, based on an *a priori* hypothesis.

To extract ROI data standard object maps in MNI space were applied. These standard object maps contain defined ROIs that have been free hand traced using ANALYZE software (Mayo clinic, MN) onto the single subject MRI in MNI space available in SPM. Details concerning the object maps employed in each study of this research project will be provided in the respective experimental chapters. The standard object maps were applied to each spatially normalized image of ^{18}F -dopa K_i or ^{11}C -DASB BP_{ND} and corresponding ADD images. Visual inspection of each plane for both images was made to ensure correct placement of the object regions over the correspondent structures. Manual corrections were made for any misalignment between K_i maps and the object maps to ensure the template was placed over the K_i

map target region, as described by Whone and colleagues (2004). Cases where misalignment was considered excessive were excluded from further analyses. After applying target regions to structures, ^{18}F -dopa Ki and ^{11}C -DASB BP_{ND} values were quantified using ANALYZE software. ^{18}F -dopa Ki and ^{11}C -DASB BP_{ND} values obtained from the different ROIs were averaged over both hemispheres for statistical analysis. Figure 3-9 shows an example of placement of ROIs on a normalized PET image of ^{18}F -dopa Ki and co-registered MRI.

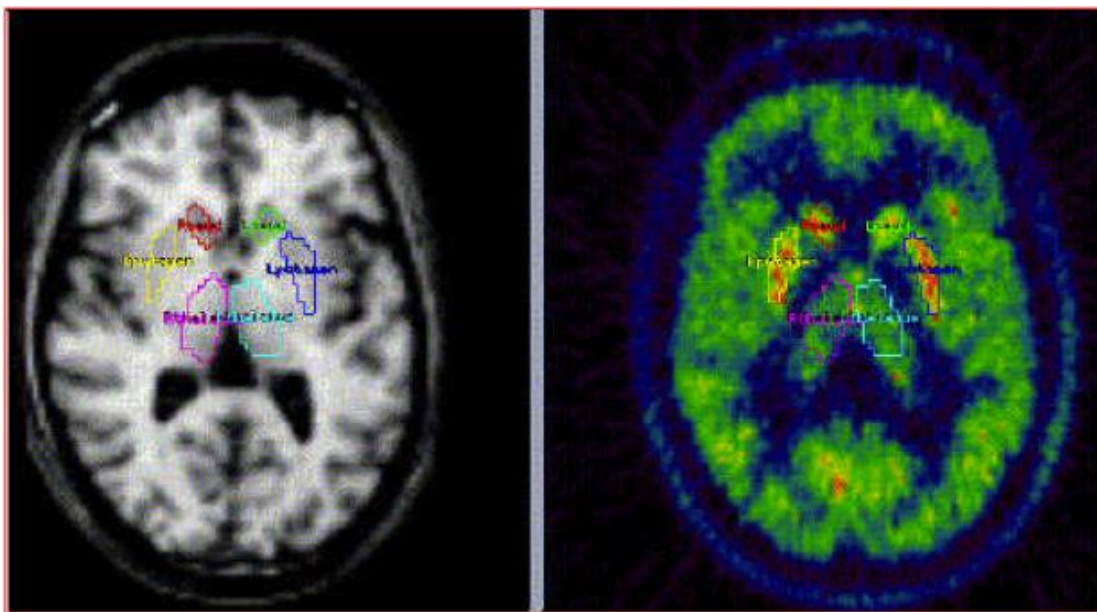


Figure 3-9 Example of standard object map applied onto a spatially normalized image of ^{18}F -dopa Ki (right) and onto a standard T1 MRI in stereotaxic space (left) to demonstrate anatomical details

3.5.5.2 Statistical parametric mapping (SPM)

SPM allows significant differences in mean tracer binding to be localised across scan datasets in voxel clusters of the brain volume and allows both between- group and within- group analyses. It requires that all the individual brain images have been spatially normalized into stereotaxic space and spatially smoothed using an isotropic

Gaussian kernel. This spatial filter accommodates inter- individual anatomic variability and improves signal to noise for the statistical analysis. After spatial smoothing the parametric images are entered into a design matrix so that parametric images are combined to form groups and subsequent comparisons can be made at a voxel level using the general linear model (Friston et al., 1995).

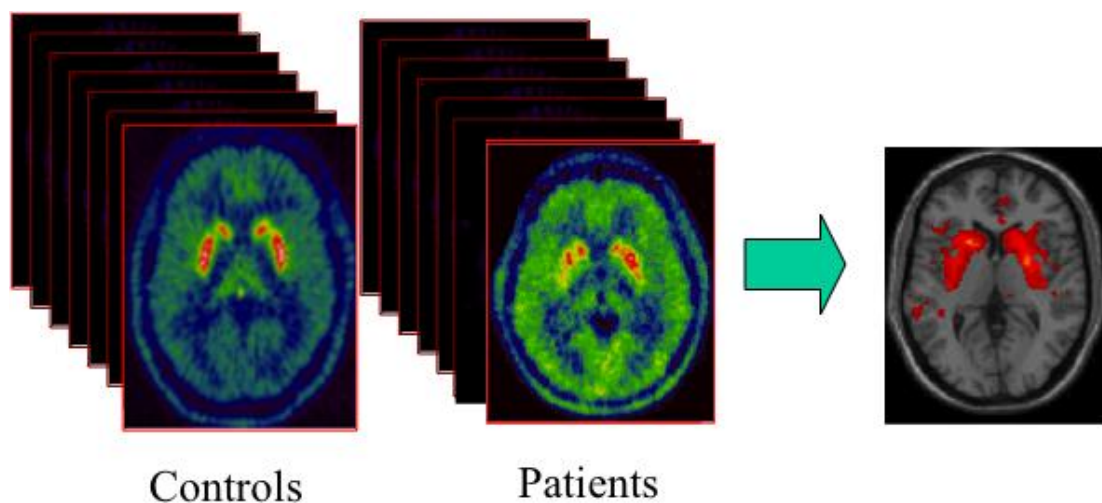


Figure 3-10. Example of a between-group comparison with statistical parametric mapping (SPM). SPM performs statistical comparisons across scan datasets in analogous voxel regions of the brain volume. Voxels with significant differences in tracer uptake are automatically overlaid onto a standard T1 MRI

More details about the type of comparison and the appropriate weighted contrasts used to localize significant decreases and/or increase in tracer uptake will be provided in the respective experimental chapters.

In general, for each contrast a t statistic is computed for each voxel to produce a SPM(t). To enable comparison between studies with different degrees of freedom the

SPM(t) is subsequently transformed to SPM(Z). Voxel based Z scores are then assembled into a 3D image, known as statistical parametric map or Z map. Significant clusters of mean group differences (or correlation) can then be identified, through the use of appropriate thresholding, and for display purposes shown projected onto a 3D glass brain or onto a single subject T1 MRI image as in figure 3-10.

In vivo assessment of brain monoamine systems in idiopathic Parkinson's disease and in *parkin* gene carriers

4

4.1 Summary of objectives

In this study, I have used ^{18}F -dopa PET to assess in vivo the involvement of extra-striatal monoaminergic pathways in a group of patients with advanced PD, and to ascertain whether idiopathic PD and monogenic forms of parkinsonism have a common pattern of non-dopaminergic dysfunction. I compared function of brain monoaminergic systems in a group of idiopathic PD patients and in a group of patients with *parkin*-linked parkinsonism matched for levels of striatal ^{18}F -dopa uptake. Additionally, I have studied the pattern of monoaminergic innervation in the brains of 12 asymptomatic heterozygote *parkin* mutation carriers and a few cases with *PINK1* mutations.

I particularly focused on: (1) hypothalamus, which contains intrinsic dopamine neurons, but also a dense innervation from midbrain raphe serotonin neurons and lateral medullary noradrenaline neurons; (2) locus coeruleus, containing noradrenergic neurons; (3) midbrain raphe serotonin neurons.

4.2 Summary of results

Compared to controls, *parkin* patients showed significant ^{18}F -dopa reductions in the

caudate, putamen, ventral striatum, locus coeruleus, midbrain raphe, and pallidum. The same structures showed reduced uptake in idiopathic PD patients, who additionally had significant reductions in hypothalamus, ventral anterior thalamus, and pineal gland. Direct comparison of *parkin* with idiopathic PD patients showed that hypothalamus was relatively targeted in idiopathic PD and midbrain raphe in *parkin* disease. Patients with *PINK1* mutations and *parkin* heterozygotes also showed monoaminergic dysfunction.

These findings suggest that *parkin* patients and idiopathic PD patients with similar levels of striatal dysfunction have different patterns of extra-striatal monoaminergic involvement, with more widespread dysfunction evident in idiopathic PD.

4.3 Scientific background

To date, only a relatively small number of imaging studies have investigated *in vivo* the involvement of extrastriatal monoaminergic systems in PD patients to complement pathological studies.

Rakshi and colleagues (1999) used a voxel-based analysis of ^{18}F -dopa uptake images to explore focal changes in dopaminergic function throughout the brain volume in patients with early and advanced PD. They found that patients with advanced PD had significant extrastriatal decreases in ^{18}F -dopa uptake in the ventral and dorsal midbrain regions compared to both controls and patients with early stages of disease. In these areas, the reduction in ^{18}F -dopa uptake could reflect degeneration of serotonergic and noradrenergic along with dopaminergic neurons. More recently, Moore and colleagues (2008) have demonstrated that ^{18}F -dopa uptake can be reliably quantitated in a variety of extra-striatal regions in patients with idiopathic PD by using a region of interest approach. In their cohort of PD patients, who had a range of disease severity, they found that extrastriatal dopamine and other monoamine systems were involved along with the nigrostriatal dysfunction, particularly in later disease.

Serotonergic and noradrenergic function have not been directly investigated in patients with mendelian forms of PD.

Neuropathological findings in these patients are distinct from idiopathic PD, with few Lewy bodies and a more uniform involvement of the substantia nigra compacta (Mori et al., 1998; Hayashi et al., 2000; van de Warrenburg et al., 2001; Farrer et al., 2001; Morales et al., 2002; Farrer et al., 2004; Sasaki et al., 2004; Zimprich et al., 2004; Khan et al., 2005a), and of striatal function imaged with PET (Khan et al., 2002a; Khan et al., 2002b; Hering et al., 2004; Scherfler et al., 2004; Khan et al., 2005a; Adams et al., 2005; Hernandez et al., 2005). However, it is not known whether neurochemical differences are limited to the dopaminergic pathways or also extend to other neurotransmitter systems. The few post-mortem studies available in parkinsonian patients with genetic mutations have shown neuronal loss and gliosis in the locus coeruleus and median raphe (Mori et al., 1998; Hayashi et al., 2000; van de Warrenburg et al., 2001; Farrer et al., 2001; Morales et al., 2002; Farrer et al., 2004; Sasaki et al., 2004; Zimprich et al., 2004; Khan et al., 2005a).

4.4 Patients and methods

4.4.1 Patients

One of my aims was to compare brain monoaminergic innervation in patients with advanced idiopathic PD and *parkin*-patients with a similar degree of striatal dopaminergic dysfunction. The CSC Neurology group has collected a large database of ^{18}F -dopa PET images of healthy volunteers and patients with idiopathic PD over the years. For this reason only parkin gene carriers underwent a PET scan for this study and patients with idiopathic PD and control subjects were selected from the Unit's database. Patients with idiopathic PD patients were carefully selected to match striatal ^{18}F -dopa uptake of *parkin*-patients.

Twelve patients with compound heterozygous *parkin* mutations (M/F= 8/4, age 45.7 ± 14 years, disease duration 18.8 ± 7.7 years, UPDRS motor score (UPDRS-3) in "off" condition 45.2 ± 23), 12 asymptomatic carriers of a single *parkin* mutation (M/F= 7/5, age 52.4 ± 10.2 years), and 4 patients with *PINK1* mutations (M/F=2/2, age 51.7 ± 16.3 years, disease duration 13.5 ± 12.1 years, UPDRS-3 in 'off' condition

33±28.9) underwent ^{18}F -dopa PET. Their PET findings were compared with those of 11 healthy volunteers (M/F= 6/5, age 66.6± 5.5 years) and 12 patients with advanced idiopathic PD matched for striatal dopaminergic dysfunction (Figure 1) (M/F= 8/4, age 60.4± 7.8 years, disease duration 9.1±3.7 years, UPDRS-3 in ‘off’ condition 41.2±8.4). None of the subjects were taking medication that would act on serotonergic and noradrenergic systems. Table 4.1 summarizes the demographics of all patients investigated.

Table 4.1. Patient Characteristics

	Healthy subjects (n=11)	Single <i>parkin</i> mutation carriers (n=12)	<i>parkin</i> patients (n=12)	IPD (n=12)	<i>PINK1</i> patients (n=4)
M/F	6/5	7/5	8/4	8/4	2/2
Age (years, mean ±SD)	66.6± 5.5	52.4±10.2	45.7±14	60.4± 7.8	51.7±16.3
Disease Duration (years, mean ±SD)	=	=	18.8±7.7	9.1±3.7	13.5±12.1
UPDRS in “off”	=	=	45.2±23	41.2±8.4	33±28.9

4.4.2 PET procedure

PET scanning took place at the Cyclotron Building, Hammersmith Hospital, Hammersmith Imanet, GE Healthcare, London, UK, and all PET scans were performed with a similar protocol and the same scanner - an ECAT EXACT HR++ (CTI/Siemens 966) camera. The high resolution, high sensitivity tomograph permits interrogation of the function of small volume structures. All patients had their

dopamine replacement therapy stopped for at least 12h (and cabergoline 3 days) before PET. Scan data were acquired in list mode (event-by-event) 3D acquisition over 95 minutes following a bolus injection of 110 MBq of ^{18}F -dopa.

4.4.3 PET analysis

The analysis was performed using an *a priori* defined ROI approach and the end-point was between-group differences in extrastriatal ^{18}F -dopa uptake, expressed as influx constants K_i .

Parametric images of ^{18}F -dopa influx constants K_i and integrated ADD images of ^{18}F -dopa uptake were created as described in Chapter 3. Both K_i and ADD images were then transformed to ANALYZE format using an in house program, ECAT_transfer. Once in ANALYZE format the parametric and ADD image from each scan were visually inspected using ANALYZE software (Mayo clinic, MN).

ROI analysis was performed on datasets transformed into standard MNI stereotaxic space using the SPM software package. First the ADD images were normalized to a smoothed ^{18}F -dopa image template in MNI space. The parametric K_i images were then spatially normalized using the same parameters created during transformation of the ADD images.

4.4.3.1 Extraction of ROI data.

Standard object maps in MNI space were used to sample ROIs for caudate nucleus, putamen, globus pallidus, ventral striatum, ventral anterior thalamus, subthalamic nucleus, hypothalamus, locus coeruleus, median midbrain raphe, red nucleus, and the pineal gland. These regions were chosen because of their monoaminergic transmitter content, post-mortem evidence of neurodegeneration and/or *in vivo* evidence of involvement in PD. The characteristics of these standard object maps have been described in Table 4-2. Briefly, the regions were traced to be within the anatomical boundaries of each structure using the single subject MRI in MNI space obtained from SPM software. In cases where tracing was not possible (subthalamic nucleus, hypothalamus, raphe, and locus coeruleus), the ROIs were rectangles placed over the structures on repeated slices.

Table 4-2 Regions of interest identified on the template object map

Caudate	ROI traced to be within the anatomical boundaries of the head of the caudate nucleus
Putamen	ROI traced to be within the anatomical boundaries of the nucleus excluding the lowest portion of the post-commissural putamen
Ventral striatum	ROI traced to include the nucleus accumbens and the lowest portion of the post-commissural putamen
Globus pallidus interna	ROI traced to be within the anatomical boundaries of the nucleus
Globus pallidus externa	ROI traced to be within the anatomical boundaries of the nucleus
Ventral ant. thalamus	ROI traced to be within the anatomical boundaries of the rostral ventral thalamus, lateral to the internal medullary lamina, ventral to the lateral dorsal nucleus
Subthalamic nucleus	ROI traced to be within the anatomical boundaries of the nucleus extending into adjacent internal capsula and Forel's field
Hypothalamus	Rectangular ROI placed over ventral diencephalon lateral to the third ventricle, medial to the internal capsule and between optic chiasm rostrally and mammillary body caudally
Locus coeruleus	Rectangular ROI placed over the lateral periventricular gray, ventral to the fourth ventricle, medial to the dentatorubrothalamic tract
Midbrain raphe	Rectangular ROI placed over the raphe from the ventral border of the aqueduct to the ventral border of the reticular formation including the dorsal and medial raphe and some adjacent medial reticular formation
Red nucleus	ROI traced to be within the anatomical boundaries of the nucleus
Pineal gland	Rectangular ROI lying dorsal to the superior colliculus and posterior commissure in the ambiens cistern

Adapted from Moore et al., 2008

The standard object map was then applied to each spatially normalized ADD and Ki image. Visual inspection of each plane for both images was made to ensure correct placement of the object region over the structure. After applying target regions to structures the Ki data for each image was then generated using ANALYZE.

4.4.4 Statistical analysis

Statistical analyses of clinical data were performed with InStat3 for Macintosh (University of Medicine and Dentistry, NJ, USA). Comparisons between groups were made using a non-parametric unpaired test (Mann-Witney statistics).

4.5 Results

Regional mean ^{18}F -dopa Ki values obtained for the normal subjects, the idiopathic PD group, the 12 *parkin* patients, and the asymptomatic carriers of a single parkin mutation, are shown in Figure 4-1, Figure 4-2, and Table 4-3.

