Clinical and neuroimaging biomarkers of early and prodromal Parkinson's disease

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University of Oxford

A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy at the University of Oxford

Supervisors: Prof. Michele T.M. Hu and Prof. Clare E. Mackay

Hilary Term 2017

Dedicated to Esther and Alfie

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Abstract

The degeneration of the substantia nigra starts many years before the motor symptoms first emerge and the diagnosis of Parkinson's disease (PD) can finally be made. Identification of patients at the early, prodromal, stage would provide insight into the earliest pathological pathways in PD and allow the targeted use of novel neuroprotective agents, slowing or even halting the progression of the disease. Patients with idiopathic REM sleep behaviour disorder (RBD) have been found to have a much higher risk of being diagnosed with PD, making them a promising group for studying prodromal PD. Resting-state functional MRI (rs-fMRI) has previously shown promise as a biomarker in early PD. The overarching aim of the research presented in this DPhil thesis was to assess the utility of rs-fMRI as a biomarker in a group representative of prodromal PD. RBD was found to be a common and under recognised symptom in patients with early PD. Although no typical motor phenotype was identified, patients with both PD and RBD suffered more frequent and severe non-motor symptoms and had a more impaired quality of life. Patients with idiopathic RBD had mild motor symptoms and the full range of non-motor symptoms associated with PD. They were also found to have pattern of visual short term memory that mirrors that seen in idiopathic PD. Analysis of the basal ganglia network using rs-fMRI demonstrated marked abnormalities in patients with early PD, not seen in Alzheimer's disease, where basal ganglia function is preserved. Patients with idiopathic RBD were indistinguishable from those with PD on rs-fMRI despite obvious differences on dopamine transporter single photon emission computerised tomography. The research presented in this DPhil thesis showed that basal ganglia connectivity, as measured using rs-fMRI, is a promising biomarker for the detection of early basal ganglia network dysfunction, and may help to identify patients at risk of developing PD in the future.

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This DPhil project would not have happened without the help and support of many people.

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Contents

List of Abbreviations

AD, Alzheimer's disease; ADC, apparent diffusion coefficient; ADL, activities of daily living; ALFF, amplitude of low frequency fluctuations; ANOVA, analysis of variance;

BDI, Beck's depression inventory; BGN, basal ganglia network; BOLD, Blood-oxygen-level dependent;

CBF, cortical blood flow; CGIC, Clinical Global Impressions Scale; CSF, cerebrospinal fluid;

DA, dopamine; DAT, dopamine transporter; DMN, default mode network; DTI, diffusion tensor imaging; DWI, diffusion weighted imaging;

EEG, electroencephalogram; EPI, echo planar imaging; ESS, Epworth sleepiness scale;

FA, fractional anisotropy; fALFF, fractional amplitude of low frequency fluctuations; FIRST, FMRIB's integrated registration and segmentation tool; fMRI, functional magnetic resonance imaging; FSL, FMRIB software library; FWHM, full-width at half-maximum;

GABA, γ-aminobutyric acid; GiV, ventral gigantocellular reticular nucleus; GLM, general linear model; GM, grey matter; GPe, external globus pallidus; GPi, internal globus pallidus; GRE, gradient echo sequence;

HAAS, Honolulu-Asia Aging Study;

ICA, independent component analysis;

KCC, Kendall's coefficient of concordance;

LADS, Leeds anxiety and depression scale; LB, Lewy body; LEDD, levodopa equivalent daily dose; LFP, local field potential;

MCI, mild cognitive impairment; MCRF, magnocellular reticular formation; MD, mean diffusivity; MEG, magnetoencephalography; MELODIC, multivariate exploratory linear optimized decomposition into independent components; MMSE, MiniMental State Examination; MNI, Montreal Neurological Institute; MoCA, Montreal cognitive assessment; MP-RAGE, magnetization prepared rapid acquisition gradient echo sequence; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; MSA, multiple system atrophy;

OCMR; Oxford Centre for Clinical Magnetic Resonance Research; OPDC, Oxford

Parkinson's Disease Centre;

PCA, principle component analysis; PD, Parkinson's disease; PE, parameter estimate; PET, positron emission tomography; PIGD, postural instability and gait disturbance; PPN, pedunculopontine nucleus; pRBD, probable REM sleep behaviour disorder; PSG, polysomnography; PSP, progressive supranuclear palsy;

QSBB, Queen Square Brain Bank;

RBD, REM sleep behaviour disorder; RBDSQ, REM sleep behaviour screening questionnaire; ReHo, regional homogeneity; REM, rapid eye movement; RF, radio frequency; ROC, receiver operating characteristic; ROI, region of interest; rs-fMRI, resting state fMRI;

SBC, seed-based correlation; SCP, slow cortical potentials; SLD, sublaterodorsal tegmental nucleus; SMN, sensorimotor network; SNc, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; SPECT, single-photon emission computed tomography; STN, subthalamic nucleus; SVM, Support Vector Machine;

TCS, transcranial sonography; TE, echo time; TFCE, threshold-free cluster enhancement; TR, repetition time;

VBM, voxel-based morphometry; VSTM, visual short-term memory;

WM, white matter;

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Publications arising from this Thesis

1. **M Rolinski**, L Griffanti, P Piccini, A A Roussakis, K Szewczyk-Krolikowski, R A L Menke, T Quinnell, Z Zaiwalla, J C Klein, C E Mackay, M T M Hu. "Basal ganglia dysfunction in idiopathic REM sleep behaviour disorder parallels that in early Parkinsons disease" ***Brain***, *139(8):22224–2234, 2016*

2. **M Rolinski**, N Zokaei, F Baig, K Giehl, T Quinnell, Z Zaiwalla, C E Mackay, M Husain, M T M Hu. "Visual short-term memory deficits in REM sleep behaviour disorder mirror those in Parkinson's disease" ***Brain***, 139(1):47–53, 2016

3. **M Rolinski**, L Griffanti, K Szewczyk-Krolikowski, R A L Menke, G K Wilcock, N Filippini, G Zamboni, M T M Hu, C E Mackay. "Aberrant functional connectivity within the basal ganglia of patients with Parkinson's disease" ***NeuroImage: Clinical***, 9(8):126–32, 2015

4. **M Rolinski**, K Szewczyk-Krolikowski, P R Tomlinson, K Nithi, Y Ben-Shlomo, M T M Hu. "REM sleep behaviour disorder is associated with worse quality of life and other non-motor features in early Parkinson's disease" ***Journal of Neurology, Neurosurgery and Psychiatry***, 85(5):560–566, 2013

Other Publications during DPhil

1. J C Klein, **M Rolinski**, L Griffanti, K Szewczyk-Krolikowski, F Baig, C Ruffmann, R A L Menke, M T M Hu, C E Mackay. "Cortical involvement in early Parkinson's disease - evidence from a multi-modal MRI study" *Submitted*

2. Z Gan-Or, J C Montplasir, J P Ross, S C Warby, S Strong, Y Dauvilliers, C S Leblond, I Arnulf, M T M Hu, **M Rolinski**, T Barber, B Högl, A Stefani, C Monaca, V Cochen De Cock, M Boivin, E Antelmi, A Heidbreder, P A Dion, A Desautels, J–F Gagnon, N Duprú, R B Postuma, G A Rouleau. "The dementia-associated *APOE* ε4 allele is not associated with REM sleep behavior disorder" ***Neurobiology of Ageing***, *In Press*

3. F Baig, M Lawton, **M Rolinski**, C Ruffmann, J Klein, K Nithi, D Okai, Y Ben-Shlomo, M T M Hu. "Personality and addictive behaviours in prodromal and early Parkinson's disease" *Submitted*

4. G Fairfoul, L I McGuire, S Pal, J W Ironside, J Neumann, S Christie, C Joachim, M Esiri, S G Evetts, **M Rolinski**, F Baig, C Ruffmann, R Wade-Martins, M T M Hu, L Parkkinen, A J E Green. "Alpha-synuclein RT-QuIC in the CSF of patients with alpha-synucleinopathies" ***Annals of Clinical and Translational Neurology***, 3:812-818, 2016

5. M Lawton, F Baig, **M Rolinski**, C Ruffmann, K Nithi, M T May, Y Ben-Shlomo, M T M Hu. "Parkinson's Disease Subtypes in the Oxford Parkinson Disease Centre (OPDC) Discovery Cohort" ***Journal of Parkinson's Disease***, 5(2):269-279, 2015

6. L Griffanti, **M Rolinski**, K Szewczyk-Krolikowski, R A Menke, N Filippini, G Zamboni, M Jenkinson, M T M Hu, C E Mackay. "Challenges in the reproducibility of clinical studies with resting state fMRI: An example in early Parkinson's disease" ***NeuroImage***, 1(124):704-713, 2015

7. F Baig, M Lawton, **M Rolinski**, C Ruffmann, K Nithi, S G Evetts, H R Fernandes, Y Ben-Shlomo, M T M Hu. "Delineating nonmotor symptoms in early Parkinson's disease and first-degree relatives" ***Movement Disorders***, 30(13):1759–1766, 2015

8. H Cousijn, E M Tunbridge, **M Rolinski**, G Wallis, G L Colclough, M W Woolrich, A C Nobre, P J Harrison. "Modulation of hippocampal theta and hippocampal-prefrontal cortex function by a schizophrenia risk gene" ***Human Brain Mapping***, 36(6):2387–2395, 2015

9. K Szewczyk-Krolikowski, R A Menke, **M Rolinski**, E Duff, G Salimi-Khorshidi, N Filippini, G Zamboni, M T M Hu, C E Mackay. "Functional connectivity in the basal ganglia network differentiates PD patients from controls" ***Neurology***, 83(3):208–214, 2014

10. M T M Hu, K Szewczyk-Krolikowski, P R Tomlinson, **M Rolinski**, C Murray, K Talbot, C E Mackay, Y Ben-Shlomo. "Predictors of cognitive impairment in an early stage Parkinson's disease cohort" ***Movement Disorders***, 29(3):351–359, 2014

Oral Presentations

1. **M Rolinski** "Widespread functional, but not structural, changes in patients with idiopathic REM Sleep Behaviour Disorder", *World Congress on Sleep Medicine*, Seoul, South Korea, 2015

2. **M Rolinski** "Motor and non-motor features of Parkinsons disease in idiopathic REM Sleep Behaviour Disorder", *World Congress on Sleep Medicine*, Seoul, South Korea, 2015

3. **M Rolinski** "RBD and Parkinson's disease findings from the Oxford Parkinson's Disease Centre", *International RBD Meeting*, Helsinki, Finland, 2014

4. **M Rolinski** "Understanding the early pathological pathways in Parkinsons disease The Oxford Parkinsons Disease (OPDC) Clinical Cohort", *Oxford Parkinson's Research Day*, Oxford, UK, 2013

5. **M Rolinski** "Parkinson's disease dementia and dementia with Lewy bodies", *Oxford Dementia Day*, Oxford, UK, 2013

Poster Presentations

1. **M Rolinski**, L Griffanti, K Szewczyk-Krolikowski, R A L Menke, N Filippini, G K Wilcock, G Zamboni, M T M Hu, C E Mackay "Aberrant resting-state functional connectivity within the basal ganglia of patients with Parkinsons disease", *Parkinson's UK Conference*, York, UK, 2014

2. L Griffanti, **M Rolinski**, K Szewczyk-Krolikowski, R A L Menke, N Filippini, G Zamboni, S M Smith, M T M Hu, C E Mackay "Exploring functional connectivity in patients with early Parkinsons using network analysis", *Parkinson's UK Conference*, York, UK, 2014

3. **M Rolinski**, L Griffanti, K Szewczyk-Krolikowski, R A L Menke, N Filippini, G K Wilcock, G Zamboni, M T M Hu, C E Mackay "Resting state fMRI discerns early Parkinson's from controls", *Association of British Neurologists Annual Meeting*, Cardiff, UK, 2014

4. E Tunbridge, H Cousijn, **M Rolinski**, G Wallis, G Colclough, M W Woolrich, A C Nober, P J Harrison "ZNF804A, a Genome-wide Supported Risk Gene, Modulates Hippocampal Theta and Hippocampus-Prefrontal Cortex Coactivation", *Society of Biological Psychiatry*, New York, USA,2014

5. **M Rolinski**, K Szewczyk-Krolikowski, P R Tomlinson, K Nithi, Y Ben-Shlomo, M T M Hu "REM sleep behaviour disorder is associated with worse quality of life and other non-motor features in early Parkinson's disease", *Oxford Parkinson's Research Day*, Oxford, UK, 2013

6. J Klein, **M Rolinski**, K Szewczyk-Krolikowski, R A L Menke, K Talbot, M T M Hu, C E Mackay "Multi-modal cortical involvement in early Parkinsons disease", *Oxford Parkinson's Research Day*, Oxford, UK, 2013

Awards

1. World Association of Sleep Medicine Young Investigator Award, 2015

Preface

This DPhil thesis contains the results of my original work carried out since the commencement of my research higher degree candidature at the University of Oxford Nuffield Department of Clinical Neurosciences. It does not include a substantial part of work that has been submitted to qualify for the award of any other degree or diploma in any university or other tertiary institution. It contains no material previously published or written by another person except where due reference has been made in the text.

Chapter 1

Introduction

Almost two hundred years ago, James Parkinson described paralysis agitans as: ***"Involuntary tremulous motion, with lessened muscular power, in parts not in action and even when supported, and to pass from a walking to a running pace: the senses and intellects being uninjured"*** (Parkinson, 2002). More than a hundred years later, thanks to the work of Constantin Nikolaevitch Tretiakoff, the role of neuronal loss within the substantia nigra of patients with Parkinson's disease (PD) was recognised (Tretiakoff, 1919). It wasn't until 1957, 140 years after James Parkinson's original description, that Carlsson and collegues discovered dopamine as a putative neurotransmitter (Carlsson et al., 1957). Three years later, Ehringer and Hornykiewicz demonstrated a marked reduction in dopamine concentration within the striatum of PD patients (Ehringer and Hornykiewicz, 1960), paving the way to the first trials of symptomatic treatment with levodopa a year later (Birkmayer and Hornykiewicz, 1961).

More than sixty years later, the introduction of levodopa still remains the greatest breakthrough in the field of PD, if not neurodegeneration as a whole. Whilst our definitions and understanding of PD may have changed a great deal since then, no better treatment has been developed. Crucially, there are still no medications that are able to cure or slow the progression of the disease. Reasons for this are plentiful, however, the lack of diagnostic, prognostic and predictive

biomarkers is likely to be an important issue. Moreover, with the diagnosis of PD only possible after the onset of motor symptoms, often many years into the disease process, we may be missing the optimal window for intervention.

1.1 Parkinson's Disease

Parkinson's disease (PD) is the second commonest neurodegenerative disorder in the world, after Alzheimer's disease. In the UK, it affects between 105 and 178 people per 100,000 of the general population (Mutch et al., 1986; Schrag et al., 2000; Hobson et al., 2005; Porter et al., 2006; Wickremaratchi et al., 2009). This corresponds to up to 120,000 cases at any one time. Typically, PD is a chronic and slowly progressive disease. It is commonly diagnosed in the sixth decade of life and has a mean duration from diagnosis to death of 15 years (Elbaz et al., 2003). Age remains the greatest risk factor for the disease, with the prevalence increasing to 700-4500 per 100,000 in those over 65 years of age (Hirtz et al., 2007). Undoubtably, as our population ages, the prevalence of PD is set to rise. Parkinson's UK (2009) estimates that by the year 2020 the number of cases in the UK will rise to 161,000. With the current cost per patient estimated at €2361 per year (Fineberg et al., 2013), this increase is likely to have a huge impact on the general society, as well as the individual patients and their families.

1.1.1 Motor symptoms

The cardinal features of PD are tremor, rigidity and bradykinesia, with the latter a prerequisite for the diagnosis of the disease (Hughes et al., 1992; Gelb et al., 1999). Although commonly considered the fourth cardinal feature, postural instability is rarely seen in early disease.

Bradykinesia

Bradykinesia is defined as slowness in initiation of voluntary movement with progressive reduction in speed and amplitude of repetitive actions (Hughes et al., 1992). By definition it is seen in all patients with PD. In the arms, bradykinesia may start with decreased arm swing or decreased dexterity in the fingers, leading to difficulty in performing simple tasks, such as doing up buttons or tying shoelaces. In the legs, bradykinesia may lead to decreased stride length or cause the patient to drag one, or both, of their feet. Other manifestations of bradykinesia include micrographia, hypomimia and hypophonia.

Rigidity

Rigidity is defined as an increased resistance to passive movement about a joint and occurs in almost all individuals with PD (Martin et al., 1973; Hughes et al., 1993; Louis et al., 1997). As is the case with bradykinesia and tremor, rigidity typically begins unilaterally. Although, eventually, rigidity will progress to the contralateral side, the signs tend to remain asymmetrical throughout the course of the disease (Scott et al., 1970). Rigidity may affect any part of the body and may contribute to reduced arm swing and the typical stooped posture associated with PD.

Tremor

Although commonly thought to be the core feature of PD, tremor is the presenting symptom for only 70% of patients, with around a fifth of patients never experiencing tremor during their disease course (Hoehn and Yahr, 1967; Martin et al., 1973; Rajput et al., 1991; Hughes et al., 1993; Louis et al., 1997). Tremor is usually in the form of the classic 4-6 Hz rest tremor. It typically lessens, or subsides completely, with movement, to reappear again after an interval when a new position of rest is achieved ('re-emergent tremor'). In PD, tremor most commonly affects the

arms, but can also involve the legs, lips, jaw and tongue (Findley et al., 1981; Hunker and Abbs, 1990).

Postural instability

Postural instability in PD is caused by the impairment of centrally-mediated postural reflexes. Patients may experience a feeling of imbalance and a tendency to fall backwards (Dimitrova et al., 2004a; Dimitrova et al., 2004b). In advanced disease, it represents one of the most disabling symptoms, associated with loss of independence and significant morbidity (Kim et al., 2013). As significant postural instability usually does not appear until later in the disease course, parkinsonism associated with early falls should lead to other conditions, such as progressive supra nuclear palsy (PSP), to be considered.

1.1.2 Non-motor symptoms

Whilst PD is still often thought of as a disease of the motor system, it is associated with a plethora of non-motor symptoms **(Table 1.1)**. These symptoms often go undiagnosed and tend to be the drivers of quality of life in patients with PD (Baig et al., 2015b). Central to the theme of this thesis, many of the non-motor symptoms precede the onset of the motor symptoms by a number of years.

Olfaction

Olfactory dysfunction eventually affects up to 90% of patients with PD (Chaudhuri et al., 2006). Most patients experience an increase in the olfactory threshold, with some alterations of identificative and discriminative functions (Tissingh et al., 2001). The dysfunction is believed to the result of degeneration of the extranigral dopaminergic neurons in the olfactory bulb and the anterior olfactory nucleus.

In 1975, Ansar and Johnson first proposed a link between the loss of olfaction

Table 1.1: The non-motor complex of Parkinson's disease

Neuropsychiatric symptoms	**Autonomic symptoms**	**Sleep disorders**	**Sensory and other symptoms**
Depression, apathy, anxiety	Cardiovascular system: orthostatic hypotension; falls related to orthostatic hypotension; bradycardia or arrhythmia	REM sleep behaviour disorder and REM loss of atonia	Pain
Compulsive-obsessive behavior (possibly drug induced), repetitive behavior	Gastrointestinal system: sialorrhoea; dysphagia and choking; reflux, vomiting, nausea; faecal constipation; faecal incontinence	Non-REM-sleep related movement disorders	Paraesthesia
Attention deficit	Urinary system: bladder disturbances; urgency and frequency; nocturia; incontinence	Sleep fragmentation and early morning wakening	Olfactory disturbance
Hallucinations, illusion, delusions	Reproductive system: sexual dysfunction; erectile impotence; hypersexuality (possibly drug induced)	Excessive daytime somnolence	Fatigue
Delirium	Thermoregulation: sweating; dry eyes (xerostomia); heat or cold intolerance	Restless legs and periodic limb movements	Weight changes
Anxiety and panic attacks		Nightmares or vivid dreams	
Dementia			

Table modified from Chaudhuri et al., 2006

and PD (Ansari and Johnson, 1975). Since then, a number of studies have suggested an association between hyposmia and the subsequent development of PD (Doty et al., 1988; Doty et al., 1992; Muller et al., 2002; Ponsen et al., 2004). Moreover, olfactory dysfunction has been reported to be associated with an increased risk of developing PD among first degree relatives of sporadic PD (Wolters et al., 2000; Ponsen et al., 2004).

The Honolulu-Asia Ageing Study (HAAS) is a population based prospective study of neurodegenerative and cerebrovascular diseases in 8006 Japanese-American men, born between 1900 and 1919 (Ross et al., 2012). Beginning in 1965, the study prospectively collected environmental, life-style and physical characteristics of the cohort. The study reported that impaired olfaction prospectively identified those who went on to develop PD with a sensitivity and specificity of 79% and 53%, respectively.

Neuropsychiatric dysfunction

In his original work, James Parkinson described the sense and intellect being uninjured. We now know this not to be the case, with a number of neuropsychiatric conditions affecting many patients with PD. These can range from anxiety, apathy and depression to frank dementia (Aarsland et al., 1999; Thanvi et al., 2003).

Depression is thought to affect between 10 and 45% of patients with PD, mainly depending on the criteria used (Burn, 2002). As well as a reactive component, there is clearly an organic link between PD and depression. Depressed PD patients have decreased concentrations of 5-hydroxyindolacetic acid, a serotonin metabolite, in the cerebrospinal fluid, and reduced 5-HT1A receptor binding compared to non-depressed patients (Brooks and Doder, 2001). It is often believed that depression associated PD results from damage to the serotoninergic neurotransmission, as well as limbic noradrenergic and dopaminergic mechanisms (Remy et al., 2005). As is

the case with depression, anxiety disorders are also very common in PD. Symptoms can range from panic attacks and phobias to generalised anxiety disorders.

Similarly to olfactory loss, anxiety and depression have been linked to the early stages of PD. In a case-control study, Shiba and colleagues, found that presence of depression and anxiety in the preceding five years was linked to the future development of the motor symptoms of PD (Shiba et al., 2000). Two years later, Schurmann and colleagues reported the outcomes of a longitudinal study which showed an increased risk of PD in subjects with depression (Schuurman et al., 2002).

Over the past 30 years, apathy, independent of depression, fatigue or somnolence, has been established as an important symptom of PD (Starkstein et al., 1992; Pluck and Brown, 2002). Patients with PD have a higher prevalence of apathy when compared to other conditions associated with similar levels of disability, again suggesting that this is not simply a reactive phenomenon (Alves et al., 2004). This has been supported by imaging studies which have found decreased reward processing in the brains of patients with PD (Kunig et al., 2000).

One of the symptoms that most strongly correlates with the need for nursing home placement and mortality in patient with PD is the onset of psychotic symptoms (Kunig et al., 2000). It is estimated that approximately 40% of patients experience visual hallucinations (Diederich et al., 2005). These tend to be benign. More troubling symptoms, such as delusions or paranoid ideation, are more commonly seen as the disease progresses. Whilst partially attributable to medication side-effects, visual hallucinations are associated with degeneration of the pedunculopontine nucleus, locus coeruleus and the raphe nuclei (Diederich et al., 2005).

