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Molecular imaging
in Parkinson's disease

Contents

Chapter 1
Introduction

1. Parkinson's disease

Parkinson's disease (PD) is a progressive neurodegenerative disorder, characterized by asymmetric onset of resting tremor, rigidity, and bradykinesia followed by postural instability. The disease usually manifests during the sixth decade and the prevalence rates increase with age. European population-based studies 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., 1995, 2000).The incidence of the disease rises steeply with age, from 17.4 in 100000 person years between 50 and 59 years of age to 93.1 in 100000 person years between 70 and 79 years, with a lifetime risk of developing the disease of 1.5% (Bower et al., 1999). The median age of onset is 60 years and the mean duration of the disease from diagnosis to death is 15 years, with a mortality ratio of 2 to 1.5 (Katzenschlager et al., 2008).

The pathological hallmark of PD is a region-specific selective loss of dopaminergic, neuromelanin-containing neurons from the pars compacta of the substantia nigra, which results in striatal dopamine deficiency. This in turn leads to increased inhibitory output activity from the basal ganglia to the ventral thalamus and frontal cortex and subsequent development of parkinsonism (Bezard et al., 2003; Obeso et al., 2008). It has been estimated that, at the time of the first motor signs of PD at least 30% of all the nerve cells in the pars compacta have already disappeared and, within 5 years of diagnosis, 50% of the neurons will have died with an annual attrition rate of 7% of surviving neurons per year. Extrapolation of the rate of nigral cell loss back beyond the date of diagnosis suggests that neuronal damage could begin at least 5–10 years before the first motor symptoms emerge (Fearnley and Lees, 1991). Cell loss in the locus coeruleus, dorsal nuclei of the vagus, raphe nuclei, nucleus basalis of Meynert, and some other catecholaminergic brain stem structures including the ventrotegmental area also occur (Damier et al., 1999). Besides nerve-cell loss, three distinctive intraneuronal inclusions can be found in PD: the Lewy body, the pale body, and the Lewy neurites. Synuclein immunocytochemistry studies reveal that intraneuronal Lewy body inclusions can be detected in the lower brainstem ahead of midbrain and

nigral involvement and later spread to limbic and association cortical areas in a predictable manner (Braak et al., 2003).

PD usually occurs as a sporadic disorder, nevertheless few environmental factors have been identified (Dick et al., 2007). Aging is the major risk factor for PD, as for other neurodegenerative diseases (Lees et al., 2009). The protective role of tobacco against PD risk is still debated (Allam, 2004; Hernàn, 2001), as also the increased risk for PD in men and postmenopausal women not taking hormone replacement, who take no or low dose of caffeine (Ascherio et al., 2003, 2004). Weak associations between Parkinson's disease and head injury, rural living, middle-age obesity, lack of exercise, well-water ingestion, and herbicide and insecticide exposure have also been reported (Elbaz et al., 2007, Thaker et l., 2008).

Genetic studies in PD identified over the past decade more than 13 loci and 9 genes, but their implication in PD is still under investigation. Alpha-synuclein point mutations, duplications and triplications cause a rare dominant form of PD in familial and sporadic cases. Autosomal dominant PD is mainly caused by mutations in the leucine-rich repeat kinase 2 (LRRK2) gene particularly in certain ethnic groups. Loss-of-function mutations in Parkin, PINK1, DJ-1 and ATP13A2 cause autosomal recessive parkinsonism with early-onset (Lesage et al., 2009).

The onset of PD motor symptoms is gradual and the early symptoms might be unnoticed and misinterpreted for a long time. The early clinical signs are often ascribed to old age or rheumatism, so that a lag of 2-3 years from first symptoms to diagnosis is not unusual. Early diagnosis of PD is desirable since in the very early stages the neural damage is presumed to be more restricted. Therefore the principal aim of PD research in the last years has been to extend diagnosis to even earlier in the disease course (Marek and Jennings, 2009).

Clinical, neuroimaging, and pathologic studies identified a variety of non motor symptoms which can precede the classic motor features of PD by years and, perhaps, even decades (Gonera et al., 1997; Abbot et al., 2003; Iranzo et al., 2006; Ross et al., 2006 et al.; Haehner, 2007). Currently, the period when these symptoms arise is commonly referred to as the premotor phase of the disease (Tolosa et al., 2009) (Figure 1.1). A prenigral involvement at the basis of non motor symptoms, preceding classic motor PD features, is supported by Braak et al.(Braak et al., 2003) recent proposal that synuclein pathology (Lewy bodies and neurites) in PD starts in the lower brainstem and progresses following a predictable caudal-rostral pattern, only reaching the substantia nigra in the mesencephalon after extensive involvement of the brainstem has occurred.
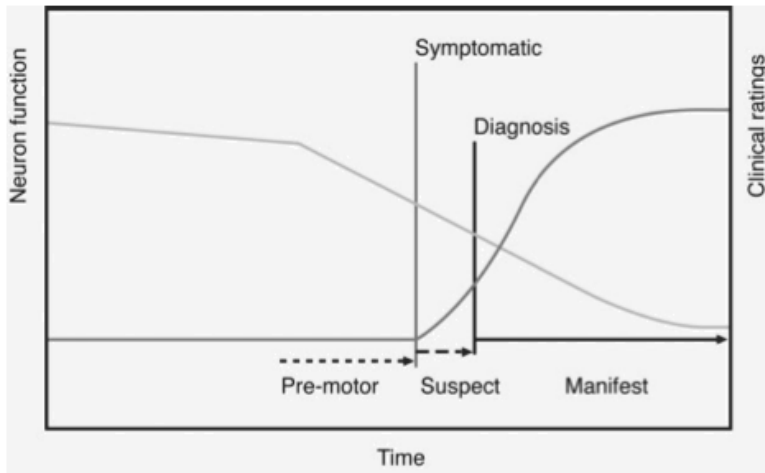
Figure 1.1 Natural history of PD (from Marek, 2009).

Among early non motor features of PD olfactory deficits and cardiac sympathetic denervation have been identified in a high proportion of patients at the time of the earliest motor signs, therefore suggesting that such features probably precede the motor signs. Other features documented in a smaller proportion of patients early in the course of the disease include pain, orthostatic hypotension, urinary urgency, and executive dysfunction found on neuropsychological testing (Lim et al., 2009).

PD is therefore characterized by insidious onset and inexorable progression, so that growing interest is directed to the identification of reliable and well-validated biomarkers to identify individuals "at risk" for PD, to accurately diagnose individuals at the threshold of clinical PD, and to monitor PD progression throughout its course (Marek and Jennings, 2009). In this perspective molecular imaging plays a key role.

2. Molecular imaging in PD

Molecular imaging refers to the use of non-invasive imaging techniques to detect signals that originate from molecules and their interaction with a specific cellular target in vivo. There are many different areas of investigation in the field of molecular imaging. Much research on neurodegenerative disorder is currently focused on detecting pre-disease state or molecular states that occur before typical symptoms of a disease are clinically manifest (Herschman, 2003).

PET and SPECT have been extensively employed to elucidate the functional changes associated with PD and other neurodegenerative parkinsonian disorders. Both modalities provide a means of assessing: (1) disease severity as reflected by presynaptic dopamine terminal dysfunction, (2) subclinical dysfunction in subjects who are at risk for PD, (3) disease progression and the effects on this of putative neuroprotective agents, and (4) changes in non-dopaminergic neurotransmission (Pavese et al., 2009).

2.1 Dopaminergic imaging

Numerous PET and SPECT studies have investigated the nigrostriatal dopami-
nergic degeneration in PD patients (Booij et al., 1999; Brooks et al., 1997). Both pre-
and post-synaptic dopaminergic functions can be measured (Figure 1.2).

Table I. Main radiopharmaceuticals used in PET and SPECT studies in PD and Parkinson plus syndromes.

Biological variable	PET radiotracer	SPECT radiotracer
Cerebral blood flow (rCBF)	$[^{15}O]H_2O$, $[^{15}O]CO_2$	$[^{99m}Tc]$-HMPAO, $[^{133}Xe]$
Oxygen extraction	$[^{15}O]O_2$	-
Glucose metabolism	$[^{18}F]$-Fluoro-desoxyglucose (FDG)	-
L-Dopa metabolism	$[^{18}F]$-6-Fluoro-L-Dopa, $[^{11}C]$-L-Dopa	-
Monoamine vesicular transport	$[^{11}C]$-Dihydro-tetrabenazine	-
Dopamine reuptake (dopamine transport; DAT)	$[^{11}C]$-Nomifensine, $[^{11}C]$-RTI-32, $[^{11}C]$-CFT, $[^{11}C]$-WIN, $[^{76}Br]$-FE-CBT, $[^{11}C]$-PE2I, $[^{11}C]$-dl-threo-methylphenidate	$[^{123}I]$-ß-CIT, $[^{123}I]$-FP-ß-CIT, $[^{123}I]$-IPT, $[^{123}I]$-Altropane, $[^{123}I]$-PE2I
D1 dopamine receptor	$[^{11}C]$-SCH-23390	-
D2 dopamine receptor	$[^{11}C]$-Raclopride, $[^{11}C]$-N-Methylspiperone, $[^{18}F]$-Fluoro-ethylspiperone, $[^{76}Br]$-Bromospiperone, $[^{76}Br]$-Lisuride, $[^{11}C]$-FLB 457	$[^{123}I]$-2-Iodospiperone, $[^{123}I]$-Iodobenzamide ($[^{123}I]$-IBZM), $[^{123}I]$-Iodolisuride, $[^{123}I]$-IBF, $[^{123}I]$-Epidepride
MAO B inhibitor	$[^{11}C]$-Deprenyl	-
Opiate receptor	$[^{11}C]$-Diprenorphine	-
Muscarinic acetylcholine receptor	$[^{11}C]$-N-Methyl-4-piperidyl-benzilate	-

Figure 1.2. PET and SPECT radiopharmaceuticals in PD (from Thobois, 2001)

Pre-synaptic aspects are mostly studied with [18F]-Fluoro-Dopa ([18F]-
FDOPA), a substrate for Dopa-decarboxylase in catecholaminergic neurons. [18F]-
FDOPA striatal uptake rate is correlated to cellular density of substantia nigra dopa-
minergic neurons and to striatal dopamine concentrations (Snow et al., 1993). Trac-
ers of dopamine pre-synaptic reuptake sites (dopamine transporter or DAT sites)
were developed more recently. The first to be used was [11C]Nomifensine, a tracer
for catecholamine uptake sites, largely replaced in recent years by the more selective
tropanes as [123I]-b-carbomethoxy-3ß-(4-iodophenyl)- tropane ([123I]-β-CIT) and
[123I-N-ω-fluoropropyl-2-β-carboxymethoxy-3β-   (4-iodophenyl)nortropane]
([123I]-FP-β-CIT), that are cocaine analogs. Post-synaptic receptor density is ex-
plored with dopamine receptor ligands, notably of the D2 type, as [11C]Raclopride
or [11C]-Nmethylspiperone or [11C]FLB 457 (Thobois et al., 2001) (Figure 1.3).
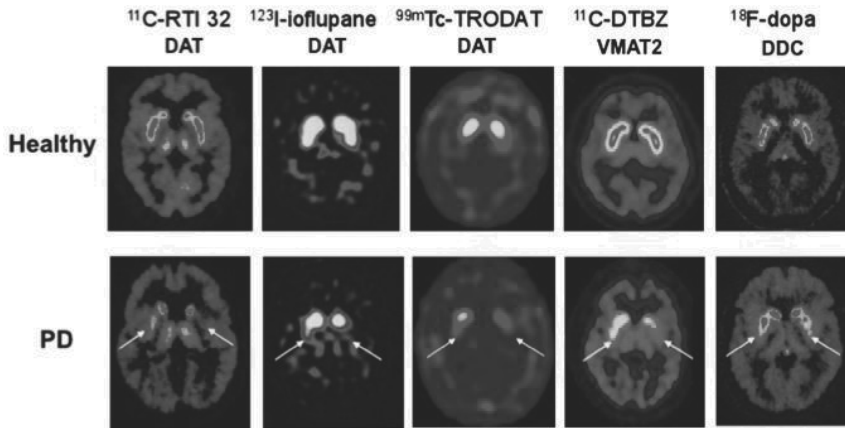
Figure 1.3.Imaging dopamine terminal function in healthy controls and early Parkinson's disease (from Pavese, 2009).

PRESYNAPTIC DOPAMINERGIC IMAGING

The assessment of functional integrity of presynaptic nigrostriatal projections is one of the main applications of molecular imaging techniques in PD. Three different markers of presynaptic dopaminergic terminals are usually employed.

<u>Markers of striatal aromatic amino acid decarboxilase activity</u>

[18F]-FDOPA radiotracer uptake reflects the dopaminergic nerve density but at the same time, the activity of the aromatic amino acid decarboxylase enzyme (AADC) that converts dopa into dopamine and the storage of dopamine (Firnau et al., 1987). This radiotracer allows the study of the integrity of the presynaptic dopaminergic system in the nigrostriatal and also the mesolimbic and mesocortical dopaminergic pathways.

