Clinical and Imaging Characteristics of Early Parkinson's Disease

Konrad Szewczyk-Krolikowski

Nuffield Department of Clinical Neurosciences

University of Oxford

Keble College

A thesis submitted
for the degree of Doctor of Philosophy
Hilary 2014

Clinical and Imaging Characteristics of Early Parkinson's Disease

Konrad Szewczyk-Krolikowski

Nuffield Department of Clinical Neurosciences

University of Oxford

Keble College

A thesis submitted for the degree of Doctor of Philosophy in Hilary 2014

Abstract

Background. Pathological processes in Parkinson's disease (PD) start long before the first symptoms appear and by the time the disease is clinically established the results of neurodegeneration may be irreversible. Efforts to prevent or stem disease progression need to start in early disease and good characterization and new markers of early PD are urgently needed.
Objectives. This thesis aims to characterize early disease stages in three projects. Firstly, clinical features of PD within 3 years of diagnosis will be explored in an incident cohort of patients and controls, using a range of tools to cover the whole breadth of clinical presentation of PD. Secondly, functional imaging studies in PD published so far will be examined through a meta-analysis to identify the most robust functional imaging markers. Thirdly, a functional MRI resting-state study in early PD will be performed to identify reproducible differences between patients and matched control subjects.
Results. The cohort analysis found that age was a strong predictor of disease severity, independent of disease duration, while gender was seen to affect disease severity depending on the body region. A meta-analysis of all published functional imaging studies across all disease stages showed abnormal activations in the Basal Ganglia but also in a wide range of motor and non-motor brain areas. Dopamine supplementation normalized activations in the Basal Ganglia and some other areas, while other circuits remained resistant to medication suggesting non-dopaminergic abnormality. In the resting-state study, the Basal Ganglia Network showed greatly reduced connectivity in early PD compared to controls, which normalized on administration of dopaminergic medication. Reduced BGN connectivity was also validated on a separate group of PD subjects achieving very good separation of patients from controls.
Conclusions. The effect of gender and age on early presentation of PD has potential significance for early diagnosis and choice of outcome measures for clinical trials. Within the realm of imaging, traditional task-based fMRI studies fail to show a clear and reproducible pattern of activations making this method unfeasible for early diagnostic testing. In contrast, resting-state fMRI connectivity in the Basal Ganglia Network appears to be a promising and reliable method even in the early stages of PD. Clinical profiling and resting imaging changes offer avenues for developing future biomarkers in early PD.

Table of Contents

Chapter I. Thesis Introduction 19

Section 1. PD pathology 19

Section 2. PD pathophysiology 23

Section 3. Epidemiology 28

Section 4. Risk factors 30

Section 5. Clinical presentation 33

Section 6. Diagnosis 34

Section 7. Treatment 37

Section 8. Biomarkers 38

Section 9. Thesis Goals 48

Chapter II. Basic clinical characteristics of early Parkinson's Disease in the Oxford Parkinson's Disease Centre cohort 49

Section 1. Introduction 49

Section 2. Methods 57

Section 3. Results 70

Section 4. Discussion 86

Chapter III. Meta-analysis of functional imaging studies in Parkinson's Disease 99

Section 1. Introduction 99

Section 2. Methods 102

Section 3. Results 119

Section 4. Discussion 126

Chapter IV. Resting-state signature of early Parkinson's Disease..... 135

Section 1. Introduction 135

Section 2. Methods 157

Section 3. Results 173

Section 4. Discussion 182

Chapter V. Thesis Conclusions 187

References 191

Appendix A. Supplemenatry data for the study of the Basic clinical characteristics of early Parkinson's Disease in the Oxford Parkinson's Disease Centre cohort 231

Appendix B. Supplementary data for the Meta-analysis of functional imaging studies in Parkinson's Disease 237

Appendix C. Supplementary data for the study of the Resting-state signature of early Parkinson's Disease 247

Appendix D. List of publications related to research performed during the thesis project..... 251

List of Figures

Figure 1. Braak staging of Parkinson's disease pathology……22

Figure 2. Basal ganglia model in health and in Parkinson's disease……25

Figure 3. Hemodynamic response curve and BOLD response curve……105

Figure 4. Identification of activated voxels……106

Figure 5. Flowchart illustrating the selection process of publications for meta-analysis……120

Figure 6. Results of PDOFF vs HC analysis, right-handed task……122

Figure 7. Effects of medication……125

Figure 8. Background and task-related BOLD activity……137

Figure 9. Major resting state brain networks……141

Figure 10. Independent Component Analysis……145

Figure 11. Single subject ICA……163

Figure 12. Schematic of group ICA analysis……166

Figure 13. Flowchart showing processing steps in the connectivity analysis….169

Figure 14. Multiple regression of parameter estimates for ICA components from subject-level timeseries……170

Figure 15. Basal Ganglia Network……174

Figure 16. Percent of variance explained by the Basal Ganglia Network……..175

Figure 17. Group comparison between Discovery group and HC group……177

Figure 18. Medication effect in the Discovery group……..178

Figure 19. Average parameter estimates values (PE) from the significantly different BGN clusters in the PD-OFF vs. HC comparison.....180

Figure 20. Average parameter estimates values (PE) from the significantly different BGN clusters in the validation cohort ……..181

List of Tables

Table 1. Basic demographics and Past Medical and Family History……..72

Table 2. Comparison of social background and pre-morbid medication history between PD and HC groups……..73

Table 3. Disease onset and motor symptoms stratified by age group and gender……76

Table 4. UPDRS (parts II and IV) and PD medication stratified by age group and gender……80

Table 5. Comparison of non-motor symptoms between PD and HC groups…….82

Table 6. Non-motor symptoms stratified by age group and gender in PD group.....84

Table 7. Large cohort studies in Parkinson's Disease. Studies in bold recruited early-stage patients with disease duration < 4 years……...87

Table 8. Demographic and clinical data of study participants……..160

Table 9. Local maxima for group comparison HC>PD-OFF. ……..179

Table 10. Local maxima for group comparison PD-ON>PF-OFF……..179

List of Abbreviations

AD	Alzheimer's Disease
ALFF	Amplitude of Low Frequency Fluctuations
ALS	Amyotrophic Lateral Sclerosis
APOE	APOlipoprotein E
ASL	Arterial Spin Labelling
ATP13A2	Adenyl TriPhosphatase type 13 A2
BB	Brain Bank
BDI	Beck's Depression Inventory
BFI	Big Five Inventory
BG	Basal Ganglia
BGN	Basal Ganglia Network
BMI	Body Mass Index
BOLD	Blood Oxygenation Level Dependent signal
CARPA	Comorbidity and Aging in Rehabilitation patients; the influence on Activities
CASL	Continuous Arterial Spin Labelling

CBMA	Coordinate-Based Meta-Analysis
CNS	Central Nervous System
COX-2	CycloOXygenase 2
CT	Computer Tomography
DAT-SPECT	Dopamine Transporter Single Photon Emission Computer Tomography
DATATOP	Deprenyl and tocopherol antioxidative therapy of parkinsonism
DBS	Deep Brain Stimulation
DMN	Default Mode Network
DNA	Deoxyribonucleic acid
DoPAMiP	Douleur et maladie de Parkinson en Midi-Pyrénées
DTI	Diffusion Tensor Imaging
ECN	Executive Control Network
EFNS	European Federation of Neurological Societies
EO	Evidence Optimization
EPI	Echo Planar Imaging
EQ-VAS	EuroQol Visual Analogue Scale
EQ5D	EuroQuol 5D scale

ESS	Epworth Sleepiness Scale
EuroPa	European network for Parkinson's disease
FDR	False Discovery Rate
FIX	Functional MRI of the Brain (FMRIB) centre's ICA-based Xnoiseifier
FLIRT	Functional MRI of the Brain (FMRIB) centre's Linear Registration Tool
fMRI	functional Magnetic Resonance Imaging
FNIRT	Functional MRI of the Brain (FMRIB) centre's Non-Linear Registration Tool
FOG	Freezing Of Gait
FSL	Functional MRI of the Brain (FMRIB) centre Software Library
FWHM	Full Width Half Maximum
GABA	Gamma Amino Butyric Acid
GEPAD	German Study on the Epidemiology of Parkinson's Disease with Dementia
GLM	General Linear Model
GP	General Practitioner
GPe	Globus Pallidus externa

GPi	Globus Pallidus interna
GPR	Gaussian Process Regression
H&Y	Hoehn and Yahr scale
HC	Healthy Controls
IBMA	Image-Based Meta-Analysis
ICA	Independent Component Analysis
ICD	Impulse Control Disorder
LB	Lewy Body
LEDD	Levodopa Equivalent Daily Dose
LRRK2	Leucine-Rich Repeat Kinase 2
MAO-B	MonoAmine Oxidase B
MCI	Mild Cognitive Impairment
MDS	Movement Disorders Society
MDS-UPDRS	Movement Disorders Society edition of the Unified Parkinson's Disease Rating Scale
MEG	MagnetoEncephaloGraphy
MELODIC	Multivariate Exploratory Linear Optimized Decomposition into Independent Components

MMSE	Mini-Mental Status Examination
MNI	Montreal Neurological Institute
MOCA	Montreal Cognitive Assessment
MP-RAGE	Magnetization Prepared RApid Gradient Echo
MPTP	1-Methyl-4-Phenyl-1,2,3,6-TetrahydroPyridine
MRI	Magnetic Resonance Imaging
MS	Multiple Sclerosis
NET-PD	Neuroprotection Exploratory Trials in Parkinson's Disease
NILS	Non-motor International Longitudinal Study
NINDS	National Institute of Neurological Disorders and Stroke
NSAID	Non-Steroidal Anti-Inflammatory Drug
OCD	Obsessive Compulsive Disorder
OPDC	Oxford Parkinson's Disease Centre
OTC	Over The Counter medication
ParkWest	Parkinson’s disease from Western and Southern Norway
PARS	Parkinson Associated Risk Study
PD	Parkinson's Disease
PD-FOG	Parkinson's Disease patient without Freezing Of Gait

PD-ON	Parkinson's Disease patients On medication
PD+FOG	Parkinson's Disease patient with Freezing Of Gait
PDOFF	Parkinson's Disease patients Off medication
PE	Parameter Estimate
PEG	Parkinson's Environment and Gene study
PET	Positron Emission Tomography
PIGD	Postural Instability and Gait Disorder
PINE	Parkinsonism Incidence in North-East Scotland
PINK1	Phosphatase and tensin homolog Induced Kinase 1
PPN	Pedunculo-Pontine Nucleus
PRIAMO	PaRkinson And non Motor symptOms
PRIPS	Prospective validation of RIsk factors for the development of Parkinson Syndromes
PROBAND	Parkinson's Repository of Biosamples and Networked Datasets
PROMS-PD	PRospective study of Mood States in Parkinson's Disease
ProPark	PROfiling PARKinson's disease
QUIP	Questionnaire for Impulsive-compulsive disorder
RBD	Rapid eye movement Behaviour sleep Disorder

RBD-SQ	Rapid eye movement disorder behaviour disease Screening Questionnaire
REM	Rapid Eye Movement sleep phase
ROC	Receiver Operating Characteristic
ROI	Region Of Interest
RS-fMRI	Resting State functional Magnetic Resonance Imaging
RSN	Resting State Network
SAA	Self Assessment of Anxiety scale
SAD	Self Assessment of Depression scale
SD	Standard Deviation
SE	Squared Exponential
SMA	Supplementary Motor Area
SN	Substantia Nigra
SNc	Substantia Nigra pars compacta
SNCA	Synuclein alpha
SNr	Substantia Nigra pars reticulata
SPECT	Single Photon Emission Computer Tomography
STN	SubThalamic Nucleus

SVM	Support Vector Machine
TE	Time of Echo
TFCE	Threshold-Free Cluster Enhancement
TH	Thalamus
TI	Time of Inversion
TR	Time of Relaxation
TREND	Tübinger Erhebung von Risikofaktoren zur Erkennung von NeuroDegeneration
UKPD-BB	United Kingdom Parkinson's Disease-Brain Bank
UPDRS	Unified Parkinson's Disease Rating Scale
VL	Ventralis Lateralis nucleus of the thalamus
VN	Visual Network

Chapter I. Thesis Introduction

I will first briefly outline the background information on PD pathogenesis and clinical aspects of the disease. Subsequently, I will identify the unmet needs in current clinical PD research and formulate the goals of this thesis.

Section 1. PD pathology

Dopamine and Substantia Nigra

The most important pathological finding that forms the basis of our understanding of PD is reduced dopamine concentration in the striatum caused by irreversible cell death of the dopaminergic neurons in the Substantia Nigra. Both those discoveries were reported by Hornykiewicz in his papers from 1963-64 (Hornykiewicz, 2010) and laid foundations for future treatment trials with levodopa. It is now known that degeneration affects predominantly the ventrolateral portion of the SN pars compacta, as opposed to normal aging where the neurons atrophy mainly in the dorsal part of SN (Fearnley and Lees, 1991). Further studies have established that typical motor symptoms occur only after 60% of SN neurons have died (Gaenslen *et al.*, 2011; Gaig and Tolosa, 2009). At that time the dopamine level in the BG is usually at 20% of that in a healthy brain (Gaig and Tolosa, 2009). For that to happen, current estimates are that the degenerative processes in dopaminergic neurons will have been progressing for 7 years (Berg *et al.*, 2013; Gaig and Tolosa, 2009).

Lewy bodies

The other pathological hallmark of Parkinson's has long been the Lewy body (LB), a neuronal intracytoplasmic inclusion made up of alpha-synuclein,

ubiquitin and around 70 other proteins (Wakabayashi *et al.*, 2007). Even though Lewy bodies form a part of pathological criteria for PD (Dickson *et al.*, 2009) their presence is not exclusive for PD and has recently been noted in other neurodegenerative conditions (Popescu *et al.*, 2004). Moreover, the exact role of LB in the pathogenetic process is a subject of debate. The traditional view of LB's as toxic to neurons has been questioned by cases of genetic PD without LB's (Poulopoulos *et al.*, 2012) and findings suggesting they may be mere bystanders (Parkkinen *et al.*, 2011) of a toxic process initiated by alpha-synuclein oligomers (Kalia *et al.*, 2013).

Non-dopaminergic pathology

Dopamine is the most important but not the only neurotransmitter affected in Parkinson disease. Almost every other transmitter system has been implicated in this condition with multiple symptoms attributed to the failure of each of the systems. The review by Barone (Barone, 2010) provides a concise summary of this rapidly developing field. Loss of cholinergic neurotransmission, resulting from degeneration in the nucleus of Meynert, together with glutamatergic dysfunction, gives rise to broadly defined cognitive problems as well as autonomic dysfunction and sleep problems. Serotonergic and adrenergic degeneration has been mostly implicated in mood disorders, pain and autonomic dysfunction. GABAergic loss may play a role in sleep and mood disorders.

Braak hypothesis

These major changes in our understanding of PD pathology at the micro scale have been paralleled by new concepts at the level of the whole brain. The traditional view of PD as a disease of the dopaminergic neurons has expanded

substantially to include almost the whole neuroaxis in a stepwise manner. The Braak model of PD pathology posits 6 stages of disease, starting at the level of the vagal nerve in the brainstem and the olfactory bulb (Braak *et al.*, 2003) (Figure 1). An even earlier involvement of the enteric plexus has been suggested (Hawkes *et al.*, 2009), putting the initial pathology outside the central nervous system. The degeneration would then progress upwards through the pons to reach Substantia Nigra only at the 3rd stage. Later, pathology could be found in the allo- and neocortex to involve even primary sensory and motor areas in the 6th stage. This revolutionary view of neurodegeneration in PD, with its multistage presymptomatic period, provided a huge impetus for studying early detection methods aimed at preventing further degeneration.

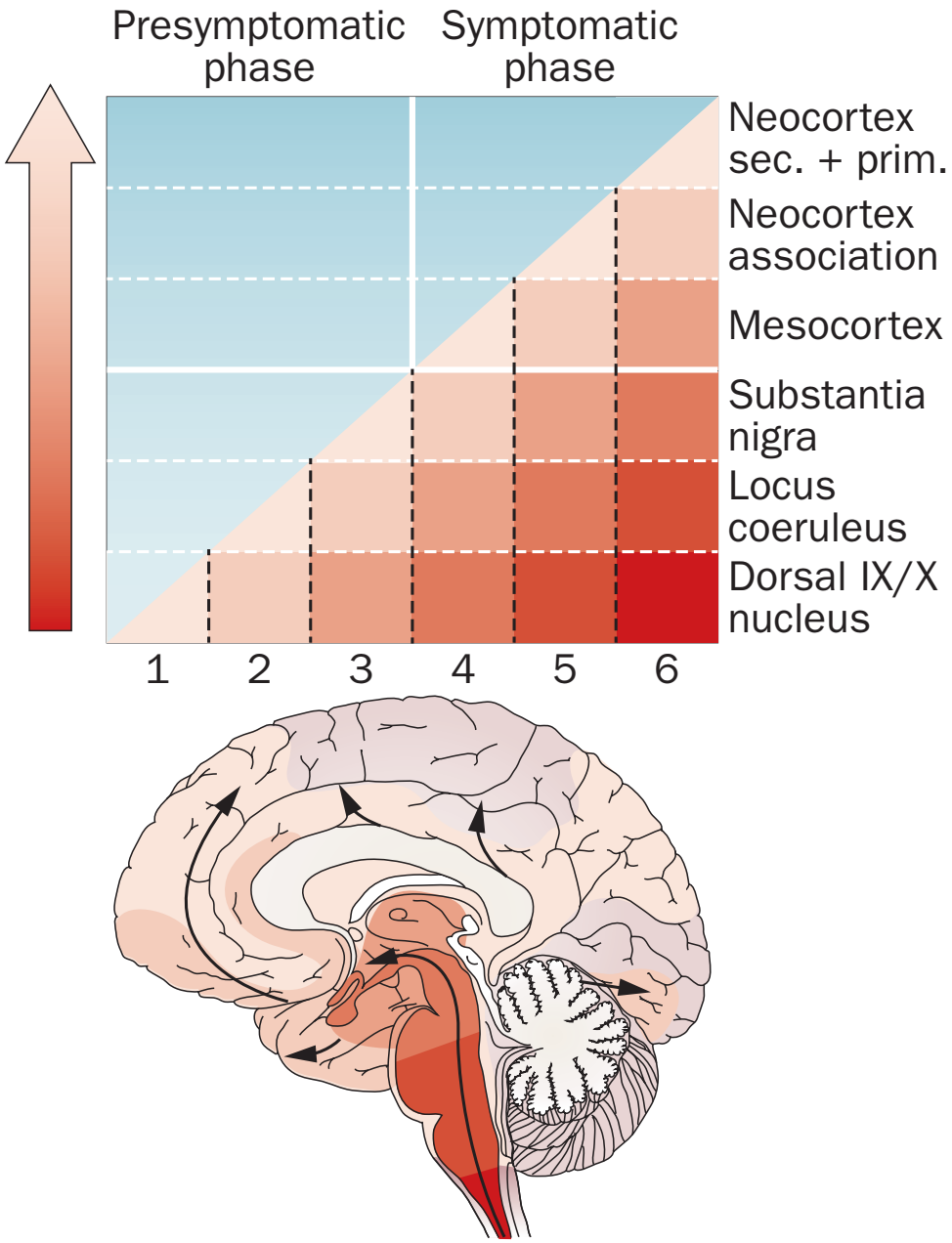


Figure 1. Braak staging of Parkinson disease pathology. PD pathology progresses in stages, starting in the dorsal nucleus of the X nerve. It advances through the brainstem affecting the locus ceruleus in stage 2, substantia nigra in stage 3, mesocortex in stage 4 and neocortex in stages 5 and 6. Disease becomes symptomatic at stage 3, after damaging substantia nigra. Reproduced from Goedert et al., 2013. Permission to reproduce this figure has been granted by the Nature Publishing Group.

Section 2. PD pathophysiology

Basal ganglia

Loss of dopaminergic neurons in a small area of the brainstem has profound consequences for the functioning of the whole brain because of the role of dopamine in a much larger system of the basal ganglia (BG) nuclei. The BG consist of the striatum (caudate nucleus and putamen), globus pallidus interna and externa (GPi, GPe), subthalamic nucleus (STN), thalamus (TH) and substantia nigra (pars compacta – SNc, and reticulata - SNr). All those structures form a densely interconnected system maintaining multiple afferent and efferent connections with the cortex (Figure 2). The main input station for cortical signals is the putamen while SNr and GPi functions as the output, inhibiting signal transduction from thalamus to the cortex (Obeso *et al.*, 2008). Three major pathways have been identified with opposing effects on the SNr/GPi inhibitory output. The direct pathway (striatum to SNr/GPi) has an inhibitory effect on SNr/GPi. The indirect pathway (striatum to GPe, STN, SNr/GPi) exerts a net excitatory effect on SNr/GPi. The hyperdirect pathway (Cortex to STN/GPi) by-passes the striatum and provides a more immediate excitation of the GPi than the indirect pathway. Dopamine from the SNc neurons has a fine-tuning effect on the system through excitation of the direct pathway and inhibition of the indirect pathway (Obeso and Lanciego, 2011; Obeso *et al.*, 2008).

The classical basal ganglia model has been developed and expanded to accommodate multiple new findings over the years (Obeso and Lanciego, 2011). Paralleling the complexity of the system, its functional interpretations have been equally multifaceted (DeLong and Wichmann, 2009). First concepts stressed

online control of speed and amplitude of movement (Georgopoulos *et al.*, 1983) and response selection (Albin *et al.*, 1989; Mink, 1996). More recent interpretations focus on switching between automatic and controlled behaviour (Hikosaka and Isoda, 2010) and mediating habitual actions and learning (Redgrave *et al.*, 2010). This variety of interpretations results not only from the constantly updated experimental findings but mainly from the background of the individual research groups: animal models versus human studies, healthy versus diseased subjects, motor versus cognitive paradigms. What it certainly proves is that the Basal Ganglia play a central role in almost any complex brain function.

Figure 2. Basal ganglia model in health (A.) and in Parkinson disease (B.). Excitatory pathways are indicated with light arrows, inhibitory – with dark. Thin lines in panel B indicate reduced activity of the relevant pathway, while thick lines signify increased activity. H – hyperdirect pathway; I – indirect pathway; D – direct pathway; GPe - globus pallidus pars externa; GPi - globus pallidus pars interna; PPN - pedunculopontine nucleus; SNc - substantia nigra pars compacta; SNr - substantia nigra pars reticularis; STN - subthalamic nucleus; VL - ventralis lateralis. Modified from Olanow et al., 2001. Permission to reproduce this figure has not been granted.

BG dysregulation in PD

The classical basal ganglia model provides a neat explanation of the effects of reduced dopamine stimulation in the parkinsonian state (DeLong and Wichmann, 2009; Obeso *et al.*, 2008) (Figure 2). In the direct pathway, lack of dopaminergic stimulation leads to underactivity of the direct pathway and disinhibition of the output nuclei SNr/GPi. Additionally, lack of inhibition in the indirect pathway produces hypoactivity in the GPe, overactivity of the STN and, again, overactivity in the output nuclei. The net effect is overactivity of SNr/GPi with overinhibition of the thalamus leading to hypo/bradykinesia. Those features of PD are also strongly correlated with level of dopaminergic damage in the striatum (Pirker, 2003), supporting the link between bradykinesia and the BG model. Conversely, tremor severity does not display such a correlation and other evidence shows that this feature is not well explained by the standard model. Correlation of tremor frequency with firing in GPe, GPi, STN and the ventralis intermedius nucleus of the thalamus (Vim) suggests that tremor may result not so much from overactivity of those structures as from increased neuronal synchrony (Obeso *et al.*, 2008). Moreover, selective abolition of tremor by lesions to the Vim, a structure linked strongly to the cerebellum, points to the role of regions beyond the standard BG model in generating tremor (Helmich *et al.*, 2012). Those inconsistencies and new findings have lead to multiple modifications of the traditional basal ganglia model in PD (Obeso and Lanciego, 2011; Obeso *et al.*, 2008).

One of the modifications of the standard model has been provided by animal studies and Deep Brain Stimulation recordings in humans, which discovered synchronized neuronal activity in various nuclei of the system and the cortex

(Eusebio and P. Brown, 2007). Of particular importance in PD are the, so called, beta-oscillations, which represent coherent neuronal activity around the 20 Hz frequency (Little and P. Brown, 2014). In the healthy brain they are suppressed with movement (Doyle *et al.*, 2005; Little and P. Brown, 2014) and rebound afterwards. In PD beta-oscillations increase off medication and are suppressed by levodopa and DBS (Little and P. Brown, 2012). It has been proposed that the function of beta-oscillations is to control information coding in motor loops (Little and P. Brown, 2014), whereby higher beta activity limits processing of novel information leading to stabilization of the current motor state. That effect is pathologically strengthened in PD producing typical symptoms of bradykinesia and rigidity.

Section 3. Epidemiology

Prevalence and incidence

Parkinson's disease is one of the leading neurodegenerative disorders affecting 122-142 people for 100,000 population in the UK (Hobson *et al.*, 2005; Wickremaratchi, Perera, *et al.*, 2009). However, figures in other countries vary greatly, with a review of good quality US and European studies suggesting a prevalence in subjects >65 years between 700-4500 per 100,000 (median 950 per 100,000) (Hirtz *et al.*, 2007). According to that study, PD takes fourth place amongst the most common neurological disorders, behind migraine, AD and stroke (respectively, 121, 67 and 10 per 100,000). Much lower prevalence has been reported in Africa, which may result from low life expectancy in that region (Wirdefeldt *et al.*, 2011).

Incidence of PD ranges from 1.5-22 per 100,000/year (Wirdefeldt *et al.*, 2011) with a median standardized incidence in developed countries of 14 per 100,000/year (Hirtz *et al.*, 2007). That figure rises dramatically with age and reaches 160 per 100,000/year in subjects >65 years old (Hirtz *et al.*, 2007). Some studies have reported a declining incidence in the very old >89 years but it is still a matter of debate whether this effect may be due to under- or misdiagnosis in subjects with multiple comorbidities (Wirdefeldt *et al.*, 2011).

Prognosis

There is no consensus in the literature with regards to the effect of PD on life expectancy. Some large studies, however, show significantly reduced life expectancy in PD compared to general population. According to a UK study (Ishihara *et al.*, 2007) patients with PD onset before 65 years live shorter by

about 10 years while those with onset above 65 years lose about 4 years. This is not to say that younger onset PD is more aggressive since in relative terms patients with onset <65 lose about 22-32% off their life expectancy whereas older onset reduces expectancy by 44%.

After reduced life expectancy, disability has the greatest impact on life of patients with PD. A landmark study from 1967 by Hoehn and Yahr (Hoehn and Yahr, 1967) showed that 25% of patients are dead or disabled within 5 years of disease onset. This increased to 67% at 9 years and 80% at 14 years. A small group was however identified lacking disability even at 20 years. A more recent study (Shulman *et al.*, 2008) from 2008 concluded that progression to disability (defined as loss of independence) occurs on average between 3 and 7 years into the disease.

Traditionally, the greatest contributor to disability has been motor dysfunction. However, recent studies have also uncovered a significant cognitive disability burden of PD with 75% of patients progressing to dementia within 10 years (Aarsland and Kurz, 2010). Closer look at early stages of disease shows that around 20% of patients have cognitive impairment at the time of diagnosis (Aarsland *et al.*, 2009; Muslimovic *et al.*, 2005). Data from the Queen Square Brain Bank further highlight the prognostic importance of cognitive disability (Kempster *et al.*, 2010). In that study, a group lead by Andrew Lees showed that time to death from onset of regular falls was on average 3.2 to 5.2 years while time from dementia to death was shorter at 2.9 to 3.8 years.

Section 4. Risk factors

There has been a great interest in defining risk factors or even a risk profile for PD (Postuma *et al.*, 2010; Postuma, Aarsland, *et al.*, 2012). Apart from a purely informational value of such research, identifying subjects at risk of PD could provide a great opportunity for disease prevention. The idea is similar to the current model of stroke and heart attack prevention. Rather then holding back treatment until a clinically obvious stroke or a myocardial infarction develops, it is now possible to give preventative medication based on presence of such risk factors as high blood pressure, high cholesterol or smoking history. The risk factors may not necessarily be diseases in their own right but rather markers of probability for developing target organ damage. Traditionally, risk factors can be divided into genetic and environmental.

Genetics

A well established method of identifying genetic versus environmental contributions to etiology of disease are twin and family studies. As monozygotic twins share 100% while dizygotic - 50% of the genetic material and both have similar early life environment, twin studies are ideally posed to answer the nature-nurture question. A large twin study in PD (Tanner *et al.*, 1999) reported 20% concordance in monozygotic twins and only a slightly smaller concordance of 12% in dizygotic pairs, indicating that genetic effects at population level are of little importance (Wirdefeldt *et al.*, 2011). However, when looking at subjects with PD onset below age 50, concordance in monozygotic pairs reached 100% while it was still only 17% in dizygotic pairs. Hence, in early onset PD genetic factors seem to play a dominant role.

An interesting twist to the story is added by considering the underlying dopaminergic dysfunction rather than the clinical manifestation of PD (Wirdefeldt *et al.*, 2011). As much as 55% of monozygotic pairs were concordant for dopaminergic dysfunction on PET and the number increased on follow-up to 70%, while dizygotic twins had stable concordance of 22% (Piccini *et al.*, 1999). This suggests that the underlying physiological susceptibility to PD is very strongly influenced by genetics and points to the environmental factors as important modulators of disease expression.

A burning question for patients with PD is the risk of disease in their relatives, especially children. The odds ratio for PD in first-degree relatives of PD patients without monogenic variants causing their PD varies greatly between 1.6 and 10.4 (Wirdefeldt *et al.*, 2011) but a meta-analysis of good quality studies calculated it at 2.9 (Thacker and Ascherio, 2008). The risk seems to be higher in a sibling than in a child or parent of the proband, which may indicate importance of shared environment or recessive genetic factors (more likely to be shared by siblings) (Wirdefeldt *et al.*, 2011).

Identification of actual genetic mutation contributing to PD has greatly accelerated in the recent decade. Seventeen genetic loci associated with the disease have been classified so far under the names PARK1-PARK18 (PARK1 and PARK4 are identical) but there may be as many as 28 candidate chromosomal locations associated with PD (Klein and Westenberger, 2012). Out of those, six genes have been clearly linked to monogenic PD: SNCA (PARK1=4), LRRK2 (PARK8), Parkin (PARK2), PINK1 (PARK6), DJ-1 (PARK7), and ATP13A2 (PARK9). However, given the large effect of environment in the development of

PD, genetic disease accounts only for 3-5% of the total patient population (Klein and Westenberger, 2012).

Age and Gender

Age is one of the most obvious factors affecting incidence of PD. Increasing incidence with age is widely known but the mechanisms behind this phenomenon still require investigation. Age can also affect the clinical presentation of disease including responsiveness to treatment and prognosis. Male gender and oophorectomy in women predisposes to PD through possible hormonal effects and by modifying environmental risks. Similar to age, it may also influence disease phenomenology.

Environment

In view of a relatively small heritability of Parkinson's disease, search for environmental risk factors is the next important frontier in the quest for prevention of PD. A list of risk factors where associations were confirmed in at least one study is very long and can be divided into protective and harmful effects. Harmful factors include: pesticides, heavy metals, organic solvents, magnetic fields, farming occupation, obesity, dairy products, infections, head trauma, diabetes, vascular disease. Protective factors may include: exposure to tobacco smoke, coffee and tea consumption, physical exercise, alcohol, antioxidants, uric acid and gout, non-steroidal anti-inflammatory drugs, estrogen (Wirdefeldt *et al.*, 2011)..

Section 5. Clinical presentation

Motor features

Motor features are the most obvious, and until recently, the only acknowledged symptoms of PD. They still form the backbone of diagnosis despite an increasing interest in a variety of non-motor features. The four main motor manifestations of PD are: brady/hypokinesia, tremor, rigidity and postural instability.

Bradykinesia is a mandatory symptom in the diagnosis of PD and is defined as "slowness in initiation of voluntary movement with progressive reduction in speed and amplitude of repetitive actions" (A. J. Hughes *et al.*, 1992). Tremor is probably the most recognizable symptom of Parkinson's but also the least disabling. Traditionally, it is defined as rest tremor of 4-6 Hz frequency, predominantly occurring in the hands (A. J. Hughes *et al.*, 1992). Rigidity is a sign defined by increased resistance and reduced extension of a joint on passive stretching. Finally, postural instability is a late symptom in the disease progression.

Non-motor features

Until recently, PD was considered a purely motor disease with few non-motor symptoms believed to develop only in the end stages of the condition. However, awareness of the existence of those problems have been there from the sixties and seventies when studies on cognitive function (Talland, 1962), smell symptoms (Ansari and Johnson, 1975) and sleep disturbance (Mouret, 1975) were published. Wider acceptance of the problem came only after large studies in the 2000's showed considerable effect of the non-motor features on quality of

life (Schrag *et al.*, 2000) and disability (Aarsland *et al.*, 2000). A true boom happened when the non-motor symptoms showed a promise for so called premotor diagnosis – as they were confirmed to predate the all important motor features (Siderowf and M. B. Stern, 2008).

Neuropsychiatric features, like cognitive impairment are, perhaps, the most feared ones by the patients (Chaudhuri *et al.*, 2006). Others from that group include depression, anxiety, apathy and impulse control behavior. Sleep disorders, such as REM behaviour sleep disorder (RBD), excessive daytime sleepiness and restless legs syndrome are important as they may predate development of typical motor features of PD (Chaudhuri *et al.*, 2006). Autonomic problems are particularly numerous and bothersome to the patient and include: constipation, fecal incontinence, urinary urgency/incontinence, impotence, excessive sweating, dribbling of saliva, difficulty swallowing, nausea (Chaudhuri *et al.*, 2006). Finally, loss of sense of smell and taste has gained a lot of attention as a premotor symptom very commonly occurring in PD (Chaudhuri *et al.*, 2006).

Section 6. Diagnosis

Despite a very characteristic symptomatology of Parkinson's disease the clinician is faced with a considerable diagnostic uncertainty when confronting real-life cases. Prior to introduction of the Queens Square Brain Bank diagnostic criteria (A. J. Hughes *et al.*, 1992) in 1992 the initial clinical diagnosis by a certified neurologist in one study, when checked against the autopsy findings, was accurate in only 65% of cases (Rajput *et al.*, 1991). That number rose to 76% when the clinicians where given the benefit of 12 years of follow-up. 76% was also the initial sensitivity of the movement disorders specialists in the landmark

study which introduced the Brain Bank criteria (A. J. Hughes *et al.*, 1992). These required that a diagnosis of PD be made only in patients presenting with bradykinesia and one of the three other symptoms of tremor, rigidity or postural instability, and be supported by a range of further clinical features with a long list of symptoms excluding the diagnosis (A. J. Hughes *et al.*, 1992). Retrospective application of those criteria to a 100 cases increased accuracy from 76% to 82%. A further study looking at prospective application of the criteria reported 91% sensitivity of the clinical diagnosis (A. J. Hughes *et al.*, 2002). All those studies investigated diagnostic accuracy in the setting of dedicated movement disorders clinics. In contrast, a study investigating accuracy of diagnosis made by GP's in the community concluded that 15% of patients diagnosed as PD did not have it according to the BB criteria while 20% were wrongly diagnosed with a different disorder (Schrag *et al.*, 2002).

All those observations paint a rather pessimistic picture of diagnostic accuracy in PD and call for improvement in diagnostic techniques. This task was recently taken up jointly by the European Federation of Neurological Societies and the European Section of the Movement Disorders Society. Their recommendations for the diagnosis of PD advise a more holistic approach and acknowledge the importance of non-motor features (Berardelli *et al.*, 2013). Moreover, for the first time, imaging methods are mentioned as facilitating the diagnosis.

Imaging in PD

Imaging has a special role in investigating and diagnosing neurological disorders. Through direct visualisation of the central nervous system in vivo,

computer tomography (CT) and magnetic resonance imaging (MRI) have enabled a neurological diagnosis to be made ante-mortem in cases where 50 years ago only post-mortem diagnosis was possible. In diseases like CNS cancer or MS, MRI has formed an essential part not only in the diagnostic process but also when deciding on treatment options. Yet in PD this trend has been rather slow to catch on. The reason for that is that structural MRI studies in PD have produced multiple inconsistent results. Atrophic changes were demonstrated in a variety of structures implicated in movement control: substantia nigra (Menke *et al.*, 2009), putamen (Tinaz *et al.*, 2011), cerebellum (Benninger *et al.*, 2009). These findings were only present in advanced disease and structural MRI in early PD has not produced a reliable biomarker. A possible reason for this failure is that cell death and structural atrophy is a late manifestation of a long degenerative process. Analogous to a model proposed for Alzheimer's disease (Jack *et al.*, 2013), atrophy is most likely preceded by a chain of functional defects and adaptations, which only later are followed by changes on structural imaging.

Functional imaging has enjoyed a much greater success in PD than structural MRI. Dopamine transporter (DaT) imaging with SPECT (Berardelli *et al.*, 2013) forms the current gold standard for diagnosing dopamine depletion in PD. The technique is very reliable and allows for tracking disease progression (Marek *et al.*, 2001), including identification of early presymptomatic stages of PD (Ponsen *et al.*, 2004; Sommer *et al.*, 2004). A major obstacle to its broad adoption, however, is low availability of SPECT scanners in clinical settings, associated high costs and use of radioactive tracers with potential health hazards.

Another functional technique, functional MRI, has not proven very successful as a diagnostic method in PD. This happened in spite of its relative availability

and simplicity of use. The main reason for its failure has been a great variability of the investigational paradigms employed and lack of clarity as to which abnormalities are the most reliable. Those themes will be expanded upon in Chapter III of this thesis, which deals specifically with findings of the functional MRI studies.

Section 7. Treatment

The currently available treatment options offer only transient improvement in some of the motor features of the disease. The most tested and effective drug, levodopa, is 50 years old (Hornykiewicz, 2010) and has not been superseded in efficacy by any of the more recently developed dopaminergic agonists. The mode of action of those drugs relies merely on alleviating symptoms by stimulating dopaminergic receptors without addressing the ongoing degeneration of dopaminergic neurons (Meissner *et al.*, 2011). The outcome for the patient is about a five year period of relatively good treatment response following which medication side effects and inexorable disease progression dominate the clinical picture (Poewe and Mahlknecht, 2009).

In view of the inability of current treatments to affect disease progression, research efforts have focused on developing compounds with neuroprotective properties. For comparison, according to a recent review (Meissner *et al.*, 2011), only one compound in current armamentarium has some effect on progression (rasagiline), while 3 drugs in phase III trials, 8 in phase II and 6 in phase I lay claim to neuroprotective effects. Testing these and other substances requires the intervention to fall into an appropriate therapeutic window, ideally, before major and irreversible damage to the Substantia Nigra neurons. This approach

demands that identification of potential candidates for treatment is made early, even before classical symptoms develop. In order to select appropriate subjects for trials of neuroprotective substances disease biomarkers are needed.

Section 8. Biomarkers

The term biomarker has been defined as:

"A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention." (Biomarkers Definitions Working Group. 2001)

In the field of Parkinson's Disease biomarkers can be used for multiple purposes, including:

- as aid in differential diagnosis of parkinsonian syndromes;
- to diagnose PD early, in the prodromal or premotor state, before the clinical diagnosis can be made;
- to track disease progression;
- for use in disease modification or neuroprotection trials. (Schapira, 2013)

The rational for their broad use in neurodegeneration is the ability to capture a complex pathological process in an easily measureable indicator. An important consideration is also a need for prediction of outcomes developing over long time periods or the ability to overcome the difficulty posed by disease fluctuations over days and months (Biomarkers Definitions Working Group., 2001)

Multiple attempts have been made to codify the requirements a reliable biomarker should fulfill. Long lists of necessary criteria have been produced by Brooks et al. (Brooks *et al.*, 2003) and McGhee et al. (McGhee *et al.*, 2013). They have been concisely subsumed by the German Society of Experimental and Clinical Neurotherapeutics (GESENT) in their 2011 paper (Gerlach *et al.*, 2011). They stipulated that the future biomarker for proof of disease-modifying therapies in PD should be:

- linked to fundamental features of PD neuropathology and mechanisms underlying neurodegeneration in PD,
- correlated to disease progression assessed by clinical rating scales,
- able to monitor the actual disease status,
- pre-clinically validated,
- confirmed by at least two independent studies conducted by qualified investigators with the results published in peer-reviewed journals.
- inexpensive, non-invasive, simple to use, and technically validated.

Considering the previously discussed long pre-diagnostic pathological disease process in PD, a further desirable feature to be added to this list is the ability to track the disease process even before PD becomes clinically manifest. This is particularly important, given that the earlier a disease-modifying intervention can be started the greater the likelihood of its success.

Depending on the nature of the measured characteristic, biomarkers used in central nervous system diseases can be divided into clinical, neuroimaging, biochemical, genetic or biochemical (Gerlach *et al.*, 2011; McGhee *et al.*, 2013; Schapira, 2013). In the following sections a brief overview will be provided for each of those groups.

Clinical biomarkers

Clinical biomarkers, can be thought of as a set of disease features identified in patient history or examination. The currently broadly used UPDRS score (Unified Parkinson's Disease Rating Scale) is an important starting point and the only widely used scale for assessment of disease progression. However, its major limitation is its investigator dependence. Hence, there has been a major push in the research community to develop more objective tests using simple timed tasks (Get-up-and-go (Shumway-Cook *et al.*, 2000), Flamingo test (Tsigilis *et al.*, 2002)) or employing equipment, e.g. electromyography (Milanov, 2002) or accelerometers (Mancini *et al.*, 2009). These tests mainly target the motor features of PD but have been recently supplemented by a multitude of scales assessing the non-motor symptoms, including olfaction, REM Behaviour Sleep Disorder or cognition. The most reliable of those markers could then be combined into a composite biomarker as suggested, for example, in the PRIPS study (Prospective validation of risk markers for the development of idiopathic Parkinson's disease) (Berg *et al.*, 2010). It is important to note that even though each of those PD symptoms becomes more pronounced as the disease progresses, most of them have also been noted before a formal PD diagnosis can be made. Hence, they have a potential to serve as prediagnostic biomarkers, so

important for neuroprotective therapy. Clinical features with a potential to serve as risk factors or biomarkers in PD will be further discussed in the introduction to Chapter II, Basic clinical characteristics of early Parkinson's Disease in the Oxford Parkinson's Disease Centre cohort.

Development of clinical biomarkers requires large clinical cohort studies recruiting patients as they are being diagnosed and assessing them with a comprehensive set of measures covering the whole breadth of phenomenological manifestations. Longitudinal follow up in such cohorts enables further assessment of disease progression and identification of disease features most fit for use as biomarkers. This study model is currently used by multiple groups investigating biomarkers in PD: Parkinson Progression Marker Initiative (Parkinson Progression Marker Initiative, 2011) in the US, PROBAND study in Scotland (www.proband.org.uk), PARS study (Siderowf *et al.*, 2012) in the US, TREND and PRIPS study in Germany (Berg, 2012). It will also be further expanded upon in Chapter II.

Neuroimaging biomarkers

Amongst various imaging techniques, four have been explored extensively as biomarkers in PD: dopamine-related imaging with PET and SPECT, Transcranial Sonography, structural MRI and functional MRI.

As reviewed in a paper by Brooks et al. (Brooks *et al.*, 2003), three radioisotope techniques are particularly promising as biomarkers in PD: vesicular monoamine transporter-2 PET (VMAT2-[11C]dihydrotetrabenazine PET), dopamine transporter SPECT (DAT- ([123I]beta-CIT) SPECT) and fluorodopa PET ([18F]fluorodopa PET). Of those, DAT SPECT has become most

widely accepted and has entered recommendations for diagnosing PD issued by the European Federation of Neurological Societies (ENFS) (Berardelli *et al.*, 2013). All those techniques rely on imaging the integrity of dopamine secreting neurons and as such reflect the basic pathological process in PD which is loss of dopaminergic neurons. They are also capable of tracking disease progression and may be abnormal before disease becomes manifest (Schapira, 2013). However, the techniques are not specific for idiopathic PD and may be abnormal in atypical parkinsonism. Moreover, exposure to radioactivity, high cost of those techniques and availability only in specialist centers makes them less attractive for wide adoption in clinical trials. (Gerlach *et al.*, 2011)

Transcranial sonography (TCS) is another candidate for an easily available and cheap biomarker in PD. Increased echogenicity of the Substantia Nigra is present in 90% of patients with PD and in 10% of healthy elderly individuals. (Berg *et al.*, 2011) Presence of that finding in asymptomatic healthy subjects was shown to increase the risk of developing PD by a factor of 17 (Berg *et al.*, 2011) It is not quite known what the finding of hyperechogenicity reflects pathologically and it does not seem to progress with disease severity. (Schapira, 2013) Hence, it is classed as a trait marker of disease. Additionally, about 10% of subjects cannot be assessed with TCS due to a so called inadequate bone window, meaning too thick or calcified temporal bone for the ultrasound beam to penetrate. (Berg *et al.*, 2010)However, despite its limitations TCS was recommended by the EFNS guidelines as a useful tool in differential diagnosis of parkinsonism and early diagnosis of subjects at risk for PD (Berardelli *et al.*, 2013).

Structural MRI provides a much more detailed picture of the brain than TCS. In spite of good spatial resolution, path to discovery of a reliable structural biomarker in PD has, so far, proven arduous and largely unsuccessful (Lehéricy *et al.*, 2012). A few recent discoveries, however, inspire hope for a more positive development in this area. High field MRI, using 7 Tesla magnets, allowed identification of a structure within the Substantia Nigra, containing densely packed dopaminergic neurons – called a nigrosome (Blazejewska *et al.*, 2013). One of the nigrosomes was shown to disappear in PD, which reliably differentiated patients from controls in blinded assessment. A follow-up study identified the nigrosome retrospectively on 3 Tesla scans, showing 100% sensitivity and 95% specificity in differentiating PD subjects from healthy participants. (Schwarz *et al.*, 2014) Another group of researchers, again using the high field 7T MRI, showed local shape changes in the SN, which, as they speculated, may be related to changes in nigrosome 1 in PD. (Kwon *et al.*, 2012) It is yet too early, however, to draw any conclusions as to whether those changes track disease progression or whether they could be used for presymptomatic diagnosis.

An MRI sequence presumed to reflect iron content in the tissue, called relaxation rate R_2^*, has been successfully applied to differentiate PD from control scans (Martin *et al.*, 2008). Importantly, increased iron content in PD detected with that method, was shown to correlate with disease severity. Those results are in agreement with pathological studies showing increased iron concentration with disease progression in PD. Another method – diffusion weighted imaging (DWI), purporting to reflect tissue integrity, has shown variable success in PD

(Lehéricy *et al.*, 2012), with some studies showing changes in the SN and basal ganglia while others failed to replicate those abnormalities. However, a meta-analysis focusing on fractional anisotropy (FA) in the SN showed significant and large reduction of FA in PD subjects (Cochrane and Ebmeier, 2013).

Further studies will need to be done to establish reliability of those findings in prospective cohorts, as well their potential use as presymptomatic biomarkers. Currently, the main and most secure use of MRI in PD is in exclusion of secondary causes of parkinsonism, e.g. vascular parkinsonism, or in differentiating idiopathic PD from MSA, PSP or CBD (Chahine and M. B. Stern, 2011).

Functional MRI represents another method of investigating brain abnormalities in PD. The main promise of this technique is to demonstrate changes beyond structural differences, and uncovering deficits and adaptations in the functioning of the brain. Many studies have now been performed looking at correlations between various task paradigms and brain activations in different stages of PD (Ballanger *et al.*, 2012; Ceballos-Baumann, 2003), including presymptomatic subjects with genetic predisposition to PD (van Nuenen, van Eimeren, *et al.*, 2009). Majority of the research has focused on motor paradigms. The main findings are reduced activation in the basal ganglia, supplementary motor area (SMA) and motor cortex with tasks including finger, hand or arm movement (Cerasa *et al.*, 2006; Helmich *et al.*, 2009; Kraft *et al.*, 2009; Prodoehl *et al.*, 2010; Rowe *et al.*, 2002). Those have been interpreted as consistent with the traditional basal ganglia model postulating reduced output from the basal ganglia as a cause for bradykinesia (Cerasa *et al.*, 2006; Prodoehl *et al.*, 2010).

Some studies have identified increased activity in frontal and cerebellar areas with simple motor tasks (Cerasa *et al.*, 2006). The proposed explanations suggested that compensatory brain activity may be required in the presence of PD pathology (Yu *et al.*, 2007).

With the focus shifting recently to the non-motor features of PD, a number of studies looked at cognitive and other non-motor paradigms. The emerging picture is rather variable, ranging from under to overactivations in a wide range of areas (Delaveau *et al.*, 2009; Ray and Strafella, 2010; Rowe *et al.*, 2008). The inconsistencies can be, at least in part, explained by the variability between the tasks themselves. Moreover, the intrinsic complexity of cognitive tasks allows for different strategies to be employed to solve them.

Task-related fMRI has shown a great potential for identifying abnormal brain activity in PD but its usefulness in clinical setting is undermined by the very defining feature of this technique: its reliance on task performance. In other words, in order to register any reliable brain activation an action has to be performed to a certain standard. This requirement poses two problems in the setting of a disease like PD. Firstly, the task may be too difficult for a patient to perform correctly. Secondly, brain activation may be strongly influenced merely by the level of performance, making it more difficult to identify the actual pathophysiological mechanisms underlying the disease.

One solution is to register fMRI brain activity at rest with no superimposed task requirement. This method is called task-free or resting-state fMRI (RS-fMRI) and has enjoyed a rapid development over the past 10 years (Biswal, 2012; Snyder and Raichle, 2012). The most popular method of analyzing the RS-fMRI data, functional connectivity, postulates that correlations between BOLD signals

from different areas of the brain are a reflection of cooperation or connectivity between those areas (Biswal *et al.*, 1995). This method has been shown to sensitively identify disease-related changes in AD (Douaud *et al.*, 2011), ALS (Douaud *et al.*, 2011), dystonia (Mohammadi *et al.*, 2012) and depression (Veer *et al.*, 2010). It has been also successfully used in PD to show abnormal connectivity between the basal ganglia and various areas of the brain (Hacker *et al.*, 2012; Helmich *et al.*, 2010), changes that responded to dopaminergic medication (T. Wu *et al.*, 2012). Functional MRI and, more specifically, resting-state fMRI will be discussed in greater detail in Chapter III and IV of this thesis.

Biochemical biomarkers

Alpha-synuclein is one of the main constituents of Lewy bodies and was one of the first potential biomarkers of PD to be studied. Alpha-synuclein oligomers were shown to be elevated in serum of PD patients (El-Agnaf *et al.*, 2006). An even better sensitivity and specificity was achieved for CSF levels of the oligomers (75% and 87.5%, respectively). That was further trumped by the ratio of the oligomers to total alpha-synuclein (89.3% and 90.6%, respectively) (Tokuda *et al.*, 2010). Those findings were further supported by other studies (Park *et al.*, 2011; Sierks *et al.*, 2011) but correlation of the marker with disease severity has yet to be established (Schapira, 2013).

Another interesting and promising biomarker may be anti-neuromelanin antibodies in serum. Since neuromelanin is released into bloodstream with destruction of dopaminergic neurons in the SN, it was hypothesized that an immune response may build up as a consequence (Gerlach *et al.*, 2011). Indeed, it has been shown that those antibodies are increased in PD patients and that

their level correlates with disease duration, although not with severity (Double *et al.*, 2009).

A completely new horizon has been opened by the so called 'omics' studies. Proteomic or metabolomics aim to analyse a wide range of proteins or metabolites at once, and identify groups or networks of substances capable of predicting a given variable. This approach has been used in PD with some success (Gerlach *et al.*, 2011; Nyhlén *et al.*, 2010) but it is too early to speculate on its applicability. Biochemical markers will not be further discussed in this thesis as its main focus are clinical and neuroimaging markers of PD.

Section 9. Thesis Goals

In this thesis early PD will be explored through three main goals, which are formulated as follows:

1. To comprehensively characterize the phenotypical picture of early Parkinson's disease. This goal will be achieved through analyzing differences in demographic features and motor and non-motor symptoms in a large incident cohort of patients with PD within 3 years of diagnosis and healthy controls. Particular attention will be paid to characterizing the effect of age and gender on the clinical picture.
2. To identify a functional imaging signature of PD in already published studies. All published functional imaging studies in PD will be analyzed to identify the most typical functional changes in the condition.
3. To characterize a resting-state functional imaging signature of early PD. A selected sample of early PD patients and healthy controls will be investigated with resting-state functional MRI to identify reproducible differences between the two groups.

Chapter II. Basic clinical characteristics of early Parkinson's Disease in the Oxford Parkinson's Disease Centre cohort

Section 1. Introduction

Over the past years the spectrum of risk factors and the very number of recognized features of Parkinson's disease has expanded considerably. There is also a growing recognition that Parkinson's disease has phenotypic heterogeneity (Ascherio *et al.*, 2004; van Rooden *et al.*, 2011) in its motor and non-motor manifestations (Chaudhuri *et al.*, 2006). Age and gender are two basic characteristics that may influence the disease phenotype, either through disease-independent factors or through differences in underlying pathology. One therefore wants to examine age and gender related differences in a large incident cohort of patients with detailed phenotypic data on both motor and non-motor features.

Oxford Parkinson's Disease Center (OPDC, http://opdc.medsci.ox.ac.uk) was established in 2009 with funding from the Parkinson's UK Monument Discovery Award and brings together world-leaders in clinical neurology, neuroepidemiology, neuroimaging, proteomics, genomics, molecular genetics, transgenic PD models, neuropharmacology, neurophysiology and neuropathology. The OPDC cohort is a prospective, longitudinal study that has recruited patients with early idiopathic Parkinson Disease, a group of healthy controls and a group of participants at risk of PD.

In the following chapter an interim analysis of the OPDC cohort will be presented. First, a review of risk factors for PD will be provided followed by a description of its motor and non-motor symptoms.

Gender

One of the most easily identifiable risk factors for PD is male gender. Multiple studies have shown male predominance in PD and two large meta-analyses calculated the male to female ratio at 1.49 (Hirtz *et al.*, 2007; Wooten, 2004) and 1.46 (Taylor *et al.*, 2007). The lifetime risk of Parkinson's has been estimated at 2% in men and 1.3% in women (Elbaz *et al.*, 2002). The suggested explanations include protective effects of oestrogens and susceptibility genes on the X chromosome (Taylor *et al.*, 2007; Wooten, 2004). Gender may also modify known environmental risk factors. Occupational exposure to pesticides or heavy metals may be lower in women due to different professional spectrum between sexes. It is also possible that the exposure has a different effect on the sexes, as suggested by a study showing detrimental effects of pesticides only in men and not in women (Baldi *et al.*, 2003). Caffeine has been shown to be protective in the all-male Health Professionals Health study (Ascherio *et al.*, 2001) but the Nurses Health Study (Ascherio *et al.*, 2001; 2004) failed to show that effect. Similar result was demonstrated by the mixed sex Cancer Prevention Study (Ascherio *et al.*, 2004).