As will be discussed in the context of the Braak hypothesis later, cognitive impairment and, ultimately, dementia is an integral part of PD. The long-term

cumulative prevalence of PD dementia is 80% (Aarsland et al., 2003) - leading to a major impact on independence, nursing home admission, psychiatric comorbidity, care-giver burden, and mortality (Levy et al., 2002; Bronnick et al., 2006; Aarsland et al., 2007). The dementia is progressive and clinically characterised by a dysexecutive syndrome and impairment of visuospatial function and memory (Emre, 2003). The loss of cholinergic neurons in the nucleus basalis is implicated and forms the rationale for treatment with cholinesterase inhibitors (Rolinski et al., 2012).

Autonomic dysfunction

Although the key characteristic of multiple system atrophy, autonomic dysfunction is very common in PD. The prevalence of symptomatic orthostatic hypotension, constipation, bladder dysfunction, erectile dysfunction, and hyperhidrosis is significantly higher in patient with PD, with approximately half of the patients stating that these symptoms affect their daily living a lot or very much (Magerkurth et al., 2005). Constipation is one of the commonest non-motor symptoms in PD (Baig et al., 2015a). Although there is evidence for the severe loss of colonic dopaminergic neurons, both centrally and peripherally, constipation does not seem to improve with dopaminergic therapy, implicating non-dopaminergic mechanisms in its pathophysiology (Singaram et al., 1995). Once again, constipation seems to precede the onset of motor symptoms, even by twenty years. Data from HAAS showed that men with less than one bowel movement per day had a 4.5-fold excess risk of developing PD versus men with more than two bowel motions per day (Abbott et al., 2001). Overall, using less then two bowel motions per day as a cut off, the onset of clinical PD could be predicted with a sensitivity and specificity of 79% and 31%, respectively (Ross et al., 2012).

Sleep disorders

Virtually all patients with PD have sleep disruption (Chaudhuri, 2003). As well as abnormalities in primary sleep architecture, patients with PD are prone to sleep disturbance from a number of contributing factors, such as urinary symptoms or sleep disordered breathing due to sleep apnea (Arnulf et al., 2002).

Excessive daytime sleepiness and involuntary dozing affects up to half of all patients with PD (Rye and Jankovic, 2002). It is thought to be caused by a combination of the disease process, the effect of nocturnal sleep disruption and antiparkinsonian drugs. In some patients, excessive daytime sleepiness has been linked to the development of sudden-onset sleep (Tracik and Ebersbach, 2001). In approximately a third of patients the sleep latency is less than five minutes (normal range 12–20 mins.) (Ulivelli et al., 2002). Similarly, polysonmographic studies have shown transition from wakefulness to sleep stage 2 within seconds, mirroring what is observed in narcolepsy (Rye and Jankovic, 2002). Although not proven in PD, the involvement of the suprachiasmatic nucleus, which regulates the sleep-wake cycle flip-flop switch, and hypocretin is likely (Saper et al., 2001; Rye and Jankovic, 2002). Once again, excessive daytime sleepiness can precede the motor symptoms of PD (Abbott, 2005). In the HAAS, its presence predicted PD with a sensitivity and specificity of 21% and 92%, respectively (Ross et al., 2012).

REM sleep behaviour disorder (RBD) is characterised by the loss of normal atonia during REM sleep, with patients moving in apparent response to their dream content. Behaviours observed during this stage are often violent, mimicking fighting and defensive mechanisms, and are thought to originate from central pattern generators of archaic threat-stimulating behaviours (Boeve et al., 2007). Whilst the estimated prevalence in the general population is only 0.5% (Ohayon et al., 1997), the symptoms of RBD are present in up to 60% of patients with PD (Rolinski et al., 2014), and 80-100% of patients with dementia of Lewy bodies (DLB)

(Boeve et al., 1998) and multiple system atrophy (MSA) (De Cock et al., 2011).

Over the past twenty years, increasing evidence has emerged for a link between RBD and the prodromal stages of a number of neurodegenerative conditions. To date, four sleep clinic (Schenck and Mahowald, 1996; Iranzo et al., 2006; Postuma et al., 2009a; Wing et al., 2012; Schenck et al., 2013), and one population based (Boot et al., 2012), studies have shown that more than 80% of patients presenting with RBD will eventually develop a clinically defined neurodegenerative syndrome. Although most patients develop PD, RBD has also been found to predate dementia with Lewy bodies and multiple system atrophy. Therefore, RBD may be considered the strongest predictor of neurodegeneration available by far (Postuma et al., 2010). Moreover, with delay between the onset of RBD and the diagnosis of PD being longer than ten years in most cases, RBD poses a large window of opportunity for the initiation of novel disease therapies before the onset of the motor symptoms.

RBD will be discussed at length throughout this thesis.

More recently, first evidence has emerged suggesting a link between REM sleep without atonia, that is the loss of atonia without the associated complex behaviours, and neurodegeneration (Stefani et al., 2015). Further research is required to fully assess this relationship.

1.1.3 Diagnosis

The diagnosis of PD remains a clinical one. Despite very characteristic motor symptomatology, clinicians are often faced with diagnostic uncertainty. Prior to the introduction of the Queen Square Brain Bank (QSBB) diagnostic criteria in 1992 (Hughes et al., 1992), the diagnostic accuracy was only slightly better than chance. When compared to post-mortem examination, certified neurologists accurately diagnosed 65% of the cases, increasing to 76% if they were given the benefit of 12 years of follow-up data (Rajput et al., 1991). An improvement came with the

Table 1.2: UK Parkinson's disease society brain bank clinical diagnostic criteria

Inclusion criteria	**Exclusion criteria**	**Supportive criteria**
Bradykinesia and at least one of the following:	History of repeated strokes with stepwise progression of parkinsonian features	(Three or more required for diagnosis of definite PD)
Muscular rigidity	History of repeated head injuries	Unilateral onset
4-6 Hz rest tremor	History of definite encephalitis	Rest tremor present
Postural instability not caused by primary visual, vestibular, cerebellar, or proprioceptive dysfunction	Oculogyric crises	Progressive disorder
	Neuroleptic treatment at onset of symptoms	Persisting asymmetry affecting side of onset most
	More than one affected relative	Excellent response (70-100%) to levodopa
	Sustained remission	Levodopa response for 5 years or more
	Strictly unilateral features after 3 years	Clinical course of 10 years or more
	Supranuclear gaze palsy	
	Cerebellar signs	
	Early severe autonomic involvement	
	Early severe dementia with disturbances of memory, language, and praxis	
	Babinski sign	
	Presence of cerebral tumour or communicating hydrocephalus on CT scan	
	Negative response to large doses of levodopa	
	MPTP exposure	

Table modified from Hughes et al., 1992

introduction of the QSBB diagnostic criteria **(Table 1.2)**. They required that a diagnosis of PD only be made in the presence of bradykinesia plus at least one of rigidity, tremor or postural instability. The diagnosis could be supported by additional features, such as unilateral onset or excellent response to levodopa, whilst features such as cerebellar signs or a supranuclear palsy acted as exclusion criteria. Retrospective applications of these criteria to a 100 cases increased the diagnostic accuracy of movement disorder specialists from 76% to 82% (Hughes et al., 1992). A prospective application of these criteria revealed a sensitivity for PD of 91% (Hughes et al., 2002).

More recently, a task force was set up by the European Federation of Neurological Societies and the European Section of the Movement Disorders Society with an aim of improving diagnostic techniques. Their advice was published in 2013, recommending a more rounded approach to the patient and including the presence of non-motor symptoms in the diagnostic process (Berardelli et al., 2013). For the first time, these recommendations have also included a role for imaging in making a diagnosis.

1.2 Basal ganglia and their dysfunction

1.2.1 The basal ganglia model

The term basal ganglia refers to the large, strongly interconnected nuclei found deep within the brain. These nuclei are instrumental to the initiation of, and the control of the postural adjustments in relation to, voluntary movement. Hence, the basal ganglia invariably lie at the heart of PD.

The basal ganglia include the neostriatum (caudate nucleus and putamen), the ventral striatum, the external and internal segments of the globes pallidus (GPe and GPi, respectively), the subthalamic nucleus (STN) and the substantia nigra pars reticulata and compacts (SNr and SNc, respectively) **(Figure 1.1)**. Input from numerous cortical areas terminates within specific basal ganglia territories, which, in turn, project to specific thalamic nuclei. The thalamic nuclei can then project back to the original cortical areas - thus forming reentrant cortico-subcortical loops (Alexander et al., 1986; Kelly and Strick, 2004). These loops can go on to form complex interconnected circuits and are able to influence a number of cortical areas, including motor, oculomotor, prefrontal associative, and limbic areas.

Let us consider the most researched of these cortico-subcortical circuits, the motor circuit, in more detail. The intrinsic circuit anatomy of this circuit is

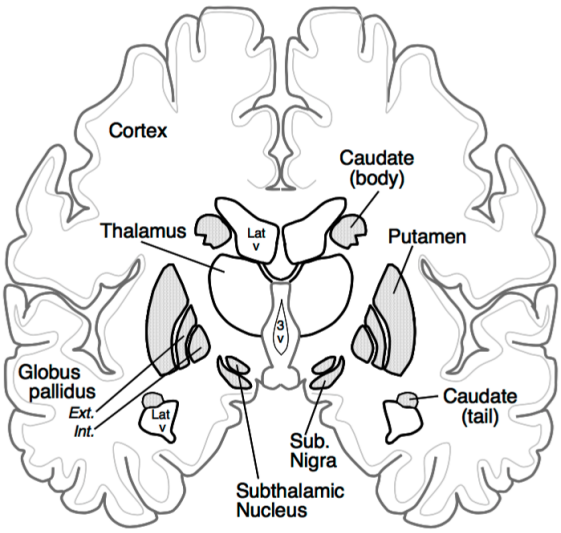

Figure 1.1: Diagram of a coronal section of the brain with the nuclei that make up the basal ganglia labelled.

summarised in **(Figure 1.2)** .The GPi and the SNr form the outputs from the basal ganglia. Neurons from these nuclei give rise to γ-aminobutyric acid (GABA)-ergic projections, which tonically inhibit the cortical projection from he ventral anterior, the ventrolateral and the intralaminar nuclei of the thalamus, and the brainstem. This tonic inhibitory signal is modulated by the balance of the direct and indirect pathways within the basal ganglia. In the direct pathway, GABA-ergic neurons from the striatum act to directly inhibit GPi/SNr, hence releasing the inhibition on the thalamus and brainstem. Conversely, the indirect pathway, involving the GPe and STN, has an overall excitatory effect on the GPi/SNr, increasing the inhibitory output from the basal ganglia. The interplay between these two pathways is modulated by dopamine from the SNc. Through opposite effects on the D1 and D2 receptors, dopamine acts to promote the direct pathway, whilst suppressing the indirect pathway. Therefore, dopamine acts to inhibit output from GPi/SNr and promote movement. A third pathway, the hyperdirect pathway, also exists. This is a direct connection from the cortex bypasses the striatum and provides a more immediate excitation of the GPi via the STN.

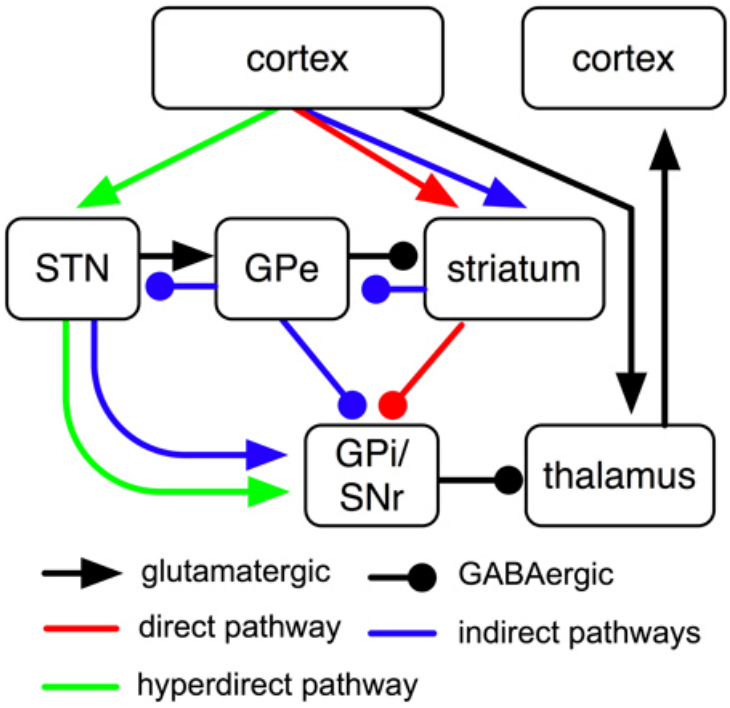

Figure 1.2: Sketch of the cortico-BG-thalamic fiber tracts and their subdivision into direct, indirect and hyperdirect pathways.

Figure taken from Schroll and Hamker, 2013

A vast oversimplification of the true circuit, this textbook model of the basal ganglia only explains a fraction of their function. Recent developments in the field suggest that the functional connections involving the basal ganglia can no longer be viewed as unidirectional cortico-basal ganglia-thalamo-cortical loops (Graybiel, 2008; Obeso et al., 2008). For example, rather than just sitting between the GPe and GPi in the indirect pathway, we now know that the STN receives afferent signal from the cerebral cortex, the thalamus and the brainstem (Inase et al., 1999; Nambu et al., 2002; Lanciego et al., 2004). The STN has also been found to directly project to the ventral thalamus (Rico et al., 2010). This would suggest a great degree of multidirectional parallel processing that goes on between these densely interconnected structures. The same applies to the dopaminergic system, which rather than just innervating the striatum also supplies the STN, GPe, GPi, the

cortex, the limbic system and the thalamus (Smith and Villalba, 2008).

1.2.2 The role of dopamine

The loss of dopaminergic (DA) neurons from the substantial nigra pars compacts (SNc) is not only the hallmark feature of PD, but is also essential for its pathological diagnosis. It is estimated that the typical motor symptoms associated with PD only emerge after 60% DA neurons within the SNc have been lost and DA levels are depleted down to approximately 20% of that found in healthy brains (Fearnley and Lees, 1991; Gaig and Tolosa, 2009). It is estimated that the degenerative process leading to neuronal loss within the SNc is likely to start approximately 7 years before the clinical diagnosis of PD is made (Gaig and Tolosa, 2009; Berg et al., 2013).

How is such a long latency period possible? It is believed that as the terminals of the DA neurons begin to degenerate, there is a reduction of high affinity DA uptake, which is coupled with an degree of redundancy in DA terminals and receptors. Therefore, function is maintained without significant disruption or the need for compensation. As the neurodegenerative process continues, the brain begins to compensate by increasing DA synthesis within the remaining terminals and the amount of DA released into the synaptic junction (Zigmond, 1994). Recent developments have also highlighter the importance of the role that non-DA-mediated mechanisms play in compensating for neuronal loss within the SNc (Bezard et al., 2003). It is only when the neuronal loss reaches a certain threshold that the compensatory mechanisms breakdown and the patient develops the motor symptoms.

The classical model of the motor circuit described above provides a helpful simplification of the effect of reduced DA, and hence, some of the motor symptoms it produces. In the direct pathway, as DA levels decrease, the resulting

under activity leads to disinhibition of the tonic inhibitory output from GPi/SNr. Furthermore, decreased activation of the D2 receptors leads to a net excitatory output acting to increase the tonic activity of the GPi/SNr. Therefore, the model would predict that decreased DA activity would lead to a greater inhibition of voluntary movement. Indeed, dopamine transporter imaging have shown that there is a strong correlation between the degree of stratal denervation and bradykinesia (Pirker, 2003). The same cannot be said of tremor which does not seem to correlate with stratal DA levels and cannot be easily explained by the classical model of the motor circuit. Instead, tremor frequency seems to correlate with firing rates within the GPe, GPi, STN and the ventralis intermedius nucleus of the thalamus (Vim), implying the role of neuronal synchrony within the basal ganglia in tremor generation (Obeso et al., 2008). It is though that deeper understanding of this synchronised neuronal activity may pave the way to better understanding of basal ganglia function (Eusebio and Brown, 2007; Little and Brown, 2012; Little and Brown, 2014).

It is worth remembering that, although DA seem to be the most important neurotransmitter responsible for motor symptoms, almost every other neurotransmitter is thought to be involved in the pathophysiology of PD (Barone, 2010). Dysfunction of these non-DA systems have been implicated in many of the non-motor symptoms described in the earlier part of this chapter. For example, cellular loss within the nucleus baseless of Maynert, and the resulting cholinergic dysfunction, has been linked with cognitive dysfunction (Candy et al., 1983; Whitehouse et al., 1983; Perry et al., 1985; Williams-Gray et al., 2006). Furthermore, impairment of the serotonin-mediated innervation originating from the brainstem raphe nuclei is linked to the affective disorders commonly described in PD (Sasaki-Adams and Kelley, 2001; Nutt, 2008). Whilst the involvement of the dopaminergic system remains contentious, the cholinergic and serotonergic systems have both been

implicated in RBD associated with PD, and will be discussed at length later (Boeve, 2010).

1.2.3 The Lewy body

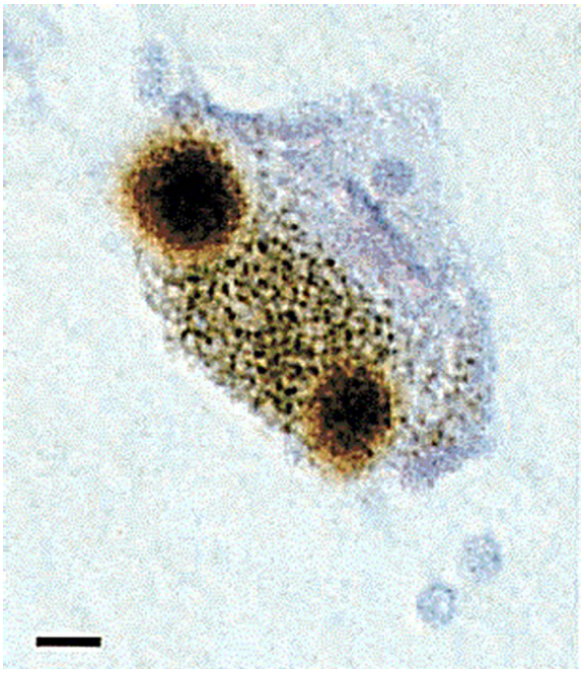

Figure 1.3: Alpha-synuclein positive Lewy body.

Post mortem tissue from the substantia nigra of a patient with Parkinson's disease stained for alpha-synuclein (brown). A single nerve cell containing two Lewy bodies is shown, with a scale bar of 8 μm. Figure taken from Spillantini et al., 1997.

The Lewy body (LB), a neuronal intracytoplasmic inclusion, is the other major pathological hallmark of PD. On histological staining, Lewy bodies have an eosinophilic core and a surrounding pale halo **(Figure 1.3)**. In 1997, a missense mutation (Ala53Thr) in the synuclein alpha (SNCA) gene was shown to cause a dominantly inherited, LB positive, form of PD (Polymeropoulos et al., 1997). This lead to the discovery that alpha-synuclein is the main component of LBs (Spillantini et al., 1997). LBs have also been found to contain approximately 70 other proteins (Wakabayashi et al., 2007), including those involved in protein

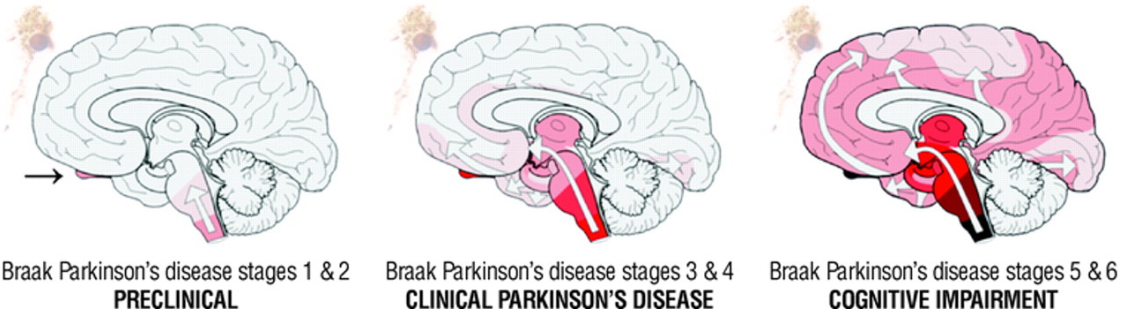

Figure 1.4: Staging of alpha-synuclein pathology thought to be associated with the evolution of PD, based on work by Braak et al., 2003.

degradation, such as ubiquitin (Kuzuhara et al., 1988).

LBs are usually observed in the brain regions associated with the most neuronal loss in PD. To date, their exact role in the pathophysiology of PD remains a contentious subject. The existence of genetic forms of parkinsonism without the presence of LBs has called into question the traditional view that LBs are themselves responsible for neuronal death (Poulopoulos et al., 2012). Instead, it has been suggested that they may merely act as bystanders to the neurotoxicity initiated by alpha-synuclein oligomers (Parkkinen et al., 2011; Kalia et al., 2013).

1.2.4 The Braak Hypothesis

In the last 15 years, the traditional view that the pathological process in PD starts with the degeneration of the DA neurons in the SNc has been challenged by Braak and colleagues (Braak et al., 2003). In their seminal work, they introduced the concept of a six-stage pathological process, starting caudally and spreading rostrally over time **(Figure 1.4)**. The model postulates that Lewy body pathology begins at the level of the vagal nerve nucleus in the brainstem, and the olfactory bulb. More recently, even earlier involvement of the enteric plexus has been found, suggesting that the disease starts outside the central nervous system (Hawkes et al., 2009).

Braak stage 2 characterises the progression of the pathology to the lower brain-

stem, affecting a number of key areas thought to be implicated in the non-motor symptoms of PD - including the raphe nucleus, the locus coeruleus and the pedunculopontine nucleus (Braak et al., 2003). It is only when stages 3 and 4 are reached, and the neurodegenerative process has affected the SNc and other deep nuclei of the midbrain and forebrain, that the classical motor symptoms of bradykinesia, rigidity and tremor emerge. Only at this stage can the clinical diagnosis of PD be made. As the Lewy bodies continue to spread rostrally, the involvement of the limbic structures and the mature neocortex marks Braak stages 5 and 6. At theses stages patients can experience visual hallucinations, cognitive impairment or frank dementia.

Braak stages 1 and 2 are often described as the presymptomatic stages of PD. As discussed previously, these stages of the disease are associated with a vast array of symptoms. Therefore, calling them presymptomatic is clearly a misnomer. In 2014, the Movement Disorders Society Task Force recommended the following terminology :

- **Preclinical PD** - the presence of neurodegenerative synucleinopathy without any clinical symptoms.
- **Prodromal PD** - presence of early symptoms and signs before the diagnosis of PD is possible.
- **Clinical PD** - diagnosis of PD can be made on the basis of the motor signs discussed before.