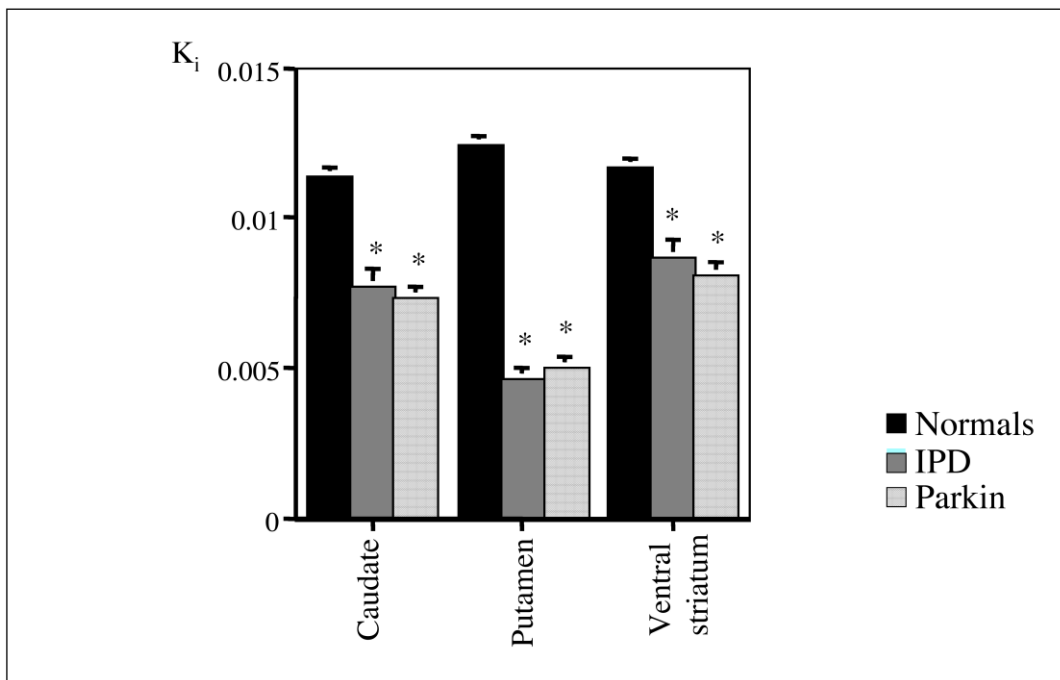


Figure 4-1. ^{18}F -dopa influx constant Ki values (mean $\pm$ standard error) (y-axis) in striatal regions. * Indicates significant differences between normal subjects and parkin-patients or patients with Idiopathic Parkinson's disease (IPD)

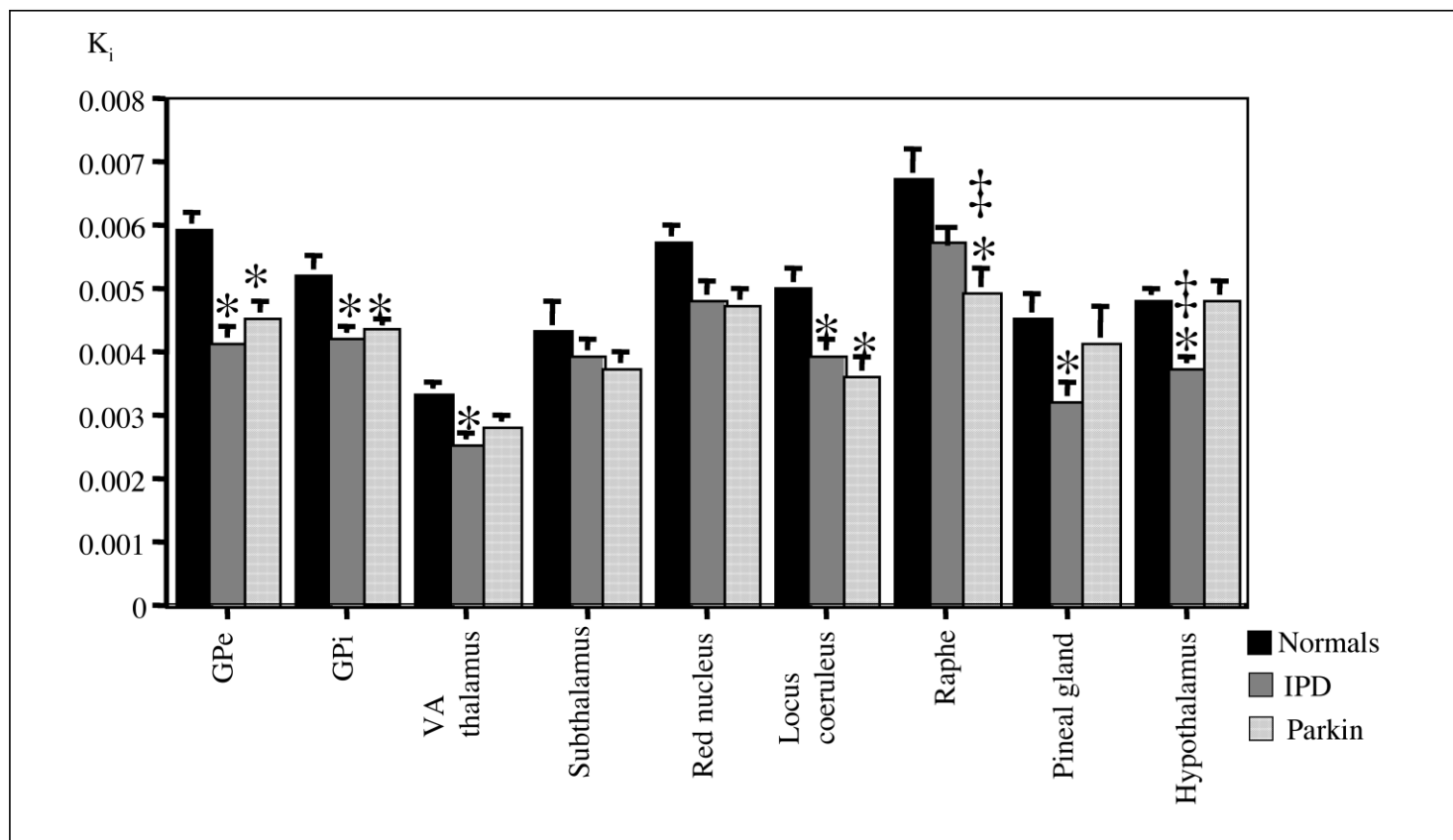


Figure 4-2. ^{18}F -dopa influx constant K_i values (mean $\pm$ standard error) (y-axis) in extrastriatal regions. * Indicates significant differences between normal subjects and parkin-patients or IPD patients; ‡ Indicates significant differences between parkin-patients and patients with Idiopathic Parkinson's disease (IPD)

Table 4.3. Regional mean ^{18}F -dopa Ki values ($\pm$ Standard Deviation) in the normal subjects, in the Idiopathic Parkinson's disease (IPD) group, in the *parkin* patients group, and in Carriers of a single *parkin* mutation.

	Healthy subjects (n=11)	Single <i>parkin</i> mutation carriers (n=12)	<i>parkin</i> patients (n=12)	IPD (n=12)
Caudate	0.0114 $\pm$ 0.0006	0.0106 $\pm$ 0.0011*	0.0074 $\pm$ 0.0013***	0.0077 $\pm$ 0.0022***
Putamen	0.0124 $\pm$ 0.0010	0.0110 $\pm$ 0.0019*	0.0050 $\pm$ 0.0012***	0.0046 $\pm$ 0.0013***
Ventral striatum	0.0117 $\pm$ 0.0009	0.0107 $\pm$ 0.0013	0.0082 $\pm$ 0.0014***	0.0087 $\pm$ 0.0022***
Globus pallidus interna	0.0052 $\pm$ 0.0010	0.0052 $\pm$ 0.0009	0.0043 $\pm$ 0.0008*	0.0042 $\pm$ 0.0009*
Globus pallidus externa	0.0059 $\pm$ 0.0008	0.0059 $\pm$ 0.0016	0.0045 $\pm$ 0.0013*	0.0041 $\pm$ 0.0011*
Ventral ant. thalamus	0.0033 $\pm$ 0.0002	0.0029 $\pm$ 0.0007	0.0028 $\pm$ 0.0006	0.0025 $\pm$ 0.0007**
Subthalamic nucleus	0.0043 $\pm$ 0.0015	0.0043 $\pm$ 0.0013	0.0037 $\pm$ 0.0011	0.0039 $\pm$ 0.0009
Hypothalamus	0.0048 $\pm$ 0.0007	0.0048 $\pm$ 0.0010	0.0046 $\pm$ 0.0008	0.0037 $\pm$ 0.0007**§
Locus coeruleus	0.0050 $\pm$ 0.0009	0.0048 $\pm$ 0.0015	0.0035 $\pm$ 0.0008**	0.0039 $\pm$ 0.0012**
Midbrain raphe	0.0068 $\pm$ 0.0015	0.0060 $\pm$ 0.0014	0.0049 $\pm$ 0.0013**§	0.0057 $\pm$ 0.0005
Red nucleus	0.0057 $\pm$ 0.0010	0.0057 $\pm$ 0.0018	0.0047 $\pm$ 0.0010	0.0048 $\pm$ 0.0009
Pineal gland	0.0045 $\pm$ 0.0014	0.0046 $\pm$ 0.0012	0.0049 $\pm$ 0.0032	0.0032 $\pm$ 0.0012*

*p<0.05 compared to healthy subjects; ** p<0.01 compared to healthy subjects; *** p<0.001 compared to healthy subjects;

§ p<0.05 Direct comparison of *parkin* patients versus IPD patients.

4.5.1 Idiopathic Parkinson's disease patients

As mentioned above, PD patients included in this study were selected from a cohort of idiopathic PD patients to match striatal ^{18}F -dopa uptake of *parkin* patients (Figure 4.1). This was done in order to assess brain monoaminergic innervation in groups of patients with similar striatal dopaminergic dysfunction. Mean putaminal ^{18}F -dopa Ki value was 0.0050 ± 0.0012 in the *parkin* patients and 0.0046 ± 0.0013 in the idiopathic PD group.

Between-group comparison of the idiopathic PD patients with the normal controls showed reduction of mean ^{18}F -dopa uptake in the striatum ($p < 0.001$ for caudate, putamen and ventral striatum), globus pallidus ($p < 0.05$), locus coeruleus ($p < 0.01$) hypothalamus ($p < 0.01$), ventral anterior thalamus ($p < 0.01$), and pineal gland ($p < 0.05$) in the idiopathic PD group. ^{18}F -dopa uptake was also reduced in the median raphe, but this did not reach significance ($p = 0.07$).

4.5.2 *Parkin* patients

Between-group comparison of the *parkin* patients with the normal controls showed reduction of mean ^{18}F -dopa uptake in the striatum ($p < 0.001$ for caudate, putamen and ventral striatum), globus pallidus ($p < 0.05$), the median raphe ($p < 0.01$), and locus coeruleus ($p < 0.01$) in the *parkin* group.

4.5.3 *Parkin* patients/ idiopathic Parkinson's disease patients comparison

The direct between-group comparison of the *parkin* with the idiopathic PD patients showed that the hypothalamus was relatively targeted in idiopathic PD and the median raphe in *parkin* disease ($p < 0.05$).

4.5.4 Asymptomatic carriers of a single *parkin* mutation

Between-group comparison of the 12 asymptomatic heterozygote carriers of a single *parkin* mutation with the normal controls showed significant reductions of mean ^{18}F -dopa uptake in striatum - caudate ($p < 0.05$) and putamen ($p < 0.05$) – but not elsewhere. Individual subject analyses revealed that two cases had significantly reduced striatal

and two others had reduced locus coeruleus ^{18}F -dopa uptake (2 SDs below the normal means).

4.5.5 *PINK1* patients

A between-group comparison of the four *PINK1* patients with the normal controls revealed a significant reduction of mean ^{18}F -dopa uptake in caudate ($p<0.01$), putamen ($p<0.01$), and ventral striatum ($p<0.05$). Individual analyses of these patients revealed that Subject 2, who had a more severe involvement of striatal regions compared to the other three subjects, also had reduced Ki values for hypothalamus, globus pallidus externa, ventral anterior thalamus, and red nucleus. Subjects 1 and 3 had a reduced ventral anterior thalamus Ki. Regional mean ^{18}F -dopa Ki values obtained for the four *PINK1* patients are shown in Table 4.4.

Table 4.4. Regional mean ^{18}F -dopa Ki values in the *PINK1* patients

	Patient 1	Patient 2	Patient 3	Patient 4	Mean value$\pm$SD
Caudate	0.0055§	0.0018§	0.0088§	0.0081§	0.0060 $\pm$ 0.0032**
Putamen	0.0040§	0.0022§	0.0104§	0.0103§	0.0067 $\pm$ 0.0043**
Ventral striatum	0.0090§	0.0060§	0.0108	0.0109	0.0092 $\pm$ 0.0023*
Globus pallidus int.	0.0047	0.0033	0.0042	0.0059	0.0045 $\pm$ 0.0011
Globus pallidus ext.	0.0050	0.0030§	0.0058	0.0068	0.0052 $\pm$ 0.0016
Ventral ant. thalamus	0.0016§	0.0025§	0.0022§	0.0040	0.0026 $\pm$ 0.0010
Subthalamic nucleus	0.0027	0.0028	0.0035	0.0045	0.0034 $\pm$ 0.0008
Hypothalamus	0.0041	0.0027§	0.0049	0.0060	0.0044 $\pm$ 0.0014
Locus coeruleus	0.0049	0.0053	0.0053	0.0057	0.0053 $\pm$ 0.0003
Midbrain raphe	0.0064	0.0044	0.0075	0.0075	0.0065 $\pm$ 0.0015
Red nucleus	0.0047	0.0026§	0.00044	0.0073	0.0048 $\pm$ 0.0019
Pineal gland	0.0047	0.0026	0.0035	0.0090	0.0050 $\pm$ 0.0028

$p<0.05$ compared to healthy subjects; ** $p<0.01$ compared to healthy subjects;

§ values 2 Standard Deviations below normal means.

	<i>Parkin</i> patients	IPD patients
GPi	↓	↓
GPe	↓	↓
VA Thalamus		↓
Hypothalamus		↓
Locus coeruleus	↓	↓
Raphe	↓	
Pineal gland		↓

Figure 4.3. Summary of results. The arrow indicate a significant decrease in mean ^{18}F -dopa Ki value compared to the healthy volunteers. IPD= Idiopathic Parkinson's disease; GPi= globus pallidus interna; GPe= globus pallidus externa; VA Thalamus= ventral anterior thalamus

4.6 Discussion

Patients with advanced idiopathic PD showed reduced ^{18}F -dopa uptake in the striatum, globus pallidus, locus coeruleus, hypothalamus, ventral anterior thalamus, and pineal gland compared to the normal controls. These results give further support to previously published findings (Moore et al., 2008).

This is the first *in vivo* PET study to focus on both striatal and extrastriatal monoaminergic structures in patients with *parkin*-linked parkinsonism and in asymptomatic carriers of a single *parkin* mutation.

The *parkin* patients showed reduced ^{18}F -dopa uptake in dopaminergic and non-dopaminergic neuronal systems when compared to normal controls. In particular, ^{18}F -dopa uptake reductions were observed in striatum, globus pallidus, locus coeruleus, and midbrain raphe. Interestingly, mean ^{18}F -dopa uptake in the hypothalamus was similar to that in the healthy controls.

When compared to idiopathic PD patients matched for striatal dysfunction, the *parkin* patients had a significantly reduced ^{18}F -dopa uptake in the median raphe. Conversely, ^{18}F -dopa uptake in the hypothalamus was significantly reduced in the PD patients.

Taken together these findings indicate different patterns of involvement of monoaminergic systems in idiopathic PD and *parkin*-linked parkinsonism. They also suggest that extrastriatal dysfunction in *parkin* patients may be more restricted than in idiopathic PD patients with a similar degree of striatal dysfunction since hypothalamus, ventral anterior thalamus, and pineal gland do not seem to be affected in the former.

In line with post-mortem studies, the locus coeruleus was the mostly affected extrastriatal area in both idiopathic PD and *parkin* patients with a similar degree of reduction in this structure in the two groups. The reduction of ^{18}F -dopa uptake in the locus coeruleus probably reflects the well-known degeneration of the noradrenaline containing-neurons that are the major neuron type in this nucleus. With its projections to most central nervous system regions, including the cerebral cortex, hippocampus, thalamus, hypothalamus, midbrain, brainstem, cerebellum, and spinal cord (Kobayashi et al., 1975; Foote et al., 1983), abnormal function of the locus coeruleus may influence symptoms in both idiopathic PD and parkinsonism due to *parkin* mutations. In animal models of PD, reduction of noradrenergic activity within the locus coeruleus alters neurochemical electrophysiological, and behavioural indices of neurotransmission in the nigrostriatal dopaminergic system, and influences the response of this system to experimental lesions (Marien et al., 2004). A large body of data indicates that the locus coeruleus is important in behavioral state control, particularly arousal (Berridge and Waterhouse, 2003), and in mechanisms of attention and response selection (Aston-Jones and Cohen, 2005) so that the pathology of the

locus coeruleus almost certainly contributes to the behavioral disturbances observed in idiopathic PD (Rommelfanger and Weinshenker, 2007).

Midbrain raphe ^{18}F -dopa uptake was severely affected in *parkin* patients. PD patients also showed reduced ^{18}F -dopa uptake in the median raphe with respect to the healthy controls, which, however, did not reach significance ($p=0.07$).

The midbrain raphe is the principal source of serotonergic innervation to the forebrain (Steinbusch, 1981), and a reduction of ^{18}F -dopa uptake can thus be attributed to impairment of serotonin terminal function though some dopamine terminals are also present. Doder and colleagues (2003) reported that severity of resting tremor in PD patients correlates with decreased 5-HT_{1A} receptor availability in median raphe, as assessed with ^{11}C -WAY 100635 PET. The more severe involvement of the midbrain raphe in our *parkin* patients might, therefore, explain the higher prevalence of tremor found in these patients (90%) (Khan et al. 2003).

The hypothalamus has a complex internal structure as it contains intrinsic dopamine neurons, but also a dense innervation from midbrain raphe serotonin neurons and lateral medullary noradrenaline neurons. In idiopathic PD patients, the reduction of ^{18}F -dopa uptake in this region could, therefore, reflect dysfunction of any or all of these pathways. Interestingly, mean ^{18}F -dopa uptake in the hypothalamus of *parkin* patients was similar to that in the healthy controls suggesting that this structure may be not involved in the pathological process associated with *parkin* mutation. This finding needs to be confirmed in larger cohorts of *parkin* patients.

Finally, I assessed the pattern of monoaminergic dysfunction in the brains of asymptomatic carriers of a single *parkin* mutation. As a group, asymptomatic heterozygous carriers of a single *parkin* mutation showed only a mild but significant reduction of ^{18}F -dopa uptake in the caudate and putamen when compared to the normal controls. However, individual analyses of these subjects revealed that two of them had reduced locus coeruleus ^{18}F -dopa uptake with normal striatal uptake. This finding suggests that, at least in some carriers, locus coeruleus might be involved independently from the dopaminergic system. It has recently been reported the rate of

nigrostriatal dysfunction progression in a subgroup of these subjects (n=6). The authors found a mean 0.56% annual reduction in putamen and 0.62 % annual reduction in caudate ¹⁸F-dopa uptake. These findings would suggest that heterozygous *parkin* gene mutations might not represent a major risk factor for late onset PD (Pavese et al., 2009).