Age

Out of 23 incidence studies reviewed by Wirdefeldt et al. (Wirdefeldt *et al.*, 2011) 22 showed a clear age-related increase of PD cases. The actual size of the increase above the age of 65 may be as high as a factor of 10, from 14 to 160 per

100,000/year (Hirtz *et al.*, 2007). Some studies have reported a declining incidence in the very old >89 years but it is still a matter of debate whether this effect may be due to under- or misdiagnosis in subjects with multiple comorbidities (Wirdefeldt *et al.*, 2011). Moreover, age modifies the phenotypical presentation of PD. Early-onset patients have more dystonia and earlier dyskinesias, slower disease progression, lower rates of dementia (Wickremaratchi, Ben-Shlomo, *et al.*, 2009) and lower rates of tremor (Wickremaratchi *et al.*, 2011) than late-onset patients. The effect of age in PD is almost taken for granted but the mechanisms behind it are not very clear. It may be that physiological ageing itself predisposes to PD, or that an accumulated exposure has to occur over time, or, perhaps, that a genetic trigger is age-dependent (Kieburtz and Wunderle, 2013).

Environmental risk factors

According to the Consensus statement of the Collaborative for Health and Environment and Parkinson's Action Network, of environmental exposures, only age has sufficient evidence for a causal relationship in PD (Bronstein *et al.*, 2009). Further two factors, smoking and coffee drinking, were deemed to have definite association with PD, with the caveat of uncertain causality. Of purely environmental factors, a range of toxins, occupational exposures and nutritional compounds have been researched but for most of them the link is equivocal.

Smoking. Ever smoking reduces the risk of PD to 0.59 compared with never smoking and the relationship depends on number of cigarettes smoked (Hernán *et al.*, 2002; Wirdefeldt *et al.*, 2011). Smokeless tobacco has also shown a less proven but strong inverse relationship with PD (Benedetti *et al.*, 2000). Causality

is difficult to establish based on those results as the picture may be complicated by an underlying addiction-aversive personality or other genetic factors common to smoking-aversion and PD. This has been partly addressed by a study showing reduced PD risk in children of smokers exposed to passive smoking (O'Reilly *et al.*, 2009) and by comparing PD subjects with their co-twins (Wirdefeldt *et al.*, 2011). A causal link seems to gain credence in the light of results showing that tobacco compounds, especially nicotine, prevents fibrillisation of alfa-synuclein – the main process leading to dopaminergic degeneration in PD (Ono *et al.*, 2007).

Coffee. Coffee drinking reduces the risk of PD to 0.69 and the risk drops with increasing amount of coffee (Hernán *et al.*, 2002; 2003). This association is valid even after controlling for smoking. The effect is modified by gender in that men enjoy a stronger protective effect than women (Ascherio *et al.*, 2004). It appears that this may be mediated by estrogen use as women who never used estrogens did show a strong protective effect of coffee (Ascherio *et al.*, 2004). Mechanisms invoked to explain the benefit of caffeine suggest both symptomatic and protective actions through antagonism of an adenosine receptor (Schwarzschild *et al.*, 2006).

Pesticides, farming and well water. Interest in a relation between pesticide exposure and PD started in the 1980s with a study reporting unusual outburst of parkinsonism in young people using heroin contaminated with MPTP – a substance similar to a herbicide paraquat (Langston *et al.*, 1983). Individual studies of occupational exposure to pesticides presented a mixed picture but a meta-analysis did show an increased risk of 1.9 (Priyadarshi *et al.*, 2000). Further studies looked at rural living, farming and well water exposure as risks

but the results were contradictory and did not allow for a definite conclusion (Wirdefeldt *et al.*, 2011). In contrast to epidemiological studies, animal and molecular experiments provided a wealth of evidence for harmful effects of high doses of pesticides on mitochondrial function and alpha-synuclein aggregation (Hatcher *et al.*, 2008).

Alcoholic drinks, uric acid, gout and NSAIDs. Adding to the list of widely condemned substances of abuse showing unexpectedly beneficial effects in PD is alcohol. Two large prospective studies showed reduced risk of PD in ever-drinkers (Grandinetti *et al.*, 1994), particularly related to beer consumption (Hernán *et al.*, 2003). However, a number of other reports failed to show a similar effect and there is no consensus on the protective effect of alcohol (Wirdefeldt *et al.*, 2011). A possible pathophysiological explanation of the protection awarded by alcoholic drinks, particularly beer, implicates uric acid (Hernán *et al.*, 2003) whose levels increase after beer consumption. This compound has well known anti-oxidant and anti-inflammatory properties and has been speculated to have neuroprotective effects in PD (X. Chen *et al.*, 2012). In support of this claim a meta-analysis found that high plasma levels of urate reduced the risk of PD. Similarly, gout, associated with high urate levels, is less prevalent among PD subjects (Alonso *et al.*, 2007).

Finally, following on the thread of inflammation in PD, non-steroidal anti-inflammatory drugs have been found to confer protection against PD (Gagne and M. C. Power, 2010). This effect may be particularly strong with ibuprofen (H. Chen *et al.*, 2003) and has not been reported with aspirin or paracetamol.

Clinical presentation: Motor features

Bradykinesia is the constitutive feature of PD. The progressive reduction in speed may be particularly important in PD and was shown to be a discriminating feature from atypical parkinsonism (Ling *et al.*, 2012). The most common site of onset for this symptom is the arm but it can be seen in the facial movements, in complex movements like writing or in leg movements. The pathophysiological mechanism underlying this symptom is deemed to be the disinhibition of the GPi and the resulting reduced recruitment of the cortical motor neurons (Rodriguez-Oroz *et al.*, 2009). Associated with bradykinesia is hypokinesia, defined as reduced frequency of spontaneous movement. Patients may have reduced blink rate and reduced facial expression, reduced arm swing when walking or generalized poverty of body movements. It has been suggested to result from disruption of cerebral pattern generators in the brainstem (Rodriguez-Oroz *et al.*, 2009). Patients may notice isolated brady/hypokinesia up to 3 years before a formal diagnosis of PD is made (Gaenslen *et al.*, 2011).

The second most important symptom is tremor and as an isolated symptom may precede diagnosis for up to 6 years (Berg *et al.*, 2013). Patients with tremor as the dominant feature of the clinical picture are known to have better prognosis and progress more slowly (Helmich *et al.*, 2012). The mechanism leading to the occurrence of this prominent sign has long been a matter of debate. A recently postulated explanation suggests that basal ganglia play a part in triggering tremor episodes while cerebello-cortical circuits determine its amplitude (dimmer-switch hypothesis) (Helmich *et al.*, 2012).

Rigidity forms an important part of examination of a parkinsonian patient and is frequently relied upon when making a diagnosis of an extrapyramidal syndrome. Prognostically, rigidity and akinesia dominant patients fare worse compared to the tremor-dominant subtype (Eggers *et al.*, 2012). However, the underlying mechanism of rigidity is only poorly explained by the classical basal ganglia model and may indeed be of cortical origin (Rodriguez-Oroz *et al.*, 2009).

Postural instability is one of the most disabling features but its occurrence early on casts doubt on the diagnosis of idiopathic PD and suggests atypical parkinsonism. If present, it portends negative prognosis with poor medication response and early disability (Post *et al.*, 2011). The putative pathology leading to instability and falls lies beyond the basal ganglia, in the pedunculo-pontine nucleus, a structure relying on cholinergic neurotransmission (S. Y. Lim *et al.*, 2009).

Clinical presentation: Non-motor features.

Recent years have seen a great expansion of research into non-motor features in PD. The most widely studied are: olfaction, cognition, depression, sleep and autonomic symptoms.

Olfaction. One of the most prevalent of those symptoms is hyposmia (reduced sense of smell) occurring in 97% of patients (Haehner *et al.*, 2009). It is present even at the very early stage, in up to 70% of patients within 4 months of formal diagnosis (Khoo *et al.*, 2013). A prospective study has shown that it may predate the diagnosis by at least 4 years and increase the risk of developing PD 5-fold (Ross *et al.*, 2008). It has also been included in the recent EFNS (European Federation of Neurological Societies) and MDS (Movement Disorders Society)

diagnostic guideline for PD (Berardelli *et al.*, 2013). The underlying pathology are most likely Lewy bodies and neurites in the olfactory bulb (Ubeda-Bañon *et al.*, 2010), a feature present at stage 1 of the Braak classification (Braak *et al.*, 2003).

Cognition. Cognitive impairment is a particularly important feature as it strongly affects the quality of life (Schrag *et al.*, 2000). According to recent longitudinal studies 75% of patients progress to dementia within 10 years (Aarsland and Kurz, 2010). Closer look at early stages of disease shows that around 20% of patients have cognitive impairment at the time of diagnosis (Aarsland *et al.*, 2009; Muslimovic *et al.*, 2005). Pathologically, cognitive impairment correlates with Lewy pathology staging outlined by Braak and also with amyloid pathology seen in Alzheimer's disease (Braak *et al.*, 2005).

Depression. Mood disorders, particularly depression, are another non-motor feature strongly affecting the quality of life (Schrag *et al.*, 2000). Depression is present in about 30-40% of PD patients (Berg *et al.*, 2013). Additionally, previous history of depressive disorder increases the risk of developing PD – the odds ratio is about 2.4 (Leentjens *et al.*, 2003). The pathology is non-dopaminergic, related to serotonergic circuits originating in the raphe nuclei in the brainstem (S. Y. Lim *et al.*, 2009), which are thought to be involved in early stages of the Braak classification.

Sleep. REM behavior sleep disorder (RBD), characterized by vivid dreams with loss of natural movement inhibition in sleep, has a particularly important place in the concept of premotor PD. It has been shown to precede PD by up to 50 years (Claassen *et al.*, 2010). The risk of PD is as high as 52% at 12 years from

RBD diagnosis (Postuma *et al.*, 2009). Importantly, PD patients with RBD have a higher risk of developing dementia (Marion *et al.*, 2008). The pathological findings in RBD include brainstem changes in the Pedunculo-Pontine Nucleus (PPN) and Locus Ceruleus (S. Y. Lim *et al.*, 2009), sites implicated early on in the Braak staging. Other commonly encountered sleep-related symptoms are: excessive daily sleepiness, periodic leg movement in sleep and restless legs syndrome (Chaudhuri *et al.*, 2006).

Autonomic symptoms. Constipation is a very common symptom in the elderly population but it may be present in up to 2/3 of PD patients (Pfeiffer, 2011). A meta-analysis calculated that the risk of developing Parkinson's is increased by a factor 2.3 in subjects with constipation (Noyce *et al.*, 2012). The putative mechanisms is damage to the colonic neurons through alpha-synuclein pathology (Shannon *et al.*, 2012) early in the disease process. Multiple other autonomic conditions have been linked to PD, including, urinary urgency, incontinence, sexual dysfunction and orthostatic hypotension (Chaudhuri *et al.*, 2006).

In the following experiment, the early PD phenotype in the OPDC was analyzed, with particular focus on the effects of age and gender.

Section 2. Methods

Participants

Study participants are recruited from neurology clinics across the Thames Valley area covering a population of 2.4 million people. Participating centers include: Oxford, Reading, Newbury, Wexham Park, High Wycombe, Aylesbury,

Milton Keynes, Kettering, Northampton, Banbury and Swindon. Neurologists, PD nurses, geriatricians and GP's from participating hospitals are asked to identify all idiopathic PD cases who were diagnosed within the last three years according to the UK PD Brain Bank criteria (UKPD-BB) by a neurologist or geriatrician with a specialist interest in PD. All participating clinicians are regularly contacted to ensure continuing screening of incident cases diagnosed since study onset. Eligible cases are approached by post and asked to contact the OPDC if they are willing to take part in the study. Exclusion criteria for participation are: non-idiopathic parkinsonism, dementia preceding PD by one year suggestive of Dementia with Lewy Bodies, cognitive impairment precluding informed consent. Atypical parkinsonian features are additionally screened for by the study neurologist using the NINDS Parkinson's tool (see Supplemental Table 1, Appendix A.).

Healthy control subjects are recruited from spouses and friends of PD participants and approached by the PD participants themselves. The PD at-risk group comprises first-degree relatives of PD patients and a smaller group of patients with REM behavior sleep disorder (RBD) diagnosed on polysomnography.

The study was undertaken with the understanding and written consent of each subject, with the approval from the Berkshire Research Ethics Committee and in compliance with national legislation and the Declaration of Helsinki. Subjects may choose to withdraw at any time and do not have to agree to all aspects of the study.

Clinical Assessment Protocol

The Study Protocol was developed by Dr Michele Hu. The choice of data items to be collected was guided by desire to cover the broadest range possible of important demographic, motor and non-motor features of the disease, while maintaining a subject and assessor friendly structure and length of the clinic visit.

Assessment of each data item was performed using previously validated clinical rating scales. Choice of the rating scales in clinical research is guided by principles stipulated in the modern test theory. Specific features of scales that require attention have been summarized concisely in publications by task force groups of the Movement Disorders Society (Evatt *et al.*, 2009; Fernandez *et al.*, 2008; Leentjens *et al.*, 2008; Schrag *et al.*, 2007) and are comprehensively explained in a paper by the Medical Outcomes Trust (Aaronson *et al.*, 2002). The last paper lists 6 major attributes requiring assessment:

- conceptual model – rational for selection of items and their relation to the measured variable;
- reliability, which comprises internal consistency (intercorrelation between items of scale at a point of time) and reproducibility (test-retest and inter-rater agreement)
- validity – degree to which the instrument measures what it purports to measure
- responsiveness – the ability to measure change over time
- interpretability – interpretation that can be assigned to the summary score of the instrument

- burden – the amount of time and effort required to administer the scale.

This theoretical framework served as guidance for selection of scales for current study. However, when comparing multiple scores available for a given clinical variable, no detailed or formal extraction of the above features was undertaken. The reasons for this decision were mainly pragmatic. In the young field of Parkinson's Disease research, particularly with regards to assessment of non-motor features, there are very few instruments that have been tested on PD populations. Moreover, economic limitations played an important part and freely available scales were favoured. Hence, the selection process was primarily constrained by the following factors:

- previous widespread use in PD research to enable comparability with existing body of research
- appropriateness for screening or diagnosis
- availability of the tool free of charge
- ease of self-administration by subject or administration by trained nurse or doctor
- conciseness.

Below follows a description of the individual decisions in the selection process.

Rater-dependent motor features assessment. There are eight major scales measuring motor symptoms and signs in PD: the Columbia University Rating Scale (CURS) (Hely *et al.*, 1993), the Parkinson's Disease Rating Scale by Webster (Webster) (Nouzeilles and Merello, 1997), and the Parkinson's Disease

Impairment Scale (PDIS) (Reynolds and G. K. Montgomery, 1987), the New York University Parkinson's disease evaluation (NYU) (Goldstein, 1980), the University of California Los Angeles scale (UCLA) (Martínez-Martín *et al.*, 1988), the Short Parkinson's Evaluation Scale (SPES) (Rabey *et al.*, 1997), the Unified Parkinson's Disease Rating Scale (UPDRS) (Goetz *et al.*, 2007) and the Scale for Outcomes in Parkinson's Disease (SCOPA) (Marinus *et al.*, 2004). UPDRS is by far the most widely used one and provides the most comprehensive assessment of all body areas. Moreover, it is available free of charge, provides access to training videos and is familiar to researchers, which ensures its easy implementation in the study. Movement Disorders Society UPDRS part III (Goetz *et al.*, 2007; 2008) has been chosen as the main tool for motor assessment in this study.

An integral part of UPDRS is a scale for assessment of motor complications, including dyskinesia and dystonia (part IV of UPDRS). A recent review by the MDS task force on scales to assess dyskinesia (Colosimo *et al.*, 2010) has identified eight scales, including: Abnormal Involuntary Movement Scale (AIMS) (Tonelli *et al.*, 2003), The Unified Parkinson's Disease Rating Scale (UPDRS) part IV (Goetz *et al.*, 2007), the Obeso Dyskinesia Rating Scale (Langston *et al.*, 1992), the Rush Dyskinesia Rating Scale (Goetz *et al.*, 1994), the Clinical Dyskinesia Rating Scale (CDRS) (Hagell and Widner, 2001), the Lang-Fahn Activities of Daily Living Dyskinesia Scale (Parkinson Study Group, 2001), the Parkinson Disease Dyskinesia Scale (PDYS-26) (Katzenschlager *et al.*, 2007), and the Unified Dyskinesia Rating Scale (UDRS) (Goetz *et al.*, 2008). Two scales were recommended for use based on available date: the AIMS scale and the Rush Dyskinesia Rating Scale. This recommendation was not available at the time of

compilation of measurement tools for this study. However, the UPDRS part IV was adopted based on the streamlined administration with the rest of the UPDRS, large body of research using this scale and its ready availability with the UPDRS package.

Rater-independent motor features assessment. In spite of its formalised way of assessing motor features, the UPDRS is very much rater dependent. A range of quantitative instruments have attempted to make the assessment of upper limb function in PD more objective. A search of pubmed.com using terms ((timed OR objective) AND "motor test" AND Parkinson) identified several methods, including: timed pronation-supination and hand-arm movements between two points (Metman *et al.*, 2004), computerised Motor Performance test Series (Pinter *et al.*, 1992), visual response time batteries (Johnson *et al.*, 2004), instrumental finger reaction time (Hayashi *et al.*, 2003), instrumental timed wrist movement task (E. B. Montgomery *et al.*, 2001) and Perdue Peg-board test (Baas *et al.*, 1993). Peg-board testing was chosen for use in this study because it has shown very good performance in assessment of PD symptoms and correlates well with UPDRSIII (Bohnen *et al.*, 2008; Haaxma *et al.*, 2008; Metman *et al.*, 2004). Moreover, is has been shown to correlate with dopaminergic deficit in DAT PET scans (Bohnen *et al.*, 2007).

Quantitative assessment tools of the lower limb function, gait and balance that have been used in PD cohorts include : modified Webster step second test (Baas *et al.*, 1993), timed 7m walk test used in the Core Assessment Program for Intracerebral Transplantation (CAPIT) and Core Assessment Program for Surgical Interventions in Parkinson's disease (CAPSIT-PD) (Defer *et al.*, 2001;

Langston *et al.*, 1992), timed 10m walk test (L. I. I. K. Lim *et al.*, 2005), dynamic gait index (Dibble and M. Lange, 2006) and timed up an go test (Lang *et al.*, 1997). The latter has been chosen for this study in view of its wide use in the literature (Dibble and M. Lange, 2006; Giladi *et al.*, 2010; Kelly *et al.*, 2006; Steffen and Seney, 2008), good clinimetric properties (L. I. I. K. Lim *et al.*, 2005; Morris *et al.*, 2001) and ease of administration.

Berg balance test (Dibble and M. Lange, 2006; L. I. I. K. Lim *et al.*, 2005) and functional reach test (Dibble and M. Lange, 2006; L. I. I. K. Lim *et al.*, 2005) are most widely used for assessment of balance in PD literature. However, Flamingo balance test (Tsigilis *et al.*, 2002) from the Eurofit battery has been used in this study in view of its ease of administration.

Cognitive assessment. A review of cognitive assessment tools in PD by the Cognitive/Psychiatric Working Group of the Parkinson Study Group, available at the time of setting up of the OPDC cohort, identified 10 scales widely used for cognitive assessment in PD: Addenbrooke's Cognitive Examination (Reyes *et al.*, 2009), Alzheimer's Disease Assessment Scale—cognitive (ADAS-cog) (R. G. Stern *et al.*, 1994), Cambridge Cognitive Assessment (CAMCOG) (Hobson and Meara, 1999; 2009), Mattis Dementia Rating Scale (DRS) (Llebaria *et al.*, 2008), Mini-Mental Parkinson (MMP) (Mahieux *et al.*, 1995), Mini-Mental State Examination (MMSE) (Hoops *et al.*, 2009), Montreal Cognitive Assessment (MoCA) (Nasreddine *et al.*, 2005), Parkinson Disease Cognitive Rating Scale (PD-CRS) (Pagonabarraga *et al.*, 2008), Parkinson Neuropsychometric Dementia Assessment (PANDA) (Kalbe *et al.*, 2008), and Scales for Outcomes in Parkinson's disease—cognition (SCOPA-cog) (Marinus, Visser, Verwey, *et al.*, 2003). Five

scales were excluded by the reviewers because their administration required more than 15 minutes (ACE, ADAS-cog, CAMCOG, DRS, and PD-CRS). MOCA was selected as the best of the other five because of its ability to cover all major cognitive domains, sensitivity to subtle cognitive impairment in PD and the fact that it has been tested in studies independent of the original developers. In view of this recommendation MOCA was adapted for use in the OPDC cohort. Additionally, MMSE was also included in the protocol by virtue its widespread use in older studies of PD (Hoops *et al.*, 2009; Kasten *et al.*, 2010; Kulisevsky and Pagonabarraga, 2009; Marinus, Visser, Verwey, *et al.*, 2003). The battery was further complemented with Semantic fluency testing as it was pointed out by the Working Group review, and evidenced in the Cambridge Parkinson Disease study (CamPaIGN) (Williams-Gray *et al.*, 2007; 2009), that this tool is particularly sensitive to transition from non-demented PD to PD dementia.

Mood. The choice of tools for mood assessment was based on two reviews produced by the Movement Disorders Society Task Force on Rating Scales for Parkinson's Disease. The review on depression rating scales (Schrag *et al.*, 2007) identified eight scales previously used in PD: Hamilton Depression Scale (Ham-D) (Hamilton, 1960), the Beck Depression Inventory (BDI) (A. Beck *et al.*, 1961), the Geriatric Depression Scale (GDS) (Yesavage *et al.*, 1982), the Zung Self-Rating Depression Scale (SDS) (Zung, 1965), the Hospital Anxiety and Depression Scale (HADS) (Zigmond and Snaith, 1983), the Montgomery-Asberg Depression Rating Scale (MADRS) (S. A. Montgomery and Asberg, 1979), the Cornell Scale for the Assessment of Depression in Dementia (CSDD) (Alexopoulos *et al.*, 1988), Centre for Epidemiologic Studies Depression Scale (CES-D) (Kirsch-Darrow *et al.*, 2006).

BDI and Ham-D were considered to be the most researched scales in PD cohorts and were also recommended as both screening tools and for assessment of severity of depression. Additionally, HADS and MADRS were thought to be appropriate for screening purposes, while MADRS and SDS were appropriate for assessment of severity. Based on this recommendation BDI was chosen as the primary tool for screening and assessment of severity of depression for the OPDC cohort.

The second review of the Movement Disorders Society Task Force on Rating Scales for Parkinson's Disease considered the selection of scales for assessment of anxiety in PD and identified six scales: Beck anxiety inventory (BAI) (A. T. Beck *et al.*, 1988), the hospital anxiety and depression scale (HADS) (Zigmond and Snaith, 1983), the Zung self-rating anxiety scale (SAS) (ZUNG, 1971), Anxiety Status Inventory (ASI) (ZUNG, 1971), the Spielberger state trait anxiety inventory (STAI) (Siemers *et al.*, 1993), and the Hamilton anxiety rating scale (HARS) (Hamilton, 2011). None of the scales was recommended for use in view of insufficient clinimetric data available in the literature. In view of lack of clear guidance a decision was made, based on personal experience of Dr Michele Hu, to include Leeds Scale for self-assessment of Anxiety and Depression (Snaith *et al.*, 1976) as the screening tool for anxiety and a supportive tool for measurement of depression in the OPDC cohort.

Personality profile. A few studies (Glosser *et al.*, 1995; MA *et al.*, 1990) and a subsequent review (Ishihara and Brayne, 2006) suggested a premorbid personality profile preceding development of PD. Various tools were used to assess personality features in previous studies and a decision was made to

include Big Five Inventory which is one of the most commonly used and freely available tool (John and Srivastava, 1999).

Olfaction. No comparative assessment has been performed to evaluate existing olfactory testing tools in Parkinson Disease. For the reason of brevity of testing only standardized tests available commercially were considered. The AAN has issued a Practice Parameter for diagnosis of new onset PD (Suchowersky *et al.*, 2006), which suggests use of one of two most commonly used instruments: University of Pennsylvania Smell Identification Test (UPSIT) (Doty *et al.*, 1984) and Sniffin' Sticks (Hummel *et al.*, 2007). For economical reasons Sniffin' Sticks was chosen for assessment of olfactory function in the OPDC cohort.

Sleep. Definite diagnosis of REM Behaviour Sleep Disorder requires overnight polysomnography, which is labour intensive, expensive and impractical in a cohort study. Using screening questionnaires for identifying clinically probable cases of RBD represents a more practicable method and one suggested and used in clinical studies (Postuma *et al.*, 2010). At the time of questionnaire selection for the OPDC cohort there were no clear guidelines as to use of RBD scales in PD. A literature review identified 7 tools that had been used for that purpose in PD studies: not validated sleep questionnaire by Comella et al. (Comella *et al.*, 1998), unstructured interview (Eisensehr *et al.*, 2001), semi-structured interview (Scaglione *et al.*, 2005) or structured interview (Gagnon *et al.*, 2002) assessing for presence of RBD criteria from International Sleep Disorders Classification symptom list (Baumann *et al.*, 2005), unvalidated Mayo Sleep Questionnaire (Ozekmekçi *et al.*, 2005), RBD non-specific sleep

questionnaire by Pacchetti et al. (Pacchetti *et al.*, 2005), unvalidated Stavanger Sleepiness Questionnaire (Gjerstad *et al.*, 2008), unvalidated questionnaire by Vibha et al. (Vibha *et al.*, 2010), validated RBD questionnaire by Stiasny-Kolster (Bugalho *et al.*, 2011). The only instrument that was validated for RBD screening was the Stiasny-Kolster (Stiasny-Kolster *et al.*, 2007) questionnaire and it was chosen for use in the OPDC cohort.

The choice of scale for day-time sleepiness assessment was based on the MDS Sleep Scale Task Force recommendations (Högl *et al.*, 2010), which identified six scales thought to be equally valid for assessing daytime sleepiness in PD: the PD Sleep Scale (PDSS) (Chaudhuri *et al.*, 2002), the Pittsburgh Sleep Quality Index (PSQI) (Buysse *et al.*, 1989), the SCOPA-sleep (SCOPA) (Marinus, Visser, van Hilten, *et al.*, 2003), the Epworth Sleepiness Scale (ESS) (Johns, 1991), the Inappropriate Sleep Composite Score (ISCS), the Stanford Sleepiness Scale (SSS) (Hoddes *et al.*, 1973). The ESS (Johns, 1991) was chosen for use in OPDC cohort based on its brevity and familiarity to the assessors in the study.

Impulse control disorders. At the time of questionnaire selection for the OPDC cohort there were no clear guidelines as to the use of questionnaires for impulse control disorders in PD. A review of the literature showed that many different approaches to screening and diagnosis of those conditions in PD had been taken, including mixing and matching of already available instruments to cover the spectrum present in PD, and devising tailor-made tools. The first group included the following instruments: Minnesota Impulsive Disorders Interview (MIDI) (Weintraub *et al.*, 2006), DSM-IV-TR criteria for pathological gambling (Dodd *et al.*, 2005), South Oaks Gambling Screen (SOGS) (Voon, Hassan,

Zurowski, Duff-Canning, *et al.*, 2006), Barratt Impulsiveness Scale (BIS-11A) (Isaias *et al.*, 2008), Maudsley Obsessional-Compulsive Inventory (MOCI) (Isaias *et al.*, 2008), compulsive shopping criteria by McElroy (Voon *et al.*, 2007), hypersexuality criteria by Caplan (Klos *et al.*, 2005), compulsive buying questionnaire by Lejoyeux (Voon, Hassan, Zurowski, de Souza, *et al.*, 2006). Instruments devised specifically for PD included: compulsive medication use in PD criteria by Giovannoni (Giovannoni *et al.*, 2000), hypersexuality in PD criteria by Voon (Voon *et al.*, 2007), punding questionnaire in PD by Evans (Evans *et al.*, 2004), hedonistic homeostatic dysregulation questionnaire in PD by Pezzella (Pezzella *et al.*, 2003) and Questionnaire for Impulsive-Compulsive Disorders in Parkinson's Disease (QUIP) (Weintraub *et al.*, 2009). The last questionnaire was chosen for the OPDC cohort in view of its broad coverage of impulsive disorders encountered in PD and good clinimetric features in this disorder. All the other methods were either unspecific for PD or did not cover the full spectrum of conditions present in PD.

Clinic visits. Patients were assessed in research clinics, by a study nurse and a doctor, using of the above presented set of questionnaires covering a spectrum of demographic information and motor and non-motor features of PD (questionnaire list in Supplemental Table 1, Appendix A.). Cognitive assessments were administered in standardized way by Dendron research nurses who had been trained by a clinical psychologist. Patients were also examined neurologically and gave a blood sample for DNA and serum biomarkers. Visits are scheduled every 18 months for 5 years initially, but the cohort is envisaged to continue for the next 10-20 years, subject to funding.

Statistical Analysis

We have presented data from an interim analysis of the PD patients and control groups including participants recruited between study onset in September 2010 up to August 2012. In the PD group, we have only included patients who were thought by the clinician to have a ≥90% likelihood of PD diagnosis. Details of recording of the raw variables are shown in the Supplementary Table 1. Basic demographic characteristics were compared using χ^2-test and unpaired *t*-test. Clinical characteristics requiring adjustment for covariates were analyzed using multivariable linear or logistic regression. To examine age and gender effects we classified PD subjects into three age groups (≤65, 65-75, >75 years) and stratified analyses by age group and gender. Most comparisons were adjusted for age and gender and, in some analyses, for additional variables that were thought to be potential confounders. Where the pattern of phenotypic features varied by age, we undertook a formal Cochrane-Armitage test for trend. A conventional threshold of 0.05 was set for statistical significance, though given the large number of comparisons any results between the 0.01 to 0.05 should be interpreted with caution as they may reflect a type I error due to multiple testing.

Candidate's contribution to the study

Oxford Parkinson's Disease Centre patient cohort was set up by Dr. Michele Hu, Honorary Clinical Lecturer at the Department of Neurology, University of Oxford. Dr. Hu selected study questionnaires and organized the clinical infrastructure. The candidate formatted study questionnaires, designed data entry forms for the study database and monitored data quality. Clinical data was collected by four study doctors, including the candidate. The candidate designed

the interim statistical analysis of the data, performed all statistical analyses, interpreted the results and wrote the original paper on which the above chapter is based.

Section 3. Results

In the analyzed period, 624 PD patients agreed to take part in our study, making up 57% of all approached subjects. Non-participants were significantly older than participants (mean (SD) age 73.0 (10.6) years, $p<0.001$), but with no gender differences. Additionally, 176 healthy controls (HC) were recruited into the study in the above period. Of the 624 PD patients, 490 had a ≥90% likelihood of PD diagnosis, as judged by the study clinician.

Demographics and General Medical History

Table 1 shows the demographics and general medical history of the PD and HC groups. The PD cohort was older and had more males than females compared to the control, but did not differ in ethnicity, handedness or marital status. We found no differences in past medical history but smoking and alcohol consumption before PD onset were less common in PD than HC whilst there were no differences seen for caffeine consumption. There were no differences in the risk of cardiovascular disease, all cancers, melanoma or gout. We could not compare the family history of PD between the two groups as control participants with any relatives with PD were re-assigned to our 'at-risk' cohort.

PD patients had lower socioeconomic status than controls (Table 2). For example, they were less educated than control subjects and less likely to live in their own accommodation or be in employment. There was no significant

difference in types of medications taken before diagnosis of PD by patients and controls (Table 2).

Table 1. Basic demographics and Past Medical and Family History. Comparison between PD and HC groups.

Dependent Variable	PD Group N=490	HC Group N=176	P value
Basic demographics			
Age, (mean ± SD)	67.9 ± 9.3	64.3 ± 9.1	<0.001
Gender, (females, %)	37.6	63.6	<0.001
Ethnicity, (White:Non-white, %)	98.4:1.6	98.3:1.7	0.57
Marital status, (married, %)	96.9	98.8	0.08[a]
Handedness, (right:left:both, %)	87.7:9.0:3.3	86.6:9.3:4.7	0.74
Past Medical History			
Vascular risk factors, (%)	45.7	43.2	0.40[a]
Cerebrovascular disease, (%)	4.3	1.7	0.18[c]
Cardiovascular disease, (%)	12.7	4.6	0.06[c]
Cancers, (%)	9.2	8.0	
Males, (%)	11.4	7.8	0.56[c]
Females, (%)	5.4	8.0	0.17[c]
Melanoma, (%)	2.1	2.8	0.11[a]
Respiratory disease, (%)	11.6	9.1	0.20[c]
Rheumatoid arthritis, (%)	1.22	0.57	0.41[a]
Gout, (%)	6.4	4.0	0.72[a]
Ovarian resection before PD, (%)	12.9	12.2	0.88[a]
Depression diagnosis before PD, (%)	18.0	22.2	0.74[b]
Anxiety diagnosis before PD, (%)	12.9	14.2	0.51[b]
Caffeine consumption before PD, cups/day, (mean ± SD)	5.3 ± 3.6	5.0 ± 2.3	0.51[c]
Smoking before PD, (%)	39.1	44.3	0.008[c]
Alcohol consumption before PD, (%)	73.7	81.1	0.03[b]
Family History			
Patients with at least one 1st degree relative with PD, (%)	16.7	N/A	N/A
Patients with at least one 2nd degree relative with PD, (%)	9.2	N/A	N/A
Patients with at least one 1st or 2nd degree relatives with PD	23.7	N/A	N/A
Subjects with at least one 1st or 2nd degree relative with any neurological disorder, (%)	45.7	54.6	0.26[a]

[a] Adjusted for age, gender
[b] Adjusted for age, gender, anxiety
[c] Adjusted for age, gender and Caffeine consumption (for Smoking) or Smoking (for Caffeine consumption, Cerebrovascular and Cardiovascular disease)
[d] Adjusted for age, gender, caffeine consumption and smoking

Table 2. Comparison of social background and pre-morbid medication history between PD and HC groups.

Dependent Variable	**PD Group N=490**	**HC Group N=176**	**P value**
Social background			
Years of education, (mean ± SD)	13.7 ± 3.58	14.9 ± 3.49	<0.001[a]
Accommodation at diagnosis, (own, %)	92.0	96.6	0.05[a]
Bedrooms at diagnosis, (≤3, %)	42.8	50.3	0.18[a]
Vehicles at diagnosis, (≤1, %)	44.1	52.8	0.24[a]
Employment at diagnosis, (employed, %)	36.1	38.6	0.02[a]
Last job, (managing people, %)	28.5	32.6	0.07[b]
Medication before PD			
OTC NSAIDS, paracetamol, aspirin; any use, (%)	69.6	66.5	0.41[a]
COX-2 inhibitors, any use, (%)	0.82	0.57	0.62[a]
Prescription NSAIDS, any use, (%)	22.9	21.0	0.42[a]
Statins, any use, (%)	33.5	26.7	0.95[a]
Hormonal treatment, any use, (%)	46.2	55.4	0.15[a]
L-type Ca antagonists, any use, (%)	5.1	2.8	0.35[a]
Amlodipine, any use, (%)	10.6	12.5	0.07[a]
Neuroleptics, any use, (%)	1.43	0.57	0.30[a]

[a] Adjusted for age, gender

[b] Adjusted for age, gender, years of education

Disease Onset, Motor Features and PD medication

The average disease duration since diagnosis was 1.74 years (Table 3) while duration since symptom onset was 3.36 years. The commonest symptom at diagnosis was bradykinesia, followed by tremor and postural problems. In 51% of patients the symptoms started on the left-hand side of the body, while only 7% had symmetrical onset. The average UPDRS-III was 27.0 with 80% of patients classified as H&Y I-II –a consequence of recruiting patients with early disease. Out of four cardinal motor features tested by UPDRS-III, bradykinesia and rigidity scores were highest with postural scores in the middle and tremor scores lowest. Greater severity of postural problems than tremor was also reflected in higher rates of PIGD-type than tremor-type. The most severely affected body region were the arms, followed by legs, face and neck. Motor complications (UPDRS-IV) were infrequent with only 5% and 4% of patients manifesting dyskinesia and motor fluctuations, respectively.

Stratification by age (Table 3) revealed that rigidity at onset was less common in older patients while rate of postural problems increased with age. The UPDRS-III score and subscores (except rigidity), annualized UPDRS-III, symmetricality of symptoms and objective measures of severity (Peg-board, Get-up-and-go, Flamingo) significantly increased with age while UPDRS-IV decreased.

Analysis of gender effects on motor features (Table 3) showed that postural problems at onset were more common in women which was also confirmed by higher average postural scores in UPDRS-III, longer time on Get-up-and-go and greater proportion of women with PIGD subtype and H&Y≥3. Bradykinesia at

onset was also more common amongst women but reached the same level as in men on the first visit. Rigidity was significantly higher in men, while total UPDRS-III showed a tendency in that direction. Men also showed significantly higher UPDRS-III in the face, neck and arms with no difference in leg scores. Greater severity in the arms was also reflected in poorer performance on all peg-board tests by men. Men had more symmetrical disease both on UPDRS-III and peg-board asymmetry index. There was no evidence that age or gender in this cohort influenced time from symptom onset to disease diagnosis, as it has been speculated that older patients and women may be slower to present to health care professionals.

Table 3. Disease onset and motor symptoms stratified by age group and gender.

Dependent Variable	Total N=490	Age stratification				Gender stratification		
		≤65 N=178	>65, ≤75 N=204	>75 N=108	P for trend	Males N=306	Females N=184	P value
Disease Onset								
Age at diagnosis, (mean ± SD)	66.1 ± 9.5	56.4 ± 6.7	68.6 ± 3.3	77.7 ± 3.8	<0.001[b]	66.1 ± 9.2	65.7 ± 9.9	0.71[a]
Age at onset, (mean ± SD)	64.5 ± 9.5	54.9 ± 6.7	66.8 ± 3.7	76.1 ± 4.1	<0.001[b]	64.8 ± 9.2	64.1 ± 10	0.29[a]
Duration since diagnosis (y), (mean ± SD)	1.74 ± 1.8	1.81	1.68	1.74	0.70[b]	1.7 ± 1.6	1.8 ± 2.2	0.71[a]
Duration since symptom onset (y), (mean ± SD)	3.36 ± 2.42	3.3 ± 2.6	3.4 ± 2.3	3.4 ± 2.4	0.70[b]	3.3 ± 2.3	3.5 ± 2.7	0.29[a]
Time from symptom onset to diagnosis (y), (mean ± SD)	1.67 ± 1.7	1.4 ± 1.4	1.8 ± 1.8	1.7 ± 1.8	0.19[b]	1.6 ± 1.7	1.7 ± 1.7	0.36[a]
Tremor at diagnosis, (%)	86.9	84.2	88	88.9	0.22[c]	87.2	86.4	0.83[d]
Rigidity at diagnosis, (%)	78.2	81.9	76.4	71	0.05[c]	76.5	81	0.24[d]
Bradykinesia at diagnosis, (%)	92.0	90.2	93.5	93.3	0.34[c]	89.9	95.6	0.05[d]
Postural problems at diagnosis, (%)	29.8	26	27.1	41	0.04[c]	25.5	36.8	0.03[d]
Symmetrical onset, (%)	6.5	3.5	6.9	5.8	0.76[c]	7.8	4.4	0.17[d]

Table 3 continued. Disease onset and motor symptoms stratified by age group and gender.

Dependent Variable	Total N=490	Age stratification				Gender stratification		
		≤65 N=178	>65, ≤75 N=204	>75 N=108	P for trend	Males N=306	Females N=184	P value
UPDRS III								
Total UPDRS III, (mean ± SD)	27.0 ± 11.1	24.4 ± 9.3	26.8 ± 11.2	31.9 ± 12.3	<0.001[e]	27.7 ± 11.2	25.9 ± 11	0.07[f]
H&Y ≥3, (%)	7.8	2.2	5.9	20.4	<0.001[e]	5.2	12	0.007[f]
Rigidity scores, (score/number of items, mean ± SD)	1.06 ± 0.51	1 ± 0.5	1 ± 0.5	1.1 ± 0.6	0.10[e]	1.1 ± 0.5	1 ± 0.5	0.002[f]
Bradykinesia scores, (score/number of items, mean ± SD)	1.18 ± 0.6	1.1 ± 0.5	1.1 ± 0.6	1.3 ± 0.7	0.02[e]	1.2 ± 0.6	1.2 ± 0.6	0.49[f]
Postural scores, (score/number of items, mean ± SD)	0.55 ± 0.48	0.4 ± 0.3	0.5 ± 0.5	0.8 ± 0.6	<0.001[e]	0.5 ± 0.4	0.6 ± 0.5	0.06[f]
Tremor scores, (score/number of items, mean ± SD)	0.38 ± 0.29	0.3 ± 0.3	0.4 ± 0.3	0.4 ± 0.3	0.008[e]	0.4 ± 0.3	0.4 ± 0.3	0.27[f]
Motor subtype, (PIGD, %)	52	48.9	47.5	67.6	0.006[e]	48.4	59.2	0.02[f]
Face and neck score, (score/number of items, mean ± SD)	0.58 ± 0.39	0.5 ± 0.33	0.58 ± 0.37	0.73 ± 0.45	<0.001[e]	0.66 ± 0.40	0.45 ± 0.32	<0.001[e]
Arms score, (score/number of items, mean ± SD)	0.97 ± 0.40	0.92 ± 0.37	0.95 ± 0.40	1.08 ± 0.46	0.005[e]	1.00 ± 0.42	0.92 ± 0.37	0.025[e]
Legs score, (score/number of items, mean ± SD)	0.75 ± 0.45	0.71 ± 0.39	0.74 ± 0.48	0.84 ± 0.45	0.29[e]	0.75 ± 0.43	0.75 ± 0.47	0.922[e]
Symmetrical disease, (%)	25	16.3	23.5	40.7	<0.001[e]	28.1	19	0.03[f]
Annualised UPDRS III (UPDRS III/disease duration), (mean ± SD)	11.5 ± 9.7	10.2 ± 7.2	11.5 ± 10.8	13.5 ± 10.7	<0.001	12	10.6	0.33

Table 3 continued. Disease onset and motor symptoms stratified by age group and gender.

Dependent Variable	Total N=490	Age stratification				Gender stratification		
		≤65 N=178	>65, ≤75 N=204	>75 N=108	P for trend	Males N=306	Females N=184	P value
Observer-independent tests								
Right arm dexterity, Peg board, (mean ± SD)	10.0 ± 2.6	11.1 ± 2.5	9.7 ± 2.5	8.9 ± 2.5	<0.001[e]	9.4 ± 2.4	11 ± 2.6	<0.001[f]
Left arm dexterity, Peg board, (mean ± SD)	9.7 ± 2.5	10.6 ± 2.5	9.5 ± 2.3	8.7 ± 2.4	<0.001[e]	9.3 ± 2.4	10.3 ± 2.5	<0.001[f]
Arm coordination, Peg board, (mean ± SD)	16.8 ± 6.2	19.4 ± 6.6	16.4 ± 5.1	13.3 ± 5.5	<0.001[e]	16 ± 6	18 ± 6.3	<0.001[f]
Arm asymmetry (absolute value of right score - left score), Peg board, (mean±SD)	0.31 ± 2.53	0.5 ± 2.7	0.2 ± 2.5	0.2 ± 2.4	0.338[e]	0.1 ± 2.5	0.6 ± 2.5	0.027f
Get-up-and-go, seconds, (mean ± SD)	10.3 ± 4.3	9.2 ± 4.2	9.9 ± 3.3	12.7 ± 5	<0.001[e]	9.8 ± 3.4	11 ± 5.3	0.001[f]
Flamingo test dichotomised passed, (%)	45.3	66.7	41.4	18.4	<0.001[e]	46	44.2	0.35[f]

[a] Adjusted for age
[b] Adjusted for gender
[c] Adjusted for gender and age at diagnosis
[d] Adjusted for age at diagnosis
[e] Adjusted for disease duration, gender
[f] Adjusted for age, disease duration

Overall, we found that the most common UPDRS-II symptom (Table 4) was tremor, followed by axial symptoms (getting out of bed; walking and balance). Stratification by age (Table 4) revealed strong effects for dressing problems, turning in bed and getting out of bed. There were also very marked differences in medication so that older patients were more likely to be on levodopa and a higher dosage and far less likely to be on an agonist, though clinical responsiveness was similar by age. There were also marked gender differences for the motor aspects of daily living activities with men reporting more problems with speech, drooling, chewing, swallowing and handwriting and leading to an overall worse total UPDRS-II. Interestingly, women were significantly more likely than men to take dopamine agonists though this result should be viewed with caution.

Non-motor symptoms

Non-motor features of the two cohorts are summarized in Table 5. All indices of cognitive performance were lower in the PD group (MMSE, MOCA, phonemic and semantic fluency). This was also reflected in more patients scoring within the MCI and dementia range. Patients had differing personality profiles (lower extraversion, conscientiousness and openness; higher neuroticism). Depression and anxiety were more common amongst patients (24.5% and 22%), even after controlling for effects of those variables on each other. Other non-motor features with significant differences between patients and controls were: hyposmia, daytime sleepiness, RBD and orthostatic hypotension. Rates of constipation were not different between groups, however use of laxatives was significantly more common in PD

Table 4. UPDRS (parts II and IV) and PD medication stratified by age group and gender.

Dependent Variable	Total N=490	Age stratification				Gender stratification		
		≤65 N=178	>65, ≤75 N=204	>75 N=108	P for trend	Males N=306	Females N=184	P value
UPDRS II, Motor Aspects of Experience of Daily Living								
Total UPDRS II, (mean ± SD)	9.4 ± 6.2	9.3 ± 6.3	8.5 ± 5.8	11.2 ± 6.6	0.07[a]	9.8 ± 6.2	8.6 ± 6.2	0.03[b]
Speech problems, (%)	40.4	47.5	34.7	44.9	0.267[a]	47.7	31.3	<0.001[b]
Saliva & drooling, (%)	50.2	49.7	49.3	57	0.23[a]	58.4	39	<0.001[b]
Chewing & swallowing, (%)	22.0	26	16.7	29	0.962[a]	26.2	17	0.02[b]
Eating problems, (%)	45.3	52.5	38.9	49.5	0.363[a]	48.5	42.3	0.12[b]
Dressing problems, (%)	59.0	56.5	58.1	69.2	0.045[a]	63	54.9	0.06[b]
Hygiene, (%)	39.4	43.5	34.5	45.8	0.983[a]	42.6	36.3	0.13[b]
Handwriting problems, (%)	60.4	66.1	54.2	67.3	0.829[a]	67.2	51.6	<0.001[b]
Hobbies, (%)	60.2	61.6	57.6	68.9	0.395[a]	62.6	59.7	0.46[b]
Turning in bed, (%)	52.2	47.7	53.7	62.6	0.014[a]	51.8	56.4	0.38[b]
Tremor, (%)	79.4	81.4	80.8	78.5	0.701[a]	80	81.3	0.80[b]
Getting out of bed, chair, (%)	67.6	58.2	70.9	81.3	<0.001[a]	68.9	68.1	0.83[b]
Walking and balance, (%)	63.1	65.5	58.4	73.8	0.362[a]	63.8	65.4	0.79[b]
Freezing, (%)	16.5	19.2	13.3	21.5	0.935[a]	16.7	18.1	0.86[b]

Table 4 continued. UPDRS (parts II and IV) and PD medication stratified by age group and gender.

Dependent Variable	Total N=490	Age stratification				Gender stratification		
		≤65 N=178	>65, ≤75 N=204	>75 N=108	P for trend	Males N=306	Females N=184	P value
UPDRS IV, Motor Complications								
Total UPDRS IV, (mean ± SD)	0.34 ± 1.37	0.6 ± 2	0.2 ± 0.8	0.2 ± 0.6	0.005[a]	0.3 ± 1.4	0.3 ± 1.4	0.63[b]
Presence of Dyskinesia, (%)	5.3	7.3	4.4	3.7	0.138[a]	5.9	4.3	0.29[b]
Presence of Motor Fluctuations, (%)	4.1	6.2	3	3.7	0.242[a]	4.6	3.8	0.42[b]
PD Medication								
Total patients on medication, (%)	88.6	87.1	90.2	88.0	0.793[a]	88.2	89.1	0.92[b]
Total LEDD, (mean ± SD)	335 ± 211	264.1 ± 229.5	307.1 ± 232.5	314.2 ± 206	0.046[a]	302.4 ± 221.1	277.5 ± 234.8	0.09[b]
Number on Levodopa, (%)	53.9	28.1	62.3	80.6	<0.001[a]	56.9	48.9	0.07[b]
Number on agonists, (%)	36.1	56.2	32.8	9.3	<0.001[a]	32.4	42.4	0.05[b]
Number on MAO-B inhibitors, (%)	26.9	34.8	28.4	11.1	<0.001[a]	28.8	23.9	0.17[b]
Response to medication, Clinical Global Impression of Change, (%)	81.7	87.9	75.1	84.4	0.262[a]	81.1	82.6	0.74[b]

[a] Adjusted for disease duration, gender

[b] Adjusted for age, disease duration.

Table 5. Comparison of non-motor symptoms between PD and HC groups.

Dependent Variable	**PD Group N=490**	**HC Group N=176**	**P value**
General health state, EQ-VAS (mean ± SD)	86.7 ± 10.7	98.5 ± 4.8	<0.001[a]
Cognitive			
MMSE, (mean ± SD)	27.3 ± 2.6	28.3 ± 1.9	<0.001[b]
MOCA, (mean ± SD)	24.9 ± 3.5	27 ± 2.4	<0.001[a]
Phonemic Fluency, (mean ± SD)	10.9 ± 3.9	12.7 ± 3.9	<0.001[c]
Semantic Fluency, (mean ± SD)	10.1 ± 3.4	12 ± 3.4	<0.001[c]
Cognitive impairment, breakdown, (normal:MCI:dementia, %)	48.2:40.0:11.9	76.0:23.4:1.1	N/A
MCI/nor	40.0:48.2	23.4:76.0	0.003[b]
Dem/nor	11.9:48.2	1.1:76.0	0.002[b]
Psychological/Psychiatric			
BFI Personality traits			
Extraversion, (mean ± SD)	23.8	27.1	<0.001[a]
Agreeableness, (mean ± SD)	36.8	37.3	0.36[a]
Conscientiousness, (mean ± SD)	35.8	36.9	0.01[a]
Neuroticism, (mean ± SD)	22.4	19.8	<0.001[a]
Openness, (mean ± SD)	34.1	36.8	<0.001[a]
Depression, Leeds SAD General, (%)	24.5	8.5	0.004[g]
Anxiety, Leeds SAA General, (%)	22.0	5.1	<0.001[g]
Any ICD, QUIP, (%)	9.8	6.3	0.54[h]
Any other OCD, QUIP, (%)	13.9	12.5	0.30[h]
Autonomic & other			
BMI, (mean ± SD)	27.4 ± 4.8	27.8 ± 5.1	0.36[a]
Hyposmia, Sniffin Sticks, (%)	74.3	9.7	<0.001[d]
Sleepiness, ESS, (%)	24.1	6.3	0.009[e]
RBD, RBD-SQ, (%)	43.5	11.4	<0.001[e]
Constipation, (%)	41.0	33.7	0.07[f]
Use of laxatives, (%)	21.8	6.3	<0.001[a]
Orthostatic hypotension, (%)	23.7	7.4	<0.001[a]
Pain, EQ5D, (%)	52.1	40.2	<0.001[a]

[a] Adjusted for age, gender
[b] Adjusted for age, gender, education
[c] Adjusted for gender, education
[d] Adjusted for age, gender, smoking
[e] Adjusted for age, gender, and RBD or ESS status
[f] Adjusted for age, gender, laxative use, exercise, fruit consumption
[g] Adjusted for age, gender and HADS depression or anxiety status
[h] Adjusted for age, gender and dopaminergic agonist use

Age influenced the presence of non-motor features (Table 6) usually worsening them though in some cases reducing the frequency. Specifically, general health state, cognitive function, use of laxatives, orthostatic hypotension, urinary problems and erectile dysfunction worsened whilst impulse control disorders, anxiety and hallucinations were better in older patients. Further adjustment for agonist use made the effect of age on hallucinations insignificant. Female patients had milder cognitive problems, daytime sleepiness, RBD features, orthostatic hypotension and sexual dysfunction but worse pain scores.

Table 6. Non-motor symptoms stratified by age group and gender in PD group.

Dependent Variable	Total N=490	Age stratification				Gender stratification		
		≤65 N=178	>65, ≤75 N=204	>75 N=108	P for trend	Males N=306	Females N=184	P value
General health state, EQ-VAS, (mean ± SD)	86.7 ± 10.7	88.2 ± 7.8	87.3 ± 11	83.1 ± 13.3	0.001[a]	87.4 ± 9.6	85.6 ± 12.2	0.09[c]
Total UPDRS I score, Non-motor symptoms, (mean ± SD)	9.0 ± 5.2	9.2 ± 5.8	8.4 ± 4.5	9.8 ± 5.5	0.49[a]	8.8 ± 5	9.4 ± 5.6	0.22[c]
Cognitive measures								
MMSE, (mean ± SD)	27.3 ± 2.6	28 ± 2.1	27.2 ± 2.2	26.5 ± 2.3	<0.001[b]	27.5 ± 2.1	27.1 ± 2.4	0.27[d]
MOCA, (mean ± SD)	24.9 ± 3.5	26.3 ± 2.7	24.7 ± 3.6	23.1 ± 3.4	<0.001[b]	24.5 ± 3.5	25.5 ± 3.4	<0.001[d]
Phonemic Fluency, (mean ± SD)	10.9 ± 3.9	11.6 ± 3.6	10.6 ± 4.3	10.4 ± 3.7	0.07[b]	10.7 ± 4	11.3 ± 3.8	0.02[d]
Semantic Fluency, (mean ± SD)	10.1 ± 3.4	10.7 ± 3.2	10 ± 3.6	9.1 ± 3.1	<0.001[b]	9.6 ± 3.4	10.8 ± 3.3	<0.001[d]
Psychological/Psychiatric								
BFI Personality traits								
Extraversion, (mean ± SD)	23.8	24.5 ± 6.6	24 ± 6.1	22.1 ± 6.4	0.01[a]	23.4 ± 6.4	24.4 ± 6.3	0.11[c]
Agreeableness, (mean ± SD)	36.8	36.6 ± 5.2	36.6 ± 5.2	37.3 ± 4.9	0.37[a]	36.2 ± 5.3	37.6 ± 4.7	0.004[c]
Conscientiousness, (mean ± SD)	35.8	35.3 ± 5.8	36.3 ± 5.5	35.5 ± 6.1	0.52[a]	35.2 ± 5.9	36.8 ± 5.3	0.004[c]
Neuroticism, (mean ± SD)	22.4	23.1 ± 6.9	22.4 ± 6	21.2 ± 7.3	0.11[a]	21.8 ± 6.7	23.4 ± 6.6	0.01[c]
Openness, (mean ± SD)	34.1	34.7 ± 7.3	34.1 ± 7.2	33 ± 6.8	0.16[a]	34.7 ± 7.3	33.1 ± 6.9	0.02[c]
Depression, Leeds SAD General, (%)	24.5	27.5	20.3	28.3	0.42[a]	22.0	29.1	0.45[c]
Anxiety, Leeds SAA General, (%)	22.0	29.4	19.3	16.2	0.007[a]	18.8	28.3	0.06[c]
Any ICD, QUIP, (%)	9.8	24.3	8.6	5.8	<0.001[g]	14	13.2	0.34[f]
Any other OCD, QUIP, (%)	13.9	26.1	14.1	4.8	<0.001[g]	17.9	14	0.07[f]
Overmedicating, QUIP, (%)	2.3	4.7	1	1	0.05[g]	2.7	1.7	0.49[c]

Dependent Variable	Total N=490	Age stratification				Gender stratification		
		≤65 N=178	>65, ≤75 N=204	>75 N=108	P for trend	Males N=306	Females N=184	P value
Fatigue in PD, UPDRS I, (%)	72.8	74.6	67.8	79.4	0.67[a]	72.4	73.6	0.83[c]
Apathy in PD, UPDRS I, (%)	18.2	23.6	14.7	15.7	0.06[a]	17.6	19	0.81[c]
Hallucinations in PD, UPDRS I, (%)	14.7	19.7	13.3	9.3	0.01[a] 0.12[g]	15.4	13.6	0.43[c]
Autonomic & other								
BMI, (mean ± SD)	27.4 ± 4.8	27.9 ± 5.4	27.1 ± 4.5	26.9 ± 4.3	0.04[a]	27.5 ± 4.3	27.1 ± 5.6	0.39[c]
Hyposmia, Sniffin Sticks, (%)	74.3	78.6	78	87.1	0.13[a]	82.3	76.7	0.16[c]
Sleepiness, ESS, (%)	24.1	25.8	21.6	27.1	0.99[a]	28.4	17.5	0.004[c]
RBD, RBD-SQ, (%)	43.5	47.5	45	44.7	0.55[a]	49	40.6	0.05[c]
Constipation, (%)	41.0	39.5	42.3	53.4	0.08[a]	41.6	47	0.19[c]
Use of laxatives, (%)	21.8	15.3	24.6	30.8	0.002[a]	23.4	21.2	0.55[c]
Orthostatic hypotension, (%)	23.7	16.9	22.8	38.9	<0.001[a]	28.3	17.4	0.008[c]
Pain in PD, UPDRS I, (%)	52.1	83.6	75.2	85	0.92[a]	77	86.3	0.02[c]
Lightheadedness, UPDRS I, (%)	44.0	44.1	41.1	49.5	0.40[a]	43.4	45.1	0.76[c]
Urinary problems, UPDRS I, (%)	64.4	59.9	63.4	73.8	0.02[a]	64.1	64.8	0.83[c]
Sexual dysfunction, (%)	19.3	18.9	21.4	16	0.70[a]	28.4	4	<0.001[c]
Erectile dysfunction, (%)	42.8	33.7	56.8	68.3	<0.001[e]	**N/A**	**N/A**	

[a] Adjusted for disease duration, gender
[b] Adjusted for years of education, disease duration, gender
[c] Adjusted for age, disease duration
[d] Adjusted for age, years of education, disease duration
[e] Adjusted for disease duration
[f] Adjusted for age at onset, gender, disease duration and dopaminergic agonist use
[g] Adjusted for disease duration, gender, use of dopaminergic agonists

Section 4. Discussion

In order to compare characteristics of our cohort with other large studies we performed a pubmed.com search which yielded 59 publications describing 35 cohorts of PD patients (Table 7). Eight of those focused on patients with early stage disease within 4 years of diagnosis/onset (bold highlight). The average age of those patients was slightly lower than in our cohort with the ParkWest (Alves *et al.*, 2009) cohort being most similar. The gender composition was comparable to our group while the UPDRS-III was considerably lower in other studies.