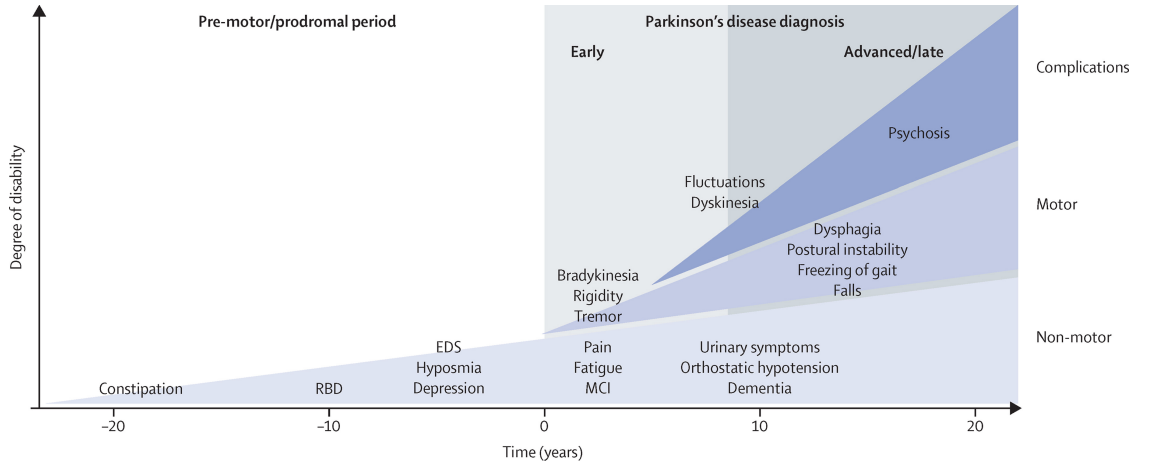

Figure 1.5: Clinical symptoms and time course of Parkinson's disease progression. Figure taken from Kalia and Lang, 2015.

1.3 The early diagnosis of PD

1.3.1 Biomarkers

As has been discussed before, and is illustrated in **Figure 1.5**, the prodromal stage poses an opportunity for therapeutic intervention before the neurodegenerative process reaches a critical threshold and the onset of motor symptoms heralds the clinical diagnosis of PD. As motor signs cannot be used to diagnose prodromal PD, there is a clear need for biomarkers to allow the diagnosis to be made.

The term biomarker is 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, 2001). In this thesis, I will concentrate on biomarkers necessary to diagnose prodromal PD. However, it it worth remembering that biomarkers are also needed to differentiate PD from other parkinsonian syndromes, track disease progression, and objectively track response to symptomatic or disease modifying therapies.

Broadly speaking, when applied to PD, biomarkers can be divided into clinical,

neuroimaging, biochemical, genetic or proteomic (Gerlach et al., 2012; McGhee et al., 2013; Schapira, 2013). Gerlach and colleagues have suggested that a biomarker should be (a) linked to the fundamental features of PD neuropathology and mechanisms underlying neurodegeneration, (b) correlated to disease progression as assessed by clinical rating scales, (c) able to monitor the exact disease status, (d) pre-clinically validated, (e) and confirmed by at least two independent investigators and published in peer-reviewed journals (Gerlach et al., 2008). Moreover, a biomarker should be inexpensive, non-invasive, simple to use, and technically validated. To date, no single biomarker exists that would satisfy these criteria. Clinical biomarkers constitute features that can be identified through history taking and clinical examination. In PD, this includes a long list of parameters, such as: rigidity, bradykinesia, tremor, postural instability, gait problems, incontinence, nocturne, sleep disturbance, hyposmia, drooling, anxiety or depression. The Unified Parkinson's Disease Rating Scale (UPDRS) (Goetz et al., 2008) incorporates many of these parameters and is the most commonly used rating scale, widely used to track disease progression. However, with the scale relying on semi-objective self- and investigator-based assessment, more objective tests have also been developed. These tend to focus on the motor symptoms and include simple times tasks, such as the Get-up-and-go (Shumway-Cook et al., 2000) or the Flamingo tests (Tsigilis et al., 2002), or the use of more advanced equipment, such as electromyography (Milanov, 2002) or accelerometers (Mancini et al., 2009). More recently, attention has also turned to the non-motor symptoms. For example, olfactory dysfunction, as tested with the University of Pennsylvania Smell Identification Test (UPSIT), was associated with excess risk of psychotic symptoms and impaired memory (Morley et al., 2011). This relationship may reflect the distribution of Lewy body pathology and, hence, could suggest the role of olfaction as a clinical biomarker. In Chapter 2, I will look at the impact that concomitant RBD has on the severity of

early PD and explore its use as a clinical biomarker further.

As discussed previously, Lewy body pathology and the neuronal loss in the SN and the locus coeruleus are necessary for the histopathological diagnosis of PD to be made (Gibb and Lees, 1988). Recent biomarker strategies have concentrated on finding and characterising alpha-synuclein in a range of tissues and bio-fluids, hoping to reflect the pathology observed in the CNS. To date, the search for blood-based biomarkers has been rather disappointing, possibly due to red blood cell containing large quantities of alpha-synuclein and, hence, rendering the theoretical difference between patients and controls irrelevant (Barbour et al., 2008). Studies in cerebrospinal fluid (CSF) have been more promising. It is believed that alpha-synuclein is sequestered in PD, resulting in lower levels being detected in the CSF of patients (Zetterberg et al., 2014). Moreover, further studies suggest that slight reductions in amyloid-beta 42, previously associated with Alzheimer's disease, may correlate with cognitive impairment, and changes in various forms of tau protein and neurofilament light chain may differentiate PD from atypical forms of parkinsonism (Parnetti et al., 2013; Magdalinou et al., 2014). Unfortunately, lumbar puncture is an invasive and costly procedure that would not be appropriate for large-scale screening of patients, unless a very robust biomarker is found.

The introduction of the Braak staging has prompted the search for alpha-synuclein outside the CNS (Lebouvier et al., 2008; Beach et al., 2010; Lebouvier et al., 2010; Shannon et al., 2012). Alpha-synuclein has been found in the gut of patients with PD, even in sample predating the onset of the motor symptoms. However, the poor correlation between the presence of alpha-synuclein and disease severity is a complicating factor (Beach et al., 2010; Lebouvier et al., 2010). Moreover, variability when it comes to the reporting of cell loss, the lack of consensus regarding the classification of Lewy bodies in the gut, and the variable staining in healthy controls means that this method remains very technically challenging (Visanji et al., 2014;

Aldecoa et al., 2015). Whilst gut tissue-based assays are unlikely to be viable on a large scale, methods looking at the faecal microbiome may be more practical (Keshavarzian et al., 2015; Scheperjans et al., 2015).

1.3.2 Neuroimaging

Over the recent years advances in molecular, structural and functional imaging have expanded our understanding of PD. By allowing us to study the brains structure and function, neuroimaging may provide us with the ideal biomarker for the early diagnosis of PD. Let us now consider some of the more commonly utilised imaging modalities in turn. Resting-state fMRI will be discussed in Chapter 3.

Positron emission tomography (PET) and single photon emission computed tomography (SPECT)

PET and SPECT are molecular imaging methods that utilise exogenous, radiolabeled molecules (Price, 2012). Broadly speaking, PET offers better spatial resolution and sensitivity than SPECT. PET, however, relies on radioisotopes such as ^{11}C, ^{18}F and ^{15}O, that have relatively short half-lives and require an on-site cyclotron. Conversely, radioisotopes such as ^{123}I or ^{99m}Tc, which are used in SPECT, may be transported from further afield. This makes SPECT less expensive and more widely available. The use of these methods includes the invasive administration of radioactive compounds which, whilst safe, nonetheless limits some of its applications. This is especially relevant to studying prodromal PD where repeated longitudinal scanning of large populations may be necessary.

One of the most commonly used ligands in PET imaging is ^{18}F-FDOPA (fluorodihydroxyphenylalanine), which targets the activity of the aromatic acid decarboxylase. This enzyme catalyses the last step in the synthesis of the monoamines dopamine, noradrenaline and serotonin. As ^{18}F-FDOPA is a substrate for aromatic

acid decarboxylase, its uptake represents the enzyme's activity and can reflect the transport and vesicular storage of the end-products. As the synthesis of the three monoamines is restricted to different brain areas, ^{18}F-FDOPA PET can be used to study dopamine function in the dorsal and ventral stratum, noradrenaline function in the locus coeruleus and serotonin function in the raphe nuclei (Hammoud et al., 2007). Other ligands can be used to target other aspects of neurotransmitter function. For example, ^{18}F-DTBZ and ^{18}F-F-AV can bind to the vesicular monoaminergic transporter VMAT and, hence, represent the transport of dopamine into pre-synaptic vesicles. ^{11}C-NNC, ^{123}I-IBZM and ^{11}C-RAC can be used to study the post-synaptic D1 and D2/D3 receptors. The reuptake of dopamine into the presynaptic cleft via the dopamine transporter (DAT) can be investigated by PET and SPECT imaging using ^{123}I-FP-CIT, ^{18}F-FP-CIT and ^{99m}Tc-TRODAT, amongst others.

Understandably, dopamine is the most frequently studied neurotransmitter in PD. Repeated observations show decreased dopamine function in the stratum of patients with PD, when compared to healthy controls (Brooks and Peever, 2011; Bajaj et al., 2013; Suwijn et al., 2015). Moreover, studies have confirmed the gradient of dopaminergic dysfunction with the earliest and greatest decrease in function occurring in the posterior putamen, followed by the anterior putamen, and then the caudate (Brooks and Peever, 2011).

PET and SPECT can also be used to directly target neurodegenerative processes. The radioligand ^{11}C-PIB has been used to image beta-amyloid plaques, most commonly found in AD but also in approximately 40% of patients with PD (Edison et al., 2013). Unfortunately, the use of this ligand observed no significant (Campbell et al., 2013) or only minor (Edison et al., 2013) differences between PD and healthy controls. Several ligands have also been used to image tau protein aggregates (Villemagne and Okamura, 2014). ^{11}C-(R)PK11195 has been used as a marker

for the mitochondrial translator protein (TSPO, tryptophan-rich sensory protein) found in microglia which are unregulated as part of the brain's inflammatory response (Iannaccone et al., 2013). Significantly increased ^{11}C-(R)PK11195 binding has been demonstrated in the temporo-parietal and occipital regions of patients with PD (Edison et al., 2013), as well as in the putamen and substantial nigra (Iannaccone et al., 2013). Unfortunately, no alpha-synuclein ligands are available to date.

Finally, PET and SPECT can be used for functional imaging of the brain. Physiological cerebral glucose metabolism can be used with ^{18}F-FDG (fluorodeoxyglucose) PET, and cerebral blood flow (CBF) or perfusion measured using ^{15}O-H_2O PET. Perfusion studies have also been performed using SPECT with the radiotracer ^{99m}Tc-EDC (ethylene cysteine dimer).

Magnetic resonance spectroscopy (MRS)

MRS, which includes single voxel MRS as well as whole-brain MRS imaging (MRSI), allows for relatively direct imaging of many biochemical compounds (Dager et al., 2008; Posse et al., 2013; Tuite et al., 2013). Proton ^{1}H-MRS and MRSI have been used to investigate a wide range of neurotransmitters in PD, including dopamine, GABA and glutamate (Emir et al., 2012; Groger et al., 2014). Gröger and colleagues were the first to use MRSI to directly observe dopamine depletion in the substantia nigra in vivo (Groger et al., 2014). Neurodegeneration can also be studied using other compounds, such as N-acetylaspartate, as a marker of healthy neurons; creatine moieties as markers of energy metabolism; and glutathione as a marker of oxidative stress. All of these have been found to be abnormal in alpha-synucleinopathies (Graff-Radford et al., 2014; Groger et al., 2014; Levin et al., 2014). Energy metabolism can be investigated by imaging high and low energy phosphate moieties (Weiduschat et al., 2014). MRS can also be used

to assess glycerophosphocholine and glycerophosphoethanolamine as markers of membrane catabolism, or myoinositol as a marker of glial activity or osmotic status.

Transcranial sonography (TCS)

TCS is a non-invasive ultrasound imaging method developed for the structural imaging of some of the regions associated with PD (Mehnert et al., 2010; Bouwmans et al., 2013; Sahuquillo et al., 2013; Alonso-Canovas et al., 2014; Stenc Bradvica et al., 2015). TCS depends on an adequate acoustic window through the skull, meaning that some individuals cannot be assessed. Moreover, this method is very operator dependant and can me difficult to employ reliably (Miller and O'Callaghan, 2015). However, its safety profile and relatively low cost when compared to the other neuroimaging methods, makes TCS a very attractive modality.

Magnetic resonance imaging (MRI)

The basics of MR imaging will be covered in more detail in the Methods section. Broadly speaking, MRI uses magnetic fields to create images by detecting the spin properties of nuclei, most commonly the ^{1}H nuclei of the hydrogen atom. Structural MRI, perfusion MRI, diffusion weighted imaging (DWI), diffusion tensor imaging (DTI) and functional MRI have all been used to study PD.

Most commonly, structural MRI has been used for morphometric studies of the sizes and shapes of brain regions in PD. The changes observed tend to be subtle and their utility as viable biomarkers has been questioned (Menke et al., 2014). More recently, neuromelanin-sensitive imaging methods have been developed, allowing better contrast in the SNc and locus coeruleus (Garcia-Lorenzo et al., 2013). Susceptibility weighted imaging has also shown refined views of the substantial nigra, including the nigrosomes (Schwarz et al., 2014), and quantitative

susceptibility mapping has shown improved imaging of the subthalamic nucleus and the globus pallidus interna (Liu et al., 2013).

Diffusion weighted MRI is based on the effects of water diffusion, which, in turn depends on microstructure characteristics of the sampled tissue (Le Bihan, 2003; Hagmann et al., 2006; Alexander et al., 2007). One measure of diffusion is the diffusion coefficient which can be used to generate apparent diffusion coefficient (ADC) maps of the brain. DTI is sensitive to the anisotropy of diffusion - that is, unequal diffusion in different directions. In the neuronal axons, the diffusion of water is hindered in the direction perpendicular to the long axis of the fibres. Therefore, DTI can be used to study the integrity of nerve fibres. DTI measures include fractional anisotropy (FA) and mean diffusivity (MD). Recently, Schwarz and colleagues performed a meta-analysis of the use of DTI in PD (Schwarz et al., 2013). The authors found insignificant disease effect in FA and MD and questioned its use as an imaging biomarker. In the case of MD, the authors argued that the method was not able to index PD-related nigral pathology. In the case of FA, it was postulated that the lack of group effect is possibly related to difficulties with reliably extracting FA values from the substantia nigra.

1.4 Aims and objectives

Let us briefly consider Alzheimer's disease (AD). AD is the commonest form of neurodegeneration, affecting an estimated 44 million people world wide (Association and others, 2011). By 2050 this number is estimated to rise to 100 million (Association and others, 2013). Despite the huge impact on the patients and their close ones, as well as the socioeconomic impact on the rest of the population, only five drugs have been approved for the treatment of AD (Hyde et al., 2012; Howard et al., 2012). No new treatments have been approved since 2003.

A study published in the past two years has explored some of the reasons behind this lack of recent developments (Cummings et al., 2014). Of the 413 therapeutic trials, representing 244 unique compounds, performed in AD between 2002 and 2012, 99.6% failed. Even more worrying, of the 221 potentially disease-modifying agents, none showed a drug-placebo difference in favour of the active treatment. As was discussed by the study authors, these trial failures may be attributable not only to a lack of efficacy or excessive side effects of the active agent, but also challenges in trial execution. The latter has been shown to be improved by better rating strategies, enhanced training and better subject-selection strategies (Cummings et al., 2011; Becker and Greig, 2012).

The issues experienced in AD research are directly transferable to PD. Although not formalised in the same way as the above AD study, problems in trial execution also impair the development of both symptomatic and disease-modifying treatments in PD. Whereas only 60% of subjects with mild cognitive impairment (MCI) transition to established AD (Manly et al., 2008), the RBD to PD transition seems to be higher. Therefore, if found to be representative of the general PD population, this prodromal population could solve some of the issues with subject selection. Moreover, more specific and sensitive biomarkers could replace the currently used rating scales, improving study endpoints.

The experiments included in this thesis set out to better characterise RBD as a prodromal stage of PD and explore the use of resting state fMRI as a biomarker in this stage of the disease. Chapter 3 describes modifications to previously reported rs-fMRI methodology, contributing to the translational pipeline for the development of reliable and clinically useful PD imaging biomarkers. The following three chapters conentrate on better characterising RBD before and after the diagnosis of PD has been made. Chapter 4 describes the prevalence and impact of concomitant RBD on the motor and non-motor phenotypes of early PD. Chapter 5 delineates

the motor and non-motor characteristics in patients with RBD, before the diagnosis of PD is made. Similarly, Chapter 6 describes the use of a visual working memory task to explore cognitive impairment associated with RBD in more detail. Lastly, Chapter 7 describes the use of rs-fMRI to detect basal ganglia dysfunction in patients with RBD before the diagnosis of PD can be made.

1.4.1 Aims

The overarching aim of the thesis was to assess the utility of a resting state fMRI–based biomarker in a group representative of prodromal Parkinson's disease.

1.4.2 Hypothesis

The starting hypotheses for the experiments in this thesis can be summarised as follows:

- The rs-fMRI analysis pipeline can be optimised to detect early PD-related basal-ganglia dysfunction.
- RBD is a common symptom in early PD, in keeping with the caudal to rostral distribution of alpha-synuclein as predicted by the Braak hypothesis.
- Idiopathic RBD is a prodromal stage of PD and is therefore associated with the non-motor and early motor symptoms of PD.
- RBD, as a prodrome to PD, is seen in idiopathic PD and, therefore, is associated with the same pattern of cognitive impairment.
- Rs-fMRI can be used to detect early basal ganglia dysfunction in the prodromal stages of PD.

Chapter 2

Methods

A plethora of research methods have been utilised in the studies that comprise this thesis. These are discussed at length in the methods section of each chapter. As the OPDC patient cohort and/or neuroimaging lie at the heart of every chapter, these are discussed in more detail here.

2.1 The OPDC patient cohort

The Oxford Parkinson's Disease Centre (OPDC; http://opdc.medsci.ox.ac.uk) is a unique multidisciplinary research centre based at the University of Oxford. It was established in 2010, supported by Parkinson's UK with funds from The Monument Trust, one of the Sainsbury Family Charitable Trusts. The OPDC brings together internationally-renowned scientists with expertise in, amongst others, clinical neurology, neuroepidemiology, neuroimaging, molecular genetics, neurophysiology and neuropathology. The core aims of the centre are to:

- Understand the progression of PD
- Predict the onset of PD
- Identify potential drug targets for PD

- Develop new treatments that will prevent the progression from the prodromal stages of the disease

  The patient cohort lies at the centre of the OPDC. One of the largest and best-characterised PD cohorts in the world, it is both prospective and longitudinal. As well as recruiting patients with early idiopathic Parkinson's disease, data is also collected with healthy controls and those at greatest risk of developing PD in the future.

2.1.1 Subject recruitment

Study participants are recruited from participating centres across the thames valley. These include hospitals in Oxford, Reading, Newbury, Wexham Park, High Wycombe, Aylesbury, Milton Keynes, Kettering, Northampton and Banbury. Additionally, patients with REM sleep behaviour disorder are also recruited from the sleep clinics at Papworth and, more recently, Sheffield Hospitals.

In the case of the PD group, neurologists, specialist nurses, geriatricians and GPs from the participating centres are asked to identify all cases of idiopathic PD diagnosed within the preceding three years. To be eligible for inclusion, the PD must have been made by a neurologist or a physician with a specialist interest in PD in accordance with the UK PD Brain Bank Criteria. All participating centres are contacted regularly to ensure all new cases are identified. Patients were excluded if an atypical parkinsonian syndrome was suspected by the referring physician. Once recruited, participants were additionally screened for atypical parkinsonian features by the study neurologists. Patients with cognitive impairment associated with their PD were included in the study, except for when the cognitive impairment preceded the onset of the motor features by one year and hence would be suggestive of a diagnosis of Dementia with Lewy Bodies, or if the cognitive impairment precluded informed consent.

Healthy control subjects are recruited from the spouses and friends of patients with PD. They are initially approached by the participants with PD themselves and asked to return an interest slip to the study administrator.

Subjects at risk of developing PD are recruited from two sources. Firstly, as in the case of healthy controls, first-degree relatives of patients with PD are approached by PD patients participating in the study. Secondly, patients with polysomnography-proven idiopathic REM sleep behaviour disorder (RBD) are recruited from the sleep centres in Oxford, Papworth and, more recently, Sheffield. Patients whose RBD is associated with a clinically-defined neurodegenerative disorder or narcolepsy or where the RBD it thought to be medication related are not eligible for the study. Medication-related RBD was defined as one when the onset of symptoms was closely related to the initiation of any of the medications previously reported to have caused symptoms of RBD (including paroxetine, fluoxetine and impipramine, venlafaxine, and mirtazapine). Eligible patients are first approached by their clinical team and asked to return an interest slip to the study administrator.

The study is undertaken with the understanding and written consent of each subject. It is performed with approval from the Berkshire Research Ethics Committee and in compliance with national legislation and the Declaration of Helsinki. Subjects are free to withdraw at any point from the study and do not have to agree to all aspects of the study protocol.

2.1.2 Clinical Assessment

Eligible participants are invited to attend clinical assessment by the study neurologist and research nurse. Participants with PD and those at risk of developing the disease are invited to attend follow-up appointments every eighteen months. Healthy controls are asked to have a telephone interview every eighteen months

to screen for symptoms of PD. Only the results from the baseline assessments are presented in this thesis.

The protocol for the clinical assessments was developed by Prof. Michele Hu. The individual part of the protocol were chosen with a desire to cover the broadest possible range of data, including subject demographics and disease-specific variables, whilst making it manageable for the subject and the research team. The scope of the assessment is summarised below. The details of the variables used for each analysis are described in the methods section of the experimental chapters.

Global and Motor Assessment

Whilst a number of scales have been designed to assess the signs and symptoms of Parkinson's disease (Ramaker et al., 2002), the Unified Parkinson's Disease Rating Scale (UPDRS) remains the most widely used. As well as providing the most comprehensive assessment of PD, the rating scale is accompanied by training videos and assessments designed to ease implementation and minimise inter-rater variability. For this reason, the most up-to-date version of the UPDRS, the Movement Disorder Society-Sponsored Revision of the Unified Parkinson's Disease Rating Scale (Goetz et al., 2008), was used to assess the subjects in the study.

The UPDRS is made up of six sections. Part I involves the evaluation of non-motor features, such as mentation, behaviour and mood. Part II requires the subjects self-evaluation of activities of daily living (ADL). ADLs are also assessed in the final section, part VI, where the overall impact is assessed using the Schwab and England scale. The most commonly referred to subsection of the UPDRS, part III is a clinician-scored comprehensive assessment of the motor features of Parkinson's. Part IV addresses complications of therapy. Part V includes the Hoehn and Yahr staging of the severity of the disease. The stages are as follows

(Hoehn and Yahr, 1967):

1. Unilateral involvement only usually with minimal or no functional disability
2. Bilateral or midline involvement without impairment of balance
3. Bilateral disease: mild to moderate disability with impaired postural reflexes; physically independent
4. Severe disabling disease; still able to walk or stand unassisted
5. Confined to bed or wheelchair unless aided

As the motor component of the UPDRS is rater dependent, further objective measures of motor performance used in the study included the Purdue Pegboard Test, the Timed Up & Go, and The Flamingo test (as described in Chapter 4).