Due to the small sample size the patients with *PINK1* mutation in the current study may not be representative of these forms of parkinsonism. Nevertheless results from the analysis of individual cases suggest a different pattern of monoaminergic involvement in these patients than idiopathic PD.

In summary, these results indicate different patterns of involvement in autosomal recessive parkinsonism and idiopathic PD involving dopaminergic, noradrenergic, and serotonergic pathways. The clinical relevance of this differential monoaminergic involvement remains to be clarified.

Progression of monoaminergic dysfunction in Parkinson's disease

5

5.1 Summary of objectives

The rate of progression of the degenerative process in extrastriatal areas in PD is still unclear. In this study, I have used serial ^{18}F -dopa PET to assess longitudinal changes in tracer uptake in brain noradrenergic, serotonergic and extrastriatal dopaminergic structures over a 3-year period in a group of early PD patients. Changes over time in ^{18}F -dopa uptake in extra-striatal structures were then compared with the rates of progression of striatal dysfunction in the same patients in order to better elucidate the time-course of the different aspects of the neurodegenerative process in PD.

5.2 Summary of results

Progressive decreases in ^{18}F -dopa Ki occurred over three years in the majority of the investigated areas, the fastest annual declines occurring in putamen (8.1%), locus coeruleus (7.8%), and globus pallidus interna (7.7%). Caudate and hypothalamus showed 6.3% and 6.1% annual Ki decline, respectively. At baseline, some structures showed increased levels of ^{18}F -dopa uptake in PD compared to controls (internal pallidum, locus ceruleus), indicating possible compensatory upregulation of monoamine turnover. These increased levels had normalised (globus pallidus interna) or become subnormal (locus coeruleus) at follow-up suggesting exhaustion of these mechanisms within the first few years of disease.

These findings suggest that loss of monoaminergic function in extrastriatal regions, as reflected by ^{18}F -dopa PET, is slower than loss of nigrostriatal dopaminergic function. When assessing the efficacy of novel neuroprotective agents on nigrostriatal dysfunction in PD, ^{18}F -dopa PET could provide supplementary information concerning function of extrastriatal monoaminergic structures.

5.3 Scientific background

Current knowledge concerning rates of PD progression in non-dopaminergic structures is limited. As discussed in Chapter 1, Braak and colleagues (2003, 2004) assessed brain patterns of abnormal immunostaining for α -synuclein and suggested that Lewy bodies and Lewy neurites first appear in the medullary dorsal nucleus of the vagus. The pathology then ascends to involve non-dopaminergic brainstem structures in the pons, such as the locus ceruleus and median raphe, antedating by several years the involvement of the substantia nigra in the midbrain and the appearance of the classical motor signs. Lewy body and Lewy neurites then progressively extend in a rostral direction to involve the nucleus basalis, the forebrain, and, in the final stages, the neocortex. However, while the pathology of PD may be ascending in nature, the associated dysfunction is highly variable from structure to structure and the rate of disease progression in individual non-dopaminergic regions and in extrastriatal dopaminergic structures remains unclear. Additionally, the relationship between decline in striatal function in PD and dysfunction in extra-striatal areas remains to be clarified.

Longitudinal imaging studies could help clarify these issues. However, no such studies have so far been conducted. There is only one cross-sectional study published (Moore et al., 2008) where ^{18}F -dopa PET was used to assess extrastriatal degeneration in PD patients with different degree of striatal dysfunction. Forty-one PD patients were arbitrarily assigned to three different groups, designed groups PD1, PD2, and PD3, based on the severity of putamen Ki reduction. PD1 patients had asymmetrical Ki decreases largely restricted to caudal putamen, PD2 patients had asymmetrical Ki decreases involving both rostral and caudal putamen, and PD3 patients had Ki

decreases throughout the entire putamen. Results indicate a progressive decline of monoaminergic function in extrastriatal areas from the PD1 to the PD3 group. However, the cross-sectional design and the large between group differences in disease duration and disability do not allow one to adequately estimate the progression rate of the degenerative process in these structures.

5.4 Patients and Methods

5.4.1 Patients

Ten patients (M/F= 6/4; mean age $\pm$ SD = 57.4 $\pm$ 7.2 years) with early stage PD (mean disease duration $\pm$ SD = 25.5 $\pm$ 7.6 months, mean UPDRS motor score in “off” condition $\pm$ SD = 17.2 $\pm$ 4.8) were enrolled in this study. A clinical diagnosis of probable idiopathic PD was made according to the UK Parkinson’s Disease Society Brain Bank diagnostic criteria for Parkinson’s disease. None of the patients had significant co-morbidity, current or previous history of other neurological conditions, dementia, or depression. Table 5-1 summarizes the demographics of all patients investigated.

Each patient was scanned with ^{18}F -dopa PET twice, at baseline and again at follow-up 37.1 $\pm$ 21.5 months later. All patients had their dopamine replacement therapy stopped for at least 12 hours before PET while cabergoline was stopped three days before scanning. On the day of each scan, disease severity in an ‘off’ state was assessed by UPDRS evaluation, which was performed half an hour before PET scanning.

Brain ^{18}F -dopa uptake values in PD patients at both baseline and follow-up were compared with those of 11 healthy volunteers from the Unit’s database (M/F= 6/5; mean age $\pm$ SD = 66.6 $\pm$ 5.5 years). None of these healthy volunteers had any significant medical history of current or previous neurological or psychiatric disorders. Three of the healthy volunteers had previously had two ^{18}F -dopa PET scans 15 days apart. Both scans were analyzed to assess reproducibility of extrastriatal ^{18}F -dopa uptake quantification.

All subjects gave their written informed consent to be scanned (approved by the Ethics Committee of Hammersmith, Queen Charlotte's & Chelsea and Acton Hospitals Trust), according to the declaration of Helsinki. Permission to administer ^{18}F -dopa was obtained from the Administration of Radioactive Substances Advisory Committee (ARSAC), UK.

Table 5-1. Patient Characteristics

	Healthy subjects (n=11)	Parkinson's disease patients (n=10)	
		At baseline	At follow-up (37.1±21.5 months)
M/F	6/5		6/4
Age (years, mean ±SD)	66.6± 5.5	57.4 ±7.2	61.1 ± 7.1
Disease Duration (months, mean ±SD)	=	25.5 ±7.6	62.6±21.3
Hoen & Yahr	=	2 ± 0.5	2.5 ± 0.5
UPDRS in "off"	=	17.2 ± 4.8	31 ± 3.6

5.4.2 PET procedure

All patients and healthy volunteers were scanned at the Cyclotron Building, Hammersmith Hospital, using an ECAT EXACT HR⁺⁺ (CTI/Siemens 966) PET scanner.

All subjects received 150mg oral carbidopa one hour prior to scanning, in order to improve ^{18}F -dopa availability to the brain (Hoffman et al., 1992).

The scanning protocol involved list mode (event-by-event) 3D acquisition over 95 minutes following a bolus injection of 110 MBq of ^{18}F -dopa.

5.4.3 PET analysis

The analysis was performed using an *a priori* ROI approach.

Parametric images of the influx constant K_i and integrated ADD images of ^{18}F -dopa uptake were created as described in Chapter 3. Both K_i and ADD images were transformed to ANALYZE format using ECAT_transfer.

ROI analysis was performed on datasets transformed into standard MNI stereotaxic space. The summed ADD images were normalized to the ^{18}F -dopa PET template in MNI space using SPM software and the individual ^{18}F -dopa K_i maps were subsequently spatially normalised by applying the transformation parameters obtained during normalisation of the corresponding summed images.

5.4.3.1 Extraction of ROI data.

Standard object maps in MNI space were used to extract ROI for eleven structures; the putamen, caudate nucleus, ventral striatum, globus pallidus interna and externa, subthalamic nucleus, red nucleus, ventral anterior thalamus, hypothalamus, midbrain raphe, and locus ceruleus. More details about the characteristics of these ROIs have been provided in paragraph 4.4.3.1 and in table 4-2.

The standard object maps were applied to the spatially normalised ^{18}F -dopa K_i maps and the values for specific ^{18}F -dopa uptake in individual regions were extracted using ANALYZE software (Mayo clinic, MN). To ensure correct placement of the ROIs, each plane of the normalised K_i maps and summed images was inspected. Manual corrections were made for any misalignment between K_i maps and the object maps to ensure the template was placed over the K_i map target region.

5.4.4 Statistical methods

In all subjects, I calculated the averaged right and left K_i for each ROI.

Percentage reductions below the averaged mean of the healthy volunteers were

calculated for each structure in individual PD patients both at baseline and follow-up according to the formula:

$$100 \times (\text{mean } ^{18}\text{F-dopa Ki healthy volunteers} - ^{18}\text{F-dopa Ki patient}) / \text{mean } ^{18}\text{F-dopa Ki healthy volunteers}$$

Individual patient values were then averaged for each structure. A negative value indicates an increase above the averaged mean of the healthy volunteers.

Percentage individual changes in ^{18}F -dopa uptake from baseline in PD patients were calculated for each structure as follows

$$100 \times (\text{baseline } ^{18}\text{F-dopa Ki} - \text{follow-up } ^{18}\text{F-dopa Ki}) / \text{baseline } ^{18}\text{F-dopa Ki}$$

Percentage changes per annum were then calculated as follows

$$12 \times (\% \text{ change } ^{18}\text{F-dopa Ki from baseline} / \text{months from baseline to follow-up})$$

Finally, individual patients values were averaged for each structure.

The Mann-Whitney U-statistic was used to compare PD patients and healthy volunteers and Wilcoxon matched-pairs to compare baseline and follow-up ^{18}F -dopa Ki values within the PD group. The relationship between the annual ^{18}F -dopa Ki decline in putamen and that in each of the other ROIs was investigated with the Spearman Rank Correlation statistic.

5.5 Results

Table 5-2 shows the means ($\pm$ standard deviation) of the K_i values obtained for each region assessed for healthy volunteers and PD patients at both baseline and follow-up,

in addition to percentage differences per annum for the PD group. Table 5-3 shows regional mean ^{18}F -dopa Ki values ($\pm$ standard deviation) in the three healthy volunteers who had two ^{18}F -dopa PET scans 15 days apart.

5.5.1 Parkinson's disease patients and healthy volunteers comparison

5.5.1.1 Baseline

When comparing PD patients at baseline with control subjects, I found decreased ^{18}F -dopa uptake in the majority of the ROIs sampled. Putamen, ventral anterior thalamus, and globus pallidus externa were the most affected structures with 41.1%, 21.5%, and 10.8% reductions below the averaged means of the healthy volunteers. Reductions were also seen in hypothalamus (10%), caudate (6.7%), subthalamic nucleus (6.5%), and ventral striatum (5.8%). However, only the ^{18}F -dopa decreases in putamen and ventral anterior thalamus reached statistical significance (Mann-Whitney U-tests $p < 0.01$ and $p < 0.05$, respectively). The globus pallidus interna, midbrain raphe, and locus coeruleus all showed raised ^{18}F -dopa uptake compared to healthy volunteers (+19.8%, +16.9%, and +2.8%, respectively). The ^{18}F -dopa Ki increases in globus pallidus interna and raphe reached statistical significance (Mann-Whitney U-tests, $p < 0.05$, in both ROIs).

5.5.1.2 Follow-up

At follow-up, PD patients showed declines in ^{18}F -dopa in all the regions sampled. Putamen Ki was most affected falling by 55.9% below the averaged mean of the healthy volunteers, followed by caudate (25.9%), hypothalamus (24.5%), ventral anterior thalamus (21.2%), globus pallidus externa (19.6%), locus coeruleus (18%), ventral striatum (17.8%), subthalamic nucleus (15.3%), red nucleus (13%), globus pallidus interna (2.9%), and median raphe (1.9%). Comparing PD patients at follow-up with control subjects revealed statistically significantly decreased K_i values in the putamen ($p < 0.001$), caudate nucleus ($p < 0.001$), ventral striatum ($p = 0.001$), globus pallidus externa ($p < 0.001$), hypothalamus ($p < 0.001$), locus coeruleus ($p < 0.05$), and ventral anterior thalamus ($p < 0.05$) with the Mann-Whitney U-statistic.

5.5.2 Baseline versus follow-up comparison in Parkinson's disease patients

The decreases in ^{18}F -dopa uptake from baseline reached statistical significance in caudate ($p<0.05$), putamen ($p<0.01$), globus pallidus interna ($p<0.01$), locus coeruleus ($p<0.01$), and hypothalamus ($p<0.01$). The most rapid annual decline in ^{18}F -dopa Ki was observed in putamen (8.1%) followed by locus coeruleus (7.8%), and globus pallidus interna (7.7%). Caudate and hypothalamus showed 6.3% and 6.1% annual declines, respectively (Table 5-2). In the remaining structures, the annual decline in ^{18}F -dopa uptake was less than 5%.

No correlations were found between the rate of annual decline in ^{18}F -dopa uptake in putamen and that of other structures. However, there was a trend towards a correlation between rate of ^{18}F -dopa decline in putamen and globus pallidus interna ($r=0.60$, $p=0.073$).

5.5.3 Comparisons between first and second scan in healthy volunteers

In the three healthy volunteers who had repeat ^{18}F -dopa PET scans, no significant changes in tracer uptake were seen in any of the sampled ROIs. Percentage changes between the two scans are reported in Table 5-3.

Table 5-2. Regional mean ^{18}F -dopa Ki values ($\pm$ Standard Deviation) in the normal subjects and in the Parkinson's disease patients at Baseline and Follow-up

	Healthy Subjects (n=11)	Parkinson's Disease Patients (n=10)		
		Baseline	Follow-up	% changes annum mean
Caudate	0.0114 $\pm$ 0.0006	0.0106 $\pm$ 0.0016	0.0084 $\pm$ 0.0022 $\downarrow$ § $\downarrow$ ***	-6.3
Putamen	0.0124 $\pm$ 0.0010	0.0073 $\pm$ 0.0023 $\downarrow$ **	0.0055 $\pm$ 0.0009 $\downarrow$ §§ $\downarrow$ ***	-8.1
Ventral striatum	0.0117 $\pm$ 0.0009	0.0110 $\pm$ 0.0014	0.0096 $\pm$ 0.0011 $\downarrow$ § $\downarrow$ ***	-4.9
Globus pallidus interna	0.0052 $\pm$ 0.0010	0.0062 $\pm$ 0.0005 $\uparrow$ *	0.0051 $\pm$ 0.0007 $\downarrow$ §§	-7.7
Globus pallidus externa	0.0059 $\pm$ 0.0008	0.0053 $\pm$ 0.0007	0.0047 $\pm$ 0.0007 $\downarrow$ ***	-2.4
Ventral ant. thalamus	0.0033 $\pm$ 0.0002	0.0026 $\pm$ 0.0006 $\downarrow$ *	0.0026 $\pm$ 0.0006 $\downarrow$ *	+0.9
Subthalamic nucleus	0.0043 $\pm$ 0.0015	0.0040 $\pm$ 0.0009	0.0036 $\pm$ 0.0007	-1.1
Hypothalamus	0.0048 $\pm$ 0.0007	0.0044 $\pm$ 0.0003	0.0037 $\pm$ 0.0004 $\downarrow$ §§ $\downarrow$ **	-6.1
Locus coeruleus	0.0050 $\pm$ 0.0009	0.0051 $\pm$ 0.0007	0.0041 $\pm$ 0.0006 $\downarrow$ §§ $\downarrow$ *	-7.8
Midbrain raphe	0.0068 $\pm$ 0.0015	0.0080 $\pm$ 0.0009 $\uparrow$ *	0.0067 $\pm$ 0.0013 $\downarrow$ §	-4.7
Red nucleus	0.0057 $\pm$ 0.0010	0.0057 $\pm$ 0.0012	0.0050 $\pm$ 0.0009	3.2

$\downarrow$ = significantly decreased compared to baseline (Wilcoxon matched-pairs signed-ranks test: § p<0.05, §§ p<0.01).

$\uparrow$ = significantly increased compared to healthy subjects, and $\downarrow$ = significantly decreased compared to healthy subjects (Mann-Whitney test: *p<0.05, ** p<0.01, and; *** p<0.001)

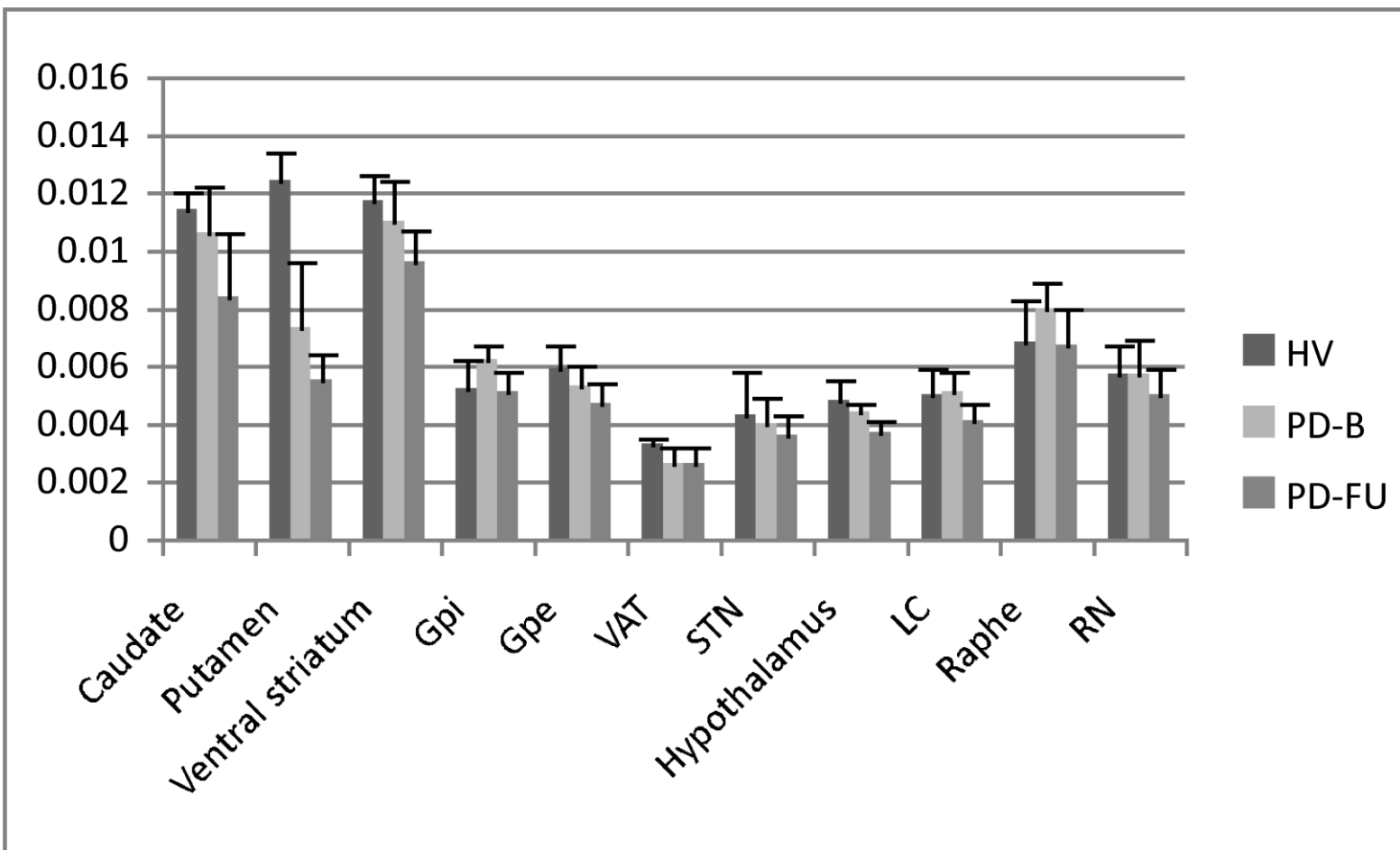


Figure 5-1. ^{18}F -dopa influx constant K_i values (mean $\pm$ standard deviation) (y-axis) in striatal and extrastriatal regions in normal subjects (HV) and patients with Parkinson's disease at baseline (PD-B) and follow-up (PD-FU). Significant differences between groups are reported in Table 5-2.