There are three major features of our study, which set us apart from other published cohorts. Firstly, the OPDC cohort is the only one of the early cohorts recruiting a control group, providing a unique opportunity to compare patient characteristics with healthy subjects. Secondly, the breadth of clinical features covered, comparable only to the ParkWest cohort (Alves *et al.*, 2009), enables us to characterize the disease picture in a lot of detail. Thirdly, our cohort has one of the highest numbers of participants with only the NINDS-PD Long Term Study 1 (Elm, The NINDS NET-PD Investigators, 2012) and the DATATOP study (Parkinson Study Group, 1989) reporting larger number of patients.

Table 7. Large cohort studies in Parkinson's Disease. Studies in bold recruited early-stage patients with disease duration < 4 years.

	Cohort Name	**Number of PD**	**Number of Controls**	**Age of PD (years)**	**Males (%)**	**Disease Duration (years)**	**Average UPDRSIII**	**Average Hoehn & Yahr**
Špica *et al.*, 2013	Belgrade, Serbia	101 / 107[a]		51.8 / 69.1[a]	74 / 68[a]	11.1 / 7.1[a]	34.6 / 35.1[a]	2.2 / 2.3[a]
Vassar *et al.*, 2012	The Parkinson's Environment and Gene Study (PEG), USA	193		72	59	5.2	25	
Elm, The NINDS NET-PD Investigators, 2012	**NINDS-PD Long Term Study 1, USA**	**1741**		**62**	**65**	**1.5**	**18**	
Perez-Lloret *et al.*, 2012	CoPark study, France	419		69	57	6		2
Shearer *et al.*, 2012	**PINE study, UK**	**162**		**72**	**57**	**2**	**24**	**2.1[b]**
Solla *et al.*, 2012	Sardinia, Italy	156		69	58	6.3		2.4
Martinez-Martin *et al.*, 2012	Non-Motor International Longitudinal Study, NILS, International	950		64	63	8		2.5[b]
Andreadou *et al.*, 2011	Athens cohort, Greece	139		70	49	8.5	25	2.1
Winter *et al.*, 2011	EuroPa, Italy	70		65	59	5.6	16	
R. G. Brown *et al.*, 2011	PROMS-PD, UK	513		68	65	6.9	26	2.5[b]
Wickremaratchi *et al.*, 2011	Cardiff, UK	380		65	63	9	27	
Winter,	EuroPa, Russia	100	100	69	38	6.7	33	2.5[b]

	Cohort Name	Number of PD	Number of Controls	Age of PD (years)	Males (%)	Disease Duration (years)	Average UPDRSIII	Average Hoehn & Yahr
Campenhausen, Popov, *et al.*, 2010								
Winter, Campenhausen, Brozova, *et al.*, 2010	EuroPa, Czech	100		64	60	7.9		2.4[b]
Winter, Campenhausen, Gasser, *et al.*, 2010	EuroPa, Austria	81		69	40	9.6	25	
Schneider *et al.*, 2010	**NET-PD, FS-1, USA**	**200**		**62**	**63**	**0.7**	**16**	
Schneider *et al.*, 2010	**NET-PD, FS-TOO, USA**	**213**		**61**	**65**	**0.7**	**16**	
Nègre-Pagès *et al.*, 2010	DoPAMiP study, France	422	98	69	57	5.4[b]	28[b]	2.2
Riedel *et al.*, 2010	GEPAD, Germany	1449		71	61	5.8		2.6[b]
Alves *et al.*, 2009	**ParkWest cohort, Norway**	**207**		**68**	**59**	**2.3**	**23**	**1.9**
Qin *et al.*, 2009	**Chinese Parkinson Study Group, China**	**391**		**64**	**66**	**3**	**24**	**2**
Beiske *et al.*, 2009	Akershus county cohort, Norway	176		69	59	7.5	22	2.1
Barone *et al.*, 2009	PRIAMO, Italy	1,072		67	60	5.1	24	2
Muslimovic *et al.*, 2008	**CARPA cohort, Netherlands**	**190**		**66**	**54**	**3.3**	**20**	**1.9[b]**
Reuther, 2008	Hessia cohort, Germany	145		67	67	9.3	31	2.8

	Cohort Name	Number of PD	Number of Controls	Age of PD (years)	Males (%)	Disease Duration (years)	Average UPDRSIII	Average Hoehn & Yahr
Reuther *et al.*, 2007								
Verbaan *et al.*, 2007	ProPark, Netherlands	420	150	61	64	10.5		2.5[b]
Wüllner *et al.*, 2007	German Competence Network, Germany	3414		66	60	8.9		2.3
Klepac *et al.*, 2007	Zagreb cohort, Croatia	111		66	47	5	25	
Korchounov *et al.*, 2005	Moscow and Schlossberg cohort, Russia	532	67	65	54	6.2		2.5
Uitti, Baba, Wszolek, *et al.*, 2005	Mayo Clinic cohort, USA	1244		70	67	7[b]	31	2.2[b]
la Levy *et al.*, 2000	Washington Heights cohort, USA	173		71	47	6.3	25	
Lyons *et al.*, 1998	Kansas Medical Centre PD Registry, USA	630		71	50	4.6	24	2.2
Tandberg *et al.*, 1995	Rogalan cohort, Norway	245		74	49	9.1	28	3.8
Starkstein *et al.*, 1992	Baltimore cohort, USA	92		66	60	9.7		2.9[b]
Giovannini *et al.*, 1991	Milan cohort, Italy	120	134	54	63	9.9		2.5
Parkinson Study Group, 1989	**DATATOP study, USA**	**800**		**61**	**66**	**1**		**1.5[b]**
OPDC, 2013	**Oxford Parkinson's Disease Centre, UK**	**490**	**176**	**67**	**63**	**1.7**	**27**	**1.7**

[a]The study recruited two cohorts: young-onset and late-onset disease; the first number refers to young-onset group, the second – to late-onset.
[b]Values are calculated based on data in the paper.

Age effect

The effect of age on clinical phenotype was of worsening severity in older patients. The performance was worse on motor indices (total UPDRS-III and subscores, H&Y, peg-board, get-up-and-go) and non-motor scales (cognition, anxiety, constipation, urinary and erectile symptoms). Those effects cannot be attributed to medication dose (LEDD increasing with age), responsiveness to medication or disease duration (similar across strata). A similar worsening in disease severity has been noted in large studies of late versus early onset PD (Diederich *et al.*, 2003; Wickremaratchi *et al.*, 2011). One possibility is that the overall disease burden in older individuals affected the severity ratings or that natural age-related slowing worsened the picture (Cooper *et al.*, 2011). However, it is also well known that late-onset PD progresses quicker than early-onset (Levy, 2007), which in our study was reflected in increased annualized UPDRS-III score with age (as proxy for disease progression). On the pathological level, higher clinical progression rate in older subjects may be caused by faster degeneration of dopaminergic terminals (la Fuente-Fernandez *et al.*, 2011). Moreover, the fact that all of the age comparisons in our study survived correction for disease duration provides a firm support for the hypothesis that age is a stronger contributor to PD progression than disease duration (Levy, 2007). Notably, the only motor feature that bucked this trend was rigidity, which is in agreement with previous work (Wickremaratchi *et al.*, 2011).

Interestingly, patient-rated disease severity (UPDRS-II) showed rather moderate changes with age, compared to objective measures (UPDRS-III, Peg-board, Get-up-and-go). This could be a result of a change with age in patients'

expectations of their own fitness (older patients expect to be less fit and are more accepting of the disease). A contributing factor could also be a mild level of disease severity in this early cohort. Other features standing out from the overall trend of age-related deterioration were motor complications, ICD's and hallucinations, which all demonstrated decreasing severity with age. Previous studies have shown that dyskinesia and ICD's occur together (Lavalaye *et al.*, 2000; Voon, Mehta, *et al.*, 2011) and that both decrease with age (Wickremaratchi *et al.*, 2011). Hallucinations, on the other hand, may increase with age (Sanchez-Ramos *et al.*, 1996) but they also increase with use of dopaminergic agonists (Biglan *et al.*, 2007). Taking both those counteracting trends into account, the difference in rate of hallucinations was not significant.

We observed increased levodopa use and decreased use of agonists with age. This effect may be related to greater disease severity requiring stronger treatment but also to avoidance of agonists in older patients for fear of provoking hallucinations (Wickremaratchi *et al.*, 2011).

Gender effect

A complex set of differences emerged from the comparison of gender-related phenotypes. In terms of motor features, a gradient of severity across body areas was identified, whereby men, compared to women, had much more advanced symptoms in the face and neck, with lesser difference in the arms and equal severity in the legs. In turn, women showed greater severity on postural scores. Importantly, the upper body symptoms differed both on examination and on patient-reported measures while postural/gait problems differed at diagnosis and on examination but not on patient-reported measures.

Most studies agree on increased prevalence of drooling (Cheon *et al.*, 2008; Perez-) and speech problems (Perez-Lloret *et al.*, 2012) in men with PD, with more variable reports on swallowing (Miller *et al.*, 2009). Gender differences in limb symptoms have not been studied, to our knowledge. However, two studies reported increased severity of postural instability in women (Baba *et al.*, 2005; Solla *et al.*, 2012) and both showed increased rigidity in men, in agreement with our findings.

The cause for gender-related dissociation of upper body symptoms from gait problems is not clear. Upper body symptoms, in particular rigidity and bradykinesia, are classical manifestations of striato-nigral pathology. Importantly, SPECT studies in healthy women (Lavalaye *et al.*, 2000; Mozley *et al.*, 2001) and female PD patients (Haaxma *et al.*, 2007) show more abundant dopaminergic terminals in the striatum than in men. Hence, we speculate that less severe upper body symptoms in women result from less pathology in the dopaminergic systems. In contrast, postural symptoms are a consequence of cholinergic degeneration in the pedunculo-pontine nucleus but we are not aware of studies investigating gender differences in this region. Alternatively, postural and gait differences between sexes could be unrelated to the disease process. It has been shown, for example, that healthy women have slower walking speed and get-up-and-go test than men (Cooper *et al.*, 2011). In support of that view, it could be argued that lack of differences on subjective assessment of gait/posture represents true lack of change compared to premorbid condition (women do not think their gait/balance is worse because they generally have poorer balance and walk slower than men). Contrary to that interpretation,

however, comparison of get-up-and-go test between genders in our controls did not show any differences (data not presented).

Another gender-related feature was greater symmetricality of symptoms in men. It may seem to contradict the previous finding of women having more postural problems as symmetry of symptoms is generally associated with axial disease. However, these are two separate features, with symmetricality referring to lateralised symptoms only and disregarding axial symptoms. Two other studies showed non-significantly increased symmetricality in men (Bliwise *et al.*, 2010; Postuma and Gagnon, 2011) although others did not find a gender effect (Gómez-Esteban *et al.*, 2010). As with the upper/lower body gradient, a cause for this phenomenon is unclear but we speculate that it may be related to greater symptom severity in men. It is widely accepted that symptoms become more symmetrical with disease progression (Gómez-Esteban *et al.*, 2010). Since in our sample men had more severe symptoms in upper limbs we can assume that the disease process was more advanced in men and, hence, greater percentage of men reached symmetrical disease stage. It is also possible that men have more symmetrical striato-nigral pathology but there are no pathological or imaging studies of this problem in the literature.

Gender showed multiple significant effects on the non-motor features. In agreement with previous studies, men had more pronounced cognitive impairment as measured by MOCA and fluency tests (Nazem *et al.*, 2009), more daytime sleepiness (Martinez-Martin *et al.*, 2012) and more RBD-related features (Romenets *et al.*, 2012). Cognitive impairment in PD has been shown to co-occur with RBD (Vendette *et al.*, 2007). Some longitudinal studies reported

quicker cognitive decline in RBD (Fantini *et al.*, 2011) and, importantly, in PD with RBD (Postuma, Bertrand, *et al.*, 2012). In view of the strong correlation of gender with both RBD and cognitive decline in our study, it would be important to investigate whether RBD is an equally strong risk factor for dementia in both sexes.

Study limitations

The study has a few limitations which affect its interpretation. Firstly, the analyses are cross-sectional, which may bias some variables through retrospective reporting. However, longitudinal data will gradually become available as patients come to follow-up.

An important caveat is that the above analyses have not been corrected for multiple comparisons. This decision was based on the nature of the study, which aims to generate hypotheses rather than confirm them. It has been argued that exploratory analyses do not require adjustment for multiple comparisons (Bender and S. Lange, 2001). Necessarily, conclusions from such studies are very guarded. They should always be followed by confirmatory studies attempting to replicate results on independent samples. As an alternative, multivariate tests, such as factor analysis or principle component analysis could be used to reduce the number of variables. This analysis is currently under way and aims at identifying clinical features contributing to PD progression. It does not, however, form a part of this thesis.

Furthermore, a great majority of the observed effect sizes in age and gender comparisons were small. Apart from a few exceptions, the differences are not clinically significant at the patient level. If confirmed, however, they do point to

potentially different pathological processes at play in different groups of PD patients. Moreover, since the changes are seen at the early disease stage, they may be augmented with disease progression, leading to clinically significant differences.

Results of the above analyses as well as similar studies published since establishing the OPDC cohort provoke reflection on potential improvements to the study protocol. Given the importance and high prevalence of mild cognitive impairment and dementia in PD, a more tailored assessment of cognitive functions in the OPDC cohort would have been desirable. The Movement Disorders Society Task Force criteria for mild cognitive impairment in PD published in 2012 (Litvan *et al.*, 2012) specify that MCI in specific cognitive domains can only be diagnosed based on abnormalities on at least two tests for a given domain. The single and multiple domain MCI may also have a different predictive value for future development of dementia. Assessment protocol in the OPDC cohort does not allow for this differentiation as it counts as level I assessment described as "abbreviated assessment" in the MDS criteria. Specifically, attention and executive function testing is sufficient in the OPDC protocol. However, according to the MDS criteria, testing in the language domain would have to complemented by Boston Naming Test or Graded Naming Test; in the memory domain – by Prose Recall test or Brief Visuo-spatial Memory Test; and in visuospatial domain – by Benton's Judgment of Line Orientation or Hooper Visual Organisation Test.

Additionally, the choice of mood assessment tools could be brought in line with the MDS recommendations by the Scales Task Force (Leentjens *et al.*,

2008). Rather than the Leeds Scale for self-assessment of Anxiety and Depression, one of the six scales suggested by the review could be used, such as: Beck Anxiety Inventory, the Hospital Anxiety and Depression Scale, the Zung Self-Rating Anxiety Scale and Anxiety Status Inventory, the Spielberger State Trait Anxiety Inventory, or the Hamilton Anxiety Rating Scale.

Similarly, the test used in OPDC for objective assessment of balance, the Flamingo Test, is not the preferred test of balance in the PD literature. Using the Berg Balance Test or the Functional Reach Test, as discussed above in the Methods section, would allow for direct comparison with published studies. More broadly, objective motor assessment tools have expanded recently with increasingly common use of wearable sensors. The devices have been successfully employed for assessment of gait (Klucken et al., 2013), freezing of gait (Moore et al., 2013) and balance (Giansanti et al., 2008). Moreover, severity of bradykinesia (Griffiths et al., 2012), dyskinesia (Griffiths et al., 2012) and tremor (Mera et al., 2012) could also be quantified in that way. Including those instruments in the OPDC protocol would add a much more precise and unbiased assessment of the main motor features.

Conclusions

Our results suggest that age is a strong predictor of disease severity even after taking into account disease duration. Gender-related motor phenotype is suggestive of a vertical split into more symmetrical upper-body disease in men and postural disease in women. Amongst the non-motor symptoms, men are more cognitively impaired and have higher rate of RBD than women. Future

research is needed to confirm the observed effects and establish whether the two genders may have different predictive value for disease progression.

Chapter III. Meta-analysis of functional imaging studies in Parkinson's Disease

Section 1. Introduction

Functional imaging in Parkinson's disease has revealed a variety of abnormal activations across the whole brain. There is, however, little agreement regarding which areas are reliably over- or underactivated, or the meaning of these changes in the disease process. The most consistent findings are: 1) reduced activation in the pre-SMA (Cunnington *et al.*, 2001; Grossman *et al.*, 2003; Haslinger *et al.*, 2001; Sabatini *et al.*, 2000), in agreement with the predictions of the classic basal ganglia model; and 2) increased activation in various other cortical areas (Dagher *et al.*, 2001; Haslinger *et al.*, 2001; Künig *et al.*, 2000; Nakamura *et al.*, 2001; Sabatini *et al.*, 2000), interpreted as compensatory recruitment of alternative brain circuits in the face of a failing basal ganglia circuit. For example, cerebellar hyper-activation has been interpreted as compensatory recruitment of the cerebello-thalamo-cortical loop in PD (Lewis *et al.*, 2007; Sen *et al.*, 2010). However, other studies using similar paradigms have failed to demonstrate these changes (Buhmann *et al.*, 2003; Samuel *et al.*, 2001). Furthermore, some studies reported coexisting hyper- and hypo-activation in the cerebellum (Cunnington *et al.*, 2001; Eckert *et al.*, 2006; Grossman *et al.*, 2003; Haslinger *et al.*, 2001; Pinto *et al.*, 2011; Sabatini *et al.*, 2000) while others found only hypo-activation in PD (Catalan, 1999; Dagher *et al.*, 2001; Hanakawa, Katsumi, *et al.*, 1999; Haslinger *et al.*, 2001; Künig *et al.*, 2000; Nakamura *et al.*, 2001; Sabatini *et al.*, 2000; Tessa *et al.*, 2010; Turner *et al.*, 2003).

The effects of dopamine supplementation on brain activity in PD are equally unclear. Some studies show levodopa exerting a normalizing effect on brain activity in both cognitive (Cools *et al.*, 2002; Fera *et al.*, 2007; Lewis *et al.*, 2007; Sen *et al.*, 2010; Tessitore *et al.*, 2002) and motor or sensory tasks (Brefel-Courbon *et al.*, 2005; Buhmann *et al.*, 2003; Cunnington *et al.*, 2001; Elsinger *et al.*, 2003; Haslinger *et al.*, 2001; Jenkins *et al.*, 1992; Kraft *et al.*, 2009; Mohammadi *et al.*, 2012; Samuel *et al.*, 2001), whereas others showed variable effects of levodopa, from relatively little effect (Dellapina *et al.*, 2011; Eckert *et al.*, 2006; Payoux *et al.*, 2010; Veer *et al.*, 2010), through mixed, over- and under-activation (Farid *et al.*, 2009; Hacker *et al.*, 2012; Helmich *et al.*, 2010; Jubault *et al.*, 2009), to increased activation over and above normal subjects (Delaveau *et al.*, 2009; T. Wu *et al.*, 2012). Two potential explanations have been suggested to accommodate the conflicting results. Firstly, dopamine supplementation may have opposing effects on brain activation depending on whether the task is of motor or cognitive nature (Mattay *et al.*, 2002). Alternatively, dopamine supplementation may affect brain activation if the task in question specifically activates the putamen, and would have no effect if putamen is not involved (Jubault *et al.*, 2009). The hypotheses introduce some clarity into the conflicting body of reports, nevertheless, some findings still defy explanation.

Conflicting results and ensuing interpretational difficulties in PD imaging, and more broadly, in the whole fMRI field, stem from three main factors: variability of activation sites, variability of employed paradigms, and low sample sizes. All three problems can be alleviated by using a meta-analytic approach. Aggregation of results from a number of studies brings out foci that are activated most frequently and with greatest intensity. Additionally, foci activated across a

range of paradigms gain in significance and can be sifted from the noisy background. Finally, power to detect significant activations is greatly increased by increasing the number of subjects in the analysis.

There are two main approaches to meta-analysis of functional imaging studies: image-based (IBMA) and coordinate-based meta-analysis (CBMA) (Salimi-Khorshidi *et al.*, 2009). In this study, we employ Gaussian Process Regression (GPR) CBMA, which was shown to be successful in the meta-analysis of highly sparse foci (collected from neuroimaging reports), and to produce more accurate results when compared with classical approaches (Salimi-Khorshidi *et al.*, 2011). GPR enables a joint analysis of activation and deactivation coordinates and their effect sizes, models the censoring of neuroimaging reports (i.e., bias towards reporting significant foci only) and their heterogeneity, infers the smoothness of the meta-analytic statistic image, and results in an accurate effect-size (e.g., T- or Z-stat) map.

To our knowledge, there has been no systematic meta-analysis of functional imaging data in PD. In order to address the above-mentioned uncertainties we asked:

Is there a simple and reproducible pattern of activations characteristic of Parkinson disease? To characterize that pattern we analyzed activations in PD patients off medication (PD-OFF) compared to healthy controls (HC) in various task paradigms: motor tasks, motor tasks with right-sided response and cognitive tasks.

What is the effect of dopaminergic medication on abnormal activations in PD? For this analysis we focused on experiments comparing PD patients on medication (PD-ON) with PD-OFF and controls.

Section 2. Methods

Basics of Functional Magnetic Resonance Imaging

Functional Magnetic Resonance Imaging (fMRI) has been used to visualize brain function sine the first publication in Science in 1991 (Belliveau *et al.*, 1991). It followed in the steps of PET and SPECT in attempting to visualize changes in brain activity related to specific actions. The way images are acquired and processed has evolved over the years but the basic fundamentals of MRI physics and image acquisition remained the same.

MRI exploits the property of the hydrogen atoms called spin, which can be visualized as precession of the atom along its own axis. Importantly, the axes of precession, or the spins, align parallel to the lines of the magnetic field. Hydrogen atoms in a water rich tissue will, thus, align their spins with the basic magnetic field of the MRI scanner, called B0 (Jezzard *et al.*, 2003). When a perpendicular magnetic field, called radiofrequency pulse (RF), is applied the atoms will align their spin with the new field. Alignment of spins in the direction perpendicular to B0 generates electric current in the MRI receiver coils. After the short RF pulse has been switched off the hydrogen atoms will return to their original position parallel to B0 and the current in the receiver coils will decay. This fluctuation of current generated by flipping of hydrogen atoms forms the basis of the, so called, T1, T2 and T2* signals and provides information on the density and properties of atoms in the sample. The T2* signal and its variation across the tissue volume is used to build a, so called, T2*-weighted image, and is of particular interest in functional MRI.

The contrast on the T2*-weighted images depends strongly, among other factors, on the level of haemoglobin oxygenation (Matthews and Jezzard, 2004). This, so called, Blood Oxygenation Level Dependent (BOLD) contrast relies on the fact that oxyhaemoglobin increases the T2* signal while deoxyhaemoglobin reduces it (Ogawa *et al.*, 1993). As the neural activity increases, as part of spontaneous fluctuation or part of a specific task, the aerobic metabolism and the cerebral rate of oxygen consumption (CMRO2) increases (Jezzard *et al.*, 2003). Paradoxically, this does not lead to increased level of deoxyhaemoglobin in the active tissue, as might be expected. High CMRO2 causes and even higher increase of the cerebral blood flow (CBF), called neurovascular coupling, which washes out deoxyhaemoglobin and replaces it with oxyhaemoglobin (Jezzard *et al.*, 2003). In effect, increased neural activity leads to higher levels of oxyhaemoglobin and higher T2* signal, enabling an indirect measurement of brain activity (Buxton *et al.*, 2004).

Neurovascular coupling is a complex and, still, not fully understood process (Buxton *et al.*, 2004). A variety of chemical and electrical mediators link neural activity with the vascular response (Iadecola, 2004). Moreover, BOLD signal is a combination of changes in cerebral blood flow (CBF) (the primary physiological feature measured in PET and SPECT), cerebral blood volume (CBV) and cerebral oxygen consumption (CMRO2) (G. G. Brown *et al.*, 2007). Changes to any of those factors can potentially affect BOLD response. Hence, methods measuring BOLD cannot disentangle the influence of cerebral oxygen consumption from changes in CBF, although the two are closely related (Logothetis, 2008). Additionally, the vascular response is sluggish, compared to the neural activity, and so the BOLD

response delays and smooths over time the neuronal response (G. G. Brown *et al.*, 2007). Another important caveat for localizing brain activity based on the BOLD signal is that the large changes in the signal may occasionally be seen around the veins draining the activated area, which may sometimes be some distance from the drained area (G. G. Brown *et al.*, 2007).

To discover which regions of the brain are active in specific tasks, a task-based fMRI paradigm is used. A participant is asked to perform a task, e.g. a finger tap, cued by the experimenter, while a T2*-weighted image is acquired. This image can then be compared with a T2* scan at rest and areas of increased activation on performing the task can be identified (G. G. Brown *et al.*, 2007). Typically, one scan acquisition takes 1-2s which puts a limit on the temporal resolution of fMRI. Practically, identifying active areas requires modeling the expected BOLD response to the task. This is required because the BOLD response is delayed by about 1-2s with respect to the neural response, then reaches a peak at 4-8s and returns back to baseline (Jezzard *et al.*, 2003). The typical response curve of the BOLD signal is called Hemodynamic Response Function (HRF) (Figure 3).

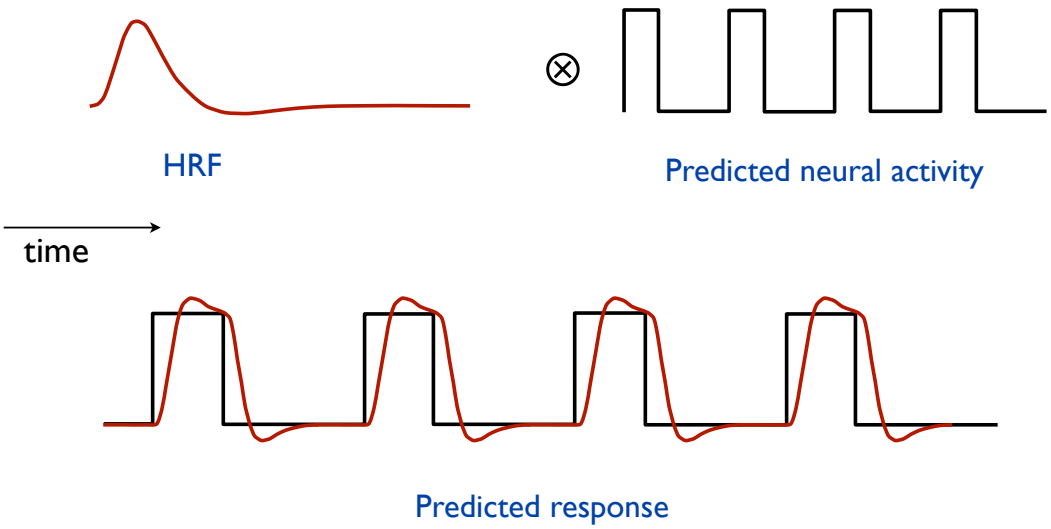


Figure 3. Hemodynamic response curve and BOLD response curve. Hemodynamic response curve is convolved with stimulus timing, or predicted neuronal activity, to produce predicted BOLD response. Image courtesy of the FMRIB Graduate Course.

In a, so called, block-design stimuli are presented in rapid succession leading to merging of individual HRF's, which increases signal intensity. To build a model of the predicted BOLD response HRF's are convolved with a stimulus timing curve as presented in Figure 3. Finally, this modeled response is compared to actual responses in each brain voxel using General Linear Model (GLM) (Figure 4). Voxels where the two curves match statistically are considered active for a given task (Jezzard *et al.*, 2003).

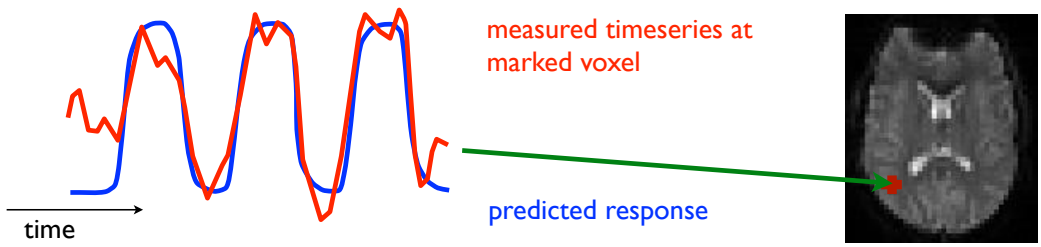


Figure 4. Identification of activated voxels. Predicted BOLD response curve (blue) is compared with the measured response from a given voxel (red). If the curves match according to the GLM then the voxel is considered active for a given task. Image courtesy of FMRIB Graduate Course.

Basics of Positron Emission Tomography

Positron Emission Tomography (PET) was historically the first technique used for studying changes in cerebral blood flow in humans and has been in use since mid 1970's (Jones, 1980). Functional PET relies on identifying areas of the brain with increased blood flow, where radioactive isotope, injected before the scan, achieves greatest concentration through diffusion from the blood (Van Heertum *et al.*, 2013). In that respect, the general theory of neurovascular coupling described in the fMRI section above applies also to PET imaging, with the caveat that the physics behind PET and the spatial and temporal resolution of the two techniques are quite different.

The source of signal in PET imaging is detection of two simultaneous photons produced by annihilation of a positron, emitted by an injected isotope, with an electron in the participants brain. Collision of a positron and an electron produces two photons travelling at 180 degrees to each other, which, when detected simultaneously by a number of detectors mounted on a ring, enable calculation of the exact location of the annihilation event. Over time, multiple

events accumulate to build a map of high and low concentration of the isotope in the brain (Van Heertum *et al.*, 2013). The accuracy of localization process is, however, reduced by three major sources of noise: positron range, scatter of photons and random events. Positron range refers to the distance the positron travels before an annihilation event and varies between a fraction of a millimeter to a few millimeters in water (Cal-González *et al.*, 2009). Scatter of photons occurs when the one or both of them scatter on the surrounding tissue before they reach the detectors, which results in incorrect location of the original event (Phelps, 2006). Random events are annihilations that occur so close in time to each other that the emitted photons cannot be reliably ascribed to any of the event (Phelps, 2006). All those can be corrected to a certain extent by specific instrumental, processing and statistical solutions. However, the ultimate spatial resolution of the technique is limited to about 10 mm, far below that of fMRI (Phelps, 2006).

A variety of isotopes can be used in PET studies, depending on the clinical application (Van Heertum *et al.*, 2013). The isotope of choice for measurement of cerebral blood flow is ^{15}O as part of a water molecule. The advantages of this method lie in high diffusivity of water in the brain tissue and a relatively short half-life of ^{15}O (2 minutes), which allows for rapid repeated acquisitions. Still, the resulting temporal resolution of such PET studies is on average 2 minutes, much poorer than in fMRI. The short half-life of the isotope requires the cyclotrone to be located in the imaging centre, resulting in high costs of the research infrastructure. Moreover, even though the radiation exposure for the patient is negligible, the scanner operators may receive a much higher dose and

are subject to strict safety regulations (Van Heertum *et al.*, 2013). Finally, PET scanning is invasive, requiring and intravenous injection – a fact that may be justified in clinical setting but may raise objections in research projects.

Due to the limitations of temporal and spatial resolution in PET studies, the construction of the final functional map differs considerably from the procedures used in fMRI. Since one acquisition lasts about 2 minutes, no detailed analysis of a task can be performed and only average activation across the whole task or even multiple tasks is possible (Toga and Mazziotta, 2002). A task scan is compared to a resting scan and the resulting difference is used to identify areas with greater or lesser activation. In view of a poor spatial resolution of the scan, the images are registered onto more detailed CT or MRI images to enable anatomical localization (Toga and Mazziotta, 2002).

Basics of Single Photon Emission Computer Tomography

The Single Photon Emission Computer Tomography (SPECT) relies on very similar basic principles to PET. Brain activation is inferred from the regional cerebral blood flow, which is estimated through intensity of radiation emitted by a radioisotope-labeled tracer injected into the bloodstream (Andreasen, 1989). Contrary to PET, the radiation emitted by the tracers is in the gamma-ray frequency and can be measured directly by detectors rotating around the patients head. To avoid contamination of the signal through rays coming from different directions, the gamma-camera is shielded by a collimator – a lead tube designed to absorb rays coming from all angles but the 90 degrees to the camera. The final image is reconstructed from a series of 2-dimensional images

taken by the rotating cameras using methods of computer tomography (Andreasen, 1989).

There are two basic types of radiotracers used in SPECT: diffusible and static (Van Heertum *et al.*, 2013). The diffusible tracers, the most commonly used being ^{133}Xe, passively diffuse from the bloodstream into the tissue and back, and their concentration depends on the regional cerebral blood flow. As the half-life of ^{133}Xe is relatively short scanning can be repeated after 15 minutes. On the other hand, the spatial resolution is only about 12 mm. The second group, static tracers, diffuse into the tissue and are retained in there after their chemical conformation is modified. The best established tracer of this type, ^{99m}Tc-HMPAO, has a half-life of 6 hours, meaning that a scan can be performed up to 6 hours after injection but consecutive scans have to be done in 1-2 day intervals. The spatial resolution is better than ^{133}Xe, at 7mm.

As with PET, the temporal resolution of SPECT scans is low and only averaged activation across multiple task iterations can be estimated. The 'resting scan' activations are then subtracted from the 'task scan' to identify task-specific activation.

Comparability of fMRI, PET and SPECT

This brief overview of the three functional imaging techniques highlights differences between them and prompts discussion about their comparability. The most prominent difference between them is the fact that PET and SPECT measure cerebral blood flow changes, while fMRI analyses a composite of CBF, CBV and cerebral oxygen consumption (CMRO2). Although CBF and CMRO2 are

closely linked, it is not entirely clear whether changes in those measures are always parallel to each other (Logothetis, 2008).

Another significant difference is low temporal resolution of PET and SPECT compared to fMRI (Van Heertum *et al.*, 2013). Consequently, PET/SPECT studies acquire only one task scan comprising one iteration of a long task or multiple iterations of a short task. The resulting activation map reflects average activation of many areas active at different time points during task performance. In contrast, fMRI studies can differentiate between activations from many short tasks and can more precisely identify brain areas involved in execution of the task. Apart from the resulting difference in precision of localization, a different number of areas may be identified or different areas may be lumped together or split apart. Varying spatial resolution between techniques may also contribute to the above effects, enhancing the lumping and splitting of foci.

Statistical procedures used to construct functional maps in the three techniques differ too. In view of much higher spatial resolution of fMRI studies, and, hence, a large number of comparisons performed, correction for multiple comparisons is of paramount importance in fMRI. Stringency of those corrections may affect results and comparability with PET/SPECT studies, where the number of data points is much smaller.

Finally, PET and SPECT studies are chronologically older techniques used mainly in the ‘80s and ‘90s, while fMRI flourished in the recent two decades. The development in imaging techniques have been paralleled by development in research paradigms, design of experiments, choice and matching of subject groups and even availability and use of treatment options for studied disorders.

All those factors are bound to have an effect on reported study results and may affect comparability between older and newer studies.

The different caveats discussed above need to be born in mind when interpreting results of this study. The rationale of any meta-analysis, however, is generalization across studies that differ in many methodological aspects but share a basic underlying assumption. The unifying principle of different imaging techniques included in this study is that they all estimate local neuronal activation based on blood flow changes in response to specific externally guided tasks. Moreover, results obtained with those techniques are often informally compared and discussed together (Chase *et al.*, 2011; Delaveau *et al.*, 2011; Postuma and Dagher, 2006), which reflects a widespread assumption that they reflect similar underlying processes. A number of published meta-analyses combined PET, SPECT and fMRI studies concluding that despite clear differences the methods yield mutually supportive results (Costafreda *et al.*, 2008; E *et al.*, 2014; Jamadar *et al.*, 2013; Liebenthal *et al.*, 2014; Phan *et al.*, 2002).

Search strategy

Articles were selected in three steps.

1. The US National Library of Medicine database (pubmed.gov) was searched for articles matching the following search criteria: (PET OR positron OR SPECT OR 'single photon' OR fMRI OR 'functional magnetic' OR 'functional imaging' OR BOLD) AND parkinson, published until March 2012.
2. All abstracts were searched for original publications reporting task-related functional neuroimaging experiments involving PD patients. The

following abstracts were excluded: reviews, editorials, letters to editors, case reports, studies not describing imaging experiments or not involving PD patients.

3. Full text articles were searched for activation coordinates from high-level, between-group contrasts (PD-OFF vs. HC, PD-ON vs. HC, PD-ON vs. PD-OFF). Additionally, references were scanned for publications missed in original search procedure. If coordinates were not reported authors were contacted and asked to supply the unpublished data. The following publications were excluded: without activation foci reported, non-task paradigms, using other imaging methods (ASL, MEG), with DBS patients, non-rCBF (not measuring regional cerebral blood flow, in case of PET and SPECT studies), non-whole brain analysis, connectivity analysis, full access unavailable, duplicates, re-analyses, only specific patients (with hallucinations, with dyskinesia, etc.), with only within-group comparisons.

Data extraction

The following meta-data were extracted from the reports: nature of the task, coordinates of activation, Z or T-scores or P-values for strength of activation, number, age and sex of subjects in study groups and use of dopaminergic medication before the scan. All activation coordinates were transformed into MNI space using GingerAle (Laird, Eickhoff, *et al.*, 2011). T-scores and P-values were transformed into Z-scores using Excel.

Experiments were divided into three groups: PD-OFF vs. HC, PD-ON vs. HC and PD-ON vs. PD-OFF. Next, experiments using specific motor, cognitive and

other tasks were identified in each group. Foci from each group were analyzed separately to build maps of activations across tasks. Due to a relatively small number of foci in each of the subanalyses, no correlation with clinical variables (UPDRS, H&Y, age, disease duration) was possible.

Additionally, to investigate which abnormal activations respond to medication, two responsiveness analyses were performed based on results of the basic analyses described above. In Responsiveness Analysis 1, results of the PD-OFF/HC (all tasks), PD-ON/PD-OFF analyses and PD-ON/HC, thresholded at $p<0.01$, False Discovery Rate (FDR) corrected (Knipe *et al.*, 2011), were overlaid to identify areas responding to medication (present in the first two analyses but absent in the last one). In Responsiveness Analysis 2, a similar procedure was performed to investigate regions not responding to medication (common areas for PD-OFF/HC and PD-ON/HC with exclusion of areas in PD-ON/PD-OFF).

Meta-analytical methods

The two main types of imaging meta-analytical methods are based on combining full images, the IBMA methods, or extracted co-ordinates of the activation foci, the CBMA methods (Salimi-Khorshidi *et al.*, 2009). The ideal IBMA analysis gathers subject-level t or z-statistics images from all included studies to build the final inference image (Lazar *et al.*, 2002). The method can account for only inter-subject differences (using fixed effects analysis, FFX) or for both inter-subject and inter-study differences (mixed effects analysis, MFX). This solution is, however, highly impractical as it requires huge computing power to simultaneously access all patients' data. Different imaging formats, resolution used, or software packages employed to process the data add a further layer of

difficulty. Moreover, obtaining subject-level scans from multiple research labs would be extremely difficult and would likely significantly limit the number of studies included in the analysis.

Hence, a more popular approach in IBMA studies is to analyse study-level z-stat images, which are a result of high-level statistics combining lower-level subject results (Salimi-Khorshidi *et al.*, 2009). Those maps can also be weighted according to the number of subjects giving the analysis additional precision. Although the computational load of this method is much more manageable than the subject-level methods, it still requires sourcing the original unpublished statistical maps from each study and tackling problems of image conversion. To make the IBMA methods more practical, large image repositories would have to be established and standards set for preferable imaging formats – measures that have already taken root in the form of various brain imaging projects.

Co-ordinate based analyses, on the other hand, rely solely on co-ordinates of peaks of activation foci, commonly published in tables as part of the original study report (Salimi-Khorshidi *et al.*, 2009; 2011). Co-ordinates represent the final result of all within and between-subject statistical processing and include the location of the highest activation in three-dimensional space as well as the strength of this activation expressed as t or z-score. The most widely used CBMA methods, reviewed and compared recently (Salimi-Khorshidi *et al.*, 2009), include: Activation Likelihood Activation (ALE) (Turkeltaub *et al.*, 2002), Kernel Density Analysis (KDA) (Wager *et al.*, 2004) and Multi-level KDA (MKDA) (Wager *et al.*, 2007).

For each reported activation focus, ALE produces a whole-brain likelihood map, with each voxel in the map assigned a value depending on its distance from the

focus (Turkeltaub *et al.*, 2002). The values are a result of Gaussian smoothing of the focus with a full width half maximum (FWHM) function. Each focus, and, consequently, each map, are considered independent of each other. Maps are then combined to yield a final ALE map, where each voxel has an activation likelihood, indicating the probability of at least one activation peak lying at that voxel. The ALE map is then formally tested against the null distribution of ALE maps produced through multiple iterations of random distribution of similar number of activation foci (with Monte-Carlo simulation).

KDA, similarly to ALE, produces a number of whole-brain likelihood maps, each one for a single focus. Instead of FWHM, the voxels are assigned a value if they are within a certain radius of the activation focus, with all other voxels equaling zero (Wager *et al.*, 2004). In the next step, all maps are simply added to form a final map, where voxel values represent probability of activation. As in ALE, this map is tested against the null distribution generated with the Monte-Carlo simulation.

Both ALE and KDA treat each focus independently, even though foci close to each other in one study are likely related and may even result from the same activation (Salimi-Khorshidi *et al.*, 2009). As a result, simple difference of thresholding between studies may result in one experiment reporting a few foci while another reports a multitude of activations. Still, ALE and KDA will treat all those results equally, consequently, giving the latter experiment much more prominence. MKDA attempts to remedy this situation by first combining only maps relating to one study to produce a single map for each experiment (Wager *et al.*, 2007). Those maps are then averaged and the final map is tested against a null set. The latter is produced by scrambling clusters of foci, rather than just

individual foci, which produces a more realistic comparison. In effect, no single study can contribute disproportionately to the final map.

All those CBMA approaches suffer from inability to take into account the effect size of the activation focus. Hence, barely significant and more noise-related foci are treated equally with highly significant ones. Accounting for activation strength forms a major advantage of the Gaussian Process Regression (GPR) method used in this study. GPR is presented in more detail below.

Gaussian Process Regression

The extracted foci in this study were analyzed with Gaussian Process Regression (GPR) (Salimi-Khorshidi *et al.*, 2011), a new method taking into account foci location and activation strength (T or Z-score).

The prior assumption of the GPR model is that the underlying statistic map that the meta-analysis attempts to estimate is drawn from a Gaussian process, such that for all voxels $\boldsymbol{v}_1, \boldsymbol{v}_2, \ldots \boldsymbol{v}_n$ in volume $\mathbf{V}$, there is a matrix $\mathbf{C}$ in $\Re^{n\times n}$, where $[f(\boldsymbol{v}_1), f(\boldsymbol{v}_2), \ldots f(\boldsymbol{v}_n)] \sim \mathcal{N}\ (\mathbf{0}, \mathbf{C})$, where $\mathbf{C}_{\boldsymbol{i,j}}$ denotes the covariance between $f(\boldsymbol{v}_i)$ and $f(\boldsymbol{v}_j)$, and $f(\boldsymbol{v}_i)$ is the underlying Z-statistic value at voxel $\boldsymbol{v}_i$. Next, the GPR model assumes that the foci that enter the meta-analysis are noisy measurements from the same map. For instance, if a study reports that the Z-stat at voxel $\boldsymbol{v}_i$ is y_i, then the GPR assumes that $y_i = f(\boldsymbol{v}_i) + \varepsilon$, where $\varepsilon \sim \mathcal{N}\ (0, \sigma_n^2)$ is the noise. The noise can be due to differences in design, sample size, analysis technique, and many other sources of heterogeneity across the studies.

Defining the mean and covariance function completely defines the Gaussian process's behavior. In our implementation of the GPR CBMA, given the Gaussian

smoothing that (almost) all Neuroimaging studies go through, and the role that spatial adjacency plays in nearby voxels' functional similarity, we assumed that f has a squared exponential covariance function (i.e., voxels that are close in space are likely to have similar Z-stat) and a zero mean (i.e., no activation unless the foci provide evidence for it).

Suppose the full-image study-level data were available, then for study *s* at voxel *k*, the contrast of parameter estimates (e.g. the study-level group effect sizes), $y_{s,k}$, can be modelled as

Equation 1: $y_{s,k} = \mu_k + w_{s,k}$,

where $w_{s,k} \sim \mathcal{N}\left(0, \sigma_{s,k}^2 + \tau_k^2\right)$, μ_k denotes the overall population mean (i.e., what the meta-analysis is expected to estimate), $\sigma_{s,k}$ is within-study standard deviation, τ_k is inter-study standard deviation, and $w_{s,k}$ is the observation/reporting error at study *s* and voxel *k*.

Typically, CBMA does not have access to the study-level contrasts of parameter estimates, $y_{s,k}$, nor their standard deviation, $\sigma_{s,k}$, at every voxel; instead it has access to sparsely sampled standardized (e.g. z-statistic) effect sizes (i.e., $z=y/\sigma$). This changes the model to

Equation 2: $z_{s,k} = \mu_k/\sigma_{s,k} + \varepsilon_{s,k}$,

where $\varepsilon_{s,k} \sim \mathcal{N}\left(0, 1 + \tau_k^2/\sigma_{s,k}^2\right)$. If we assume that every study has the same σ image (i.e., studies are similarly reliable in their effect-size estimates), then the model can be rewritten as

Equation 3: $z_{s,k} = m_k + \varepsilon_{s,k}$,

where $m_k = \mu_k/\sigma_k$, $\varepsilon_{s,k} \sim \mathcal{N}(0, 1 + v_k^2)$ and $v_k^2 = \tau_k^2/\sigma_k$.

Even though CBMA only has access to *n* sparsely-located samples of the Z-stat image ($\boldsymbol{z} = (z_1, z_2, \dots z_n)$) with their corresponding voxel coordinates $\mathbf{V} = \{\boldsymbol{v}_1, \boldsymbol{v}_2, \dots \boldsymbol{v}_n\}$ €, we can employ GPR to model the unobserved voxels' standardized mean effect size *m*. Under GPR, *m* is assumed to be a sample from a Gaussian process, i.e., $m \sim \mathcal{N}(0, \mathbf{C})$ with **C** denoting the covariance matrix of the GP. We employ a squared exponential (SE) covariance function whose shape can be described with two hyperparameters $\boldsymbol{\sigma}_f$ (describing *m*'s variance) and $\boldsymbol{\lambda}$ (describing *m*'s smoothness). Assuming that *z* is sampled from *m* with an additive Gaussian noise of $\mathcal{N}(0, \sigma_n^2)$, results in

Equation 4: $z_k \sim \mathcal{N}(m_k, \sigma_n^2)$,

where σ_n corresponds to $1 + \tau_k^2/\sigma_{s,k}^2$.

In the first step of this solution (inference), the model's hyperparameters ($\boldsymbol{\sigma}_n$, $\boldsymbol{\sigma}_f$, and $\boldsymbol{\lambda}$) are estimated using evidence optimization (EO). These estimates are used in the second step (prediction) to predict the full *m* map. We incorporate our prior knowledge about statistic images' smoothness by employing a Gamma prior on $\boldsymbol{\lambda}$ in order to minimize the likelihood of an extremely high or low smoothness. This Gamma prior has a shape parameter of 7.7 and a scale parameter of 0.3 (i.e., 90% chance of image's smoothness in FWHM being between 0 and 8 mm).

Candidate's contribution to the study

The Candidate designed the study, performed literature search and extracted the data. Data analysis was performed in collaboration with Dr. Salimi-Khorshidi, a Research Fellow at the FMRIB Centre, University of Oxford, using a

method developed by Dr. Salimi-Khorshidi. The candidate interpreted the results and wrote a manuscript on which the above chapter is based.

Section 3. Results

Search results

Consecutive steps of the selection process of publications to be included in the meta-analysis are shown in Figure 5. The original pubmed.com search yielded 3,873 abstracts. Abstract search produced 346 publications which were then screened in the full text form. The final number of articles included in the meta-analysis was 60, of which 39 employed functional MRI imaging, 19 – task-related H_2O^{15}-PET and 2 – task-related SPECT. Overall, they involved experiments performed on 607 PD patients and 561 controls reporting 777 activation foci. Table I in Appendix B. shows detailed characteristics of the analyzed studies. A list in Appendix B shows studies excluded after the full-text search stage. The selected papers reported 130 between-group contrasts, 74 of which compared PD-OFF patients to HC, 27 – PD-ON to HC and 26 – PD-ON to PD-OFF. In the PD-OFF to HC comparison, motor tasks were investigated in 39 experiments and the largest subgroup comprised right-handed motor tasks with 28 experiments. Cognitive tasks in PD-OFF to HC comparisons were investigated in 30 experiments. Analysing PD-OFF/PD-ON and PD-ON/HC comparisons by task was not possible due to small number of experiments in each of the subgroups. A breakdown of studies, experiments, foci and subjects included in each of the 6 main analyses is presented in Table II, Appendix B.

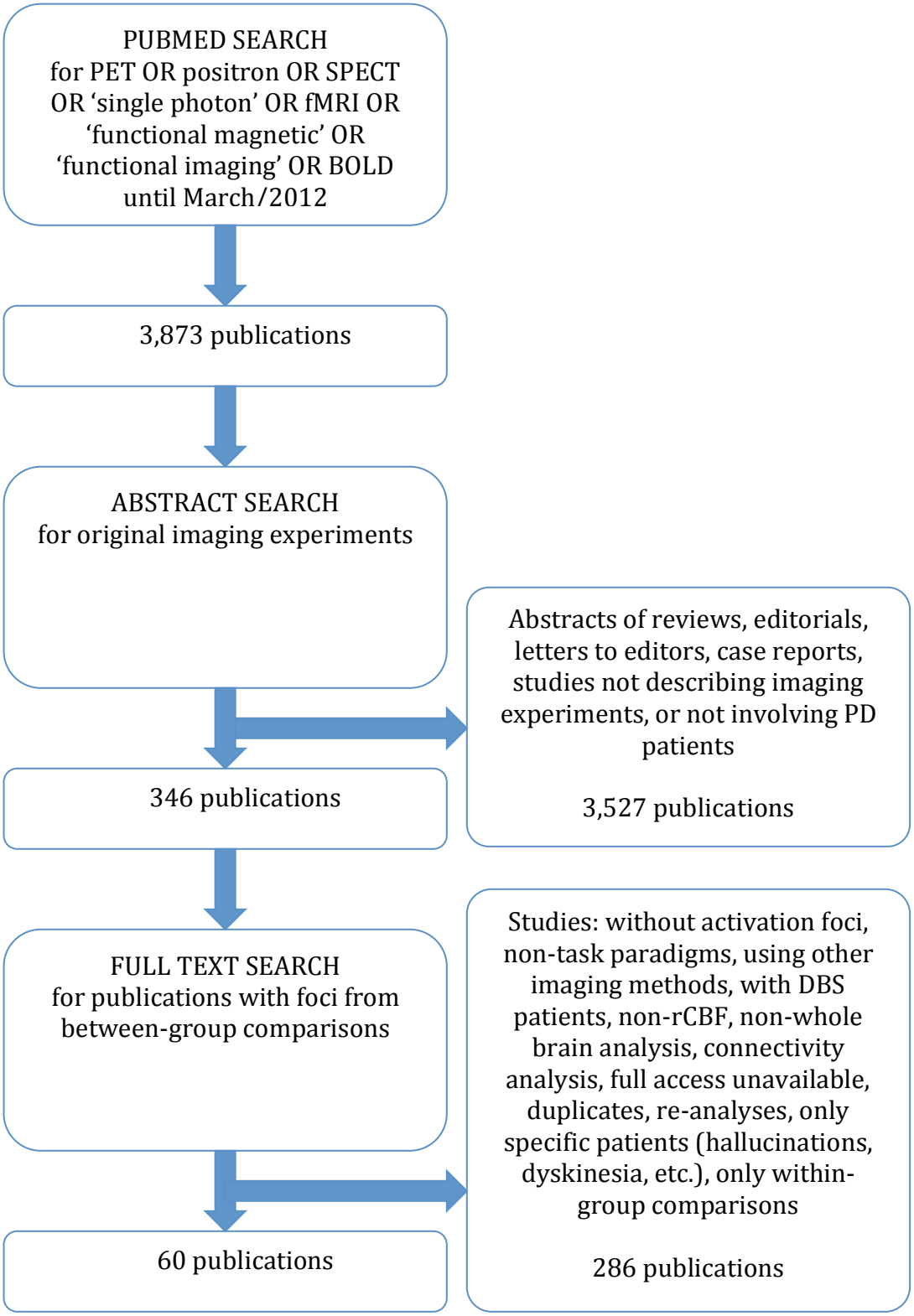


Figure 5. Flowchart illustrating the selection process of publications for meta-analysis.

Patterns of activations in PD

We focused our analysis on a group of the most prevalent paradigms in the literature – right-handed motor tasks (202 foci from 28 experiments). The resulting pattern revealed complex hyper- and hypoactivations bilaterally (Figure 6). The changes were more prominent in the left hemisphere and comprised a relatively symmetrical pattern of hypoactivations in the basal ganglia, secondary sensory cortex and Supplementary Motor Area with hyperactivations in the primary and secondary motor areas and occipito-parietal junction. Cerebellar changes comprised of mainly medial and lateral hyperactivations and lateral hypoactivations.