The density of the axonal terminal plexus and the AADC activity is estimated by the uptake of [18F]-FDOPA in the striatal nuclei, measured by an influx constant Ki over 90 minutes. [18F]-FDOPA uptake in the striatum of patients with PD is influenced by the number of remaining dopaminergic cells. Pathological studies, indeed, demonstrated that levels of striatal [18F]-FDOPA uptake correlated with nigral cell counts in PD experimental models (Snow et al., 1993; Pate et al., 1993). In the early stages, however, because of the presence of compensatory up-regulation of AADC [18F]-FDOPA PET may underestimate the degenerative process (Ribeiro et al., 2002).

Correlations have been found between [18F]-FDOPA putaminal uptake and clinical severity of rigidity and bradykinesia as measured by the Unified Parkinson's Disease Rating Scale (UPDRS). Tremor severity was instead demonstrated not to be related with [18F]-FDOPA uptake, suggesting that either non-nigrostraital and/or non-dopaminergic pathways are involved in the pathogenesis of this symptom (Brooks et al., 1990; Vingerhoets et al., 1997; Broussolle et al., 1999).

[18F]-FDOPA uptake reduction seems to follow a gradient along the rostro-caudal axis of the striatum. In patients with hemiparkinsonism dorsal posterior putamen is characterized by the greatest reduction in [18F]-FDOPA uptake. As the disease progress to bilateral parkinsonism, the ventral and anterior putamen and the dorsal caudate show additional reductions, and finally in the advanced stages reductions extends to the ventral head of the caudate (Morrish et al., 1995). [18F]-FDOPA findings are in line with post mortem studies reporting an uneven pattern of dopamine loss in PD striatum. Dopaminergic cell loss occur first in the ventrolateral subregions of SNpc, which projects to dorsal putamen (Kish et al., 1988).

The annual decrement in the [18F]-FDOPA influx has been estimated to be of 9% to 13% in putamen and 3% in caudate nucleus (Morrish et al., 1998; Nurmi et al., 2000). These data suggest that the degeneration of dopaminergic neurons is an active process during both the preclinical period and the symptomatic phase of disease.

Increased [18F]-FDOPA uptake has been reported in early PD in dorsolateral prefrontal cortex, anterior cingulate and globus pallidus pars interna. Increased extra-striatal [18F]-FDOPA uptake in early PD is presumably due to compensatory up-regulation of AACD (Rakshi et al., 1999; Kaasinen et al., 2001; Whone et al., 2003).

Markers of presynaptic dopamine transporter binding

The presynaptic dopamine transporter (DAT), a plasma membrane transporter with high affinity for dopamine, is uniquely found in dopaminergic dendrites and axons; it is therefore a potential marker of nigrostriatal projections integrity. DAT availability is commonly measured by several PET ligands ([11C]2-beta-carbomethoxy-3beta-(4-fluorophenyl) tropane ([11C]-CFT), [18F](2--carbomethoxy-3-(4-fluorophenyl)tropane ([18F]-CFT, [18F] N-(3-fluoropropyl)-2beta-carbon ethoxy-3beta-(4-iodophenyl) nortropane ([18F]-FP-CIT), and [11C]-RTI-32) and SPECT tracers (such as [123I]-β-CIT, 123I-FP-CIT, [123I]-altropane, [11C]-methylphenidate, and 99mTc-TRODAT-1).

The SPECT tracer [123I]-β-CIT, a tropane derivative, binds to DAT, noradrenergic (NART), and serotonergic (SERT) transporters. DAT binding is estimated by striatal uptake at 24 h post-injection, whereas SERT binding by brainstem uptake at 1 h post-injection. Striatal [123I]-β-CIT uptake, as [18F]-FDOPA uptake, correlates with disease stage and symptom severity in PD, particularly with bradykinesia but not rest tremor (Seibyl et al., 1995; Brücke et al., 1997; Pirker, 2003). The main disadvantage of [123I]-β-CIT is its slow striatal uptake kinetics. Since after administration, it takes 24 h to equilibrate in the striatum, SPECT must be delayed to the following day. Recently, new SPECT tracers such as [123I]-FP-CIT, 123I-altropane, and [123I]-PE21 have been developed; they have faster uptake kinetics (2 to 3 hours) though they give higher non-specific signals. Another SPECT tracer the 99mTc-TRODAT-1 has the advantage of being technetium based and so available in kit form, however its specific signal is lower than the [123I] based SPECT tracers.

DAT markers, as [18F]-FDOPA PET, differentiate early PD from normal subjects with a sensitivity of around 90%. In early PD, as a response to dopamine cell loss, remaining DAT may be down-regulated in surviving neurons in order to preserve dopamine level. DAT markers may therefore overestimate the reduction of

terminal density in early PD. DAT binding, alike [18F]-FDOPA, decreases with age (Pirker et al., 2000; van Dyck et al., 1995).

Measurement of the vesicular monoamine transporter 2

The type-2 vesicular monoamine transporter (VMAT2), exclusively expressed in the brain, is responsible for the uptake of monoamines from the cytoplasm into the secretory vesicles in dopamine neurons (Liu and Edwards, 1997). Lee et al. compared striatal uptake of [11C]-dihydrotetrabenazine ([11C]DTBZ), a PET tracer that binds to VMAT2, [18F]-FDOPA, and the DAT ligand [11C]-methylphenidate in PD. They found in the parkinsonian striatum, [18F]-FDOPA Ki relatively less reduced than the [11C]DTBZ binding potential, and [11C]DTBZ binding less reduced than [11C]-methylphenidate binding (Lee et al., 2000). This finding supports the presence of relative AADC up-regulation and DAT down-regulation in PD striatum in order to increase dopamine turnover and diminish its re-uptake.

POST-SYNAPTIC DOPAMINERGIC IMAGING

At least five dopamine receptor subtypes have been described so far, which are classified into two main categories: the D1 type receptors (D1 and D5), which stimulate the enzyme adenylate cyclase, and the D2 type receptors (D2, D3, D4), which are inhibitory. Post-synaptic dopaminergic PET and SPECT studies in PD have mainly focused on D2 dopamine receptors.

[11C]-Raclopride, a low affinity dopaminergic D2 receptor ligand has been used in most PET studies to study postsynaptic dopamine receptors. In de novo, drug-naïve PD patients, [11C]-Raclopride binding is either normal or increased in the putamen and normal in the caudate nucleus (Dentresangle et al., 1999; Rinne et al., 1993). The increase of the putaminal binding at disease onset is usually interpreted in the frame of compensatory mechanism involving receptor up-regulation. In advanced PD, [11C]-Raclopride binding normalizes in the putamen and most often decreases in the caudate (Antonini et al., 1994; Brooks et al., 1990, 1992; Dentresangle et al., 1999; Turjanski et al., 1997).

The expression of D2 and D3 receptors has been followed by the dopaminergic ligand, [11C]-FLB 457. They are reduced in the anterior cingulate, dorsolateral prefrontal and temporal cortex and the thalamus in late stage PD, suggesting lesions of the mesolimbic dopaminergic pathways that may be involved in the cognitive and emotional deficits in PD patients (Kaasinen et al., 2000, 2003).

The striatal binding of [123I]-iodobenzamide (IBZM), a SPECT ligand of dopamine D2 receptors was found not to be altered in idiopathic drug-naïve PD patients (Schwarz et al., 1996).

[11C]-SCH23390, a D1 receptor ligand, is normally taken up in the putamen of de novo drug-naïve PD patients and its binding may either be normal or reduced in more advanced and treated patients (Shinotoh et al., 1993; Turjanski et al., 1997).

2.2 Non-dopaminergic imaging and extrastriatal systems

PD is a multi-system disease and the involvement of extrastriatal pathways in the disease process is now well established (Lang, 2007). Serotoninergic, noradrenergic and cholinergic neurons have been shown to be target of neurodegeneration soon in the disease process, indeed Lewy body pathology in noradrenergic, serotoninergic and cholinergic systems seems to be responsible for the onset of some non-motor symptoms in PD (Rodriguez-Oroz et al., 2009).

SEROTONINERGIC AND NORADRENRGIC SYSTEMS

Serotoninergic and noradrenergic systems have been investigated by [18F]-FDOPA PET. AADC is present in all monoaminergic neurons terminals, therefore [18F]-FDOPA uptake in extrastriatal areas reflects the density serotoninergic and no-radrenergic together with dopaminergic terminals. A recent [18F]-FDOPA cross-sectional study on extrastriatal dopamine dysfunction in PD, showed in early PD raised [18F]-FDOPA uptake in midbrain raphe and reduced tracer uptake in locus coeruleus, while in advanced patients [18F]-FDOPA uptake decreased in both nuclei. Uptake into the red nucleus, subthalamus, ventral thalamus and pineal gland was also targeted in more advanced patients, whereas limbic areas, except for hypothalamus, were spared even in late disease (Moore et al., 2008).

Serotoninergic system has been explored by [11C]-WAY-100635 PET, a marker of serotonin 5-HT1A receptors which act as autoreceptors on the soma of serotoner-gic neurones in the median raphe. PD patients compared to healthy controls showed a 25% reduction in binding of [11C]-WAY-100635 in the midbrain raphe, but the magnitude of reduction was independent of the presence of depression (Doder et al., 2000). [11C]-DASB PET, a marker of brain serotonin transporter binding, revealed in advanced, non-depressed PD patients a binding reduction of 20–30% in all ex-amined brain areas compatible with a modest, widespread loss of brain serotonergic innervation (Guttman, 2007). These studies, therefore, do not support a major role for serotonergic loss in PD depression. Noradrenergic and dopaminergic neurotransmis-sion has been recently assessed in PD patients with depression, by means of [11C]-RT132 PET. Depressed PD patients showed lower [11C]-RT132 binding in locus coeruleus and areas of the limbic system than non-depressed PD patients. In areas with low dopaminergic innervation such as locus coeruleus, [11C]-RT132 uptake mainly reflects the density of noradrenergic neurons. In limbic areas, such as the ven-tral striatum and amygdala, [11C]-RT132 uptake reflects function of both dopami-nergic and noradrenergic pathways (Remy et al., 2005). An important role for nora-drenaline and limbic dopaminergic rather than serotonergic dysfunction in the patho-genesis of depression in PD patients was therefore suggested.

CHOLINERGIC SYSTEM

Cholinergic deficiency in demented PD patients has been assessed by PET and SPECT. [123I]-BMV SPECT, an in vivo marker of the vesicular acetylcholine transporter, showed in non demented PD patients reduced tracer uptake in the parietal and occipital cortex, whereas in demented PD a more severe global reduction of cortical binding similar to that seen in Alzheimer's disease patients occurs (Kuhl et al., 1996). Cortical acethylcholinesterase activity in PD has been assessed by [11C]-MP4A PET, revealing a loss of cholinergic function throughout the cortex in parallel with the loss of striatal dopaminergic function, demented PD cases showing the most severe reductions (Hilker et al., 2005). [11C]-PMP PET was also used to assay cortical acethylcholinesterase activity in PD with and without dementia. As expected, PD patients with dementia (PDD) were characterized by the greatest reduction in cortical acetylcholinesterase activity. As compared to Alzheimer's disease patients PDD patients, although matched for dementia severity, showed a lesser reduction in cholinergic innervation, the lateral temporal cortex being selectively targeted (Bohnen et al., 2003).

2.3 Brain metabolic imaging

Molecular imaging techniques to localize and quantify abnormalities of brain energy metabolism have been used for many years to study the pathophysiology of neurodegenerative disorders. (Slosman and Pellerin, 2004; Herholz et al., 2004).

In Parkinson's disease, presynaptic dopamine loss has been shown to be associated with broader functional abnormalities involving cortico–striato–pallido–thalamocortical (CSPTC) loops and related pathways (De Long and Wichmann, 2007). Thus, nigrostriatal dysfunction, even if highly localized, is associated with disease-specific alterations on regional cerebral metabolism across the whole brain.

[18F]FDG-PET, thanks to its capability to assess local synaptic activity as well as biochemical maintenance process, has been widely employed in the study of the pathophysiology of PD (Pellerin et al., 2007, Eidelberg, 2009).

The first [18F]FDG-PET studies revealed 1) in hemiparkinsonian patients at an early stage of the disease, an increased metabolism in the lentiform nucleus (putamen and Globus pallidus) on the opposite side of the affected hemibody (Wolfson et al., 1985) 2) in more severely affected patients with dementia, a decrease in cortical glucose utilization, particularly in posterior and temporal parietal associative regions (Kuhl et al., 1984; Wolfson et al., 1985).

Afterwords, network analysis of functional imaging data in PD revealed a specific abnormal spatial covariance pattern involving metabolic changes at key nodes of the CSPTC loops and related pathways (Eidelberg et al., 1994, 1997; Moeller et al., 1999; Ma et al., 2007).