Table 5-3. Regional mean ^{18}F -dopa Ki values ($\pm$ Standard Deviation) in the normal subjects with two scans

	Healthy subjects	Healthy subjects with two scans		
	(n=11)	First scan	Second scan	% Changes mean (absolute value)
Caudate	0.0114 $\pm$ 0.0006	0.0105 $\pm$ 0.0006	0.0112 $\pm$ 0.0007	3.7
Putamen	0.0124 $\pm$ 0.0010	0.0125 $\pm$ 0.0011	0.0127 $\pm$ 0.0012	1.6
Ventral striatum	0.0117 $\pm$ 0.0009	0.0128 $\pm$ 0.0007	0.0130 $\pm$ 0.0004	2.4
Globus pallidus interna	0.0052 $\pm$ 0.0010	0.0051 $\pm$ 0.0005	0.0049 $\pm$ 0.0003	5.1
Globus pallidus externa	0.0059 $\pm$ 0.0008	0.0058 $\pm$ 0.0008	0.0059 $\pm$ 0.0009	1.7
Ventral ant. thalamus	0.0033 $\pm$ 0.0002	0.0027 $\pm$ 0.0002	0.0028 $\pm$ 0.0004	4.8
Subthalamic nucleus	0.0043 $\pm$ 0.0015	0.0047 $\pm$ 0.0009	0.0049 $\pm$ 0.0014	6.3
Hypothalamus	0.0048 $\pm$ 0.0007	0.0043 $\pm$ 0.0008	0.0043 $\pm$ 0.0005	5.5
Locus coeruleus	0.0050 $\pm$ 0.0003	0.0051 $\pm$ 0.0015	0.0050 $\pm$ 0.0002	5.9
Midbrain raphe	0.0068 $\pm$ 0.0015	0.0069 $\pm$ 0.0012	0.0065 $\pm$ 0.0008	6.1
Red nucleus	0.0057 $\pm$ 0.0010	0.0056 $\pm$ 0.0007	0.0059 $\pm$ 0.0007	5.0

5.6 Discussion

This is the first longitudinal, prospective, *in vivo* study to report the rate of disease progression in extrastriatal monoaminergic structures in early stages of PD.

Using serial ^{18}F -dopa PET imaging, a marker of AADC activity in monoaminergic neurons, I found that the putamen had the fastest annual decline in ^{18}F -dopa uptake (8.1%) followed by locus coeruleus (7.8%), and globus pallidus interna (7.7%).

Caudate and hypothalamus showed a 6.3% and 6.1% decline per annum respectively, whereas the remaining structures showed less than 5% annual decline in ^{18}F -dopa uptake (Table 5-2). Additionally, I found no significant correlations between the rate of annual decline in ^{18}F -dopa uptake in putamen and that in other structures. These findings suggest that the degeneration in extrastriatal monoaminergic structures in PD occurs independently from nigrostriatal degeneration and at a slower rate. These results are in line with several post-mortem studies, which have shown that in advanced PD cases, the cell loss in non-dopaminergic nuclei ranges between 30 and 50%, whereas it is close to 80% in the nigral region (Jellinger et al., 1991; Zarow et al., 2003; Thannickal et al., 2007).

In agreement with previous studies (Rakshi et al., 1999; Whone et al., 2003; Moore et al., 2008), baseline mean ^{18}F -dopa uptake in globus pallidus interna and midbrain raphe was significantly raised in our early PD patients. As discussed in previous chapters of this thesis, increases in ^{18}F -dopa uptake in these structures is likely to reflect upregulation of AADC activity and dopamine turnover in surviving terminals and could represent a compensatory adaptive mechanism to preserve functionality. The longevity of these compensatory mechanisms is unknown. In cross-sectional studies, advanced PD patients showed significant reductions in globus pallidus interna and raphe ^{18}F -dopa uptake compared to both controls and PD patients with early disease, suggesting that compensatory mechanisms involving these structures eventually fail during the time course of the disease. In this longitudinal study, tracer uptake in these two nuclei fell slightly below the control mean value after a three-year follow up, suggesting that compensatory hyperactivity reverses within the first years

of disease. The annual declines observed in ^{18}F -dopa uptake in globus pallidus interna and midbrain raphe could, therefore, simply reflect the loss of their compensatory upregulation rather than cellular loss. Longer follow-ups with three or more imaging assessments are required to assess the effective rate of disease progression in these structures once the compensatory mechanisms have disappeared.

The locus ceruleus showed the second highest mean annual ^{18}F -dopa decline in the PD group. At follow-up, our patients had significantly lower ^{18}F -dopa uptake in the locus coeruleus than controls ($p < 0.05$) with a mean 18% reduction below the averaged mean of the healthy volunteers. Reduced ^{18}F -dopa Ki in this nucleus most likely reflects the progressive loss of noradrenergic terminal function. Interestingly, at baseline, PD patients had higher locus coeruleus ^{18}F -dopa uptake than the healthy volunteers, although the difference between the two groups was not statistically significant. Lewy body pathology in the locus coeruleus is a well-documented post-mortem finding in PD (Zarow, et al., 2003) and appears in stage 2 of the disease (Braak et al., 2004). The preservation of ^{18}F -dopa uptake in the locus coeruleus of PD patients at baseline observed in this study could indicate that AADC upregulation also occurs in this nucleus. Alternatively, it could imply that Lewy body pathology is not necessarily associated with neuronal dysfunction in this area in the early phases of the disease. The hypothalamus is commonly affected in PD. Lewy body inclusions have been demonstrated in every nucleus of the hypothalamus, particularly the tuberomammillary nucleus and the lateral and posterior hypothalamic nuclei (Langston and Forno, 1978; Sandyk et al., 1987, Wakabayashi and Takahashi, 1997). Reduced hypothalamic ^{18}F -dopa uptake has previously been reported in PD patients (Moore et al., 2008). The cohort of patients with advanced PD investigated in the experimental study described in chapter 4 of this thesis also showed reduced hypothalamic ^{18}F -dopa Ki compared to both controls and patients with *parkin*-linked parkinsonism. Moreover, our group has recently reported a significant reduction in dopamine D2 receptor availability, as measured by ^{11}C -raclopride PET, in the hypothalamus of PD patients compared with age-matched healthy volunteers (Politis et al., 2007). In this study, I found no significant difference in mean ^{18}F -dopa uptake in the hypothalamus between PD patients and healthy volunteers at baseline although it was reduced by 10%. Three

years later, the PD patients showed a significant 20.2% reduction ($p < 0.05$) below the controls' mean value in ^{18}F -dopa uptake with an annual 6.2% decline from baseline. These findings confirm that hypothalamic involvement is common in PD and suggest that this structure could be targeted at very early stages of the disease with a fast progression rate once degeneration has started.

The hypothalamus has a complex internal structure including intrinsic dopaminergic neurons and a dense innervation from serotonergic midbrain raphe and lateral medullary noradrenergic neurons. In PD patients, the reduction of ^{18}F -dopa uptake in this region could reflect dysfunction in any or all of these pathways. Further post-mortem and *in vivo* studies, preferably with PET ligands specific for each of these neurotransmitter systems, are required to better understand the pathogenesis of hypothalamic involvement in PD.

The mean annual decline in putamen and caudate ^{18}F -dopa Ki noted in this study is comparable with data from previous studies (Morrish et al., 1996, 1998). The rate of progression of dopaminergic dysfunction in the ventral striatum has not been assessed previously. The ventral striatum, which encompasses the nucleus accumbens and the ventromedial parts of caudate and putamen, is generally considered a limbic center and has widespread connections with other limbic structures including the amygdala, hippocampus, and the prefrontal cortex (Groenewegen and Trimble, 2007). The ventral striatum is strongly innervated by dopaminergic fibers from the midbrain ventral tegmental area. In PD, the ventral tegmental area shows a less pronounced loss of dopaminergic neurons than the contiguous substantia nigra pars compacta. In line with these observations, I found a relatively slow mean annual decline (4.9%) in ^{18}F -dopa uptake in ventral striatum compared to both putamen (8.1%) and caudate (6.3%).

Annual decline in ^{18}F -dopa uptake in red nucleus, and in the remaining extrastriatal monoaminergic structures assessed in this study was less than 5%, suggesting that degeneration occurs at a slow rate in these structures.

Finally, this study provides evidence that uptake of ^{18}F -dopa in extrastriatal structures can be measured reliably and with good reproducibility. In the three healthy volunteers who had repeat ^{18}F -dopa PET scans, no significant differences in tracer K_i between the first and second scans were seen in any of the sampled ROIs. The mean percentage of change (absolute value) between the two scans ranged from 1.6 % (putamen) to 6.3% (subthalamic nucleus).

^{18}F -dopa PET and the dopamine transporter marker ^{123}I - β -CIT SPECT have been used as surrogate markers of nigrostriatal degeneration in clinical trials of putative neuroprotective drugs in PD (Oertel et al., 2000; Rakshi et al., 2002; Rascol et al., 2000; Parkinson Study Group, 2002; Whone et al., 2003; Pavese et al., 2005). Unfortunately, no unequivocal evidence of neuroprotection has been demonstrated for any drugs tested so far. Interpretation of data generated by these trials has also been limited by adaptive compensatory effects upregulating dopamine turnover and downregulating transporter binding over time, direct effects on receptor availability and enzyme activities by the drug itself, and placebo effects. All these confounding factors potentially can interfere with the imaging outcome and must be addressed when designing new clinical trials of neuroprotective agents in PD patients. In this study, I have shown that ^{18}F -dopa PET can be successfully used to assess disease progression in serotonergic and noradrenergic extrastriatal areas along with nigrostriatal degeneration. The technique could, therefore, provide supplementary information on the progression of the degenerative process in extrastriatal monoaminergic structures when assessing the effects of novel neuroprotective agents in PD.

PET investigation of chronic fatigue in Parkinson's disease

6

6.1 Summary of objectives

I used ^{18}F -dopa PET and ^{11}C -DASB PET to study the role of aberrant dopaminergic and serotonergic neurotransmission in the pathophysiology of fatigue in PD.

The overall objective of this study was to test the hypothesis that chronic fatigue in PD is due to reduced serotonergic and dopaminergic innervation of the non-motor components of the basal ganglia including the ventral striatum, and associated limbic circuits. These are the structures that facilitate the link between emotion/motivation (limbic system) and motor response. The hypothesis is that patients with PD who experience central fatigue have pathology causing reductions in serotonin and dopamine turnover within the basal ganglia and limbic circuits.

6.2 Summary of results

Using a region of interest analytical approach, I found that PD patients with fatigue had significantly lower serotonin transporter binding than patients without fatigue in caudate, putamen, ventral striatum and thalamus. Striatal ^{18}F -dopa uptake was similar in the fatigued and non-fatigued groups. Voxel-based analysis localised further relative serotonin transporter binding reductions in cingulate and amygdala of the fatigue group, and ^{18}F -dopa uptake reductions in caudate and insula. In conclusion, fatigue in

Parkinson's disease is associated with reduced serotonergic function in basal ganglia and limbic structures. Insular dopaminergic dysfunction could also play a role. These findings imply that strategies to increase brain level of serotonin would be a rational approach for relieving fatigue symptoms in Parkinson's disease and may also be relevant to alleviating fatigue in other clinical conditions.

6.3 Scientific background

Disabling fatigue is a common non-motor symptom of PD and is estimated to occur in about one-third of patients. (Karlsen et al., 1999; Abe et al., 2000; Friedman and Friedman, 2001; Lou et al., 2001; Herlofson and Larsen, 2002). Sometimes the symptom antedates the development of motor disturbances. Hagell and Brundin (2009) have recently reported that in a cohort of 118 consecutive PD patients, 18 (15.3%) had fatigue before any motor disturbance.

The PRIAMO study (Barone et al., 2009) reported that fatigue occurred in 58.1% of a cohort of 1072 patients ranging from 37.7% in HY stage 1 patients to 81.6% in stages 4-5. Affected patients typically complain of a feeling of constant exhaustion or tiredness, either mental, physical, or both (Brown et al., 2005). However, severity of mental fatigue does not correlate well with physical fatigue, suggesting that the two conditions are caused by independent mechanisms in PD (Lou et al., 2001).

The reason for the high prevalence of fatigue in PD remains poorly understood. In most studies, the presence and severity of fatigue does not correlate with disease duration or degree of motor disability. Additionally, no direct relationship between presence of fatigue and type, dosage, and duration of anti-parkinsonian medication has been found (Abe et al., 2000; Friedman and Friedman, 2001; Hagell and Brundin, 2009). Conversely, the presence of depression, nocturnal sleep disturbance, and autonomic impairment are all thought to exacerbate the subjective experience of fatigue (Karlsen et al., 1999; Lou et al., 2001; Alves et al., 2004; Hagell and Brundin, 2009). However, these factors cannot completely explain the high prevalence of fatigue in PD, as the symptom is common in non-depressed patients and is also

experienced by patients with no complaint of sleep disorders (Friedman and Friedman, 1993; Karlsen et al., 1999; Lou et al., 2001).

It has been proposed that basal ganglia dysfunction plays a role in the mechanism of fatigue in diseases of the central nervous system (Chaudhuri et al., 2000; Chaudhuri et al., 2003; Chaudhuri et al., 2004). Several observations would support this view: In patients with multiple sclerosis, fatigue has been reported to be associated with reduced glucose metabolism in the frontal cortex and the basal ganglia (Roelcke et al., 1997). Bilateral posteroventral pallidotomy for treatment of PD can also lead to profound fatigue, sleepiness, changes in behaviour, and poor initiative in executive functions despite improvement in motor control (Ghika et al., 1999). However, a recent study among levodopa-naïve PD patients found that patients with fatigue had similar striatal dopamine transporter uptake compared to patients without fatigue (Schifitto et al., 2008). These findings indicate that non-dopaminergic basal ganglia pathways are most likely to be involved in fatigue generation in PD or, if dopaminergic dysfunction plays a role, it involves extrastriatal projections.

Several studies have suggested that serotonergic deficiency is a pathophysiological factor in chronic fatigue syndrome without parkinsonism (Narita et al., 2003; Yamamoto et al., 2004; Cleare et al., 2005; Katafuchi et al., 2006). Accordingly, reduced serotonin transmission could have a role in the occurrence of fatigue in patients with PD.

6.4 Patients and methods

6.4.1 Patients

Twenty PD patients with a clinical diagnosis of idiopathic PD (UK Parkinson's Disease Society Brain Bank diagnostic criteria for Parkinson's disease) were recruited for this study. Patients with dementia [Mini Mental State Examination (Folstein et al., 1975) <23], depression [Hamilton Rating Scale for Depression (Hamilton, 1960) >16 and Beck Depression Inventory (Beck et al., 1961) >9], and sleep disturbances, specifically excessive daytime sleepiness (EDS) [Epworth Sleepiness Scale Johns, 1991) >8] were not enrolled.

The patients were divided into two groups according to the presence ($n = 10$) or absence ($n = 10$) of fatigue. A presence of fatigue was defined as a score ≥ 8 on the Parkinson Fatigue Scale (PFS-16) (Brown et al., 2005). The PFS-16 has specifically been designed to measure fatigue in a PD population taking in account possible overlapping with other motor and non-motor symptoms of PD. In particular, it provides a measure of fatigue, which is independent of affective, sleep, and cognitive disturbances. The scale contains 16 questions. Seven of them are related to the presence or absence of subjective experience of fatigue, whereas the remaining nine measure the impact of fatigue on daily activity. Each item has five response options from 1 (strongly disagree) to 5 (strongly agree). In this paper I scored each item using a binary scoring method. In this method, the response "agree" and "strongly agree" are scored 1, and all other responses are scored 0. The total score ranges from 0- 16, 16 being worse score and 8 the cut-off score to identify patients who perceive fatigue to be a problem (Brown et al., 2005).

The two groups of PD patients with and without fatigue were matched for age, disease duration, daily intake of levodopa equivalent units (LEU), and UPDRS scores in both "off" and "on" conditions. Table 6.1 summarizes the demographics of all patients investigated. All the subjects had a stable response to their dopamine replacement therapy and none were taking medication targeting serotonergic and noradrenergic

systems. LEUs were calculated as follows: 100 mg levodopa = 2mg cabergoline = 1mg pramipexole or 1 mg pergolide = 5 mg ropinirole = 4 mg rotigotine patch = 0.5 mg rasagiline. The conversion factors below were used for controlled release levodopa and levodopa/carbidopa/entecapone combination: 125 mg controlled release levodopa = 65 mg levodopa; 50 mg levodopa/carbidopa/entecapone combination = 65 mg levodopa.