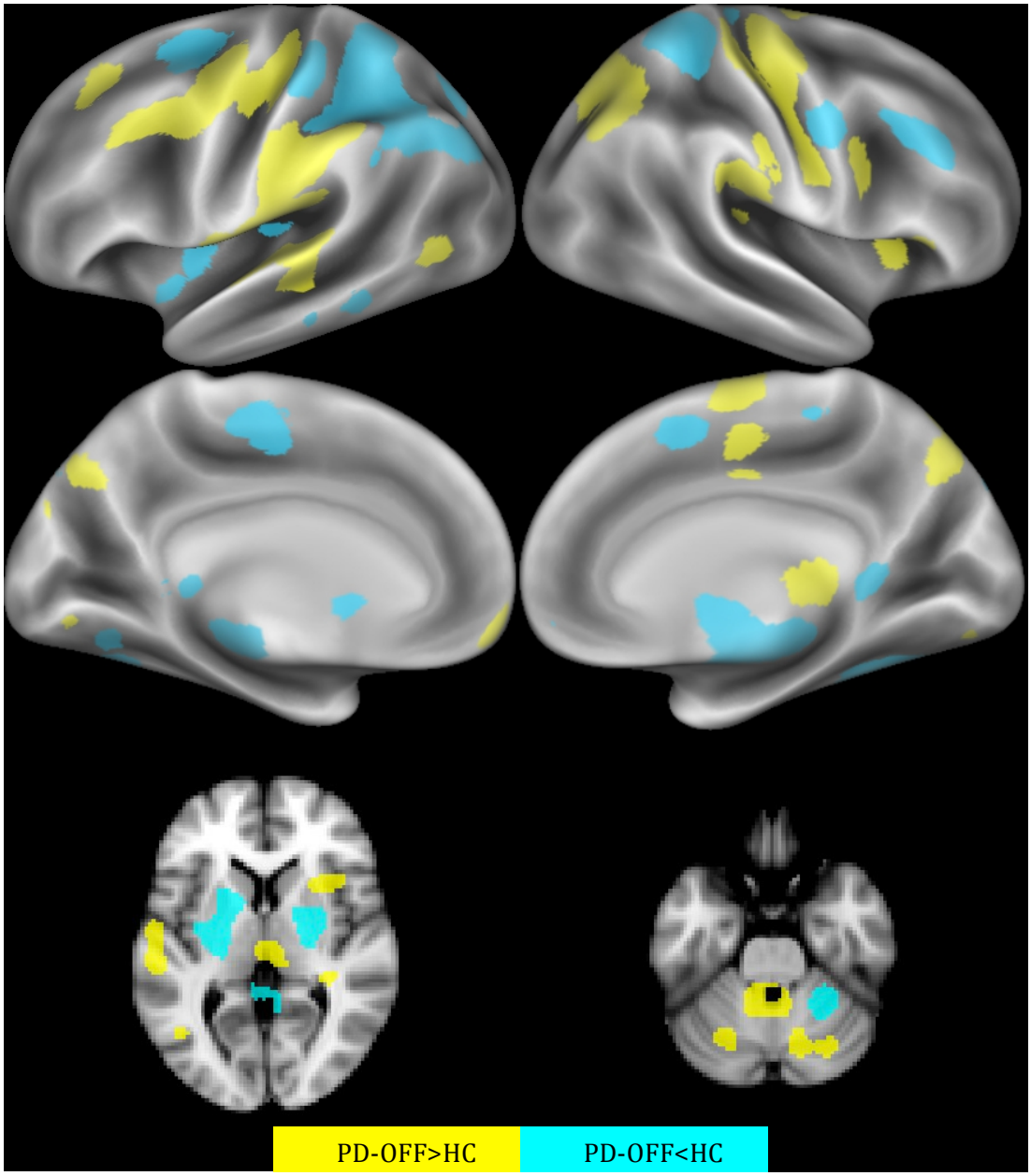


Figure 6. Results of PDOFF vs HC analysis, right-handed tasks. Upper panel shows surface projections in Caret. Lower panel shows changes in the basal ganglia and the cerebellum. Activations are thresholded at $p<0.01$, FDR corrected. Yellow color represents hyperactivations, blue color represents hypoactivations.

Extending the analysis to all motor tasks (results not shown) showed a very similar pattern to the one described above, reflecting the fact that the majority of motor tasks were right-handed. Analysis of cognitive paradigms resulted in very small and scattered changes, resulting from a large variability of foci locations and tasks employed (results not shown). Further extending the scope to all tasks (motor and non-motor, 498 foci, 74 experiments) in the PD-OFF to HC comparison provided a more symmetrical picture, with the pattern of right-handed tasks preserved and more areas in mainly prefrontal areas present (Fig. 7A).

Effect of medication

In view of a small number of experiments investigating PD-ON/PD-OFF and PD-ON/HC comparisons (27 experiments for each comparison) we could only perform aggregated analyses across all tasks. Direct comparison of PD-ON and PD-OFF states (Figure 7B) showed hyperactivations in the SMA, basal ganglia and bilateral anterior insula and hypoactivations in premotor, medial frontal and parietal areas. No changes were seen in the cerebellum. PD-ON/HC analysis (Figure 7C) showed symmetrical areas of hypoactivations in the primary and secondary somatosensory areas, the thalamus and cerebellum, coupled with hyperactivations in the primary motor areas, pre-SMA and cerebellum.

In order to clarify which of the original abnormalities present in PD-OFF state respond to medication, Responsiveness Analysis 1 was performed. This showed that medication responsive changes (both hyper- and hypoactivations) were located in the SMA, the left superior parietal lobule and the basal ganglia (red regions in Figure 7D). The medication unresponsive changes from

Responsiveness Analysis 2 were found in the somato-sensory cortex bilaterally, the left motor cortex, the pre-SMA and in the cerebellum (blue regions in Figure 7D).

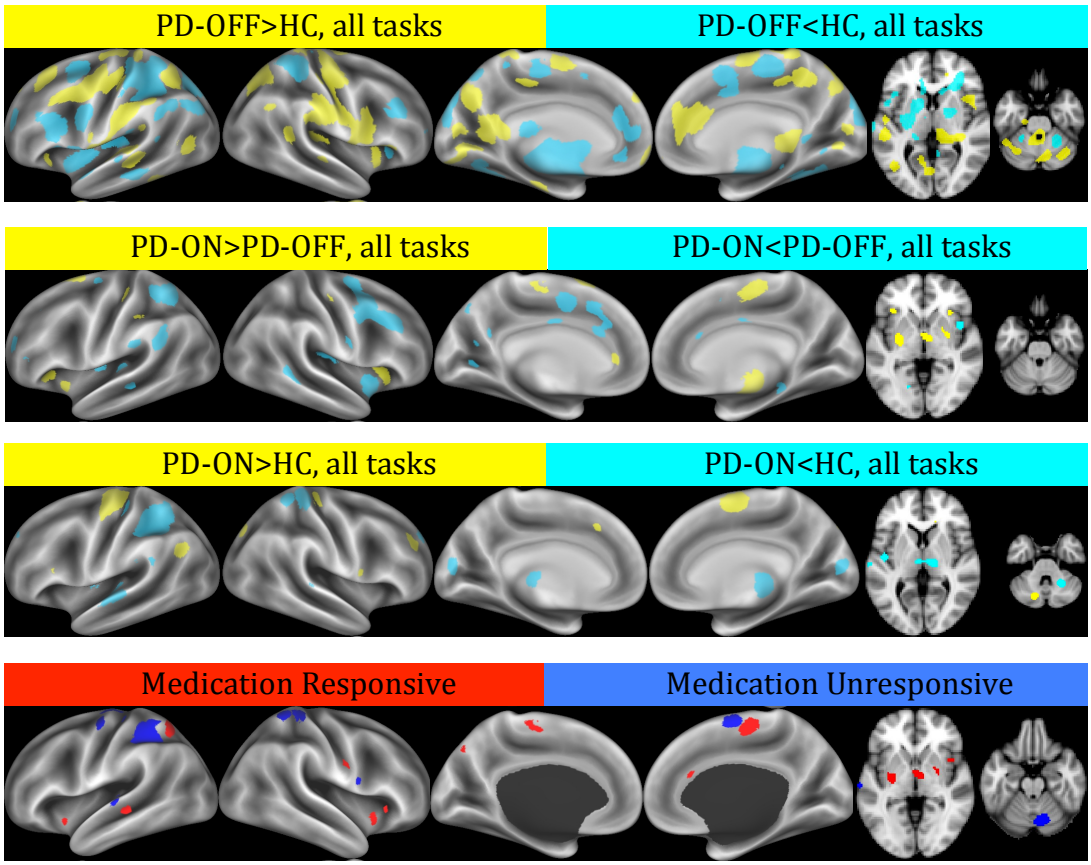


Figure 7. Effects of medication. Activations are thresholded at p<0.01, FDR corrected.

A. PD-OFF vs. HC analysis across all tasks.

B. PD-ON vs. PD-OFF analysis across all tasks.

C. PD-ON vs. HC analysis across all tasks.

D. Responsiveness Analyses: Medication Responsive areas (red) and Medication Unresponsive areas (blue).

Section 4. Discussion

This is the first coordinate-based meta-analysis (CBMA) of functional imaging studies in Parkinson's Disease. Our results show that, even with a simple right-handed task, PD patients off medication present a complex pattern of hyper- and hypoactivations compared to control subjects. Direct comparison of the two medication states suggests that dopamine supplementation may both increase and decrease activation. Based on response to dopamine supplementation two sets of activation patterns can be discerned: medication-responsive areas directly linked to nigro-striatal dopamine loss, and medication-unresponsive regions whose function may depend on other neurotransmitter systems.

Patterns of activations in PD

There are two striking general features of the right-handed motor task analysis: coexistence of hyper- and hypoactivations and bilateral spread of the abnormalities. The first one cannot be easily explained by the traditional basal ganglia model, which only mentions reduced cortical activation in PD (DeLong and Wichmann, 2009; Obeso and Lanciego, 2011). To address the presence of hyperactivations the concept of functional compensation has been invoked (Grafton, 2004). It has been suggested that in the presence of failing basal ganglia circuits other brain areas may need to increase their activity, or completely new circuits may need to be recruited to maintain the same behavioural output (Haslinger *et al.*, 2001; Künig *et al.*, 2000; Sabatini *et al.*, 2000).

Presence of symmetrical bilateral changes in both the cortex and the basal ganglia is surprising, given the unilateral nature of the right-handed tasks. This clearly indicates a pathophysiological effect of PD on the function of the whole brain, even in tasks as simple as hand movements. The finding is consistent with the compensation hypothesis but it may also suggest that pathological activations 'spread' to the opposite hemisphere through symmetrical brain networks. Furthermore, it suggests a need for a more holistic, network-based approach to the analysis of individual experiments – moving beyond a focus on individual areas and structures. Interestingly, recent studies have shown that many of the resting state networks (RSN) are symmetrical, including the Sensori-Motor Network (Biswal *et al.*, 2010; 2009; Laird, P. M. Fox, *et al.*, 2011), the Basal Ganglia Network (Robinson *et al.*, 2009) or the Default Mode Network (Damoiseaux *et al.*, 2006).

I will now discuss specific changes in three areas implicated in movement control: the basal ganglia and SMA proper, the sensori-motor cortex and the cerebellum.

The basal ganglia are central to PD pathophysiology as areas suffering the greatest reductions in dopamine projections. The SMA, in turn, is an integral part of the sensorimotor BG-thalamocortical circuit (DeLong and Wichmann, 2009). Activation in the basal ganglia has been shown to inversely correlate with dopamine-responsive motor symptoms in various motor tasks (Damier *et al.*, 1999; 2011; Prodoehl *et al.*, 2010; T. Wu, Chan, *et al.*, 2010; T. Wu, Wang, *et al.*, 2010). Similarly, SMA activity (T. Wu and Hallett, 2008) and connectivity (Cao *et al.*, 2011; T. Wu, Wang, *et al.*, 2011) have been found to closely reflect disease

symptoms in various task paradigms. Hence, reduced activation in those two regions is most consistent with the classical BG model in PD.

In the sensori-motor area, a bilateral pattern of precentral hyperactivation and postcentral hypoactivation was identified. As will be discussed later, those areas featured strongly in the medication effect analyses too. Changes in the postcentral area would be expected primarily in sensory tasks (Cao *et al.*, 2011), but their presence in purely motor analysis suggests an abnormal proprioceptive or kinesthetic control of movement (Mima *et al.*, 1999; Szameitat *et al.*, 2012). There is strong evidence for proprioceptive deficits in arm or leg movements in PD (Konczak *et al.*, 2007; 2009; Maschke *et al.*, 2003; Putzki *et al.*, 2006) which may cause post-central hypoactivations (Boecker *et al.*, 1999; Foki *et al.*, 2010; Parkinson Progression Marker Initiative, 2011) and fail to respond to PD treatment (Jobst *et al.*, 1997; Maschke *et al.*, 2003; 2006; O'Suilleabhain *et al.*, 2001). The precentral hyperactivation may be a form of compensatory response to reduced proprioceptive input.

Finally, the cerebellum showed a mixture of hyper- and hypoactivations. Cerebellar hyperactivations have received most prominence in PD (Cerasa *et al.*, 2006; Lewis *et al.*, 2007; 2011; Rascol *et al.*, 1997; Sen *et al.*, 2010) and have been interpreted as compensatory recruitment of the cerebello-striato-cortical network in the face of a failing striato-cortical network. It has also been suggested that cerebellum may be the source of tremor in PD, which has been explained as a side effect of the compensatory overactivation of that structure (Helmich *et al.*, 2012). Presence of hypoactivations in our meta-analysis adds another layer of complexity to the picture and requires a modification to that theory.

Effect of medication

In view of a small number of experiments investigating effects of medication we could not perform separate analyses for different task groups. Thus, our conclusions can only be very general. The PD-ON/HC analysis identified both hypo- and hyperactivations in the dopamine-replete state. This is contrary to a common conclusion in PD imaging studies stating that dopaminergic medication normalises aberrant activations. In fact, dopamine supplementation may induce overstimulation of circuits not affected by dopaminergic degeneration (Cools *et al.*, 2007; Delaveau *et al.*, 2009) and, as a consequence, may impair performance in complex cognitive tasks. Furthermore, looking closely at the effect of medication in direct comparison of PD-ON and PD-OFF groups, we can see areas with increasing (Basal Ganglia and SMA) and decreasing (prefrontal cortex) activity as well as regions with no identifiable medication effect (sensori-motor area and cerebellum). This, in turn, suggests that not all activation changes are linked to dopamine loss, making space for other neurotransmitters recently implicated in PD (Rolinski *et al.*, 2012; Yarnall *et al.*, 2011). In order to more precisely delineate medication responsiveness in our meta-analysis two responsiveness analyses were performed.

In Responsiveness Analysis 1 we identified areas of off-medication hypoactivations, which were shown to normalise on treatment: the basal ganglia, the SMA proper and an area in the superior parietal lobule. As discussed above, BG and SMA are central to the basal ganglia model. It is then likely that dopamine-responsiveness of those regions is a reflection of the primary nigro-striatal degeneration.

In Responsiveness Analysis 2, abnormal activations in the off-state were identified which did not improve on medication. Firstly, large hypoactivations around the sensory regions were seen. This is in agreement with proprioceptive abnormalities failing to respond to PD treatment (Jobst *et al.*, 1997). Secondly, the pre-SMA showed persistently increased activation regardless of medication state. This region has a more associative function (Nachev *et al.*, 2008) than SMA and connects with the caudate and anterior putamen (Lehericy, 2004), areas that do not suffer from nigro-striatal dopamine depletion in PD. Persistent hyperactivation in the pre-SMA may represent compensatory changes due to underactivation of the SMA proper. Finally, cerebellar hypo- and hyperactivations also failed to respond to medication.

It is possible that the three dopamine resistant patterns in our analyses are not directly related to the nigro-striatal dopaminergic loss central to Parkinsonian symptoms. Instead, they may represent a broader neurodegenerative process, likely involving other neurotransmitter systems. Alternatively, they could be involved in compensatory processes deployed to make up for the failing basal ganglia circuit.

Limitations

The GPR meta-analysis method uses coordinates of peak activations to reconstruct possible activation levels in adjacent voxels. A major limitation of this method, as well as any CBMA method, as opposed to IBMA, is its use of highly processed and simplified results of very complex studies. Firstly reconstruction of each focus based on the co-ordinate of its peak is bound to be imperfect. Activation foci in real studies are usually highly asymmetrical and

spread over variably large areas – something that cannot be inferred from simple co-ordinates. As a result, a reconstructed study map will underestimate activation in some areas while overestimating it in others. Secondly, accounting for thresholding variability between studies is imprecise when using only effect sizes. This will, inevitably, skew the results towards the more leniently thresholded experiments, albeit to a lesser degree than with other popular methods, like ALE or MKDA. There is also no way to account for differences in other statistical procedures used in each study (e.g., whether FFX or MFX method was used). Thirdly, the vast majority of publications report only positive activations for within-group contrasts (group A, task activation > rest activation), omitting deactivations (group A, rest activation > task activation) in their results. That produces an imbalance, where, in extreme situation, an area may be assigned a positive activation by the meta-analysis, as a result of a few reported positive foci and omitted reporting of negative foci in other studies. This danger is mitigated by focusing the analysis on high-level, between-group contrasts. These analyses invariably report both positive and negative contrasts (activation in group A > group B, activation in group B > group A). For this reason, only group-level contrasts were included in this study. Fourthly, none of the CBMA methods, including GPR, can as yet account for the sample size of each study. This is an important consideration as underpowered studies with small numbers of patients are much more prone to reporting noisy activations. With large variability of sample numbers, the low powered studies may gain disproportionate significance as all their foci, including the noisy ones, will be treated equally with more accurate foci from larger studies.

However, since an overwhelming majority of published results are only available as activation foci, use of coordinate-based method represents an acceptable trade-off between accuracy and the number of analysed studies. Compared to other CBMA methods, the method used here (GPR) has the advantage of accounting for the strength of activation, which enables more realistic reconstruction of the original results.

With regards to specific limitations of the GPR method, the assumption that the map is drawn from the Gaussian process relates to a specific feature of the covariance function f. It assumes that all spatially proximate voxels in the functional data set form a continuum along the surface of the cortex, along which brain activity slowly dissipates as a function of distance from the focus. However, it is an oversimplification as it does not take into account the convolution of the cortex and the fact that two adjacent areas (e.g., adjacent rims of two gyri) may be discontinuous and anatomically separate (in this case, separated by the cortex of the sulcus). In consequence, the relationship between activations in the two gyri violates this assumption. This fact would be a major problem in high resolution studies, where activations in the adjacent gyri could be clearly distinguished, like the 7 Tesla MRI studies. However, in the case of most fMRI studies and certainly all PET and SPECT studies, the available resolution does not allow for such fine differentiation. Additionally, the smoothing function applied in all imaging studies to increase signal-to-noise ratio further contributes to reducing the resolution of the final image.

Meta-analysis requires generalizing across various aspects of experiments, which may be considered important for interpretation of individual results. In Parkinson Disease, for instance, it has been claimed that whether a task is

internally or externally paced may have an important effect on its performance and resulting brain activations. Similarly, the age of participants or disease severity may affect the results. Moreover, different studies used different medication groups in the ON state scans, ranging from levodopa, through agonists and apomorphine, to rasagiline and amantadine. Effect of those drugs on activation sites and strength may be variable and is difficult to account for. This introduces another source of variability into the meta-analysis. It has to be said, however, that all bar 3 studies used levodopa as well as other medications, which makes the effect of medication effects more comparable. While ignoring these differences between studies, meta-analysis focuses only on the common denominator and so its outcomes must be interpreted with caution.

Conclusion

Our meta-analysis reveals a complex pattern of abnormal brain activations in Parkinson disease proving that pathological and compensatory changes spread far beyond the basal ganglia circuit. Dopamine supplementation has a normalizing effect on some of the abnormal patterns, while other circuits seem resistant to medication suggesting development of compensatory activity or involvement of non-dopaminergic pathology. A clearer understanding of the interaction of these systems may help guide treatment for the variety of motor and non-motor symptoms experienced by PD patients.

Chapter IV. Resting-state signature of early Parkinson's Disease

Section 1. Introduction

Fundamentals of resting-state fMRI

As noted in a recent review (Raichle, 2011) resting brain activity was probably first recorded in humans by Hans Berger in 1929 by means of electroencephalography. He also commented that identifying "intellectual work" with this method may be difficult as it contributes little activity compared to the background activity of the brain. An overwhelming majority of studies have since then attempted to eliminate that activity, which has been perceived as noise and contrasted with specific task-related changes. Just how important is this resting activity to the functioning of the brain can be realized when studying energy consumption of the brain. While comprising just 2% of body mass, brain is responsible for 20% of energy consumption in the body (M.D. Fox and Greicius, 2010). However, increases of metabolism accounting for task performance do not usually reach 5% of the resting metabolism, indicating that "idle brain" is already working at full capacity (M.D. Fox and Greicius, 2010).

The first study that brought the attention of the fMRI community to the resting activity of the brain was carried out by Biswal in 1995 (Biswal *et al.*, 1995). The researchers used principles of fMRI, described in Chapter III, to investigate how BOLD signal changes while their subjects were at rest, rather than, as in the classical task-related fMRI studies, during multiple repetitions of a task. They took a raw BOLD time course from a hand area of the cortex and

correlated it with activity of all the rest of brain voxels. The result was a map of voxels where time courses were very similar to the original one from the hand area. That map looked almost exactly the same as one obtained from a traditional task experiment with bilateral finger movements. The authors' conclusion was that "functionally related brain regions exhibit correlation of […] fluctuations in the resting state" (Biswal *et al.*, 1995). In other words, distinct and separate areas concerned with a specific function (like finger movement) have synchronized activity even at rest.

This study has clearly shown that resting brain activity is much more than just a background noise for the task-related activity. The overwhelming power of the background signal over task changes in fMRI was later visualized by Fox (M. D. Fox *et al.*, 2006; M. D. Fox and Greicius, 2010), confirming findings discussed earlier in the context of EEG and metabolic studies. In that study subjects were pressing a button with their right hand (Figure 8.). Extracting a BOLD time-series from the left motor cortex (controlling the right hand), one cannot easily identify the exact moment of the button press and the curve appears as extremely noisy (Figure 8 A.). However, overlying this time-series with the corresponding one from the right motor cortex shows how strikingly similar they are, in spite of the random appearance (Figure 8 B.). Only further subtraction of the right time-series from the left brings out the peak of activation corresponding to the button press (orange curve, Figure 8 C.). Even when focusing only on the time of the button press, the BOLD change responsible for the task activity accounts for only 20% of the variance, with 80% resulting from background activity.

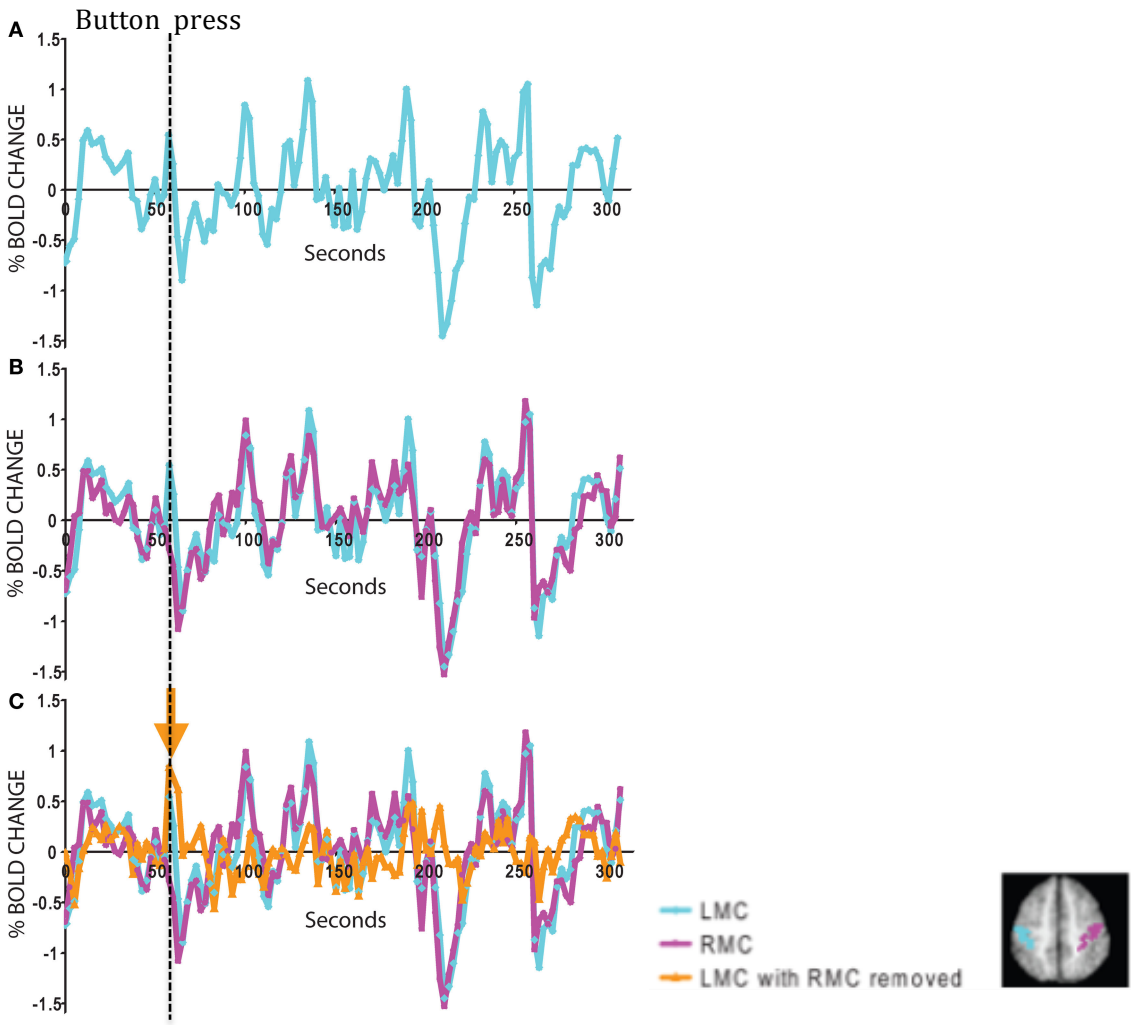


Figure 8. Background and task BOLD activity. Pressing a button accounted for little BOLD variability compared to the background BOLD fluctuations.

A. BOLD time-course from the left motor cortex (LMC, blue) before, during and after a single button press with the right hand (indicated by dotted line). Peak relating to task is drowned by background activity.

B. BOLD time-courses from left (blue) and right motor cortex (RMC, magenta) overlaid on one another. The background activity dominating the recording is highly correlated in the motor cortex across hemispheres.

C. Subtraction of RMC BOLD curve from LMC (orange).

Figure modified from M.D. Fox and Greicius, 2010 and reproduced under Creative Commons Attribution License.

Since its discovery, researches have attempted to characterize the resting BOLD signal in more detail. According to a review by Raichle, it has a few basic features (Raichle, 2011). Firstly, the activity is present under different levels of consciousness, including anaesthesia and different stages of sleep. Hence, it cannot simply represent unconstrained conscious cognition. Secondly, patterns of coherence in the resting activity go beyond simple monosynaptic anatomical connections. Thus, they cannot be simply explained by Hebb's rule "cells that fire together, wire together". Third, background variability of the BOLD signal has been shown to affect intensity of the evoked BOLD changes and influence behavioural outcomes. One of the hypotheses suggests that it does so through coordinating cortical excitability, a theory that will be explained below.

One of the essential questions when characterizing the nature and function of the resting BOLD fluctuations is what is its neurophysiological correlate. Both task related and resting BOLD signal has been shown to correlate best with local field potentials (LFP), which correspond to integrated electrical potentials from pre- and postsynaptic terminals recorded with microelectrodes (Logothetis, 2008). LFP's can be classified according to different frequency bands: infraslow fluctuations (ISF) - <1Hz; delta – 1-4 Hz; theta – 4-8 Hz; alpha – 8-12 Hz; beta – 12-24 Hz and gamma - >24 Hz. The ISF's and delta band, often called slow cortical potentials (SCP), are closest in frequency spectrum to resting and background BOLD fluctuations, which are most prominent in the 0.01-4.0 Hz range (Raichle, 2011). Studies have shown, perhaps not unexpectedly, that resting BOLD fluctuations best correlate with LFP's in the SCP range (He and Raichle, 2009). SCP's have been hypothesized, in turn, to affect higher frequency LFP's, through a mechanism called "cross-frequency, phase-amplitude coupling"

(Raichle, 2011). It posits that amplitudes of the higher frequency LFP's vary depending on the phase of SCP's, in that a certain SCP phase promotes higher amplitudes while a different phase reduces them. Broadly speaking, SCP's, detectable in fMRI as resting-state BOLD fluctuations, modulate cortical excitability. Thus, sharing the same SCP-mediated fluctuations enables distant brain areas to form networks tasked with processing incoming stimuli in a similar manner (Raichle, 2011).

Functional connectivity

The first resting-state fMRI study by Biswal (Biswal *et al.*, 1995) discovered that BOLD activity in the left-sided hand area correlated with activity in the right-sided hand area and also the Supplementary Motor Area. This phenomenon of sharing of similar BOLD activity across different but functionally related areas was called "functional connectivity" as was defined as: "temporal correlation of a neurophysiological index measured in different brain areas" (Biswal *et al.*, 1995). Whereas anatomical connectivity refers to physical neuronal pathways connecting remote regions, functional connectivity represents intermittent and synchronised interactions between spatially separate brain areas (Margulies *et al.*, 2010).

The simplest way of interrogating functional connectivity is seed-based correlation (SBC). This method requires a selection of a voxel, a cluster of voxels or region of interest (ROI) from an anatomical atlas, from which a BOLD time-series is extracted. The time-series is then linearly correlated with another ROI or with all other voxels in the brain to produce a map of areas significantly correlated with the seed (Cole *et al.*, 2010). A major advantage of SBC is that it

provides a simple answer to a direct question using a statistically transparent method: which brain regions have correlated activity with the seed region. Following in steps of Biswal et al. (Biswal *et al.*, 1995) further studies have shown interconnectivity between primary motor areas in both hemispheres and sensory, supplementary and premotor regions (Xiong *et al.*, 1999). Seeds in visual cortex were found to be correlated across hemispheres (Cordes *et al.*, 2000) as were areas of auditory processing (Cordes *et al.*, 2000). Finally, a set of areas, called default mode network, comprising precuneus, medial prefrontal cortex and bilateral parietal cortex was found to be highly interconnected and consistently underactive during cognitive processing and hyperactive at rest (Greicius *et al.*, 2003).

A common conclusion from all those seed based experiments is that resting BOLD fluctuations bind multiple, often remote brain regions (called nodes), to form resting state networks (RSN). In this model, each RSN consists of a spatial map of discreet brain regions which share a common BOLD time-series. The networks are always based in grey matter and are thought to support separate perceptual and cognitive processes (Cole *et al.*, 2010). Moreover, RSN's reflect coactivations seen in task-related studies, although the latter are usually smaller parts of larger RSN's (Biswal *et al.*, 1995; Cordes *et al.*, 2000).

The most well known and characterized networks are shown in Figure 9 and include: Default Mode Network (DMN) (Greicius *et al.*, 2003), Visual Network (VN) (Y.-C. Li *et al.*, 2011), Auditory Network (AN), Sensorimotor Network, Executive Control Network (ECN) (Seeley *et al.*, 2007), Salience Network (SN) and Dorsal Attention Network (DAN).

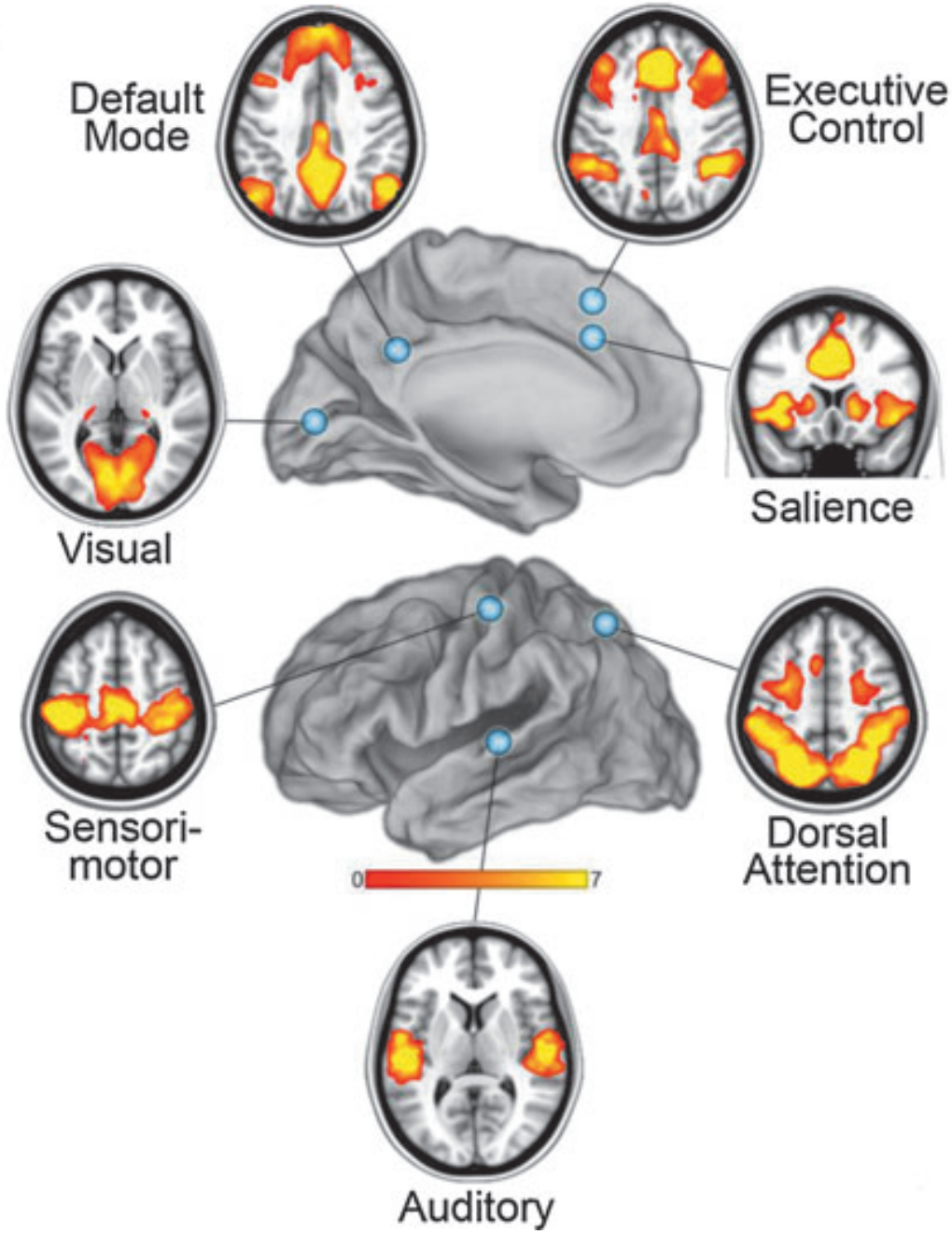


Figure 9. Major resting state brain networks. The red-yellow areas indicate brain regions connected by a common low frequency BOLD fluctuation. Reproduced from Raichle, 2011. Permission to reproduce this figure has been granted by Mary Ann Liebert Inc. Publishers.

The relative simplicity of the resting-state fMRI protocol is a major advantage in studying disease populations. The method has been particularly widely used in the study of dementia, thanks to the purported role in cognition of one of the most widely researched networks, the DMN. Greicius et al. (Greicius *et al.*, 2004) have shown that reduced connectivity in the hippocampus and the cingulate in the DMN has a potential as a disease biomarker and can differentiate patients from controls. Another study, by Filippini et al. (Filippini *et al.*, 2009), demonstrated that functional connectivity may be reduced in the DMN even prior to development of cognitive impairment in subjects with a genetic risk factor for dementia.

A disease with largely motor phenomenology, Amyotrophic Lateral Sclerosis, have also showed abnormal connectivity in the DMN and, more importantly, in the Sensorimotor Network (SMN) (Mohammadi *et al.*, 2009). Changes in the latter network have been demonstrated in a recent study to differentiate between patients and controls (Douaud *et al.*, 2011).

Studies in psychiatric diseases, including depression and schizophrenia, also showed changes in functional connectivity. Whitfield-Gabrieli et al. (Whitfield-Gabrieli *et al.*, 2009) showed increased connectivity at rest in the DMN in patients with schizophrenia which correlated with disease severity. A similar change, but to a lesser degree, was seen in relatives of those patients (Whitfield-Gabrieli *et al.*, 2009).

For all its success, the seed based method has multiple limitations. A major problem with SBC is subjectivity in the choice of seeds. It is mostly a well known anatomical region or an activation focus from a task study. There is, however, no

clear reason why a seed should be limited to a particular anatomical area or be of particular size. It has been shown that, indeed, a small change in the size or location of the seed area may lead to large changes in the resulting map (Cole *et al.*, 2010). Additionally, seeding the network from one of its other nodes may result in a significantly different RSN configuration (Cole *et al.*, 2010). Another problem with SBC is that the original time-series may be contaminated by physiological (cardiac, respiratory, CSF related) or mechanical (head motion) noise (Margulies *et al.*, 2010). Investigators attempt to clean the seeding time-series by regressing well known sources of noise. This procedure can only account for the known noise and will necessarily ignore contaminants not included by the investigator (Cole *et al.*, 2010). Finally, SBC cannot separate networks that share a common node (Cole *et al.*, 2010). If the seed area is involved in different networks through different BOLD time-series then the resulting seed-based connectivity map may contain parts of two separate networks. In an attempt to remedy the limitations of the seed based analysis some researchers turned to methods enabling observer-independent isolation of resting state networks.

Independent Component Analysis

Independent component analysis (ICA) assumes that the brain is organized into functionally separate networks and attempts to identify BOLD time-series unique to each of them, forgoing the observer-dependent selection of a seed region (Margulies *et al.*, 2010). ICA is often explained as a solution to the cocktail party problem (G. D. Brown *et al.*, 2001). The problem refers to a task of separating multiple streams of conversation in a room filled with people all

talking simultaneously. The situation is presented in Figure 10, where multiple sources of signal (people) recorded and mixed by a number of detectors (microphones) have to be unmixed and identified as independent components. In this analogy individual RSN BOLD timeseries are equivalent to multiple signal sources. The signals are recorded by detectors placed in every brain voxel. Inevitably, each detector/voxel receives a mix of different BOLD signals from different RSN's in its vicinity. ICA algorithm takes the input signals from all the voxels and unmixes it into independent components thought to represent the original RSN time-series. Those time series can be further regressed against the original BOLD timeseries in each voxel to produce spatial maps of individual RSN's. A detailed technical description of the algorithm and its implementation in the analysis of RS-fMRI can be found in Beckmann et al. (Beckmann, DeLuca, Devlin and Smith, 2005b)

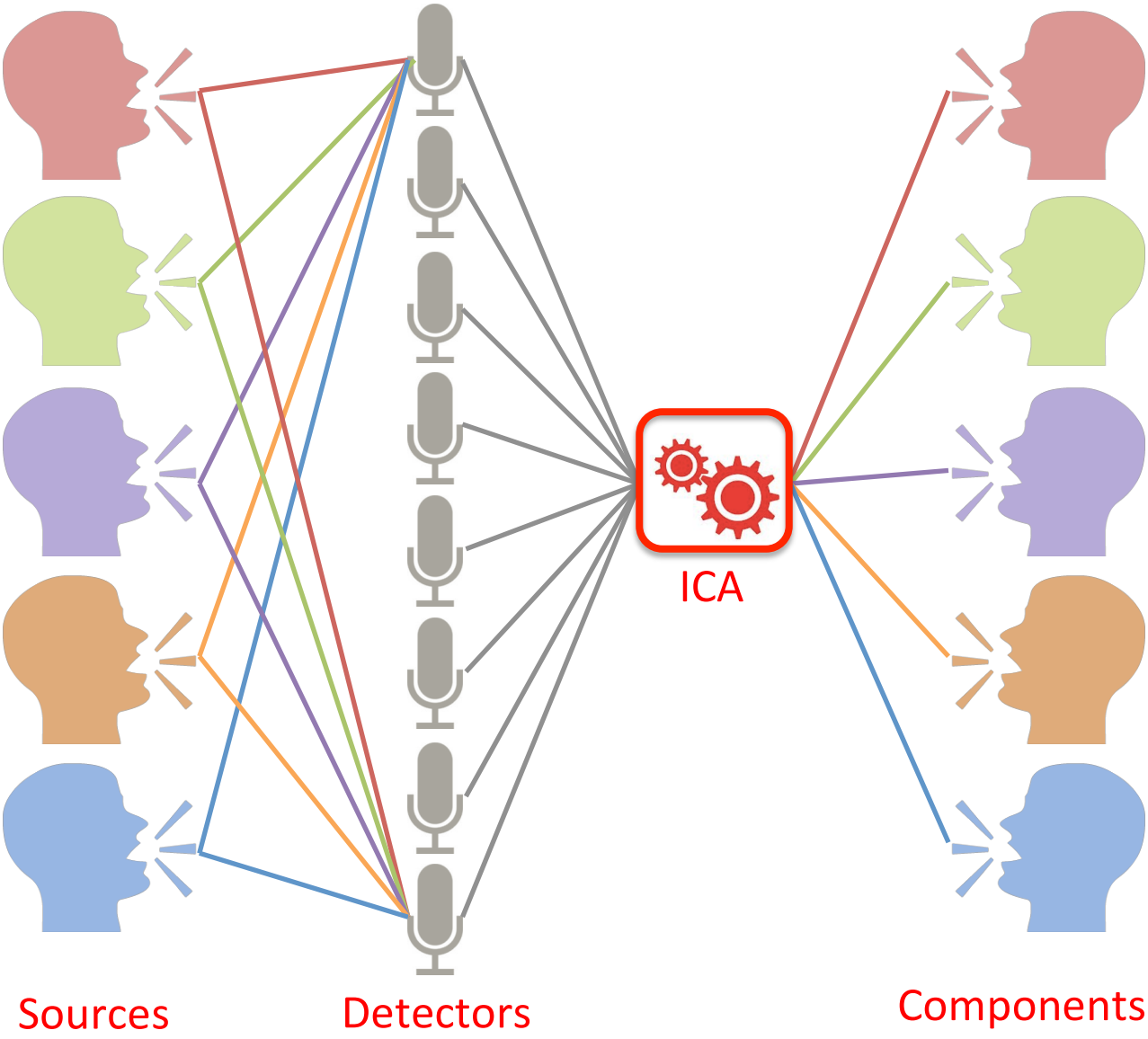


Figure 10. Independent Component Analysis. Multiple sources of signal (people) recorded and mixed by a number of detectors (microphones) are unmixed and identified as independent components.

The ICA method makes following basic assumptions (G. D. Brown *et al.*, 2001):

- signal sources are independent and stationary,
- the mixing process is linear and
- the number of sources cannot be larger than the number of detectors.

Independence refers to spatial discreetness of the networks. This requirement does not actually exclude spatial overlapping of the networks, which happens on the borders between neighboring RSN's. Rather, it specifies that the networks cannot be highly co-linear (Cole *et al.*, 2010). The requirement of linearity for the original mixing process cannot be guaranteed but it is likely the best approximation a biological process (G. D. Brown *et al.*, 2001). Equally, it is difficult to establish the exact number of sources but the most reproducible networks are obtained with dimensionality (specified number of networks for extraction) of 20-60, well below the standard number of voxels in modern fMRI studies (Beckmann, DeLuca, Devlin and Smith, 2005b).

The major advantage of ICA over seed-based correlations is its observer-independence in the process of seed estimation. RSN nodes are not selected but, rather, emerge from the process of finding the most spatially independent BOLD time-series in the data. This helps reduce potential bias and variability between studies (Margulies *et al.*, 2010). It also avoids the network being heavily centered on one node, which may happen with SBC, as discussed above (Cole *et al.*, 2010). Further advantage of ICA is its ability to separate structured noise (Cole *et al.*, 2010; Margulies *et al.*, 2010). Head movement artefacts or cardiac noise is segregated into separate independent components leaving the RSN components 'cleaner'. This is potentially more effective than regressing head

motion registered externally (as done in SBC), as ICA identifies the actual effect of the noise within the recorded BOLD signal. Moreover, various types of noise can be identified above the usual set included in the SBC (magnetic field irregularities, scanner specific noise). Finally, seed-based methods provide a measure of total connectivity of a given region ignoring the fact that the same area may be involved in different neural networks (De Luca *et al.*, 2006; Joel *et al.*, 2011). Superiority of ICA lies in the ability to investigate connectivity within specified networks, even when they partly overlap spatially (Joel *et al.*, 2011).

In spite of its relative automation of signal processing, ICA has its limitations. Firstly, the number of components (called dimensionality) has to be established prior to running the algorithm. The most commonly used ICA algorithms provide an option for automatic dimensionality estimation, however, many researches choose to specify it themselves (Margulies *et al.*, 2010). That introduces subjectivity and potential bias, which many authors hope to avoid by referring to previous literature to support their choices. A consequence of the dimensionality problem is a, so called, 'networks within networks problem' (Cole *et al.*, 2010). Performing the analysis with two different dimensionalities will identify smaller sub-networks in the higher dimensionality analysis, which have been hiding in the larger networks of the lower dimensionality. That presents a question of biological significance of the super- and sub-networks. Having acknowledged this difficulty, it is worth noting that biological systems, including human brain, often naturally display multilevel hierarchical organization, e.g., the visual cortex can be divided into primary and secondary areas, the secondary area has regions for motion and colour perception, which

can be further subdivided down to cortical columns, etc. (G. D. Brown *et al.*, 2001). Another potential limitation of the ICA method is observer-dependence for separation of relevant RSN's from artefactual components. The actual task is, however, practically much simpler than might be predicted. The artifact components have specific power distributions and recognizable maps mostly centered outside the grey matter (Beckmann, DeLuca, Devlin and Smith, 2005b). On the other hand, RSN's have their own typical shapes, known, among others, from previous seed based studies. Finally, whereas SBC-based maps can be simply explained as displaying functional connectivity with a specific region, the ICA maps may seem difficult to interpret, especially in studies without a predefined hypothesis. The 'broad' or basic function of each RSN can be gleaned, however, from studies comparing them with results of task-related studies or even seed-based connectivity studies. In that sense, sensori-motor network would be important for motor function, while visual or auditory RSN's are essential in respective sensory processing. Greater difficulty arises with more 'abstract' RSN's, such as executive control, default mode or salience networks.

Having discussed the limitations of independent component analysis, it is worth reviewing evidence for biological significance of ICA-derived networks. The issue can be broadly divided into problems of reproducibility and problems of comparability of the ICA networks with results of other studies. As for reproducibility, it is important to note that ICA networks are highly consistent across different sessions in the same subjects (S. Chen *et al.*, 2008) as well as between different participants (Damoiseaux *et al.*, 2006) and, importantly, different studies (Cole *et al.*, 2010). Different imaging methods have replicated

RSN networks found in fMRI studies, including perfusion fMRI (De Luca *et al.*, 2006), magnetoencephalography (Brookes *et al.*, 2011) and cortical neurophysiological recordings (He and Raichle, 2009). Similar sets of ICA-derived RSN's are also found across different age groups, e.g. in infants (Fransson *et al.*, 2007) and in elderly subjects (Greicius *et al.*, 2004). What is crucial for future clinical use of the method, consistent ICA RSN's are present in different disease states, including amyotrophic lateral sclerosis (Mohammadi *et al.*, 2009), dystonia (Mohammadi *et al.*, 2012), Alzheimer's disease (Greicius *et al.*, 2004), depression (Greicius *et al.*, 2007) and epilepsy (Widjaja *et al.*, 2013).

With regards to comparability of the standard ICA-derived RSN's, those same networks can be found in task-related experiments. Laird et al. (Laird, P. M. Fox, *et al.*, 2011) performed a meta-analysis of a large number of task-fMRI experiments and found very good correspondence between task-fMRI networks and those derived via ICA from resting-state studies. Networks identified included the primary processing areas (Visual, Audtory and Sensorimotor Networks) as well as association areas forming Default Mode, Executive Control or Salience Networks. Correlation of respective networks with tasks that activated them showed that DMN was mainly associated with theory of mind and social cognition tasks, ECN – with language and executive tasks while SN– with a variety of tasks linking emotional responses to cognition (Laird, P. M. Fox, *et al.*, 2011). As mentioned before, ICA RSN's are also strikingly similar to networks obtained with seed-based methods (Cole *et al.*, 2010; Rosazza and Minati, 2011).

Independent Component Analysis in fMRI has been rapidly developing since its first use in task fMRI by McKeown et al. in 1998 (McKeown *et al.*, 1998) and then in resting-state fMRI by Kiviniemi et al. in 2003 (Kiviniemi *et al.*, 2003). Many popular software packages are available and a number of variations of the method have been explored. More detailed discussion of the method used in this study and rationale for its selection will be presented in the Methods section below.

RS-fMRI in PD

In PD, resting-state fMRI has been used since 2009, with a variety of analytical approaches. BOLD analysed simply as a record of amplitude variability in different brain areas is called Amplitude of Low Frequency Fluctuations (ALFF) and can be thought of as activity map of the brain (Skidmore *et al.*, 2011). In PD, Skidmore et al. (Skidmore *et al.*, 2011) found largely reduced ALFF in SMA, nodes of the DMN and the cerebellum which agreed with previous studies using PET (Moeller *et al.*, 1999) and CASL (Continuous Arterial Spin Labeling) (Ma *et al.*, 2006). Moreover, a classification analysis based on ALFF values yielded 92% sensitivity and 87% specificity for differentiating PD patients from controls. Two later studies using ALFF (Kwak *et al.*, 2012; Zhang *et al.*, 2013), however, reported almost diametrically opposite findings to Skidmore et al., with increased ALFF in many regions which partly normalized after levodopa (Kwak *et al.*, 2010; 2012). The contradictions were ascribed to methodological differences.

A different method of probing functional connectivity at rest investigates local correlation of BOLD timeseries. Called Regional Homogeneity (ReHo) it

looks at how well a signal in a given voxel is synchronized with its neighbours. The assumption is that within a functional cluster the synchronization of activity, or local connectivity, will be similar allowing for delineation of regions working together (T. Wu *et al.*, 2009; 2012; Zang *et al.*, 2004). Wu et al. (T. Wu *et al.*, 2009) found that in PD patients off medication ReHo was reduced in the putamen and SMA which, they interpreted as consistent with reduced activity in those areas found in task-related fMRI studies. Increased homogeneity in the primary and premotor areas and the cerebellum was seen as consistent with increased activity in similar regions in some task-fMRI studies, which has been suggested to have compensatory functions. Administration of levodopa partly normalized the disturbed ReHo and was seen as evidence for dopaminergic changes as a cause for the observed abnormalities in PD. A very similar result was recently reported by Yang et al. (Yang *et al.*, 2013), except for the effect of levodopa, which was not investigated in that study. Regional homogeneity method may be particularly sensitive to noise as neighboring voxels will loose coherence with dropping signal to noise ratio (Zhu *et al.*, 2013). However, the method has been shown to have high reproducibility with different simulated noise levels, as long as the neighborhood size used to calculate ReHo is above 20 voxels (Z. Li *et al.*, 2012) (standard size used in above studies was 26).

Functional connectivity between remote brain areas can be investigated with a so called 'seed-based' method. The method looks at simple covariation of BOLD activity in a predefined seed with BOLD timecourse in each voxel across the whole brain. Many studies in PD focused, unsurprisingly, on the striatum. Helmich et al. (Helmich *et al.*, 2010) investigated seed-based functional

connectivity between the anterior and posterior putamen and the rest of the brain. The researchers found that compared to controls, in PD patients off medication posterior putamen has reduced connectivity with the parietal cortex while connectivity of the anterior putamen with this area of the cortex increases. The authors concluded that, since anterior and posterior putamen belong to different cortico-striatal loops, PD leads to reduction of spatial segregation between different loops, which may underlie deficient sensorimotor integration seen in PD (Helmich *et al.*, 2010). In their opinion, anterior putamen, less affected by dopamine depletion, compensates for the more affected posterior putamen. This study did not, however, assess the effect of dopamine supplementation on the observed abnormalities.

A study by Hacker et al. (Hacker *et al.*, 2012), in turn, looked only at PD patients in the on medication state. They showed a similar effect of increased connectivity in parietal regions with anterior putamen, however, the posterior putamen did not have reduced connectivity with that area. A much more striking result of that study is a greatly reduced connectivity between all regions of the striatum and the brainstem and cerebellum. To explain this phenomenon, the authors tentatively allude to a broad range of concepts concerning the role of brainstem in PD: the Braak hypothesis, the importance of PPN in RBD and gait problems, and the role of the cerebellum in tremor.

A third study using seed-based correlations from the putamen investigated both on and off medication patients (Kwak *et al.*, 2010). In the off medication state compared to controls, all striatal areas showed only increased connectivity with various temporal and frontal structures, which is in stark contrast to the

two other studies. Levodopa strikingly reduced connectivity in PD to the level below that in controls, particularly in the sensorimotor cortex. This finding is particularly difficult to conceptualise given that levodopa only partially alleviates clinical deficits.

Other researchers looked at extra-striatal parts of the basal ganglia. Substantia nigra (T. Wu *et al.*, 2012) showed a very mixed pattern of connectivity in PD off medication versus controls, with both increased and decreased connectivity present in multiple areas across the whole brain. In their discussion, however, authors stressed only the reduced connectivity and pointed to the fact that it normalized with administration of levodopa. Reduced connectivity was explained as a phenomenon paralleling dopamine deficiency in PD.

Subthalamic Nucleus (STN) functional connectivity, a basal ganglia nucleus important for Deep Brain Stimulation in PD, was investigated by Baudrexel et al. (Baudrexel *et al.*, 2011). They found that STN in PD patients off medication has increased connectivity with the motor cortex, a result that was interpreted as consistent with a recently proposed 'hyperdirect' pathway in the Basal Ganglia model (Nambu *et al.*, 2002; Obeso and Lanciego, 2011). Moreover, tremor-dominant patients showed particularly strong connectivity with the primary motor hand area while rigidity-dominant subjects had additionally increased connectivity with the SMA.

Differences between tremor-dominant and rigidity-dominant subtypes in PD was also explored by a study looking at connectivity of the cerebellar structures, thought to play a role in generation of tremor (Liu *et al.*, 2013). Tremor-

dominant subjects displayed reduced connectivity with various cerebellar areas. Additionally, PD in general had altered connectivity with the nodes of the DMN, which the authors interpreted as compensatory mechanisms.

Only one study investigated cortical structures involved in movement generation as seed regions for connectivity analysis. Wu et al. (T. Wu, X. Long, *et al.*, 2011) found that in the off-medication state SMA has reduced connectivity with a broad range of areas, including the putamen and parietal cortex. Conversely, little connectivity changes were found in primary motor areas. The authors concluded that SMA, as a region involved in movement planning and preparation known to be impaired in PD, suffers greater changes than areas concerned with movement execution.