The Parkinson's disease-related pattern (PDRP) is characterized by increased pallido–thalamic and pontine metabolic activity associated with relative reductions in premotor cortex, supplementary motor area (SMA), and in parietal association regions (Figure 4) . PDRP has been detected in several patient population (Eckert et al.,

2007), it shows a good test-retest reproducibility in individual subjects over hours to weeks (Ma et al., 2007), and its expression correlates with standardized motor ratings in different patient cohorts (Asanuma et al., 2006). Further PDRP expression discriminates between PD, atypical parkinsonian syndromes, and healthy control subjects in prospective FDG PET scans (Eckert et al., 2008).

Network analysis was further used to explore pathophysiological changes at the basis of cognitive dysfunction in PD.(Huang et al., 2007). A PD-related cognitive pattern (PDCP) characterized by metabolic reductions mainly in medial frontal and parietal association regions, and relative increases in cerebellar cortex and dentate nuclei was identified (Figure 1.4). This cognitive pattern correlated with performance on neuropsychological tests of memory and executive functioning. The expression of the PDCP was found to increase stepwise in PD patients categorized by clinical criteria for mild cognitive impairment (MCI) (Huang et al., 2008).
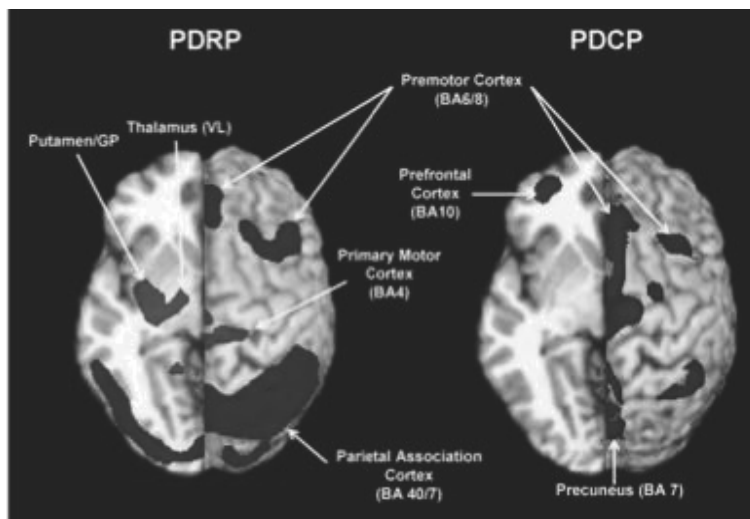
Figure 1.4. PDRP and PDCP (from Poston, 2009)

Besides network analysis, region of interest (ROI) analysis and voxel based multivariate analysis by Statistical Parametric Mapping (SPM), were used to explore brain metabolism in PD. Frontal and temporo-parietal hypometabolism has been documented in PD patients (Hu et al., 2000; Juh et al., 2005), although recent studies suggested that patients without cognitive impairment had limited areas of hypometabolism in the frontal and occipital cortices, whereas in patients with mild cognitive impairment there were extensive areas of hypometabolism in the posterior cortical regions, including the temporo-parieto-occipital junction, medial parietal, and inferior temporal cortices (Huang et al., 2008; Hosokai et al., 2009).

3. Molecular imaging biomarkers in PD

PD distinctive motor features are characterized by an insidious onset and an inexorable but variable progression. Thus, in recent years much effort are directed to determine reliable and well validated biomarkers for PD to identify individuals "at risk" before motor symptoms onset, accurately diagnose individuals at the threshold of clinical PD, and monitor PD progression throughout its course. Biomarkers of such a type, would dramatically improve patient care and accelerate research into both PD cause and therapeutics (Marek et al., 2008).

Biomarkers are broadly defined as characteristics that are objectively measured and evaluated as indicators of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention (Biomarkers Definitions Working Group, 2001; De Gruttola et al., 2001).

Biomarkers in clinical research and clinical care can be employed to the following aims: 1) to measure the biologic activity of a disease, therefore providing useful information about the disease process; 2) as a diagnostic tools or to determine risk and prognosis; 3) to provide information about an intervention's mechanism or biologic activity in in-vitro models, animal studies, or early human clinical trials in therapy development and evaluation; 4) as surrogate endpoints (i.e. a subset of biomarkers intended to substitute for clinical endpoints).

Biomarkers for PD are critical to the understanding of disease cause and disease progression. In particular, biomarkers may provide a window to the premotor period of PD before typical symptoms are manifest, but while degeneration has already begun (Marek and Jennings, 2009). Both pathology and imaging studies, indeed, demonstrated that PD is characterized by a long period during which vulnerable neuronal populations are degenerating, but when typical motor symptoms have not yet developed. At the threshold of motor symptoms about 40–60% of dopamine neuronal markers have been lost (Fearnley and Lees,1991; Marek, 1999; Morrish et al., 1998). The duration of the premotor period is unclear, however imaging and pathology data suggest that it may last from 5 to 20 years.

3.1 Dopaminergic imaging

Molecular imaging of the presynaptic nigrostriatal function, because of its capability to assess the ongoing dopaminergic degeneration, has been widely used as biomarker in studies assessing early PD. Dopaminergic degeneration has been explored either with [18F]-FDOPA and/or with DAT tracers (Brooks et al., 1990; Asenbaum et al., 1997; Mozley et al., 2000; Frey et al., 1996; Booij et al., 1998; Innis et al., 1993; Vingerhoets et al., 1994). A good correlation has been found between dopamine neuron loss and [18F]-FDOPA uptake, although in a very small sample (Snow et al., 1993). In postmortem PD brain, DAT density has been shown to be reduced in the striatum (Kaufman et al., 1991; Niznik et al., 1991; Wilson et al., 1996).

Consistent with the expected PD pathology, reductions in [18F]-FDOPA, vesicular monoamine transporter type 2 and DAT ligand uptake have been found. In details, these imaging studies demonstrate asymmetric loss of dopaminergic uptake, with a greater loss in the putamen as compared to the caudate, and the imaging loss correlates with worsening clinical symptoms in cross-sectional evaluation (Huang et al., 2001; Booij et al., 2001; Fischman et al., 1998; Sawle et al., 1994; Seibyl et al., 1995; Frey et al., 2001; Scherfler et al., 2007).

Many studies have demonstrated that imaging markers can identify individuals with typical motor PD, even in its early stages, however, if the main future application of imaging biomarkers would be to assess premotor PD, studies must move "backwards" to focus on subjects at risk but not yet diagnosed. (Marek and Jennings, 2009) (Figure 1.1).

At present, the challenge is how to find at risk subjects. Individuals with one or more symptoms of parkinsonism and therefore with suspected, but not diagnosed, PD constitutes one way to identify subjects at risk for PD. Several studies have examined the accuracy of DAT imaging in subjects with nondiagnostic motor symptoms (Catafau and Tolosa, 2004; Jennings et al., 2004; Tolosa et al., 2007). Imaging was compared with a longitudinal "gold standard" clinical diagnosis. Individuals with suspected PD and reduced DAT density were demonstrated to be at very high risk for a clinical diagnosis of PD. These imaging markers, however, cannot distinguish between idiopathic PD and other parkinsonisms such as progressive supranuclear palsy, multiple system atrophy, cortical basal ganglionic degeneration, and diffuse Lewy body disease. To this aim, [18F]FDG PET by revealing changes in neural circuit directly or indirectly related to dopaminergic dysfunction, may be more useful in distinguishing PD from these Parkinson syndromes (Eckert et al., 2008).

Dopaminergic imaging has also demonstrated its utility in clinical trials on very early PD. In untreated recently diagnosed PD, in vivo dopaminergic imaging has identified about 10–15% of enrolled subjects with scans in the normal range—termed scans without evidence of dopaminergic deficit (SWEDD) (Whone et al., 2002; Parkinson Study Group, 2004, 2007). Follow-ups subsequently demonstrated that those patients with SWEDD are unlikely to have PD, whereas those with DAT deficit are very likely to have PD (Marek et al., 2003) Therefore, dopaminergic imaging accurately distinguishes subjects unlikely to have PD from those with an eventual clinical diagnosis of PD.

Before the development of motor symptoms, early non-motor symptoms may reliably identify subjects at risk for PD, so that combined with imaging markers they may aid further defining premotor PD. Among early non motor features of PD, the ones more often described include loss of olfactory function, sleep disturbances (e.g., RBD), autonomic dysfunction such as constipation, and behavioral and cognitive changes (Langston, 2006; Siderowf and Stern, 2006; Tolosa et al., 2007).

To identify premotor PD, early clinical manifestations and dopaminergic imaging have been recently considered together. Among PD relatives showing olfactory deficit, DAT imaging identifies a sub-group with increased risk of developing PD (Ponsen et al., 2004). Similar findings have been reported by combining RBD and dopaminergic imaging (Stiasny-Kolster et al., 2005).

Recent advances in molecular genetics, by uncovering several genes for PD, has provided new possibilities to identify at risk and premotor PD population (Klein and Schlossmacher, 2007; Pankratz et al., 2007). Genes mutations previously described generally account for a very small number of PD patients, on the contrary LRRK2 mutations may identify approximately 20–35% of the PD patients in populations such as the Ashkenazi Jewish or in regional populations of Spain or North Africa (Ozelius et al., 2006; Saunders-Pullman et al., 2006; Lesage et al., 2006). In addition, thanks to initiatives as the International LRRK2 Consortium genetic, clinical, and imaging assessment have been combined to shed new light on premotor PD in genetic PD subsets (Healy et al., 2008). Dopaminergic imaging has been demonstrated to be altered in unaffected family members of patients with PD who are gene-positive for LRRK2 and other Park gene abnormalities (Adams et al., 2005; Khan et al., 2002).

3.2 Non-dopaminergic imaging

Recent growing interest is directed towards nondopaminergic imaging biomarkers for premotor PD, aimed to explore specific potential underlying pathophysiology of PD such as inflammation, α-synuclein deposition, mitochondrial dysfunction, or protein misfolding (Hirsch et al., 2003; Olanow and McNaught, 2006; Schapira et al., 1998; Singleton et al., 2003).

Inflammation has been proposed as a risk biomarker for PD by recent pathologic, imaging and epidemiologic studies, as a consequence imaging markers of inflammation may be an early marker of PD risk (Chen et al., 2005, 2008; McGeer and McGeer, 2004). Increased microglial activation in PD has been shown by [11C]-PK11195 PET studies (Gerhard et al., 2006; Ouchi et al., 2005).

Molecular genetics showed how increased α-synuclein could be a risk biomarker for PD (Winkler, 2007; Cookson and van der Brug, 2008; Ahn et al., 2008). Changes in the plasma and cerebral spinal fluid of PD patients have been revealed by α-synuclein assays (El-Agnaf et al., 2006; Tokuda et al., 2006). Unfortunately, α-synuclein imaging is as yet an unfulfilled goal.

Early non-motor features of PD, such as olfactory deficits, sleep disturbances, autonomic dysfunction, and behavioral and cognitive changes, are most often not due to dopamine loss, but may involve pathology in norepinephrine, serotonin, cholinergic, or other neuronal systems. Nondopaminergic imaging biomarkers for early non motor clinical manifestations have begun to emerge. For example, cardiac imaging with [123I]-metaiodobenzylguanidine may identify early norepinephrine dysfunction in autonomic neurons (Spiegel et al., 2005; Oka et al., 2007). Serotonin dysfunction has been shown to be associated with depression in PD both by imaging and pathologic studies (Guttman et al., 2007).

The growing burden of dementia in PD patients, has turned the interest to imaging biomarkers to identify early cognitive dysfunction in PD. Markers of β-amyloid burden, glucose metabolism, or nicotinic receptor binding, have been suggested as tools that might identify prodromal sign of dementia in PD (Rowe et al., 2007; Fujita et al., 2006; Huang et al., 2007; Maetzler et al., 2008).

3.3 Brain metabolic imaging

Metabolic brain imaging with [18F]FDG PET, thanks to its capability to assess regional synaptic activity has become an prominent biomarker for PD (Eidelberg et al., 1997). [18F]FDG-PET can be used both to study the pathophysiology of PD and to assess the treatment effects.

Since localized dopaminergic pathology can alter functional connectivity across the entire brain in a disease-specific manner, spatial covariance methods have been used to identify metabolic abnormalities at the network level in PD (Eckert and Eidelberg, 2005; Eckert al., 2007).

Network analysis of [18F]FDG-PET identified the previously described PDRP, which has been detected in seven independent patient populations (e.g., Moeller et al., 1999; Feigin.et al., 2002; Lozza et al., 2004; Eckert et al., 2007). Its expression in individual subjects has been demonstrated to be highly reproducible (Ma et al., 2007). PDRP scores have also been used to discriminate between patients with PD and atypical parkinsonism (Ma et al., 2007; Eckert et al., 2007, 2008). In PD patients, pattern expression has been found to correlate with standardized motor ratings (Asanuma et al., 2006; Eidelberg et al., 1994, 1995), symptoms duration (Moeller and Eidelberg, 1997; Huang et al., 2007b), and intraoperative measurements of subthalamic nucleus (STN) firing rate (Lin et al., 2008). PDRP has therefore been proposed as a biomarker to differentiate PD patients from patients with atypical parkinsonism, and to assess longitudinal changes in disease-related motor disability.