Non-motor symptoms

Cognitive impairment A task force formed by the Parkinson Study Group Cognitive/Psychiatric Working Group identified ten rating scales widely used for the cognitive assessment of patients with PD (Chou et al., 2010). Based on the time taken to administer and its ability to cover all major cognitive domains, as well as sensitivity to subtle cognitive changes in PD and the independent verification, the Montreal Cognitive Assessment (MoCA) (Nasreddine et al., 2005) was chosen to be used in this study. Moreover, by virtue of its widespread use in older PD studies, the mini-mental state examination (MMSE) (Folstein et al., 1975) was also included in the protocol.

As demonstrated in the Cambridgeshire Parkinsons Incidence from GP to Neurologist (CamPaIGN) study, verbal fluency testing is particularly sensitive to transition from non-demented PD to PD dementia (Williams-Gray et al., 2007;

Williams-Gray et al., 2009). Consequently, semantic and phonemic fluency testing was added to the clinical assessment.

Affect The available tools for the assessment of anxiety and depression in PD have previously been scrutinised by the Movement Disorders Society Task Force on Rating Scales for Parkinsons disease. Schrag and colleagues (Schrag et al., 2007) reviewed the depression scales used in PD. These included the Hamilton Depression Scale (Ham-D) (Hamilton, 1960), the Beck Depression Inventory (BDI)(Beck, 1961), the Geriatric Depression Scale (GDS) (Yesavage et al., 1983), 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) (Montgomery and Asberg, 1979), the Cornell Scale for the Assessment of Depression in Dementia (CSDD) (Alexopoulos et al., 1988), and the Centre for Epidemiologic Studies Depression Scale (CES-D) (Kirsch-Darrow et al., 2006).

The available anxiety depression scores were considered by Leentjens and colleagues (Leentjens et al., 2008).These included the Beck Anxiety Inventory (BAI) (Beck et al., 1988), the Hospital Anxiety and Depression Scale (HADS) (Zigmond and Snaith, 1983), the Zung Self-Rating Anxiety Scale (SDS) (Zung, 1971), the 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, 1959).

Of the depression scales available, the BDI and the Ham-D were considered to be the most researched scales in the context of PD. Both scales were recommended for both screening and severity assessment. Hence, the BDI was chosen to screen for and asses the severity of depression in the OPDC cohort. Unfortunately, none of the anxiety rating scales were recommended for use because of paucity of

evidence to support their use. In view of this, the Leeds Scale for Self-Assessment of Anxiety and Depression (Snaith et al., 1976) was used to assess anxiety in he cohort, based on the personal experience of the PI.

Olfaction At the time of the study protocol being formulated, no comprehensive comparisons of olfactory testing methods in PD were available. The two standardised and commercially available methods, as suggested by the American Association of Neurology (Suchowersky et al., 2006), were the University of Pennsylvania Smell Identification Test (UPSIT) (Doty et al., 1984) and Sniffin Sticks (Hummel et al., 2007). For practical and monetary reasons, the Sniffin Sticks were used to assess olfactory function in the cohort.

Sleep As is discussed throughout this thesis, overnight polysomnography (PSG) is the gold standard for the diagnosis of REM Sleep Behaviour Disorder. However, the required resources, manpower and the associated costs, make the use of PSG in large cohort studies impractical (Postuma et al., 2010). Therefore, a screening questionnaire was used in this study.

At the time the study protocol was being written, a number of means of screening for RBD in the PD population were found. These included:

- Sleep questionnaire (unvalidated) (Comella et al., 1998)
- Unstructured interview (Eisensehr et al., 2001)
- Semi-structured interview (Scaglione et al., 2005)
- Structured interview (Gagnon et al., 2002)
- Mayo Sleep Questionnaire (unvalidated) (Boeve et al., 2002)
- Sleep questionnaire (not RBD-specific) (Pacchetti et al., 2005)

- Sleepiness Questionnaire (unvalidated) (Gjerstad et al., 2008)
- Sleep questionnaire (unvalidated) (Vibha et al., 2011)
- The RBD Screening Questionnaire (RBDSQ; validated) (Stiasny-Kolster et al., 2007; Nomura et al., 2011)

As the RBDSQ was the only questionnaire to have been validated, it was chosen to be included in the study protocols. The full details of the RBDSQ are discussed in chapter 4.

2.2 Neuroimaging

As alluded to in the introduction, MRI relies on the natural properties of hydrogen ions, primarily found in the water molecules that make up approximately 80% of the human body. The properties of the hydrogen ions and how they behave within a MRI scanner depend on the composition of the tissue within which they are found. That is, the signal generated from these ions not only depends on how much water is within the tissue of interest, but also how freely the water molecules can move. Therefore, the hydrogen ions found within the CSF will behave differently from the found in the grey or white matter. So why does this happen and how can we exploit this property in neuroimaging? And how can these properties can be used to study brain function? To understand this, we must first consider the principles behind MRI.

2.2.1 The Principles of Magnetic Resonance Imaging

The nucleus of an hydrogen atom consists of a single proton precessing on its own axis. This may be graphically represented as a spinning top, as shown in **(Figure 2.1)**. The object is spinning with an angular frequency of ω radians per

second. As the top is spinning about an axis it has no net linear momentum. The angular momentum (often referred to as spin), however, describes the fact that every point in the top is moving with a certain velocity. The direction of the angular momentum vector can be found pointing along the axis of the spinning top, with the magnitude of the vector proportional to the speed of rotation. The angular momentum vector is labeled L on the figure. As the hydrogen atom is positively charged, its precession about its axis generates a magnetic field, called the magnetic moment. In biological samples, the hydrogen atoms are randomly orientated meaning that the individual magnetic moments cancel each other out and the net magnetic moment for the sample is zero (M=0; **(Figure 2.2)**).

Let us now assume that the sample is placed in an external magnetic field (B0), such as the one created by a MRI scanner. The angular momentum vector of the proton will try to align itself with the direction of the field so that it precesses around the direction of B0. In a magnetic field the energy levels for a nucleus with a spin number I will split into (2I + 1) discrete energy levels. Therefore, the magnetic moment of a proton (I=1/2) can be orientated in two ways: parallel or anti-parallel (where the anti-parallel orientation requires slightly more energy). The frequency of electromagnetic radiation necessary to switch between these two states is known as the Larmor frequency and is directly proportional to B0. By introducing a small gradient to the external magnetic field in the x-, y- and -z directions, this property can be utilised in imaging to allow localisation within a three-dimensional space.

Once all of the angular momentum vectors of the hydrogen atoms have aligned with B0 (by convention, this is displayed in the z direction), the sum of all of the vectors is known as the net magnetisation (M0, **(Figure 2.2)**). It is this net magnetisation that is the signal that can be measured with MRI. M0 is of the order of μT where as most modern MRI scanners generate a B0 of 3T, therefore, when

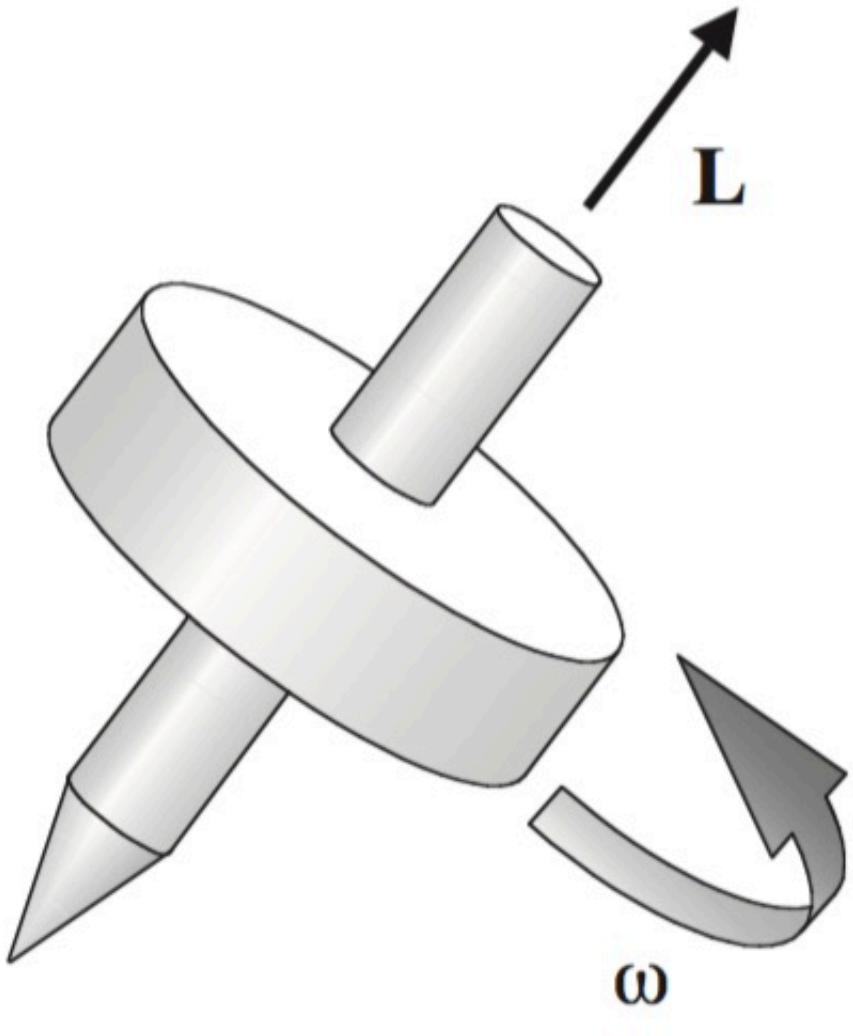

Figure 2.1: A spinning top rotating with an angular frequency ω radians per second has an angular momentum **L** pointing along the axis of the top. Adapted from Jezzard et al., 2003.

M0 is parallel to B0 it cannot be measured accurately. To get round this problem, M0 is tipped into the transverse, x-y, plane using a radiofrequency (RF) pulse. The RF necessary to do this is the Larmor frequency with a flip angle of 90 degrees. As long as the RF pulse is on, M0 will now be perpendicular to B0. In turn, this induces a current in the receiver coil designed to only be sensitive to magnetisation in the transverse plane. Crucially, the RF pulse causes a phase coherence of the

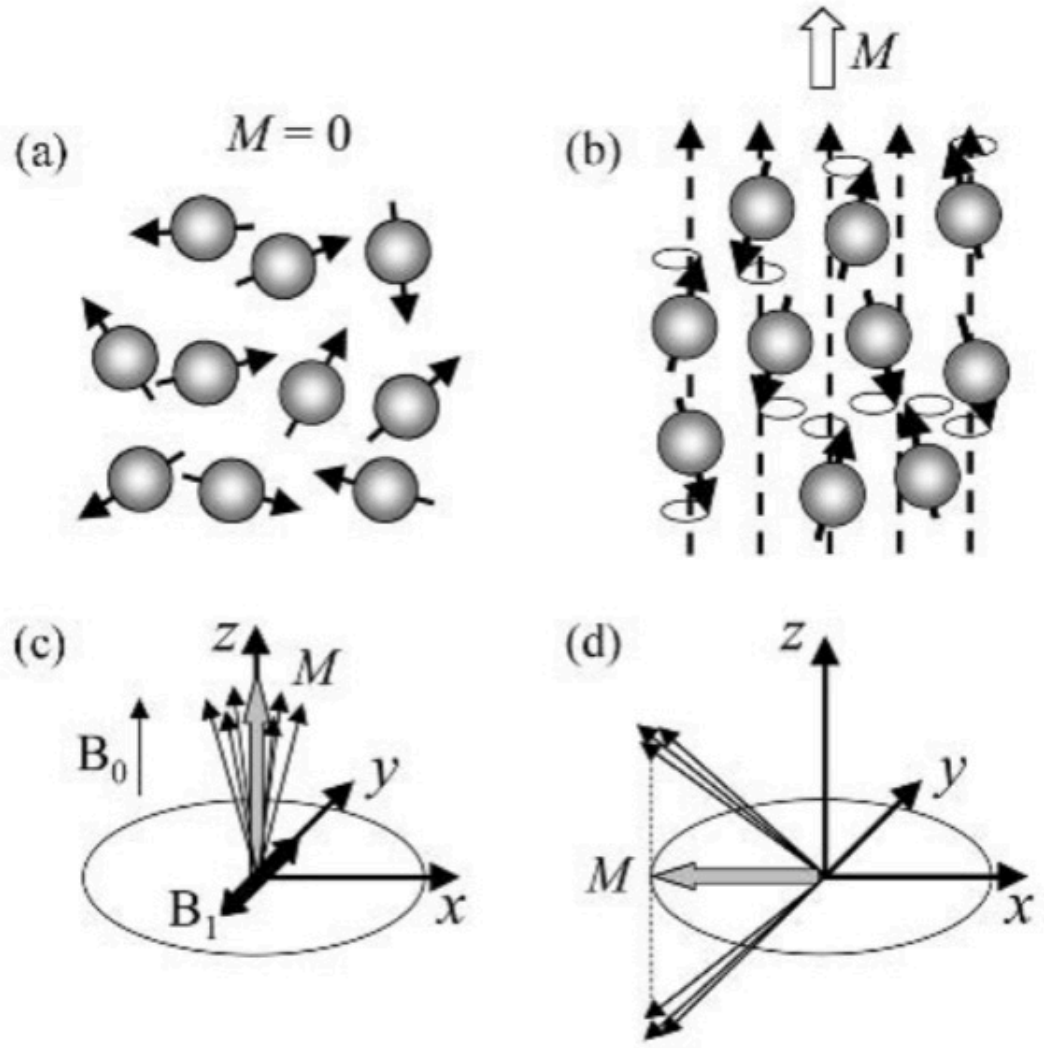

Figure 2.2: (a) Behaviour of a sample when placed in a strong magnetic field. (b) The nuclear magnetic moments are initially randomly oriented. (c) Gradually the moments align either with the field or against it. The slight preferential alignment along the direction of the field acts like a single magnetization vector M. (d) An oscillating B1 magnetic field can change the orientation of some of the nuclear moments until there is a net magnetization vector in the x-y plane. Adapted from Jezzard et al., 2003.

spins. Therefore, the amplitude of the signal picked up by the receiver coil is greatest just after excitation, after which, the signal decays exponentially. This decay is secondary to the realignment of the angular momentum vectors with B0 and spins dephasing because of random field fluctuations. It is this decay in the

signal that gives contrast to MR images.

Contrast

As well as flipping M0 to the transverse plane, the RF pulse also causes a phase coherence of the spins. Therefore, the amplitude of the signal picked up by the receiver coil is greatest just after excitation, after which the signal decays exponentially. This decay is secondary to the realignment of the angular momentum vectors with B0 and spins dephasing. As both of these processes depend on the properties of the tissue within which the spinning hydrogen atoms are found, it is this signal decay that gives contrast to MR images.

The time it takes for the hydrogen ions to align with B0 is called the spin-lattice relaxation time. T1 is defined as the time it takes for 63% of M0 to be restored along the z-axis following a 90 degree RF pulse. As the angular momentum vectors of the individual protons realign with B0, the energy absorbed from the RF pulse is released and absorbed by the surrounding environment (lattice). The energy transfer is lattice-specific and depends on how freely the hydrogen atoms can move within their environment. For example,the relatively free water found in CSF means that energy transfer is very inefficient and T1 is very long. Conversely, in white matter where the hydrogen atoms are more constrained within the myelin sheaf, energy transfer is much more rapid, leading to a considerably shorter T1 **(Table 2.1)**.

Due to the properties described above, the net magnetisation restored along the z-axis (Mz) a short time after the RF pulse will be highest in white matter, followed by grey matter and then CSF. If a second RF pulse is applied before Mz is fully restored, a higher restored Mz will lead to a higher magnetisation in the transverse plane and therefore a higher signal intensity. This means that if a second RF is applied before Mz = M0, contrast between the tissues can be generated. The time

Table 2.1: Relaxation times and proton densities of different brain tissues at 1.5T and 3.0T

Field strength (T)	Tissue	T1 (ms)	T2 (ms)	T2* (ms)	Proton density
1.5	White matter	510	67	78	0.61
	Gray matter	760	77	69	0.69
	Arterial blood	1441	290	55	0.72
	CSF	2650	280	n.a.	1.0
3.0	White matter	1080	70	50	0.61
	Gray matter	1820	100	50	0.69
	Arterial blood	1932	275	46	0.72
	CSF	3817	1442	n.a.	1.0

Table modified from MacIntosh and Graham, 2013.

between two RF pulses is called the repetition time (TR). As illustrated in **Figure 2.3**), by shortening the TR one can generate good contrast between the white matter (highest signal and hence appears bright), CSF (low signal and hence dark) and grey matter (intermediate). These principles form the basis of the T1-weighted structural imaging used in this thesis.

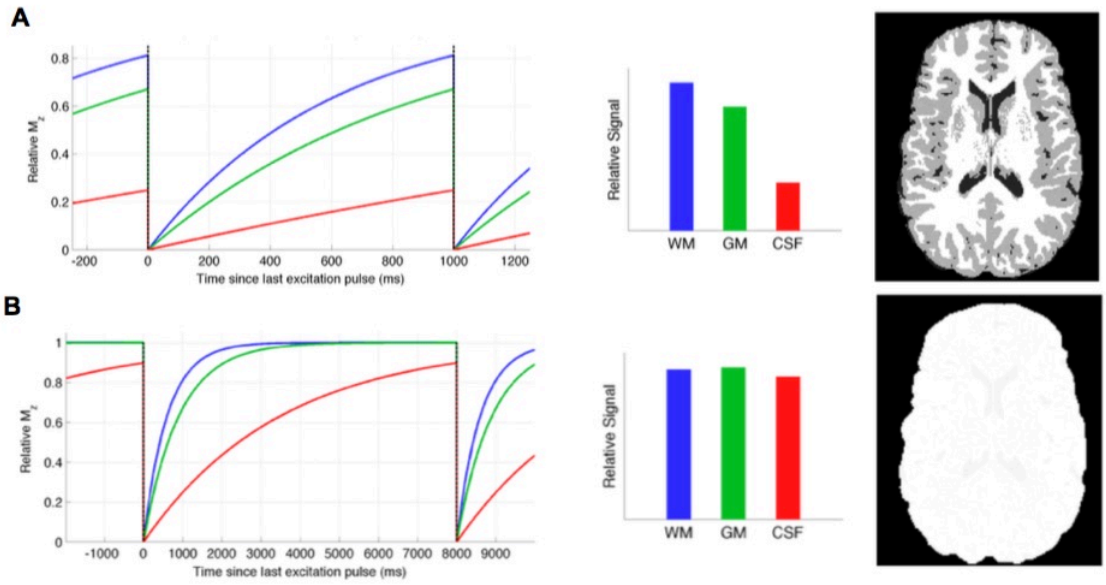

Figure 2.3: T1-weighted imaging. (A) WM (blue) has a shorter T1 and recovers more than GM (green) if a short TR is chosen. (B) A long TR leads to full recovery of Mz in WM and GM and does not produce any contrast. (Figure from FMRIB graduate course).

Let us now consider dephasing as the second contribution to signal decal. Dephasing occurs due to small differences in the precession frequencies of the spinning hydrogen atoms **(Figure 2.4)**. The rate of dephasing, dependent on random field inhomogeneities and spin-spin interactions, is referred to as spin-spin relaxation time or T2. By convention, T2 is the time taken for the transverse magnetisation to drop to 37% of its initial value. Similarly to spin-lattice relaxation, the energy transfer between spins is most efficient in white matter (short T2) and least efficient in CSF (long T2) **(Table 2.1)**. T2-weighted imaging makes use of spin-echo sequences. Here, a certain period after the initial 90 degree RF pulse, a second RF pulse is transmitted, this time with a flip angle of 180 degrees. This pulse does not stop the dephasing, instead reversing the phase angles. This means that, after a time equal to the delay between the 90 degree and the 180 degree RF pulse, the spins come into phase again. This leads to a second signal peak in the receiver coil, called an echo. The time between the initial 90 degree RF pulse and the echo is called the echo time (TE).

In order to curtail artefact, T2-weighted imaging is designed to minimise the effects of magnetic filed inhomogeneities and magnetic susceptibilities (the measure of how magnetised a tissue becomes when it is placed in a strong magnetic field). However, it is these properties that allow us to study brain function.

Any air pockets found inside the body, for example sinuses, have very low magnetic susceptibility. By contrast, blood has very high susceptibility because of the iron-rich haemoglobin. This difference in magnetic susceptibilities generates a small magnetic gradient at tissue boundaries, leading to more rapid dephasing on either side of the boundary. The relaxation time which takes into account magnetic filed inhomogeneities and magnetic susceptibility is denoted as T2*. T2*-weighted images are generated in a similar fashion to T2-weighted ones except for rather than using a RF pulse to refocus the spins, a magnetic gradient is applied instead

to initially dephase and then rephase the spins. This is referred to as a gradient echo (GRE) sequence.

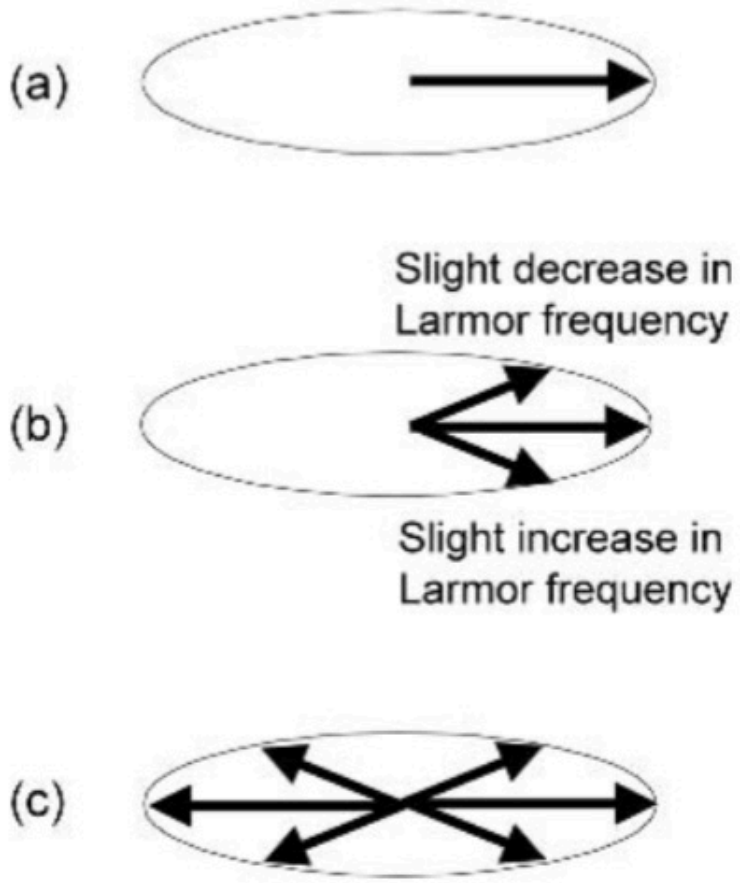

Figure 2.4: Decay of transverse magnetization. (a) Initially all the signal is in phase. (b) Random field fluctuations mean that some nuclei lag behind the system resonant frequency and some lead ahead. (c) Eventually the spread of frequencies means that the signal is no longer coherently pointing along one direction and there is no signal detected. Adapted from Jezzard et al., 2003.