An interesting attempt at combining all the above methods (ALFF, ReHo and seed-based connectivity) to differentiate PD from controls has been described by Long et al. (D. Long *et al.*, 2012). The authors used a Support Vector Machine (SVM) method, a mathematical algorithm automatically selecting most relevant features for separating two groups of subjects. They found that using only functional connectivity methods PD patients can be differentiated from controls with 58% sensitivity and 85% specificity. Adding grey matter and white matter quantification measures improved the classification to 79% and 93%, respectively. The results are impressive, however, closer look at the study raises doubts about the validity of the classification. A low number of subjects, 19 PD and 27 controls, and lack of validation group calls into question the reproducibility of the finding. Surprisingly, the automatic classifier did not include any of the regions previously implicated in PD pathophysiology, e.g.

putamen, SMA or M1. Moreover, 10 of the 20 brain regions in the final set are white matter content changes in the cortex – a feature that has not been previously shown to be affected in PD and is most likely an artefactual finding. The seemingly high accuracy is probably a result of overfitting, a known problem with SVM (De Martino *et al.*, 2008) when the number of subjects used is lower than the number of features available for classification.

Independent Component Analysis in PD

So far four studies have used ICA in PD. Tessitore et al. (Tessitore, Esposito, *et al.*, 2012) found that PD patients on medication compared to controls had reduced connectivity in the Default Mode Network and that connectivity in the DMN correlated with various cognitive measures. However, a previous study by Krajcovicova et al. (Krajcovicova *et al.*, 2012) failed to show any differences in this network despite imaging a group of PD patients on medication with similar age and disease duration. Potentially important differences between those studies were a stronger scanner used by Tessitore et al. (3T versus 1.5T) and greater cognitive impairment of patients in their study (MMSE 27.6 versus 29.6). Perhaps more importantly, Tessitore et al. did not match their groups for cognition and, in fact, cognitive scores in their control group are not available. If, indeed, the groups differed significantly in that regard then the reported results may be simply down to cognitive mismatch and not specific to Parkinson's Disease.

Tessitore et al. (Tessitore, Amboni, *et al.*, 2012) reported in a different paper on results in attention and visual networks in PD patients on medication. They did not identify any differences between patients and controls but once the PD

group was split according to presence of freezing of gate (FOG), the PD+FOG had reduced connectivity in both networks compared to PD-FOG. The findings were interpreted within a concept of competition for attentional resources between locomotion and cognition.

The most recent study employing ICA in PD was published by the same group and looked at Sensori-Motor Network (SMN) in 10 drug-naïve patients (Esposito *et al.*, 2013). They found that PD patients had reduced connectivity in Supplementary Motor Area within the SMN when compared with healthy controls. Importantly, this deficit normalized after levodopa. The authors concluded that underconnectivity in the SMA rather than primary motor areas is consistent with abnormality in motor control and planning seen in PD.

From the above studies it could be tentatively suggested that PD tends to have reduced connectivity in various networks and that the deficits may be responsive to levodopa. However, the number of studies employing ICA in PD is too small to draw any robust conclusions. Moreover, none of the results have been validated on separate samples making false positive findings more likely.

None of the studies so far has looked at the Basal Ganglia Network (BGN). The BGN has been identified as a separate resting network in healthy controls (Robinson *et al.*, 2009), as well as in a meta-analysis of task-related fMRI (Laird, P. M. Fox, *et al.*, 2011) studies. Recently, abnormal connectivity in this network has been demonstrated in epilepsy (Luo *et al.*, 2012). Patients presented greater connectivity in the BGN than controls, which was interpreted as consistent with a strong modulatory function of the basal ganglia in the process of generalization in epilepsy.

The aim of the following experiment was to identify changes in the Basal Ganglia Network in patients with early Parkinson's Disease. The study was planned in three stages. First, a resting-state template containing a BG network was developed on a large group of elderly healthy controls. Secondly, a discovery cohort was used to identify changes in the BGN and the effect of dopaminergic medication on its connectivity. In the last step, a validation cohort was employed to test reproducibility of the findings in a separate group of patients.

Section 2. Methods

Subjects

Thirty-two right-handed patients with early Parkinson's disease were recruited from the Oxford Parkinson's Disease Centre (OPDC) cohort. The OPDC recruits patients from the Thames Valley area with a diagnosis of idiopathic PD within the last 3 years according to UK PD Society Brain Bank criteria. Participants undergo assessment in designated research clinics covering: medical interview, characterization of the motor and non-motor features of PD (including the new MDS-UPDRS (Goetz *et al.*, 2007)) and cognitive assessment. PD subjects in our study were divided into a discovery cohort and a validation cohort. The discovery cohort consisted of 19 medicated patients scanned both off medication (at least 12h after the last dose of dopaminergic medication) and on medication (at least 1h after taking patients own dopaminergic medication). The validation cohort comprised 13 patients: 5 drug-naïve and 8 medicated, all of whom were scanned off medication. Of the total of 27 medicated patients 12 were taking levodopa formulations, 8 – ropinirole, 4 – pramipexole, 2 –

rotigotine, 2 amantadine, and 13 MAO-B inhibitors (selegiline, rasagiline). Of those, 14 were on only one medication while 13 where on 2 or more.

Twenty right-handed healthy controls (HC), matched for age and sex to the discovery cohort were recruited from the OPDC control group. The HC participants were unrelated to PD patients and were mostly spouses or friends of the PD participants.

Inclusion criteria for all participants in the study were: right-handedness and ability to give informed consent. Exclusion criteria were: standard contraindications to MRI scanning, other neurological or psychiatric diseases, and more than one risk factor for cerebrovascular disease (hypertension, diabetes mellitus, hypercholesterolaemia, cardiovascular disease). Specific exclusion criteria for the PD groups were: physician-rated certainty of diagnosis <90%, more than mild tremor in ON-medication state (>2 on any tremor item of UPDRS-III) and presence of dyskinesia or dystonia to avoid motion artifacts during imaging. A specific exclusion criterion for controls was having first or second degree relatives with PD. One healthy control was excluded due to large ventricles, which made registration to standard space problematic. Basic demographic characteristics of study participants are provided in Table 8 and subject specific details can be found in Supplemental Table 1, Appendix C.

The healthy ICA template (see below) was developed using 80 elderly healthy controls - 19 from the OPDC cohort (described above) and 61 healthy control scans from two previously published studies that used the same scanner and acquisition protocol (see below): 22 participants from Zamboni et al. (Zamboni *et al.*, 2013) and 39 participants from Filippini et al. (Filippini *et al.*,

2011; 2012). Inclusion criteria for those subjects were: right-handedness and MMSE ≥ 29. Exclusion criteria were: significant brain atrophy, enlarged ventricles or intracranial pathology (stroke, tumor, cyst) on structural scans. Basic details of those subjects can be found in Supplemental Table 2, Appendix C.

The experiments were undertaken with the understanding and written consent of each subject, with the approval from the local ethics committee, and in compliance with national legislation and the Declaration of Helsinki.

Table 8. Demographic and clinical data of study participants.

	Group			Group differences			
	Discovery	Validation	Healthy controls	Difference between three groups (P value)	Discovery vs. Healthy control (P value)	Discovery vs. Validation (P value)	Validation vs. Healthy controls (P value)
Number	19	13	19	-	-	-	-
Gender (f:m)	10:9	5:8	8:11	0.692a	-	-	-
Age (years)	58.9 (10.8)	66.8 (11.4)	60.6 (7.7)	0.098b	0.867c	0.091c	0.22c
MMSE (total score)	29.0 (0.9)	27.8 (1.9)	29.4 (1.4)	0.01b	0.715c	0.057c	0.01c
Duration of diagnosis (months)	30.7 (11.8)	21.3 (15.1)	-	-	-	0.731d	-
UPDRS III, motor score	23.9 (9.6)	29.7 (14.7)	-	-	-	0.432d	-
Hoehn and Yahr score	1.8 (0.4)	2.1 (0.5)	-	-	-	0.670d	-
LEDD (mg)	382 (205)	402 (167)e	-	-	-	0.647d	-

[a]Chi-square test

[b]One-way ANOVA

[c]post-hoc Tukey test

[d]t-test

[e]excluding drug-naïve patients

Image acquisition

All MRI data were acquired with a 3T Siemens (Erlangen, Germany) Trio MR scanner with a 12-channel head coil. For each subject T1-weighted images were obtained using a 3D Magnetization Prepared-Rapid Acquisition Gradient Echo (MP-RAGE) sequence (192 axial slices, flip angle: 8°, 1×1×1 mm voxel size, TE/TR/TI = 4.7ms/2040ms/900ms).

Functional images were acquired using gradient echo planar imaging (EPI) (TR=2000ms, TE=28ms, flip angle=89°, resolution=3×3×3.5mm). Thirty-four axial slices were acquired per volume, covering both hemispheres with incomplete coverage of the cerebellum; 180 repetitions were acquired in 6 min. Participants were instructed to remain still and awake with eyes open.

Image analysis

Analysis of the resting-state scans was performed using MELODIC v3.0 (Beckmann, DeLuca, Devlin and Smith, 2005b), part of the FSL software package (Woolrich *et al.*, 2009). The images were motion corrected and unwarped using a fieldmap.

It has recently been shown that head motion during a resting-state scan may affect functional connectivity (J. D. Power *et al.*, 2012; Van Dijk *et al.*, 2012). We, therefore, took particular care to account for any effects of motion in our analyses. Firstly, we investigated group differences in average head motion, defined as averaged absolute translations in x, y and z directions, estimated from the motion correction procedure (Van Dijk *et al.*, 2012). As shown in the table e-3, healthy controls did not show significantly increased motion compared to the discovery PD group, in neither off nor on medication state. Secondly, to account

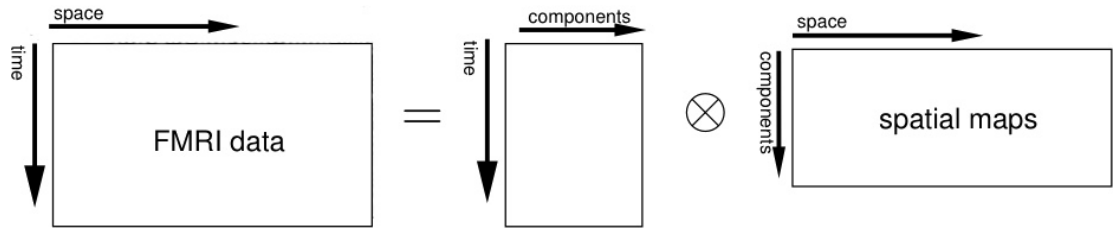
for effect of motion and other noise (various types of physiological noise), we employed an ICA-based denoising approach similar to the one described by Smith et al., 2012 (Smith *et al.*, 2012). Subject-level ICA with automatic dimensionality estimation was performed to identify noise components. An automated component classification method, called FIX, was then used to classify and regress the noise time series from the data (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FIX). This method regresses unique variance related to the noise components and motion confounds from the preprocessed datasets.

The following standard processing steps were applied: high-pass filtering (150s), masking of non-brain voxels, voxel-wise demeaning, normalization of the voxel-wise variance, and spatial smoothing using a Gaussian kernel of FWHM=6.0 mm. The preprocessed data were then linearly registered to the structural image using FLIRT (Jenkinson *et al.*, 2002) with optimization using Boundary-Based Registration (Greve and Fischl, 2009) and registered to MNI space with non-linear registration (FNIRT).

Choice of ICA method

The simplest implementation of ICA in rs-fMRI, called single subject ICA, involves analysing each subject separately. As shown in Figure 11, a single subject's session can be represented as a two dimensional matrix $X_{v,t}$ of BOLD signal values in each voxel $v_{1...m}$ and at time points $t_{1...n}$. ICA decomposes the data into matrix $A_{k,t}$, containing timeseries for each component $k_{1...p}$, and matrix $S_{v,k}$, including spatial maps (value for each voxel $v_{1...m}$) for each component k (Cole *et al.*, 2010). The resulting component maps can be sorted into genuine RSN's and

artefactual components, reflecting structured noise. The benefit of this method is that each decomposition is unique to the subject, a feature that has been exploited for identifying noise components in data denoising. It is much more problematic, however, to statistically compare individual components between subjects or groups. Although RSN's are very reproducible between subjects, there is no certainty that they will be computed in exactly the same configuration in each individual (Cole *et al.*, 2010). Some components may not exist in all subjects at the same level of dimensionality (isolating Basal Ganglia Component may require dimensionality of 20 in one subject and 40 in another). Others can be merged with other components or may be split into two. This problem may be simply due to the amount of noise in the data and its effect on the ICA process (Cole *et al.*, 2010). The only solution are various types of matching of individual components to a standard set (Cole *et al.*, 2010; Margulies *et al.*, 2010). This is most reliable for the strongest components, like DMN or SM, but is much more fallible for lower ranking ones, as Salience Network or Basal Ganglia Network (Zuo *et al.*, 2010).



$X_{v,t}$ $A_{k,t}$ $S_{v,k}$

Figure 11. Single subject ICA. Single subject's data presented as a matrix $X_{v,t}$ are decomposed into a component timeseries matrix $A_{k,t}$ and a component spatial maps matrix $S_{v,k}$ .Modified from FMBRIB course material.

This problem has been the main motivation for developing the group ICA method (Beckmann, DeLuca, Devlin and Smith, 2005a; 2005b; Calhoun *et al.*, 2001). As shown in the Figure 12, it is a modification of the single ICA. In the first step, the subject data are combined into a large group dataset. This happens through concatenation in time, whereby BOLD timeseries from corresponding voxels $v_{1...m}$ from each patient are joined together 'back to back'. Next, one set of component maps is produced for the group as a whole, employing the ICA algorithm similar to the single ICA. Finally, the subject specific timeseries and maps are derived from the group components in the process called 'dual regression' (Filippini *et al.*, 2009). Here, the group-level component maps (spatial regression) are fitted within the General Linear Model (GLM) against the original subject datasets to yield the subject-level component timeseries. Next, these timeseries are regressed against the original datasets (temporal regression) to form subject-level component maps (Cole *et al.*, 2010; Filippini *et al.*, 2009).

In addition to providing a solution to the matching problem, group ICA benefits from a much higher signal to noise ratio compared to single ICA. When analysed together, the signal specific to RSN's is amplified while unstructured noise is reduced (Cole *et al.*, 2010). That leads to further two major advantages over single ICA: firstly, much more detailed and better delineated components, and, secondly, ability to increase dimensionality. The first feature considerably increases accuracy of the RSN selection process, as group components are much easier to recognize than single subject ones. High dimensionality, on the other hand, allows for reliable isolation of networks that would likely merge with others in the single ICA (Cole *et al.*, 2010). Moreover, group ICA with dual

regression has been shown to be more reproducible over time than single ICA (Zuo *et al.*, 2010).

A potential problem with group ICA is the effect individual subjects or subject groups may have on group components. It may be argued that if a specific RSN is strongly expressed in one or more subjects then it may bias the process to produce a stronger component for this RSN at a group level. This may be of particular importance when merging subjects from two different groups, e.g. healthy and diseased subjects. The implicit assumption in the published studies has been that the group level RSN's will be an average of the two groups, especially if the subject numbers are equal in both (unpublished discussions on FMRIB methods forum). This has not, however, been formally tested and the exact effects of this phenomenon are not known. For this reason, the group ICA approach has been modified in this study. Instead of including the disease group in the group component development, the group ICA was only performed on a large group of healthy controls. The subject-level RSN's where then derived from this set using dual regression. That way the resulting template for healthy group RSN's is guaranteed to be unbiased towards the disease group and comes from a relatively uniform set of subjects. Additionally, the template can be reused for other experiments, including a validation experiment described below. This method is open to criticism that some information contained in the disease group data can be lost. For example, the disease group might have influenced the group decomposition to produce slightly different RSN's. However, the authors thought that, on balance, the template approach was the most beneficial.

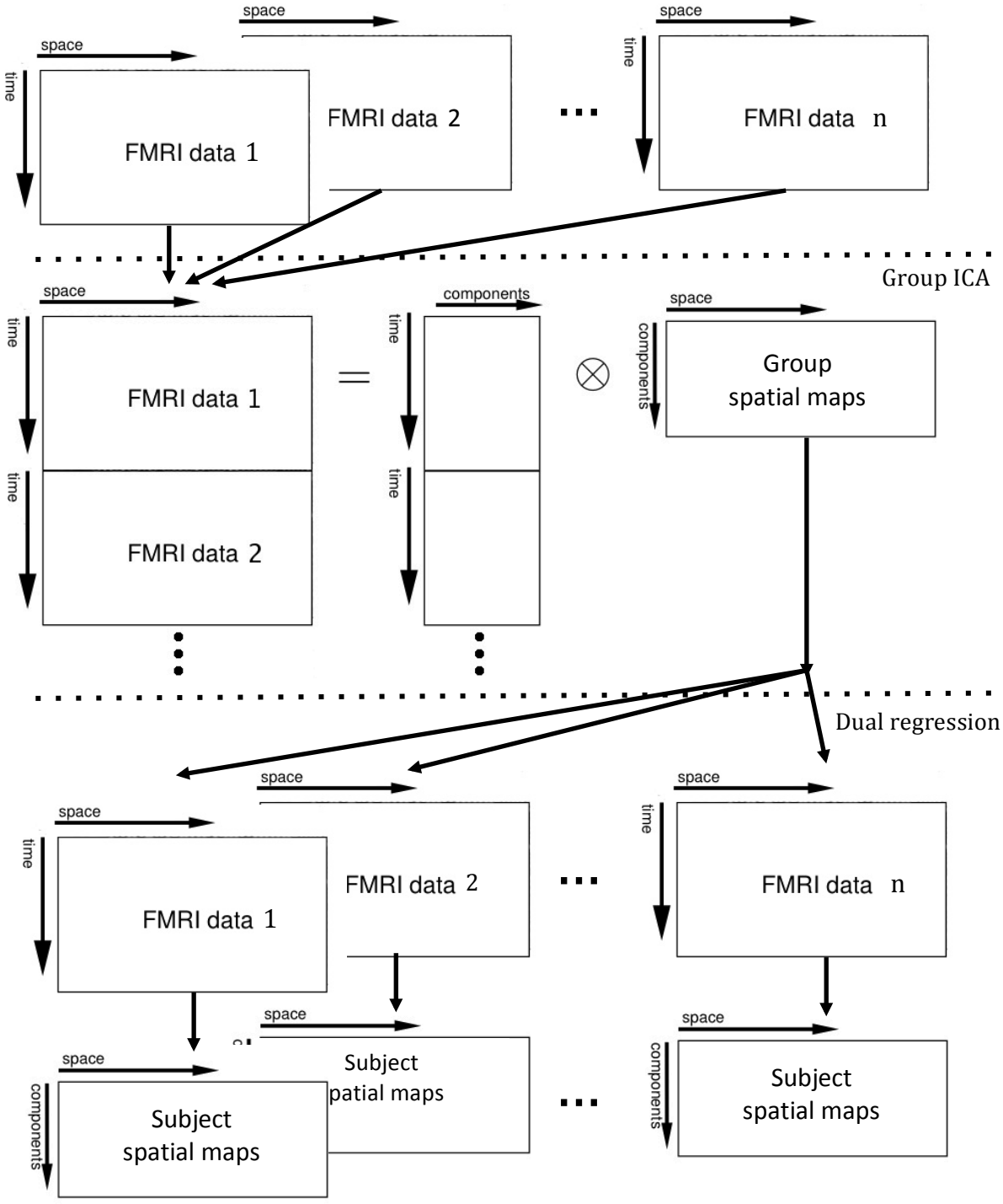


Figure 12. Schematic of group ICA analysis. In the first step, single subject data are temporally concatenated. In the Group ICA, the whole group data set undergoes ICA to yield Group spatial maps. In the Dual regression the group spatial maps are regressed against single subject data sets to produce Subject spatial maps. Modified from FMBRIB course material.

Connectivity analysis

The process of connectivity analysis followed in this study in presented in Figure 13. In order to create an unbiased template of resting state networks typical for healthy elderly subjects, a group ICA implemented in Melodic was performed on all 80 controls (19 from OPDC cohort and 61 from two previous studies). Basal Ganglia Network is not usually present in individual or group ICA results with low dimensionality. In fact, previous studies have identified it with ICA of 40 or more components (dimensionality > 40) (Luo *et al.*, 2012; Robinson *et al.*, 2009). In view of the latter, dimensionality was incrementally increased from 25 until a Basal Ganglia Network was visually identified at a level of 50 components. Further increase of dimensionality to 70 did not produce any appreciable improvement in delineation of the BG component and dimensionality of 50 was chosen for further analysis. The amount of variance explained by the BGN was then calculated.

Twenty-nine components (including the BGN) were classified as relating to functional connectivity according to the following criteria: main network nodes located in the grey matter, low PE values in the skull, CSF and white matter, similarity to standard RSN's identified by Biswal et al., 2010 (Biswal *et al.*, 2010) and Beckmann et al., 2005 (Beckmann, DeLuca, Devlin and Smith, 2005b). The remaining 21 components were identified as residual noise. Since the focus of the study was the BGN network, only the BGN maps were analysed further.

Differences in BG resting functional connectivity across cohorts was analysed using the Dual-Regression method (Filippini *et al.*, 2009), a method described above. This approach allowed for assessment of specific changes in the connectivity of the basal ganglia, controlling for differences in correlation

associated with global signal and artefacts. Spatial maps of the BGN and noise components were projected using GLM onto each subject's functional images to extract subject-specific time-courses for each component. Next, the BGN and the 21 noise timeseries were used to estimate BGN component maps in a linear model fit against the individual fMRI data sets. This last step is presented in Figure 14. Voxels of these component maps contain parameter estimates (PE), which are regression coefficients for the BGN and noise components timeseries. Biologically, PE for a given component indicates how much the component contributes to the BOLD timeseries in that voxel. A large PE in a group of voxels suggests that the group shares the component timeseries to a high degree and, hence, that it is functionally connected.

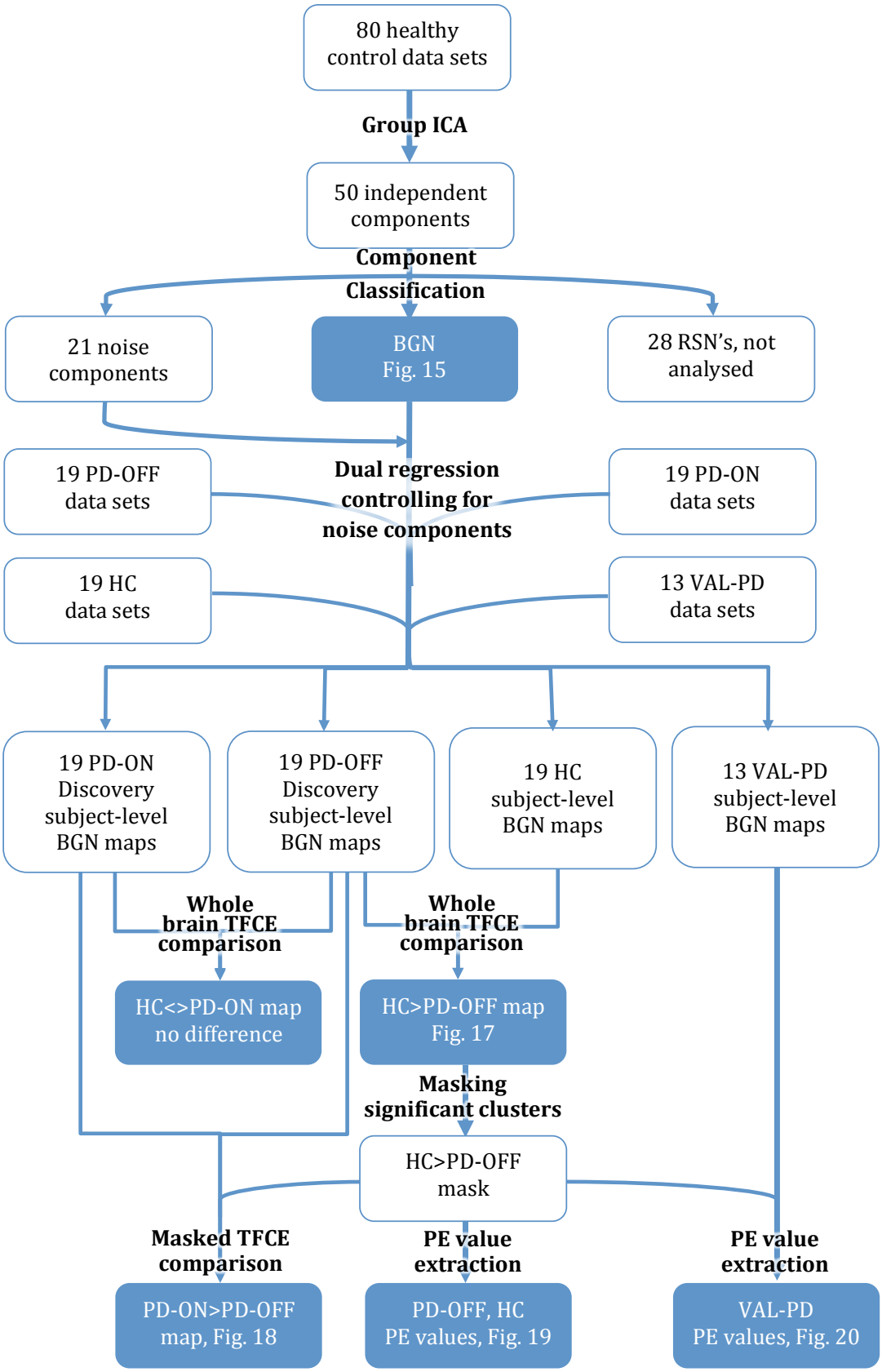


Figure 13. Flowchart showing processing steps in the connectivity analysis. The blue rectangles represent results and figures included in the manuscript.

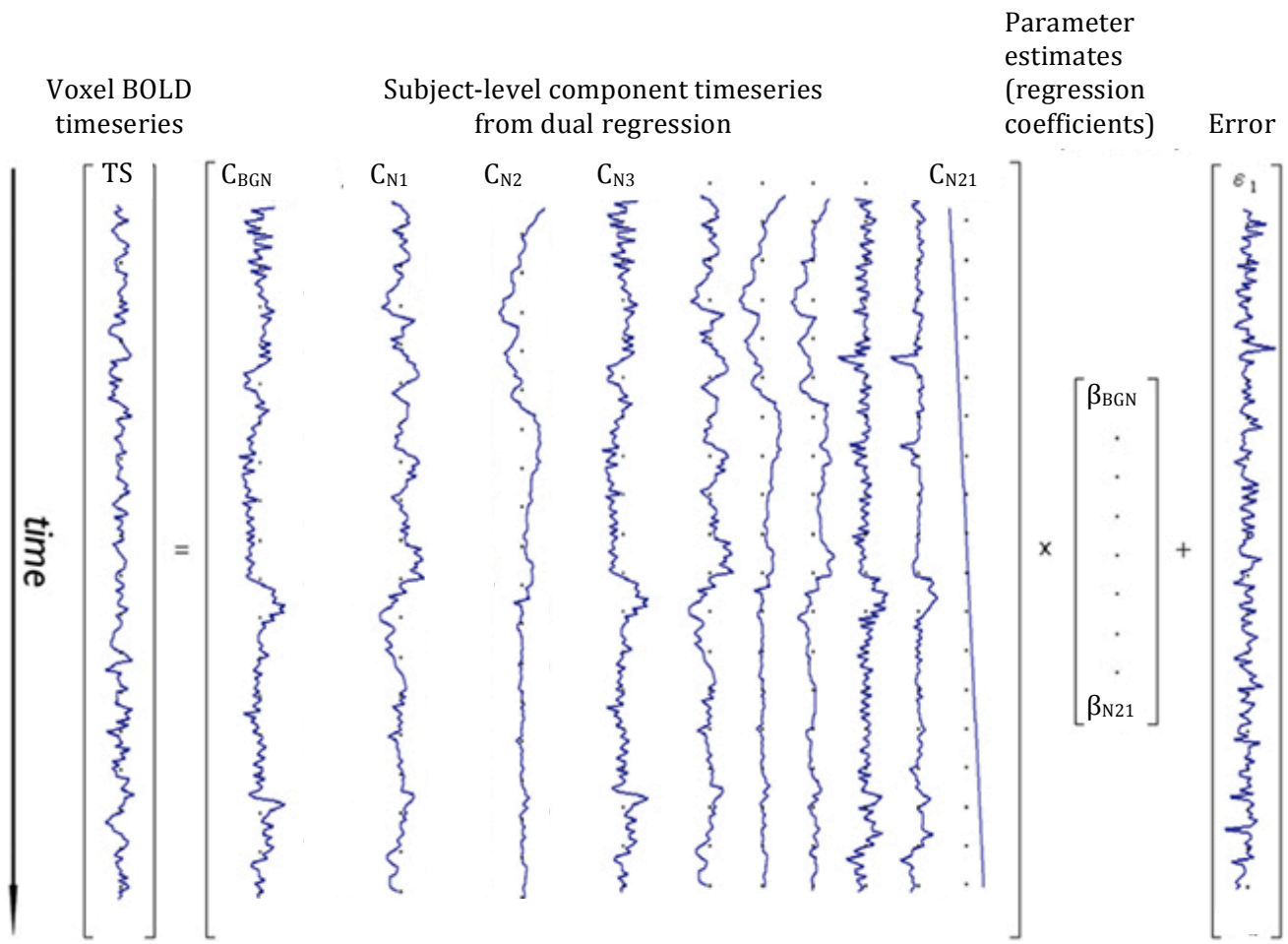


Figure 14. Multiple regression of parameter estimates for ICA components from subject-level timeseries. Each voxel timeseries (TS) in the original subject data set is regressed against subject-level component timeseries (C_{BGN}, C_{N1} – C_{N21}) from first step of the dual regression. The results are parameter estimates (PE) or regression coefficients (β_{BGN}, β_{N1} – β_{N21}) for each component in that voxel and an error term (ε).

At the discovery stage, spatial BGN connectivity maps of 19 PD-OFF subjects were compared to 19 age and sex matched HC subjects using unpaired t-test implemented in the FSL tool Randomize (v2.1). Randomise provides exact false positive control using a permutation testing implemented in Threshold-Free Cluster Enhancement (TFCE) (Smith and Nichols, 2009) which enhances sensitivity to spatially limited effects. Significant clusters (at $p<0.05$ after FWE correction) were then used as a mask to identify medication effects in a paired t-test comparison of 19 PD-OFF and 19 PD-ON subjects. The significance was set at $p<0.05$, FDR corrected within the above mask. PD-ON patients were also compared to HC subjects with an unpaired t-test, using TFCE in randomize.

In order to characterize connectivity changes between PD-OFF and HC in more detail, a post-hoc analysis was performed on averaged parameter estimate (PE) values from significantly different clusters. A receiver-operator characteristic (ROC) curve was generated to determine an optimal threshold for separating the two groups based on average connectivity.

At the validation stage, average connectivity was extracted from 13 PD patients from the validation cohort, using a mask of significant clusters from the discovery stage. A threshold identified in the ROC analysis was applied to the validation cohort and its accuracy in this group was determined.

Calculation of explained variance

In view of the high number of components in the group ICA the amount of variance per component compared to total variance in the whole data set was expected to be low. However, since the components were spatially independent, variance was predicted to vary considerably across brain regions. Specifically,

percentage of variance explained by the component was expected to be high in the main nodes of the networks and low in areas not contributing to the component, resulting in low average but high local variance. To visualize that effect a voxel-wise distribution of variance explained by the BGN network relative to total variance in the 4D dataset was calculated. This was done for each of the 80 subjects included in the template, and then averaged across subjects. A simple relationship between variances was assumed:

$V_{total} = V_{BGN} + V_{other}$,

where:

V_{total} – total subject-level variance in an individual dataset,

V_{BGN} – subject-level variance explained by the BGN timecourse,

V_{other} – subject-level variance explained by all other independent components (28 RSN's and 21 noise components) and residual variance not explained by the ICA decomposition.

Percentage of explained variance by the BGN was calculated according to:

$$\%V_{expl} = 100 * \left(\frac{V_{total} - V_{other}}{V_{total}}\right)$$

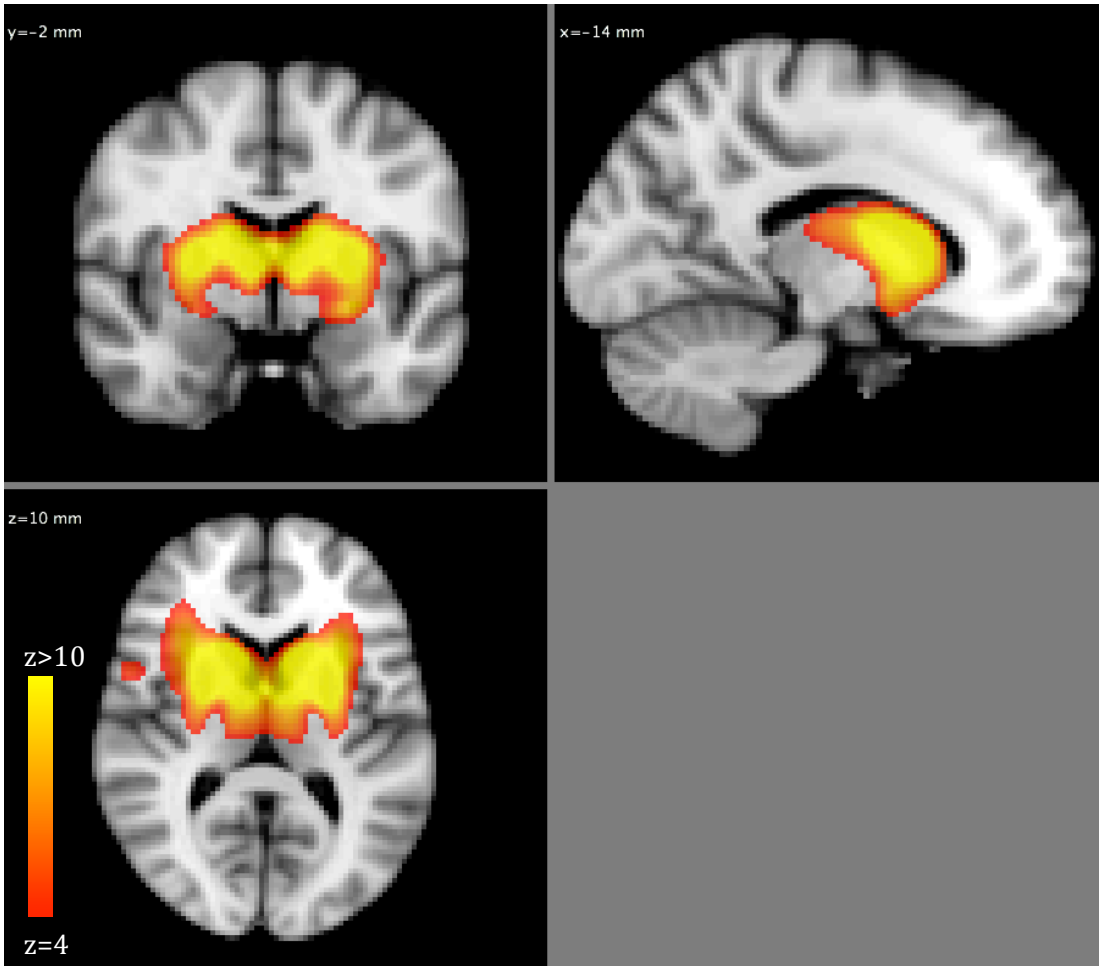
V_{total} was a 3D image with voxel values indicating total variance in the voxel's time series and was calculated using fslmaths, FSL. V_{noise} was calculated by regressing the individual BGN timecourse from each individual dataset (using fslregfilt, FSL) and then calculating voxel-wise variance in the whole image (fslmaths, FSL). The result was a 3D image with value in each voxel corresponding to the variance in that voxel. All $\%V_{expl}$ were then averaged in a voxel-wise manner across all 80 subjects.

Candidate's contribution to the study

The candidate designed the study. Data collection was performed in collaboration with Dr. Ricarda Menke, a Research Fellow at the FMRIB Centre, University of Oxford. The candidate analysed the data, interpreted the results and wrote the manuscript on which the above chapter is based.

Section 3. Results

ICA consistently identified a basal ganglia network including the putamen and caudate bilaterally as well as anterior parts of the thalamus (Figure 15). This network explained 30-60% of the variance of voxels within the basal ganglia (Figure 16).


y=-2 mm
x=-14 mm
z=10 mm
z>10
z=4

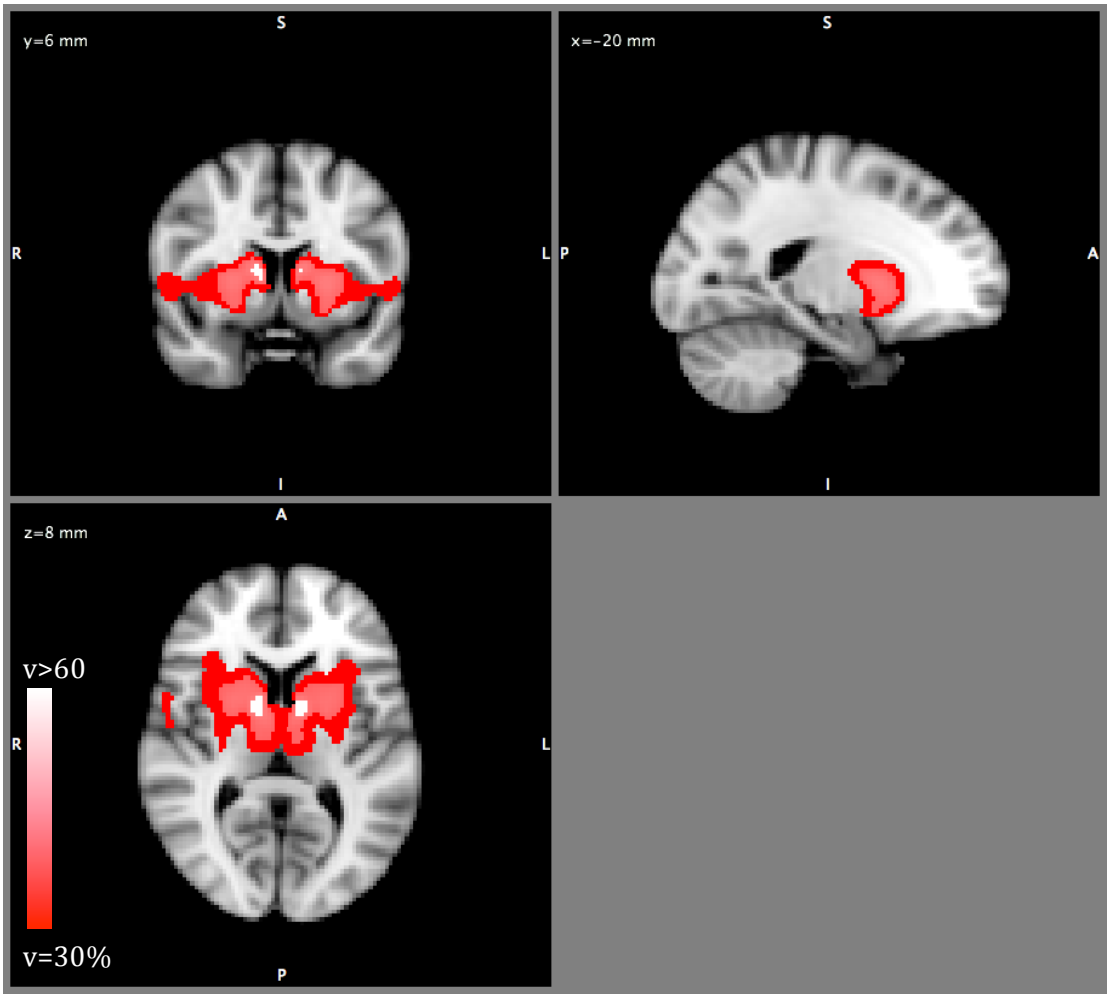


Figure 16. Percent of variance explained by the Basal Ganglia Network relative to total variance from 80 subjects in the ICA template. Intensity of each voxel reflects percentage of explained variance as per the colour bar. The image is overlaid onto the MNI152 brain.

At the discovery stage, voxel-wise comparison of 19 PD-OFF patients to 19 age and sex matched controls demonstrated reduced BGN connectivity in PD-OFF group in 16,273 voxels spread across 9 clusters (Table 9) (Figure 17): putamen and caudate bilaterally, midbrain, superior temporal gyrus bilaterally, dorso-lateral prefrontal cortex bilaterally, medial prefrontal cortex and precuneus.

Voxel-wise comparison of those same 19 PD patients in off and on medication states revealed significantly increased connectivity in the on state

affecting mostly the basal ganglia (Table 10) (Figure 18). PD-ON patients did not show any significant difference in connectivity when compared to the HC group.

In ROC analysis of averaged PE's from the significant BGN clusters in PD-OFF/HC comparison, the area under the curve was 0.972 (95% CI 0.921-1.0). The cutoff value of 3.88 differentiated the PD-OFF from HC group with 100% sensitivity and 89.5% specificity (Figure 19).

Validation Cohort

Applying the threshold of average connectivity in the significant BGN clusters derived from the discovery group correctly identified PD subjects in 85% of cases (11 cases of 13) (Figure 20). One drug-naïve subject and one medicated patient demonstrated average connectivity values above the threshold.

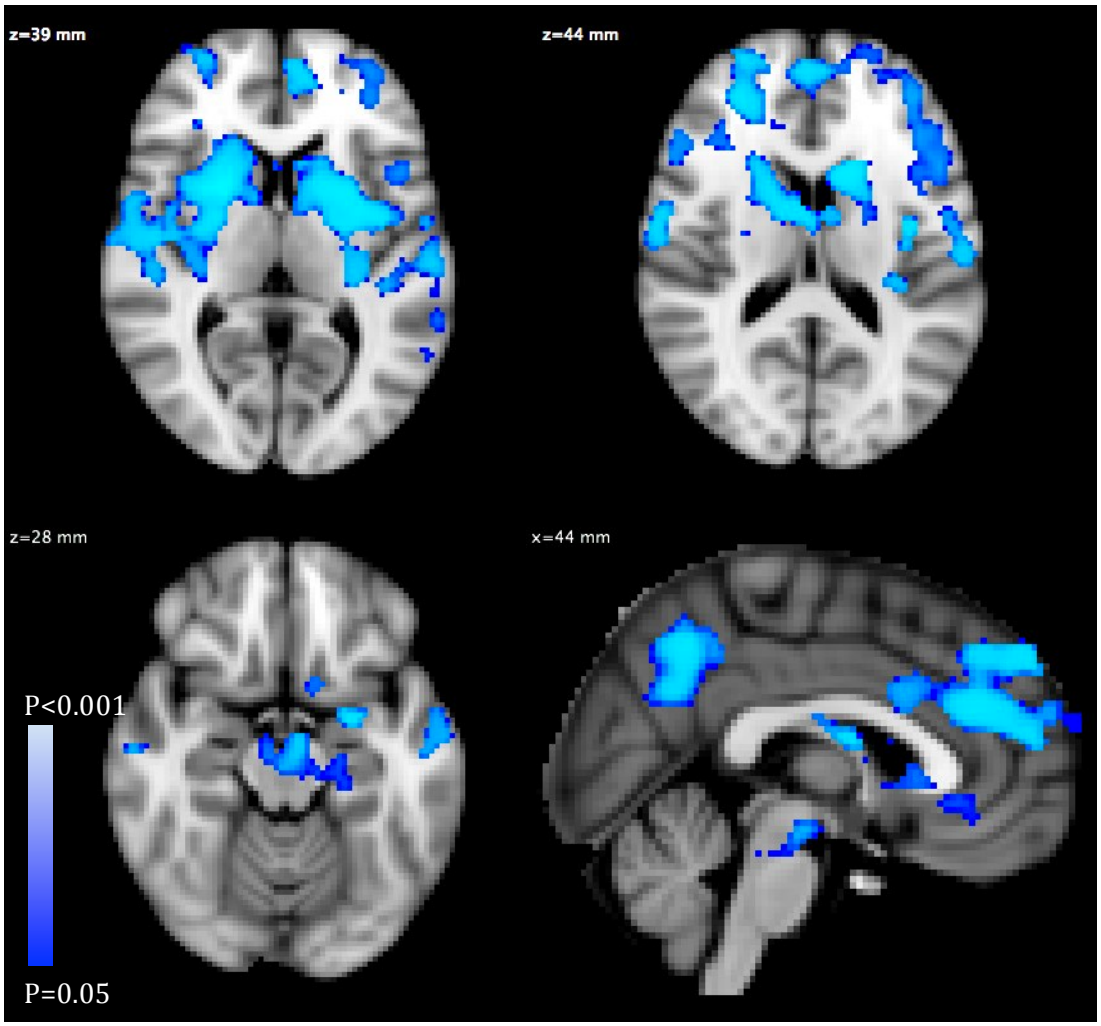


Figure 17. Group comparison between discovery group (PD patients off medication) and HC group. Significant clusters are located in the putamina bilaterally, medial frontal area, bilateral prefrontal areas, and precuneus. Images are displayed in radiological convention (right is left). Slice location is displayed in MNI coordinates. Clusters are thresholded at $p<0.05$ after correction for family-wise error.

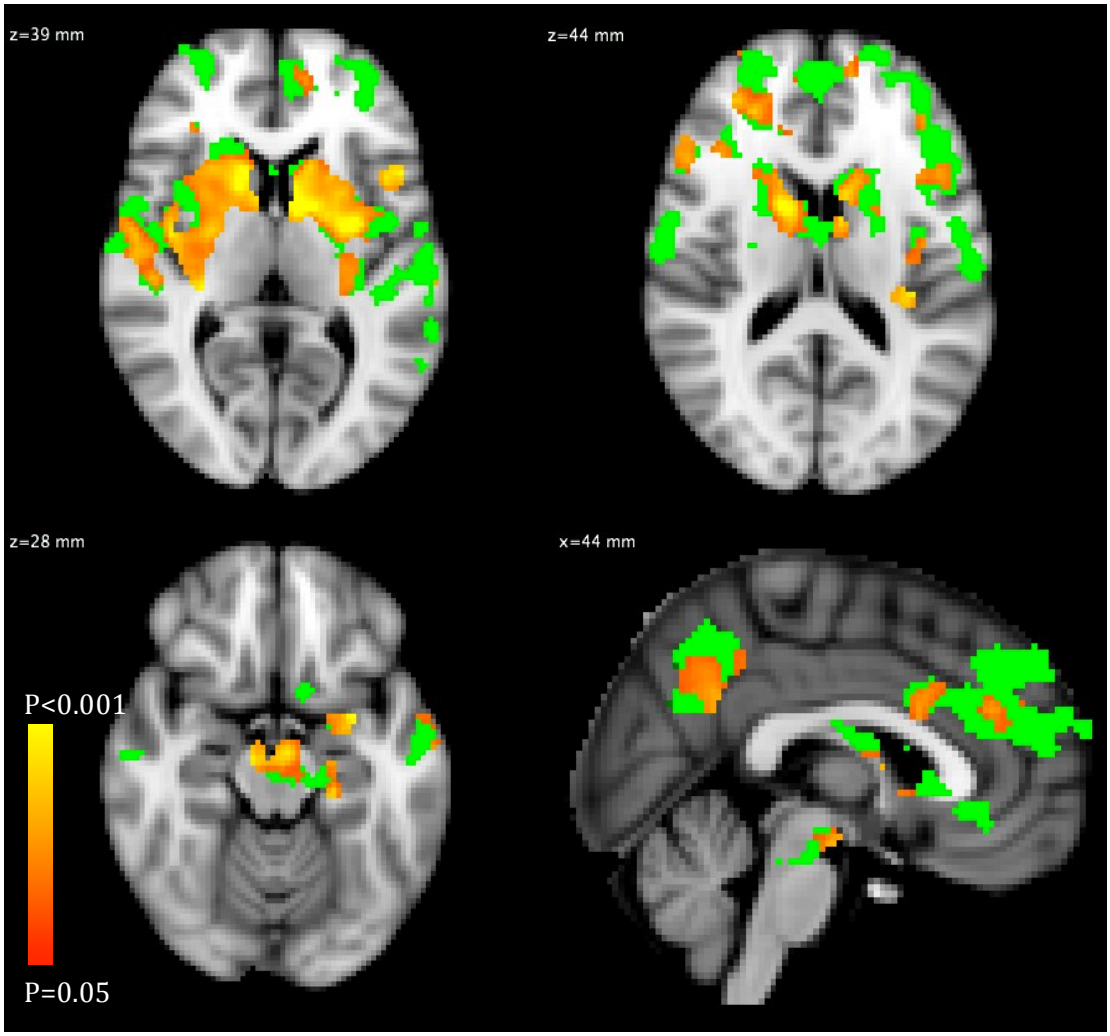


Figure 18. Medication effect in the Discovery group. Clusters with increased connectivity after medication (red-yellow) are shown on the background of the mask of significantly different clusters from the PD-OFF vs. HC comparison (green). Images are displayed in radiological convention (right is left). Slice location is displayed in MNI coordinates. Clusters are thresholded at $p<0.05$ after false discovery rate correction.

Table 9. Local maxima for group comparison HC>PD-OFF. Coordinates are listed in MNI space. P values denote significance after FWE correction at p<0.05.

Anatomical Region	MNI Coordinates			p value
	X	Y	Z	
Right putamen	26	6	-8	0.001
Right precuneus	6	-55	44	0.002
Right middle frontal gyrus	24	30	58	0.02
Right intraparietal sulcus	32	-62	44	0.04
Right paracingulate gyrus	4	40	24	0.005
Left putamen	-22	6	2	0.005
Left middle temporal gyrus	-56	-42	-6	0.03
Left middle frontal gyrus	-46	22	16	0.03
Left brainstem	-4	-14	-16	0.01

Table 10. Local maxima for group comparison PD-ON>PF-OFF. Coordinates are listed in MNI space. P values denote significance after FDR correction at p<0.05 within a mask of significant cluster from HC>PD-OFF comparison.

Anatomical Region	MNI Coordinates			p value
	X	Y	Z	
Right putamen	-10	10	-2	<0.001
Right middle frontal gyrus	16	52	22	0.001
Right precuneus	12	-50	40	0.002
Left hippocampus	-22	-26	-16	<0.001
Left frontal operculum	-50	18	6	0.005
Left frontal cingulate	-2	40	0	0.001
Left frontal cingulate	-4	12	28	0.007
Left temporal pole	-62	-10	-22	0.01

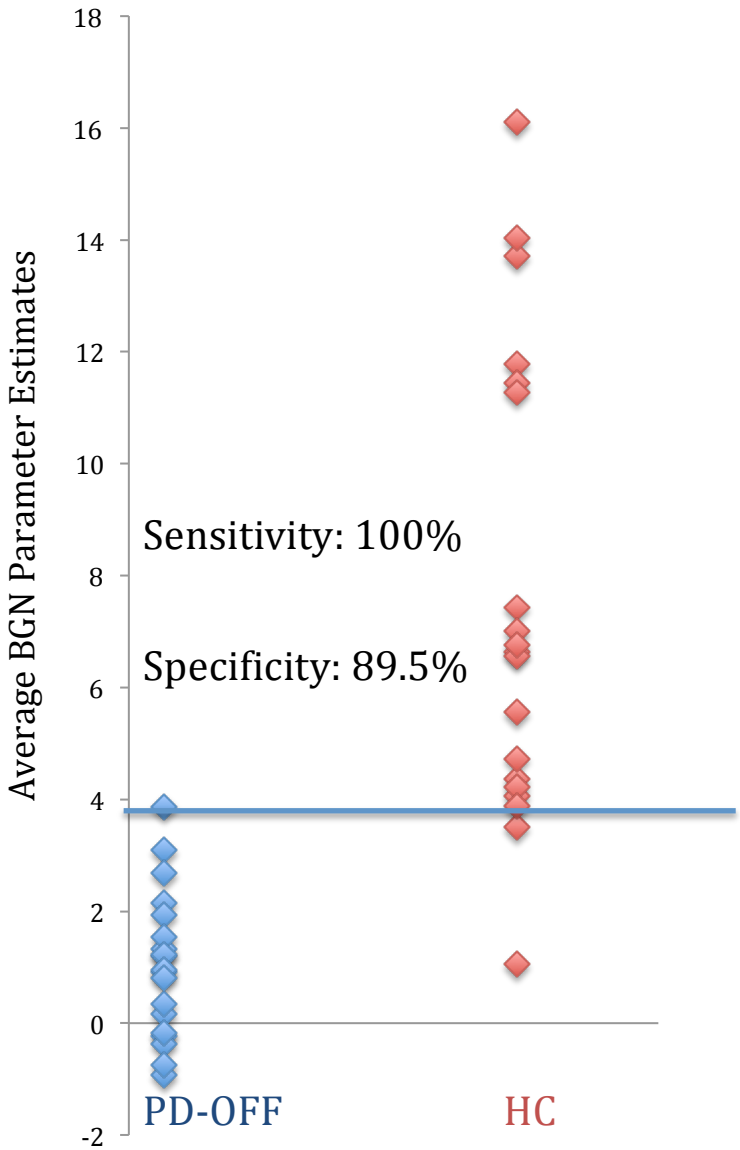


Figure 19. Average parameter estimate values (PE) from the significantly different BGN clusters in the PD-OFF vs. HC comparison. The blue line indicates a PE threshold with 100% sensitivity and 89.5% specificity for differentiating PD-OFF from HC.

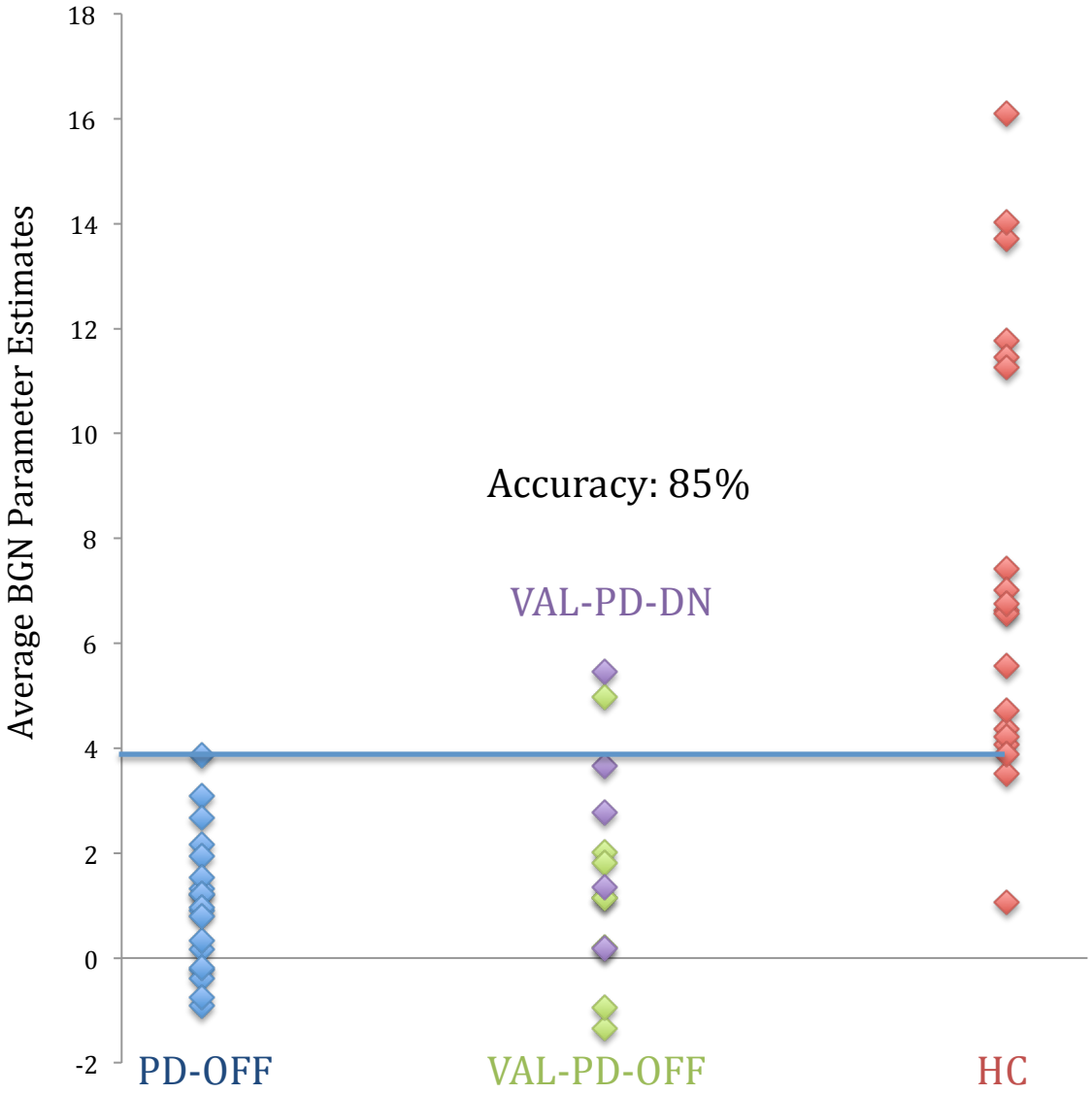


Figure 20. Average parameter estimate values (PE) from the significantly different BGN clusters in the validation cohort (violet - drug naïve patients; green – PD-OFF patients). Fitting the previously derived threshold provides 85% accuracy for classifying PD patients.