In addition to motor symptoms, voxel-based spatial covariance analysis of FDG PET has been used to identify a distinct PD-related metabolic pattern associated with cognitive dysfunction in non-demented patients, the PDCP, previously described (Huang et al., 2007a). Correlations between PDCP expression and performance in neuropsychological tests assessing memory and executive functions have been found (Huang et al., 2007a). The expression of this pattern has been shown to be greater in PD patients with mild cognitive impairment (MCI) compared to cognitively normal PD (Huang et al., 2008). PDCP, as PDRP, has been found to be highly reproducible in individual patients. However, unlike the PDRP, PDCP activity is not modulated by symptomatic treatment with levodopa or STN stimulation (Huang et al., 2007a; Hirano et al., 2008). Moreover, the time course of PDCP expression in early stage disease is different from that of the PDRP (Huang et al., 2007b), with a slower, non-linear trajectory that is unrelated to concurrent changes in motor function or striatal dopamine transporter (DAT) binding (Eckert et al., 2007b). The PDCP has been therefore suggested as a complementary network biomarker with specificity for early cognitive dysfunction in patients with PD.

[18F]FDGF-PET has been used as biomarker to measures the pathologic processes associated with clinical disease progression. In a recent longitudinal study, [18F]FDG-PET was used in conjunction with dopaminergic imaging and UPDRS assessments to follow the change in network PDRP and PDCP activity in patients with early PD (Huang, 2007b). The development of PDRP expression over time was found to occur in parallel with progressive deterioration in UPDRS motor ratings and putamen DAT binding, whereas the progression of PDCP was found to progressively increase over time but with a slower and less significant progression rate as com-

pared to PDRP(Huang, 2007b). Further, changes in PDCP activity did not correlate with concurrent declines in striatal DAT binding or increases in UPDRS motor ratings. Thus, the two network measures of disease progression, the PDRP and the PDCP, have been regarded as distinct. These findings suggest that different underlying pathophysiological networks could be associated with motor and cognitive disease progression in PD, and therefore the use of the PDCP has been proposed as a biomarker to assess cognitive changes in PD patients.

The quantification of network activity was used also in the assessment of the clinical response to therapeutic intervention in PD (Eckert and Eidelberg, 2005). Informations obtained by comparing the changes in metabolic network activity during treatment, can indeed be used as biomarkers to identify the underlying pathological processes involved in observed clinical changes. Applications of such a type are fundamental in the development of novel PD treatments and in the assessment of intervention efficacy.

During dopaminergic treatment changes in both pallidal metabolism and PDRP network activity was found to correlated with clinical improvement in the UPDRS motor rating score (Feigin et al., 2001; Asanuma et al., 2006). During neurosurgical modulation of subthalamic nucleus (STN) activity a significant correlation between PDRP expression and intraoperative recordings of STN firing rate was found (Lin et al., 2008). The activity of this metabolic network may therefore be modulated by surgical interventions in this region. Indeed, FDG-PET studies have revealed sustained reductions in PDRP expression following therapeutic STN lesioning (Su et al., 2001; Trošt et al., 2003) as well as in PD cohorts treated with STN DBS (Asanuma et al., 2006; Trošt et al., 2006; Hirano et al., 2008). Overall, the metabolic changes reported with STN DBS reflect normalization of the regional changes observed in untreated PD patients. Specifically, STN stimulation has been found to increase metabolism in parietal association cortex and, to a lesser degree, in the prefrontal cortex (Trošt et al., 2006; Asanuma et al., 2006), regions with significant reduced metabolism in untreated PD (Moeller et al., 1999; Eckert et al., 2007b). This suggests that the baseline metabolic reductions observed in these cortical regions are to some degree reversible. This may be attributable to a correction of overactive signaling in inhibitory pallidal projections to a variety of thalamic relay nuclei (Taktakishvili et al., 2002).

Interestingly, the effects of levodopa and STN DBS on PDRP expression were not found to be additive (Asanuma et al., 2006). A "floor effect'" occurs, which does not allow to lower network activity beyond a naturally defined level determined by the effects of therapeutic STN lesioning. These findings suggest that upper and lower thresholds exist for therapeutic PDRP modulation. These bounds may define the minimum and maximum functional changes that are achievable with current interventions. Whether interventions at other key PDRP nodes like the pedunculopontine nucleus (PPN) (Stefani et al., 2007) can further reduce network activity is a topic of ongoing investigation.

Chapter 2
Aim

The aim of this thesis was to explore brain function in drug-naïve Parkinson's disease patients by means of molecular imaging techniques.

In detail:

[18F]FDG-PET was employed to identify in drug-naïve PD patients brain metabolic alteration uniquely related to disease process and not modulated by antiparkinsonian therapeutic intervention. To this aim changes in cerebral metabolic rate of glucose (CMRglc) in drug-naïve PD patients as compared to a group of normal controls (NL) were assessed (Study 1).

[18F]FDG-PET and [123I]FP-CIT SPECT were employed together to explore the early functional changes in brain function related to dopaminergic depletion in the putamen (Study 2) and in the caudate nucleus (Study 3).

Chapter 3
Methods

1. Subjects

Thirty-one consecutive PD patients (males 20, females 11; mean age 65,87 ± 6,18 years; mean disease duration 18,58 ± 9,14 months) from the Neurological Clinic of the University of Florence were enrolled in this study.

The inclusion criteria were 1) clinical diagnosis of possible PD according to Gelb criteria (Gelb, 1999) 2) striatal decrease in DAT density as measured with [123I]FP-CIT SPECT. The exclusion criteria were 1) current or past assumption of any anti-parkinsonian drugs 2) moderate cognitive impairment (Mini Mental State Examination < 24, MMSE; Folstein, 1975) 3) diagnosis of major depression 4) white matter microvascular disease and atrophy on brain MRI.

Seventeen patients had predominant right-sided limb involvement, fourteen patients had predominant involvement of the left limbs.

Before the beginning of a specific therapy for PD, patients underwent clinical assessment, neuropsychological assessment, MRI, [123I]FP-CIT SPECT, [18F]FDG PET within a period of one month at our University Institution.

2. Clinical and neuropsychological assessment

Clinical severity of disease was assessed with Hoehn & Yahr (H&Y) scores (Hoehn and Yahr, 1967) and the motor part (part III) of the Unified Parkinson's Disease Rating Scale (UPDRS III) (Fahn et al., 1987).

The neuropsychological assessment explored the following cognitive domains: executive functions (EF), attention and inhibition processes, memory and visuoconstructional abilities. MMSE was administered to evaluate global cognitive functioning and to exclude patients with moderate cognitive decline. EF, attention and inhibition processes were explored by the Modified Card Sorting Test (MCST) (Nelson, 1976), letter fluency (Novelli et al., 1986) and the Stroop Color Word Test (SCWT) (Spreen and Strauss, 1991). Visual short term memory (STM) was assessed by the Corsi Block Tapping Test (CBTT), verbal STM by Two Syllabic Word Repetition Test (TSWRT) and by the recency effect of the Serial Position Curve Test (SPCT), verbal long term memory (LTM) was assessed by the primacy effect of the SPCT

(Spinnler and Tognoni, 1987). Visuoconstructional abilities were assessed by Fragmented Figures Assembling Test (FFAT) (Spinnler and Tognoni, 1987).

3. MRI

MRI brain scan was performed on Gyroscan NT Intera (Philips Medical System, Europe) with T1-, T2-weighted, and FLAIR sequences (matrix 512x512, voxel size 0,49x0,49x6,60mm). Images were reconstructed into 20 slices with 6 mm slice thickness in axial, coronal and sagittal orientation.

4. [123I]FP-CIT SPECT

All subjects underwent a [123I]FP-CIT SPECT scan. SPECT images were acquired 3 hours after intravenous administration of [123I]FP-CIT (185 MBq) with a 3-headed camera (IRIX, Philips Medical System, Europe) equipped with UHR collimators. Images were acquired over 360° (120 angles, 40 projection/head,128 X 128 matrix, bin size 2,33 mm); scanning time was of 60 sec/step and lasted 40 minutes. Image data were reconstructed using OSEM algorithm into 128 slices (128x128 matrix, 2.33 mm/pixel, 2.33 mm slice thickness). Attenuation correction was performed using Chang algorithm.

5. [18F]-FDG PET

Patients were injected with a dose of 370 MBq ^{18}F-FDG, in resting state in a dimly lighted room with minimal background noise. Thirty minutes after [18F]-*FDG* administration, a scan lasting 20 minutes was acquired, using a GE PET device. A polycarbonate head holder was used to reduce head movements during the scan. Emission scans were acquired in two dimensional mode with an axial FOV of 15 cm and a planar FOV of 55 x 55 cm. Images were reconstructed by OSEM iterative algorithm (2 iterations, 21 subsets). A transmission scan with ^{68}Ge-sources was used for photon attenuation correction. Thirty-five PET slices were acquired using a 256x256 matrix with a pixel dimension of 2.15 mm and slice thickness of 4.25 mm.

6. Statistical Parametric Mapping (SPM)

PET images were converted from DICOM into Analyze format using the MIPAV software and then processed using SPM2 (Study1) or SPM5 (Study 2 & 3) (Wellcome Department of Cognitive Neurology, Institute of Neurology, London) running on Matlab 7 (Mathworks, Inc., Natick, MA). A 12- parameter linear affine transformation and a non-linear three-dimensional deformation were applied to each subject scan to realign and spatially normalize images to a reference stereotactic template (Montreal Neurological Institute -MNI-, McGill University, Montreal, Quebec). The normalized data were then smoothed using a Gaussian kernel at FWHM 12

mm to account for individual variability in structure–function relation and to enhance signal to noise ratio. Global normalization was performed using the proportional scaling routine in SPM. The gray matter threshold was set to 0.8 of the mean and global calculation was obtained with respect to the mean voxel value.

The appropriate SPM design was used for each study, in the context of the general linear model. Coordinates of local maxima were converted from MNI to Talairach space using the "mni2tal" function (**http://www.mrc-cbu.cam.ac.uk/Imaging/Common/mnispace.shtml)** and labeled according to the Talairach and Tournoux space (Talairach and Tournoux, 1988) by using the Talairach Daemon Client Version 2.0 (**http://ric.uthscsa.edu/projects/talairachdaemon.html**).

Patients informed consent was obtained according to the Declaration of Helsinki and the local Ethical Committee approved the study.

Chapter 4
Study 1 Cerebral brain metabolism in drug-naïve Parkinson's disease patients

1. Background

In recent years, attention has turned towards the potential applications of functional neuroimaging in Parkinson's disease (PD).

Dopaminergic imaging has become a prominent diagnostic tool in PD, but nigral cell loss is just one of several events in the disease progression and it may parallel the effects of disease in other brain areas (Michell et al., 2004; Braak et al., 2003). In this scenario, [18F]fluoro-deoxyglucose ([18F]FDG) PET, because of its capability to assess changes in physiologically relevant neural systems, has been proposed as biomarker for PD, particularly to monitor disease progression and modulation effect of antiparkisonian therapy (Eckert et al., 2007; Asanuma et al., 2006).

Until now, [18F]FDG-PET studies examining brain metabolic changes in PD mainly included, besides few drug-naïve patients, already treated patients in the "off-medication" condition (Asanuma et al., 2006; Ma et al., 2007). However in these washout designs a persistent medication effect on brain metabolism could not be completely ruled out.

Thus, to assess the possible role of [18F]FDG-PET as a biomarker to monitor treatment effect, it is important to evaluate brain metabolic alterations in drug-naïve PD patients. Therefore this [18F]FDG-PET study explored alterations in cerebral metabolic rate of glucose (CMRglc) in drug-naïve PD patients.

2. Methods

2.1 Subjects

The whole PD sample was included in study 1,i.e. thirty-one consecutive PD patients (Table 4.1).

Twenty–five healthy and unmedicated sex- and age-matched normal subjects (NL) were recruited at New York University (NYU) (Table 4.1). Brain disease or cognitive disorders were excluded by MRI and detailed clinical and neuropsychological examinations.

Table 4.1. Clinical characteristics of the study sample

	NL (n=25)	PD (n=31)	p
Gender	15 males, 10 females	21 males, 10 females	n.s
Age	64.13 ± 6.99	65.87 ± 6.18	n.s
MMSE	29.40 ± 0.80	28.56 ± 1.58	< 0.05
Disease duration (months)	/	18.58 ± 9.14	
Side of symptoms onset	/	17 right, 14 left	
Prevalent motor symptom	/	21 tremor dominant 10 non tremor dominant	
UPDRSIII	/	13.58 ± 5.27	
H&Y (range)	/	1-2	

Note: Values are mean ± SD; NL, normal subjects; PD, Parkinson's disease patients; MMSE, Mini Mental State Examination; UPDRS, Unified Parkinson's Disease Rating Scale; H&Y, Hoehn and Yahr.