Eight PD patients with fatigue (PD-F) and seven PD patients without fatigue (PD-NF) had both ^{18}F -dopa and ^{11}C -DASB PET. The remaining 5 patients had only one scan as they withdraw their consent after the first PET session. In summary, ten PD-F patients and nine PD-NF patients had ^{18}F -dopa PET and eight PD-F patients and eight PD-NF patients had ^{11}C -DASB PET (Table 6.1).

The study received approval from the Ethics Committee of Hammersmith, Queen Charlotte's & Chelsea and Acton Hospitals Trust. Permission to administer ^{18}F -dopa and ^{11}C -DASB was obtained from the Administration of Radioactive Substances Advisory Committee (ARSAC), UK.

Since the main aim of this project was to compare function between PD patients with and without fatigue no healthy control subjects were scanned during this study. However, ^{11}C -DASB PET findings for the two PD groups were compared with those of 9 healthy volunteers (all males, age 44.6 ± 5.5 years) from the Unit's database. None of these healthy volunteers had any significant medical history including current or previous medical history of neurological or psychiatric disorders.

6.4.2 PET protocol

PET scanning took place at the Cyclotron Building, Hammersmith Hospital, Hammersmith Imanet, GE Healthcare, London, UK, and all PET scans were performed using an ECAT EXACT HR+ (CTI/Siemens 962) camera. All patients had their dopamine replacement medication stopped for at least 12h before PET.

The scanning protocol for ^{18}F -dopa involved 3D acquisition over 94 minutes and 30 seconds following a bolus injection of 185 MBq of ^{18}F -dopa.

The scanning protocol for ^{11}C -DASB involved 3D acquisition over 90 minutes and 30 seconds following a bolus injection of 450 MBq of ^{11}C -DASB.

6.4.3 PET analysis

Parametric images of the influx constant K_i and integrated ADD images of ^{18}F -dopa uptake were created as described in Chapter 3. Both K_i and ADD images were then transformed to ANALYZE format using an in house program, ECAT_transfer. Once in ANALYZE format the parametric and ADD image from each scan were visually inspected using ANALYZE software (Mayo clinic, MN).

Spatial normalization of ADD images and K_i maps into standard MNI stereotaxic space was performed using the SPM software package implemented in Matlab5. First the ADD images were normalized to a smoothed ^{18}F -dopa template in MNI space. Then the transformation parameters were applied to the respective ^{18}F -dopa K_i maps.

As described in Chapter 3, parametric images of specific ^{11}C -DASB BP_{ND} were generated on a voxel wise basis for the whole brain using a basis function implementation of the simplified reference region compartmental model with the cerebellum providing the reference tissue input function (Gunn et al., 1997). Integrated ADD images of ^{11}C -DASB uptake were also created for spatial normalization purposes. ADD images and parametric images of ^{11}C -DASB BP_{ND} were then normalized, using SPM software, to a smoothed ^{11}C -DASB ADD image template.

6.4.3.1 Extraction of ROI data

To extract ROI data I used a standard object map in MNI space. The standard object map contained regions defined for caudate nucleus, putamen, ventral striatum, thalamus, and median raphe. The same standard object map was applied to each spatially normalized image of ^{18}F -dopa K_i or ^{11}C -DASB BP_{ND} and corresponding ADD images. Visual inspection of each plane for both images was made to ensure

correct placement of the object regions over the correspondent structures. After applying target regions to structures, ^{18}F -dopa Ki and ^{11}C -DASB BP_{ND} values were quantified using ANALYZE software. ^{18}F -dopa Ki and ^{11}C -DASB BP_{ND} values obtained from the different ROIs were averaged over both hemispheres for statistical analysis.

6.4.3.2 Voxel-based analysis

SPM software was used to localize significant changes in tracer uptake between PD-NF and PD-F patients at a voxel level. Normalized parametric images of ^{18}F -dopa Ki and ^{11}C -DASB BP_{ND} were spatially smoothed using a 6 x 6 x 6 mm (full-width at half maximum) isotropic Gaussian kernel.

For each tracer, between-group comparisons were performed by using weighted contrasts to localize significant decreases in mean voxel tracer uptake in PD-F patients compared to PD-NF patients. The contrast was used to generate maps of Z scores on a voxel basis using the general linear model (Friston et al., 1995). The statistical maps were visualized at a threshold $p < 0.01$ (Z score = 2.33), and any resulting cluster with a $p < 0.05$ is reported.

6.4.4 Statistical analysis

Statistical analyses of clinical data were performed with SPSS 16 for Windows (SPSS Inc., Chicago, Illinois).

Comparisons between groups were made using a non-parametric unpaired test (Mann-Whitney U-test) or non-parametric ANOVA (Kruskal-Wallis test) as appropriate. *Post Hoc* comparisons after Kruskal-Wallis test were performed using two-tailed Mann-Whitney U-test. Resulting p values were corrected according to the Bonferroni method.

Correlations between variables were tested with Spearman rank correlation.

Table 6-1. Patient Characteristics

		¹¹ C-DASB PET	¹⁸ F-Dopa PET	Age (yrs)	Disease Duration (yrs)	LEU	PFS-16 score	UPDRS off	UPDRS on
PD-NF patients									
#1	M	X	X	53	7	450	1	30	15
#2	M	X	X	71	4	200	1	42	25
#3	M	//	X	63	3	590	2	32	28
#4	F	X	X	46	3	750	3	33	15
#5	F	X	X	76	4	915	5	36	27
#6	M	X	X	52	2	835	5	26	19
#7	M	X	X	77	6	715	6	40	25
#8	M	X	//	70	4	550	6	36	20
#9	F	//	X	59	4	550	7	32	18
#10	F	X	X	69	4	250	7	28	17
Mean ± SD				63.6±10.7	4.1±1.4	580±235	4.3±2.4	33.5±5.1	20.9±4.9
<i>PD-F patients</i>									
#11	M	X	X	69	5	750	10	35	25
#12	F	X	X	57	3	785	10	22	14
#13	F	X	X	66	7	1480	11	42	22
#14	M	//	X	59	5	550	13	42	25
#15	M	X	X	55	3	280	13	32	17
#16	F	X*	X	58	6	635	14	36	17
#17	M	X	X	77	8	600	14	38	21
#18	M	X	X	70	3	1000	15	34	23
#19	M	X	X	66	11	1135	16	38	31
#20	F	//	X	70	4	520	16	30	18
Mean ± SD1				64.7±7.2	5.5±2.6	773±348	13.2±2.3	34.9±6	21.3±5

* In this patient the spatial normalization of the individual parametric image to the ¹¹C-DASB template was unsuccessful and the patient was excluded from the analysis. x= performed; // = non performed

6.5 Results

Severity of fatigue in the whole group of 20 PD patients did not correlate with age (Spearman $r = 0.24$), disease duration (Spearman $r = 0.28$), LEU intake (Spearman $r = 0.32$), UPDRS motor subscale-3 score in off stage (Spearman $r = 0.32$), or UPDRS-3 score in on stage (Spearman $r = 0.08$).

6.5.1 ^{18}F -dopa findings

Regional mean ^{11}F -dopa Ki values in PD-F patients and PD-NF patients are shown in Table 6-2.

Table 6-2. Regional mean ^{18}F-dopa Ki values ($\pm$ Standard Deviation) in Parkinson's disease patients with (PD-F) and without (PD-NF) fatigue		
	PD-NF	PD-F
	(n=9)	(n=10)
Caudate	0.0082 $\pm$ 0.0014	0.0071 $\pm$ 0.0016†
Putamen	0.0051 $\pm$ 0.0010	0.0045 $\pm$ 0.0009
Ventral striatum	0.0089 $\pm$ 0.0013	0.0084 $\pm$ 0.0013
Thalamus	0.0026 $\pm$ 0.0008	0.0025 $\pm$ 0.0008
Raphe	0.0038 $\pm$ 0.0007	0.0040 $\pm$ 0.0008
Mann-Whitney U-test: †p=0.094		

PD-F patients and PD-NF patients had similar levels of mean ^{18}F -dopa uptake in putamen, ventral striatum, thalamus, and median raphe. PD-F patients showed a trend towards lower mean ^{18}F -dopa uptake in caudate than PD-NF patients ($p=0.095$).

Categorical comparisons with SPM between PD-F patients and PD-NF patients localized clusters of relative significant decreases in ^{18}F -dopa Ki in caudate bilaterally and left insula (Table 6-3, Figure 6-1) in the cohort of PD-F patients.

Table 6-3. Areas of significant decreases in ^{18}F -dopa Ki in Parkinson's disease patients with fatigue compared Parkinson's disease patients without fatigue

Z score	MNI co-ordinates			Area	p value (corrected)
	x	y	z		
3.68	-38	-28	18	L Insula	P<0.001
3.50	-13	6	14	L caudate	P<0.001
2.75	15	15	7	R caudate	P<0.01

Coordinates are Montreal Neurological Institute (MNI) space; R=right; L=left

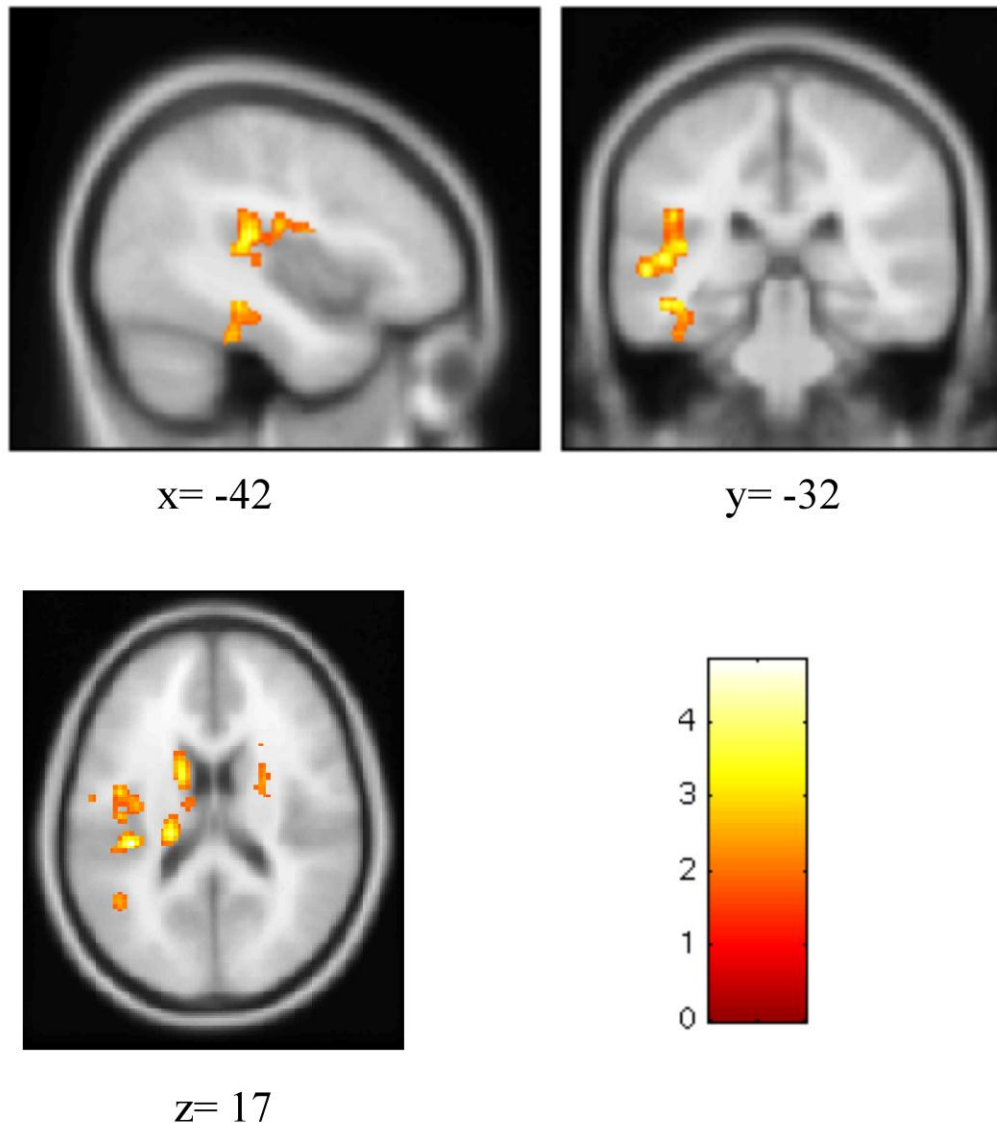


Figure 6-1. Reduced ^{18}F -dopa uptake in Parkinson's disease patients with fatigue. Projections of statistical parametric maps showing areas of significant decreases in ^{18}F -dopa Ki in the patients with Parkinson's disease with fatigue ($n=9$) compared to patients with Parkinson's disease without fatigue ($n=10$). In the sagittal and coronal images, decreases are seen in the left insula and left temporal gyrus. Only the cluster in the left insula, however, is statistically significant. In the transaxial image, decreases are seen in the left caudate and left insula. The colorstripe indicates t values. Areas are superimposed on the averaged T1 MRI image in MNI space available in SPM

6.5.2 ^{11}C -DASB findings

For one PD-F patient spatial normalization of their parametric BP_{ND} image to the ^{11}C -DASB template was unsuccessful so this case was excluded from analysis. Regional mean ^{11}C -DASB BP_{ND} values in normal subjects, PD-F patients, and PD-NF patients are shown in Table 6-4 and Figure 6-2. Figure 6-3 shows ^{11}C -DASB ADD images from a normal subject, a PD-NF patient, and a PD-F patient. Kruskal-Wallis one-way analysis of variance showed a statistically significant effect of group (controls, PD-F, and PD-NF patients) on ^{11}C -DASB BP_{ND} values for caudate ($p<0.001$), putamen ($p<0.001$), ventral striatum ($p<0.001$), and thalamus ($p<0.001$). There was no statistically significant effect of group on ^{11}C -DASB BP_{ND} for median raphe ($p=0.26$). *Post hoc* analyses with the Mann-Whitney U-test and Bonferroni adjustment showed that control subjects had significant higher ^{11}C -DASB BP_{ND} values than both groups of PD patients in the caudate, putamen, and ventral striatum and significantly higher ^{11}C -DASB BP_{ND} values than PD-F patients in thalamus. In all these brain regions, PD-NF patients had significantly higher BP_{ND} values than PD-F patients.

Table 6-4. Regional mean ^{11}C -DASB binding potential values ($\pm$ Standard Deviation) in the normal subjects and in Parkinson's disease patients with (PD-F) and without (PD-NF) fatigue

	Healthy subjects (n=10)	PD-NF (n=8)	PD-F (n=7)
Caudate	1.68 $\pm$ 0.87	0.80 $\pm$ 0.17**[-52%]	0.28 $\pm$ 0.16**§§ [-83%]
Putamen	2.10 $\pm$ 0.9	1.09 $\pm$ 0.15* [-48%]	0.51 $\pm$ 0.26**§§ [76%]
Ventral striatum	1.86 $\pm$ 0.63	1.06 $\pm$ 1.17**[43%]	0.48 $\pm$ 0.34**§ [-74%]
Thalamus	1.49 $\pm$ 0.37	1.13 $\pm$ 0.21 [32%]	0.5 $\pm$ 0.29**§ [-66%]
Raphe	1.49 $\pm$ 0.31	1.53 $\pm$ 0.33 [0%]	0.89 $\pm$ 0.74 [-40%]

Mann-Whitney U-test, p values corrected with Bonferroni method for multiple comparison: * $p<0.05$ compared to healthy subjects; ** $p<0.01$ compared to healthy subjects; Direct comparison PD-NF and PD-F: § $p<0.05$; §§ $p<0.01$; The percentage in brackets represents percentage of reduction from normal controls' mean value

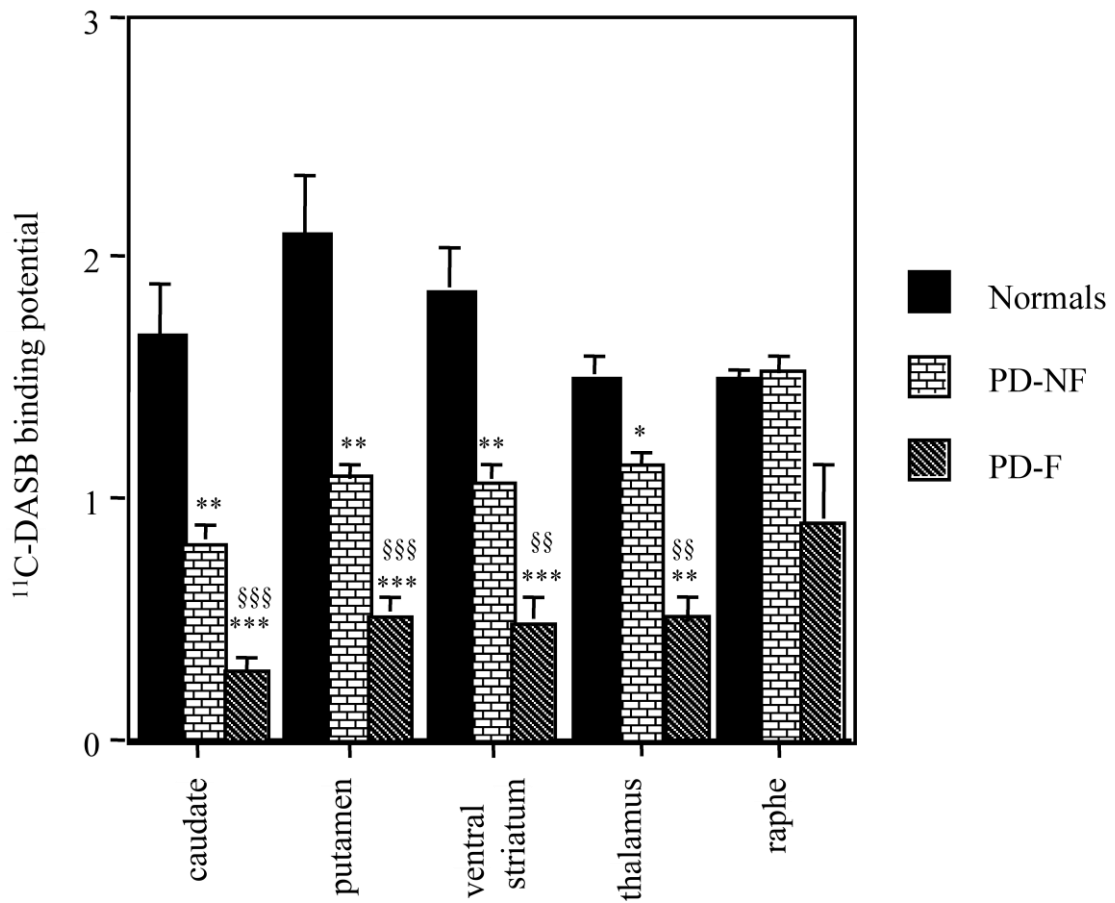


Figure 6-2. ^{11}C -DASB binding potential (mean $\pm$ standard error) (y-axis) in striatal and extrastriatal regions. * Indicates significant differences between normal subjects and Parkinson's disease patients with (PD-F) or without (PD-NF) fatigue; § Indicates significant differences between PD-NF and PD-F patients