Section 4. Discussion

This is the first study to demonstrate widely reduced functional connectivity in the Basal Ganglia Network in Parkinson's disease. The difference in connectivity reliably separates the PD group off medication from controls. Administration of dopaminergic medication improves deficient connectivity in this network. Moreover, the abnormal connectivity is validated on a separate group of drug-naïve and medicated patients.

Reduced connectivity in the Basal Ganglia Network

Changes in the BGN in the PD-OFF vs. HC analysis were focused in the putamen bilaterally (Figure 17). Decreased functional connectivity in the putamen, understood as reduced coherence of the BOLD signal, are in line with findings of Wu et al. who reported reduced regional homogeneity (T. Wu *et al.*, 2009) and reduced degree of connectivity (T. Wu *et al.*, 2012) in a few voxels in the putamen. Helmich et al. examined the functional connectivity of the caudate and anterior and posterior putamen with the rest of the brain (Helmich *et al.*, 2010). They did not find reduced connectivity within the seeds themselves but did report a difference in the right insula, immediately next to our focus of reduced connectivity in right putamen. A recent study by Hacker et al. (Hacker *et al.*, 2012) using a similar technique of seed-based analysis of BG connectivity showed generally reduced connectivity with the cerebellum and particularly brainstem – a finding also confirmed by our study. However, comparison of seed-based studies with ICA-based analyses needs to be done with caution since correlations in the former are based on a simple averaging of time-series from

the ROI of choice while in the latter – only variance specific to the component of interest is taken into account (Joel *et al.*, 2011).

The physiological significance of reduced connectivity of the basal ganglia may be clarified by reference to the traditional task-related fMRI and PET studies. Abnormal activations have been reported in the basal ganglia in a wide range of paradigms: simple hand movements (Cerasa *et al.*, 2006; Rowe *et al.*, 2002; T. Wu, Wang, *et al.*, 2010), working memory and planning (Owen *et al.*, 1998), set shifting and feedback (Monchi *et al.*, 2004; 2007) and temporal processing (Harrington *et al.*, 2011; Jahanshahi *et al.*, 2010). Of note, only three out of the studies cited above (Cerasa *et al.*, 2006; Jahanshahi *et al.*, 2010; Owen *et al.*, 1998) showed increased activations in the basal ganglia in PD patients, with the great majority reporting reduced activity. It is not clear yet how the task fMRI findings relate to the resting state changes. We hypothesize, however, that reduced functional connectivity in the basal ganglia, as shown in our resting state experiment, may lead to reduced recruitment of this network seen in task fMRI.

Connectivity values in healthy controls showed a larger spread relative to PD subjects (Figure 19 and 20). This phenomenon could be tentatively interpreted as a floor effect of connectivity indices in the PD group. A large number of the PD values are close to or below zero, indicating a complete disconnection of large parts of the basal ganglia from the BGN component. Thus, the fact that the PD values have a much smaller spread may suggest that the connectivity cannot drop below a certain level since it would mean a complete disintegration of the network. Conversely, large spread of the healthy subject values could point to

adaptability of the BGN as long as a certain minimal level of BGN activity is maintained.

Medication Effect

Administration of dopaminergic medication clearly improved connectivity as shown in the PD-OFF/PD-ON comparison, leading to disappearance of any significant differences between PD and HC. This finding suggests that reduced connectivity in the BGN is a functional rather than structural phenomenon and is related to dopamine-dependent process.

The mechanism of this effect is not clear. The two main explanations are: a direct effect of dopaminergic supplementation on motor-related circuits, and an indirect, more generalized effect through increased arousal, increased impulsivity or increased cognitive responsiveness, all of which are known to result from dopaminergic stimulation. The exact mechanism cannot be elucidated based on results from this study as none of those effects of dopamine has been investigated after the scanning sessions.

It is also not clear, whether the medication effect is specific to PD patients. It is possible that healthy controls exposed to dopaminergic supplementation would show a similar increase in BGN connectivity. One study in the literature investigated that effect (Cole, Beckmann, *et al.*, 2013), using the same group ICA method as in current study. The authors found that healthy controls exposed to levodopa both increased and decreased connectivity in the Basal Ganglia Network. The changes were very small and located outside the BGN network. Those results differ significantly from those reported here. Although it is not possible to draw far-reaching conclusions from this one publication, it does

suggest that response to dopaminergic supplementation in healthy controls may be different than that in PD.

Since the PD-ON and PD-OFF sessions may differ in the amount of tremor or bradykinesia, this could have potentially introduced noise into the functional scans. Hence, great care was taken to maximise the signal to noise ratio (SNR). Firstly, group ICA was used to identify RSN's, as it is known to possess a much higher SNR than single ICA (Cole *et al.*, 2010). Secondly, individual data sets were preprocessed with Fix to remove noise related to scanning or patient movement. Thirdly, SNR was further increased by removing group-level noise at the stage of dual regression. Finally, the contamination from tremor is highly unlikely as 7 out of 19 patients did not have any resting tremor (Supplementary Table 3, Appendix C). Moreover, lack of significant differences in motion between the groups speaks against a strong influence of head movements on the results.

A recent study by Esposito et al. (Esposito *et al.*, 2013) also showed increase in connectivity in PD patients after levodopa. The authors investigated the Sensori-motor network but the fact that connectivity off and on medication changed in the same direction as in our study suggests a common underlying pathological mechanism in both networks.

Validation

Reduced connectivity in the BGN separated the PD-OFF from the NC group with high sensitivity and specificity (100% and 89.5% respectively). A particularly strong finding from this study is a confirmation of this result in the validation cohort, 85% of which showed results below the derived threshold.

However, the method did not achieve a complete separation of the PD subjects from controls. Further modification may improve the overall accuracy, e.g. through inclusion of other networks or clinical characteristics in the model. The fact that 4 of 5 drug-naïve patients also showed reduced BGN connectivity increases robustness of the finding speaks against it being just a simple effect of short-term medication withdrawal.

Methodological Considerations

Development of the elderly healthy control ICA template enabled us to isolate resting state networks which were unbiased by the pathological changes in PD. The approach has been successfully used previously (Cole, Oei, *et al.*, 2013). Moreover, the template can be reused to test reproducibility of original findings on a separate group, as done in our study, and may serve to identify pathological changes in at risk groups in the future.

Conclusions

Functional connectivity at rest, analysed with an observer-independent method, showed clear abnormalities in PD patients in areas relevant to disease pathophysiology. Reliability of this finding was demonstrated on a separate group of drug-naïve and medicated patients. Additionally, connectivity changes dramatically improved after dopaminergic medication. Application of this technique to a larger baseline cohort, and longitudinal scanning will help critically evaluate the role of resting-state functional MRI as a potential imaging biomarker for early motor Parkinson's disease.

Chapter V. Thesis Conclusions

The overarching goal of this thesis is to characterize idiopathic Parkinson disease in its early stages, across a broad range of manifestations. This was realized in three steps. Firstly, demographic and clinical features of PD were explored in an incident cohort of patients three years from diagnosis. Age was found to be a strong predictor of disease severity, independent of disease duration, while gender was seen to affect disease severity depending on the body region. Secondly, the typical changes on functional imaging in PD were identified based on a meta-analysis of all published studies across all disease stages. Abnormal activations were found in the Basal Ganglia but also in a wide range of motor and non-motor brain areas. Dopamine supplementation normalized activations in the Basal Ganglia and some other areas, while other circuits remained resistant to medication suggesting non-dopaminergic abnormality. Thirdly, the resting-state functional connectivity approach was used to explore the most robust differences between early PD patients and healthy controls. Basal Ganglia Network showed greatly reduced connectivity, which normalized on administration of dopaminergic medication. Reduced BGN connectivity was also validated on a separate group of PD subjects achieving very good separation of the two groups.

With the main focus of PD research shifting towards presymptomatic detection of early disease states, above results may facilitate selection of the most sensitive tests for that task. The effect of gender on early presentation of PD, for example, has potential significance for efforts at early diagnosis. Male subjects, presenting with mainly upper body, symmetrical disease may be more

easily identified with tests focusing on those features, e.g., Peg-board test. Additionally, cognitive testing, RBD features and some autonomic symptoms may be a more sensitive tool for men then women. Conversely, identification of early female sufferers may be aided by procedures testing lower limbs and gait. Differential sensitivity of various tests depending on gender may also inform choice of outcome measures in clinical trials. Significance of age for disease severity may need to be factored in too, as younger subjects, who progress more slowly, may need longer time to show measureable change on disease outcomes than older participants.

Within the realm of imaging, traditional task-based functional MRI appears to be very dependent on the nature of the task and the level of its performance. The meta-analysis of functional studies failed to show a clear pattern of strong abnormal activations and resulted, instead, in a complex map of areas across the whole brain – likely too complex to be used in early diagnostic testing.

Against that backdrop, resting-state connectivity provides a much simpler alternative. Lack of task requirement eliminates the complication of choosing from a myriad of available motor and cognitive paradigms. Measuring BGN connectivity at rest is a physiologically plausible method, not only because basal ganglia play a crucial role in PD, but also because dopaminergic medication normalizes the pathological changes. The finding appears robust in a cross-validation test, even including subjects who were medication naïve.

Further research will be needed to more closely characterize the nature of reduced connectivity in the Basal Ganglia Network in PD. Firstly, the biological relevance of the connectivity changes will need to be explored. For that purpose,

correlation of BGN connectivity with established measures of dopamine deficiency, e.g. through DAT-SPECT scanning, could elucidate the underlying pathogenesis of underconnectivity in PD.

Secondly, the natural history of connectivity changes throughout the disease course will need to be characterized. The onset of the process could be investigated on the 'at risk' groups for developing PD, including asymptomatic carriers of LRRK2 and GBA mutations as well as patients with REM sleep behavior disorder. Additionally, longitudinal imaging follow up of already diagnosed PD subjects will help explore how the connectivity changes with disease progression

Thirdly, specificity of the BGN changes for Parkinson's Disease should be studied. In the broader context of neurodegeneration, comparison of connectivity between PD and disorders like Alzheimer's Disease or Multiple Sclerosis will be important to establish whether the BGN connectivity is a phenomenon related strictly to basal ganglia disorders or a part of more generalized degenerative processes. Narrowing the focus to basal ganglia disorders, comparison of connectivity between idiopathic PD and Progressive Supranuclear Palsy and Multiple System Atrophy, will help determine the exact specificity of BGN changes for differential diagnosis.

References

Aaronson N, Alonso J, Burnam A, Lohr KN, Patrick DL, Perrin E, et al. Assessing health status and quality-of-life instruments: attributes and review criteria. Qual Life Res 2002; 11: 193–205.

Aarsland D, Bronnick K, Larsen JP, Tysnes OB, Alves G, Norwegian ParkWest study group. Cognitive impairment in incident, untreated Parkinson disease: the Norwegian ParkWest study. Neurology 2009; 72: 1121–1126.

Aarsland D, Kurz MW. The epidemiology of dementia associated with Parkinson disease. J Neurol Sci 2010; 289: 18–22.

Aarsland D, Larsen JP, Tandberg E, Laake K. Predictors of nursing home placement in Parkinson's disease: a population-based, prospective study. J Am Geriatr Soc 2000; 48: 938–942.

Albin RL, Young AB, Penney JB. The functional anatomy of basal ganglia disorders. Trends in Neurosciences 1989; 12: 366–375.

Alexopoulos GS, Abrams RC, Young RC, Shamoian CA. Cornell Scale for Depression in Dementia. Biological Psychiatry 1988; 23: 271–284.

Alonso A, Rodríguez LAG, Logroscino G, Hernán MA. Gout and risk of Parkinson disease: a prospective study. Neurology 2007; 69: 1696–1700.

Alves G, Muller B, Herlofson K, HogenEsch I, Telstad W, Aarsland D, et al. Incidence of Parkinson's disease in Norway: the Norwegian ParkWest study. J Neurol Neurosurg Psychiatry 2009; 80: 851–857.

Anders S, Sack B, Pohl A, Münte T, Pramstaller P, Klein C, et al. Compensatory premotor activity during affective face processing in subclinical carriers of a single mutant Parkin allele. Brain 2012; 135: 1128–1140.

Andreadou E, Anagnostouli M, Vasdekis V, Kararizou E, Rentzos M, Kontaxis T, et al. The impact of comorbidity and other clinical and sociodemographic factors on health-related quality of life in Greek patients with Parkinson's disease. Aging Ment Health 2011; 15: 913–921.

Andreasen NC. Brain Imaging. American Psychiatric Pub; 1989.

Ansari KA, Johnson A. Olfactory function in patients with Parkinson's disease. Journal of chronic diseases 1975; 28: 493–497.

Ascherio A, Weisskopf MG, O'Reilly EJ, McCullough ML, Calle EE, Rodriguez C, et al. Coffee consumption, gender, and Parkinson's disease mortality in the cancer prevention study II cohort: the modifying effects of estrogen. Am. J. Epidemiol. 2004; 160: 977–984.

Ascherio A, Zhang SM, Hernán MA, Kawachi I, Colditz GA, Speizer FE, et al. Prospective study of caffeine consumption and risk of Parkinson's disease in men and women. Ann Neurol 2001; 50: 56–63.

Baas H, Stecker K, Fischer PA. Value and appropriate use of rating scales and apparative measurements in quantification of disability in Parkinson's disease. J Neural Transm Gen Sect 1993; 5: 45–61.

Baba Y, Putzke JD, Whaley NR, Wszolek ZK, Uitti RJ. Gender and the Parkinson's disease phenotype. J Neurol 2005; 252: 1201–1205.

Baglio F, Blasi V, Falini A, Farina E, Mantovani F, Olivotto F, et al. Functional brain changes in early Parkinson's disease during motor response and motor inhibition. Neurobiology of Aging 2011; 32: 115–124.

Baldi I, Lebailly P, Mohammed-Brahim B, Letenneur L, Dartigues J-F, Brochard P. Neurodegenerative diseases and exposure to pesticides in the elderly. Am. J. Epidemiol. 2003; 157: 409–414.

Ballanger B, Baraduc P, Broussolle E, Le Bars D, Desmurget M, Thobois S. Motor urgency is mediated by the contralateral cerebellum in Parkinson's disease. J Neurol Neurosurg Psychiatry 2008; 79: 1110–1116.

Ballanger B, Poisson A, Broussolle E, Thobois S. Functional imaging of non-motor signs in Parkinson's disease. J Neurol Sci 2012; 315: 9–14.

Barone P, Antonini A, Colosimo C, Marconi R, Morgante L, Avarello TP, et al. The PRIAMO study: A multicenter assessment of nonmotor symptoms and their impact on quality of life in Parkinson's disease. Mov Disord 2009; 24: 1641–1649.

Barone P. Neurotransmission in Parkinson's disease: beyond dopamine. Eur J Neurol 2010; 17: 364–376.

Baudrexel S, Witte T, Seifried C, Wegner von F, Beissner F, Klein JC, et al. Resting state fMRI reveals increased subthalamic nucleus-motor cortex connectivity in Parkinson's disease. Neuroimage 2011; 55: 1728–1738.

Baumann C, Ferini-Strambi L, Waldvogel D, Werth E, Bassetti CL. Parkinsonism with excessive daytime sleepiness--a narcolepsy-like disorder? J Neurol 2005; 252: 139–145.

Beck A, Ward C, Mendelson M. Beck depression inventory (BDI). Arch Gen Psychiatry 1961

Beck AT, Epstein N, Brown G, Steer RA. An inventory for measuring clinical anxiety: Psychometric properties. Journal of Consulting and Clinical Psychology 1988; 56: 893–897.

Beckmann CF, DeLuca M, Devlin JT, Smith SM. Investigations into resting-state connectivity using independent component analysis. Philosophical Transactions of the Royal Society B: Biological Sciences 2005a; 360: 1001–1013.

Beckmann CF, DeLuca M, Devlin JT, Smith SM. Investigations into resting-state connectivity using independent component analysis. Philosophical Transactions of the Royal Society B: Biological Sciences 2005b; 360: 1001–1013.

Beiske AG, Loge JH, Rønningen A, Svensson E. Pain in Parkinson's disease: Prevalence and characteristics. Pain 2009; 141: 173–177.

Belliveau J, Kennedy D, McKinstry R, Buchbinder B, Weisskoff R, Cohen M, et al. Functional mapping of the human visual cortex by magnetic resonance imaging. Science 1991; 254: 716–719.

Bender R, Lange S. Adjusting for multiple testing--when and how? J Clin Epidemiol 2001; 54: 343–349.

Benedetti M, Bower J, Maraganore D. Smoking, alcohol, and coffee consumption preceding Parkinson's disease. Neurology 55, 1350–1358 (2000).

Benninger DH, Thees S, Kollias SS, Bassetti CL, Waldvogel D. Morphological differences in Parkinson's disease with and without rest tremor. J Neurol 2009; 256: 256–263.

Berardelli A, Wenning GK, Antonini A, Berg D, Bloem BR, Bonifati V, et al. EFNS/MDS-ES/ENS [corrected] recommendations for the diagnosis of Parkinson's disease. Eur J Neurol 2013; 20: 16–34.

Berg D, Lang AE, Postuma RB, Maetzler W, Deuschl G, Gasser T, et al. Changing the research criteria for the diagnosis of Parkinson's disease: obstacles and opportunities. Lancet Neurol 2013; 12: 514–524.

Berg D, Seppi K, Behnke S, Liepelt I, Schweitzer K, Stockner H, et al. Enlarged substantia nigra hyperechogenicity and risk for Parkinson disease: a 37-month 3-center study of 1847 older persons. Arch Neurol-Chicago 2011; 68: 932–937.

Berg D, Seppi K, Liepelt I, Schweitzer K, Wollenweber F, Wolf B, et al. Enlarged hyperechogenic substantia nigra is related to motor performance and olfaction in the elderly. Mov Disord 2010; 25: 1464–1469.

Berg D. Is pre-motor diagnosis possible?--The European experience. Parkinsonism Relat. Disord. 2012; 18 Suppl 1: S195–8.

Biglan KM, Holloway RG, McDermott MP, Richard IH, Parkinson Study Group CALM-PD Investigators. Risk factors for somnolence, edema, and hallucinations in early Parkinson disease. Neurology 2007; 69: 187–195.

Biomarkers Definitions Working Group. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin. Pharmacol. Ther. 2001; 69: 89–95.

Biswal B, Yetkin FZ, Haughton VM, Hyde JS. Functional connectivity in the motor cortex of resting human brain using echo-planar MRI. Magn Reson Med 1995; 34: 537–541.

Biswal BB, Mennes M, Zuo X-N, Gohel S, Kelly C, Smith SM, et al. Toward discovery science of human brain function. Proc Natl Acad Sci U S A 2010; 107: 4734–4739.

Biswal BB. Resting state fMRI: a personal history. Neuroimage 2012; 62: 938–944.

Blazejewska AI, Schwarz ST, Pitiot A, Stephenson MC, Lowe J, Bajaj N, et al. Visualization of nigrosome 1 and its loss in PD: pathoanatomical correlation and in vivo 7 T MRI. Neurology 2013; 81: 534–540.

Bliwise DL, Trotti LM, Greer SA, Juncos JJ, Rye DB. Phasic muscle activity in sleep and clinical features of Parkinson disease. Ann Neurol 2010; 68: 353–359.

Boecker H, Ceballos-Baumann A, Bartenstein P, Weindl A, Siebner H, Fassbender T, et al. Sensory processing in Parkinson‘s and Huntington’s disease. Brain 1999; 122: 1651.

Bohnen NI, Kuwabara H, Constantine GM, Mathis CA, Moore RY. Grooved pegboard test as a biomarker of nigrostriatal denervation in Parkinson's disease. Neurosci Lett 2007; 424: 185–189.

Bohnen NI, Studenski SA, Constantine GM, Moore RY. Diagnostic performance of clinical motor and non-motor tests of Parkinson disease: a matched case–control study. Eur J Neurol 2008; 15: 685–691.

Braak H, Del Tredici K, Rüb U, de Vos RAI, Jansen Steur ENH, Braak E. Staging of brain pathology related to sporadic Parkinson's disease. Neurobiology of Aging 2003; 24: 197–211.

Braak H, Rüb U, Jansen Steur ENH, Del Tredici K, de Vos RAI. Cognitive status correlates with neuropathologic stage in Parkinson disease. Neurology 2005; 64: 1404–1410.

Brefel-Courbon C, Payoux P, Thalamas C, Ory F, Quelven I, Chollet F, et al. Effect of levodopa on pain threshold in Parkinson's disease: a clinical and positron emission tomography study. Mov Disord 2005; 20: 1557–1563.

Bronstein J, Carvey P, Chen H, Cory-Slechta D, DiMonte D, Duda J, et al. Meeting report: consensus statement-Parkinson‘s disease and the environment: collaborative on health and the environment and Parkinson’s Action Network (CHE PAN) conference 26-28 June 2007. 2009. p. 117–121.

Brookes MJ, Woolrich M, Luckhoo H, Price D, Hale JR, Stephenson MC, et al. Investigating the electrophysiological basis of resting state networks using magnetoencephalography. Proc Natl Acad Sci U S A 2011; 108: 16783–16788.

Brooks DJ, Frey KA, Marek KL, Oakes D, Paty D, Prentice R, et al. Assessment of neuroimaging techniques as biomarkers of the progression of Parkinson's disease. Experimental Neurology 2003; 184 Suppl 1: S68–79.

Brown GD, Yamada S, Sejnowski TJ. Independent component analysis at the neural cocktail party. Trends in Neurosciences 2001; 24: 54–63.

Brown GG, Perthen JE, Liu TT, Buxton RB. A primer on functional magnetic resonance imaging. Neuropsychol Rev 2007; 17: 107–125.

Brown RG, Landau S, Hindle JV, Playfer J, Samuel M, Wilson KC, et al. Depression and anxiety related subtypes in Parkinson's disease. J Neurol Neurosurg Psychiatry 2011; 82: 803–809.

Bugalho P, da Silva JA, Neto B. Clinical features associated with REM sleep behavior disorder symptoms in the early stages of Parkinson's disease. J Neurol 2011; 258: 50–55.

Buhmann C, Binkofski F, Klein C, Buchel C, van Eimeren T, Erdmann C, et al. Motor reorganization in asymptomatic carriers of a single mutant Parkin allele: a human model for presymptomatic parkinsonism. Brain 2005; 128: 2281–2290.

Buhmann C, Glauche V, Sturenburg HJ, Oechsner M, Weiller C, Buchel C. Pharmacologically modulated fMRI--cortical responsiveness to levodopa in drug-naive hemiparkinsonian patients. Brain 2003; 126: 451–461.

Buxton RB, Uludağ K, Dubowitz DJ, Liu TT. Modeling the hemodynamic response to brain activation. Neuroimage 2004; 23 Suppl 1: S220–33.

Buysse DJ, Reynolds CF, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry Res 1989; 28: 193–213.

Cal-González J, Herraiz JL, España S, Desco M, Vaquero JJ, Udías JM. Positron range effects in high resolution 3D PET imaging. 2009: 2788–2791.

Calhoun VD, Adali T, Pearlson GD, Pekar JJ. A method for making group inferences from functional MRI data using independent component analysis. Hum Brain Mapp 2001; 14: 140–151.

Cao H, Xu X, Zhao Y, Long D, Zhang M. Altered brain activation and connectivity in early Parkinson disease tactile perception. American Journal of Neuroradiology 2011; 32: 1969–1974.

Cardoso EF, Fregni F, Maia FM, Melo LM, Sato JR, Cruz AC, et al. Abnormal visual activation in Parkinson's disease patients. Mov Disord 2010; 25: 1590–1596.

Catalan MJ. A PET study of sequential finger movements of varying length in patients with Parkinson's disease. Brain 1999; 122: 483–495.

Ceballos-Baumann AO. Functional imaging in Parkinson's disease: activation studies with PET, fMRI and SPECT. J Neurol 2003; 250 Suppl 1: I15–23.

Cerasa A, Hagberg GE, Peppe A, Bianciardi M, Gioia MC, Costa A, et al. Functional changes in the activity of cerebellum and frontostriatal regions during externally and internally timed movement in Parkinson's disease. Brain Research Bulletin 2006; 71: 259–269.

Chahine LM, Stern MB. Diagnostic markers for Parkinson's disease. Curr Opin Neurol 2011; 24, 309–317 .

Chase HW, Eickhoff SB, Laird AR, Hogarth L. The neural basis of drug stimulus processing and craving: an activation likelihood estimation meta-analysis. Biological Psychiatry 2011; 70: 785–793.

Chaudhuri KR, Healy DG, Schapira AHV, National Institute for Clinical Excellence. Non-motor symptoms of Parkinson's disease: diagnosis and management. Lancet Neurol 2006; 5: 235–245.

Chaudhuri KR, Pal S, DiMarco A, Whately-Smith C, Bridgman K, Mathew R, et al. The Parkinson's disease sleep scale: a new instrument for assessing sleep and nocturnal disability in Parkinson's disease. J Neurol Neurosurg Psychiatry 2002; 73: 629–635.

Chen H, Zhang SM, Hernán MA, Schwarzschild MA, Willett WC, Colditz GA, et al. Nonsteroidal anti-inflammatory drugs and the risk of Parkinson disease. Arch Neurol-Chicago 2003; 60: 1059–1064.

Chen S, Ross TJ, Zhan W, Myers CS, Chuang K-S, Heishman SJ, et al. Group independent component analysis reveals consistent resting-state networks across multiple sessions. Brain Res 2008; 1239: 141–151.

Chen X, Wu G, Schwarzschild MA. Urate in Parkinson's disease: more than a biomarker? Curr Neurol Neurosci Rep 2012; 12: 367–375.

Cheon S-M, Ha M-S, Park MJ, Kim JW. Nonmotor symptoms of Parkinson's disease: prevalence and awareness of patients and families. Parkinsonism Relat. Disord. 2008; 14: 286–290.

Claassen DO, Josephs KA, Ahlskog JE, Silber MH, Tippmann-Peikert M, Boeve BF. REM sleep behavior disorder preceding other aspects of synucleinopathies by up to half a century. Neurology 2010; 75: 494–499.

Cochrane CJ, Ebmeier KP. Diffusion tensor imaging in parkinsonian syndromes: a systematic review and meta-analysis. Neurology 2013; 80: 857–864.

Cole DM, Beckmann CF, Oei NYL, Both S, van Gerven JMA, Rombouts SARB. Differential and distributed effects of dopamine neuromodulations on resting-state network connectivity. Neuroimage 2013; 78: 59–67.

Cole DM, Oei NYL, Soeter RP, Both S, van Gerven JMA, Rombouts SARB, et al. Dopamine-dependent architecture of cortico-subcortical network connectivity. Cereb Cortex 2013; 23: 1509–1516.

Cole DM, Smith SM, Beckmann CF. Advances and pitfalls in the analysis and interpretation of resting-state FMRI data. Front Syst Neurosci 2010; 4: 8.

Colosimo C, Martinez-Martin P, Fabbrini G, Hauser RA, Merello M, Miyasaki J, et al. Task force report on scales to assess dyskinesia in Parkinson's disease: critique and recommendations. Mov Disord 2010; 25: 1131–1142.

Comella CL, Nardine TM, Diederich NJ, Stebbins GT. Sleep-related violence, injury, and REM sleep behavior disorder in Parkinson's disease. Neurology 1998; 51: 526–529.

Cools R, Lewis SJG, Clark L, Barker RA, Robbins TW. L-DOPA disrupts activity in the nucleus accumbens during reversal learning in Parkinson's disease. Neuropsychopharmacology 2007; 32: 180–189.

Cools R, Stefanova E, Barker RA, Robbins TW, Owen AM. Dopaminergic modulation of high-level cognition in Parkinson's disease: the role of the prefrontal cortex revealed by PET. Brain 2002; 125: 584–594.

Cooper R, Hardy R, Aihie Sayer A, Ben-Shlomo Y, Birnie K, Cooper C, et al. Age and gender differences in physical capability levels from mid-life onwards: the harmonisation and meta-analysis of data from eight UK cohort studies. Plos One 2011; 6: e27899.

Cordes D, Haughton VM, Arfanakis K, Wendt GJ, Turski PA, Moritz CH, et al. Mapping functionally related regions of brain with functional connectivity MR imaging. Am J Neuroradiol 2000; 21: 1636–1644.

Costafreda SG, Brammer MJ, David AS, Fu CHY. Predictors of amygdala activation during the processing of emotional stimuli: a meta-analysis of 385 PET and fMRI studies. Brain Res Rev 2008; 58: 57–70.

Cunnington R, Egan GF, O'Sullivan JD, Hughes AJ, Bradshaw JL, Colebatch JG. Motor imagery in Parkinson's disease: a PET study. Mov Disord 2001; 16: 849–857.

Dagher A, Owen AM, Boecker H, Brooks DJ. The role of the striatum and hippocampus in planning: a PET activation study in Parkinson's disease. Brain 2001; 124: 1020–1032.

Dalrymple-Alford JC, MacAskill MR, Nakas CT, Livingston L, Graham C, Crucian GP, et al. The MoCA: Well-suited screen for cognitive impairment in Parkinson disease. Neurology 2010; 75: 1717–1725.

Damier P, Hirsch EC, Agid Y, Graybiel AM. The substantia nigra of the human brain. II. Patterns of loss of dopamine-containing neurons in Parkinson's disease. Brain 1999; 122 (Pt 8): 1437–1448.

Damoiseaux JS, Rombouts SARB, Barkhof F, Scheltens P, Stam CJ, Smith SM, et al. Consistent resting-state networks across healthy subjects. Proc Natl Acad Sci U S A 2006; 103: 13848–13853.

De Luca M, Beckmann CF, De Stefano N, Matthews PM, Smith SM. fMRI resting state networks define distinct modes of long-distance interactions in the human brain. Neuroimage 2006; 29: 1359–1367.

De Martino F, Valente G, Staeren N, Ashburner J, Goebel R, Formisano E. Combining multivariate voxel selection and support vector machines for mapping and classification of fMRI spatial patterns. Neuroimage 2008; 43: 44–58.

Defer G-L, Widner H, Mari R-M, R my P, Levivier M. Core assessment program for surgical interventional therapies in Parkinson's disease (CAPSIT-PD). Mov Disord 2001; 14: 572–584.

Delaveau P, Jabourian M, Lemogne C, Guionnet S, Bergouignan L, Fossati P. Brain effects of antidepressants in major depression: a meta-analysis of emotional processing studies. Journal of Affective Disorders 2011; 130: 66–74.

Delaveau P, Salgado-Pineda P, Witjas T, Micallef-Roll J, Fakra E, Azulay J-P, et al. Dopaminergic modulation of amygdala activity during emotion recognition in patients with Parkinson disease. Journal of Clinical Psychopharmacology 2009; 29: 548–554.

Dellapina E, Gerdelat-Mas A, Ory-Magne F, Pourcel L, Galitzky M, Calvas F, et al. Apomorphine effect on pain threshold in Parkinson's disease: a clinical and positron emission tomography study. Mov Disord 2011; 26: 153–157.

DeLong M, Wichmann T. Update on models of basal ganglia function and dysfunction. Parkinsonism Relat. Disord. 2009; 15 Suppl 3: S237–40.

Desrosiers J, Hébert R, Bravo G, Dutil E. The Purdue Pegboard Test: normative data for people aged 60 and over. Disabil Rehabil 1995; 17: 217–224.

Dibble LE, Lange M. Predicting falls in individuals with Parkinson disease: a reconsideration of clinical balance measures. J Neurol Phys Ther 2006; 30: 60–67.

Dickson DW, Braak H, Duda JE, Duyckaerts C, Gasser T, Halliday GM, et al. Neuropathological assessment of Parkinson's disease: refining the diagnostic criteria. Lancet Neurol 2009; 8: 1150–1157.

Diederich NJ, Moore CG, Leurgans SE, Chmura TA, Goetz CG. Parkinson disease with old-age onset: a comparative study with subjects with middle-age onset. Arch Neurol-Chicago 2003; 60: 529–533.

Dirnberger G, Frith CD, Jahanshahi M. Executive dysfunction in Parkinson's disease is associated with altered pallidal-frontal processing. Neuroimage 2005; 25: 588–599.

Dodd ML, Klos KJ, Bower JH, Geda YE, Josephs KA, Ahlskog JE. Pathological gambling caused by drugs used to treat Parkinson disease. Arch Neurol-Chicago 2005; 62: 1377–1381.

Doty RL, Shaman P, Dann M. Development of the University of Pennsylvania Smell Identification Test: a standardized microencapsulated test of olfactory function. Physiol. Behav. 1984; 32: 489–502.

Douaud G, Filippini N, Knight S, Talbot K, Turner MR. Integration of structural and functional magnetic resonance imaging in amyotrophic lateral sclerosis. Brain 2011; 134: 3470–3479.

Double KL, Rowe DB, Carew-Jones FM, Hayes M, Chan DKY, Blackie J, et al. Anti-melanin antibodies are increased in sera in Parkinson's disease. Experimental Neurology 2009; 217: 297–301.

Doyle LMF, Yarrow K, Brown P. Lateralization of event-related beta desynchronization in the EEG during pre-cued reaction time tasks. Clin Neurophysiol 2005; 116: 1879–1888.

E K-H, Chen S-HA, Ho M-HR, Desmond JE. A meta-analysis of cerebellar contributions to higher cognition from PET and fMRI studies. Hum Brain Mapp 2014; 35: 593–615.

Eckert T, Peschel T, Heinze HJ, Rotte M. Increased pre-SMA activation in early PD patients during simple self-initiated hand movements. J Neurol 2006; 253: 199–207.

Eggers C, Pedrosa DJ, Kahraman D, Maier F, Lewis CJ, Fink GR, et al. Parkinson subtypes progress differently in clinical course and imaging pattern. Plos One 2012; 7: e46813.

Eisensehr I, Lindeiner von H, Jäger M, Noachtar S. REM sleep behavior disorder in sleep-disordered patients with versus without Parkinson's disease: is there a need for polysomnography? J Neurol Sci 2001; 186: 7–11.

El-Agnaf OMA, Salem SA, Paleologou KE, Curran MD, Gibson MJ, Court JA, et al. Detection of oligomeric forms of alpha-synuclein protein in human plasma as a potential biomarker for Parkinson's disease. FASEB J. 2006; 20: 419–425.

Elbaz A, Bower JH, Maraganore DM, McDonnell SK, Peterson BJ, Ahlskog JE, et al. Risk tables for parkinsonism and Parkinson's disease. J Clin Epidemiol 2002; 55: 25–31.

Elm JJ, The NINDS NET-PD Investigators. Design innovations and baseline findings in a long-term Parkinson‘s trial: The national institute of neurological disorders and stroke exploratory trials in Parkinson’s Disease Long-Term Study-1. Mov Disord 2012; 27: 1513–1521.

Elsinger CL, Rao SM, Zimbelman JL, Reynolds NC, Blindauer K, Hoffman RG. Neural basis for impaired time reproduction in Parkinson's disease: an fMRI study. J Int Neuropsychol Soc 2003; 9: 1088–1098.

Esposito F, Tessitore A, Giordano A, De Micco R, Paccone A, Conforti R, et al. Rhythm-specific modulation of the sensorimotor network in drug-naive patients with Parkinson's disease by levodopa. Brain 2013; 136: 710–725.

Eusebio A, Brown P. Oscillatory activity in the basal ganglia. Parkinsonism Relat. Disord. 2007; 13: S434–S436.

Evans AH, Katzenschlager R, Paviour D, O'Sullivan JD, Appel S, Lawrence AD, et al. Punding in Parkinson's disease: its relation to the dopamine dysregulation syndrome. Mov Disord 2004; 19: 397–405.

Evatt ML, Chaudhuri KR, Chou KL, Cubo E, Hinson V, Kompoliti K, et al. Dysautonomia rating scales in Parkinson‘s disease: sialorrhea, dysphagia, and constipation--critique and recommendations by movement disorders task force on rating scales for Parkinson’s disease. Mov Disord 2009; 24: 635–646.

Fantini ML, Farini E, Ortelli P, Zucconi M, Manconi M, Cappa S, et al. Longitudinal study of cognitive function in idiopathic REM sleep behavior disorder. Sleep 2011; 34: 619–625.

Farid K, Sibon I, Guehl D, Cuny E, Burbaud P, Allard M. Brain dopaminergic modulation associated with executive function in Parkinson's disease. Mov Disord 2009; 24: 1962–1969.

Fearnley JM, Lees AJ. Ageing and Parkinson's disease: substantia nigra regional selectivity. Brain 1991; 114 (Pt 5): 2283–2301.

Feigin A, Ghilardi MF, Fukuda M, Mentis MJ, Dhawan V, Barnes A, et al. Effects of levodopa infusion on motor activation responses in Parkinson's disease. Neurology 2002; 59: 220–226.

Fera F, Nicoletti G, Cerasa A, Romeo N, Gallo O, Gioia MC, et al. Dopaminergic modulation of cognitive interference after pharmacological washout in Parkinson's disease. Brain Research Bulletin 2007; 74: 75–83.

Fernandez HH, Aarsland D, Fénelon G, Friedman JH, Marsh L, Tröster AI, et al. Scales to assess psychosis in Parkinson's disease: Critique and recommendations. Mov Disord 2008; 23: 484–500.

Filippini N, Ebmeier KP, MacIntosh BJ, Trachtenberg AJ, Frisoni GB, Wilcock GK, et al. Differential effects of the APOE genotype on brain function across the lifespan. Neuroimage 2011; 54: 602–610.

Filippini N, MacIntosh BJ, Hough MG, Goodwin GM, Frisoni GB, Smith SM, et al. Distinct patterns of brain activity in young carriers of the APOE-epsilon4 allele. Proc Natl Acad Sci U S A 2009; 106: 7209–7214.

Filippini N, Nickerson LD, Beckmann CF, Ebmeier KP, Frisoni GB, Matthews PM, et al. Age-related adaptations of brain function during a memory task are also present at rest. Neuroimage 2012; 59: 3821–3828.

Foki T, Pirker W, Klinger N, Geissler A, Rath J, Steinkellner T, et al. FMRI correlates of apraxia in Parkinson's disease patients OFF medication. Experimental Neurology 2010; 225: 416–422.

Folstein MF, Folstein SE, McHugh PR. 'Mini-mental state'. A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975; 12: 189–198.

Fox MD, Greicius M. Clinical applications of resting state functional connectivity. Front Syst Neurosci 2010; 4, 19.

Fransson P, Skiöld B, Horsch S, Nordell A, Blennow M, Lagercrantz H, et al. Resting-state networks in the infant brain. Proc Natl Acad Sci U S A 2007; 104: 15531–15536.

Gaenslen A, Swid I, Liepelt-Scarfone I, Godau J, Berg D. The patients' perception of prodromal symptoms before the initial diagnosis of Parkinson's disease. Mov Disord 2011; 26: 653–658.

Gagne JJ, Power MC. Anti-inflammatory drugs and risk of Parkinson disease: a meta-analysis. Neurology 2010; 74: 995–1002.

Gagnon JF, Bédard MA, Fantini ML, Petit D, Panisset M, Rompré S, et al. REM sleep behavior disorder and REM sleep without atonia in Parkinson's disease. Neurology 2002; 59: 585–589.

Gaig C, Tolosa E. When does Parkinson's disease begin? Mov Disord 2009; 24 Suppl 2: S656–64.

Genovese CR, Lazar NA, Nichols T. Thresholding of statistical maps in functional neuroimaging using the false discovery rate. Neuroimage 2002; 15: 870–878.

Georgopoulos AP, Delong MR, Crutcher MD. Relations between parameters of step-tracking movements and single cell discharge in the globus pallidus and subthalamic nucleus of the behaving monkey. J. Neurosci. 1983; 3: 1586–1598.

Gerlach M, Maetzler W, Broich K, Hampel H, Rems L, Reum T, et al. Biomarker candidates of neurodegeneration in Parkinson's disease for the evaluation of disease-modifying therapeutics. J Neural Transm 2011; 119, 39–52.

Giansanti D, Macellari V, Maccioni G. Telemonitoring and telerehabilitation of patients with Parkinson's disease: health technology assessment of a novel wearable step counter. Telemed J E Health 2008; 14: 76–83.

Giladi N, Fichtner A, Poewe W, Boroojerdi B. Rotigotine transdermal system for control of early morning motor impairment and sleep disturbances in patients with Parkinson's disease. J Neural Transm 2010; 117: 1395–1399.

Gill DJ, Freshman A, Blender JA, Ravina B. The Montreal cognitive assessment as a screening tool for cognitive impairment in Parkinson's disease. Mov Disord 2008; 23: 1043–1046.

Giovannini P, Piccolo I, Genitrini S, Soliveri P, Girotti F, Geminiani G, et al. Early-onset Parkinson's disease. Mov Disord 1991; 6: 36–42.

Giovannoni G, O'Sullivan JD, Turner K, Manson AJ, Lees AJ. Hedonistic homeostatic dysregulation in patients with Parkinson's disease on dopamine replacement therapies. J Neurol Neurosurg Psychiatry 2000; 68: 423–428.

Gjerstad MD, Boeve B, Wentzel-Larsen T, Aarsland D, Larsen JP. Occurrence and clinical correlates of REM sleep behaviour disorder in patients with Parkinson's disease over time. J Neurol Neurosurg Psychiatry 2008; 79: 387–391.

Glosser G, Clark C, Freundlich B, Kliner-Krenzel L, Flaherty P, Stern M. A controlled investigation of current and premorbid personality: characteristics of Parkinson's disease patients. Mov Disord 1995; 10: 201–206.

Goedert M, Spillantini MG, Del Tredici K, Braak H. 100 years of Lewy pathology. Nature Reviews Neurology 2013; 9, 13–24.

Goerendt IK, Lawrence AD, Brooks DJ. Reward processing in health and Parkinson's disease: neural organization and reorganization. Cereb Cortex 2004; 14: 73–80.

Goetz CG, Fahn S, Martinez-Martin P, Poewe W, Sampaio C, Stebbins GT, et al. Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS): Process, format, and clinimetric testing plan. Mov Disord 2007; 22: 41–47.

Goetz CG, Nutt JG, Stebbins GT. The Unified Dyskinesia Rating Scale: presentation and clinimetric profile. Mov Disord 2008; 23: 2398–2403.

Goetz CG, Stebbins GT, Shale HM, Lang AE, Chernik DA, Chmura TA, et al. Utility of an objective dyskinesia rating scale for Parkinson's disease: inter- and intrarater reliability assessment. Mov Disord 1994; 9: 390–394.

Goldstein M. Ergot Compounds and Brain Function. Lippincott Williams & Wilkins; 1980.

González-García N, Armony JL, Soto J, Trejo D, Alegría MA, Drucker-Colín R. Effects of rTMS on Parkinson's disease: a longitudinal fMRI study. J Neurol 2011; 258: 1268–1280.

Gómez-Esteban JC, Tijero B, Ciordia R, Berganzo K, Somme J, Lezcano E, et al. Factors influencing the symmetry of Parkinson's disease symptoms. Clin Neurol Neurosurg 2010; 112: 302–305.

Grafton ST. Contributions of functional imaging to understanding parkinsonian symptoms. Curr. Opin. Neurobiol. 2004; 14: 715–719.

Grandinetti A, Morens DM, Reed D, MacEachern D. Prospective study of cigarette smoking and the risk of developing idiopathic Parkinson's disease. Am. J. Epidemiol. 1994; 139: 1129–1138.

Greicius MD, Flores BH, Menon V, Glover GH, Solvason HB, Kenna H, et al. Resting-state functional connectivity in major depression: abnormally increased contributions from subgenual cingulate cortex and thalamus. Biological Psychiatry 2007; 62: 429–437.

Greicius MD, Krasnow B, Reiss AL, Menon V. Functional connectivity in the resting brain: a network analysis of the default mode hypothesis. Proc Natl Acad Sci U S A 2003; 100: 253–258.

Greicius MD, Srivastava G, Reiss AL, Menon V. Default-mode network activity distinguishes Alzheimer's disease from healthy aging: evidence from functional MRI. Proc Natl Acad Sci U S A 2004; 101: 4637–4642.

Greve DN, Fischl B. Accurate and robust brain image alignment using boundary-based registration. Neuroimage 2009; 48: 63–72.

Griffiths RI, Kotschet K, Arfon S, Xu ZM, Johnson W, Drago J, et al. Automated assessment of bradykinesia and dyskinesia in Parkinson's disease. J Parkinsons Dis 2012; 2: 47–55.

Grossman M, Cooke A, DeVita C, Lee C, Alsop D, Detre J, et al. Grammatical and resource components of sentence processing in Parkinson's disease: an fMRI study. Neurology 2003; 60: 775–781.

Haaxma CA, Bloem BR, Borm GF, Horstink MWIM. Comparison of a timed motor test battery to the Unified Parkinson‘s Disease Rating Scale-III in Parkinson’s disease. Mov Disord 2008; 23: 1707–1717.

Haaxma CAC, Bloem BRB, Borm GFG, Oyen WJGW, Leenders KLK, Eshuis SS, et al. Gender differences in Parkinson's disease. J Neurol Neurosurg Psychiatry 2007; 78: 819–824.

Hacker, C. D., Perlmutter, J. S., Criswell, S. R., Ances, B. M. & Snyder, A. Z. Resting state functional connectivity of the striatum in Parkinson's disease. Brain 135, 3699–3711 (2012).

Haehner A, Boesveldt S, Berendse HW, Mackay-Sim A, Fleischmann J, Silburn PA, et al. Prevalence of smell loss in Parkinson's disease--a multicenter study. Parkinsonism Relat. Disord. 2009; 15: 490–494.

Hagell P, Widner HK. Clinical rating of dyskinesias in Parkinson's disease: Use and reliability of a new rating scale. Mov Disord 2001; 14: 448–455.

Hamilton M. A rating scale for depression. J Neurol Neurosurg Psychiatry 1960; 23: 56.

Hamilton M. The Assessment Of Anxiety States By Rating. British Journal of Medical Psychology 2011; 32: 50–55.

Hanakawa T, Fukuyama H, Katsumi Y, Honda M, Shibasaki H. Enhanced lateral premotor activity during paradoxical gait in Parkinson's disease. Ann Neurol 1999; 45: 329–336.

Hanakawa T, Katsumi Y, Fukuyama H, Honda M, Hayashi T, Kimura J, et al. Mechanisms underlying gait disturbance in Parkinson's disease: a single photon emission computed tomography study. Brain 1999; 122 (Pt 7): 1271–1282.

Harrington DL, Castillo GN, Greenberg PA, Song DD, Lessig S, Lee RR, et al. Neurobehavioral mechanisms of temporal processing deficits in Parkinson's disease. Plos One 2011; 6: e17461.

Haslinger B, Erhard P, Kämpfe N, Boecker H, Rummeny E, Schwaiger M, et al. Event-related functional magnetic resonance imaging in Parkinson's disease before and after levodopa. Brain 2001; 124: 558–570.

Hatcher JM, Pennell KD, Miller GW. Parkinson's disease and pesticides: a toxicological perspective. Trends Pharmacol. Sci. 2008; 29: 322–329.

Hawkes CH, Del Tredici K, Braak H. Parkinson's disease: the dual hit theory revisited. Ann. N. Y. Acad. Sci. 2009; 1170: 615–622.

Hayashi R, Hashimoto T, Tada T, Ikeda S-I. Effects of unilateral pallidotomy on voluntary movement, and simple and choice reaction times in Parkinson's disease. Mov Disord 2003; 18: 515–523.

He BJ, Raichle ME. The fMRI signal, slow cortical potential and consciousness. Trends Cogn. Sci. (Regul. Ed.) 2009; 13: 302–309.

Helmich RC, Aarts E, de Lange FP, Bloem BR, Toni I. Increased dependence of action selection on recent motor history in Parkinson's disease. J. Neurosci. 2009; 29: 6105–6113.

Helmich RC, Derikx LC, Bakker M, Scheeringa R, Bloem BR, Toni I. Spatial remapping of cortico-striatal connectivity in Parkinson's disease. Cereb Cortex 2010; 20: 1175–1186.

Helmich RC, Helmich RC, Hallett M, Hallett M, Deuschl G, Deuschl G, et al. Cerebral causes and consequences of parkinsonian resting tremor: a tale of two circuits? Brain 2012; 135: 3206–3226.

Hely MA, Chey T, Wilson A, Williamson PM, O'Sullivan DJ, Rail D, et al. Reliability of the Columbia scale for assessing signs of Parkinson's disease. Mov Disord 1993; 8: 466–472.

Hernán MA, Chen H, Schwarzschild MA, Ascherio A. Alcohol consumption and the incidence of Parkinson's disease. Ann Neurol 2003; 54: 170–175.

Hernán MA, Takkouche B, Caamaño-Isorna F, Gestal-Otero JJ. A meta-analysis of coffee drinking, cigarette smoking, and the risk of Parkinson's disease. Ann Neurol 2002; 52: 276–284.

Hikosaka O, Isoda M. Switching from automatic to controlled behavior: cortico-basal ganglia mechanisms. Trends Cogn. Sci. (Regul. Ed.) 2010; 14: 154–161.

Hirtz D, Thurman DJ, Gwinn-Hardy K, Mohamed M, Chaudhuri AR, Zalutsky R. How common are the 'common' neurologic disorders? Neurology 2007; 68: 326–337.

Hobson P, Gallacher J, Meara J. Cross-sectional survey of Parkinson's disease and parkinsonism in a rural area of the United Kingdom. Mov Disord 2005; 20: 995–998.

Hobson P, Meara J. The detection of dementia and cognitive impairment in a community population of elderly people with Parkinson's disease by use of the CAMCOG neuropsychological test. Age Ageing 1999; 28: 39–43.

Hoddes E, Zarcone V, Smythe H, Phillips R, Dement WC. Quantification of sleepiness: a new approach. Psychophysiology 1973; 10: 431–436.

Hoehn MM, Yahr MD. Parkinsonism: onset, progression and mortality. Neurology 1967; 17: 427–442.

Holden A, Wilman A, Wieler M, Martin WRW. Basal ganglia activation in Parkinson's disease. Parkinsonism Relat. Disord. 2006; 12: 73–77.

Hoops S, Nazem S, Siderowf AD, Duda JE, Xie SX, Stern MB, et al. Validity of the MoCA and MMSE in the detection of MCI and dementia in Parkinson disease. Neurology 2009; 73: 1738–1745.

Hornykiewicz O. A brief history of levodopa. J Neurol 2010; 257: S249–52.

Högl B, Arnulf I, Comella C, Ferreira J, Iranzo A, Tilley B, et al. Scales to assess sleep impairment in Parkinson's disease: critique and recommendations. Mov Disord 2010; 25: 2704–2716.

Hughes AJ, Daniel SE, Ben-Shlomo Y, Lees AJ. The accuracy of diagnosis of parkinsonian syndromes in a specialist movement disorder service. Brain 2002; 125: 861–870.

Hughes AJ, Daniel SE, Kilford L, Lees AJ. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. J Neurol Neurosurg Psychiatry 1992; 55: 181–184.

Hughes LE, Barker RA, Owen AM, Rowe JB. Parkinson's disease and healthy aging: independent and interacting effects on action selection. Hum Brain Mapp 2010; 31: 1886–1899.

Hummel T, Kobal G, Gudziol H, Mackay-Sim A. Normative data for the 'Sniffin' Sticks' including tests of odor identification, odor discrimination, and olfactory thresholds: an upgrade based on a group of more than 3,000 subjects. Eur Arch Otorhinolaryngol 2007; 264: 237–243.

Iadecola C. Neurovascular regulation in the normal brain and in Alzheimer's disease. Nat. Rev. Neurosci. 2004; 5: 347–360.

Isaias IU, Siri C, Cilia R, De Gaspari D, Pezzoli G, Antonini A. The relationship between impulsivity and impulse control disorders in Parkinson's disease. Mov Disord 2008; 23: 411–415.

Ishihara L, Brayne C. What is the evidence for a premorbid parkinsonian personality: A systematic review. Mov Disord 2006; 21: 1066–1072.

Ishihara LS, Cheesbrough A, Brayne C, Schrag A. Estimated life expectancy of Parkinson's patients compared with the UK population. J Neurol Neurosurg Psychiatry 2007; 78: 1304–1309.

Jack CR, Knopman DS, Jagust WJ, Petersen RC, Weiner MW, Aisen PS, et al. Tracking pathophysiological processes in Alzheimer's disease: an updated hypothetical model of dynamic biomarkers. Lancet Neurol 2013; 12: 207–216.

Jahanshahi M, Jones CRG, Zijlmans J, Katzenschlager R, Lee L, Quinn N, et al. Dopaminergic modulation of striato-frontal connectivity during motor timing in Parkinson's disease. Brain 2010; 133: 727–745.

Jamadar SD, Fielding J, Egan GF. Quantitative meta-analysis of fMRI and PET studies reveals consistent activation in fronto-striatal-parietal regions and cerebellum during antisaccades and prosaccades. Front Psychol 2013; 4: 749.

Jenkins IH, Fernandez W, Playford ED, Lees AJ, Frackowiak RS, Passingham RE, et al. Impaired activation of the supplementary motor area in Parkinson's disease is reversed when akinesia is treated with apomorphine. Ann Neurol 1992; 32: 749–757.

Jenkinson M, Bannister P, Brady M, Smith S. Improved optimization for the robust and accurate linear registration and motion correction of brain images. Neuroimage 2002; 17: 825–841.

Jezzard P, Matthews PM, Smith SM. Functional Magnetic Resonance Imaging. Oxford University Press; 2003.

Jobst EE, Melnick ME, Byl NN, Dowling GA, Aminoff MJ. Sensory perception in Parkinson disease. Arch Neurol-Chicago 1997; 54: 450–454.

Joel SE, Caffo BS, van Zijl PCM, Pekar JJ. On the relationship between seed-based and ICA-based measures of functional connectivity. Magn Reson Med 2011; 66: 644–657.

John OP, Srivastava S. The Big Five trait taxonomy: History, measurement, and theoretical perspectives. Handbook of personality: Theory and research 1999; 2: 102–138.

Johns MW. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. Sleep 1991; 14: 540–545.

Johnson AM, Vernon PA, Almeida QJ, Grantier LL, Singarayer R, Jog MS. Screening for Parkinson's disease with response time batteries: a pilot study. BMC Med Inform Decis Mak 2004; 4: 14.