BOLD Signal

T2*-weighted imaging forms the basis of functional MRI (fMRI). The physiological basis behind fMRI is as follows. Neuronal activity requires energy in the form of glucose and oxygen. This is supplied to the neurons by a network of blood

vessels. Increased neuronal activity requires an increase in blood supply, leading and increase in blood flow, volume and oxygenation. All three of these variables influence the blood oxygen level dependent (BOLD) signal. This is because they alter the ratio of diamagnetic (essentially non-magnetic) oxyhemoglobin (oxygenated blood) to paramagnetic deoxyhaemoglobin (deoxygenated blood). During increased neuronal activity, these three factors outweigh the oxygen utilised by the neurons. This results in decreased concentration of deoxygenated haemoglobin and leads to a decrease in magnetic susceptibility, a longer T2* and a higher MR signal.

2.2.2 Resting-state fMRI

The neuroimaging studies presented in this thesis made use of resting-state fMRI (rs-fMRI). Unlike the classical task-based fMRI paradigms where a subject is asked to perform a task within the scanner, rs-fMRI records neuronal activation while the subject is lying in the scanner with their eyes open and not doing a particular task. This makes rs-fMRI ideal when studying populations where performing a task within the scanner may be difficult.

Resting brain activity was probably first recorded using electroencephalography (EEG) by Hans Berger in 1929 (Berger, 1929). He reflected that identifying intellectual work using resting EEG may prove difficult as its contribution is insignificant compared to the background activity of the brain. Since then, the majority of studies, whether fMRI, EEG or magnetoencephalography (MEG), have strived to minimise background brain activity, dismissing it simply as noise. The breakthrough came in 1995 when Biswal and colleagues (Biswal et al., 1995) investigated spontaneous fluctuations in BOLD signal in subjects at rest. The authors extracted raw BOLD time-courses from the cortical hand area and correlated it with the activity throughout the rest of the brain. The resulting spatial map closely resem-

bled one obtained from classical task-based experiments, prompting the authors to conclude that distinct and separate areas associated with a specific function are synchronised, even at rest.

Since then, the spontaneous fluctuation in the resting brain activity have been characterised further allowing some basic principles to be established (Raichle, 2011). Background activity has been found to be present during the different stages of sleep and even when consciousness is impaired. Therefore, the recorded signal cannot simply represent an unconstrained conscious cognition. Moreover, the patterns of resting activity coherence extend beyond simple monosynaptic connections. Thus, they must represent more extensive functional networks. Lastly, the variability in the resting BOLD signal has been demonstrated to influence the intensity of the evoked BOLD response and affect behavioural outcomes. Therefore, it must signify an underlying physiological role. It is postulated that the resting BOLD activity is made up of the slow cortical potentials (SCP) that can be recorded from pre- and post-synaptic terminals using microelectrodes (Logothetis, 2008). It is believed that SCP allow neurons to vary the amplitudes of local field potentials (LFP) and modulate cortical excitability. Coherence of SCP-mediated fluctuations enables brain areas to form functional networks tasked with processing incoming stimuli in a similar manner (Raichle, 2011). The most well known and best characterised resting state networks are summarised in **Figure 2.5**.

Measuring functional connectivity

Functional connectivity is defined as the temporal correlation of a neurophysiological index measured in different brain areas (Biswal et al., 1995). Whereas anatomical connectivity refers to physical neuronal pathways, functional connectivity represents intermittent and synchronised interactions between spatially distinct brain regions (Margulies et al., 2010).

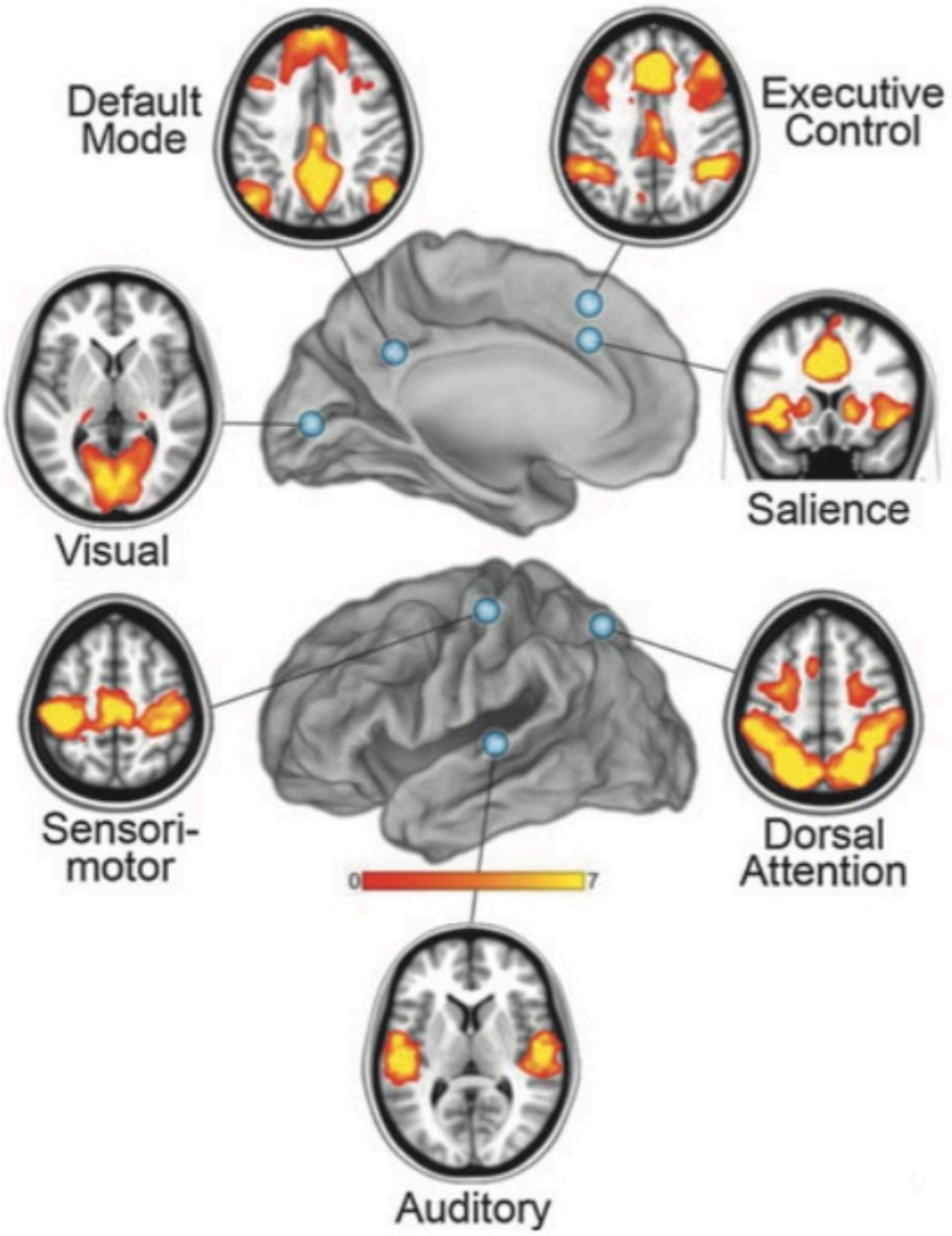

Figure 2.5: Major resting state networks. The red-yellow areas indicate brain regions connected by common low frequency BOLD fluctuations. Adapted from Raichle, 2011.

Seed-based correlation (SBC) methods form the simples means of studying functional connectivity. Here a voxel, cluster of voxels or a region of interest (ROI) is selected a priori and a mean BOLD time-series extracted. This is then linearly

correlated with either another ROI or will all other voxels in the brain. A spacial map can then be generated, displaying voxels that are significantly correlated with the original seed (Cole et al., 2010). This method is a suitable tool for testing a priori assumptions. Hierarchical clustering has been used to expand seed-based approaches by using a correlation matrix developed from the use of multiple seeds to determine which regions are most closely related (Cordes et al., 2002). An advantage of SBC analysis methods it that the results are focused on specific ROIs and may be easier to interpret in relation to neuroanatomy and a priori hypotheses. However, extracting a single mean time-series from the seed ROI means that one must assume that the structure is functionally homogeneous.

Regional homogeneity (ReHo) is another method used for resting-state fMRI analysis. ReHo uses Kendalls coefficient concordance (KCC) to measure the similarity of a given voxel with its nearest neighbours based on the BOLD time-series (Zang et al., 2004). As is the case with SBC methods, RoHo is relatively easy to calculate and understand. Moreover, it is relatively insensitive to possible region-to-region and trial-to-trial variability. However, it too is open to potential bias introduced by selecting a priori regions of interest.

Amplitude of low-frequency fluctuations (ALFF) is an index that reflects the intensity of spontaneous regional brain activity. This is done by calculating the voxel-wise magnitude of the fluctuation within a certain frequency range. As well as means of analysis functional connectivity, useful diagnostic information about neural processes may be present in the oscillatory amplitude envelopes (Yu-Feng et al., 2007; Zuo et al., 2010).Unfortunately, ALFF is particularly sensitive to physiological noise. In order to minimise this, fractional ALFF (fALFF) calculates the ratio of the power spectrum of a low frequency (0.01-0.08 Hz) to that of the entire frequency range. Although questions remain over the susceptibility of this method to physiological noise, independent testing have revealed moderate to

high levels of reliability and consistency in terms of the spatial maps generated (Zuo et al., 2010).

More recently, graph theory has been applied to the analysis of fMRI. Graph theory is a mathematical theory and approach to studying graphs made up of nodes and edges. This method allows one to interrogate how these nodes, inter-connected by edges, interact with each other (Sporns, 2003). Applying this methodology to neuroimaging, the brain is analogous to a graph in which voxels represent the nodes and the edges are the connections between them. Unlike more conventional univariate methods of resting-state fMRI analysis, graph theory can directly describe and compare different brain networks using topological parameters, such as clustering-coefficient, characteristic path length, degree of connectivity, centrality, and modularity (Van Den Heuvel and Pol, 2010). Whilst this makes this method very attractive, the results can be very difficult to interpret.

Independent Component Analysis (ICA)

Akin to graph theory, Independent Component Analysis (ICA) does not require observer-based selection of a region of interest. Instead, ICA assumes the at the brain is organised into functionally separate networks and sets out to identify the BOLD time-series unique to each of them (Margulies et al., 2010). This is often best explained using the cocktail party problem (Brown et al., 2001). Let us assume one is at a busy cocktail party with the room filled with many people all talking simultaneously. The problem described here refers to the task of separating the sound recorders from the whole room, back into the individual conversation streams.

When this analogy is applied to neuroimaging, one can think of the time-series from the individual resting-state networks as a single conversation stream and the data acquired during the scan as the sound recording from the room. During the

process of acquiring the data, the signal measured from each voxel is a mixture of the BOLD signal from the individual networks to which it belongs. The ICA algorithm aims to decode the mixed signal back into its individual components and generate the corresponding time-series. This information can then be used to generate spatial maps of the individual networks (Beckmann et al., 2005).

In order for ICA to take place, a number of important assumptions much be satisfied (Brown et al., 2001). Firstly, the signal sources much be independent and stationary. Whilst the spatial overlapping of the networks is permitted, they must not be highly co-linear (Cole et al., 2010). Secondly, the mixing of the BOLD signal within the individual voxels must be linear. Although this cannot be guaranteed, linearity of the mixing process is likely to be the best approximation of a biological process (Brown et al., 2001). Lastly, in order for ICA to generate a trustworthy result, the number of sources cannot exceed the number of detectors. Although the number of individual resting-state networks that can be extracted from a rs-fMRI study remains contentious, most studies extract between 20 and 60 networks. This is significantly fewer than the number of voxels in a single brain scan.

The major advantage of ICA is observer independence. Unlike other methods, the regions-of-interest are intrinsically derived by the analysis method rather than selected by the user. This reduces potential bias and variability within and between studies (Margulies et al., 2010). Another significant advantage of ICA is its ability to distinguish biological signal from structured noise (Salimi-Khorshidi et al., 2014). The noise from head movements or arterial pulsation, amongst others, can be isolated into their own components, improving the noise-to-signal ratio in the remaining networks (Griffanti et al., 2016). Lastly, as mentioned above, ICA allows a degree of spatial overlap between the networks. Therefore, unlike seed based methods, for example, ICA can be used to study the connectivity of a specific region within a number of separate networks.

Despite its merits, the shortcomings of ICA must also be considered. Firstly, the decomposition of the signal into the individuals components is obtained by means of iterative optimisation. This process introduces a degree of run-to-run variability and means that repeated runs on the same data may result in slightly different results. This type of variability can be reduced when selecting more stringent convergence criteria.

As already discussed, one must decide on the number of networks that will be extracted by ICA. This is referred to as the dimensionality of the ICA. Although automated methods for dimensionality selection exist (Beckmann et al., 2001), there is no one single way of doing this to represent the underlying neurophysiology. Valid reasons for the use of both high and low dimensionality exists and selection can be guided by a priori hypotheses about the networks being studied. As the precise features of the individual networks depend on the number of dimensions used, comparing results across different studies may be difficult.

Choosing an incorrect dimensionality for the analysis may make the results difficult to interpret. For example, when working with a high dimensionality model, a functional network can be split into a number of sub-networks. This can result in a large number of components which may be difficult to identify and classify (Tohka et al., 2008). Moreover, the connectivity of a single region may, over time, share varying connectivity patterns with different networks. This is referred to as nonstationarity of that region. If that particular region is shared across a number of networks, this varying pattern of connectivity may be lost during the decomposition process.

Despite some of the short-comings of the method which have to be considered during its application, ICA is one of the leading methods of rs-fMRI analysis. Based its merits, its application in PD, and local expertise, ICA was used to analyse the data presented in this thesis.

Chapter 3

Early Parkinson's disease and the resting brain

The study presented in this chapter has been published as a paper.

M Rolinski, L Griffanti, K Szewczyk-Krolikowski, R A L Menke, G K Wilcock, N Filippini, G Zamboni, M T M Hu, C E Mackay. "Aberrant functional connectivity within the basal ganglia of patients with Parkinson's disease" ***NeuroImage: Clinical***, 9(8):126–32, 2015

3.1 Introduction

Before testing a biomarker in the prodromal stages of PD, we must first show its utility in established disease. In this chapter I will present an analysis of resting-state functional connectivity in patients with early Parkinson's disease. I will follow on from work previously carried out by our lab, working towards a neuroimaging biomarker that may be translated into clinical practice. By including a disease control group, in this case patients with Alzheimer's disease, I will describe the sensitivity of this method to basal ganglia dysfunction.

3.1.1 Regional changes

Previous studies have adopted numerous methodologies to study the impact of PD on the resting brain. In 2011, Skidmore and colleagues (Skidmore et al., 2013) used Amplitude of Low Frequency Fluctuation (ALFF) to study patients with PD. ALFF is thought to reflect the intensity of spontaneous brain activity and, in line with previous PET (Moeller et al., 1999) and and continuous arterial spin labelling (Ma et al., 2007) studies, the authors demonstrated decreased activity in the supplementary motor area (SMA), nodes of the default mode network (DMN) and the cerebellum. Moreover, the authors used this method to correctly classify the groups with a sensitivity and specificity of 92% and 87%, respectively. Since then two studies have reported increased ALFF in patients with PD which partially corrected on the administration of levodopa (Kwak et al., 2012; Zhang et al., 2013). The supposedly conflicting results have been, at least partially, attributed to methodological differences.

Regional homogeneity (ReHo) is a voxel-based measure of brain activity which evaluates how well a signal in a given voxel is synchronised with its nearest neighbours. The aim of this method is to identify functional clusters (Zang et al., 2004). In 2009, Wu and colleagues reported reduced ReHo in the putamen and SMA of PD patients off medications (Wu et al., 2009). Furthermore, increased ReHo was observed the primary and the pre-motor areas, and the cerebellum, leading the authors to conclude that these changes may have a compensatory role. Similar results have more recently also been reported by Yang and colleagues (Yang et al., 2013).

3.1.2 Remote communications

Seed-based methods have classically been employed to study functional connectivity between remote brain regions. Here, covariation of the average BOLD timecourse from a predefined seed region with every voxel across the whole brain is calculated. Hence, distant functional connections can be studies. Helmich and colleagues studied the connectivity between the anterior and posterior putamen and the rest of the brain (Helmich et al., 2010) in PD patients off dopaminergic medications. Whilst they found decreased connectivity between the posterior putamen and the parietal cortex, increased connectivity was found between the anterior putamen and the same cortical region. This seemingly compensatory increase in the connectivity has since been observed in patients on levodopa (Hacker et al., 2012). Importantly, this study also showed reduced connectivity between the striatum and the brainstem and cerebellum.

Long and colleagues used a Support Vector Machine (SVM) method in an attempt to combine ALFF, ReHo and seed-based analysis to best differentiate patients with PD from healthy controls (Long et al., 2012). Only using functional connectivity measures, the groups could be differentiated with a sensitivity of 58% and a specificity of 85%. These could be increased to 79% and 93%, respectively, once quantitative measures of grey and white matter were added. It should be noted that the automatic classifier did not include any of the regions previously implicated in PD by the studies discussed above, instead selecting numerous cortical white matter regions. The relatively low number of subjects included in the study means that the model used was particularly susceptible to overfitting and the results should be interpreted with caution (De Martino et al., 2008).

3.1.3 ICA

As discussed in the methods section, seed-based methods require the identification of a pre-defined region of interest and assume functional homogeneity within that volume. Therefore, ICA-based analysis methods may provide a more data-driven approach. Tessitore and colleagues (Tessitore et al., 2012) were the first to use this approach in PD and found decreased connectivity in regions of the DMN in patients with PD. Activity in these regions correlated with clinical measures of cognitive function, such as word recall, the Corsi block span and a clock-drawing test. These results could not be replicated by Krajcovicova and colleagues (Krajcovicova et al., 2012). Although the difference in the results could be attributed to the different magnet field strength used in the two studies, it is likely that patient selection had the bigger impact. PD patients in the Tessitore study were more cognitively impaired and, hence, the results may be the reflection of this, rather than the presence of PD per se.

More recently, Esposito and colleagues (Esposito et al., 2013) studied the sensorimotor network (SMN), comparing ten drug-nave PD patients to eighteen healthy controls. The authors reported decreased connectivity within the SMA, which normalised on administration of levodopa.

3.1.4 The Basal Ganglia Network

Basal ganglia dysfunction invariably lies at the heart of PD (Eidelberg et al., 1990). The basal ganglia network (BGN) was first identified as an independent functional network in healthy controls (Robinson et al., 2009) and was subsequently shown in a meta-analysis of resting-state fMRI studies (Laird et al., 2011). In a study recently published by our group (Szewczyk-Krolikowski et al., 2014), we have shown a dramatic decrease of connectivity within the BGN of patients with early

PD scanned after an overnight withdrawal of their dopaminergic medications. Not only did these changes almost normalise when patients were given their medications, but they could be used to differentiate the PD patients from the healthy controls with specificity and sensitivity of 100% and 89.5%, respectively. When tested on an independent validation sample, the diagnostic accuracy of the method for PD was 85%.

The study of the BGN shows great promise for the development of a viable neuroimaging biomarker. However, the use of a data-driven method to study the whole of the BGN potentially opens this approach to interference from non-PD-specific group differences that may have an impact on the overall accuracy of the method when replicated using greater sample sizes. Moreover, it is not clear whether changes observed are specific to PD or whether they are representative of a more global process.

3.1.5 Aims

The aims of the study presented in this chapter were twofold:

1. To test whether a priori confines may be placed upon the analysis to establish a functional imaging signature for PD that is separate from the ageing process as a whole, as well as other forms of neurodegeneration known to have an impact on resting state networks; and

2. To assess whether any of the changes are specific to PD or relate to other forms of neurodegeneration.

3.2 Methods

3.2.1 Participants

Thirty-two patients with early PD (within 3.5 years of diagnosis) and 19 healthy-controls were recruited from the Oxford Parkinson's Disease Centre (OPDC) cohort. These participants were the same as those in our previous study (Szewczyk-Krolikowski et al., 2014). Prior to recruitment into the imaging arm of the study, all participants underwent extensive assessment, including a structured general medical interview, detailed characterisation of motor and non-motor features, and cognitive assessment. Only patients that met the UK PD Society Brain Bank Criteria for clinically probable idiopathic Parkinson's disease (Hughes et al., 1992), as assessed by the study neurologist, were included in the PD group. Moreover, in order to select a clinically homogeneous group and minimise the effect of movement artefact, only the participants with minimal tremor were selected. PD subjects were scanned in a clinically defined off-state, a minimum of 12 hours after the withdrawal of their dopaminergic medications. The healthy control group consisted of subjects with no family history of parkinsonism, recruited largely from the spouses and friends of the PD participants. Healthy controls were not receiving any medications known to affect the dopaminergic system. Both groups only included subjects classified as cognitively healthy, as defined by a Mini-Mental State Examination (MMSE) $>$26 and no subjective complaint of memory problems.

A further thirty-one patients with clinically probable Alzheimer's disease were recruited from the Oxford Project to Investigate Memory and Ageing and from the Memory Assessment Clinic at the John Radcliffe Hospital in Oxford, United Kingdom (Zamboni et al., 2013). Patients with AD met both the DSM-IV for dementia and the National Institute of Neurological and Communicative Disease and Stroke/Alzheimer's Disease and Related Disorders Association criteria for

probable AD dementia and had never taken cholinesterase inhibitors. These patients were selected as they have already been scanned using an identical imaging protocol on the same MRI scanner.

The study was undertaken with the understanding and written consent of each subject, with the approval of the local NHS ethics committee, and in compliance with national legislation and the Declaration of Helsinki.

3.2.2 Neuroimaging data acquisition

Scanning was performed at the Oxford Centre for Clinical Magnetic Resonance Research (OCMR) using a 3T Trio Siemens MRI scanner (Erlangen, Germany) equipped 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, 1x1x1 mm3 voxel size, TE/TR/TI = 4.7ms/2040ms/900ms). Acquisition time for the MP-RAGE was 6 minutes. Functional images were acquired using gradient echo planar imaging (EPI) (TR=2000ms, TE=28ms, flip angle=89, resolution=333.5mm). 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 their eyes open. Field maps were also acquired to reduce EPI distortion due to magnetic field inhomogeneity (TR=488ms, TE=5.19ms and 7.65ms).

3.2.3 Brain volume measurements

Brain tissue volume, normalised for subject head size, was estimated with SIENAX (Smith et al., 2002). Tissue-type segmentation with partial volume estimation was carried out (Zhang et al., 2001) in order to calculate grey matter volumes.

Volumes of the subcortuical structures were estimated using FMRIB's Integrated Registration and Segmentation Tool (FIRST) (Patenaude et al., 2011). The volume of each subcortical structure was adjusted for individual head size differences via multiplication by the volumetric scaling factor derived from SIENAX.