2.2 Statistical analysis

Chi-square test and independent t-test were used to compare demographic and clinical data between PD and NL, and between PD subgroups (Right onset PD vs left onset PD, tremor dominant vs nontremor dominant PD), as appropriate. One-way ANOVA was used to determine if significant demographic and clinical differences existed between patients at different stages of PD. According to the Modified Hohen & Yahr stages (Jankovic et al., 1990) three groups were identified (i.e.Stage 1 = Unilateral disease; Stage 1.5 = Unilateral plus axial involvement; Stage 2 = Bilateral disease, without impairment of balance.) P values <0.05 were considered significant. All statistical analysis were made using SPSS 15.0 (SPSS Inc., Chicago, IL).

2.3 SPM

The Two sample t-test was employed to explore CMRglc differences between PD patients and NL, and between PD subgroups (i.e. right-onset, left-onset, tremor-dominant, non tremor-dominant) and NL.

One-way ANOVA was performed to explore the main effect of disease severity, using disease stage as condition according to the Modified H&Y scale. Age was set as nuisance variable. The significance threshold was set to a corrected value of p < 0.01 (False Discovery Rate, FDR, corrected). Coordinates of local maxima were labeled according to the Talairach and Tournoux space.

3. Results

3.1 Demographic and clinical findings

There were no demographic differences between PD patients and NL (Table 4.1).

There were no differences between right- and left-onset PD subgroups for motor dominant symptom, mean disease duration, UPDRSIII and H&Y scores, while left-onset PD were older than right-onset PD. In the right-onset PD group there were more males than females (p<0.05). There were no differences between tremor-dominant and non tremor-dominant PD patients for age, gender distribution, side of symptoms onset, mean disease duration, UPDRSIII and H&Y scores. As expected, PD patients at different H&Y stages showed significant increases in UPDRSIII at advanced H&Y stages, while there were not demographic differences between groups.

3.2 SPM results

As compared to NL, PD patients showed hypometabolism in premotor area (PM, BA 6), dorsolateral prefrontal cortex (DLPFC, BA 46, 47), inferior temporal gyrus (ITG, BA 37), superior and inferior parietal lobule (SPL and IPL, BA 7, 39), inferior and middle occipital gyrus (IOG and MiOG, BA 18, 19) bilaterally (p<0.01 FDR corrected). As compared to NL, PD patients showed hypermetabolism in lentiform nucleus (LN), primary motor area (PMA, BA 4) and cerebellum of both hemispheres. The same pattern of CMRglc changes was observed in the comparisons between right-onset PD versus NL, and left-onset PD versus NL, but with a greater cluster extent in brain regions contralateral to the first affected body side. Both tremor-dominant and non tremor-dominant PD, as compared to NL, respectively showed the CMRglc alterations seen in the whole group analysis. The one-way ANOVA confirmed a significant main effect of disease severity bilaterally in PM, PMA, DLPFC, ITG, SPL, IPL, IOG, MiOG, LN and cerebellum. Post hoc analysis revealed a progressive CMRglc reduction in PM, DLPFC, ITG, SPL, IPL, IOG, MiOG and a progressive CMRglc increase in PMA, LN and cerebellum (Table 4.2, Figure 4.1).

Table 4.2. Brain regions showing significant CMRglc differences in PD patients as compared to NL subjects.

Cluster extent	Anatomic area	Functional area	Talairach Coordinates			z
			x	y	z	
PD < NL						
9696	Left Inferior Parietal Lobule	BA 39	-50	-71	11	6.16
	Left Inferior Temporal Gyrus	BA 37	-56	-58	-4	6.03
	Left Inferior Temporal Gyrus	BA 37	-50	-67	3	5.91
	Inferior Occipital Gyrus	BA 18	-35	-90	-8	4.41
4679	Right Precentral Gyrus	BA 6	62	4	24	5.21
	Right Middle Frontal Gyrus	BA 46	52	27	25	5.08
	Right Middle Frontal Gyrus	BA 47	50	38	-6	4.91
4763	Left Inferior Frontal Gyrus	BA 47	-42	26	-9	4.99
	Left Precentral Gyrus	BA 6	-45	0	24	4.85
	Left Middle Frontal Gyrus	BA 47	-45	40	-5	4.64
4752	Right Inferior Temporal Gyrus	BA 37	48	-63	1	4.74
	Middle Occipital Gyrus	BA 19	45	-70	-4	4.01
1009	Right Superior Parietal Lobule	BA 7	14	-71	41	4.22
PD > NL						
9895	Left cerebellum	Culmen	-6	-41	-18	5.12
	Right cerebellum	Culmen	14	-52	-15	4.80
1970	Right Lentiform Nucleus		29	-17	11	5.01
894	Left Lentiform Nucleus		-26	-17	12	4.83
54	Right Precentral Gyrus	BA 4	24	-15	48	3.68
181	Left Precentral Gyrus	BA 4	-12	-22	64	3.67
P < 0.01 FDR corrected						

Note: Values are mean ± SD; NL, normal subjects; PD, Parkinson's disease patients; MMSE, Mini Mental State Examination; UPDRS, Unified Parkinson's Disease Rating Scale; H&Y, Hoehn and Yahr.

Figure 4.1. Brain metabolic alterations in drug-naïve PD patients as compared to normal sub-jects.
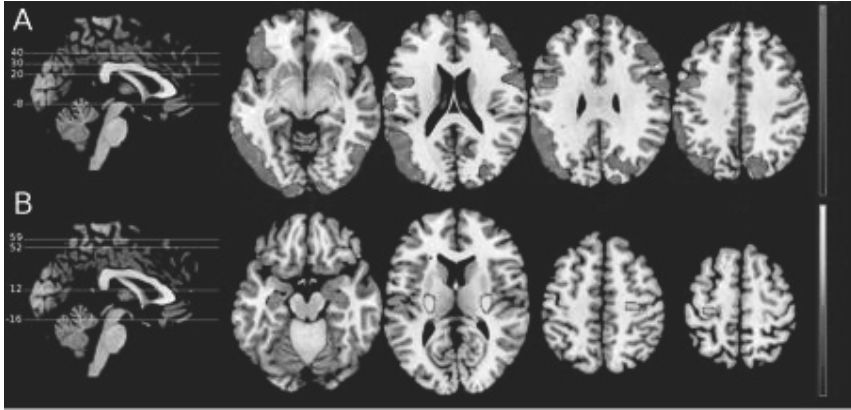
Legend: Statistical parametric maps showing CMRglc reductions (a) and increases (b) in drug-naïve PD patients as compared to normal controls. Areas of hypometabolism and hypermetabolism are displayed on a standardized spatially normalized MRI.

Chapter 5
Study 2 Brain metabolic correlates of dopaminergic degeneration in de novo idiopathic Parkinson's disease

1. Background

The cardinal pathological feature of PD is the degeneration of dopaminergic nigrostriatal neurons in the substantia nigra pars compacta, resulting in a loss of dopaminergic terminals in the striatum (Fearnley and Lees, 1991). SPECT and PET imaging has been widely used to reveal abnormalities in dopaminergic transmission, particularly in the diagnostic phase (Seibyl et al., 2003). Nigrostriatal dopaminergic neuronal loss in PD is, however, the cardinal of several neuro-functional steps in the pathologic progression of the disease(Bezard et al., 2003; Braak et al., 2003; Obeso et al., 2004). Previous [18F]-FDG PET studies showed a characteristic pattern of regional glucose metabolism (rCMRglc) in patients with PD compared to controls, involving frontal and temporo-parietal-occipital hypometabolism, together with increased pallido-thalamic, pontine and cerebellar activity (Eckert et al., 2005b; Eidelberg et al., 1994; Fukuda et al., 2001; Huang et al., 2007b). Besides, several studies showed the correlation between reduced rCMRglc and severity of motor impairment in PD, demonstrating an inverse correlation involving in particular anterior cingulate gyrus, orbitofrontal and occipitotemporal regions (Nagano-Saito et al., 2004).

The study of the relation between the severity of dopaminergic degeneration, as assessed with [123I]-FP-CIT SPECT, and metabolic dysfunction in PD patients, as assessed by [18F]FDG-PET might reveal in which regions rCMRglc changes are directly related to the dopaminergic neuronal loss. To this aim, an accurate measurement of dopaminergic degeneration plays a key role and the enrolment of drug naïve PD patient is mandatory. Indeed, in de novo PD patients the pathological processes are not yet affected by pharmacological agents (Fahn et al., 2004), allowing a more accurate insight of PD processes.

The present study evaluates in drug-naïve PD patients the relation between the brain metabolic changes, measured with [18F]-FDG PET, and the severity of dopaminergic degeneration, assessed with [123I]-FP-CIT SPECT. Striatal [123I]-FP-CIT uptake was quantified with the Binding Potential measurement (Tissingh et al., 1998; Innis et al., 2007), estimated by the Least Squares (LS) method, an algorithm previously developed in our Institute, which allows to obtain BP estimates that are less affected by partial volume effects (PVE) and its reliability has been validated in phantom (Vanzi et al., 2007) and clinical studies (Berti et al., 2008).

2. Methods

2.1 Subjects

Twenty-six PD patients of the whole drug-naïve PD sample were included in this study (17 males and 9 females, mean age: 65.3±6.4 years) (Table 5.1). They were examined with MRI scan, [123I]-FP-CIT SPECT and [18F]-FDG PET.

Table 5.1. Patients' demographic and clinical data

Demographics	Value
Age (years)	65 ± 6 [53-77]
Sex	17 males, 9 females
Side of motor symptoms onset	13 right, 13 left
Symptom at onset	20 tremor, 6 brad/rig
Disease duration (months)	19 ± 9 [3-36]
UPDRS III score	14 ± 5 [7-27]
H&Y score	1.6 ± 0.4 [1-2.5]

Note: values are mean ± standard deviation [range]

2.2 Image analysis

Each MRI scan was coregistered with the corresponding SPECT scan reconstructed with the OSEM algorithm by using a 3D method based on rigid transformation with correlation ratio as cost function implemented in Medical Image Processing, Analysis and Visualization (MIPAV version 3.1.3, National Institutes of Health, NIH, Bethesda, MD). Coregistered MRI/SPECT images consisted in axial sections of 128 slices (128x128 matrix, 2.33 mm/pixel, 2.33 mm slice thickness).

ROIs were manually segmented on the coregistered MRI images on right and left caudate nucleus (NC) and right and left putamen, and on cerebellum. These ROIs were rigidly repositioned on [123I]FP-CIT SPECT and used to quantify striatal BP by means of Least Squares (LS) method (Formiconi et al., 1993; Vanzi et al., 2007).

Striatal BP was estimated as the ratio of a region of specific binding (striatum) to a region of non-specific binding (cerebellum). We estimated BP for right and left caudate nucleus and right and left putamen separately.

Among all striatal regions, putaminal BP was identified as the most accurate measure to determine the severity of dopaminergic degeneration (Spiegel et al., 2007; Isaias et al., 2007) and therefore used to test the correlation with the clinical scales of disease severity and with rCMRglc.

2.3 SPM

The Multiple Regression Design was employed to explore regions in which rCMRglc correlates negatively with UPDRS III and positively with putaminal BP. The SPM{t} maps were obtained at a height threshold of p < 0.005, cluster extent ≥ 50 voxels. Correction for multiple comparisons was conducted using SPM's Small-Volume-Correction (SVC) facility (Worsley et al., 1996) across the frontal lobe, based on the a priori knowledge of the physiology of fronto-striatal circuits (Alexander et al., 1986, 1990) and of the regional pattern of metabolic changes in PD, which involves prefrontal regions (Fukuda et al., 2001). The significance threshold was set to a corrected value of p<0.05 (False Discovery Rate, FDR, corrected).

3. Results

Caudate and putaminal BP values are shown in Table 5.2.

Table 5.2. Striatal BP values

Striatal region	BP
Right caudate nucleus	6.98 ± 1.88
Left caudate nucleus	6.86 ± 1.90
Right putamen	4.59 ± 1.33
Left putamen	4.54 ± 1.33
Ipsi caudate nucleus	7.20 ± 1.82
Contr caudate nucleus	6.64 ± 1.91
Ipsi putamen	5.05 ± 1.36
Contr putamen	4.09 ± 1.36

Note: values are mean ± standard deviation [range]

SPM analysis demonstrated a negative correlation between UPDRS III scores and rCMRglc in bilateral premotor cortex (PMC, Brodmann Area-BA 6) (Table 5.3). The clusters did not survive after SVC correction for multiple comparisons.

Right putaminal BP positively correlated with rCMRglc in bilateral DLPFC (BA 9), bilateral PMC (BA 6) and bilateral anterior prefrontal cortex (APFC, BA 10) (Table 5.3) (Figure 5.1). After SVC correction for multiple comparison, right putaminal BP positively correlated with rCMRglc in right DLPFC (BA 9), right PMC (BA 6) and right APFC (BA 10).