Jones T. Positron emission tomography and measurements of regional tissue function in man. Br. Med. Bull. 1980; 36: 231–236.

Jubault T, Monetta L, Strafella AP, Lafontaine A-L, Monchi O. L-dopa medication in Parkinson's disease restores activity in the motor cortico-striatal loop but does not modify the cognitive network. Plos One 2009; 4: e6154.

Kalbe E, Calabrese P, Kohn N, Hilker R, Riedel O, Wittchen H-U, et al. Screening for cognitive deficits in Parkinson's disease with the Parkinson neuropsychometric dementia assessment (PANDA) instrument. Parkinsonism Relat. Disord. 2008; 14: 93–101.

Kalia LV, Kalia SK, McLean PJ, Lozano AM, Lang AE. α-Synuclein oligomers and clinical implications for Parkinson disease. Ann Neurol 2013; 73: 155–169.

Kasten M, Bruggemann N, Schmidt A, Klein C. VALIDITY OF THE MoCA AND MMSE IN THE DETECTION OF MCI AND DEMENTIA IN PARKINSON DISEASE. Neurology 2010; 75: 478–479.

Katschnig P, Schwingenschuh P, Jehna M, Švehlík M, Petrovic K, Ropele S, et al. Altered functional organization of the motor system related to ankle movements in Parkinson's disease: insights from functional MRI. J Neural Transm 2011; 118: 783–793.

Katzenschlager R, Schrag A, Evans A, Manson A, Carroll CB, Ottaviani D, et al. Quantifying the impact of dyskinesias in PD: the PDYS-26: a patient-based outcome measure. Neurology 2007; 69: 555–563.

Kelly VE, Samii A, Slimp JC, Price R, Goodkin R, Shumway-Cook A. Gait changes in response to subthalamic nucleus stimulation in people with Parkinson disease: a case series report. J Neurol Phys Ther 2006; 30: 184–194.

Kempster PA, O'Sullivan SS, Holton JL, Revesz T, Lees AJ. Relationships between age and late progression of Parkinson's disease: a clinico-pathological study. Brain 2010; 133: 1755–1762.

Khoo TK, Yarnall AJ, Duncan GW, Coleman S, O'Brien JT, Brooks DJ, et al. The spectrum of nonmotor symptoms in early Parkinson disease. Neurology 2013; 80: 276–281.

Kieburtz K, Wunderle KB. Parkinson's disease: evidence for environmental risk factors. Mov Disord 2013; 28: 8–13.

Kirsch-Darrow L, Fernandez HH, Fernandez HF, Marsiske M, Okun MS, Bowers D. Dissociating apathy and depression in Parkinson disease. Neurology 2006; 67: 33–38.

Kiviniemi V, Kantola J-H, Jauhiainen J, Hyvärinen A, Tervonen O. Independent component analysis of nondeterministic fMRI signal sources. Neuroimage 2003; 19: 253–260.

Klein C, Westenberger A. Genetics of Parkinson's disease. Cold Spring Harb Perspect Med 2012; 2: a008888.

Klepac N, Pikija S, Kraljić T, Relja M, Trkulja V, Juren S, et al. Association of rural life setting and poorer quality of life in Parkinson's disease patients: a cross-sectional study in Croatia. Eur J Neurol 2007; 14: 194–198.

Klos KJ, Bower JH, Josephs KA, Matsumoto JY, Ahlskog JE. Pathological hypersexuality predominantly linked to adjuvant dopamine agonist therapy in Parkinson's disease and multiple system atrophy. Parkinsonism Relat. Disord. 2005; 11: 381–386.

Klucken J, Barth J, Kugler P, Schlachetzki J, Henze T, Marxreiter F, et al. Unbiased and mobile gait analysis detects motor impairment in Parkinson's disease. Plos One 2013; 8: e56956.

Knipe MDW, Wickremaratchi MM, Wyatt-Haines E, Morris HR, Ben-Shlomo Y. Quality of life in young- compared with late-onset Parkinson's disease. Mov Disord 2011; 26: 2011–2018.

Konczak J, Corcos DM, Horak F, Poizner H, Shapiro M, Tuite P, et al. Proprioception and motor control in Parkinson's disease. J Mot Behav 2009; 41: 543–552.

Konczak J, Krawczewski K, Tuite P, Maschke M. The perception of passive motion in Parkinson's disease. J Neurol 2007; 254: 655–663.

Korchounov A, Kessler KR, Yakhno NN, Damulin IV, Schipper HI. Determinants of autonomic dysfunction in idiopathic Parkinson's disease. J Neurol 2005; 252: 1530–1536.

Kraft E, Loichinger W, Diepers M, Lule D, Schwarz J, Ludolph AC, et al. Levodopa-induced striatal activation in Parkinson's disease: a functional MRI study. Parkinsonism Relat. Disord. 2009; 15: 558–563.

Krajcovicova L, Mikl M, Marecek R, Rektorova I. The default mode network integrity in patients with Parkinson's disease is levodopa equivalent dose-dependent. J Neural Transm 2012; 119: 443–454.

Kulisevsky J, Pagonabarraga J. Cognitive impairment in Parkinson's disease: tools for diagnosis and assessment. Mov Disord 2009; 24: 1103–1110.

Künig G, Leenders KL, Martin-Sölch C, Missimer J, Magyar S, Schultz W. Reduced reward processing in the brains of Parkinsonian patients. Neuroreport 2000; 11: 3681–3687.

Kwak Y, Peltier S, Bohnen NI, Müller MLTM, Dayalu P, Seidler RD. Altered resting state cortico-striatal connectivity in mild to moderate stage Parkinson's disease. Front Syst Neurosci 2010; 4: 143.

Kwak Y, Peltier SJ, Bohnen NI, Muller MLTM, Dayalu P, Seidler RD. L-DOPA changes spontaneous low-frequency BOLD signal oscillations in Parkinson's disease: a resting state fMRI study. Front Syst Neurosci 2012; 6: 52.

Kwon D-H, Kim J-M, Oh S-H, Jeong H-J, Park S-Y, Oh E-S, et al. Seven-tesla magnetic resonance images of the substantia nigra in Parkinson disease. Ann Neurol 2012; 71: 267–277.

la Fuente-Fernandez de R, Schulzer M, Kuramoto L, Cragg J, Ramachandiran N, Au WL, et al. Age-specific progression of nigrostriatal dysfunction in Parkinson's disease. Ann Neurol 2011; 69: 803–810.

Laird AR, Eickhoff SB, Fox PM, Uecker AM, Ray KL, Saenz JJ, et al. The BrainMap strategy for standardization, sharing, and meta-analysis of neuroimaging data. BMC Res Notes 2011; 4: 349.

Laird AR, Fox PM, Eickhoff SB, Turner JA, Ray KL, McKay DR, et al. Behavioral interpretations of intrinsic connectivity networks. J Cognitive Neurosci 2011; 23: 4022–4037.

Lang AE, Martíanez-Martín P, Urra DG, Gómez JB. Timed tests in parkinson's disease evaluation1. Mov Disord 1997; 12: 127–128.

Lang AE. A critical appraisal of the premotor symptoms of Parkinson's disease: Potential usefulness in early diagnosis and design of neuroprotective trials. Mov Disord 2011; 26: 775–783.

Langston JW, Ballard P, Tetrud JW, Irwin I. Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. Science 1983; 219: 979–980.

Langston JW, Widner H, Goetz CG, Brooks D, Fahn S, Freeman T, et al. Core assessment program for intracerebral transplantations (CAPIT). Mov Disord 1992; 7: 2–13.

Lavalaye J, Booij J, Reneman L, Habraken JB, van Royen EA. Effect of age and gender on dopamine transporter imaging with [123I]FP-CIT SPET in healthy volunteers. Eur J Nucl Med 2000; 27: 867–869.

Lazar NA, Luna B, Sweeney JA, Eddy WF. Combining brains: a survey of methods for statistical pooling of information. Neuroimage 2002; 16: 538–550.

Leentjens AFG, Dujardin K, Marsh L, Martinez-Martin P, Richard IH, Starkstein SE, et al. Anxiety rating scales in Parkinson's disease: critique and recommendations. Mov Disord 2008; 23: 2015–2025.

Leentjens AFG, Van den Akker M, Metsemakers JFM, Lousberg R, Verhey FRJ. Higher incidence of depression preceding the onset of Parkinson's disease: a register study. Mov Disord 2003; 18: 414–418.

Lehericy S. 3-D Diffusion Tensor Axonal Tracking shows Distinct SMA and Pre-SMA Projections to the Human Striatum. Cereb Cortex 2004; 14: 1302–1309.

Lehéricy S, Sharman MA, Santos Dos CL, Paquin R, Gallea C. Magnetic resonance imaging of the substantia nigra in Parkinson's disease. Mov Disord 2012; 27: 822–830.

Levy G, Tang MX, Cote LJ, Louis ED, Alfaro B, Mejia H, et al. Motor impairment in PD: relationship to incident dementia and age. Neurology 2000; 55: 539–544.

Levy G. The relationship of Parkinson disease with aging. Arch Neurol-Chicago 2007; 64: 1242–1246.

Lewis MM, Du G, Sen S, Kawaguchi A, Truong Y, Lee S, et al. Differential involvement of striato- and cerebello-thalamo-cortical pathways in tremor- and akinetic/rigid-predominant Parkinson's disease. Neuroscience 2011; 177: 230–239.

Lewis MM, Slagle CG, Smith AB, Truong Y, Bai P, McKeown MJ, et al. Task specific influences of Parkinson's disease on the striato-thalamo-cortical and cerebello-thalamo-cortical motor circuitries. Neuroscience 2007; 147: 224–235.

Li Y-C, Chen C-C, Chen J-H. Impact of visual repetition rate on intrinsic properties of low frequency fluctuations in the visual network. Plos One 2011; 6: e18954.

Li Z, Kadivar A, Pluta J, Dunlop J, Wang Z. Test-retest stability analysis of resting brain activity revealed by blood oxygen level-dependent functional MRI. J. Magn. Reson. Imaging 2012; 36: 344–354.

Liebenthal E, Desai RH, Humphries C, Sabri M, Desai A. The functional organization of the left STS: a large scale meta-analysis of PET and fMRI studies of healthy adults. Front Neurosci 2014; 8: 289.

Lim LIIK, van Wegen EEH, de Goede CJT, Jones D, Rochester L, Hetherington V, et al. Measuring gait and gait-related activities in Parkinson's patients own home environment: a reliability, responsiveness and feasibility study. Parkinsonism Relat. Disord. 2005; 11: 19–24.

Lim SY, Fox SH, Lang AE. Overview of the Extranigral Aspects of Parkinson Disease. Arch Neurol-Chicago 2009; 66: 167–172.

Ling H, Massey L, Lees A, Brown P. Hypokinesia without decrement distinguishes progressive supranuclear palsy from Parkinson's disease. Brain 2012; 135, 1141–1153.

Little S, Brown P. What brain signals are suitable for feedback control of deep brain stimulation in Parkinson's disease? Ann. N. Y. Acad. Sci. 2012; 1265: 9–24.

Little S, Brown P. The functional role of beta oscillations in Parkinson's disease. Parkinsonism Relat. Disord. 2014

Litvan I, Goldman JG, Tröster AI, Schmand BA, Weintraub D, Petersen RC, et al. Diagnostic criteria for mild cognitive impairment in Parkinson's disease: Movement Disorder Society Task Force guidelines. Mov Disord 2012; 27: 349–356.

Liu H, Edmiston EK, Fan G, Xu K, Zhao B, Shang X, et al. Altered resting-state functional connectivity of the dentate nucleus in Parkinson's disease. Psychiatry Res 2013; 211: 64–71.

Llebaria G, Pagonabarraga J, Kulisevsky J, García-Sánchez C, Pascual-Sedano B, Gironell A, et al. Cut-off score of the Mattis Dementia Rating Scale for screening dementia in Parkinson's disease. Mov Disord 2008; 23: 1546–1550.

Logothetis NK. What we can do and what we cannot do with fMRI. Nature 2008; 453: 869–878.

Long D, Wang J, Xuan M, Gu Q, Xu X, Kong D, et al. Automatic classification of early Parkinson's disease with multi-modal MR imaging. Plos One 2012; 7: e47714.

Lotze M, Reimold M, Heymans U, Laihinen A, Patt M, Halsband U. Reduced ventrolateral fMRI response during observation of emotional gestures related to the degree of dopaminergic impairment in Parkinson disease. J Cognitive Neurosci 2009; 21: 1321–1331.

Luo C, Li Q, Xia Y, Lei X, Xue K, Yao Z, et al. Resting state basal ganglia network in idiopathic generalized epilepsy. Hum Brain Mapp 2012; 33: 1279–1294.

Lyons KE, Hubble JP, Tröster AI, Pahwa R, Koller WC. Gender differences in Parkinson's disease. Clin Neuropharmacol 1998; 21: 118–121.

Ma Y, Tang C, Spetsieris PG, Dhawan V, Eidelberg D. Abnormal metabolic network activity in Parkinson's disease: test–retest reproducibility. J Cereb Blood Flow Metab 2006; 27: 597–605.

Mahieux F, Michelet D, Manifacier MJ, Boller F, Fermanian J, Guillard A. Mini-Mental Parkinson: first validation study of a new bedside test constructed for Parkinson's disease. Behav Neurol 1995; 8: 15–22.

Mallol R, Barrós-Loscertales A, López M, Belloch V, Parcet MA, Avila C. Compensatory cortical mechanisms in Parkinson's disease evidenced with fMRI during the performance of pre-learned sequential movements. Brain Res 2007; 1147: 265–271.

Mancini M, Zampieri C, Carlson-Kuhta P, Chiari L, Horak FB. Anticipatory postural adjustments prior to step initiation are hypometric in untreated Parkinson's disease: an accelerometer-based approach. Eur J Neurol 2009; 16: 1028–1034.

Marek K, Innis R, van Dyck C, Fussell B, Early M, Eberly S, et al. [123I]beta-CIT SPECT imaging assessment of the rate of Parkinson's disease progression. Neurology 2001; 57: 2089–2094.

Margulies DS, Böttger J, Long X, Lv Y, Kelly C, Schäfer A, et al. Resting developments: a review of fMRI post-processing methodologies for spontaneous brain activity. Magn Reson Mater Phy 2010; 23: 289–307.

Marinus J, Visser M, Stiggelbout AM, Rabey JM, Martínez-Martín P, Bonuccelli U, et al. A short scale for the assessment of motor impairments and disabilities in Parkinson's disease: the SPES/SCOPA. J Neurol Neurosurg Psychiatry 2004; 75: 388–395.

Marinus J, Visser M, van Hilten JJ, Lammers GJ, Stiggelbout AM. Assessment of sleep and sleepiness in Parkinson disease. Sleep 2003; 26: 1049–1054.

Marinus J, Visser M, Verwey NA, Verhey FRJ, Middelkoop HAM, Stiggelbout AM, et al. Assessment of cognition in Parkinson's disease. Neurology 2003; 61: 1222–1228.

Marion M-H, Qurashi M, Marshall G, Foster O. Is REM sleep behaviour disorder (RBD) a risk factor of dementia in idiopathic Parkinson's disease? J Neurol 2008; 255: 192–196.

Martin WR, Wieler M, Gee M. Midbrain iron content in early Parkinson disease: a potential biomarker of disease status. Neurology 2008; 70: 1411–1417.

Martinez-Martin P, Falup Pecurariu C, Odin P, Hilten JJ, Antonini A, Rojo-Abuin JM, et al. Gender-related differences in the burden of non-motor symptoms in Parkinson’s disease. J Neurol 2012; 259: 1639–1647.

Martínez-Martín P, Carrasco de la Peña JL, Ramo C, Antigüedad AR, Bermejo F. [Study of inter-observer reliability in the use of qualitative scales assessing Parkinson disease (II)]. Arch Neurobiol (Madr) 1988; 51: 287–291.

Maschke M, Gomez CM, Tuite PJ, Konczak J. Dysfunction of the basal ganglia, but not the cerebellum, impairs kinaesthesia. Brain 2003; 126, 2312–2322.

Maschke M, Tuite PJ, Krawczewski K, Pickett K, Konczak J. Perception of heaviness in Parkinson's disease. Mov Disord 2006; 21: 1013–1018.

Mattay VS, Tessitore A, Callicott JH, Bertolino A, Goldberg TE, Chase TN, et al. Dopaminergic modulation of cortical function in patients with Parkinson's disease. Ann Neurol 2002; 51: 156–164.

Matthews PM, Jezzard P. Functional magnetic resonance imaging. J Neurol Neurosurg Psychiatry 2004; 75: 6–12.

McGhee DJM, Royle PL, Thompson PA, Wright DE, Zajicek JP, Counsell CE. A systematic review of biomarkers for disease progression in Parkinson's disease. BMC Neurol 2013; 13: 35.

McKeown MJ, Makeig S, Brown GG, Jung TP, Kindermann SS, Bell AJ, et al. Analysis of fMRI data by blind separation into independent spatial components. Hum Brain Mapp 1998; 6: 160–188.

Meissner WG, Frasier M, Gasser T, Goetz CG, Lozano A, Piccini P, et al. Priorities in Parkinson's disease research. Nat Rev Drug Discov 2011; 10: 377–393.

Menke RA, Scholz J, Miller KL, Deoni S, Jbabdi S, Matthews PM, et al. MRI characteristics of the substantia nigra in Parkinson's disease: a combined quantitative T1 and DTI study. Neuroimage 2009; 47: 435–441

Menza MA, Forman NE, Goldstein HS, Golbe LI. Parkinson's disease, personality, and dopamine. J Neuropsychiatry Clin Neurosci 1990; 2: 282–287.

Mera TO, Heldman DA, Espay AJ, Payne M, Giuffrida JP. Feasibility of home-based automated Parkinson's disease motor assessment. J Neurosci Meth 2012; 203: 152–156.

Metman LV, Myre B, Verwey N, Hassin-Baer S, Arzbaecher J, Sierens D, et al. Test-retest reliability of UPDRS-III, dyskinesia scales, and timed motor tests in patients with advanced Parkinson's disease: an argument against multiple baseline assessments. Mov Disord 2004; 19: 1079–1084.

Milanov I. Correlation between tremor parameters. Funct. Neurol. 2002; 17: 19–23.

Miller N, Allcock L, Hildreth AJ, Jones D, Noble E, Burn DJ. Swallowing problems in Parkinson disease: frequency and clinical correlates. J Neurol Neurosurg Psychiatry 2009; 80: 1047–1049.

Mima T, Sadato N, Yazawa S, Hanakawa T, Fukuyama H, Yonekura Y, et al. Brain structures related to active and passive finger movements in man. Brain 1999; 122 (Pt 10): 1989–1997.

Mink JW. The basal ganglia: Focused selection and inhibition of competing motor programs. Prog. Neurobiol. 1996; 50: 381–425.

Moeller JR, Nakamura T, Mentis MJ, Dhawan V, Spetsieres P, Antonini A, et al. Reproducibility of regional metabolic covariance patterns: comparison of four populations. J. Nucl. Med. 1999; 40: 1264–1269.

Mohammadi B, Kollewe K, Samii A, Krampfl K, Dengler R, Münte TF. Changes of resting state brain networks in amyotrophic lateral sclerosis. Experimental Neurology 2009; 217: 147–153.

Mohammadi B, Kollewe K, Samii A. Changes in resting-state brain networks in writer's cramp. Hum Brain Mapp 2012; 33: 840–848.

Monchi O, Petrides M, Doyon J, Postuma RB, Worsley K, Dagher A. Neural Bases of Set-Shifting Deficits in Parkinson's Disease. J. Neurosci. 2004; 24: 702–710.

Monchi O, Petrides M, Mejia-Constain B, Strafella AP. Cortical activity in Parkinson's disease during executive processing depends on striatal involvement. Brain 2007; 130: 233–244.

Monchi O, Petrides M, Strafella AP, Worsley KJ, Doyon J. Functional role of the basal ganglia in the planning and execution of actions. Ann Neurol 2006; 59: 257–264.

Montgomery EB, Koller WC, LaMantia TJK, Newman MC, Swanson-Hyland E, Kaszniak AW, et al. Early detection of probable idiopathic Parkinson's disease: I. development of a diagnostic test battery. Mov Disord 2001; 15: 467–473.

Montgomery SA, Asberg M. A new depression scale designed to be sensitive to change. Br J Psychiatry 1979; 134: 382–389.

Monti MM. Statistical Analysis of fMRI Time-Series: A Critical Review of the GLM Approach. Front. Hum. Neurosci. 2011; 5: 28.

Moody TD, Bookheimer SY, Vanek Z, Knowlton BJ. An implicit learning task activates medial temporal lobe in patients with Parkinson's disease. Behav Neurosci 2004; 118: 438–442.

Moore ST, Yungher DA, Morris TR, Dilda V, MacDougall HG, Shine JM, et al. Autonomous identification of freezing of gait in Parkinson's disease from lower-body segmental accelerometry. J Neuroeng Rehabil 2013; 10: 19.

Morris S, Morris ME, Iansek R. Reliability of measurements obtained with the Timed ‘Up & Go’ test in people with Parkinson disease. Phys Ther 2001; 81: 810–818.

Mouret J. Differences in sleep in patients with Parkinson's disease. Electroencephalogr Clin Neurophysiol 1975; 38: 653–657.

Mozley LH, Gur RC, Mozley PD, Gur RE. Striatal dopamine transporters and cognitive functioning in healthy men and women. Am J Psychiatry 2001; 158: 1492–1499.

Muslimovic D, Post B, Speelman JD, Schmand B, de Haan RJ, CARPA Study Group. Determinants of disability and quality of life in mild to moderate Parkinson disease. Neurology 2008; 70: 2241–2247.

Muslimovic D, Post B, Speelman JD, Schmand B. Cognitive profile of patients with newly diagnosed Parkinson disease. Neurology 2005; 65: 1239–1245.

Nachev P, Kennard C, Husain M. Functional role of the supplementary and pre-supplementary motor areas. Nat. Rev. Neurosci. 2008; 9: 856–869.

Nakamura T, Ghilardi MF, Mentis M, Dhawan V, Fukuda M, Hacking A, et al. Functional networks in motor sequence learning: abnormal topographies in Parkinson's disease. Hum Brain Mapp 2001; 12: 42–60.

Nambu A, Tokuno H, Takada M. Functional significance of the cortico–subthalamo–pallidal 'hyperdirect' pathway. Neuroscience Research 2002; 43: 111–117.

Nasreddine ZS, Phillips NA, Bédirian V, Charbonneau S, Whitehead V, Collin I, et al. The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. J Am Geriatr Soc 2005; 53: 695–699.

Nazem S, Siderowf AD, Duda JE, Have TT, Colcher A, Horn SS, et al. Montreal cognitive assessment performance in patients with Parkinson's disease with "normal" global cognition according to mini-mental state examination score. J Am Geriatr Soc 2009; 57: 304–308.

Nègre-Pagès L, Grandjean H, Lapeyre-Mestre M, Montastruc JL, Fourrier A, Lépine JP, et al. Anxious and depressive symptoms in Parkinson's disease: the French cross-sectionnal DoPaMiP study. Mov Disord 2010; 25: 157–166.

Nouzeilles MI, Merello M. Correlation between results of motor section of UPDRS and Webster Scale. Mov Disord 1997; 12: 613.

Noyce AJ, Bestwick JP, Silveira-Moriyama L, Hawkes CH, Giovannoni G, Lees AJ, et al. Meta-analysis of early nonmotor features and risk factors for Parkinson disease. Ann Neurol 2012; 72: 893–901.

Nyhlén J, Constantinescu R, Zetterberg H. Problems associated with fluid biomarkers for Parkinson's disease. Biomark Med 2010; 4: 671–681.

O'Reilly EJ, Chen H, Gardener H, Gao X, Schwarzschild MA, Ascherio A. Smoking and Parkinson's disease: using parental smoking as a proxy to explore causality. Am. J. Epidemiol. 2009; 169: 678–682.

O'Suilleabhain P, Bullard J, Dewey RB. Proprioception in Parkinson's disease is acutely depressed by dopaminergic medications. J Neurol Neurosurg Psychiatry 2001; 71: 607–610.

Obeso JA, Lanciego JL. Past, present, and future of the pathophysiological model of the Basal Ganglia. Front Neuroanat 2011; 5: 39.

Obeso JA, Marin C, Rodriguez-Oroz C, Blesa J, Benitez-Temiño B, Mena-Segovia J, et al. The basal ganglia in Parkinson's disease: current concepts and unexplained observations. Ann Neurol 2008; 64 Suppl 2: S30–46.

Ogawa S, Menon RS, Tank DW, Kim SG, Merkle H, Ellermann JM, et al. Functional brain mapping by blood oxygenation level-dependent contrast magnetic resonance imaging. A comparison of signal characteristics with a biophysical model. Biophys. J. 1993; 64: 803–812.

Olanow CW, Watts RL, Koller WC. An algorithm (decision tree) for the management of Parkinson's disease (2001): treatment guidelines. Neurology 2001; 56: S1–S88.

Ono K, Hirohata M, Yamada M. Anti-fibrillogenic and fibril-destabilizing activity of nicotine in vitro: implications for the prevention and therapeutics of Lewy body diseases. Experimental Neurology 2007; 205: 414–424.

Owen AM, Doyon J, Dagher A, Sadikot A, Evans AC. Abnormal basal ganglia outflow in Parkinson's disease identified with PET. Implications for higher cortical functions. Brain 1998; 121 (Pt 5): 949–965.

Ozekmekçi S, Apaydin H, Kiliç E. Clinical features of 35 patients with Parkinson's disease displaying REM behavior disorder. Clin Neurol Neurosurg 2005; 107: 306–309.

Pacchetti C, Manni R, Zangaglia R, Mancini F, Marchioni E, Tassorelli C, et al. Relationship between hallucinations, delusions, and rapid eye movement sleep behavior disorder in Parkinson's disease. Mov Disord 2005; 20: 1439–1448.

Pagonabarraga J, Kulisevsky J, Llebaria G, García-Sánchez C, Pascual-Sedano B, Gironell A. Parkinson's disease-cognitive rating scale: a new cognitive scale specific for Parkinson's disease. Mov Disord 2008; 23: 998–1005.

Park MJ, Cheon S-M, Bae H-R, Kim S-H, Kim JW. Elevated levels of α-synuclein oligomer in the cerebrospinal fluid of drug-naïve patients with Parkinson's disease. J Clin Neurol 2011; 7: 215–222.

Parkinson Progression Marker Initiative. The Parkinson Progression Marker Initiative (PPMI). Prog. Neurobiol. 2011; 95: 629–635.

Parkinson Study Group. DATATOP: a multicenter controlled clinical trial in early Parkinson's disease. Parkinson Study Group. Arch Neurol-Chicago 1989; 46: 1052–1060.

Parkinson Study Group. Evaluation of dyskinesias in a pilot, randomized, placebo-controlled trial of remacemide in advanced Parkinson disease. Arch Neurol-Chicago 2001; 58: 1660–1668.

Parkkinen L, O'Sullivan SS, Collins C, Petrie A, Holton JL, Revesz T, et al. Disentangling the Relationship between Lewy Bodies and Nigral Neuronal Loss in Parkinson's Disease. J Parkinsons Dis 2011; 1: 277–286.

Payoux P, Brefel-Courbon C, Ory-Magne F, Regragui W, Thalamas C, Balduyck S, et al. Motor activation in multiple system atrophy and Parkinson disease: a PET study. Neurology 2010; 75: 1174–1180.

Perez-Lloret S, Nègre-Pagès L, Ojero-Senard A, Damier P, Destée A, Tison F, et al. Oro-buccal symptoms (dysphagia, dysarthria, and sialorrhea) in patients with Parkinson's disease: preliminary analysis from the French COPARK cohort. Eur J Neurol 2012; 19: 28–37.

Pezzella FR, Di Rezze S, Chianese M, Fabbrini G, Vanacore N, Colosimo C, et al. Hedonistic homeostatic dysregulation in Parkinson's disease: a short screening questionnaire. Neurol. Sci. 2003; 24: 205–206.

Pfeiffer RF. Gastrointestinal dysfunction in Parkinson's disease. Parkinsonism Relat. Disord. 2011; 17: 10–15.

Phan KL, Wager T, Taylor SF, Liberzon I. Functional neuroanatomy of emotion: a meta-analysis of emotion activation studies in PET and fMRI. Neuroimage 2002; 16: 331–348.

Phelps ME. PET. Springer Science & Business Media; 2006.

Piccini P, Burn DJ, Ceravolo R, Maraganore D, Brooks DJ. The role of inheritance in sporadic Parkinson's disease: evidence from a longitudinal study of dopaminergic function in twins. Ann Neurol 1999; 45: 577–582.

Pinter MM, Helscher RJ, Nasel COJ, Riedl E, Schnaberth G. Quantification of motor deficit in Parkinson's disease with a motor performance test series. J Neural Transm Gen Sect 1992; 4: 131–141.

Pinto S, Mancini L, Jahanshahi M, Thornton JS, Tripoliti E, Yousry TA, et al. Functional magnetic resonance imaging exploration of combined hand and speech movements in Parkinson's disease. Mov Disord 2011; 26: 2212–2219.

Pirker W. Correlation of dopamine transporter imaging with parkinsonian motor handicap: how close is it? Mov Disord 2003; 18 Suppl 7: S43–51.

Poewe W, Mahlknecht P. The clinical progression of Parkinson's disease. Parkinsonism Relat. Disord. 2009; 15: S28–S32.

Ponsen MM, Stoffers D, Booij J, van Eck-Smit BLF, Wolters EC, Berendse HW. Idiopathic hyposmia as a preclinical sign of Parkinson's disease. Ann Neurol 2004; 56: 173–181.

Popescu A, Lippa CF, Lee VMY, Trojanowski JQ. Lewy bodies in the amygdala: increase of alpha-synuclein aggregates in neurodegenerative diseases with tau-based inclusions. Arch Neurol-Chicago 2004; 61: 1915–1919.

Post B, Muslimovic D, van Geloven N, Speelman JD, Schmand B, de Haan RJ, et al. Progression and prognostic factors of motor impairment, disability and quality of life in newly diagnosed Parkinson's disease. Mov Disord 2011; 26: 449–456.

Postuma RB, Aarsland D, Barone P, Burn DJ, Hawkes CH, Oertel W, et al. Identifying prodromal Parkinson‘s disease: pre-motor disorders in Parkinson’s disease. Mov Disord 2012; 27: 617–626.

Postuma RB, Bertrand J-A, Montplaisir J, Desjardins C, Vendette M, Rios Romenets S, et al. Rapid eye movement sleep behavior disorder and risk of dementia in Parkinson's disease: A prospective study. Mov Disord 2012; 27: 720–726.

Postuma RB, Dagher A. Basal ganglia functional connectivity based on a meta-analysis of 126 positron emission tomography and functional magnetic resonance imaging publications. Cereb Cortex 2006; 16: 1508–1521.

Postuma RB, Gagnon J-F. Symmetry of Parkinson's disease and REM sleep: one piece of the puzzle. Ann Neurol 2011; 69: 905–author reply 906.

Postuma RB, Gagnon JF, Montplaisir J. Clinical prediction of Parkinson's disease: planning for the age of neuroprotection. J Neurol Neurosurg Psychiatry 2010; 81: 1008–1013.

Postuma RB, Gagnon JF, Vendette M, Fantini ML, Massicotte-Marquez J, Montplaisir J. Quantifying the risk of neurodegenerative disease in idiopathic REM sleep behavior disorder. Neurology 2009; 72: 1296–1300.

Poulopoulos M, Levy OA, Alcalay RN. The neuropathology of genetic Parkinson's disease. Mov Disord 2012; 27: 831–842.

Power JD, Barnes KA, Snyder AZ, Schlaggar BL, Petersen SE. Spurious but systematic correlations in functional connectivity MRI networks arise from subject motion. Neuroimage 2012; 59: 2142–2154.

Priyadarshi A, Khuder SA, Schaub EA, and Shrivastava S. A meta-analysis of Parkinson's disease and exposure to pesticides. Neurotoxicology 2000; 21: 435–440.

Prodoehl J, Spraker M, Corcos D, Comella C, Vaillancourt D. Blood oxygenation level-dependent activation in basal ganglia nuclei relates to specific symptoms in de novo Parkinson's disease. Mov Disord 2010; 25: 2035–2043.

Putzki N, Stude P, Konczak J, Graf K, Diener H-C, Maschke M. Kinesthesia is impaired in focal dystonia. Mov Disord 2006; 21: 754–760.

Qin Z, Zhang L, Sun F, Fang X, Meng C, Tanner C, et al. Health related quality of life in early Parkinson's disease: impact of motor and non-motor symptoms, results from Chinese levodopa exposed cohort. Parkinsonism Relat. Disord. 2009; 15: 767–771.

Rabey JM, Bass H, Bonuccelli U, Brooks D, Klotz P, Korczyn AD, et al. Evaluation of the Short Parkinson‘s Evaluation Scale: a new friendly scale for the evaluation of Parkinson’s disease in clinical drug trials. Clin Neuropharmacol 1997; 20: 322–337.

Ragonese P, Salemi G, Morgante L, Aridon P, Epifanio A, Buffa D, et al. A case-control study on cigarette, alcohol, and coffee consumption preceding Parkinson's disease. Neuroepidemiology 2003; 22: 297–304.

Raichle ME. The restless brain. Brain Connect 2011; 1: 3–12.

Rajput AH, Rozdilsky B, Rajput A. Accuracy of clinical diagnosis in parkinsonism--a prospective study. Can J Neurol Sci 1991; 18: 275–278.

Ramírez-Ruiz B, Martí M-J, Tolosa E, Falcón C, Bargalló N, Valldeoriola F, et al. Brain response to complex visual stimuli in Parkinson's patients with hallucinations: a functional magnetic resonance imaging study. Mov Disord 2008; 23: 2335–2343.

Rascol O, Sabatini U, Fabre N, Brefel C, Loubinoux I, Celsis P, et al. The ipsilateral cerebellar hemisphere is overactive during hand movements in akinetic parkinsonian patients. Brain 1997; 120 (Pt 1): 103–110.

Ray N, Strafella AP. Dopamine, Reward, and Frontostriatal Circuitry in Impulse Control Disorders in Parkinson's Disease: Insights from Functional Imaging. Clin Eeg Neurosci 2010; 41: 87–93.

Redgrave P, Rodriguez M, Smith Y, Rodriguez-Oroz MC, Lehericy S, Bergman H, et al. Goal-directed and habitual control in the basal ganglia: implications for Parkinson's disease. Nat. Rev. Neurosci. 2010; 11: 760–772.

Reuther M, Spottke EA, Klotsche J, Riedel O, Peter H, Berger K, et al. Assessing health-related quality of life in patients with Parkinson's disease in a prospective longitudinal study. Parkinsonism Relat. Disord. 2007; 13: 108–114.

Reyes MA, Perez-Lloret S, Lloret SP, Roldan Gerschcovich E, Gerscovich ER, Martin ME, et al. Addenbrooke‘s Cognitive Examination validation in Parkinson’s disease. Eur J Neurol 2009; 16: 142–147.

Reynolds NC, Montgomery GK. Factor analysis of Parkinson's impairment. An evaluation of the final common pathway. Arch Neurol-Chicago 1987; 44: 1013–1016.

Riedel O, Klotsche J, Spottke A, Deuschl G, Förstl H, Henn F, et al. Frequency of dementia, depression, and other neuropsychiatric symptoms in 1,449 outpatients with Parkinson's disease. J Neurol 2010; 257: 1073–1082.

Robinson S, Basso G, Soldati N, Sailer U, Jovicich J, Bruzzone L, et al. A resting state network in the motor control circuit of the basal ganglia. BMC Neurosci. 2009; 10: 137.

Rodriguez-Oroz MC, Jahanshahi M, Krack P, Litvan I, Macias R, Bezard E, et al. Initial clinical manifestations of Parkinson's disease: features and pathophysiological mechanisms. Lancet Neurol 2009; 8: 1128–1139.

Rolinski M, Fox C, Maidment I, McShane R. Cholinesterase inhibitors for dementia with Lewy bodies, Parkinson‘s disease dementia and cognitive impairment in Parkinson’s disease. Cochrane Database Syst Rev 2012; 3: CD006504.

Romenets SR, Gagnon J-F, Latreille V, Panniset M, Chouinard S, Montplaisir J, et al. Rapid eye movement sleep behavior disorder and subtypes of Parkinson's disease. Mov Disord 2012; 27: 996–1003.

Rosazza C, Minati L. Resting-state brain networks: literature review and clinical applications. Neurol Sci 2011; 32: 773–785.

Ross GW, Petrovitch H, Abbott RD, Tanner CM, Popper J, Masaki K, et al. Association of olfactory dysfunction with risk for future Parkinson's disease. Ann Neurol 2008; 63: 167–173.

Rowe J, Stephan KE, Friston K, Frackowiak R, Lees A, Passingham R. Attention to action in Parkinson's disease: impaired effective connectivity among frontal cortical regions. Brain 2002; 125: 276–289.

Rowe JB, Hughes L, Ghosh BCP, Eckstein D, Williams-Gray CH, Fallon S, et al. Parkinson's disease and dopaminergic therapy--differential effects on movement, reward and cognition. Brain 2008; 131: 2094–2105.

Sabatini U, Boulanouar K, Fabre N, Martin F, Carel C, Colonnese C, et al. Cortical motor reorganization in akinetic patients with Parkinson's disease: a functional MRI study. Brain 2000; 123 (Pt 2): 394–403.

Salimi-Khorshidi G, Nichols TE, Smith SM, Woolrich MW. Using Gaussian-process regression for meta-analytic neuroimaging inference based on sparse observations. IEEE Trans Med Imaging 2011; 30: 1401–1416.

Salimi-Khorshidi G, Smith SM, Keltner JR, Wager TD, Nichols TE. Meta-analysis of neuroimaging data: A comparison of image-based and coordinate-based pooling of studies. Neuroimage 2009; 45: 810–823.

Samuel M, Ceballos-Baumann AO, Blin J, Uema T, Boecker H, Passingham RE, et al. Evidence for lateral premotor and parietal overactivity in Parkinson's disease during sequential and bimanual movements. A PET study. Brain 1997; 120 (Pt 6): 963–976.

Samuel M, Ceballos-Baumann AO, Boecker H, Brooks DJ. Motor imagery in normal subjects and Parkinson's disease patients: an H215O PET study. Neuroreport 2001; 12: 821–828.

Sanchez-Ramos JR, Ortoll R, Paulson GW. Visual hallucinations associated with Parkinson disease. Arch Neurol-Chicago 1996; 53: 1265–1268.

Sawamoto N, Honda M, Hanakawa T, Aso T, Inoue M, Toyoda H, et al. Cognitive slowing in Parkinson disease is accompanied by hypofunctioning of the striatum. Neurology 2007; 68: 1062–1068.

Scaglione C, Vignatelli L, Plazzi G, Marchese R, Negrotti A, Rizzo G, et al. REM sleep behaviour disorder in Parkinson's disease: a questionnaire-based study. Neurol. Sci. 2005; 25: 316–321.

Schapira AHV. Recent developments in biomarkers in Parkinson disease. Curr Opin Neurol 2013; 26: 395–400.

Schneider JS, Elm JJ, Parashos SA, Ravina BM, Galpern WR, NET-PD Investigators. Predictors of cognitive outcomes in early Parkinson disease patients: The National Institutes of Health Exploratory Trials in Parkinson Disease (NET-PD) experience. Parkinsonism Relat. Disord. 2010; 16: 507–512.

Schonberg T, O'Doherty JP, Joel D, Inzelberg R, Segev Y, Daw ND. Selective impairment of prediction error signaling in human dorsolateral but not ventral striatum in Parkinson's disease patients: evidence from a model-based fMRI study. Neuroimage 2010; 49: 772–781.

Schott BH, Niehaus L, Wittmann BC, Schütze H, Seidenbecher CI, Heinze H-J, et al. Ageing and early-stage Parkinson's disease affect separable neural mechanisms of mesolimbic reward processing. Brain 2007; 130: 2412–2424.

Schrag A, Barone P, Brown RG, Leentjens AFG, McDonald WM, Starkstein S, et al. Depression rating scales in Parkinson's disease: critique and recommendations. Mov Disord 2007; 22: 1077–1092.

Schrag A, Ben-Shlomo Y, Quinn N. How valid is the clinical diagnosis of Parkinson's disease in the community? J Neurol Neurosurg Psychiatry 2002; 73: 529–534.

Schrag A, Jahanshahi M, Quinn N. What contributes to quality of life in patients with Parkinson's disease? J Neurol Neurosurg Psychiatry 2000; 69: 308–312.

Schwarz ST, Afzal M, Morgan PS, Bajaj N, Gowland PA, Auer DP. The 'swallow tail“ appearance of the healthy nigrosome - a new accurate test of Parkinson”s disease: a case-control and retrospective cross-sectional MRI study at 3T. Plos One 2014; 9: e93814.

Schwarzschild MA, Agnati L, Fuxe K, Chen J-F, Morelli M. Targeting adenosine A2A receptors in Parkinson's disease. Trends in Neurosciences 2006; 29: 647–654.

Seeley WW, Menon V, Schatzberg AF, Keller J, Glover GH, Kenna H, et al. Dissociable intrinsic connectivity networks for salience processing and executive control. J. Neurosci. 2007; 27: 2349–2356.

Sen S, Kawaguchi A, Truong Y, Lewis MM, Huang X. Dynamic changes in cerebello-thalamo-cortical motor circuitry during progression of Parkinson's disease. Neuroscience 2010; 166: 712–719.

Shannon KM, Keshavarzian A, Mutlu E, Dodiya HB, Daian D, Jaglin JA, et al. Alpha-synuclein in colonic submucosa in early untreated Parkinson's disease. Mov Disord 2012; 27: 709–715.

Shearer J, Green C, Counsell CE, Zajicek JP. The impact of motor and non motor symptoms on health state values in newly diagnosed idiopathic Parkinson's disease. J Neurol 2012; 259: 462–468.

Shulman LM, Gruber-Baldini AL, Anderson KE, Vaughan CG, Reich SG, Fishman PS, et al. The evolution of disability in Parkinson disease. Mov Disord 2008; 23: 790–796.

Shumway-Cook A, Brauer S, Woollacott M. Predicting the probability for falls in community-dwelling older adults using the Timed Up & Go Test. Phys Ther 2000; 80: 896–903.

Siderowf A, Jennings D, Eberly S, Oakes D, Hawkins KA, Ascherio A, et al. Impaired olfaction and other prodromal features in the Parkinson At-Risk Syndrome Study. Mov Disord 2012; 27: 406–412.

Siderowf A, Stern MB. Premotor Parkinson's disease: clinical features, detection, and prospects for treatment. Ann Neurol 2008; 64 Suppl 2: S139–47.

Siemers ER, Shekhar A, Quaid K, Dickson H. Anxiety and motor performance in Parkinson's disease. Mov Disord 1993; 8: 501–506.

Sierks MR, Chatterjee G, McGraw C, Kasturirangan S, Schulz P, Prasad S. CSF levels of oligomeric alpha-synuclein and beta-amyloid as biomarkers for neurodegenerative disease. Integr Biol (Camb) 2011; 3: 1188–1196.

Skeie GO, Muller B, Haugarvoll K, Larsen JP, Tysnes OB. Differential effect of environmental risk factors on postural instability gait difficulties and tremor dominant Parkinson's disease. Mov Disord 2010; 25: 1847–1852.

Skidmore FM, Yang M, Baxter L, Deneen von KM, Collingwood J, He G, et al. Reliability analysis of the resting state can sensitively and specifically identify the presence of Parkinson disease. Neuroimage 2011; 75, 249–261.

Smith SM, Miller KL, Moeller S, Xu J, Auerbach EJ, Woolrich MW, et al. Temporally-independent functional modes of spontaneous brain activity. Proc Natl Acad Sci U S A 2012; 109: 3131–3136.

Smith SM, Nichols TE. Threshold-free cluster enhancement: addressing problems of smoothing, threshold dependence and localisation in cluster inference. Neuroimage 2009; 44: 83–98.

Snaith RP, Bridge GW, Hamilton M. The Leeds scales for the self-assessment of anxiety and depression. Br J Psychiatry 1976; 128: 156–165.

Snyder AZ, Raichle ME. A brief history of the resting state: the Washington University perspective. Neuroimage 2012; 62: 902–910.

Solla P, Cannas A, Ibba FC, Loi F, Corona M, Orofino G, et al. Gender differences in motor and non-motor symptoms among Sardinian patients with Parkinson's disease. J Neurol Sci 2012; 323: 33–39.

Sommer U, Hummel T, Cormann K, Mueller A, Frasnelli J, Kropp J, et al. Detection of presymptomatic Parkinson's disease: combining smell tests, transcranial sonography, and SPECT. Mov Disord 2004; 19: 1196–1202.

Spraker MB, Prodoehl J, Corcos DM, Comella CL, Vaillancourt DE. Basal ganglia hypoactivity during grip force in drug naïve Parkinson's disease. Hum Brain Mapp 2010; 31: 1928–1941.

Starkstein SE, Mayberg HS, Leiguarda R, Preziosi TJ, Robinson RG. A prospective longitudinal study of depression, cognitive decline, and physical impairments in patients with Parkinson's disease. J Neurol Neurosurg Psychiatry 1992; 55: 377–382.

Steffen T, Seney M. Test-Retest Reliability and Minimal Detectable Change on Balance and Ambulation Tests, the 36-Item Short-Form Health Survey, and the Unified Parkinson Disease Rating Scale in People With Parkinsonism. Phys Ther 2008; 88: 733–746.

Stern RG, Mohs RC, Davidson M, Schmeidler J, Silverman J, Kramer-Ginsberg E, et al. A longitudinal study of Alzheimer's disease: measurement, rate, and predictors of cognitive deterioration. Am J Psychiatry 1994; 151: 390–396.

Stiasny-Kolster K, Mayer G, Schäfer S, Möller JC, Heinzel-Gutenbrunner M, Oertel WH. The REM sleep behavior disorder screening questionnaire--a new diagnostic instrument. Mov Disord 2007; 22: 2386–2393.

Suchowersky O, Reich S, Perlmutter J, Zesiewicz T, Gronseth G, Weiner WJ, et al. Practice Parameter: diagnosis and prognosis of new onset Parkinson disease (an evidence-based review): report of the Quality Standards Subcommittee of the American Academy of Neurology. Neurology 2006; 66: 968–975.

Szameitat AJ, Shen S, Conforto A, Sterr A. Cortical activation during executed, imagined, observed, and passive wrist movements in healthy volunteers and stroke patients. Neuroimage 2012; 62: 266–280.

Špica V, Pekmezović T, Svetel M, Kostić VS. Prevalence of non-motor symptoms in young-onset versus late-onset Parkinson's disease. J Neurol 2013; 260: 131–137.

Takeda A, Saito N, Baba T, Kikuchi A, Sugeno N, Kobayashi M, et al. Functional imaging studies of hyposmia in Parkinson's disease. J Neurol Sci 2010; 289: 36–39.

Talland GA. Cognitive Function in Parkinson's Disease J Nerv Ment Dis 1962; 135: 196-205.

Tandberg E, Larsen JP, Nessler EG, Riise T, Aarli JA. The epidemiology of Parkinson's disease in the county of Rogaland, Norway. Mov Disord 1995; 10: 541–549.

Tanner CM, Ottman R, Goldman SM, Ellenberg J, Chan P, Mayeux R, et al. Parkinson disease in twins: an etiologic study. JAMA 1999; 281: 341–346.

Taylor KSM, Cook JA, Counsell CE. Heterogeneity in male to female risk for Parkinson's disease. J Neurol Neurosurg Psychiatry 2007; 78: 905–906.

Tessa C, Lucetti C, Diciotti S, Baldacci F, Paoli L, Cecchi P, et al. Decreased and increased cortical activation coexist in de novo Parkinson's disease. Experimental Neurology 2010; 224: 299–306.

Tessitore A, Amboni M, Esposito F, Russo A, Picillo M, Marcuccio L, et al. Resting-state brain connectivity in patients with Parkinson's disease and freezing of gait. Parkinsonism Relat. Disord. 2012; 18: 781–787.

Tessitore A, Esposito F, Vitale C, Santangelo G, Amboni M, Russo A, et al. Default-mode network connectivity in cognitively unimpaired patients with Parkinson disease. Neurology 2012

Tessitore A, Hariri AR, Fera F, Smith WG, Chase TN, Hyde TM, et al. Dopamine modulates the response of the human amygdala: a study in Parkinson's disease. J. Neurosci. 2002; 22: 9099–9103.

Thacker EL, Ascherio A. Familial aggregation of Parkinson's disease: a meta-analysis. Mov Disord 2008; 23: 1174–1183.

Thaler A, Mirelman A, Helmich RC, van Nuenen BFL, Rosenberg-Katz K, Gurevich T, et al. Neural correlates of executive functions in healthy G2019S LRRK2 mutation carriers. Cortex 2013

Tinaz S, Courtney MG, Stern CE. Focal cortical and subcortical atrophy in early Parkinson's disease. Mov Disord 2011; 26: 436–441.

Toga AW, Mazziotta JC. Brain Mapping. Academic Press; 2002.

Tokuda T, Qureshi MM, Ardah MT, Varghese S, Shehab SAS, Kasai T, et al. Detection of elevated levels of α-synuclein oligomers in CSF from patients with Parkinson disease. Neurology 2010; 75: 1766–1772.

Tomlinson CL, Stowe R, Patel S, Rick C, Gray R, Clarke CE. Systematic review of levodopa dose equivalency reporting in Parkinson's disease. Mov Disord 2010; 25: 2649–2653.

Tonelli H, Tonelli D, Poiani GR, Vital MABF, Andreatini R. Reliability and clinical utility of a Portuguese version of the Abnormal Involuntary Movements Scale (AIMS) for tardive dyskinesia in Brazilian patients. Braz. J. Med. Biol. Res. 2003; 36: 511–514.

Tsigilis N, Douda H, Tokmakidis SP. Test-retest reliability of the Eurofit test battery administered to university students. Percept Mot Skills 2002; 95: 1295–1300–1300.

Turkeltaub PE, Eden GF, Jones KM, Zeffiro TA. Meta-analysis of the functional neuroanatomy of single-word reading: method and validation. Neuroimage 2002; 16: 765–780.

Turner RS, Grafton ST, McIntosh AR, DeLong MR, Hoffman JM. The functional anatomy of parkinsonian bradykinesia. Neuroimage 2003; 19: 163–179.

Ubeda-Bañon I, Saiz-Sanchez D, la Rosa-Prieto de C, Argandoña-Palacios L, Garcia-Muñozguren S, Martinez-Marcos A. alpha-Synucleinopathy in the human olfactory system in Parkinson's disease: involvement of calcium-binding protein- and substance P-positive cells. Acta Neuropathol. 2010; 119: 723–735.

Uitti RJ, Baba Y, Whaley NR, Wszolek ZK, Putzke JD. Parkinson disease: handedness predicts asymmetry. Neurology 2005; 64: 1925–1930.

Uitti RJ, Baba Y, Wszolek ZK, Putzke DJ. Defining the Parkinson's disease phenotype: initial symptoms and baseline characteristics in a clinical cohort. Parkinsonism Relat. Disord. 2005; 11: 139–145.

van Dijk KRA, Sabuncu MR, Buckner RL. The influence of head motion on intrinsic functional connectivity MRI. Neuroimage 2012; 59: 431–438.

van Eimeren T, Ballanger B, Pellecchia G, Miyasaki JM, Lang AE, Strafella AP. Dopamine agonists diminish value sensitivity of the orbitofrontal cortex: a trigger for pathological gambling in Parkinson's disease? Neuropsychopharmacology 2009; 34: 2758–2766.

van Eimeren T, Pellecchia G, Cilia R, Ballanger B, Steeves TDL, Houle S, et al. Drug-induced deactivation of inhibitory networks predicts pathological gambling in PD. Neurology 2010; 75: 1711–1716.

van Heertum RL, Tikofsky RS, Ichise M. Functional Cerebral SPECT and PET Imaging. Lippincott Williams & Wilkins; 2013.

van Nuenen BFL, van Eimeren T, van der Vegt JPM, Buhmann C, Klein C, Bloem BR, et al. Mapping preclinical compensation in Parkinson's disease: an imaging genomics approach. Mov Disord 2009; 24 Suppl 2: S703–10.

van Nuenen BFL, Weiss MM, Bloem BR, Reetz K, van Eimeren T, Lohmann K, et al. Heterozygous carriers of a Parkin or PINK1 mutation share a common functional endophenotype. Neurology 2009; 72: 1041–1047.

van Rooden SM, Colas F, Martinez-Martin P, Visser M, Verbaan D, Marinus J, et al. Clinical subtypes of Parkinson's disease. Mov Disord 2011; 26: 51–58.

Vassar SD, Bordelon YM, Hays RD, Diaz N, Rausch R, Mao C, et al. Confirmatory factor analysis of the motor unified Parkinson's disease rating scale. Parkinsons Dis 2012; 719: 167.

Veer IM, Beckmann CF, van Tol M-J, Ferrarini L, Milles J, Veltman DJ, et al. Whole brain resting-state analysis reveals decreased functional connectivity in major depression. Front Syst Neurosci 2010; 4

Vendette M, Gagnon JF, Decary A, Massicotte-Marquez J, Postuma RB, Doyon J, et al. REM sleep behavior disorder predicts cognitive impairment in Parkinson disease without dementia. Neurology 2007; 69: 1843–1849.

Verbaan D, Marinus J, Visser M, van Rooden SM, Stiggelbout AM, Middelkoop HAM, et al. Cognitive impairment in Parkinson's disease. J Neurol Neurosurg Psychiatry 2007; 78: 1182–1187.

Vibha D, Shukla G, Singh S, Goyal V, Srivastava AK, Behari M. Lower prevalence of sleep disturbances in familial versus sporadic Parkinson's disease: a questionnaire based study. J Neurol Sci 2010; 295: 27–30.

Voon V, Gao J, Brezing C, Symmonds M, Ekanayake V, Fernandez H, et al. Dopamine agonists and risk: impulse control disorders in Parkinson's disease. Brain 2011; 134: 1438–1446.

Voon V, Hassan K, Zurowski M, de Souza M, Thomsen T, Fox S, et al. Prevalence of repetitive and reward-seeking behaviors in Parkinson disease. Neurology 2006; 67: 1254–1257.

Voon V, Hassan K, Zurowski M, Duff-Canning S, de Souza M, Fox S, et al. Prospective prevalence of pathologic gambling and medication association in Parkinson disease. Neurology 2006; 66: 1750–1752.

Voon V, Mehta AR, Hallett M. Impulse control disorders in Parkinson's disease: recent advances. Curr Opin Neurol 2011; 24: 324–330.

Voon V, Thomsen T, Miyasaki JM, de Souza M, Shafro A, Fox SH, et al. Factors associated with dopaminergic drug-related pathological gambling in Parkinson disease. Arch Neurol-Chicago 2007; 64: 212–216.

Wager TD, Jonides J, Reading S. Neuroimaging studies of shifting attention: a meta-analysis. Neuroimage 2004; 22: 1679–1693.

Wager TD, Lindquist M, Kaplan L. Meta-analysis of functional neuroimaging data: current and future directions. Soc Cogn Affect Neurosci 2007; 2: 150–158.

Wakabayashi K, Tanji K, Mori F, Takahashi H. The Lewy body in Parkinson's disease: molecules implicated in the formation and degradation of alpha-synuclein aggregates. Neuropathology 2007; 27: 494–506.

Weder B, Azari NP, Knorr U, Seitz RJ, Keel A, Nienhusmeier M, et al. Disturbed functional brain interactions underlying deficient tactile object discrimination in Parkinson's disease. Hum Brain Mapp 2000; 11: 131–145.