3.2.4 Analysis of rs-fMRI

Resting state analysis was performed using probabilistic independent component analysis (ICA) as implemented in the Multivariate Exploratory Linear Optimized Decomposition into Independent Component FSL tool (MELODIC) (Beckmann and Smith, 2004), part of the FSL software package (Woolrich et al., 2009). Individual pre-statistical processing consisted of motion correction, brain extraction, unwarping using fieldmap data, spatial smoothing using Gaussian kernel of FWHM of 6mm, and high-pass temporal filtering of 150s. To account for the effect of motion, non-neural physiology, scanner artefacts and other confounds, we employed a previously described ICA–based de–noising approach. Briefly, after performing subject-level ICA with automated dimensionality estimation, FIX tool was used to automatically classify the obtained components into signal or noise (Salimi-Khorshidi et al., 2014). The contribution of noise was then regressed out from the data, based on the unique variance related to the noise components and motion confounds from the preprocessed datasets (Griffanti et al., 2014).

Once preprocessed, data was linearly registered to the corresponding structural image using FLIRT (Jenkinson et al., 2002), optimised using Boundary-Based Registration, and registered to Montreal Neurological Institute (MNI) space using non-linear registration.

In order to allow direct comparison across the three cohorts, a resting state template, including the basal ganglia network (BGN) and 21 residual noise components, generated from 80 healthy elderly subjects (Szewczyk-Krolikowski et al.,

2014) was used **(Figure 3.1)**. The 21 residual noise components that were included were not fully removed by FIX and were identified as residual noise based on the identification of standard noise components (Beckmann, 2012) and location of signal peaks in non-grey matter areas (e.g. white matter, CSF, skull).

The dual regression approach (Filippini et al., 2009) was used to identify individual temporal dynamics and the associated spatial maps of the BGN. In the first step of dual regression, the template including the BGN and 21 noise components maps was used in a general linear model (GLM) fit (as spatial regressors) against the separate fMRI data sets previously cleaned with FIX, the output being the corresponding temporal dynamics for each component and subject. Secondly, these time–courseswere used in a second GLM fit (as temporal regressors) against the cleaned fMRI datasets to estimate subject–specific spatial maps. Finally, the BGN maps were collected across subjectsand tested voxel–wise for statistically significant differences between groups using non–parametric permutation testing with randomise (v. 2.1; part of FSL). The statistical analysis was confined to the basal ganglia as defined by the Harvard–Oxford Subcortical Atlas (Mazziotta et al., 2001), in order to test intra–basal ganglia functional connectivity. Results were corrected for age, sex and total grey matter volume, anddefined as significant at $P<0.05$ fully corrected for multiple comparisons using the Threshold–Free Cluster Enhancement (TFCE) approach (Smith and Nichols, 2009).

3.2.5 Subcortical regions of interest

Subcortical masks were created from the Harvard–Oxford Subcortical Atlas (Mazziotta et al., 2001). The generated masks were used to extract mean parameter estimates (P.E.), representing the connectivity of a given voxels with the timecourse of the whole network, from subject–specific BGN spatial maps, from the following ROIs: caudate, pallidum and the posterior and anterior putamen, bilaterally.

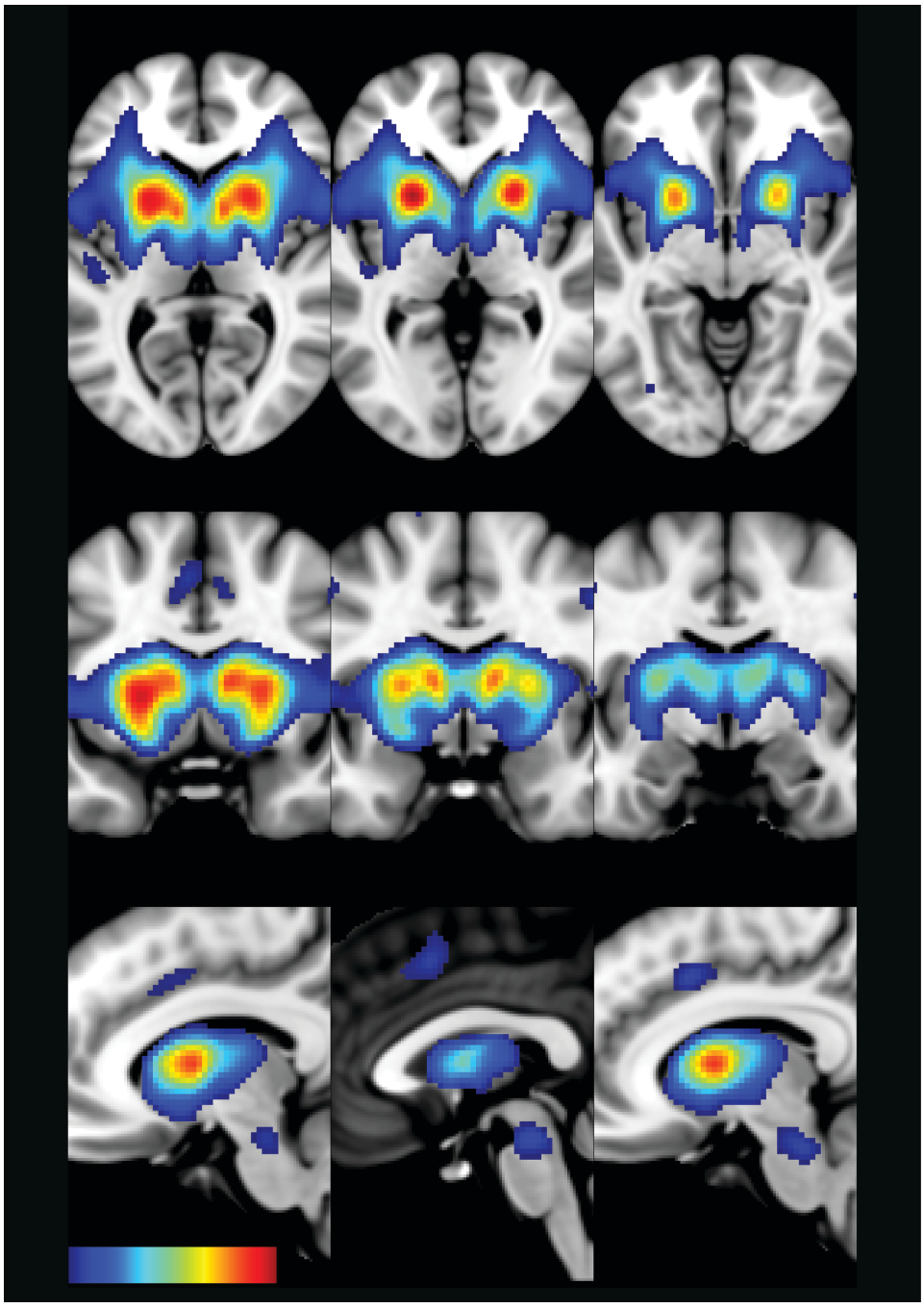

Figure 3.1: An unbiased template of the resting state basal ganglia network (BGN) generated from 80 healthy controls (Szewczyk-Krolikowski et al., 2014). The spatial map is thresholded at z>2.6.

The boundary between the anterior and posterior putamen was taken to be the posterior aspect of the fornix on the axial plane.

3.2.6 Statistical analysis

All statistical analyses in this study, other than the statistical analyses included in the MRI analysis tools, were performed using Statistical Package for the Social Sciences version 22.0 (SPSS, Chicago, IL, USA). Demographic, clinical and volumetric MRI continuous data were statistically compared between the groups using analysis of variance (ANOVA); the Bonferroni method was used to correct for multiple comparisons. The Pearson χ^2 test was to compare ordinal variables. A principal component analysis (PCA)-based dimension reduction method was used to combine values extracted from the ROIs of all subjects into a single factor. The alpha was set at 0.05.

3.2.7 Candidate's contribution to the study

The candidate designed the study. Data collection for the HC and PD groups was performed in conjunction with Drs Griffanti, Szewczyk-Krolikowski and Menke. Data for the AD group was provided by Dr Zamboni and Prof. Wilcock. The candidate analysed the data, interpreted the results and wrote the manuscript on which this chapter is based.

3.3 Results

3.3.1 Participants

Mean disease duration for patients with PD (n=32) was 25.0 (13.9) months. There were no significant differences in age, sex, MMSE, total grey matter or subcortical volumes between healthy controls and subjects with PD **(Table 3.1)**. Subjects with AD were older and had reduced whole-brain grey matter volume and the volume of the putamen.

Table 3.1: Comparisons of demographic and anatomical characteristics.

	Healthy Controls (HC)	Subjects with Alzheimer's disease (AD)	Subjects with Parkinson's disease (PD)	Group comparisons
Demographic characteristics				
n	19	31	32	-
Age, years	60.6 (7.7)	74.5 (6.5)	62.1 (11.9)	AD-HC[a], AD-PD[a]
Gender, F:M	08:11	15:16	14:18	ns
Disease duration, months	-	-	25.0 (13.9)	-
Mean LEDD, mg	-	-	332 (226)	-
UPDRS III (OFF)	-	-	27.0 (12.6)	-
MMSE	29.4 (1.4)	20.7 (5.7)	28.4 (1.6)	AD-HC[a], AD-PD[a]
Anatomical characteristics				
Corrected total grey matter volume, cm^3	705.2 (47.1)	640.6 (38.1)	697.5 (47.9)	AD-HC[a], AD-PD[a]
Corrected volume of the caudate, cm^3	4.4 (0.6)	4.6 (0.5)	4.5 (0.4)	ns
Corrected volume of the pallidum, cm^3	2.3 (0.3)	2.2 (0.5)	2.3 (0.4)	ns
Corrected volume of the putamen, cm^3	6.0 (0.6)	5.2 (0.7)	5.9 (0.8)	AD-HC[a], AD-PD[a]

F, female; M, male; MMSE, Mini-Mental State Examination; ns, non-significant. [a]Post hoc test significant at $p<0.0005$

Table 3.2: Mean absolute head motion

	HC (n=19)	AD (n=31)	PD (n=32)	Group comparisons
Mean absolute head motion, mm (SD)	0.55 (0.53)	0.59 (0.43)	0.30 (0.15)	AD-PDa
Mean proportion of components removed as noise, % (SD)	40.8 (8.0)	45.6 (12.8)	38.2 (12.6)	AD-PD[a]

[a]Post hoc test significant at $p<0.05$

3.3.2 rs-fMRI analysis

Details of mean absolute head motion and the proportion components identified as noise by FIX are shown in **Table 3.2**. No subjects had to be excluded on the basis of excessive head motion. On average, patients with AD had more head motion, and had a marginally larger proportion of components identified as noise, when compared to subjects with PD but not healthy controls. The mean connectivity maps for each group are shown in **Figure 3.2**.

3.3.3 Voxel-wise analysis

Voxel-wise comparisons restricted to the basal ganglia demonstrated widespread decrease in connectivity within the network in subjects with PD when compared to healthy controls, subjects with AD, and healthy controls and patients with AD, combined **(Figure 3.3)**. Reduced connectivity was observed in the caudate, pallidum and the putamen, bilaterally. No differences within the basal ganglia network were found between the HC and AD groups.

3.3.4 Region of interest analysis

As demonstrated in the voxel-wise analysis, measures of functional connectivity within the regions of interest were markedly altered in the Parkinson's disease group **(Table 3.3 and Figure 3.4)**. Mean parameter estimates (P.E.) were significantly lower in all of the subcortical regions, with the greatest decrease in P.E., compared to the healthy control group, observed in the posterior putamen

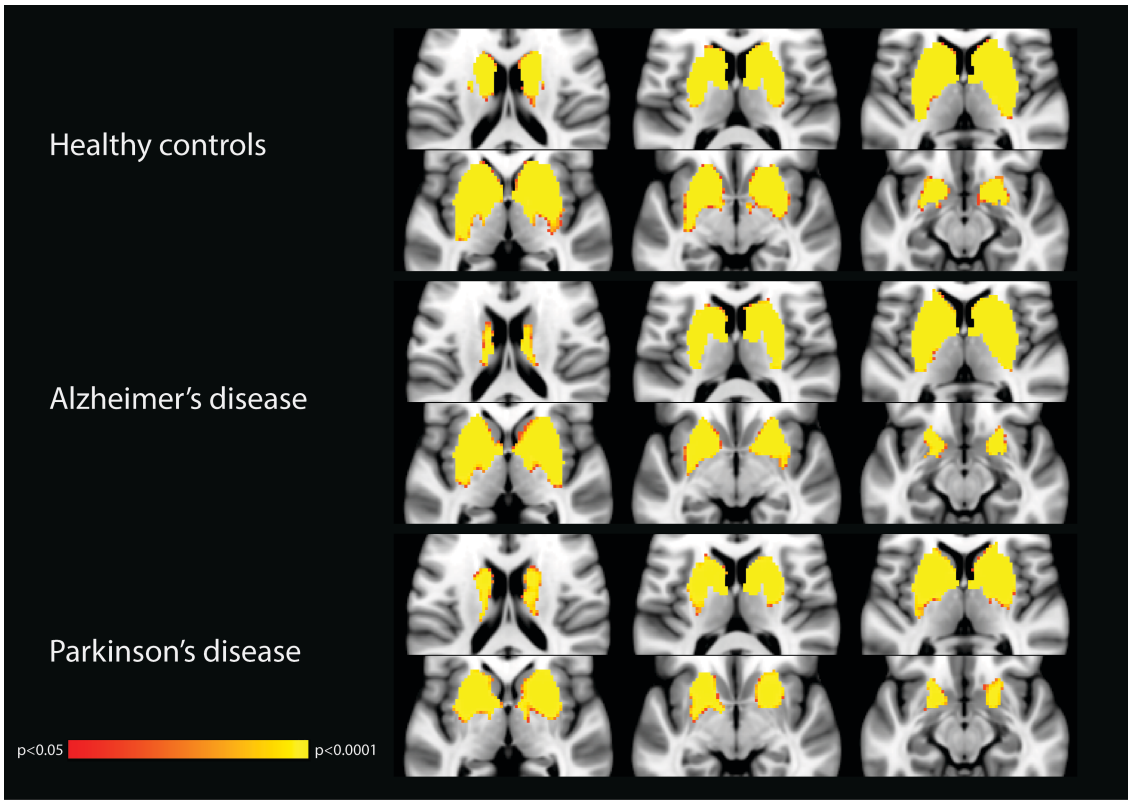

Figure 3.2: Group-level map of the BGN for each study group. Images are displayed in radiological convention (right is left). Clusters are thresholded at P>0.05 after FWE correction.

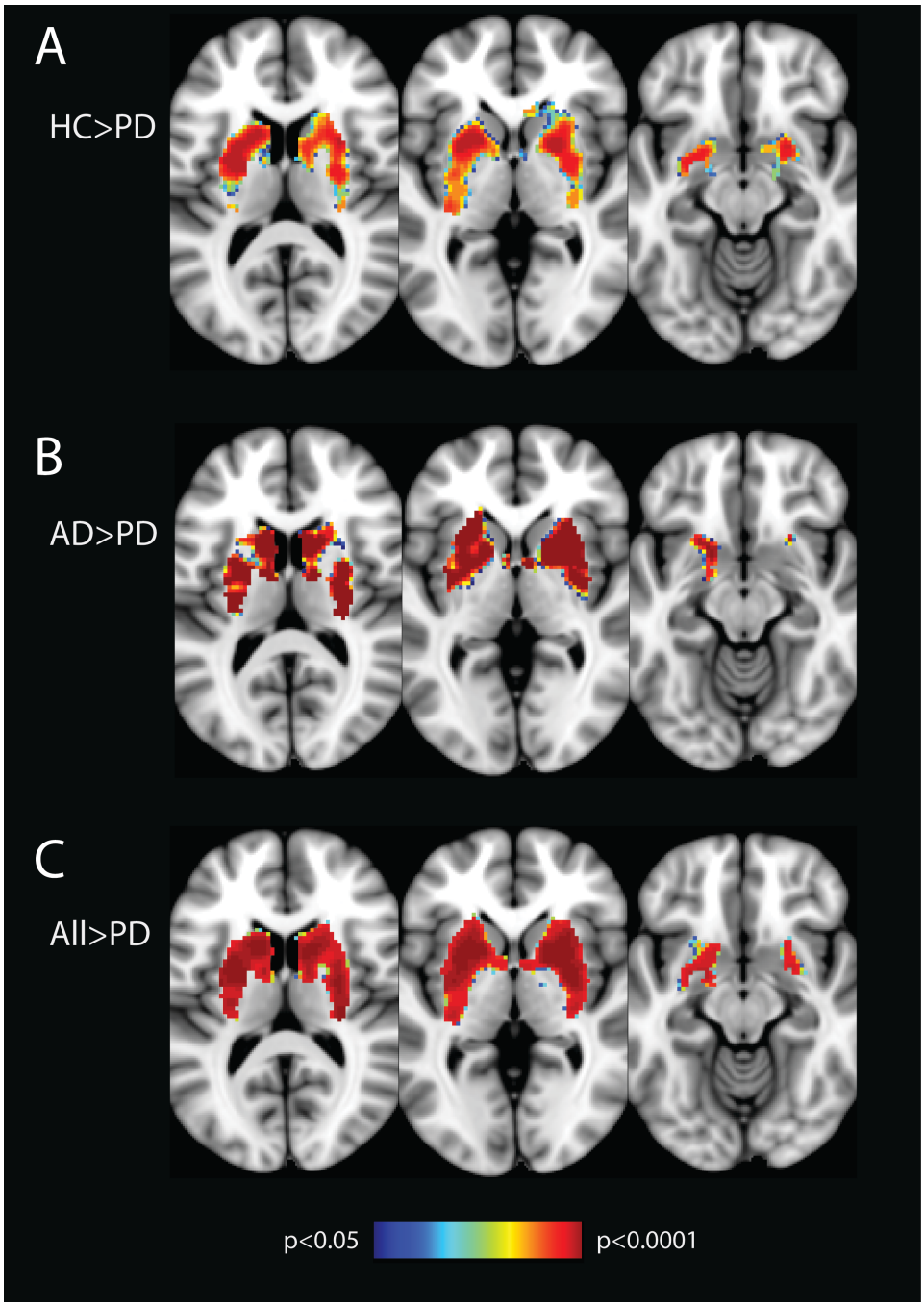

Figure 3.3: Voxel-wise analysis confined to the basal ganglia showing decreased connectivity in PD patients compared to a) healthy controls; b) patients with Alzheimer's disease; and c) patients with Alzheimer's disease and healthy controls, combined. Results show significantly reduced connectivity in PD in all regions of the basal ganglia. In image c, voxels showing reduced connectivity make up 68.9%, 99.0% 98.3% and 99.7% of the total volume of the caudate, pallidum, anterior putamen and the posterior putamen, respectively. Images are displayed in radiological convention (right is left). Clusters are thresholded at P>0.05 after FWE correction.

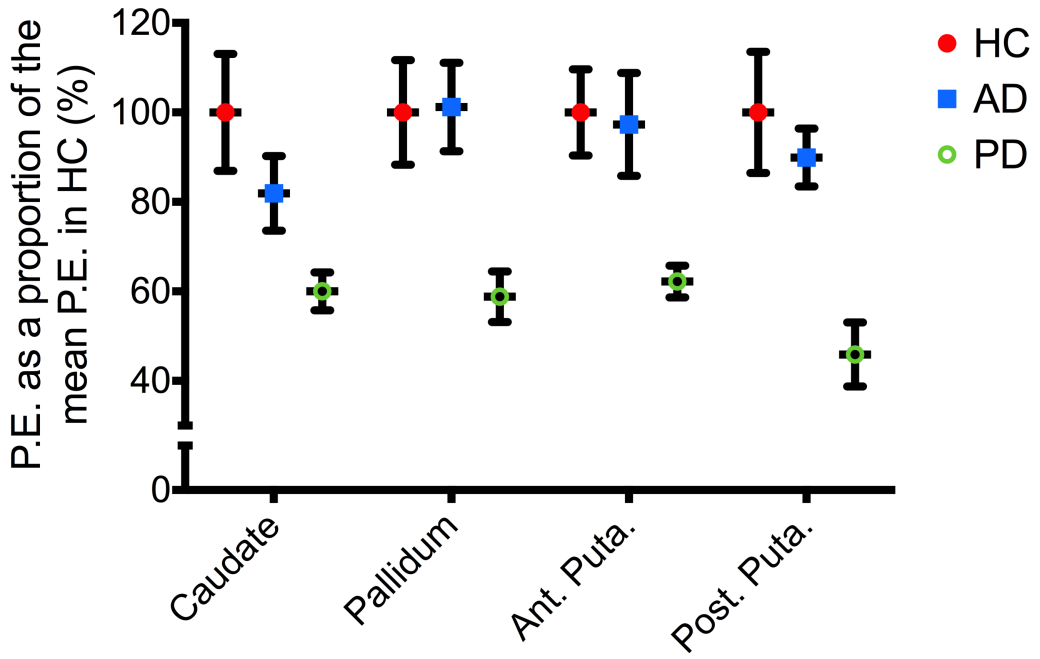

Figure 3.4: Regions of interest analysis. Average of the mean P.E. extracted bilaterally from the regions of interest, expressed as a proportion of the mean P.E. for that region in healthy controls (mean ± standard error of the mean). Results demonstrate significantly reduced P.E. in all regions in PD, compared to healthy controls and patients with Alzheimer's disease. HC, healthy controls; AD, Alzheimer's disease; PD, Parkinson's disease; P.E., parameter estimates; Ant. Puta., anterior putamen; Post. Puta., posterior putamen.

($p<0.005$). Although the mean P.E. in the caudate of subjects with AD was lower than that of healthy controls, this difference did not reach statistical significance (95% confidence interval -45.7 to 8.1).

Table 3.3: Mean parameter estimates extracted from regions of interest within the basal ganglia.

	Healthy Controls (HC)	Subjects with Alzheimer's disease (AD)	Subjects with Parkinson's disease (PD)	Group comparisons
Caudate	27.6 (15.6)	22.6 (12.0)	16.0 (7.0)	AD-PD[b], HC-PD[b]
Pallidum	17.0 (9.7)	17.2 (10.6)	10.0 (6.3)	AD-PC[c], HC-PD[c]
Anterior putamen	36.5 (14.4)	35.5 (14.4)	22.7 (7.4)	AD-PD[a], HC-PD[a]
Posterior putamen	21.8 (11.0)	19.6 (11.1)	10.0 (7.9)	AD-PD[b], HC-PD[b]

[a]Post hoc test significant at $p<0.0005$; [b]Post hoc test significant at $p<0.005$; [c]Post hoc test significant at $p<0.05$

3.3.5 The diagnostic utility of resting state connectivity

In order to ascertain the diagnostic utility of resting state connectivity analyses, and represent basal ganglia activity as a whole, the mean parameter estimates across the four regions of interest were coalesced into a single component score using principal component analysis (PCA)-based dimensionality reduction. The component accounted for 68.3% of the total variance in the data. As was the case for the individual regions of interest, the mean PCA component score was significantly lower in Parkinson's disease than that in healthy subjects and subjects with Alzheimer's disease **(Figure 3.5a)**. The mean component score did not correlate with disease severity (UPDRS III motor score) or disease duration. The Receiver Operating Characteristic (ROC) area under the curve (AUC) was 0.81 (95% C.I. 0.71 to 0.90), when comparing the PD group to the group including both HC and AD **(Figure 3.5b)**. The mean component score was a better discriminator than when the individual mean parameter estimates from the four regions of interest were used. The mean component score for PD patients scanned in the clinically defined on-state was significantly higher than those in the off-state ($p<0.0001$). There was no statistically significant difference between PD patients taking dopaminergic medication, patients with Alzheimer's disease and healthy controls ($p=0.4$).