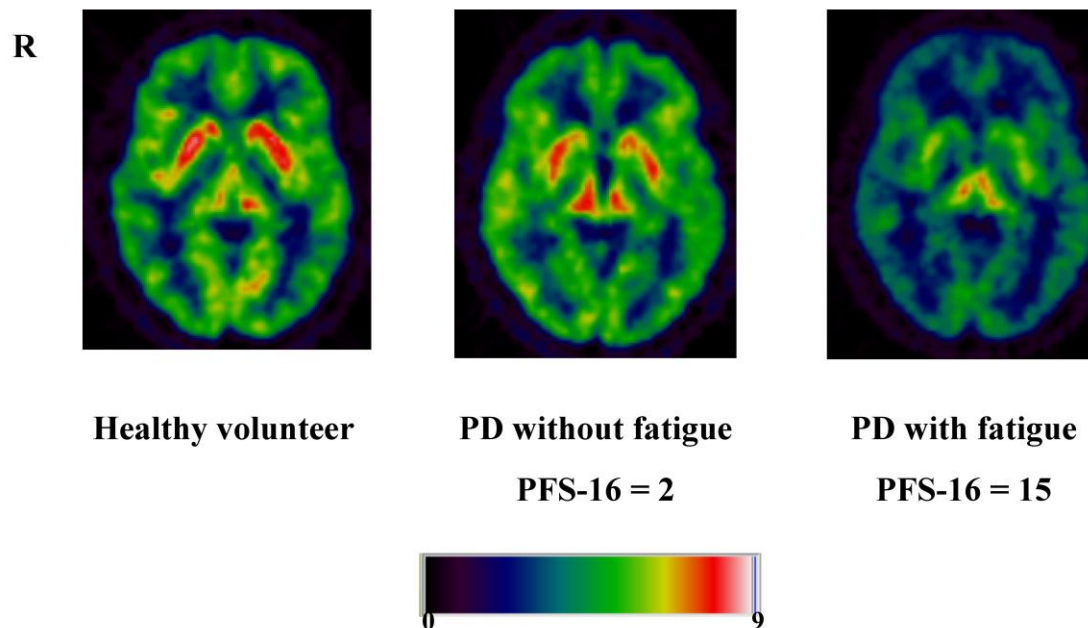


Figure 6-3. ADD images of ^{11}C -DASB binding potential from a normal subject, a Parkinson's disease patients without fatigue, and a Parkinson's disease patients with fatigue. The colorstripe indicate binding potential values for ^{11}C -DASB binding potential

Categorical comparisons with SPM between PD-F patients and PD-NF patients localized clusters of significant relative bilateral decreases in ^{11}C -DASB BP_{ND} in caudate, putamen, ventral striatum, thalamus, cingulate gyrus, and amygdala (Table 6-5, Figure 6-4) in the cohort of PD-F patients.

6.5.3 Clinical correlations

Correlation analyses between clinical characteristics of PD patients and regional ^{11}C -DASB BP_{ND} or ^{18}F -dopa Ki are summarized in Table 6-6 and Table 6-7.

Table 6-5. Areas of significant decreases in ^{11}C -DASB binding potential in Parkinson's disease patients with fatigue compared to Parkinson's disease patients without fatigue

Z score	MNI co-ordinates			Area	p value (corrected)
	x	y	z		
5.13	25	5	9	R putamen	p<0.001
4.83	-26	2	8	L putamen	p<0.001
4.80	14	9	12	R caudate	p<0.001
4.60	-14	12	6	L caudate	p<0.001
4.40	-34	-12	20	L insula	p<0.001
4.40	-10	6	40	L cingulate BA24/32	p<0.001
4.07	4	-14	42	R cingulate BA24/32	p<0.001
3.83	8	-8	10	R thalamus	p<0.001
3.58	-10	-14	6	L thalamus	p<0.001
3.24	4	-28	46	R paracentral lobule BA 31	p<0.01
3.20	18	-2	-22	L amygdala	p<0.01

Coordinates are Montreal Neurological Institute (MNI) space; R=right; L=left; BA= Brodmann area

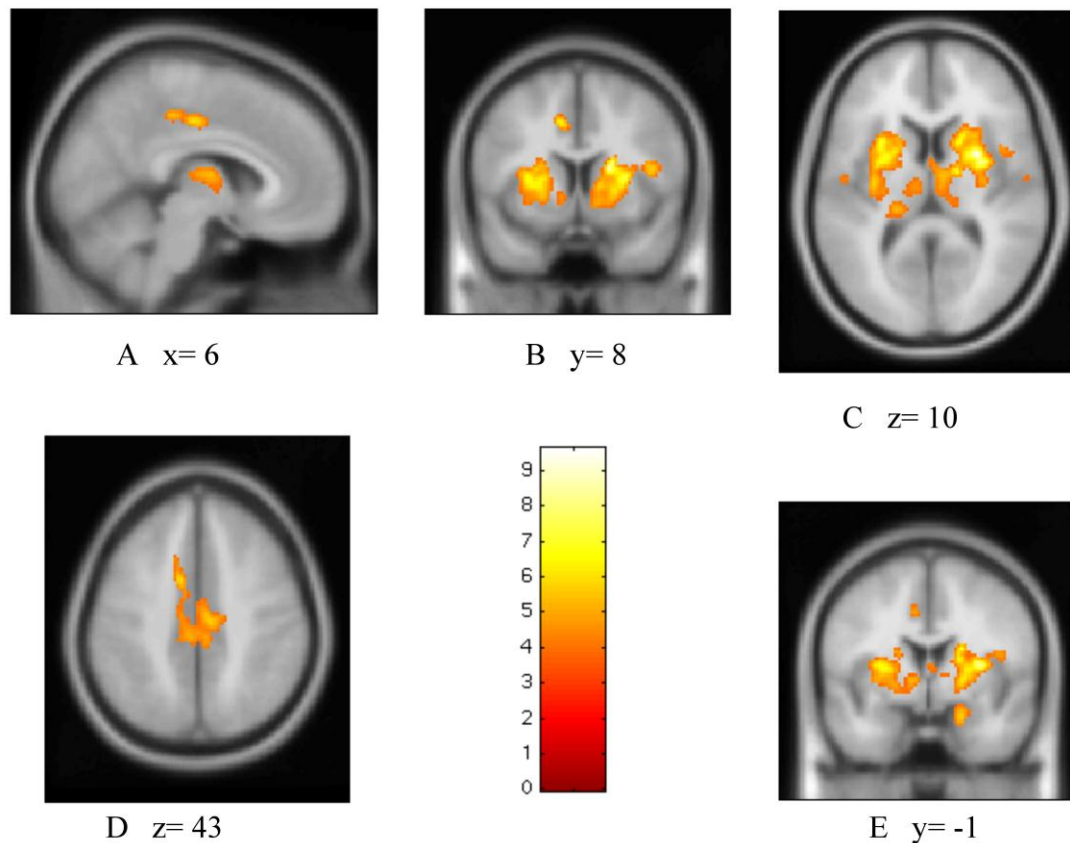


Figure 6-4. Reduced ^{11}C -DASB binding potential (BP) in Parkinson's disease patients with fatigue. Projections of statistical parametric maps showing areas of significant decreases in ^{11}C -DASB BP in the patients with Parkinson's disease with fatigue ($n=7$) compared to patients with Parkinson's disease without fatigue ($n=8$). Areas are superimposed on the averaged T1 MRI image in MNI space.

A. Sagittal image showing clusters of reduced ^{11}C -DASB BP in right cingulate and right thalamus.

B. Coronal image showing clusters of reduced ^{11}C -DASB BP in right and left striatum and left cingulate.

C. Transaxial image showing clusters of reduced ^{11}C -DASB BP in right and left striatum and right and left thalamus.

D. Transaxial image showing clusters of reduced ^{11}C -DASB BP in right and left cingulate.

E. Coronal image showing clusters of reduced ^{11}C -DASB BP in right and left striatum, and left amygdala. The colorstripe indicates T values

Table 6-6. Correlation analysis for regional ^{11}C -DASB binding potential and clinical characteristics in the total group of Parkinson's disease patients (n=15)

	Spearman Rank correlation coefficient (rho)				
	Caudate	Putamen	Ventral Striatum	Thalamus	Raphe
Age	-0.29	-0.32	-0.27	-0.48	-0.61*
Disease Duration	-0.38	-0.29	-0.40	-0.31	-0.54*
UPDRS off	-0.40	-0.38	-0.35	-0.42	-0.46

*p<0.05

Table 6-7. Correlation analysis for regional ^{18}F -Dopa Ki and clinical characteristics in the total group of Parkinson's disease patients (n=19)

	Spearman Rank correlation coefficient (rho)				
	Caudate	Putamen	Ventral Striatum	Thalamus	Raphe
Age	-0.33	-0.05	-0.19	0.23	-0.25
Disease Duration	-0.07	-0.10	-0.04	0.04	-0.21
UPDRS off	-0.56*	-0.51*	-0.28	-0.26	-0.33

*p<0.05

6.6 Discussion

This study has shown that the presence of fatigue in PD is associated with relative serotonergic denervation in the basal ganglia and associated limbic circuits compared to non-fatigued cases. With an ROI analytical approach, I found that SERT availability, as reflected by ^{11}C -DASB binding, was significantly reduced in both dorsal and ventral striatum, and in the thalamus of PD-F patients compared to PD-NF patients.

Voxel based analysis comparing PD-NF and PD-F groups confirmed and extended the findings from the ROI analysis. In the PD-F group, clusters of significant decreases in ^{11}C -DASB BP_{ND} were localized in the whole striatum, in the thalami, and in additional cortical regions, including cingulate gyrus (Brodmann areas 24 and 32 bilaterally), right paracentral lobule (Brodmann area 31), left insula, and right amygdala relative to non-fatigued cases (Figure 6-4).

An explorative voxel-based comparison between ^{18}F -dopa images of PD-NF and PD-F patients localized significant ($p < 0.01$) ^{18}F -dopa uptake reductions in the caudate and left insula in the latter group (Figure 6-1). An ROI analysis showed a trend for lower ^{18}F -dopa K_i values in the caudate (averaged right and left) of PD-F patients compared to PD-NF patients ($p = 0.094$) but no other differences between the two groups. This finding is in line with a recent SPECT study showing similar striatal dopamine transporter binding in early PD patients with and without fatigue (Schifitto et al., 2008). Taken together, these findings are against dopaminergic nigrostriatal degeneration per se being a factor in the pathogenesis of fatigue in PD. However, reduced ^{18}F -dopa uptake in the insular cortex of PD-F patients suggests a possible link between fatigue and loss of extrastriatal dopaminergic function.

The PD-NF and PD-F patients were carefully matched for gender, age, disease duration, daily LEU, and severity of motor symptoms, as measured by UPDRS, in both “off” and “on” condition. PD patients with dementia, overt depression, and EDS,

a common confounder of fatigue, were not included in the study and so the differential PET findings observed in the PD-F groups should be specific for fatigue in PD.

¹¹C-DASB has high specificity and sensitivity for SERT (nanomolar affinity) and much lower affinity for the dopamine and norepinephrine transporters (micromolar affinity) (Houle et al., 2000). It is likely that decrease of ¹¹C-DASB binding reflects a functional loss of SERT-expressing terminals in the corresponding areas of the brain due to degeneration of the serotonergic pathways. It could be argued that low ¹¹C-DASB binding simply reflects downregulation of SERT expression. However, several post-mortem studies have now demonstrated depletion of serotonin and SERT levels in the striatum as well as in extrastriatal areas in PD patients (Scatton et al., 1983; Shannak et al., 1994; Kish et al., 2008). In particular, Kish and colleagues (2008) have recently reported that SERT and other serotonergic markers, including serotonin itself, were significantly reduced in the striatum of PD patients compared to controls. There was a preferential loss of SERT and serotonin in caudate compared with the putamen. These workers also found that changes in striatal SERT binding in PD brains reflected an actual reduction in levels of SERT protein.

The basal ganglia receive sensory and motor input from all cortical areas and are a relay in neuronal pathways processing emotional, motivational, associative and cognitive functions. A major role of the basal ganglia is to integrate sensorimotor, associative, limbic and motor information into efficient thought and action (Bolam et al., 2000). It has been suggested that structural lesions and/or changes in the neurotransmitter balance within the basal ganglia and associated structures disrupt the process of integration of limbic input concerning emotional status and consequent motor output. Dissociation of motivation from executive motor movement could lead to a reluctance to act and a feeling of fatigue (Chaudhuri et al., 2000). In line with this, Hagell and Brundin (2009) have recently reported that lack of motivation is a significant predictor of fatigue in PD patients. These findings provide strong evidence in support of this pathophysiological model of fatigue in PD. PD-F patients showed reduced SERT expression in the main components of the basal ganglia including the

ventral striatum where the limbic-to-motor integration take place (Mogenson et al., 1980).

Decreases of ^{11}C -DASB binding in PD-F patients were also found in limbic structures including: (i) Brodmann areas 24 and 31, corresponding to the ventral portion and the isthmus of the cingulate gyrus respectively, both involved in emotion processing; (ii) Brodmann area 32, corresponding to the dorsal anterior cingulate cortex anterior, which is involved in decision making processes; and (iii) the amygdala, which is involved in the processing of emotional reactions and emotional memory as well as spatial and motor learning.

The insular cortex is also a component of the limbic system. It has reciprocal connections with anterior cingulate, amygdala, basal ganglia and prefrontal cortex and could represent a center of multi-modal sensory-motor-limbic integration (Agustine, 1996). It is known to play a role in regulating autonomic functions. Changes in ^{11}C -DASB binding and ^{18}F -dopa uptake were observed in the left insular cortex of PD-F patients. An asymmetrical involvement of the insular cortex is unexpected and this apparent lateralization of PET findings could reflect lack of power due to the relatively small number of patients in this study.

To date the DAT blocker, methylphenidate, at a dose of 10 mg three times a day, is the only drug that has shown significant improvement of fatigue in PD patients (Mendonca et al., 2007) in controlled studies while open label studies have indicated beneficial effect of some dopaminergic agents such as levodopa and apomorphine infusion (Honig et al., 2009; Naidu et al., 2009). Selective serotonin reuptake inhibitors (SSRIs) are commonly used in the treatment of chronic fatigue syndrome (CFS) and are often used in PD patients with history of fatigue. These medications increase synaptic levels of serotonin by inhibiting SERT activity. However, if this finding of low ^{11}C -DASB binding reflects an effective loss of SERT protein, as observed in post-mortem parkinsonian brain, SSRIs are less likely to be efficacious in these patients. Alternative strategies to increase brain level of serotonin and

serotonergic transmission in non-depressed PD patients with fatigue should be tested.

Fatigue is a common symptom in other diseases including CFS, multiple sclerosis, and stroke. It is possible that a dysfunction of brain serotonergic pathways in the basal ganglia/limbic systems may be common to the occurrence of fatigue in these conditions. Preliminary reports of a serotonergic dysfunction in CFS patients without PD support this hypothesis. Using the PET ligand ^{11}C -(+)McN5652, Yamamoto and colleagues (2004) have reported reduced SERT binding in the rostral subdivision of anterior cingulate (Brodmann areas 24/32) in CFS patients compared with controls. Cleare and colleagues (2005) found widespread reduction of ^{11}C -WAY-100635, a specific radioligand for serotonin 5-HT_{1A} receptors, in CFS patients. Reductions in ^{11}C -WAY-100635 binding were particularly marked in the hippocampus bilaterally. Other limbic areas, including amygdala, insular, and anterior cingulate, also showed significant tracer binding reduction in the CFS group. Although serotonergic function has not been investigated in vivo in multiple sclerosis patients with fatigue, an early PET study (Roelcke et al., 1997) demonstrated that these patients had reduced glucose metabolism in basal ganglia and in lateral and medial prefrontal cortex, the same areas where serotonergic dysfunction was seen in my cohort of PD patients with fatigue. Future research is required to clarify the role of serotonergic dysfunction in the pathogenesis of fatigue in these clinical conditions.

There are a few more findings of this study that need further consideration:

In line with other PET studies suggesting raphe involvement in advanced PD (Rakshi et al., 1999; Moore et al., 2008), ^{11}C -DASB BP_{ND} values in the raphe inversely correlated with disease duration in the cohort of PD patients. Yet, raphe median ^{11}C -DASB BP_{ND} values in PD-NF and PD-F patients were similar and were not significantly different from the control subjects. Since the majority of the serotonin innervation of the brain arises from the raphe nuclei (Hornung, 2003), one might have expected more severe raphe involvement in PD-F patients to parallel the striatal/cortical changes. However, my findings are in agreement with previous reports.

Albin and colleagues (2008), who used ^{11}C - DASB PET to assess brain serotonergic pathway function in patients with early PD, also found that the magnitude of SERT binding reductions in PD patients compared to controls was greater in the forebrain than in brainstem regions. Additionally, receptor autoradiography with ^3H -citalopram, a marker of SERT, showed a normal density of ^3H -citalopram binding sites in the median raphe of PD patients, despite severe binding reductions in both the basal ganglia and cerebral cortex (Chinaglia et al., 1993). A possible explanation for these findings is that the degenerative process in the raphe-striatal neurons in PD targets the nerve terminals rather than the cell bodies.