Weintraub D, Hoops S, Shea JA, Lyons KE, Pahwa R, Driver-Dunckley ED, et al. Validation of the questionnaire for impulsive-compulsive disorders in Parkinson's disease. Mov Disord 2009; 24: 1461–1467.

Weintraub D, Siderowf AD, Potenza MN, Goveas J, Morales KH, Duda JE, et al. Association of dopamine agonist use with impulse control disorders in Parkinson disease. Arch Neurol-Chicago 2006; 63: 969–973.

Werheid K, Zysset S, Müller A, Reuter M, Cramon von DY. Rule learning in a serial reaction time task: an fMRI study on patients with early Parkinson's disease. Brain Res Cogn Brain Res 2003; 16: 273–284.

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

Whitfield-Gabrieli S, Thermenos HW, Milanovic S, Tsuang MT, Faraone SV, McCarley RW, et al. Hyperactivity and hyperconnectivity of the default network in schizophrenia and in first-degree relatives of persons with schizophrenia. Proc Natl Acad Sci U S A 2009; 106: 1279–1284.

Wickremaratchi MM, Ben-Shlomo Y, Morris HR. The effect of onset age on the clinical features of Parkinson's disease. Eur J Neurol 2009; 16: 450–456.

Wickremaratchi MM, Knipe MDW, Sastry BSD, Morgan E, Jones A, Salmon R, et al. The motor phenotype of Parkinson's disease in relation to age at onset. Mov Disord 2011; 26: 457–463.

Wickremaratchi MM, Perera D, O'Loghlen C, Sastry D, Morgan E, Jones A, et al. Prevalence and age of onset of Parkinson's disease in Cardiff: a community based cross sectional study and meta-analysis. J Neurol Neurosurg Psychiatry 2009; 80: 805–807.

Widjaja E, Zamyadi M, Raybaud C, Snead OC, Smith ML. Abnormal functional network connectivity among resting-state networks in children with frontal lobe epilepsy. American Journal of Neuroradiology 2013; 34: 2386–2392.

Williams-Gray CH, Evans JR, Goris A, Foltynie T, Ban M, Robbins TW, et al. The distinct cognitive syndromes of Parkinson's disease: 5 year follow-up of the CamPaIGN cohort. Brain 2009; 132: 2958–2969.

Williams-Gray CH, Foltynie T, Brayne CEG, Robbins TW, Barker RA. Evolution of cognitive dysfunction in an incident Parkinson's disease cohort. Brain 2007; 130: 1787–1798.

Winter Y, Campenhausen von S, Arend M, Longo K, Boetzel K, Eggert K, et al. Health-related quality of life and its determinants in Parkinson's disease: results of an Italian cohort study. Parkinsonism Relat. Disord. 2011; 17: 265–269.

Winter Y, Campenhausen von S, Brozova H, Skoupa J, Reese JP, Bötzel K, et al. Costs of Parkinson's disease in eastern Europe: a Czech cohort study. Parkinsonism Relat. Disord. 2010; 16: 51–56.

Winter Y, Campenhausen von S, Gasser J, Seppi K, Reese JP, Pfeiffer K-P, et al. Social and clinical determinants of quality of life in Parkinson's disease in Austria: a cohort study. J Neurol 2010; 257: 638–645.

Winter Y, Campenhausen von S, Popov G, Reese JP, Balzer-Geldsetzer M, Kukshina A, et al. Social and clinical determinants of quality of life in Parkinson's disease in a Russian cohort study. Parkinsonism Relat. Disord. 2010; 16: 243–248.

Wirdefeldt K, Adami H-O, Cole P, Trichopoulos D, Mandel J. Epidemiology and etiology of Parkinson's disease: a review of the evidence. Eur. J. Epidemiol. 2011; 26 Suppl 1: S1–58.

Woolrich MW, Jbabdi S, Patenaude B, Chappell M, Makni S, Behrens T, et al. Bayesian analysis of neuroimaging data in FSL. Neuroimage 2009; 45: S173–86.

Wooten GF. Are men at greater risk for Parkinson's disease than women? J Neurol Neurosurg Psychiatry 2004; 75: 637–639.

Wu T, Chan P, Hallett M. Effective connectivity of neural networks in automatic movements in Parkinson's disease. Neuroimage 2010; 49: 2581–2587.

Wu T, Hallett M. A functional MRI study of automatic movements in patients with Parkinson's disease. Brain 2005; 128: 2250–2259.

Wu T, Hallett M. Neural correlates of dual task performance in patients with Parkinson's disease. J Neurol Neurosurg Psychiatry 2008; 79: 760–766.

Wu T, Long X, Wang L, Hallett M, Zang Y, Li K, et al. Functional connectivity of cortical motor areas in the resting state in Parkinson's disease. Hum Brain Mapp 2011; 32: 1443–1457.

Wu T, Long X, Zang Y, Wang L, Hallett M, Li K, et al. Regional homogeneity changes in patients with Parkinson's disease. Hum Brain Mapp 2009; 30: 1502–1510.

Wu T, Wang J, Wang C, Hallett M, Zang Y, Wu X, et al. Basal ganglia circuits changes in Parkinson's disease patients. Neuroscience Letters 2012; 524, 55–59.

Wu T, Wang L, Hallett M, Chen Y, Li K, Chan P. Effective connectivity of brain networks during self-initiated movement in Parkinson's disease. Neuroimage 2011; 55: 204–215.

Wu T, Wang L, Hallett M, Li K, Chan P. Neural correlates of bimanual anti-phase and in-phase movements in Parkinson's disease. Brain 2010; 133: 2394–2409.

Wu X, Li R, Fleisher AS, Reiman EM, Guan X, Zhang Y, et al. Altered default mode network connectivity in Alzheimer's disease--a resting functional MRI and Bayesian network study. Hum Brain Mapp 2011; 32: 1868–1881.

Wüllner U, Schmitz-Hübsch T, Antony G, Fimmers R, Spottke A, Oertel WH, et al. Autonomic dysfunction in 3414 Parkinson‘s disease patients enrolled in the German Network on Parkinson’s disease (KNP e.V.): the effect of ageing. Eur J Neurol 2007; 14: 1405–1408.

Xiong J, Parsons LM, Gao JH, Fox PT. Interregional connectivity to primary motor cortex revealed using MRI resting state images. Hum Brain Mapp 1999; 8: 151–156.

Yang H, Zhou XJ, Zhang M-M, Zheng X-N, Zhao Y-L, Wang J. Changes in spontaneous brain activity in early Parkinson's disease. Neurosci Lett 2013; 549: 24–28.

Yarnall A, Rochester L, Burn DJ. The interplay of cholinergic function, attention, and falls in Parkinson's disease. Mov Disord 2011; 26: 2496–2503.

Yesavage JA, Brink TL, Rose TL, Lum O, Huang V, Adey M, et al. Development and validation of a geriatric depression screening scale: a preliminary report. J Psychiatr Res 1982; 17: 37–49.

Yu H, Sternad D, Corcos DM, Vaillancourt DE. Role of hyperactive cerebellum and motor cortex in Parkinson's disease. Neuroimage 2007; 35: 222–233.

Zamboni G, Wilcock GK, Douaud G, Drazich E. Resting functional connectivity reveals residual functional activity in Alzheimer's disease. Biological Psychiatry 74, 375–383 (2013).

Zang Y, Jiang T, Lu Y, He Y, Tian L. Regional homogeneity approach to fMRI data analysis. Neuroimage 2004; 22: 394–400.

Zhang J, Wei L, Hu X, Zhang Y, Zhou D, Li C, et al. Specific frequency band of amplitude low-frequency fluctuation predicts Parkinson's disease. Behav Brain Res 2013

Zhu S, Fang Z, Hu S, Wang Z, Rao H. Resting state brain function analysis using concurrent BOLD in ASL perfusion fMRI. Plos One 2013; 8: e65884.

Zigmond AS, Snaith RP. The hospital anxiety and depression scale. Acta Psychiatr Scand 1983; 67: 361–370.

Zung WW. A self-rating depression scale. Arch Gen Psychiatry 1965; 12: 63–70.

Zung WW. A Rating Instrument For Anxiety Disorders. Psychosomatics 1971; 12: 371–379.

Zuo XN, Kelly C, Adelstein JS, Klein DF, Castellanos FX, Milham MP. Reliable intrinsic connectivity networks: Test-retest evaluation using ICA and dual regression approach. Neuroimage 2010; 49: 2163–2177.

EuroQol--a new facility for the measurement of health-related quality of life. The EuroQol Group. Health Policy 1990; 16: 199–208.

PROBAND Website [Internet]. probandorguk[cited 2012 Jul 18] Available from: http://www.proband.org.uk

unpublished discussions on FMRIB methods forum. https://www.jiscmail.ac.uk/cgi-bin/webadmin?A0=FSL;

commondataelements.ninds.nih.gov [Internet]. [cited 2013 Jan 20] Available from: http://www.commondataelements.ninds.nih.gov

Appendix A. Supplementary data for the study of the Basic clinical characteristics of early Parkinson's Disease in the Oxford Parkinson's Disease Centre cohort

Supplemental Table 1. Questionnaires and Derived Variables.

Patient-Completed Questionnaires	
Demographics	Basic demographic information
Big Five Inventory (John and Srivastava, 1999)	Personality profiling
Epworth Sleepiness Scale (Johns, 1991)	Daytime sleepiness questionnaire
Sleepiness, (%)	dichotomised to 'unusual level of sleepiness - ESS >=11' versus 'normal results - EPS <=10'
RBD Questionnaire (Stiasny-Kolster *et al.*, 2007)	Patient reported features of RBD
RBD, (%)	dichotomised to 'positive result - RBD >=5' versus 'normal - RBD<=4'
Health-Related Quality of Life EQ5D and EQ-VAS ('EuroQol--a new facility for the measurement of health-related quality of life. The EuroQol Group.', 1990)	General assessment of quality of life
Pain, EQ5D, (%)	pain score from EQ-5D health questionnaire dichotomised to 'no pain or discomfort' versus 'moderate or extreme pain or discomfort'
Constipation Questionnaire	Assessment of frequency of bowel movements
Constipation, (%)	average daily bowel movement frequency in the past week dichotomised to 'constipation - <1' versus 'normal - >=1'
Use of laxatives, (%)	use of laxatives in the past week dichotomised to 'any' versus 'none'
UPDRS I, Non-motor aspects of daily living (Goetz *et al.*, 2007)	Assessment of basic non-motor symptoms in PD in the last week. Each item was dichotomised as 'present – score 1-4' versus 'absent – score 0'.
UPDRS II, Motor aspects of daily living (Goetz *et al.*, 2007)	Assessment of basic motor symptoms in PD in the last week. Each item was dichotomised as 'present – score 1-4' versus 'absent – score 0'.
Social background	Information about education, wealth and employment.
Accommodation at diagnosis, (own, %)	Dichotomised to 'own' versus 'renting'
Bedrooms at diagnosis, (≤3, %)	Dichotomised to '≤3' versus '>3'
Vehicles at diagnosis, (≤1, %)	Dichotomised to '≤1' versus >1'

Supplemental Table 1. Questionnaires and Derived Variables.

Employment at diagnosis, (employed, %)	Dichotomised to 'employed in any capacity' versus not employed
Last job, (managing people, %)	Dichotomised to 'job involving managing people' versus 'job not involving managing people'
Leeds Depression and Anxiety General Scales (Snaith *et al.*, 1976)	Assessment of level of depression and anxiety in undiagnosed cases.
Depression, Leeds SAD General, (%)	dichotomised to 'case - >=7' versus 'non-case - <7' (Snaith *et al.*, 1976)
Anxiety, Leeds SAA General, (%)	dichotomised to 'case - >=7' versus 'non-case - <7' (Snaith *et al.*, 1976)
Beck Depression Inventory (BDI) (A. Beck *et al.*, 1961)	Extensive assessment of symptoms of depression.
Questionnaire for Impulsive-Compulsive Disorders in Parkinson's Disease (QUIP Anytime Short) (Weintraub *et al.*, 2009)	Assessment of impulsive and compulsive disorders in PD occurring anytime in life.
Any ICD, QUIP, (%)	dichotomised score for any main impulsive disorder (gambling, sex, buying or eating)
Any other OCD, QUIP, (%)	dichotomised score for any other compulsive behaviour (organised activities, ordering, purposeless walking or driving)
Overmedicating, QUIP, (%)	dichotomised score for any excessive parkinsonian medication use
Nurse-Administered Questionnaires	
General Exam	Weight, height, lying and standing blood pressure.
BMI	Body Mass Index calculated as: weight in kg divided by square of height in meters.
Orthostatic hypotension, (%)	Orthostatic hypotension defined as difference between lying and standing blood pressure greater than 20 (SBP) or 10 (DBP).
MOCA (Nasreddine *et al.*, 2005;	Assessment of general cognition. Additional point was added for patients with <=12 years of education.
Cognitive impairment, (normal:MCI:dementia, %)	Defined based on MOCA scores: normal cognition 26-30, MCI 21-25, dementia <=20 (Dalrymple-Alford *et al.*, 2010; Gill *et al.*, 2008; Nazem *et al.*, 2009;
MMSE (Folstein *et al.*, 1975;	Assessment of general cognition.
Phonemic and Semantic Fluency	Assessment of fluency scored according to population norms.
Peg board tests (Desrosiers *et al.*, 1995)	Test of manual dexterity. Test results indicate number of pegs/items inserted into peg board within 30s (1min for 'Arm coordination')

Supplemental Table 1. Questionnaires and Derived Variables.

Arm asymmetry (absolute value of right score - left score), Peg board, (mean±SD)	absolute difference between right and left hand scores reflecting asymmetry of disease
Get-up-and-go (Shumway-Cook *et al.*, 2000;	Test of walking speed. The result is an average in seconds of three trials of timed 3m walk.
Flamingo test (Tsigilis *et al.*, 2002)	Test of balancing on one leg for 30s. The result is dichotomised to 'subjects who completed 30s trial' versus 'subjects who failed before 30s'.
Doctor Administered Questionnaires	
Past Medical History	Review of medical history covering most common conditions.
Vascular risk factors, (%)	Per cent of patients with any of the following: high blood pressure, high cholesterol level, diabetes mellitus
Cerebro-vascular disease, (%)	Per cent of patients with any of the following: stroke or TIA
Cardio-vascular disease, (%)	Per cent of patients with any of the following: angina, heart attack, heart failure
Cancers, (%)	Per cent of patients with any of the following: lung, bowel, breast, prostate, melanoma, or any other cancer reported by patient
Respiratory disease, (%)	Per cent of patients with any of the following: asthma, chronic bronchitis, emphysema
Mini Environmental Risk Questionnaire for PD Baseline (MERQ-PD B) (*commondataelements.ninds.nih.gov*)	Assessment for exposure to commonly accepted risk factors for PD.
Smoking before PD, (%)	Dichotomised to 'smoking at least one cigarette per day for at least 6 months' versus 'non-smoker by that definition'.
Alcohol consumption before PD, (%)	Dichotomised to 'drinking any alcohol' versus 'non-drinker'.
Medication (different types) before PD	Dichotomised to 'any use' versus 'no use'.
Modified Schwab & England Activities of Daily Living	Assessment of activities of daily living.
PD Features (*commondataelements.ninds.nih.gov*)	Presence of typical features of PD at diagnosis as reported by the patient and in the notes.
Family History (*commondataelements.ninds.nih.gov*)	Extensive family history including occurrence of PD and other neurological disorders in the family.
PD Medication	List of current and previous medication taken for PD.
Levodopa equivalent daily dose (LEDD) (Tomlinson *et al.*, 2010)	Calculated from all current dopaminergic medication.

Supplemental Table 1. Questionnaires and Derived Variables.

Clinical Global Impression of Change (CGI)	Eight-level scale assessing change in symptoms after starting PD medication.
Other Medication (*commondataelements.ninds.nih.gov*)	List of medication unrelated to PD.
Sniffin Sticks (Hummel *et al.*, 2007;	Assessment of hyposmia. Sniffin Sticks was score dichotomised at 10th percentile according to normal values for age and sex.
UPDRS I Non-motor features of daily living (Goetz *et al.*, 2007;	Clinician-assessed non-motor symptoms of PD.
Fatigue in PD, (%)	Dichotomised score from item 1.13 of MDS-UPDRS I: 'any fatigue' versus 'no fatigue'
Apathy in PD, (%)	Dichotomised score from item 1.5 of MDS-UPDRS I: 'any apathy' versus 'no apathy'
Hallucinations in PD, (%)	Dichotomised score from item 1.2 of MDS-UPDRS I: 'any hallucinations' versus 'no hallucinations'
UPDRS III Motor Examination (Goetz *et al.*, 2007)	Standardised assessment of PD signs.
Rigidity score (score/number of items)	Sum of scores from item 3.3 MDS-UPDRS III divided by 5
Bradykinesia scores, (score/number of items)	sum of scores from items 3.4, 3.5, 3.6, 3.7, 3.8, divided by 10
Postural scores, (score/number of items)	Sum of scores from items 3.9, 3.10, 3.11, 3.12, 3.13, divided by 5
Tremor scores, (score/number of items)	Sum of scores from items 3.15, 3.16, 3.17, divided by 9
Motor subtype, (PIGD:Mix:Tremor, %)	Sum of scores from all MDS-UPDRS tremor items (2.10, 3.15, 3.16, 3.17) divided by the sum of the postural and gait items (2.12, 2.13, 3.10, 3.11, 3.12) gives a Tremor/PIGD index; index <=1 defines PIGD subtype, >=1.5 - tremor dominant subtype, between 1-1.5 - mixed subtype (Skeie *et al.*, 2010)
Face and neck score, (score/number of items)	Sum of scores from MDS-UPDRS III items for face and neck symptoms (3.1, 3.2, 3.3 Neck, 3.17 Lip/Jaw) divided by 4
Arms score, (score/number of items)	Sum of scores from MDS-UPDRS III items for upper extremities (3.3 RUE, 3.3 LUE, 3.4, 3.5, 3.6, 3.15, 3.16, 3.17 RUE, 3.17 LUE) divided by 14
Legs score, (score/number of items)	Sum of scores from MDS-UPDRS III items for lower extremities (3.3 RLE, 3.3 LLE, 3.7, 3.8, 3.17 RLE, 3.17 LLE) divided by 8

Supplemental Table 1. Questionnaires and Derived Variables.

Symptom laterality, (right : left : symmetrical, %)	Right-dominant symptoms: difference between all right-sided scores and all left-sided scores >=4; left-dominant symptoms: difference between all left-sided scores and all right-sided scores >=4; symmetrical: intermediate scores (Uitti, Baba, Whaley, *et al.*, 2005)
Annualised UPDRS III (UPDRS III/disease duration)	Total UPDRS III score divided by disease duration in years since symptom onset, reflecting symptomatic progression since disease onset
UPDRS IV Motor Complications (Goetz *et al.*, 2007)	Assessment of dyskinesia, motor fluctuations and dystonia.
Presence of Dyskinesia, (%)	Dichotomised score from item 4.1 of MDS-UPDRS IV: 'No dyskinesia' versus 'Any dyskinesia'
Presence of Motor Fluctuations, (%)	Dichotomised score from item 4.3 of MDS-UPDRS IV: 'No OFF time' versus 'Any OFF time'
Specific Clinical Features (*commondataelements.ninds.nih.gov*)	Assessment of atypical parkinsonian features not fitting with idiopathic PD.
Sexual dysfunction, (%)	Significant change in sexual functioning in the past year
Erectile dysfunction, (%)	Ability to maintain erection without treatment dichotomised to 'very poor or poor' versus 'fair, good or very good'
Likelihood of Diagnosis	Clinician-assessed likelihood of diagnosis of idiopathic PD from 0%-100%.

Appendix B. Supplementary data for the Meta-analysis of functional imaging studies in Parkinson's Disease

Supplemental Table I. Studies selected for meta-analysis with breakdown of number of foci according to contrasts investigated. * Medication administered in ON state scanning: 1- levodopa, 2- apomorphine, 3- MAO-B inhibitors (selegiline, rasagiline, 4- dopamine agonists (pramipexole, ropinirole, cabergoline, bromocriptine), 5- amantadine, 6- entecapone, 7- not reported.

Publication	Modality	Medication in ON state*	Number of subjects		Number of foci in each experiment according to subject group								
					PDOFF<>NC			PDON<>NC			PDON<>PDOFF		
			PD	NC	cognitive	motor	other	cognitive	motor	other	cognitive	motor	other
Samuel *et al.*, 1997	PET		6	6		17							
Owen *et al.*, 1998	PET		6	6	17								
Boecker *et al.*, 1999	PET		8	8			13						
Catalan, 1999	PET		13	13		7							
Hanakawa T., Fukuyama H. et al., 1999	SPECT		10	10		3							
Hanakawa T., Katsumi Y. et al, 1999	SPECT		10	10		14							
Sabatini *et al.*, 2000	fMRI		6	6		15							
Weder *et al.*, 2000	PET		12	12		11							
Cunnington *et al.*, 2001	PET	1,2	6	3		3						3	
Dagher *et al.*, 2001	PET		6	6	20								
Haslinger *et al.*, 2001	fMRI	1	8	8		7			8			10	
Samuel *et al.*, 2001	PET		6	6		12							
Cools *et al.*, 2002	PET	1, 3, 4, 5, 6	11	6							6		
Feigin *et al.*, 2002	PET	1	7	0								4	
Mattay *et al.*, 2002	fMRI	1, 4	10	0							13	7	
Rowe *et al.*, 2002	fMRI		12	12	5	2							
Tessitore *et al.*, 2002	fMRI	1, 4	10	10	5			4			3		
Buhmann *et al.*, 2003	fMRI	1	8	10		2						2	
Grossman *et al.*, 2003	fMRI	7	7	9				41					
Turner *et al.*, 2003	PET		12	12		9							
Werheid *et al.*, 2003	fMRI	1, 4, 5	7	7				2					

Goerendt *et al.*, 200	PET		9	9	8								
Monchi *et al.*, 2004	fMRI		8	9	18								
Moody *et al.*, 2004	fMRI	1, 3, 4, 5	6	7				5					
Brefel-Courbon *et al.*, 2005	PET	1, 4	9	9			3			1			2
Dirnberger *et al.*, 2005	PET		6	6	2								
T. Wu and Hallett, 2005	fMRI		12	12		12							
Eckert *et al.*, 2006	fMRI	1	9	9		18			9			4	
Cerasa *et al.*, 2006	fMRI		10	11		11							
Holden *et al.*, 2006	fMRI		6	7		4							
Monchi *et al.*, 2006	fMRI		7	7	27								
Fera *et al.*, 2007	fMRI	1	12	10	11			3					
Mallol *et al.*, 2007	fMRI		13	11		13							
Sawamoto *et al.*, 2007	PET		7	9	6								
Schott *et al.*, 2007	fMRI		11	19	7								
Ballanger *et al.*, 2008	PET		8	8		5							
Ramírez-Ruiz *et al.*, 2008	fMRI	1, 4	10	10				1					
Westermann *et al.*, 2008	fMRI	7	12	16						8			
Delaveau *et al.*, 2009	fMRI	1	14	13							35		
Farid *et al.*, 2009	fMRI	1	9	9	5			2			4		
Jubault *et al.*, 2009	fMRI	1, 3, 4, 6	11	0							3		
Kraft *et al.*, 2009	fMRI	1	12	12		25			12			8	
Lotze *et al.*, 2009	fMRI		9	9	2								
van Eimeren *et al.*, 2009	fMRI	1, 4	8	0							3		
Cardoso *et al.*, 2010	fMRI	1, 3, 4, 5	16	18				3					
L. E. Hughes *et al.*, 2010	fMRI	1, 3, 4, 5, 6	16	14					10			5	
Jahanshahi *et al.*, 2010	PET	1, 2, 3, 4, 6	8	8		14						18	
Payoux *et al.*, 2010	PET	1	8	10		3						1	
Schonberg *et al.*, 2010	fMRI	1, 3, 4, 5	7	17				2					
Spraker et al., 2010	fMRI		14	14		23							
Takeda et al., 2010	fMRI		9	7			7						
Tessa et al., 2010	fMRI		15	11		12							
van Eimeren et al., 2010	PET	2	7	0							12		
Wu et al., 2010	fMRI		15	15		35							
Baglio et al., 2011	fMRI		15	11	7	6							
Harrington et al., 2011	fMRI	1, 4	21	19	24						6		

González-García et al., 2011	fMRI	1, 4, 5	17	10					13				
Katschnig et al., 2011	fMRI		17	20		3							
Pinto et al., 2011	fMRI		9	15		15	15						
Voon et al., 2011	fMRI	1, 4	14	0							1		

List of publications excluded after the full text search process.

Aasly JO. et al. Neurology 2012 Jan 3;78 (1):55-61
Abe N. et al. Brain 2009 May;132 (Pt 5):1386-95
Abe Y. et al. J Neurol Neurosurg Psychiatry 2003 Apr;74 (4):419-22
Agniel A. et al. J Neurol Neurosurg Psychiatry 1991 Sep;54 (9):783-6
Antonelli F. et al. Expert Rev Neurother 2010 Dec;10 (12):1827-38
Antonini A. et al. Expert Rev Neurother 2008 Dec;8 (12):1853-64
Antonini A. et al. Mov Disord 1998 Mar;13 (2):268-74
Antonini A. et al. Neurol Sci 2001 Feb;22 (1):45-6
Antonini A. et al. Neurology 1998 Sep;51 (3):803-10
Appel-Cresswell S. et al. Curr Opin Neurol 2010 Aug;23 (4):407-12
Arahata Y. et al. J Neurol Sci 1999 Mar 1;163 (2):119-26
Argyelan M. et al. J Neurosci 2008 Oct 15;28 (42):10687-95
Bartels AL. et al. Mov Disord 2008;23 Suppl 2:S461-7
Bartrés-Faz D. et al. 2007 Aug;6 (6):588-92 Epub 2006 Nov 27
Baumann CR. et al. Parkinsonism Relat Disord 2012 Jan;18 Suppl 1:S90-2
Beauchamp MH. et al. Brain Cogn 2008 Nov;68 (2):134-43
Berding G. et al. Mov Disord 2001 Nov;16 (6):1014-22
Berti V. et al. Eur J Nucl Med Mol Imaging 2010 Mar;37 (3):537-44
Bhidayasiri R. et al. Parkinsonism Relat Disord 2012 Feb 7
Bissessur S. et al. J Neural Transm Suppl 1997;50:25-30
Black KJ. et al. Neuropsychopharmacology 2005 Mar;30 (3):590-601
Bohlhalter S. et al. Behav Brain Funct 2009 Dec 15;5:49
Bohlhalter S. et al. Exp Neurol 2011 Jan;227 (1):24-5
Bohnen NI. et al. J Nucl Med 2011 Jun;52 (6):848-55
Bohnen NI. et al. Neurology 1999 Feb;52 (3):541-6
Borghammer P. et al. Brain Struct Funct 2010 May;214 (4):303-17
Borghammer P. et al. Neuroimage 2009 Apr 1;45 (2):249-57
Borghammer P. et al. Neuroimage 2009 Oct 1;47 (4):1514-21
Bosman T. et al. Eur J Nucl Med Mol Imaging 2003 Jan;30 (1):16-24
Brooks DJ. et al. Parkinsonism Relat Disord 2007;13 Suppl 3:S268-75
Brooks DJ. et al. Semin Neurol 2008 Sep;28 (4):435-45
Broussolle E. et al. Mov Disord 1993 Oct;8 (4):459-62
Brusa L. et al. Neurol Sci 2002 Sep;23 Suppl 2:S61-2
Buhmann C. et al. Brain 2005 Oct;128 (Pt 10):2281-90
Cameron IG. et al. Neuropsychologia 2010 Jun;48 (7):1948-57
Carbon M. et al. Curr Opin Neurol 2003 Aug;16 (4):475-80
Carbon M. et al. Hum Brain Mapp 2003 Jul;19 (3):197-211
Carbon M. et al. J Neurol Sci 2006 Oct 25;248 (1-2):72-7
Carbon M. et al. Neuroimage 2004 Apr;21 (4):1497-507
Carbon M. et al. Neuroimage 2007 Jan 1;34 (1):361-70
Cardoso EF. et al. Int J Neuropsychopharmacol 2008 Mar;11 (2):173-83
Cardoso EF. et al. Neuroimage 2009 Aug 15;47 (2):467-72
Ceballos-Baumann AO. et al. Adv Neurol 1997;74:127-39
Ceballos-Baumann AO. et al. J Neurol 2003 Feb;250 Suppl 1:I15-23
Chae Y. et al. Mov Disord 2009 Sep 15;24 (12):1794-802
Cheesman AL. et al. J Neurol Neurosurg Psychiatry 2005 Sep;76 (9):1204-10
Cilia R. et al. Arch Neurol 2008 Dec;65 (12):1604-11

Cilia R. et al. Mov Disord 2011 Feb 1;26 (2):225-33
Cools R. et al. Neuropsychopharmacology 2007 Jan;32 (1):180-9
Costa DC. et al. J Cereb Blood Flow Metab 1988 Dec;8 (6):S109-15
Cuberas-Borros G. et al. Clin Nucl Med 2011 Nov;36 (11):991-6
Deblieck C. et al. Rev Neurol Dis 2008 Summer;5 (3):125-33
Defebvre L. et al. Acta Neurol Scand 1995 Sep;92 (3):235-41
Defebvre LJ. et al. J Nucl Med 1999 Jun;40 (6):956-62
del Olmo MF. et al. Parkinsonism Relat Disord 2006 Apr;12 (3):155-64
Delaveau P. et al. Eur Neuropsychopharmacol 2010 Nov;20 (11):784-92
Delaveau P. et al.J Clin Psychopharmacol. 2007 Dec;27 (6):692-7.
Dellapina E. et al. Mov Disord 2011 Jan;26 (1):153-7
Démonet JF. et al. J Cereb Blood Flow Metab 1994 May;14 (3):431-8
Derejko M. et al. Nucl Med Commun 2006 Dec;27 (12):945-51
Derejko M. et al. Nucl Med Rev Cent East Eur 2001;4 (2):123-7
Desmurget M. et al. Eur J Neurosci 2004 May;19 (10):2871-80
Dethy S. et al. Mov Disord 1998 Mar;13 (2):275-80
Duffau H. et al. Exp Brain Res 1996;107 (3):453-62
Eberling JL. et al. Neurobiol Aging 1994 May-Jun;15 (3):329-35
Eckert T. et al. Eur J Nucl Med Mol Imaging 2007 Apr;34 (4):496-501
Eckert T. et al. Lancet Neurol 2007 Oct;6 (10):926-32
Eckert T. et al. Mov Disord 2007 Jan 15;22 (2):167-73
Eggers C. et al. J Neurol Sci 2009 Jan 15;276 (1-2):27-30
Eidelberg D. et al. J Cereb Blood Flow Metab 1994 Sep;14 (5):783-801
Eidelberg D. et al. Mov Disord 1990;5 (3):203-13
Eidelberg D. et al. Neurol Clin 1992 May;10 (2):421-33
Eidelberg D. et al. Neurology 1995 Nov;45 (11):1995-2004
Eidelberg D. et al. Trends Neurosci 2009 Oct;32 (10):548-57
Elsinger CL. et al. J Int Neuropsychol Soc 2003 Nov;9 (7):1088-98
Emir UE. et al. PLoS One 2012;7 (1):e30918
Fabre N. et al. Mov Disord 1998 Jul;13 (4):677-83
Feigin A. et al. Neurology 2001 Dec 11;57 (11):2083-8
Feigin A. et al. Neurology 2003 Jun 10;60 (11):1744-9
Feng T. et al. Neurol Res 2008 May;30 (4):377-82
Firbank MJ. et al. Neuroimage 2003 Oct;20 (2):1309-19
Fitzpatrick T. et al. Clin EEG Neurosci 2010 Jul;41 (3):119-26
Foki T. et al. Exp Neurol 2010 Oct;225 (2):416-22
Frosini D. et al. Mov Disord 2010 Oct 30;25 (14):2449-53
Fukuda M. et al. Brain 2001 Aug;124 (Pt 8):1601-9
Fukuda M. et al. Parkinsonism Relat Disord 2001 Oct;8 (2):91-4
Gaudiello F. et al. Radiol Med 2006 Mar;111 (2):284-90
Gemmell HG. et al. Psychiatry Res 1989 Sep;29 (3):327-9
Goerendt IK. et al. J Neural Transm 2006 Jan;113 (1):75-86
Grossman M. et al. Cereb Cortex 1992 Nov-Dec;2 (6):513-25
Haslinger B. et al. Neuroimage 2005 Nov 15;28 (3):598-606
Hayashi R. et al. 2009 Dec 15;287 (1-2):264-6 Epub 2009 Sep 13
Helmich RC. et al. Hum Brain Mapp 2011 Jun 14
Helmich RC. et al. J Neurosci 2009 May 13;29 (19):6105-13
Helmich RC. et al. Neuropsychologia 2007 Jun 11;45 (10):2201-15
Herzog J. et al. Brain 2008 Jan;131 (Pt 1):132-45

Hirano S. et al. Mov Disord 2009;24 Suppl 2:S725-31
Hirato M. et al. 1993;60:511-4 PubMed PMID: 8420180
Hirato M. et al. Acta Neurochir Suppl 1995;64:5-8
Holroyd S. et al. J Neuropsychiatry Clin Neurosci 2006 Summer;18 (3):402-4
Hosey LA. et al. Clin Neuropharmacol 2005 Jan-Feb;28 (1):18-27
Hsu JL. et al. Eur J Nucl Med Mol Imaging 2007 Sep;34 (9):1458-66
Hu MT. et al. Brain 2000 Feb;123 (Pt 2):340-52
Huang C. et al. Neuroimage 2007 Jan 15;34 (2):714-23
Huang C. et al. Neurology 2008 Apr 15;70 (16 Pt 2):1470-7
Huang Y. et al. Int J Neurosci 2009;119 (8):1190-7
Huang Y. et al. Int J Neurosci 2010 Feb;120 (2):150-4
Hummel T. et al. J Neurol Sci 2010 Feb 15;289 (1-2):119-22
Ibarretxe-Bilbao N. et al.Neuroimage. 2011 Jul 15;57 (2):589-97
Ikawa M. et al. Nucl Med Biol 2011 Oct;38 (7):945-51
Imamura K. et al. Acta Neurol Scand 2011 Jul;124 (1):28-39
Imamura K. et al. Brain Nerve 2009 Jun;61 (6):683-90
Imamura K. et al. Neurocase 2008;14 (3):271-5
Imon Y. et al. J Nucl Med 1999 Oct;40 (10):1583-9
Isaias IU. et al. Front Hum Neurosci 2011;5:179
Isaias IU. et al. J Neurosci 2011 Oct 12;31 (41):14458-62
Jagust WJ. et al. Brain 1992 Apr;115 (Pt 2):521-37
Jahanshahi M. et al. Brain 1995 Aug;118 (Pt 4):913-33
Jenkins IH. et al. Ann Neurol 1992 Dec;32 (6):749-57
Jokinen P. et al. Parkinsonism Relat Disord 2010 Dec;16 (10):666-70
Juh R. et al. Eur J Radiol 2004 Sep;51 (3):223-33
Juh R. et al. Neurosci Res 2005 Jul;52 (3):211-9
Kaasinen V. et al. J Neurol 2006 Mar;253 (3):340-8
Kajimoto Y. et al. Int Rev Neurobiol 2010;90:48-62
Kalmar Z. et al. Eur Neurol 2011;66 (4):220-6
Kamagata K. et al. J Magn Reson Imaging 2011 Apr;33 (4):803-7
Karachi C. et al. J Clin Invest 2010 Aug 2;120 (8):2745-54
Kataoka H. et al. Mov Disord 2009 Feb 15;24 (3):471-3
Kato T. et al. Radiat Med 1992 May-Jun;10 (3):87-93
Kawabata K. et al. J Geriatr Psychiatry Neurol 1991 Oct-Dec;4 (4):194-203
Kawai Y. et al. Neurology 2008 Apr 15;70 (16 Pt 2):1390-6
Keitz M. et al. Brain Res 2008 Oct 21;1236:225-33
Kikuchi A. et al. J Neurol Sci 2001 Dec 15;193 (1):29-36
Kimura N. et al. Mov Disord 2011 Aug 9
Klein JC. et al. Neurology 2012 Feb 29
Kobari M. et al. J Neurol Sci 1995 Feb;128 (2):212-8
Kramberger MG. et al. Psychiatr Danub 2010 Sep;22 (3):446-9
Künig G. et al. Neuroreport 2000 Nov 27;11 (17):3681-7
Kwon KY. et al. Eur J Neurol 2008 Oct;15 (10):1043-9
Kwon KY. et al. Mov Disord 2007 Dec;22 (16):2352-8
Labudda K. et al. Behav Neurol 2010;23 (3):131-43
Lee PH. et al. J Nucl Med 2008 Dec;49 (12):2006-11
Lee PW. et al. Conf Proc IEEE Eng Med Biol Soc 2007;2007:5235-8
Leenders KL. et al. Brain 1985 Mar;108 (Pt 1):171-91
Leh SE. et al. Neuropsychopharmacology 2010 Jan;35 (1):70-85

Lemke MR. et al. Eur J Neurol 2008 Apr;15 Suppl 1:21-5
Lewis MM. et al. Neuroscience 2007 Jun 15;147 (1):224-35
Lewis SJ. et al. J Neurosci 2003 Jul 16;23 (15):6351-6
Li J. et al. Conf Proc IEEE Eng Med Biol Soc 2007;2007:5992-5
Li J. et al. Conf Proc IEEE Eng Med Biol Soc 2008;2008:4617-20
Li J. et al. Neuroimage 2008 Jun;41 (2):398-407
Liepelt I. et al. Mov Disord 2009 Jul 30;24 (10):1504-11
Lin TP. et al. Brain 2008 May;131 (Pt 5):1373-80
Liotti M. et al. Neurology 2003 Feb 11;60 (3):432-40
Lozza C. et al. Hum Brain Mapp 2004 Jul;22 (3):236-45
Lyoo CH. et al. Eur Neurol 2010;64 (2):65-73
Ma AJ. et al. Zhonghua Yi Xue Za Zhi 2008 Oct 14;88 (37):2623-8
Ma Y. et al. 2009 Apr 1;45 (2):260-6 Epub 2008 Oct 18
Ma Y. et al. J Cereb Blood Flow Metab 2007 Mar;27 (3):597-605
Ma Y. et al. J Cereb Blood Flow Metab 2010 Mar;30 (3):505-9
Ma Y. et al. Mol Imaging Biol 2007 Jul-Aug;9 (4):223-33
Macrì MA. et al. Magn Reson Imaging 2006 May;24 (4):419-24
Marié RM. et al. J Neuroimaging 2007 Oct;17 (4):277-85
Marklund P. et al. Brain 2009 Feb;132 (Pt 2):336-46
Markus HS. et al. J Neurol Neurosurg Psychiatry 1994 Feb;57 (2):180-5
Markus HS. et al. Mov Disord 1995 Mar;10 (2):179-87
Martin WR. et al. Can J Neurol Sci 1987 Aug;14 (3 Suppl):448-51
Matsui H. et al. Intern Med 2007;46 (21):1765-9
Matsui H. et al. J Geriatr Psychiatry Neurol 2006 Mar;19 (1):41-5
Matsui H. et al. Parkinsonism Relat Disord 2006 Jun;12 (5):273-8
Matsui H. et al. Parkinsonism Relat Disord 2007 Apr;13 (3):174-81
Matsui H. et al. Sleep 2006 Jul;29 (7):917-20
Mattfeld AT. et al. Learn Mem 2011 Oct 21;18 (11):703-11
Mayberg HS. et al. Ann Neurol 1990 Jul;28 (1):57-64
McClure SM. et al. Neuroscientist 2004 Jun;10 (3):260-8
Melamed E. et al. J Neurol Neurosurg Psychiatry 1986 Nov;49 (11):1301-4
Melzer TR. et al. Brain 2011 Mar;134 (Pt 3):845-55
Mentis MJ. et al. Mov Disord 2006 Apr;21 (4):549-55
Mentis MJ. et al. Neurology 2003 Feb 25;60 (4):612-9
Miletich RS. et al. J Cereb Blood Flow Metab 1994 Jan;14 Suppl 1:S106-14
Moeller JR. et al. Brain 1997 Dec;120 (Pt 12):2197-206
Moessnang C. et al. Cereb Cortex 2011 Jun;21 (6):1246-53
Monchi O. et al. Brain 2007 Jan;130 (Pt 1):233-44
Monchi O. et al. Clin EEG Neurosci 2010 Apr;41 (2):76-81
Monchi O. et al.Neural Netw. 2000 Oct-Nov;13 (8-9):953-73.
Montastruc JL. et al. Mov Disord 1987;2 (4):279-89
Moraschi M. et al. Magn Reson Imaging 2010 Oct;28 (8):1152-8
Müller JL. et al. Psychiatry Clin Neurosci 2003 Dec;57 (6):562-8
Mure H. et al. Neuroimage 2011 Jan 15;54 (2):1244-53
Nagamachi S. et al. Ann Nucl Med 2008 Aug;22 (7):557-64
Nakamura S. et al. Eur Neurol 1988;28 Suppl 1:24-8
Nakamura T. et al. Hum Brain Mapp 2001 Jan;12 (1):42-60
Nandhagopal R. et al. Neurology 2008 Apr 15;70 (16 Pt 2):1478-88
Narayana S. et al. Hum Brain Mapp 2010 Feb;31 (2):222-36

Ng B. et al. Hum Brain Mapp 2010 Jan;31 (1):88-97
Nishioka K. et al. Mov Disord 2009 Sep 15;24 (12):1811-9
Nobili F. et al. Mov Disord 2009 Feb 15;24 (3):414-21
Oh M. et al. J Nucl Med 2012 Feb 9
Oishi K. et al. Eur Neurol 2004;52 (3):151-5
Oishi M. et al. Int J Neurosci 1996 Mar;85 (1-2):147-54
Oishi N. et al. Neurology 2005 Dec 13;65 (11):1708-15
Osaki Y. et al. Mov Disord 2009 Jul 30;24 (10):1475-80
Otsuka M. et al. Ann Nucl Med 1997 Aug;11 (3):251-7
Otsuka M. et al. J Neurol Sci 1996 Dec;144 (1-2):77-83
Pålhagen SE. et al. J Neurol 2009 Sep;256 (9):1510-8
Palmer SJ. et al. Eur J Neurosci 2009 Jun;29 (11):2187-96
Palmer SJ. et al. Neuroscience 2009 Jan 23;158 (2):693-704
Pappatà S. et al. Neurology 2011 Oct 4;77 (14):1357-62
Park HK. et al. Mov Disord 2009 Jan 30;24 (2):237-45
Parker F. et al. Brain Res 1992 Jul 3;584 (1-2):11-7
Parkinson A. et al. Neuropsychologia 2010 Sep;48 (11):3228-34
Paschali A. et al. Eur J Nucl Med Mol Imaging 2009 Nov;36 (11):1851-8
Paschali A. et al. Eur J Nucl Med Mol Imaging 2010 Jun;37 (6):1128-40
Peppard RF. et al. J Neurosci Res 1990 Dec;27 (4):561-8
Péran P. et al. Cortex 2009 Sep;45 (8):960-71
Peters S. et al. Neuroreport 2003 May 6;14 (6):809-12
Piert M. et al. J Nucl Med 1996 Jul;37 (7):1115-22
Pizzolato G. et al. J Cereb Blood Flow Metab 1988 Dec;8 (6):S101-8
Playford ED. et al. Adv Neurol 1993;60:506-10
Playford ED. et al. Ann Neurol 1992 Aug;32 (2):151-61
Pourfar MH. et al. J Neurosurg 2012 Jan;116 (1):114-8
Powers WJ. et al. J Bioenerg Biomembr 2009 Dec;41 (6):505-8
Prodoehl J. et al. Mov Disord 2010 Oct 15;25 (13):2035-43
Rao H. et al. Mov Disord 2010 Aug 15;25 (11):1660-9
Rascol O. et al. Arch Neurol 1992 Feb;49 (2):144-8
Rascol O. et al. Brain 1998 Mar;121 (Pt 3):527-33
Rascol O. et al. J Neurol Neurosurg Psychiatry 1994 May;57 (5):567-71
Rascol OJ. et al. Adv Neurol 1993;60:419-21
Ray N. et al. Clin EEG Neurosci 2010 Apr;41 (2):87-93
Rektorova I. et al.Mov Disord. 2007 Oct 31;22 (14):2043-51.
Rieger JW. et al. Clin EEG Neurosci 2008 Oct;39 (4):169-74
Ring HA. et al. 1994 Sep;165 (3):333-9 PubMed PMID: 7994502
Rougemont D. et al. J Neurol Neurosurg Psychiatry 1984 Aug;47 (8):824-30
Rowe JB. et al. 2010 Sep;52 (3):1015-26 Epub 2010 Jan 4
Rowe JB. et al. Brain 2008 Aug;131 (Pt 8):2094-105
Sabatini U. et al. Br J Clin Pharmacol 1991 Aug;32 (2):229-34
Sachin S. et al. J Neurol Sci 2008 Oct 15;273 (1-2):51-6
Saint-Cyr JA. et al. J Int Neuropsychol Soc 2003 Jan;9 (1):103-27
Santens P. et al. Eur Neurol 2003;49 (4):238-42
Sen S. et al. Neuroscience 2010 Mar 17;166 (2):712-9
Sestini S. et al. Eur J Nucl Med Mol Imaging 2010 Jan;37 (1):12-22
Shibasaki H. et al. Clin Neurophysiol 2011 Sep 7
Shine JM. et al. J Clin Neurosci 2011 Jun;18 (6):807-10

Sidtis JJ. et al. J Cereb Blood Flow Metab 2012 Jan;32 (1):41-9
Smith FW. et al. Br J Radiol 1988 Oct;61 (730):914-20
Snijders AH. et al. Brain 2011 Jan;134 (Pt 1):59-72
Song IU. et al. Alzheimer Dis Assoc Disord 2012 Feb 17
Spampinato U. et al. J Neurol Neurosurg Psychiatry 1991 Sep;54 (9):787-92
Spetsieris PG. et al. Comput Med Imaging Graph 1995 May-Jun;19 (3):295-306
Spetsieris PG. et al. Conf Proc IEEE Eng Med Biol Soc 2010;2010:2906-9
Spetsieris PG. et al. Neuroimage 2009 May 1;45 (4):1241-52
Stebbins GT. et al. Neurology 2004 Oct 26;63 (8):1409-16
Stoessl AJ. et al. Neurotherapeutics 2011 Jan;8 (1):72-81
Stoessl AJ. et al. Parkinsonism Relat Disord 2009 Dec;15 Suppl 3:S13-6
Subramanian L. et al. J Neurosci 2011 Nov 9;31 (45):16309-17
Tachibana H. et al. Dementia 1995 Mar-Apr;6 (2):89-93
Tachibana H. et al. Neuroradiology 1994 May;36 (4):276-80
Takahashi R. et al. J Neurol Sci 2010 Jan 15;288 (1-2):88-91
Tang CC. et al. J Neurosci 2010 Jan 20;30 (3):1049-56
Tatsch K. et al. Neurodegener Dis 2010;7 (5):330-40
Thiel A. et al. J Neural Transm 2003 Nov;110 (11):1289-301
Thobois S. et al. Neuroimage 2004 Sep;23 (1):1-16
Thobois S. et al. Neurology 2000 Oct 10;55 (7):996-1002
Thobois S. et al. Neuroreport 2000 Mar 20;11 (4):785-9
Tinaz S. et al. Neurobiol Aging 2008 Mar;29 (3):397-407
Turjanski N. et al. J Neural Transm Suppl 1997;51:37-48
Ukmar M. et al. Radiol Med 2006 Feb;111 (1):104-15
van Eimeren T. et al. Arch Neurol 2009 Jul;66 (7):877-83
van Eimeren T. et al. Parkinsonism Relat Disord 2010 Jul;16 (6):384-7
van Nuenen BF. et al. Mov Disord 2009;24 Suppl 2:S703-10
van Nuenen BF. et al. Neurology 2009 Mar 24;72 (12):1041-7
Vander Borght T. et al. J Nucl Med 1997 May;38 (5):797-802
Wallin A. et al. Acta Neurol Scand 2007 Dec;116 (6):347-54
Wang L. et al. Neurosci Lett 2000 Mar 24;282 (3):133-6
Wang SJ. et al. Eur J Nucl Med 1993 Apr;20 (4):339-44
Waragai M. et al. J Neurol Sci 2007 Sep 15;260 (1-2):57-64
Welge-Lüssen A. et al. Neuroscience 2009 Aug 18;162 (2):537-43
Williams-Gray CH. et al. Brain 2008 Feb;131 (Pt 2):397-408
Williams-Gray CH. et al. J Neurosci 2007 May 2;27 (18):4832-8
Wolfson LI. et al. Neurology 1985 Oct;35 (10):1399-405
Wu JC. et al. Neuroreport 2000 Jul 14;11 (10):2139-44
Wu T. et al. Neuroimage 2010 Feb 1;49 (3):2581-7
Wu T. et al. Neuroimage 2011 Mar 1;55 (1):204-15
Yong SW. et al. Eur J Neurol 2007 Dec;14 (12):1357-62
Yoritaka A. et al. 2009;61 (3):164-70 Epub 2009 Jan 8
Yu H. et al. Neuroimage 2007 Mar;35 (1):222-33

Supplemental Table 2. Summary of studies included in the six meta-analyses.

Analysis	Number of Studies	Number of Experiments	Number of Foci	Number of PD subjects	Number of HC subjects
PD-OFF vs. HC, All tasks	43	74	498	427	434
PD-OFF vs. HC, Motor tasks	25	39	301	253	259
PD-OFF vs. HC, Right-handed motor tasks	24	28	202	242	244
PD-OFF vs. HC, Cognitive tasks	15	30	159	148	151
PD-ON vs. HC, All tasks	16	27	124	167	175
PD-ON vs. PD-OFF, All tasks	21	27	155	218	-
All groups, All tasks	60	130	777	607	561

Appendix C. Supplementary data for the study of the Resting-state signature of early Parkinson's Disease

Supplemental Table 1. Subject specific demographic and clinical data.

Subject Number	Group	Gender	Age (years)	MMSE	Duration of diagnosis (months)	UPDRS III, motor score	Hoehn and Yahr score	LEDD
1	discovery	m	69.0	29	20.8	32	2	100
2	discovery	m	71.5	30	16.2	35	2	300
3	discovery	m	42.5	28	21.7	39	2	100
4	discovery	f	72.9	27	5.6	30	2	100
5	discovery	m	63.6	28	11.6	13	1	450
6	discovery	m	66.8	30	32.1	29	2	180
7	discovery	f	50.1	29	25.6	20	2	40
8	discovery	f	39.1	30	37.4	17	2	550
9	discovery	f	41.4	30	39.3	19	2	680
10	discovery	m	69.2	29	36.6	23	2	550
11	discovery	m	66.8	29	26.3	26	2	450
12	discovery	f	57.3	30	31.4	30	2	680
13	discovery	f	47.4	29	29.7	11	1	180
14	discovery	m	71.5	28	12.5	18	2	400
15	discovery	f	62.2	30	42.3	34	2	640
16	discovery	f	51.6	29	33.9	41	2	400
17	discovery	f	65.4	28	42.4	7	1	500
18	discovery	f	60.5	28	12.1	18	1	400
19	discovery	m	50.4	30	47.0	13	2	565
20	validation	m	73.5	29	15.3	71	2	300
21	validation	f	40.1	30	19.0	24	2	180
22	validation	f	81.6	29	50.9	22	2	620
23	validation	m	88.2	27	54.2	47	3	550
24	validation	m	66.9	28	23.2	29	2	470
25	validation	f	65.7	30	6.1	23	3	200
26	validation	m	69.3	23	24.3	24	2	600
27	validation	f	69.5	27	26.2	40	2	300
28	validation	m	55.8	29	2.8	16	2	0
29	validation	m	69.9	26	13.5	26	2	0
30	validation	m	69.3	29	16.4	19	1	0
31	validation	m	62.1	26	4.5	30	2	0
32	validation	f	57.0	28	20.1	15	2	0

33	controls	f	54.2	30	-	-	-	-
34	controls	f	63.7	30	-	-	-	-
35	controls	f	66.5	30	-	-	-	-
36	controls	m	75.2	30	-	-	-	-
37	controls	f	58.6	30	-	-	-	-
38	controls	m	48.4	24	-	-	-	-
39	controls	f	41.8	30	-	-	-	-
40	controls	m	61.8	28	-	-	-	-
41	controls	f	65.5	28	-	-	-	-
42	controls	m	58.1	30	-	-	-	-
43	controls	m	75.0	30	-	-	-	-
44	controls	m	65.7	30	-	-	-	-
45	controls	f	57.0	29	-	-	-	-
46	controls	f	55.6	30	-	-	-	-
47	controls	m	59.2	29	-	-	-	-
48	healthy controls	m	60.5	30	-	-	-	-
49	healthy controls	m	64.0	30	-	-	-	-
50	healthy controls	m	61.6	30	-	-	-	-
51	healthy controls	m	59.0	30	-	-	-	-

Supplemental Table 2. Demographic details of two cohorts used to develop the resting-state template.

	Filippini et al.	Zamboni et al.
Number	39	22
Gender (f:m)	24:15	12:10
Age (SD)	65.3 (7.1)	75.0 (7.7)
MMSE (SD)	29.9 (0.4)	30 (0.6)

Supplemental Table 3. Subject specific motion parameters.

PD subject number	Mean motion off medication (mm)	Mean motion on medication (mm)	Highest score for resting tremor off medication	HC subject number	Mean motion (mm)
1	0.077	0.071	1	33	0.050
2	0.091	0.075	2	34	0.120
3	0.092	0.043	2	35	0.084
4	0.039	0.051	2	36	0.616
5	0.116	0.060	1	37	0.095
6	0.125	0.138	0	38	0.285
7	0.068	0.088	0	39	0.037
8	0.053	0.071	0	40	0.035
9	0.115	0.082	0	41	0.029
10	0.204	0.099	2	42	0.125
11	0.288	0.080	0	43	0.157
12	0.091	0.113	1	44	0.292
13	0.048	0.085	1	45	0.031
14	0.074	0.073	1	46	0.102
15	0.086	0.128	0	47	0.126
16	0.224	0.099	1	48	0.326
17	0.100	0.059	1	49	0.477
18	0.107	0.142	1	50	0.141
19	0.135	0.118	0	51	0.116
Average	0.112[a]	0.088[b]	0.842	Average	0.171

[a]No significant difference with HC group (p=0.373, Mann-Whitney U Test); no significant difference with on medication session (p=0.277, related-samples Wilcoxon Signed Rank Test)

[b]No significant difference with HC group (p=0.133, Mann-Whitney U Test)

Appendix D. List of publications related to research performed during the thesis project.

The influence of age and gender on motor and non-motor features of early Parkinson's disease: Initial findings from the Oxford Parkinson Disease Center (OPDC) discovery cohort. Szewczyk-Krolikowski K, Tomlinson P, Nithi K, Wade-Martins R, Talbot K, Ben-Shlomo Y, Hu MT. Parkinsonism Relat Disord. 2013 Oct 12. pii: S1353-8020 (13)00356-8. doi: 10.1016/j.parkreldis.2013.09.025.

Functional connectivity in the Basal Ganglia network differentiates PD patients from Controls. Szewczyk-Krolikowski K, Menke R, Rolinski M, Duff E, Salimi-Khorshidi G, Filippini N, Zamboni G, Hu MT, Mackay CE. Under revision by Neurology.