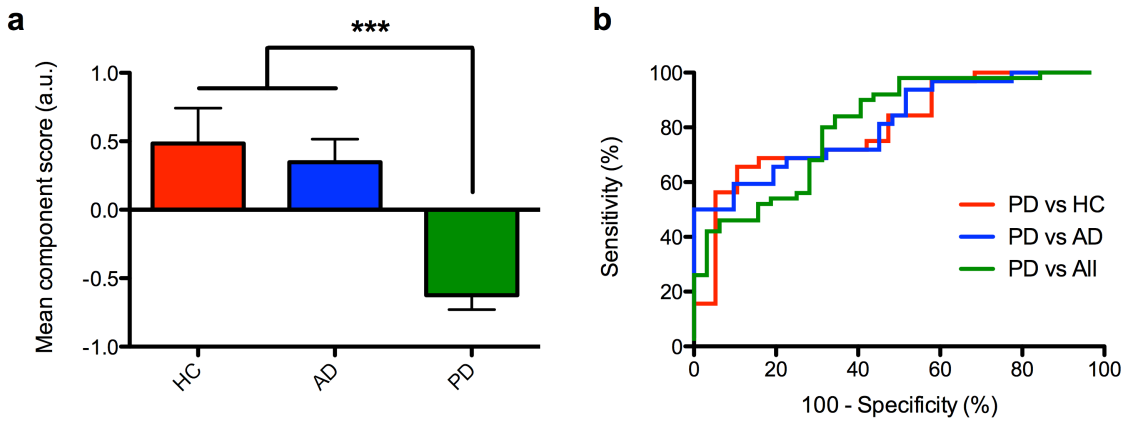

Figure 3.5: The diagnostic utility of resting state connectivity. a) Mean component scores were calculated using a data-driven principal component analysis-based dimensionality reduction method by combining the mean parameter estimates from each of the regions of interest. Results demonstrate a significantly lower score in patients with PD, when compared to patients with Alzheimer's disease and healthy controls. ***$p<0.0001$. b) Individual component scores were used to generate receiver operating characteristics (ROC) curves. Area under the curve was 0.80 (95% C.I. 0.68-0.93), 0.81 (95% C.I. 0.71-0.92) and 0.81 (95% C.I. 0.71-0.90) for the PD vs HC, PD vs AD and PD vs All comparisons, respectively.

3.4 Discussion

Using resting state fMRI we have previously shown connectivity differences in patients with PD relative to controls. Using a refined methodology, here we reproduce this finding and show that aberrant connectivity within the basal ganglia network is not a widespread feature of neurodegeneration, being absent in AD. Moreover, by extracting mean parameter estimates from a priori defined regions of interest within the basal ganglia, we have shown that resting-connectivity of the basal ganglia may have a potential role as a diagnostic biomarker for PD. Hence, by moving away from a single study-specific design and by including disease controls, we contribute to the translational pipeline for the development of reliable and clinically useful imaging biomarkers.

3.4.1 Resting state connectivity reflects basal ganglia dysfunction

In line with previous task based (Monchi et al., 2007; Wu et al., 2010) and resting state studies (Hacker et al., 2012; Wu et al., 2012; Szewczyk-Krolikowski et al., 2014), we have comprehensively shown reduced connectivity within the basal ganglia network in PD subjects, including the caudate, putamen and pallidum, when compared to healthy controls. Importantly, we have also shown that the reduction in resting state connectivity is apparent in the PD group not only when compared to a healthy aged population, but also to patients with another diffuse neurodegenerative condition, in this case, Alzheimer's disease, which is typically associated with changes within the default mode network (DMN) (Greicius et al., 2004). At face value, this may seem somewhat surprising given the evidence for basal ganglia involvement in AD from pathophysiological (Ikonomovic et al., 2008) and imaging studies (de Jong et al., 2008; Madsen et al., 2010). Indeed, we have shown reduced total grey matter volume, as well as the volume of the putamen, in patients with AD, when compared to those with PD. Despite this, however, dopamine and homovanillic acid levels seem not to be altered in AD (Langlais et al., 1993), suggesting that overall function is preserved. This may explain why there are no statistically significant differences in resting state connectivity between patient with AD and healthy controls. Therefore, resting state connectivity seems to reflect basal ganglia function independently from non-specific neurodegeneration.

3.4.2 Heterogeneous pattern of basal ganglia involvement in PD

As can be seen from the voxel-wise analyses (Figure 2), the involvement of the basal ganglia in PD is not uniform, with the caudate relatively spared. The difference in mean PE's is particularly striking between the caudate and posterior putamen (Figure 3). This is in agreement with previous post-mortem (Kish et al., 1988;

Wilson et al., 1996; Goldstein et al., 2011) and radiotracer imaging (Morrish et al., 1998; Nurmi et al., 2001) studies showing a heterogeneous pattern of basal ganglia involvement in PD, with the greatest loss of dopaminergic function in the posterior putamen, and caudate the least affected. Moreover, a recent seed-based resting state connectivity study (Hacker et al., 2012) showed a graded pattern of striatal functional connectivity with the brainstem of patient with PD and healthy controls. The authors speculated that this gradient represents the underlying susceptibility to dopaminergic dysfunction in PD.

3.4.3 Utility as a neuroimaging biomarker

When combined into a single component score using a data-driven dimensionality reduction technique, reduced connectivity scores within the basal ganglia separated patients with PD from the other participants with a diagnostic sensitivity of 81%, similar to the 85% diagnostic accuracy using the method previously described by our group (Szewczyk-Krolikowski et al., 2014). The method used herein may, however, have a number of advantages. Firstly, parameter estimates were extracted from whole regions of interest selected a priori on the basis that they are known to play a key role in the pathophysiology of Parkinson's disease. This is in contrast to the previous method where parameter estimates were extracted using a mask of voxels in the PD group that showed reduced connectivity when compared to a healthy control group. As this mask was created from a relatively small number of participants, the results obtained using it may not be transferrable to other patient groups and MRI scanners. Clearly, both methods of obtaining measures of basal ganglia function must be validated on a completely separate group of PD patients and healthy controls. Secondly, by focussing on regions of interest, this method allows us to study the function of individual constituents of the basal ganglia. The pattern of decreased connectivity resembles that obtained using radiotracer

imaging and has the potential to further our understanding of the pathophysiology of Parkinson's disease.

3.4.4 Limitations and future work

Further testing of larger independent study groups is necessary to validate our results. Moreover, in order to reduce imaging artefacts and conceivable confounding effects on the basal ganglia network, the generalizability of our findings may have been limited by excluding subjects with tremor-dominant disease. Whilst previous studies have not found any difference in basal ganglia connectivity in patients with and without tremor (Helmich et al., 2010), and tremor does not appear to be correlated with basal ganglia dysfunction (Eidelberg et al., 1995; Benamer et al., 2003; Isaias et al., 2007), further studies are necessary to confirm that the results of this study are transferrable to the tremor-dominant PD group. Lastly, the diagnostic accuracy of 81% using the method described herein is less than the diagnostic accuracy of a clinical assessment carried out by an experienced clinician (Hughes et al., 1992). Current work is concentrating on the integration of multiple brain networks to improve the sensitivity and specificity for PD. Moreover, further studies are required to ascertain whether this method is sensitive enough to pick up basal ganglia dysfunction in prodromal PD, for it to have a role as a potential imaging biomarker. This will be explored further throughout this thesis.

3.4.5 Conclusions

Results of this indicate that resting-state functional MRI can be used to demonstrate the aberrant functional connectivity within the basal ganglia of patients with early Parkinson's disease. These changes are not seen in Alzheimer's disease, suggesting that they are representative of true basal ganglia dysfunction, and

not just generalised neurodegeneration. Although further validation is necessary, extracting measures of functional connectivity from a priori regions of interest within the basal ganglia shows promise as a diagnostic biomarker of PD.

Chapter 4

Impact of REM sleep behaviour disorder on concomitant Parkinson's disease

The study presented in this chapter has been published as a paper.

M Rolinski, K Szewczyk-Krolikowski, P R Tomlinson, K Nithi, Y Ben-Shlomo, M T M Hu. "REM sleep behaviour disorder is associated with worse quality of life and other non-motor features in early Parkinson's disease" ***Journal of Neurology, Neurosurgery and Psychiatry***, 85(5):560–566, 2013

4.1 Introduction

In the previous chapter I have proposed a potential method for developing a novel neuroimaging biomarker for the diagnosis of early and prodromal PD. Across the next few chapters I will concentrate on developing an enriched at-risk cohort in which biomarkers of prodromal PD can be studied. As discussed before, we know that patients with idiopathic RBD are at an increased risk of developing PD. In this chapter I will present an analysis of the impact of concomitant RBD on the clinical phenotype of early PD.

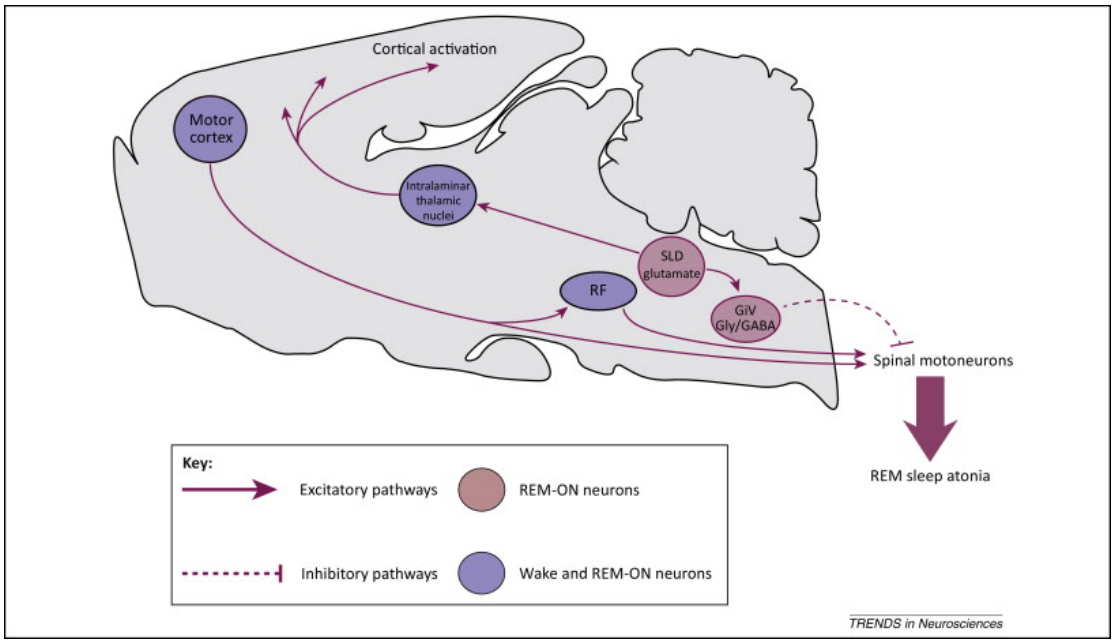

Figure 4.1: Circuitry responsible for motor control in rapid eye movement (REM) sleep and its potential involvement in REM sleep behaviour disorder (RBD). During REM sleep, the descending REM-ON glutamatergic neurons of the sublaterodorsal tegmental nucleus (SLD) excite the REM-ON GABA- and glycine-releasing neurons in the ventral gigantocellular reticular nucleus (GiV). These GiV neurons project to and inhibit skeletal motoneurons, which causes REM sleep atonia. Another population of ascending SLD neurons induce activation of the cortex during REM sleep (including the motor cortex) by exciting intralaminar thalamocortical neurons. In healthy REM sleep, the SLD-GiV circuit inhibits motoneurons, which prevents pyramidal neurons in the motor cortex from producing movement. However, in patients with RBD, degeneration of the SLD-GiV circuit releases motoneurons from their normal source of inhibition, which allows excitatory projections from the motor cortex (via brainstem reticular neurons) to produce motor behaviours during REM sleep. Image from Peever et al., 2014.

4.1.1 The pathophysiology of RBD

A number of case reports have suggested that lesions, typically either ischaemic or inflammatory, within or near the mesencephalic and pontine tegmentum are associated with the development of RBD in humans (Kimura et al., 2000; Plazzi and Montagna, 2002; Zambelis et al., 2002; Provini et al., 2004; Tippmann-Peikert et al., 2006). Although, not clearly understood, a number of animal studies, mainly in cats (Hendricks et al., 1982; Lai and Siegel, 1988; Lai and Siegel, 1990; Shouse and Siegel, 1992; Lai and Siegel, 1997; Morrison, 1998) and rats (Boissard et al., 2002;

Boissard et al., 2003; Lu et al., 2006), have allowed us to speculate as to the neuronal circuitry that underlies RBD.

It is believed that the normal loss of muscle tone during REM sleep is the result of two mechanism, one passive and one active (Siegel, 2006). Therefore, RBD may result from dysfunction of either or both of these pathways. The passive pathway relies on serotonergic neurons, which project to motor nuclei, reducing their firing rate as a person moves from light to deep non-REM sleep. As the individual enters REM sleep, these neurons cease to fire resulting in atonia (McGinty and Harper, 1976; Trulson and Jacobs, 1979). A cat model of RBD has been used to show that the serotonin cells in the dorsal raphe fail to switch off during REM sleep (Trulson et al., 1981). The involvement of the serotonergic pathway in generating REM atonia may explain the association between the use of common antidepressants and the onset of the symptoms of RBD (Winkelman and James, 2004; Parish, 2007; Teman et al., 2009).

Additionally to the passive mechanism, an active process which occurs specifically during REM sleep has been identified. This mechanism is summarised in **Figure 4.1**. Although the pathways and structures discussed here relate to mouse brains, similar regions are likely to be involved in humans. neurons of the sublaterodorsal tegmental nucleus (SLD) are referred to as REM-ON as they are more active during REM sleep than during non-REM sleep and the awake period. SLD neurons are glutaminergic (Clement et al., 2011) and are thought to induce atonia by activating the GABA and glycinergic neurons within the ventral and alpha magnocellular reticular formation. In turn, these directly inhibit the skeletal motoneurons (Lai and Siegel, 1988; Vetrivelan et al., 2009). Further to this, Lu and colleagues (Lu et al., 2006) have suggested that the SLD can act directly in the on the spinal cord, inducing atonia by activating inhibitory spinal interneurons.

Although the pathways described above are required to induce atonia during

REM sleep, other circuits are necessary to drive the complex movements observed in patients with RBD. The red nucleus, laterodorsal tegmental, and pedunculopontine nucleus (PPN) are thought to be involved in generating phasic movements during REM sleep, with cells in these nuclei showing synchronous discharges with muscle twitches during REM sleep (Gassel et al., 1966; Karlsson et al., 2005; Lim et al., 2007). Furthermore, a number of possible candidate brain regions, including the ventrolateral periaqueductal grey, locus coeruleus, dorsal raphe, substantia nigra, lateral hypothalamus, amygdala, thalamus and basal forebrain, are thought to be involved in modulating complex behaviours (Brown et al., 2012). Pathology in many of these regions has previously been described in patients with RBD (Gagnon et al., 2006; Boeve et al., 2007; Mathis et al., 2007).

4.1.2 PD and concomitant RBD

The prevalence of concomitant RBD in patients with established PD varies greatly, depending on patient selection and the means of diagnosing RBD. Predictions range from 15% to 64%, depending on the clinical definition of the disorder, if video telemetry is used, and the bed partners are systematically interviewed (Arnulf, 2012). However, with the majority of these studies performed in the advanced stages of PD, less is known about the association between RBD and the early motor stages of PD.

All of the evidence to date suggest a number of brainstem structures have a role in the development of RBD in humans. The most likely candidates include the SLD, the magnocellular reticular formation (MCRF), the dorsal raphe nucleus and the peduncolopontine nucleus (PPN) (Peever et al., 2014). As these brainstem structures correspond to Stage 2 of the Braak pathophysiological classification of PD (Braak et al., 2003), one would expect RBD to be closely associated with early PD.

4.1.3 Aims

The aims of the study presented in this chapter were twofold:

1. To assess the prevalence of concomitant RBD in patients with early motor PD.

2. To assess whether concomitant RBD has an impact on the motor and non-motor phenotype of PD.

4.2 Methods

4.2.1 Patient selection

A consecutive sample of early PD patients (time from diagnosis 3.5 years) was prospectively recruited as part of the Oxford Parkinson's Disease Centre (OPDC) cohort. Participants were recruited between September 2010 and August 2012. Patients were eligible for study inclusion if they met the UK Parkinson's Disease Society Brain bank criteria (Hughes et al., 1992) for the diagnosis of idiopathic Parkinson's disease, as judged by a neurologist. Patients with secondary parkinsonism due to head trauma or medication use, or features of atypical parkinsonism syndromes, such as multiple system atrophy, progressive supranuclear palsy, corticobasal degeneration or dementia with Lewy bodies, were excluded. The study was undertaken with the understanding and written consent of each subject, with the approval of the local NHS ethics committee, and in compliance with national legislation and the Declaration of Helsinki.

4.2.2 The REM sleep behaviour disorder screening questionnaire

Participants were asked to complete the REM Sleep Behaviour Disorder Screening Questionnaire (RBDSQ) (Stiasny-Kolster et al., 2007) prior to attending the study clinic. The RBDSQ is a patient self-rating instrument with ten questions (yes/no) assessing various aspects of sleep behaviour **(Appendix 1)**. The input of the patient's bed partner is encouraged but is not necessary. The questions are as follows:

- Questions 1 to 4 assess the content and frequency of dreams and their relationship to nocturnal movements and behaviour;
- Question 5 asks about potential nocturnal injuries sustained by the patient or their bed partner;
- Question 6 is divided into four sub-sections and is designed to assess nocturnal motor behaviour, e.g. vocalization, sudden limb movements, complex movements or bedding items that fall down;
- Questions 7 and 8 deal with nocturnal awakenings;
- Question 9, deals with disturbed sleep in general; and
- Question 10 asks about presence of any neurological disorder.

The maximum total score of the RBDSQ is 13, with a higher score suggesting more features associated with RBD. A cut-off score of 5 was reported by the authors as most useful when differentiating patients with idiopathic RBD from controls (Stiasny-Kolster et al., 2007). As this cut-off is only associated with a specificity of 0.56 (Stiasny-Kolster et al., 2007), sensitivity analyses were also performed using a RBDSQ cut-off of 6 and 7, in light of subsequent validation studies that found

stronger test performance characteristics using these higher cut-off (Nomura et al., 2011; Chahine et al., 2013).

As a formal polysomnography study remains necessary to formally diagnose RBD, the RBDSQ only allows the selection of those with probable RBD (pRBD).

4.2.3 Patient evaluation

A study neurologist and a trained research nurse carried out comprehensive evaluation of each patient blinded to the results of the RBDSQ. The following measures of interest were included:

1. **Patient demographics**, including age, sex and smoking history. A comprehensive past medical history was taken. Disease duration was calculated from the date the diagnosis was made. The delay to diagnosis was defined as the number of months between the onset of motor symptoms and the date of diagnosis. As the date of symptom onset was based on participant relocation, and thus open to bias, only disease duration was included in further analyses. A detailed history of all medication use was taken from the patients and, where available, medication records were reviewed. For dopaminergic medications, the levodopa equivalent daily dose (LEDD) was calculated. The response to PD medications was assessed using the patient-rated Clinical Global Impression of Change Scale (CGIC) (Goetz et al., 2008). Concomitant use of medications associated with symptoms of RBD was recorded (anti-depressants and bisoprolol (Iranzo and Santamaria, 1999)). Where available, clinic letters were reviewed for the presence of resting tremor, rigidity, bradykinesia and postural instability at the time of first presentation.

2. **Motor features of PD**, including parts II, III and IV of the Movement Disorders Society (MDS) revised Unified Parkinson's Disease Rating Scale (UPDRS)

(Goetz et al., 2008). Tremor and postural instability/gait disorder (PIGD) scores were calculated as the sum of all tremor (maximum sub-score of 36) and postural instability and gait disorder (maximum sub-score of 8) scorings on UPDRS III (Sixel-Doring et al., 2011). Hand dexterity, motor speed and coordination were assessed using the Purdue Pegboard Test; the total number of pegs inserted using each hand individually and then both hands over a total of 90 seconds, and the total number of components assembled during the 60 second assembly task were used as the outcome measures. The Timed Up & Go (the average time over three attempts to get up from sitting and walk 3m) and the Flamingo tests (testing the ability of the patient to balance on one leg for 30s) were also performed to assess gait and postural stability. All motor assessments were performed while the subject was taking their usual PD medications in a clinically defined on state.

3. **Non-motor features of PD**, including UPDRS I. Daytime sleepiness was assessed using the Epworth Sleepiness scale (ESS). Olfaction was assessed using the Sniffin' Sticks odor identification test. Participants were presented with 16 felt-tip pens scented with 16 common odors and asked to identify each one from a choice of four (maximum score of 16). Cognition was assessed using the Mini-Mental State examination (MMSE) and the Montreal Cognitive Assessment (MoCA). Scores on both tests were normalized for years of education (calculated as the number of years at school plus years of further education). Cognitive impairment was defined according to the MMSE and MoCA screening cut-offs ($<$26/30) (Dalrymple-Alford et al., 2010). For phonemic and semantic fluency, the total number of words generated beginning with F, A and S, and animal and boys' names category respectively over 60 seconds each was counted. The Beck Depression Inventory was used to evaluate depression (Beck et al., 1996). The Questionnaire for Impulsive-

Compulsive Disorders in Parkinson's Disease (QUIP Anytime Short) was used to test for impulse control disorders (gambling, sexual, buying and eating behaviors) and compulsive behaviors (punding, hobbyism and walk-about (excessive, aimless wandering)) (Weintraub et al., 2009). A postural drop in blood pressure was defined as the difference in the systolic blood pressure measured with the patient lying on the examination couch for three minutes, and again two minutes after standing. In relation to constipation, participants were asked about their bowel frequency and laxative use.

4. **Health-related quality of life** was assessed using the EQ-5D questionnaire (EuroQol, 1990), with patients asked to assess their quality of life according to five domains (mobility, self-care, activities, pain and anxiety), as well as scoring their health out of 100 on a visual analogue scale.

4.2.4 Statistical analysis

Analysis of descriptive variables was performed using two-tailed unpaired t tests and χ^2 tests for continuous and ordinal variables, respectively. Multivariable linear and logistic regression analyses were used to determine the independent relationship between pRBD and the dependent variable, controlling for age, sex, disease duration and smoking history (number of pack-years, where one pack-year equates to smoking 20 cigarettes per day for one year). We have presented both the crude results (means and percentages) as well as the adjusted difference in means and odds ratios (95% confidence intervals and p-values) for continuous and binary variables respectively. The total years of education was also used as a covariate when comparing the MMSE and MoCA. Cut-off for statistical significance was defined as $p<0.05$. No formal adjustment was made for multiple testing but p-values between 0.01-0.05 were treated with caution, unless previously reported,

given the possibility of type I errors.