While PD-NF and PD-F patients were age-matched, the healthy control subjects used for the ^{11}C - DASB PET comparisons were 20 years younger than the PD patients. An age-related decline in the availability of SERT when measured with PET and SPECT has been reported. Buchert and colleagues have recently reviewed this topic (2006). The percentage reduction in SERT availability per decade across published series varies from 2% to 10.5% (median 50th percentile 4.5%). Based on these observations, the maximum decline in ^{11}C - DASB binding in my two PD cohorts that could be ascribed to aging, is around 21%. This is far less than the decline observed in this study, as the PD-F patients had a reduction close to 80% of the control mean in most of the regions assessed by ROI approach (table 6-4). Therefore, the reduction in ^{11}C - DASB binding observed in the PD-F groups is unlikely to be explained by aging alone.

While findings from this study indicate that the level of dopaminergic nigrostriatal dysfunction does not differentiate PD patients with and without fatigue, they do not exclude loss of striatal dopamine playing a role in its pathogenesis. Although fatigue is associated with more severe involvement of the serotonergic system in PD patients, it is likely that changes in the serotonin/dopamine balance within the basal ganglia also contribute.

Finally, by excluding patients with dementia, depression, and EDS I have reduced the effects of possible confounding factors and shed light on the pathogenesis of “primary” fatigue in PD. However, all these other factors are known to exacerbate the subjective experience of fatigue and in some cases could be the predominant cause for fatigue like symptoms. Distinct neurochemical substrates could underlie these situations and need to be investigated carefully so that focused interventions can optimize the treatment of fatigue in PD patients.

PET markers of serotonin function in Parkinson's disease - a combined ^{18}F -dopa and ^{11}C -DASB PET study

7

7.1 Summary of objectives

The overall objective of this study was to verify the hypothesis that ^{18}F -dopa PET can be used to evaluate the distribution and the function of serotonergic systems in the brain of patients with PD. This was achieved by assessing the relationship between ^{18}F -dopa uptake and measurements of SERT availability by ^{11}C -DASB PET within brain serotonergic structures.

I particularly focused on: (1) The raphe nuclei complex in the midline of the brainstem where serotonergic neurons are predominantly located. (2) The hypothalamus, which contains intrinsic dopamine neurons, but also a dense innervation from midbrain raphe serotonin neurons. (3) The anterior cingulate, a region of very high SERT density in autopsy human brains (Mantere et al., 2002).

7.2 Summary of results

^{18}F -dopa uptake in the raphe nuclei complex of PD patients was significantly correlated with SERT availability in the same structure. Trends towards significant correlations between ^{18}F -dopa K_i values and ^{11}C -DASB binding values were also

observed in the hypothalamus and the anterior cingulate cortex, suggesting a large serotonergic contribution to ^{18}F -dopa uptake in these regions. Conversely, no correlations were found in brain structures with mixed dopaminergic, serotonergic, and noradrenergic innervations, or with predominant dopaminergic innervation. These findings provide evidence that ^{18}F -dopa PET represents a reliable marker for raphe serotonergic function in PD and validate previous studies where ^{18}F -dopa PET has been used to assess serotonergic function in PD.

7.3 Scientific background

As described in previous chapters of this thesis, ^{18}F -dopa uptake when expressed as an influx constant, K_i , reflects the activity of the enzyme AADC in the axonal plexus. Since this enzyme is present in high concentration in all catecholaminergic neurons and in even greater concentrations in serotonergic neurons (Jaeger et al., 1984), ^{18}F -dopa uptake in different brain structures is likely to reflect the overall pattern of distribution of monoamine neuronal systems in those areas, including the dopamine, noradrenaline and serotonin innervation (Brown et al., 1999; Moore et al., 2003). In a PET study in ten healthy subjects, Brown and colleagues (1999) quantified, for the first time, specific ^{18}F -dopa uptake in both striatal and extrastriatal regions in the human brain, including regions with no substantial dopaminergic innervation. Interestingly, they found a wide range of ^{18}F -dopa regional uptake rate constants (K_c) values even for regions with low dopamine concentrations. The authors also assessed the relationship between regional ^{18}F -dopa K_c values and regional amine neurotransmitter concentrations and AADC activity in the human brain, obtained from post-mortem studies in the published literature. They found a poor correlation between regional ^{18}F -dopa K_c values and regional dopamine concentrations throughout the brain, whereas, regional ^{18}F -dopa K_c values correlated well with both AADC activity and the sum of local concentrations of dopamine, norepinephrine, epinephrine, and serotonin. These findings provide support for the proposition that ^{18}F -dopa PET is marker for AADC activity and overall brain monoaminergic function. However, in areas in which a definite innervation predominates, specific ^{18}F -dopa uptake is likely

mainly to reflect the activity and density of the predominant neuronal population. Indeed ^{18}F -dopa uptake in the striatum - where dopamine innervation from the nigral complex is the major monoaminergic component - correlates with striatal dopamine levels and also with nigrostriatal cell counts in post-mortem and animal studies (Snow et al., 1993; Pate et al., 1993).

Similar considerations may apply to brain structures with extensive serotonergic or noradrenergic components such as the raphe nuclei complex and the locus coeruleus respectively. However, the relationship between ^{18}F -dopa uptake in these structures and other markers of serotonergic or noradrenergic function has not yet been assessed.

7.4 Patients and methods

7.4.1 Patients

Eighteen patients with a clinical diagnosis of idiopathic PD (UK Parkinson's Disease Society Brain Bank diagnostic criteria) were included in this study. Fourteen of them were participants in the study described in chapter 6 (six had a clinical complaint of fatigue). Four additional PD patients were recruited for this study. Table 7.1 summarizes the demographics of all patients investigated.

Table 7-1. Patient Characteristics

	Parkinson's disease patients (n=18)
M/F	12/6
Age (years, mean $\pm$ SD)	67.8 $\pm$ 10.5
Disease Duration (years, mean $\pm$ SD)	9.3 $\pm$ 9.8
UPDRS in "off"	32.3 $\pm$ 12.0

None of the patients had significant co-morbidity, previous history of psychiatric or neurological conditions (e.g. depression, cognitive impairment, stroke, head injury, epilepsy). All the patients had a stable response to their dopamine replacement therapy and none were taking medication targeting serotonergic and noradrenergic systems.

All subjects gave their written informed consent to be scanned (approved by the Ethics Committee of Hammersmith, Queen Charlotte's & Chelsea and Acton Hospitals Trust), according to the declaration of Helsinki. Permission to administer ^{18}F -dopa and ^{11}C -DASB was obtained from the Administration of Radioactive Substances Advisory Committee (ARSAC), UK.

7.4.2 PET protocol

PET scanning took place at the Cyclotron Building, Hammersmith Hospital, Hammersmith Imanet, GE Healthcare, London, UK, and all PET scans were performed using an ECAT EXACT HR+ (CTI/Siemens 962) camera. All patients had their dopamine replacement medication stopped for at least 12h before PET.

The scanning protocol for ^{18}F -dopa involved 3D acquisition over 94.5 minutes following a bolus injection of 185 MBq of ^{18}F -dopa. The scanning protocol for ^{11}C -DASB involved 3D acquisition over 90.5 minutes following a bolus injection of 450 MBq of ^{11}C -DASB.

7.4.3 PET analysis

Parametric images of the influx constant K_i and integrated ADD images of ^{18}F -dopa uptake were created as described in Chapter 3. Both K_i and ADD images were then transformed to ANALYZE format using an in house program, ECAT_transfer. Once in ANALYZE format the parametric and ADD image from each scan were visually inspected using ANALYZE software.

Spatial normalization of ADD images and K_i maps into standard MNI stereotaxic space was performed using SPM software package implemented in Matlab5. First the

ADD images were normalized to a smoothed ^{18}F -dopa template in MNI space. Then the transformation parameters were applied to the respective ^{18}F -dopa Ki maps.

As described in Chapter 3, parametric images of specific ^{11}C -DASB BP_{ND} were generated on a voxel wise basis for the whole brain using a basis function implementation of the simplified reference region compartmental model with the cerebellum providing the reference tissue input function (Gunn et al., 1997). Integrated ADD images of ^{11}C -DASB uptake were also created for spatial normalization purposes. ADD images and parametric images of ^{11}C -DASB BP_{ND} were then normalized, using SPM software, to a smoothed ^{11}C -DASB ADD image template.

7.4.3.1 Extraction of ROI data

Standard object maps in MNI space were used to extract ROI data. The standard object map containing regions defined for caudate nucleus, putamen, ventral striatum, thalamus, hypothalamus, and raphe has been described in details in paragraph 4.4.3.1 and in Table 4-2. An additional standard object map was employed to extract ROIs values for amygdala, anterior cingulate, posterior cingulate, and prefrontal cortex. The characteristics of this standard object maps have been described in Table 7-2.

Table 7-2 Additional regions of interest identified on the template object map

Amygdala	ROI traced to include the entire amygdaloid complex rostral to the ventral hippocampus
Anterior cingulate	ROI traced to include the frontal part of the cingulate cortex dorsal to corpus callosum, Brodmann areas 24, 33, and 32
Posterior cingulate	ROI traced to include the posterior part of the cingulate cortex dorsal to corpus callosum, lying behind the anterior cingulate cortex. Brodmann areas 23 and 31
Prefrontal cortex	ROI traced to include the anterior part of the frontal lobes of the brain, lying in front of the motor and premotor areas

The standard object maps were applied to each spatially normalized image of ^{18}F -dopa Ki or ^{11}C -DASB BP_{ND} and corresponding ADD images. Visual inspection of each plane for both images was made to ensure correct placement of the object regions over the correspondent structures. After applying target regions to structures, ^{18}F -dopa Ki and ^{11}C -DASB BP_{ND} values were quantified using ANALYZE software. ^{18}F -dopa Ki and ^{11}C -DASB BP_{ND} values obtained from the different ROIs were averaged over both hemispheres for statistical analysis.

7.4.4 Statistical analysis

Statistical analyses of clinical data were performed with SSPS 16 for Windows (SPSS Inc., Chicago, Illinois). The Kolmogorov-Smirnov test was used to test data sets for normality. Correlations between variables were tested with Pearson rank correlation.

7.5 Results

All data sets passed the normality test. Correlation analyses between regional ^{11}C -DASB BP_{ND} and ^{18}F -dopa Ki are summarized in Table 7-3.

There was a significant correlation ($p=0.042$) between ^{18}F -dopa Ki values and ^{11}C -DASB BP_{ND} values in the raphe complex (Table 7-3 and Figure 7-1A). A trend towards a significant correlation between ^{18}F -dopa Ki values and ^{11}C -DASB BP_{ND} values was also observed in the hypothalamus ($p=0.063$) and the anterior cingulate cortex ($p=0.078$) (Table 7-3 and Figure 7-1B and C). No correlations were found between uptake of the two radiotracers in any of the remaining regions assessed in this study (Table 7-3)

PET images in figure 7-3 show examples of ^{18}F -dopa Ki uptake and ^{11}C -DASB binding in the median raphe of two patients with PD.

Table 7-3. Correlation analysis for regional ^{11}C -DASB binding potential and ^{18}F -dopa Ki in Parkinson's disease patients (n=18)

	Correlation coefficient (r)	Coefficient of determination (r squared)	p value
Caudate	0.211	0.044	p=0.401
Putamen	0.151	0.023	p=0.549
Ventral Striatum	0.060	0.004	p=0.814
Thalamus	0.123	0.015	p=0.626
Hypothalamus	0.426	0.181	p=0.063
Raphe	0.483	0.234	p=0.042
Amygdala	0.157	0.025	p=0.535
Anterior cingulate	0.425	0.181	p=0.078
Posterior cingulate	-0.171	0.029	p=0.496
Prefrontal cortex	0.115	0.013	p=0.650

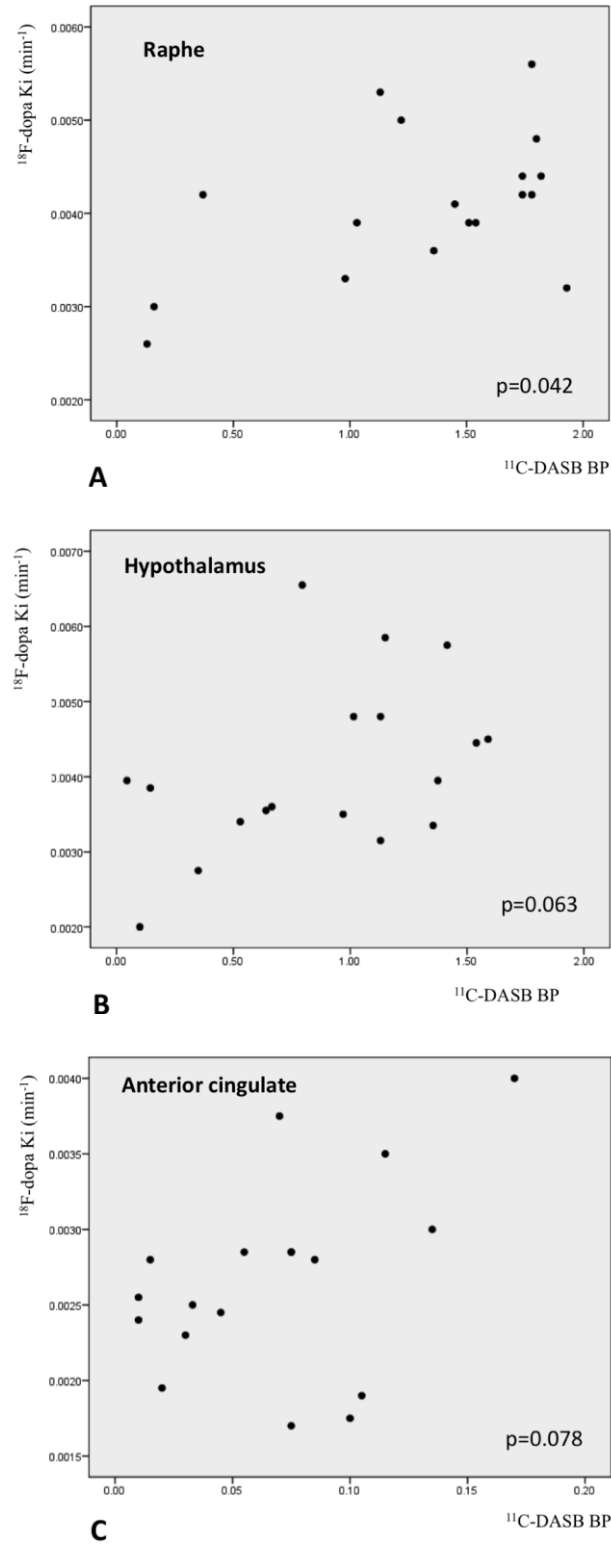
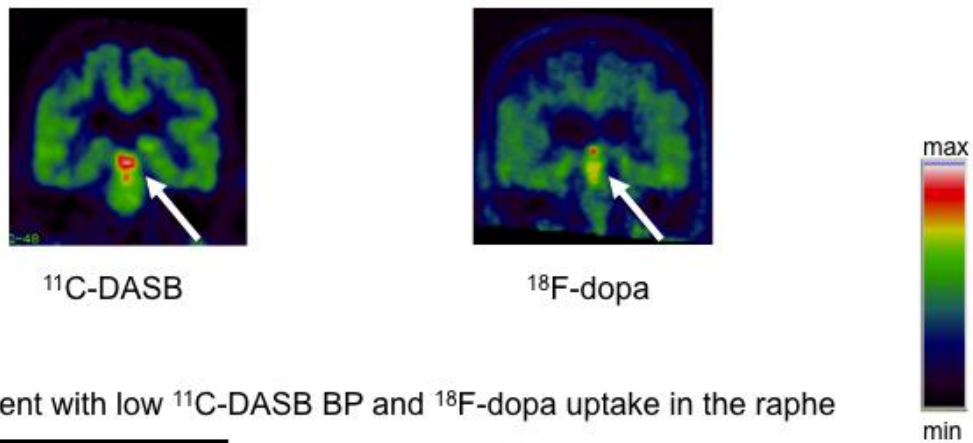


Figure 7-1. Scatterplots of individual ^{11}C -DASB BP_{ND} values and ^{18}F -dopa Ki values in raphe nuclei complex (A), hypothalamus (B), and anterior cingulate (C)

PD patient with high ^{11}C -DASB BP and ^{18}F -dopa uptake in the raphe



PD patient with low ^{11}C -DASB BP and ^{18}F -dopa uptake in the raphe



Figure 7-2. ADD images of ^{11}C -DASB binding and ^{18}F -dopa uptake from two Parkinson's disease (PD) patients. The patient depicted at the top shows high ^{11}C -DASB binding and ^{18}F -dopa uptake in the raphe nuclei complex (indicated by the white arrow), whereas the patient depicted at the bottom shows low ^{11}C -DASB binding and ^{18}F -dopa uptake in the raphe nuclei complex. The same color scale was used to display ^{11}C -DASB binding and ^{18}F -dopa Ki uptake

7.6 Discussion

This is the first study to assess in vivo the relationship between ^{18}F -dopa uptake and serotonin terminal function in human brains. ^{11}C -DASB PET was used to obtain an index of regional brain serotonin presynaptic terminal function to correlate with local ^{18}F -dopa uptake. As previously mentioned in chapter 3, ^{11}C -DASB has high specificity and sensitivity for SERT and much lower affinity for the dopamine and norepinephrine transporters and therefore represents a specific marker of serotonergic innervation (Houle et al., 2000). Additionally, ^{11}C -DASB binding potential values are remarkably consistent under test-retest conditions for most brain regions (Kim et al., 2006).

In the cohort of 18 patients with PD examined in this study, there was a significant correlation ($p=0.042$) between ^{18}F -dopa Ki values and ^{11}C -DASB BP_{ND} values in the raphe nuclei complex (Figure 7-1A). No other significant correlations were found between the two radiotracers in any of the remaining regions assessed in this study (Table 7-3). However, a trend towards a significant correlation between ^{18}F -dopa Ki values and ^{11}C -DASB binding values was observed in the hypothalamus ($p=0.063$) and the anterior cingulate cortex ($p=0.078$) (Figure 7-1B and C).