Left putaminal BP positively correlated with rCMRglc in bilateral DLPFC (BA 9), bilateral PMC (BA 6), bilateral APFC (BA 10) and bilateral orbitofrontal cortex (OFC, BA 11) (Table 5.3) (Figure 5.1). After SVC correction for multiple compari-

son, left putaminal BPLS positively correlated with rCMRglc in left DLPFC (BA 9), left PMC (BA 6), left APFC (BA 10) and left OFC (BA 11).

Table 5.3. List of local maxima of the most significant correlations with rCMRglc.

	Cluster extent	Anatomic area	Functional area	Talairach Coordinates			t
				x	y	z	
UPDRS III negative							
	94	Left Frontal lobe	GFm BA 6	-26	15	62	3.91
		Left Frontal lobe	GFs BA 6	-16	22	60	3.67
		Left Frontal lobe	GFs BA 6	-18	13	64	3.17
	113	Right Frontal lobe	GFs BA 6	22	-6	70	3.62
		Right Frontal lobe	GFs BA 6	16	3	68	3.51
Right putaminal BP$_{LS}$ positive							
	2552	Right Frontal lobe	GFs BA 9	16	48	25	4.93*
		Right Frontal lobe	GFd BA 10	2	51	9	4.47*
		Right Frontal lobe	GFs BA 10	12	66	2	4.30*
	2035	Right Frontal lobe	GFi BA 6	55	0	24	4.77*
		Right Frontal lobe	GFm BA 9	50	25	32	4.72*
	1251	Left Frontal lobe	GPrC BA 9	-44	21	36	4.63
		Left Frontal lobe	GPrC BA 6	-53	0	35	4.43
	178	Left Frontal lobe	GFm BA 10	-38	53	10	4.15
Left putaminal BP positive							
	1332	Left Frontal lobe	GPrC BA 9	-46	21	36	5.62*
		Left Frontal lobe	GFm BA 9	-24	32	26	4.69*
		Left Frontal lobe	GPrC BA 6	-48	0	33	4.62*
	454	Right Frontal lobe	GFs BA 11	8	59	-21	4.98
		Left Frontal lobe	GFs BA 11	-2	57	-21	3.77*
		Left Frontal lobe	GFs BA 11	-12	66	-10	3.63
	125	Left Frontal lobe	GFm BA 10	-38	51	9	3.77*
	362	Right Frontal lobe	GPrC BA 6	55	2	31	3.75
	175	Left Frontal lobe	GFs BA 9	-18	58	27	3.65
		Left Frontal lobe	GFs BA 10	-12	61	23	3.42
	675	Right Frontal lobe	GFd BA 10	4	51	5	3.47
		Right Frontal lobe	GFd BA 9	20	42	22	3.43
	101	Right Frontal lobe	GR BA 11	4	32	-20	3.31

Note: * Survived at SVC correction for multiple comparisons (p<0.05).

Abbreviations: GFm=middle frontal gyrus; GFs=superior frontal gyrus; GFi=inferior frontal gyrus; GPrC=precentral gyrus; GFd=medial frontal gyrus; GR=rectal gyrus.
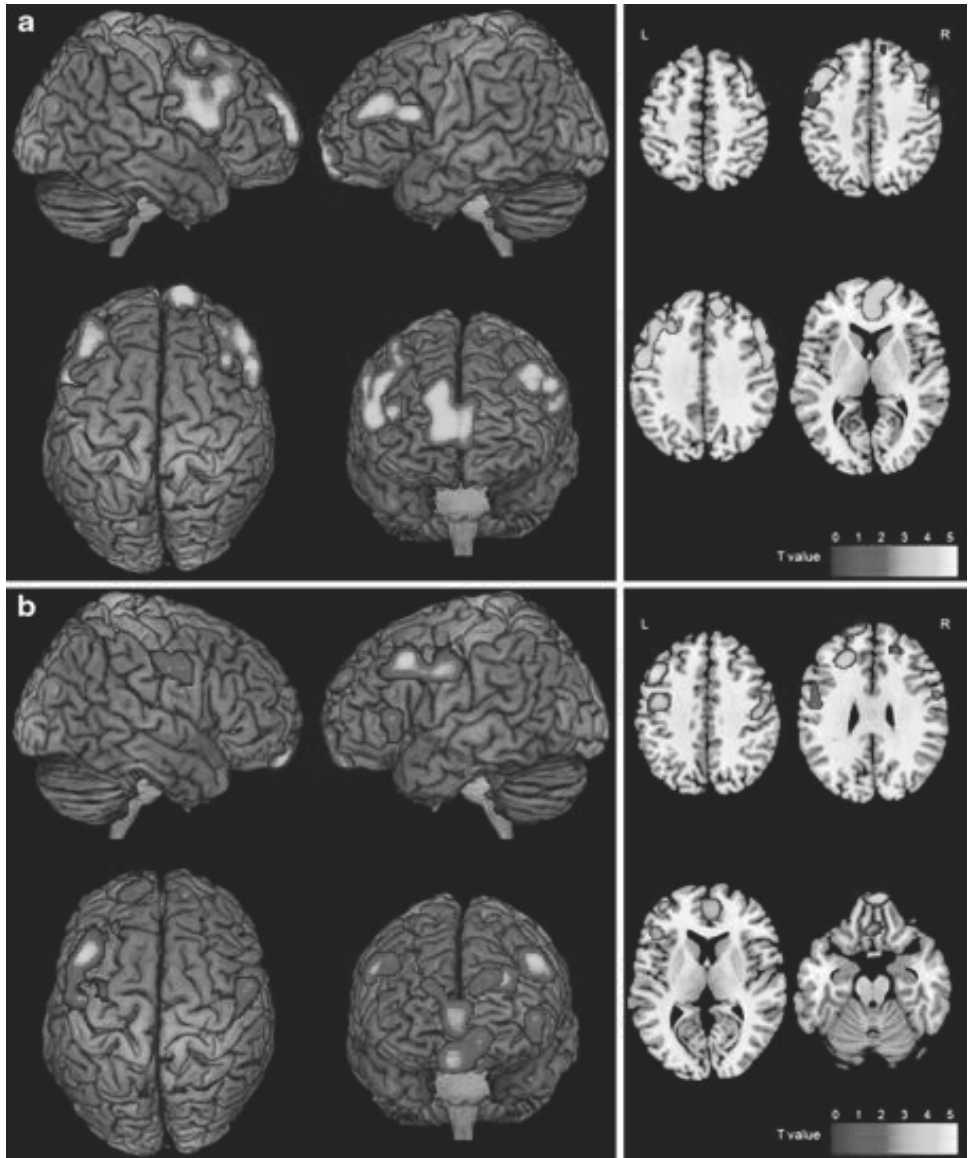
Figure 5.1 Regions with significantly positive correlation with right (a) and left (b) BP in the [18F]-FDG study

Chapter 6
Study 3 Functional changes related to dopamine depletion in the caudate nucleus in early Parkinson's disease

1. Background

PD is a neurodegenerative disorder, in which, besides characteristic motor symptoms, cognitive deficits ranging from mild cognitive impairment to genuine dementia often occur (Caviness et al., 2007; Riedel et al., 2008). Functional neuroimaging plays a key role in the understanding of mechanisms underlying both motor and non motor dysfunction in PD (Nandhagopal et al., 2008).

The cardinal neuropathological feature of PD is the degeneration of nigrostriatal dopaminergic (DA) neurons, resulting in a loss of DA terminals in the striatum (Fearnley and Lees, 1991). [123I]FP-CIT SPECT imaging is the diagnostic technique most widely used to clinically assess DA system impairment (Booij et al., 2001). Besides, in PD patients correlations have been found between DA depletion in the caudate nucleus (NC) and the degree of dementia (Rinne et al., 1989) and neuropsychological performance (Brück et al., 2001, 2005; Marie et al., 1999), although these findings have not been always confirmed (Broussolle et al., 1999; Rinne et al, 2000).

On the other side, [18F]FDG PET, has been used to identify regional changes in brain function that differentiate PD patients with and without cognitive dysfunction (Huang et al., 2008). Several studies identified a metabolic pattern associated with cognitive dysfunction in PD characterized by metabolic reductions in frontal and parietal association areas with relative increases in the cerebellar vermis and dentate nuclei (Huang et al., 2007).

DA transporter imaging (FP-CIT SPECT imaging) and brain metabolic imaging (FDG PET imaging) have been broadly employed to explore the biological substrate of PD, but the information of these two widespread molecular imaging techniques has been long considered apart. In study 2 we investigated the relation between the severity of DA degeneration in the putamen and metabolic dysfunction in early drug naïve PD patients (Berti et al., 2009), thus focusing on the functional changes occurring in motor basal ganglia cortical circuit. Besides the metabolic changes in expected motor cortical regions, we found changes in associative and limbic regions, such as dorsolateral prefrontal, anterior prefrontal and orbitofrontal areas, hence suggesting an early involvement of non motor frontostriatal circuit in PD.

Given the central role of NC in cognitive dysfunction in PD (Grahn et al., 2008), the study of the direct relation between DA depletion severity in NC, brain metabo-

lism and cognitive functions in early drug naïve PD patients could reveal the early functional changes directly related to NC DA depletion in associative frontostriatal circuit, in the absence of the modulation effect of anitiparkinsonian treatment both on brain metabolism (Asanuma et al, 2006) and cognitive functions (Cools, 2006).

Therefore in the present study, we investigate the direct relation between DA degeneration severity in NC, assessed by [123I]FP-CIT SPECT NC BP, brain metabolic dysfunction, assessed by [18F]FDG PET rCMRglc, and cognitive dysfunction in de novo PD patients.

2. Methods

2.1 Subjects

Twenty five drug naïve non demented PD patients of the original PD sample were enrolled in this study.

Demographic data, disease duration, motor symptoms onset side, UPDRS III scores, H&Y scores, neuropsychological data are shown in Tables 6.1, 6.2.

Table 6.1. Patients characteristics

No of subjects	25
Sex	17 males, 8 females
Age (years)	65.60 ± 6.34 [53-77]
Education	7.44 ± 3.30 [5-18]
Side of motor symptoms onset	12 right, 13 left
Disease duration (months)	19.52 ± 9.44 [3-36]

Note: values are mean ± standard deviation [range]

Table 6.2 Clinical and neuropsychological data

UPDRS III	14.44 ± 5.48 [7-27]
H&Y	1.6 ± 0.4 [1-2.5]
MMSE	28.69 ± 1.63 [24.27-30]
MCST PE	5.09 ± 7.63 [0-35]
MCST NPE	3.52 ± 4.79 [0-16]
MCST TE	9.04 ± 10.34 [0-36]
MCST Categories	5.08 ± 1.60 [2-6]
Letter fluency	31.32 ± 9.14 [16-48.5]
SCWT A	19.68 ± 7.11 [11-44]
SCWT B	27.96 ± 10.07 [11-47]
SCWT C	42.56 ± 16.00 [19-80]
CBTT	5.42 ± 1.01 [4-7.25]
TSWRT	4.35 ± 0.72 [3-5.63]
SPCT Primacy effect	3.67 ± 2.10 [0-7.50]
SPCT Recency effect	13.64 ± 4.65 [3-20]
FFAT	9.06 ± 3.31 [0.75-13.75]

Note: Values are mean ± standard deviation [range]

UPDRS III, Unified Parkinson's Disease Rating Scale part III; H&Y, Hoehn and Yahr; MMSE, Mini Mental State Examination; MCST PE, NPE, TE, Modified Card Sorting Test perseverative errors, non perseverative errors, total errors; SCWT A, B, C, Stroop Color Word Test, part A, part B, part C; CBTT, Corsi Block Tapping Test; TSWRT, Two Syllabic Word Repetition Test; SPCT, Serial Position Curve Test; FFAT, Fragmented Figures Assembling Test.

2.2 Image analysis

Each MRI scan was coregistered with the corresponding SPECT scan reconstructed with the OSEM algorithm by using a 3D method based on rigid transformation with correlation ratio as cost function implemented in Medical Image Processing, Analysis and Visualization (MIPAV version 3.1.3, National Institutes of Health, NIH, Bethesda, MD). Coregistered MRI/SPECT images consisted in axial sections of 128 slices (128x128 matrix, 2.33 mm/pixel, 2.33 mm slice thickness).

ROIs were manually segmented on the coregistered MRI images on right and left NC and right and left putamen, and on cerebellum. These ROIs were rigidly repositioned on [123I]FP-CIT SPECT and used to quantify striatal BP by means of Least Squares (LS) method. The LS method, a reconstruction algorithm developed in our Institute (Formiconi, 1993), validated in phantom (Vanzi et al., 2007) and clinical studies (Berti et al., 2008), was used to estimate striatal BP since it allows a quantification of striatal [123I]FP-CIT BP scarcely affected by partial volume effects (Vanzi et al., 2007), and it was demonstrated to be a sensitive parameter to explore correlations with rCMRglc relative to traditional reconstruction algorithms (Berti et al., 2009).

Striatal BP was estimated as the ratio of a region of specific binding (striatum) to a region of non-specific binding (cerebellum). We estimated BP for right and left NC and right and left putamen separately.

Among striatal regions, right and left NC BP separately were used to test the correlation with neuropsychological test scores and with rCMRglc, according to the classical model of basal ganglia functional organization which maintains NC as the striatal relais of non motor associative loop (Alexander et al, 1986).

2.3 Statistical analysis

For normality testing, Kolmogorov-Smirnov test and Shapiro-Wilk test were used, as appropriate. Independent t test was used to compare NC and putamen BP. Spearman's rho correlation coefficient was used to determine the relationship between NC BP values and neuropsychological tests scores. Values of $p < 0.05$ were considered statistically significant.

2.4 SPM

The Multiple Regression Design was employed to explore regions in which rCMRglc correlates with neuropsychological test scores and with right and left NC BP separately. Among neuropsychological test, the following tests were selected for the SPM multiple regression analysis: MCST perseverative errors and letter fluency for EF, SCWT C for attention and inhibition process, SPCT primacy effect for LTM, SPCT recency effect for STM, FFAT for visuoconstructional abilities. The SPM{t} maps were obtained at a height threshold of $p < 0.005$, cluster extent ≥ 50 voxels. Correction for multiple comparisons was conducted using SPM's Small-Volume-Correction (SVC) facility (Worsley et al., 1996) across dorsolateral prefrontal cortex

(DLPFC), anterior prefrontal cortex (APFC), orbitofrontal cortex (OFC), based on the a priori knowledge of the physiology of fronto-striatal non motor circuits (Alexander et al, 1986; Alexander and Crutcher, 1990) and across prefrontal, posterior parietal and cerebellar regions based on the regional pattern of metabolic changes associated with cognitive dysfunction in PD (Huang et al., 2007). The significance threshold was set to a corrected value of $p < 0.05$ (False Discovery Rate, FDR, corrected).

3. Results

3.1 [123I]FP-CIT SPECT and neuropsychological findings

NC and putaminal BP values are shown in Table 6.3.

Table 6.3 [123I]FP-CIT SPECT Binding Potential (BP)

Right NC BP	6.86 ± 1.82 [3.58-9.94]
Left NC BP	6.73 ± 1.81 [3.24-9.87]
Ipsilateral NC BP	7.20 ± 1.76 [4.03-9.94]
Contralateral NC BP	6.39 ± 1.78 [3.24-9.87]
Right putaminal BP	4.48 ± 1.48 [0.26-6.87]
Left putaminal BP	4.45 ± 1.31 [1.33-6.89]
Ipsilateral putaminal BP	4.96 ± 1.30 [1.33-6.89]
Contralateral putaminal BP	3.98 ± 1.29 [0.26-6.23]

Note: Values are mean ± standard deviation [range]

NC: caudate nucleus.

There is no significant difference between BP values of right and left NC and of right and left putamen. Considering the first and most affected side of motor symptoms, the BP values in the contralateral putamen to the most affected body side (3.98 ± 1.29) were significantly lower than BP values in the ipsilateral putamen (4.96 ± 1.30) ($p < 0.01$). Contralateral NC BP values had a lower mean as compared to ipsilateral NC BP values (6.39 ± 1.78 vs 7.20 ± 1.76), however this difference did not reach statistical significance ($p = 0.11$). Putaminal BP values were significantly lower than NC BP values, both in the ipsilateral and contralateral side (4.96 ± 1.30 vs 7.20 ± 1.76, $p < 0.0001$, and 3.99 ± 1.29 vs 6.39 ± 1.78 $p < 0.0001$ respectively). There was a significant correlation between right NC and right putamen ($r = 0.769$, $p < 0.0001$), left NC and left putamen ($r = 0.699$, $p < 0.0001$), ipsilateral NC and ipsilateral putamen ($r = 0.693$, $p < 0.0001$), contralateral NC and contralateral putamen ($r = 0.791$, $p < 0.0001$).

None of the patients complained of subjective cognitive deficits, even so compared to available normative data impairment in both EF and memory domains were observed (14 patients), as well as selective deficits in EF (one patients) and memory

(7 patients). Only three patients showed no cognitive impairment, nevertheless no significant correlation was found between right and left NC BP and neuropsychological test scores.

3.2 Correlation with rCMRglc

As concerns the correlation with neuropsychological measures SPM analysis revealed that rCMRglc correlated: (1) negatively with MCST perseverative errors in right premotor cortex (PMC, BA 6); (2) positively with letter fluency in bilateral OFC (BA 10), DLPFC (BA 9, BA 46), right primary motor area (PMA, BA 4) and PMC (BA 6), (3) negatively with SCWT part C in left PMC (BA 6), right PMA (BA 4) and right primary auditory area (BA 41); (4) positively with the SPCT primacy effect in left extrastriate visual association area (BA 19), and in right cerebellar posterior lobe; (5) positively with SPCT recency effect in right postcentral gyrus (BA 43), PMC (BA 6) and in left insula (BA 13); (6) positively with FFAT in left thalamus and left OFC (BA 47). None of these clusters survived FDR SVC correction for multiple comparisons (Table 6.4).

Table 6.4 List of local maxima of the most significant correlations between neuropsychological test scores and rCMRglc.

	Cluster extent	Anatomic and functional area	Talairach Coordinates (mm)			
			x	y	z	t
1. MCST EP Negative correlation	128	R Superior Frontal Gyrus BA 6	18	15	60	3.69
2. Letter fluency Positive correlation	226	R precentral Gyrus BA 4	53	-6	41	3.90
		R precentral Gyrus BA 4	48	-5	52	2.97
	86	R Middle Frontal Gyrus BA46	44	44	25	3.32
	96	R Middle Frontal Gyrus BA 10	40	58	-10	3.21
	121	L Superior Frontal Gyrus BA 10	-20	66	23	4.00
	143	L Middle Frontal Gyrus BA 9	-36	48	27	3.56
		L Superior Frontal Gyrus BA 9	-12	52	34	3.20
		L Superior Frontal Gyrus BA 10	-26	52	25	3.04
2. SCWT C Negative correlation	55	R Precentral Gyrus BA 4	55	-12	36	3.22
	90	R Superior temporal gyrus BA 41	45	-34	13	3.36
	54	L Middle Frontal Gyrus BA 6	-38	14	59	3.57
3. SPCT Primacy effect Positive correlation	154	R cerebellar posterior lobe Tuber	48	-67	-29	3.47
	124	L Superior occipital gyrus BA 19	48	-67	-29	3.47
4. SPCT Recency effect Positive correlation	674	R Postcentral gyrus BA 43	53	-16	16	4.88
		R Precentral Gyrus BA 6	54	1	11	4.48
	57	L Insula BA 13	-43	-24	16	3.19
5. FFAT Positive correlation	243	L Thalamus	-4	-2	2	4.03
	237	L Orbital Gyrus BA 47	-12	20	-26	3.94

p value < 0.005 uncorrected

Note: MCST PE, Modified Card Sorting Test perseverative errors; SCWT C, Stroop Color Word Test part C; SPCT, Serial Position Curve Test; FFAT, Fragmented Figures Assembling Test; R, Right; L, Left.

As concerns NC BP correlation, SPM analysis revealed a positive correlation between right NC BP and rCMRglc in bilateral anterior cingulate cortex (ACC, BA 32), OFC (BA 10, 11, 47), DLPFC (BA 9), superior and middle temporal gyrus (BA 38, 21), while left NC BP correlated with rCMRglc in bilateral ACC (BA 32), OFC (BA 10, 11), DLPFC (BA 9) and right middle temporal gyrus (BA 38). All these clusters survived the FDR SVC correction for multiple comparisons (Table 6.5, Figures 6.1 and 6.2). Middle temporal regions were not included in our a priori hypothe-

sis based on frontostriatal circuits so a post hoc SVC correction was performed based on network connecting NC and ventral striatum to middle temporal regions, demonstrated by recent MRI tractography studies (Leh et al., 2007).

Table 6.5 List of local maxima of the most significant correlations between NC BP and rCMRglc.

	Cluster extent	Anatomic and functional area	Talairach Coordinates (mm)			
			x	y	z	t
1. Right NC BP						
Positive correlation	3160	L Anterior Cingulate BA 32	0	41	5	6.43
		R Superior Frontal Gyrus BA 11	14	61	-18	4.76
		R Rectal Gyrus BA 11	4	34	-20	4.66
	359	R Superior Frontal Gyrus BA 9	46	40	31	4.08
	108	R Inferior Frontal Gyrus BA 47	42	21	-4	4.07
	201	L Middle Frontal Gyrus BA 9	-44	35	31	3.89
	50	L Middle Frontal Gyrus BA 10	-44	35	31	3.34
	335	R Superior Temporal Gyrus BA 38	40	10	-34	5.19
	219	L Middle Temporal Gyrus BA 21	-42	8	-34	3.37
2. Left NC BP						
Positive correlation	114	L Cingulate Gyrus BA 32	-12	23	34	4.46
	518	R Superior Frontal Gyrus BA 11	12	59	-21	4.85
		R Rectal Gyrus BA 11	2	32	-20	4.22
	942	R Medial Frontal Gyrus BA 10	10	43	11	4.76
	341	R Superior Frontal Gyrus BA 9	46	40	31	3.80
	537	L Superior Frontal Gyrus BA 9	-40	36	29	3.87
		L Middle Frontal Gyrus BA 10	-36	51	10	3.43
	190	R Middle Temporal Gyrus BA 38	42	8	-36	4.71

p value < 0.05 FDR SVC

Note: NC, caudate nucleus; BP, Binding Potential; R, Right; L, Left.

Figure 6.1 Direct correlation between rCMRglc and right NC BP. (A) Significant clusters projected on the standardized MNI MRI render surface, (B) Significant cluster projected on standardized MNI MRI axial slices, (C-F) Regression analysis of rCMRglc response as dependent variable and right caudate BP as independent variable at most significant clusters.NC; caudate nucleus.
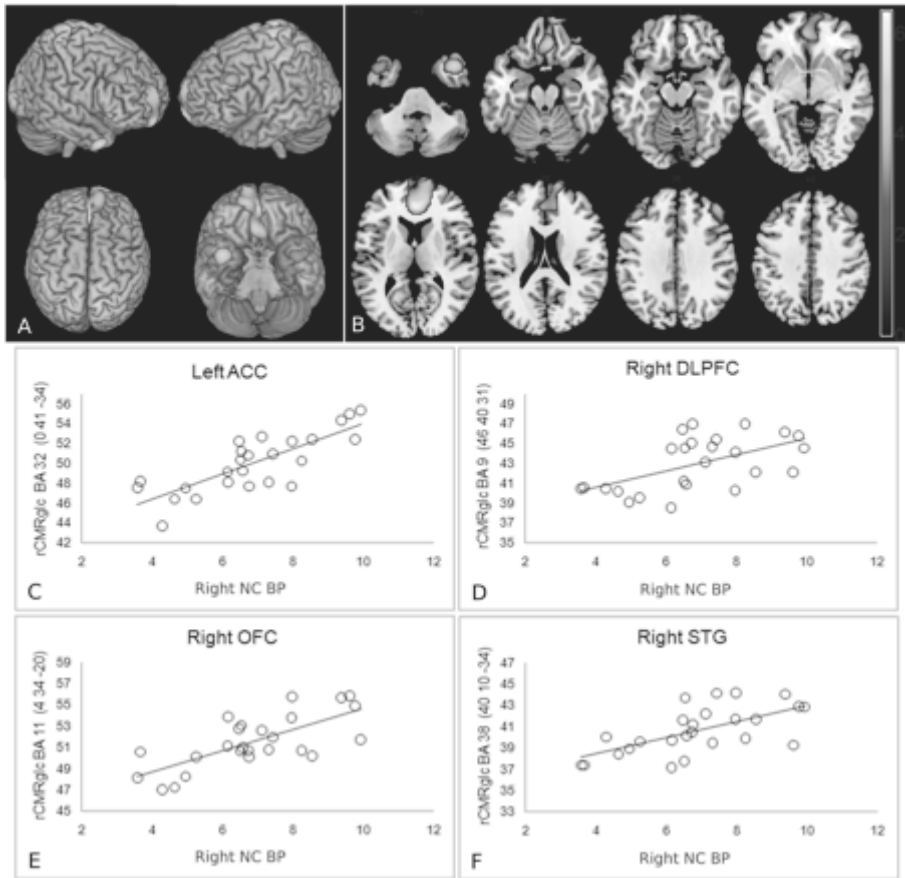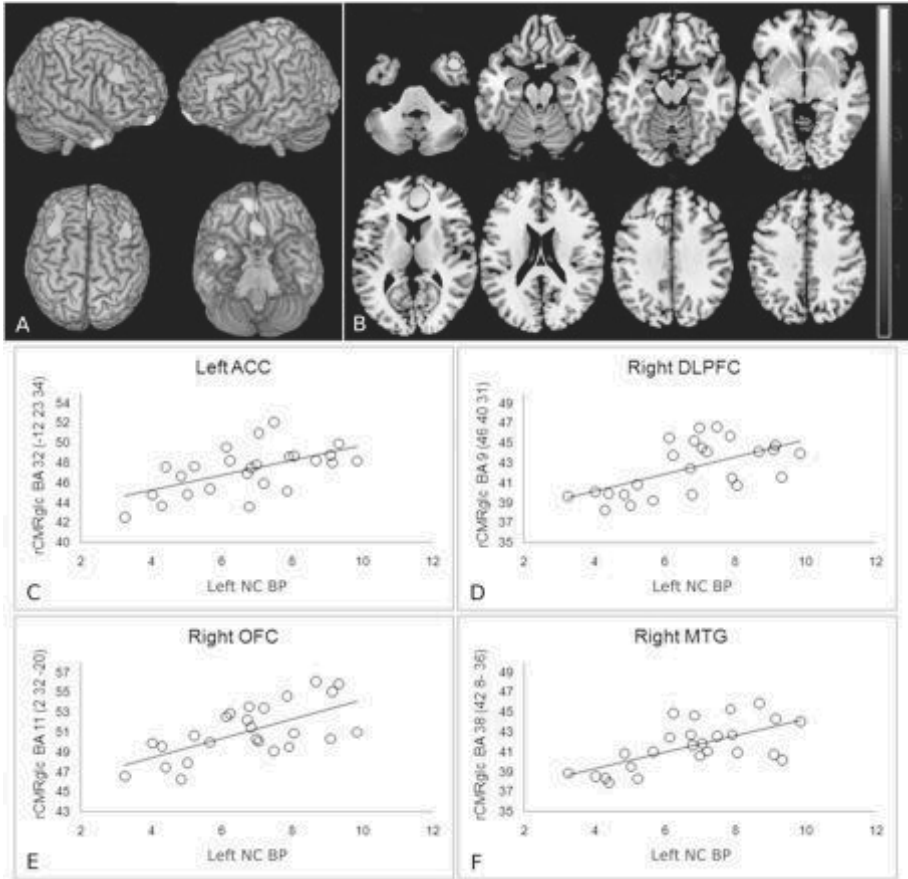
Figure 6.2 Direct correlation between rCMRglc and left NC BP. (A) Significant clusters projected on the standardized MNI MRI render surface, (B) Significant clusters projected on standardized MNI MRI axial slices, (C-F) Regression analysis of rCMRglc response as dependent variable and left caudate BP as independent variable at most significant clusters. NC, caudate nucleus.