The raphe nuclei complex in the midline of the brainstem contains the majority of the brain serotonergic neurons (Dahlström and Fuxe, 1964). The rostral cell groups in the pons and midbrain, which includes the dorsal raphe nucleus, project extensively to the forebrain, whereas the caudal raphe nuclei in the medulla project towards the spinal cord and brainstem. In this study, the ROI placed over the raphe mainly included the dorsal and medial raphe and some adjacent medial reticular formation. In this region serotonin neurons are largely represented, whereas catecholaminergic neurons are much less numerous and are exclusively located in the rostral part of the dorsal raphe nucleus (Baker et al., 1991). In line with these anatomical findings, results from this study show that ^{18}F -dopa uptake in the raphe of PD patients mirrors local measurement of SERT availability, indicating that ^{18}F -dopa uptake in this

structure mostly reflects density and AADC activity of serotonergic terminals. Therefore, ^{18}F -dopa PET can be used as a reliable marker of raphe serotonergic function in PD.

The hypothalamus and the anterior cingulate cortex also have a significant serotonergic innervation (Bogdanski et al., 1957; van de Kar and Lorens, 1979; Mantere et al., 2002; Takano et al., 2010). The observed trend towards a significant correlation between ^{18}F -dopa K_i values and ^{11}C -DASB binding values in hypothalamus and anterior cingulate cortex may indicate a large serotonergic contribution to ^{18}F -dopa uptake in these regions.

No correlation between ^{18}F -dopa K_i values and ^{11}C -DASB BP_{ND} values was found in caudate, putamen, and ventral striatum. This result is not surprising considering that the striatum receives a very dense dopaminergic innervation originating from the substantia nigra pars compacta and the ventral tegmental area (Lindvall and Björklund, 1978; Gerfen, 1987) and a more moderate serotonergic innervation from the dorsal raphe nucleus (Azmitia and Segal, 1978; Steinbusch, 1984). Additionally, previous results from both human and animal studies have shown that ^{18}F -dopa K_i values correlate well with striatal dopamine levels and with nigrostriatal cell counts (Snow et al., 1993; Pate et al., 1993).

Finally, no correlations were found between the two tracers in brain structures with mixed dopaminergic, serotonergic and noradrenergic innervations. Reductions in ^{18}F -dopa uptake in these structures, as observed in PD (Moore et al., 2003 and also in chapter 4 and 5 of this thesis), are likely to reflect dysfunction in any or all of these pathways. Nevertheless, ^{18}F -dopa uptake in these regions still represents a useful marker of overall monoaminergic function and may provide the rationale for further investigations with more specific PET ligands if the involvement of a particular neurotransmitter is suspected.

In this study, the relationship between ^{18}F -dopa uptake and ^{11}C -DASB binding values was assessed in patients with PD rather than healthy volunteers. This allows one to assess the regional correlations between the two tracers in subjects who might have different degrees of serotonergic impairment, as reported in both post-mortem and imaging studies (Kish et al., 2008; Politis et al., 2010). The strict correlation between ^{18}F -dopa K_i and ^{11}C -DASB BP_{ND} values in the raphe in this cohort of PD patients suggest that ^{18}F -dopa PET is as sensitive as ^{11}C -DASB in detecting pathological and or functional changes in this structure.

Review of major findings and Conclusions

8

8.1 Summary of main findings

The experiments described in this thesis were undertaken with a view to improve understanding of the degenerative process in non-dopaminergic structures in idiopathic PD and in monogenic forms of parkinsonism.

Both pathological and neurochemical studies have demonstrated that serotonergic, noradrenergic, and cholinergic neurons are significantly affected in PD. However, the extent and time course of degeneration of non-dopaminergic pathways in living patients with PD and atypical forms of parkinsonism remains to be fully elucidated. To date, only a relatively small number of imaging studies have been performed to investigate the involvement of extrastriatal monoaminergic structures in PD patients and no such studies have been performed in patients with monogenic forms of parkinsonism or in asymptomatic heterozygote carriers of a recessive mutation. Degeneration and/or dysfunction of these monoaminergic nuclei could be responsible for the occurrence of non-motor symptoms including fatigue and depression. However, the physiopathogenetic mechanisms and the neurochemical substrates of non-motor symptoms in PD have not been extensively investigated.

The rate of progression of the degenerative process in extrastriatal areas also remains unclear. Several functional imaging studies with both PET and SPECT have been

performed to assess *in vivo* disease progression in PD patients. However, all of them have exclusively focused on the progression of nigrostriatal degeneration.

In this thesis, I have used PET imaging to clarify the extent and time course of the involvement of striatal and extrastriatal monoaminergic structures in PD and to elucidate the pathogenesis of fatigue, a disabling non-motor symptom which is reported by a significant number of PD patients.

The main findings of this thesis can be summarized as follows:

- 1) *Parkin* and idiopathic PD patients with similar levels of striatal dysfunction have different patterns of extra-striatal monoaminergic involvement, more widespread dysfunction being evident in idiopathic PD.
- 2) Compared to controls, asymptomatic heterozygote carriers of a single *parkin* mutation have significant reductions of mean ^{18}F -dopa uptake in striatum but not elsewhere. However, individual subject analyses revealed that two cases had significantly reduced locus coeruleus ^{18}F -dopa uptake.
- 3) Loss of monoaminergic function in extrastriatal regions, as reflected by ^{18}F -dopa uptake, is slower than loss of nigrostriatal dopaminergic function.
- 4) Compensatory upregulation of monoamine turnover in extrastriatal regions during the early phase of PD disappears within a few years of disease.
- 5) Fatigue in PD is associated with reduced serotonergic function in the basal ganglia and limbic structures.
- 6) ^{18}F -dopa PET can be used as a reliable marker of raphe serotonergic function in PD.

8.2 Implications of findings

This PhD programme has provided several novel observations, expanding our knowledge of the pathological process in PD. One of the novel findings has been the demonstration that, despite a similar pattern of reduction in striatal ^{18}F -dopa uptake in *parkin* and idiopathic PD patients, the two groups have distinct patterns of extrastriatal monoaminergic involvement, with more widespread dysfunction in idiopathic PD. Interestingly, while ^{18}F -dopa uptake in the raphe nuclei complex was severely affected in *parkin* patients, it was only mildly reduced in idiopathic PD patients. Since reductions in ^{18}F -dopa uptake in this region reflect impairment of serotonin innervation, as demonstrated in chapter 7, *parkin* patients seem to have a greater serotonergic dysfunction than idiopathic PD patients. Having said that, both pathological and neuroimaging studies in PD patients have reported that SERT binding reductions in PD patients compared to controls are greater in the basal ganglia and cerebral cortex than in brainstem regions (Albin et al., 2008; Chinaglia et al., 1993), suggesting that the degenerative process targets the serotonergic presynaptic terminals rather than the cell bodies. It is therefore possible that the degenerative process targets the serotonergic presynaptic terminals in idiopathic PD and the perikarya in *parkin* patients. Taken together these findings could indicate that the neurochemical abnormalities in idiopathic PD and in *parkin*-linked parkinsonism are different. Future research should elucidate the clinical relevance of the different monoaminergic involvement in the two conditions.

In addition to these data, I have also assessed for the first time the pattern of monoaminergic dysfunction in the brains of asymptomatic carriers of a single *parkin* mutation. Confirming previous studies from this Unit, I found a mild but significant reduction of ^{18}F -dopa uptake in the caudate and putamen in the asymptomatic heterozygous carriers compared to the normal controls. No other significant differences were found between the two groups in any of the assessed monoaminergic structures, although two heterozygous carriers had reduced locus coeruleus ^{18}F -dopa

uptake with normal striatal uptake. This finding suggests that, at least in some carriers, locus coeruleus might be involved independently from the dopaminergic system. Kay and colleagues (2007), have recently reported that heterozygous *parkin* point mutations are as common in control subjects as in PD patients against the hypothesis that heterozygous *parkin* sequence variants are risk factors for PD. Additionally, the rate of nigrostriatal dysfunction progression seems to be very slow in asymptomatic heterozygous carriers (Pavese et al., 2009). Taken together these findings would suggest that heterozygous *parkin* gene mutations are not a major risk factor for late onset PD.

The study described in chapter 5 has produced several new findings regarding the time course of the monoaminergic degeneration in PD patients. Using serial ^{18}F -dopa PET scans, I found that putamen had the fastest annual decline in ^{18}F -dopa uptake followed by locus coeruleus and globus pallidus interna. Caudate and hypothalamus showed a 6.3% and 6.2% decline per annum respectively, whereas the remaining structures showed less than a 5% annual decline in ^{18}F -dopa uptake. No significant correlations were found between the rate of annual decline in ^{18}F -dopa uptake in putamen and that in any of the other assessed regions of interest. Taken together these findings indicate that the degeneration in extrastriatal monoaminergic structures in PD occurs independently from nigrostriatal degeneration and at a slower rate. Of particular importance is the observation that hypothalamic involvement is common in PD and is targeted at very early stages of the disease with a fast progression rate once degeneration has started. Hypothalamic dysfunction may contribute to sleep disorders, endocrine dysfunction and weight loss in PD patients.

Confirming findings of previous studies, I found increased ^{18}F -dopa uptake in globus pallidus interna and midbrain raphe in patients with early PD, which is likely to represent a compensatory upregulation of AADC activity and dopamine turnover in these regions. The longitudinal assessment of these patients reported in chapter 5 showed that tracer uptake in these two nuclei fell slightly below the control mean value after a three-year follow up, suggesting that compensatory hyperactivity reverses within the first years of disease.

In conclusion, the study in chapter 5 has shown that with serial ^{18}F -dopa PET scans it is possible to assess disease progression in serotonergic and noradrenergic extrastriatal areas along with nigrostriatal degeneration. This could be relevant in the choice of an imaging biomarker when designing future clinical trial to assess the effects of novel neuroprotective agents in PD.

One of the major findings of this thesis has been the demonstration that the presence of fatigue in PD is associated with relative serotonergic denervation in the basal ganglia and associated limbic circuits. In the study described in chapter 6, I found that PD patients with fatigue had significantly lower SERT binding than patients without fatigue in caudate, putamen, ventral striatum and thalamus. Additionally, in the fatigue group, voxel-based analysis localised ^{18}F -dopa uptake reductions in caudate and insula, and further SERT reductions in cingulate and amygdala.

These findings have shed light on the neurobiology of fatigue in PD and might have important therapeutic implication as they provide the rationale for strategies to increase brain level of serotonin and serotonergic transmission in the treatment of this common complaint among PD patients.

Finally, another important observation in this thesis is the good relationship between ^{18}F -dopa uptake and SERT availability in the raphe nuclei complex of PD patients.

This evidence suggests that ^{18}F -dopa PET represents a reliable marker of serotonergic function in the raphe and validate previous studies where ^{18}F -dopa PET has been used to assess serotonergic function in PD. ^{18}F -dopa PET imaging is also used to assess the involvement of the dopaminergic system in several psychiatric diseases, including depression and schizophrenia. Based on the results of this thesis, it appears that the technique could provide supplementary information on the function of serotonergic function in these conditions.

8.3 Strengths and limitations

One of the considerable strengths of this study is that I have used PET scanners with high resolution and high sensitivity that permit interrogation of the function of small volume structures.

I have also used a rigorous methodological approach to analyze PET images. In order to minimize the number of possible volumetric variations when drawing ROIs on individual PET images, I used standard ROI maps of predefined volumes of interest in MNI space that were applied to each spatially normalized parametric image. This technique has several advantages. First, the method allows the same cubic volume to be interrogated in all scans. Second, the normalization of baseline and follow-up images to the same orientation and space increases the confidence that the same anatomical structures are assessed in longitudinal studies.

Special attention was given to patients' selection. In chapter 4, for example the main aim of that study was to investigate brain monoaminergic innervation in *parkin*-patients and patients with idiopathic PD. However, I wanted to exclude that a different extrastriatal monoaminergic pattern in the two conditions could be related to the different degrees of striatal involvement in the two groups. Therefore only *parkin* gene carriers underwent a PET scan for this study and patients with idiopathic PD were carefully selected from the Unit's database to match striatal ^{18}F -dopa uptake of parkin-patients.

In chapter 6, the two groups of PD patients with and without fatigue were matched for all possible confounding factors, including age, disease duration, daily intake of LEU, and UPDRS scores in both "off" and "on" conditions. Additionally, since I wanted to elucidate neurochemistry of primary fatigue, patients with dementia, depression, and EDS were excluded from the study.

However, a number of methodological weaknesses and limitations to the experimental studies reported in this thesis also need to be acknowledged. Some of these limiting issues are specific to the study design in each of the thesis' experiments whereas others are generally applicable to the field of brain imaging.

PET is a very expensive modality and the cost of a PET scan is quite high particularly in a research context. The impact of irradiating healthy volunteers is also relevant as it raises important ethical issues. Considering that the CSC Neurology group has

collected a large database of ^{18}F -dopa and ^{11}C -DASB PET images of healthy volunteers over the years, it was decided to not scan new healthy volunteers for the studies of this thesis.

Whilst striatal ^{18}F -dopa does not appear to be age-dependent in healthy controls (Sawle et al., 1990), an age-related decline in the availability of SERT when measured with PET and SPECT has been reported (Buchert et al., 2006).

In the study in chapter 6, PD patients with and without fatigue were age-matched, whereas the healthy control subjects used for the ^{11}C -DASB PET comparisons were 20 years younger than the PD patients. Therefore, the effect of age on the ^{11}C -DASB reductions in the two cohorts of PD patients cannot be completely excluded. However, the reductions in ^{11}C -DASB binding that I found in the PD patients with fatigue (about 80% of the control mean in most of the regions assessed by ROI approach) is much bigger than the maximum decline (around 21%) that could be ascribed to aging on the basis of previous studies. Additionally, I found no significant correlations between age and tracer binding in caudate, putamen, ventral striatum, and thalamus in the cohort of 15 PD patients.

Therefore, the reduction in ^{11}C -DASB binding observed in PD patients with fatigue is unlikely to be explained by aging alone.

Probably the most notable weakness in this thesis is the lack of clinical details on the differences in non-motor symptoms between idiopathic PD and *parkin*-patients in Chapter 4. Unfortunately, as the idiopathic PD patients were selected from our data base to match striatal ^{18}F -dopa uptake in *parkin*-patients, data on sleep problems or other non-motor symptoms in these patients were not always available. Therefore, I was not able to compare the different groups on the basis of their non-motor symptoms. The clinical relevance of the different monoaminergic involvement in the two conditions remains to be elucidated.

In all the experiments of this thesis ^{18}F -dopa PET has been used as a marker of monoaminergic terminals. In areas in which a definite innervation predominates, specific ^{18}F -dopa uptake is likely to reflect the activity and density of the predominant

neuronal population (eg dopamine innervation in the striatum and serotonergic innervation in the raphe). However, in brain structures like the hypothalamus, the red nucleus, and the pineal gland that have a mixed dopaminergic, serotonergic and noradrenergic innervations, ^{18}F -dopa uptake probably reflects the combined effect of activity and density of different groups of monoaminergic afferents to these structures. Therefore, the reductions of ^{18}F -dopa uptake in these regions that I have observed in PD patients could reflect dysfunction in any or all of these pathways. Nevertheless, ^{18}F -dopa uptake in these regions still represents a useful marker of overall monoaminergic function and may provide the rationale for further investigations with more specific PET ligands if the involvement of a particular neurotransmitter is suspected.

Another possible limitation of this thesis is that the studies were not performed using a partial volume effect correction. In imaging, the partial volume effect is the loss of contrast between two adjacent tissues in an image caused by insufficient resolution that results in some voxels containing both tissues. As a consequence, the signal in these voxels is an average of the signal of both tissues.

Partial volume effect in my studies could have led to a decrease of the absolute K_i or BP_{ND} values. However, partial volume effect is particularly relevant in the case of brain atrophy, such as in Alzheimer's disease. In PD, gross brain morphology is not altered by the disease process. Additionally, my ROIs were drawn well within the anatomical boundaries of each structure to avoid overlapping with adjacent tissues. Therefore, it is unlikely that relative regional difference in K_i and BP_{ND} values between controls and PD patients in this thesis have been greatly altered by partial volume effects.

8.4 Possible directions for future work

The experimental work presented in this thesis has important implications for future work in the field. Some possible directions for future research are outlined below.

Future research should elucidate the clinical relevance of the different monoaminergic involvement in idiopathic PD and in *parkin*-linked parkinsonism.

Particular attention should be given to non-motor symptoms in both conditions.

Monoaminergic involvement in a larger number of patients with *PINK1* mutation and in other genetic form of parkinsonism should also be pursued. I believe that a better understanding of the similarities and differences between idiopathic PD and genetic forms of parkinsonism may shed light on the neurobiology of these conditions.

The study in Chapter 5 has shown that ^{18}F -dopa PET can be successfully used to assess disease progression in serotonergic and noradrenergic extrastriatal areas along with nigrostriatal degeneration. However, the annual declines observed in ^{18}F -dopa uptake in some structures (globus pallidus interna, midbrain raphe, and locus coeruleus) could simply reflect the loss of their compensatory upregulation rather than cellular loss. Longer follow-ups with three or more imaging assessments are required to assess the effective rate of disease progression in these structures once the compensatory mechanisms have disappeared.

The part of the study dealing with reproducibility in healthy volunteers only included three subjects scanned twice and needs to be extended.

Further studies can be designed to establish whether serial ^{18}F -dopa PET scans can be used as an imaging biomarker of disease progression in serotonergic and noradrenergic extrastriatal areas along with nigrostriatal degeneration in clinical trials to assess the effects of novel neuroprotective agents in PD.

Fatigue is very common in many neurological conditions (eg, multiple sclerosis, stroke) as well as non-neurological disorders (eg, CFS) and there is no real evidence to suggest that this symptom is fundamentally different between diagnoses. My finding of reduced serotonergic activity in PD patients with fatigue can therefore be seen in a wider perspective. Future research should be directed at determining the role of serotonergic dysfunction in the pathogenesis of fatigue in these clinical conditions. In particular, it would be interesting to assess serotonergic function in patients with CFS. However, it is likely that it is the change in the serotonin/dopamine balance

within the basal ganglia that contributes to fatigue in PD. Therefore, I believe that the imaging assessment of patients with CFS should be performed combining dopaminergic and serotonergic PET ligands.

It would also be interesting to design and carry out clinical trials to assess the beneficial effect of serotonin agents such as 5-Hydroxytryptophan on fatigue in PD patients.

The imaging approach used in this thesis could also be applied to elucidate physiopathological mechanisms underlying other non-motor symptoms including sleep disorders, depression, and psychosis in PD. It would also be extremely valuable to correlate *in vivo* PET measures with neuropathological changes seen at post mortem if the patients and their relatives agreed to prospective brain donation.

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