Chapter 7
Discussion

The aim of this thesis was to explore the early functional brain changes characteristic of drug naïve PD patients by means of molecular imaging techniques, and specifically [123I]FP-CIT SPECT and [18F]FDG PET.

1. Brain metabolism in drug naïve PD patients

In study 1 we investigated brain metabolism in drug-naïve PD patients, in order to explore CMRglc alterations uniquely related to disease process and not modulated by antiparkinsonian treatment.

Drug-naïve PD patients showed several CMRglc alterations as compared to NL, characterized by hypometabolism in frontal (PM, DLPFC) and temporo-parieto-occipital regions (ITG, SPL, IPL, IOG, MiOG), and hypermetabolism in motor (PMA), lentiform and cerebellar regions. Interestingly, these metabolic changes were confirmed independently of symptoms onset side and of prevalent motor symptom. The progressive effect of disease severity on CMRglc in frontal, temporo-parieto-occipital, motor, lentiform and cerebellar regions was demonstrated by post hoc comparisons between NL and PD patients at increasing H&Y stages.

The proposal to explore brain metabolism in drug-naïve PD patients rose from the recent growing interest for imaging biomarkers, but also from the fact that [18F]FDG-PET studies supporting brain metabolism as a PD biomarker mainly examined already treated patients in the off-medication condition. The drawbacks of the washout designs were previously addressed for DAT-imaging studies, about which contrasting evidences concerning drug-induced striatal DAT regulation emerged (Guttman et al., 2001; Boij et al., 2008). Moreover, after antiparkinsonian therapies withdrawal the effect of medication on clinical symptoms persists for over two days, and therefore it could not be ruled out a possible persistent effect also on brain metabolic activity (Fahn et al., 2004).

Our findings are consistent with previous [18F]FDG-PET studies, enrolling both drug-naïve and off-medication PD patients, which demonstrated in PD patients CMRglc reductions in frontal and temporo-parieto-occipital areas, and CMRglc increases in subcortical regions and cerebellum (Asanuma et al., 2006; Ma et al., 2007; Juh et al., 2005; Hosokai et al., 2009).

So far, this [18F]FDG-PET study confirms in drug-naïve patients brain metabolic alterations in PD-related regions, therefore fulfilling a preliminary verification to the use of brain metabolism as a PD biomarker to monitor disease progression and treatment modulation effect. The brain metabolic changes found in drug-naïve PD patients motivate further research on the effect of therapeutic intervention on brain metabolism, in order to assess whether and in which PD-related regions pharmacological treatment could modify brain activity.

2. Early functional changes related to dopamine depletion in drug naïve PD patients

In studies 2 and 3 we explored the direct relation between the severity of striatal DA degeneration, brain metabolism, motor and cognitive impairment in early drug-naïve PD patients in order to look for the early functional changes related to striatal DA depletion in PD.

2.1 [123I]FP-CIT SPECT and clinical findings

[123I]FP-CIT SPECT revealed, consistent with previous SPECT findings, lower NC DA impairment compared to the putamen, and a greater impairment in the putamen contralateral to the first and most affected body side compared to the ipsilateral one (Booij et al., 1997). DA depletion in contralateral NC was greater than in ipsilateral NC, although not reaching statistical significance. Contralateral NC BP were however strongly correlated with contralateral putaminal BP; this finding suggests that at early disease stages NC has already got involved in the disease process, as demonstrated by previous SPECT findings (Winogrodzka et al., 2003) and by PD animal models (Pérez-Otaño et al., 1994), in which a parallel loss of DA terminals in putamen and NC was observed, although firstly and to a greater extent in the putamen.

As concerns motor impairment, we recently demonstrated that [123I]FP-CIT SPECT BP measured with the LS method allows to find a correlation between putaminal DA impairment and clinical ratings of PD severity (Berti et al., 2008). On the other hand the LS method does not succeed in finding the correlation between DA impairment in the CN and early cognitive deficits in drug-naïve PD patients.

In line with previous reports (Muslimović et al., 2005; Williams-Gray et al., 2007), the present PD sample is characterized by cognitive deficits mainly in attention, executive and memory domains, nevertheless no significant correlation was found between NC BP and neuropsychological test scores. Our results are in disagreement with previous studies which found correlations between cognitive measures and striatal BP (Duchesne et al., 2002; Müller et al., 2000); however, they differ from the present one for several reasons. First of all some of them used a combined NC/putaminal BP (Duchesne, 2002). Further PD samples enrolled in these studies included patients already under antiparkinsonian treatment, at different disease stages, and with a wider range of cognitive deficits. The aim of study 3 was to explore the early functional and cognitive changes related to the severity of DA depletion in NC, thus we selected only patients at early disease stages, who had never been phar-

macologically treated before. The contrasting evidences about the effect of DA replacement treatment on cognitive functions in PD given (Cools, 2006), this latter point became fundamental. The drawback of this accurate patients selection was that we reduced variability between patients, hence not ensuring enough variability for a correlation analysis between a biological parameter and cognitive variables. Therefore the lack of a correlation between NC BP and cognitive dysfunction in our study may be due to the narrow range of neuropsychological test scores or, not less important, to the small PD sample size, this latter point confirming the need for larger patients sample to obtain significant results relative to cognitive measure (Alexander et al., 2002).

2.2 Brain metabolic changes related to DA degeneration in early PD

In study 2 we found putaminal DA degeneration to be related to functional activity in premotor, dorsolateral prefrontal, anterior prefrontal, and orbitofrontal regions. The correlation between disease severity, as measured by putaminal BP, and rCMRglc in prefrontal cortical areas confirms the classical model of the basal ganglia circuit organization, derived from anatomical and electrophysiological studies in non-human primates (Albin et al., 1989, Obeso et al., 2000).

The concept of frontostriatal loops has been widely used to explain the functional organization of frontostriatal circuits, by emphasizing the functional interrelationships between the neocortex and the striatum. According to this model, five parallel topographically organized functional circuits has been described: two motor circuits (i.e., the motor circuit & oculomotor circuit) and three complex (i.e., nonmotor) circuits, originating from the dorsolateral prefrontal cortex (DLPFC), the anterior cingulate cortex (ACC), and the orbitofrontal cortex OFC (Alexander et al., 1986).

In study 2 we found a positive correlation between putaminal BP and rCMRglc not only in frontal regions belonging to the motor loop, but also in dorsolateral, anterior prefrontal and orbitofrontal regions, which are part of the associative and limbic loop (Alexander, 1986). Indeed, the loss of dopaminergic projections in PD is not confined to the putamen but at the same time occurs also in the caudate nucleus and in ventral striatum, which are the striatal relais of associative and limbic frontostriatal loops, therefore connected with DLPFC, APFC and OFC.

This finding led us to further investigate the direct relation between DA degeneration in the NC and brain metabolism. As expected we found caudate DA depletion to be related to functional changes in brain regions belonging to associative and limbic frontostriatal circuits, i.e. DLPFC, OFC and ACC. Therefore, besides motor circuits, we further confirm the partially anatomical and functional segregation of non motor frontostriatal circuits too. Recent MRI tractography studies, by exploring NC connections in vivo, in humans, showed that the head of NC was connected to medial, ventral and dorsolateral prefrontal cortex, prefrontal pole, and supplementary motor area (Lehericy et al., 2004).

The essential pathophysiological characteristic of the PD state is increased neuronal firing activity in the output nuclei of the basal ganglia leading to excessive inhibition of thalamocortical motor system. The correlation between the severity of DA degeneration in the putamen and brain metabolic alteration in several prefrontal areas

therefore confirm pathopshysiological changes occurring in the functional organization of the basal ganglia in PD. Indeed, the excessive inhibition of thalamocortical projections may be at the basis of reduced prefrontal rCMRglc correlating with reduced BP. Whether thalamocortical inhibition extends to different cortico-striatal pallidal loops is not yet known, since most studies in PD and animal models have focused on the motor features associated with DA depletion and replacement, and have not addressed the molecular and physiological changes that occur in the different basal ganglia subcircuits (Obeso et al., 2008). The finding of a correlation between NC DA depletion and dorsolateral, orbitofronatal, anterior cingulate activity however, suggest that reduced prefrontal rCMRglc correlating with reduced NC BP may be due to the excessive inhibition of thalamocortical projections in associative and limbic regions.

Besides DLPFC, OFC and ACC regions which are directly involved in classical frontostriatal circuits, we found a positive correlation between NC DA depletion and rCMRglc reduction in superior and middle temporal regions, and specifically in the temporal pole (TP). In support of a direct connection between temporal lobe and NC, the temporal lobe has been demonstrated to be a target of basal ganglia outputs in functional connectivity studies (Postuma and Dagher, 2006), in anatomical studies in animals (Middleton and Strick, 1996), and in recent MRI tractography study (Leh et al., 2007). In turn, the TP, thanks to its anatomical position, is highly interconnected with OFC and with the amygdala (Olson, 2007), and anatomical and functional connections between ventral striatum and the basolateral amygdala have been reported (Cardinal et al., 2002; Postuma and Dagher., 2006).

The correlation between 123I-FP-CIT putaminal binding potential and rCMRglc, demonstrates a direct association between a biomarker of dopaminergic degeneration severity and cortical metabolic reduction in several frontal and prefrontal areas, such as PMC, DLPFC, APFC and OFC. Our findings, hence, turn the attention on [18F]-FDG uptake in prefrontal regions as a potential biomarker in PD. A biomarker is a characteristic that is objectively measured and evaluated as an indicator of normal or pathogenic biological processes (Biomarkers Definition Working Group, 2001). According with that, prefrontal [18F]-FDG uptake might fulfil the basic requirements for a biomarker, since it can be objectively measured and it is associated with the PD pathogenic process, i.e. the dopaminergic degeneration as demonstrated in study 2.

On the other hand the correlation between NC DA impairment and reduced rCMRglc in associative and limbic regions (study 3) gives further evidences of an early involvement of non motor circuit in PD. More interestingly, the metabolic reduction in temporolimbic regions was uniquely related to DA depletion in NC. The early involvement of temporolimbic regions in PD disease progression has been demonstrated by neuropathological studies (Braak et al., 2003), which proposed the temporolimbic pathology to parallel occur to the degeneration of nigrostriatal DA neurons. Our finding of a selective correlation between NC DA degeneration and temporolimbic hypometabolism might suggest additional early functional changes in temporolimbic regions more directly mediated by NC DA depletion.

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