4.2.5 Candidate's contribution to the study

The candidate designed the study. The clinical data was collected by four study neurologists, including the candidate. The candidate performed all statistical analyses, interpreted the results and wrote the manuscript on which this chapter is based.

4.3 Results

A total of 481 individuals participated in the study. Six subjects were excluded due to an incomplete RBDSQ, leaving 475 for analysis, of whom 292 were male (61.5%). The mean (SD) age was 67.7 (9.5) years and mean (SD) disease duration was 1.5 (1.0) years. According to patient reports, none of the participants had previously been given the diagnosis, either clinical or PSG-confirmed, of RBD. Only four participants were on medications used to treat the symptoms of RBD (one participant was receiving melatonin and three were on clonazepam). A total of 224 subjects with PD (47.2%, 95% confidence interval [CI] 42.7 to 51.9%) were identified to have a diagnosis of pRBD using the RBDSQ at the recommended cut-off score of five. One hundred and seventy six (37.1%, 95% CI 32.8 to 41.7%) and 141 (29.7%, 95% CI 25.6 to 33.4%) subjects were diagnosed with concomitant pRBD using a RBDSQ cut-off of six and seven, respectively. The results using a RBDSQ cut-off of five are described herein.

4.3.1 Basic demographics

Table 4.1 shows the demographics and medication details of the PD-nonRBD and PD-RBD groups. Both groups had similar ages, disease duration and duration of

Table 4.1: Demographic and medication data in subjects with Parkinson's disease (PD-nonRBD) and Parkinson's disease and probable REM sleep behavior disorder (PD-RBD).

		PD-nonRBD (n=251)	**PD-RBD (n=224)**	**p-value**
Age, y		67.9 ± 9.5	67.5 ± 9.4	0.6
Sex, % male		57.4	66.1	0.05
Disease duration, y		1.4 ± 1.0	1.6 ± 1.0	0.14
Delay to diagnosis, y		1.7 ± 1.7	1.6 ± 1.7	0.8
Taking PD medications	Any, %	90	93.8	0.14
	Levodopa, %	51	58	0.12
	Dopamine agonist, %	40.6	38.8	0.7
	LEDD, mg/d	322.9 ± 196.7	345.2 ± 189.6	0.2
Patients reporting improvement on medication, %		77.6	85.6	0.03
Taking medications associated with symptoms of RBD, %		12.7	21.4	0.01
Smoking history, %		34.3	44.8	0.02

symptoms prior to the formal diagnosis of PD being made. The PD-RBD group was more likely to be male, although there was only weak evidence against the null hypothesis (p=0.05). The use of PD medications did not differ between the two groups, with no difference between the numbers of patients on levodopa or dopamine agonists. While the levodopa equivalent daily dose (LEDD) was the same in both groups, subjects with pRBD were more likely to report benefit from their PD medications on the CGIS (77.6% vs. 85.6%, p=0.03). Both use of medications previously associated with symptoms of RBD and smoking was more common in the PD-RBD group. The presence of resting tremor, rigidity, bradykinesia and postural instability at the time of diagnosis was comparable in the two groups (clinic letters from the time of diagnosis were available for 337 of the 475 participants (70.9%)).

4.3.2 Severity of motor symptoms

There was no statistically significant difference in the quantitative motor measures between patients with PD with and without pRBD **(Table 4.2)**. The total UPDRS III motor score was the same in the two groups, as was the total tremor and postural

instability/gait disorder (PIGD) score, and Hoehn and Yahr stage. Participants with pRBD performed similarly well on the Purdue Pegboard, Timed Up and Go and Flamingo tests. There was no difference in the frequency of reported dyskinesia or motor fluctuations. Participants with RBD more frequently reported problems with every motor aspect of experience of daily living included in UPDRS II (score > 0), with problems with speech, chewing and swallowing, turning in bed, and walking and balance reaching statistical significance. Freezing was reported more than two times more commonly by subjects with concomitant pRBD ($p<0.001$), but no difference in gait freezing was observed as part of UPDRS III (PD 7/251 vs. PD/RBD 9/224, p=0.5).

4.3.3 Non-motor symptoms

Participants with pRBD scored significantly higher on the Epworth Sleepiness Scale, reported fewer bowel motions per day and had a larger drop in systolic blood pressure on standing from a lying position **(Table 4.3)**. Although subjects without pRBD tended to score higher on the Sniffin Smell Test, this was consistent with chance (p=0.07). Higher depression scores were seen using the Beck Depression Inventory in the pRBD group. A statistically significant difference in baseline cognition was evident on the MMSE (p=0.03), but not the MoCA (p=0.2), with no evident difference in phonemic and semantic fluency. There was no difference in the frequency of impulsive-compulsive disorders in the two groups.

Patients with concomitant PD and pRBD had a poorer outcome in almost every non-motor domain of UPDRS I. Patients with pRBD reported a statistically significant increase in the frequency of cognitive impairment, hallucinations, depressed and anxious mood, and apathy. Moreover, they suffered more pain, urinary problems, constipation and light-headedness on standing.

Table 4.2: Motor symptoms in subjects with Parkinson's disease (PD-nonRBD) and Parkinson's disease and probable REM sleep behavior disorder (PD-RBD)

Continuous measures		**PD-nonRBD (n=251)**	**PD-RBD (n=224)**	**Adjusted difference in means* (PD-RBD - PD-nonRBD) (95% CI)**	**p-value***
UPDRS III	Total	26.9 ± 11.2	26.8 ±,10.6	-0.12 (-2.08, 1.84)	0.9
	Tremor score	3.3 ±,2.4	3.4 ±,2.8	0.03 (-0.44, 0.50)	0.9
	PIDG score	1.2 ±,1.2	1.3 ±,1.3	0.17 (-0.05, 0.39)	0.14
	Hoehn & Yahr	1.9 ±,0.5	1.9 ±,0.5	0.15 (-0.26, 0.56)	0.5
Purdue Pegboard, s	Total	28.7 ±,6.9	28.0 ±,6.9	-0.57 (-1.72, 0.59)	0.3
	Assembly	17.0 ±,6.2	17.0 ±,6.1	0.07 (-0.97, 1.11)	0.9
Timed Up and Go, s		10.0 ±,4.5	10.6 ±,4.9	0.79 (-0.05, 0.63)	0.07
Binary measures				**Adjusted odds ratio* (95% CI)**	
UPDRS IV, %	Dyskinesia	0.05	0.05	0.89 (0.38, 2.02)	0.8
	Motor fluctuations	0.04	0.03	0.68 (0.24, 1.97)	0.5
UPDRS II, %	Speech	32.1	45.7	1.52 (1.03, 2.25)	0.03
	Saliva and drooling	46.4	55	1.30 (0.89, 1.88)	0.16
	Chewing and swallowing	16.8	38.1	1.80 (1.45, 2.83)	0.01
	Eating tasks	46.4	46.6	0.98 (0.68, 1.42)	0.9
	Dressing	54.8	64.3	1.42 (0.98, 2.06)	0.07
	Hygiene	35.6	44.3	1.39 (0.96, 2.02)	0.09
	Handwriting	60	63.3	1.05 (0.71, 1.56)	0.8
	Doing hobbies and other activities	59.2	63.8	1.14 (0.78, 1.65)	0.5
	Turning in bed	47.2	60.6	1.77 (1.22, 2.57)	0.003
	Tremor	79.6	80.5	1.05 (0.66, 1.68)	0.8
	Getting out of bed	65.2	72.4	1.40 (0.93, 2.12)	0.1
	Walking and balance	59.6	69.5	1.48 (1.00, 2.19)	0.05
	Freezing	10.4	23.5	2.64 (1.65, 4.22)	<0.001
Flamingo, %		45.7	42.9	0.80 (0.53, 1.21)	0.3

*Adjusted for age, sex, disease duration and smoking history

Table 4.3: Non-motor symptoms in subjects with Parkinson's disease (PD-nonRBD) and Parkinson's disease and probable REM sleep behavior disorder (PD-RBD).

Continuous measures		**PD-nonRBD (n=251)**	**PD-RBD (n=224)**	**Adjusted difference in means* (PD-RBD - PD-nonRBD) (95% CI)**	**p-value***
Sniffin Smell Test		7.5 ± 3.0	7.0 2.9	-0.51 (-1.06, 0.04)	0.07
Orthostatic systolic BP drop, mmHg		4.7 ± 16.4	9.3 16.7	4.58 (1.60, 7.56)	0.003
Beck Depression Inventory		8.1 ± 5.5	10.9 7.3	2.84 (1.61, 4.07)	<0.001
MMSE		27.5 ± 2.1	27.1 2.4	-0.44 (-0.83, -0.05)**	0.03**
MoCA		24.8 ± 3.5	24.2 3.7	-0.42 (-1.05, 0.21)**	0.2**
Fluency	Phonemic	38.8 ± 14.0	38.7 14.1	0.02 (-2.51, 2.55)	1
	Semantic	35.4 ± 9.1	34.1 9.2	-1.16 (-2.71, 0.39)	0.14
Binary measures				**Adjusted odds ratio* (95% CI)**	
Epworth Sleepiness Scale >9, %		38.3	55.8	2.03 (1.35, 3.07)	0.001
<1 bowel motion/day, %		38.2	47	1.48 (1.01, 2.17)***	0.043***
QUIP, %	Gambling	1.7	1.4	0.84 (0.19, 3.63)	0.8
	Sex	2.9	5.9	1.90 (0.71, 5.05)	0.2
	Buying	3.7	6.5	1.58 (0.64, 3.90)	0.3
	Eating	5.3	8.1	1.63 (0.76, 3.50)	0.2
	Hobbyism	11.7	15.3	1.26 (0.71, 2.22)	0.4
	Punding	3.7	8.1	2.27 (0.98, 5.27)	0.05
	Walkabout	0.8	1.4	1.48 (0.24, 9.14)	0.7
	Medication Use	2.1	2.7	1.32 (0.38, 4.55)	0.7
UPDRS I, %	Cognitive impairment	30.8	47.3	1.93 (1.33, 2.81)	0.001
	Hallucinations	8.8	19.2	2.39 (1.35, 4.21)	0.002
	Depressed mood	16.4	26.8	1.99 (1.27, 3.13)	0.003
	Anxious mood	27.9	35.3	1.51 (1.02, 2.23)	0.05
	Apathy	13.5	22.8	1.93 (1.19, 3.16)	0.008
	Features of DDS	2.4	5.8	2.46 (0.91, 6.68)	0.08
	Sleep problems	63.2	82.3	2.72 (1.77, 4.18)	<0.001
	Daytime sleepiness	77.5	84.5	1.57 (0.96, 2.56)	0.07
	Pain	76.8	85	1.77 (1.08, 2.89)	0.02
	Urinary problems	59.6	70	1.65 (1.11, 2.44)	0.01
	Constipation	40.7	58.8	2.10 (1.44, 3.04)	<0.001
	Light headedness on standing	35.7	52.5	1.97 (1.36, 2.86)	<0.001
	Fatigue	69.5	76.5	1.40 (0.93, 2.12)	0.1

*Adjusted for age, sex, disease duration and smoking history; **Adjusted for age, sex, disease duration, smoking history and years of education; ***Adjusted for age, sex, disease duration and laxative use.

Table 4.4: The impact of health on the activities of daily living in subjects with Parkinson's disease (PD-nonRBD) and Parkinson's disease and probable REM sleep behavior disorder (PD-RBD).

EQ-5D	Continuous measures	PD-nonRBD (n=251)	PD-RBD (n=224)	Adjusted difference in means* (PD-RBD - PD-nonRBD) (95% CI)	p-value*
	Patient-reported health score	71.9 ± 16.9	66.4 ± 19.6	-5.05 (-8.32, -1.78)	0.003
	Binary measures			**Adjusted odds ratio* (95% CI)**	
	Problems with mobility, %	44.2	54.3	1.46 (1.01, 2.12)	0.04
	Problems with self-care, %	18.4	27.8	1.72 (1.11, 2.64)	0.02
	Problems with activities, %	39.8	53.2	1.68 (1.16, 2.44)	0.006
	Problems with pain, %	55.8	64.9	1.52 (1.03, 2.25)	0.03
	Problems with anxiety and depression, %	33.5	51.6	2.16 (1.49, 3.13)	<0.001

*Adjusted for age, sex, disease duration and smoking history.

4.3.4 Health-related quality of life

Participants with concomitant probable RBD more commonly reported impairment in each domain of the EQ-5D and reported a lower health score overall **(Table 4.4)**.

4.3.5 Sensitivity analyses

We repeated all the main analyses using the higher RBDSQ cut-off points of 6 and 7 and found the pattern of results to be qualitatively similar. Using a cut-off of 6, a significant difference between the two groups could now be observed in the MoCA score (24.8 ± 3.5 vs 24.0 ± 3.7, p=0.034), and the percent of participants reporting daytime sleepiness and fatigue on UPDRS I (77.8% vs 86.0%, p=0.035 and 69.0 vs 79.2, p=0.023, respectively). Conversely, any differences in the number of participants reporting urinary problems on UPDRS I (p=0.073), speech problems on UPDRS II (p=0.1), <1 bowel motion per day (p=0.1), and mobility problems on the EQ-5D questionnaire (p=0.1), could now be attributed to chance. Using a cut-off of 7, the null hypothesis could not be rejected when comparing the percentage of participants with a smoking history (p=0.15) or reporting <1 bowel motion per day (p=0.3) or pain and urinary problems on UPDRS I (p=0.06 and 0.1, respectively), in

the two groups. This was also the case for participants reporting speech or walking and balance problems on UPDRS II (p=0.08 and p=0.3, respectively), or mobility problems on EQ-5D (p=0.3). Interestingly, when using a cut-off of 7, participants with pRBD were more likely to report punding behaviour on QUIP (4.0% vs 10.0%, p=0.02) and symptoms of dopamine dysregulation syndrome on UPDRS I (2.4% vs 7.8%, p=0.01).

4.3.6 Post hoc analysis

We performed a post hoc analysis to exclude cases where the symptoms of RBD could have been attributed to medication use. Eighty of the initial 475 participants (16.8%) were found to have current or historical use of medications previously associated with symptoms of RBD and were, therefore, excluded from further analyses. Exclusion of these participants had very little effect on the results described above (See Appendix 2 for full details).

4.4 Discussion

To our knowledge, this is the largest comprehensive study of patients with early PD with and without probable RBD (pRBD). We have found that, not only is pRBD very common in PD, but it is also associated with more profound non-motor symptoms, even in the early stages of the disease.

4.4.1 Prevalence of RBD in patients with PD

In our study, the frequency of probable RBD was 47.2%, which is similar to that found in more advanced PD **(Table 4.5)**. In line with previously published studies, we have shown that participants with and without probable RBD were of a similar age (De Cock et al., 2007; Gjerstad et al., 2008; Postuma et al., 2008; Vibha et al.,

Table 4.5: The prevalence of REM sleep behaviour disorder in patients with Parkinson's disease.

Study	Participants with PD	Disease duration in years (SD)	Method of RDB diagnosis	Percentage of patents with concomitant RBD (95% confidence interval)
Our study	475	1.5 (1)	Questionnaire	47.2 (42.7-51.9)
De Cock, 2007	100	7 (4)	PSG	41.0 (31.4-50.6)
Postuma, 2008	36	7.7 (6.2)	PSG	58.3 (42.2-74.4)
Vibha, 2011	134	5.5 (4.1)	Questionnaire	19.4 (12.7-26.4)
Gjerstad, 2008	231	9.0 (5.6)	Questionnaire	14.7 (10.1-19.3)
Scaglione, 2005	195	8.1 (5.1)	Questionnaire	32.8 (26.2-39.4)
Lavault, 2010	61	6.9 (4.7)	Questionnaire	63.4 (51.3-75.5)
Lee, 2010	447	6.1 (4.6)	Questionnaire	36.7 (32.2-41.7)
Pacchetti, 2005	289	8.4 (5.4)	Questionnaire	26.6 (21.5-31.7)
Yoritaka, 2009	150	6.4 (4.6)	Questionnaire	54.0 (46.0-62.0)
Chahine, 2013	75	4.0 (4.0)	PSG	41.3 (30.2-52.4)
Bugalho, 2010	75	2.8 (1.4)	Questionnaire	54.6 (43.3-65.9)
Poryazova, 2013	417	11 (7)	Questionnaire	41.2 (36.5-45.9)

2011), gender (De Cock et al., 2007; Postuma et al., 2008; Sixel-Doring et al., 2011; Vibha et al., 2011) and had a similar disease duration (Scaglione et al., 2005; Vibha et al., 2011). We also observed that the delay to diagnosis from the time the patient first noticed any motor symptoms was the same in both groups, suggesting that these patients do not have a significantly more rapid progression of their motor symptoms leading to a quicker diagnosis. This finding supports that of Lavault and colleagues (Lavault et al., 2010) who found no specific worsening of motor disability scores after a period of follow up in patients with RBD, when compared to those without.

4.4.2 RBD and motor symptoms

We found that patients with pRBD were as likely to be on PD medications and had a comparable LEDD, but, somewhat surprisingly, were more likely to report an improvement on their medications (p=0.03) when compared to patients without RBD. If true, this finding would be in direct contrast to previous reports showing a higher levodopa dose and lower levodopa sensitivity in patients with RBD

(Postuma et al., 2008; Sixel-Doring et al., 2011). Moreover, as we did not observe a difference in objective motor disability scores, such as UPDRS III, Hoehn and Yahr and the Purdue Pegboard, despite patients with RBD reporting more problems with some motor tasks, such as walking and balance, turning in bed and freezing, it is possible that this finding represents a type I error due to multiple comparisons.

The presence of RBD has previously been associated with non-tremor-dominant phenotype of PD (Kumru et al., 2007; Postuma et al., 2008). In this study, we have shown that not only was there no difference in the tremor and PIDG scores at the time the patients were examined, but there was also no difference in the presence of the core signs of parkinsonism at the time the diagnosis was first made. Given the relatively early clinical stage of the participants included in this study, it is possible that the different motor phenotypes are yet to develop. Longitudinal follow up will asses whether patients' signs will progress at different rates, and thus result in a more non-tremor dominant phenotype over time.

4.4.3 RBD and non-motor symptoms

Unlike the motor scores, the non-motor scores were comprehensively worse in the PD-RBD group. We have replicated the findings of previous studies, which have demonstrated an association between RBD and increased frequency of hallucinations (Pacchetti et al., 2005; Gjerstad et al., 2008; Goetz et al., 2010; Sixel-Doring et al., 2011; Vibha et al., 2011), daytime sleepiness (Yoritaka et al., 2009; Vibha et al., 2011), constipation (Yoritaka et al., 2009) and orthostatic hypotension (Postuma et al., 2008; Gagnon et al., 2009), and confirmed that it exists in early PD. Contrary to some previous reports (De Cock et al., 2007; Gjerstad et al., 2008; Lavault et al., 2010; Vibha et al., 2011), we were able to demonstrate a greater prevalence of depression in patients with pRBD when compared to those without, using both clinical rating scales and patient reporting. These other negative studies

may have been underpowered to demonstrate an association. Our findings would support the theory that the presence of RBD in PD is representative of a more diffuse disease process early in its evolution, perhaps explained by the proximity of the serotoninergic raphe and other brainstem nuclei to the nuclei implemented in the pathophysiology of RBD (Boeve et al., 2007).

Previous studies have reported an increased prevalence of mild cognitive impairment (MCI) in patients with RBD and more established PD (Sinforiani et al., 2006; Gagnon et al., 2009). Here, for the first time, we have shown a significant difference in the cognitive scores of patients with early PD, with and without concomitant pRBD. Whilst the difference in the total MMSE score was modest and could be the result of multiple comparisons (p=0.03), the difference in the number of subjects in each group satisfying the screening criteria for cognitive impairment was much higher in the PD-RBD group (14.9% vs. 56.7%; p=0.006).

Collectively, we have shown that pRBD was associated with worse non-motor symptom severity scores, a higher prevalence of mood disorders, daytime sleepiness and cognitive impairment, and a greater impact on activities of daily living. By replicating the results after excluding patients with possible RBD secondary to medication use, we have shown that the observed differences were likely to be due to the underlying pathology. These findings go some way to support previous theories that RBD marks a more diffuse and complex phenotype of PD (Postuma et al., 2012). However, we cannot specify whether it is the pRBD itself that is responsible for this phenotype or whether this is due to an association with other non-motor symptoms, for example, a greater prevalence of depression or cognitive impairment.

4.4.4 Limitations and future work

A clear limitation of our study is that the diagnosis of RBD was based on a questionnaire and not confirmed by objectively polysomnography (PSG). Whilst PSG inevitably remains the gold standard, studies have supported the use of RBDSQ in screening for pRBD and its role in clinical studies (Nomura et al., 2013). Importantly, it must also be noted that whilst the RBDSQ does not require the input from a bed partner, thus making it ideal for screening large populations where this information may not be available, this may lead to the under-diagnosis of pRBD. A further limitation of this study is the use of screening questionnaires for conditions such as depression, excessive sleepiness and cognitive impairment. Although these questionnaires can give an insight into the prevalence certain patient-reported symptoms, further information and assessment is required to allow a clinical diagnosis to be made.

4.4.5 Conclusions

Findings from early PD confirm that RBD is common throughout its natural history, consistent with neuropathological staging. Patients with early PD and concomitant pRBD have a different phenotype with greater prevalence of some non-motor features. At this stage, we cannot speculate on their rate of motor progression compared to non pRBD patients. Further follow-up will enable us to explore whether pRBD should be routinely collected as a potential biomarker for clinical progression in PD.

Chapter 5

Clinical characteristics of idiopathic REM sleep behaviour disorder

5.1 Introduction

In the previous chapter I explored the impact of concomitant RBD on the clinical phenotype of established PD. However, if RBD is truly representative of the early stages of PD, patients with RBD must exhibit some other features of the disease. In this chapter I will explore the presence of motor and non-motor features of PD in patients with RBD, and compare them to patients with established PD and healthy controls.

5.1.1 RBD in the context of prodromal PD

As discussed in the previous chapters, presumed anatomical substrates of RBD correlate to stage 2 of Braak's hypothesis of alpha-synuclein spread in PD. Therefore, one would expect the presence of other non-motor features in these patients. Indeed, progression to stages 3 and 4 may result in early motor features that are yet unnoticeable to the individuals.

In one of his seminal papers, Postuma and colleagues (Postuma et al., 2009b) looked for markers of neurodegeneration in 68 subjects with RBD, drawing a

comparison to patients with established PD and healthy controls. Compared to health controls, the authors reported impairment of olfaction, colour vision, autonomic function and cognitive function in patents with RBD. Mirroring the pattern observed in established PD (Lawton et al., 2015), patients with RBD demonstrated a great deal of heterogeneity - many of the patients showed severe impairment, whereas others scored in the normal ranges. Additionally, patients with RBD demonstrated early motor impairment akin to, but not as severe as, PD.

Whilst cementing the place of RBD as a marker of prodromal PD, the study described below had certain shortcomings. Firstly, only certain non-motor symptoms were screened for. Mood disturbance, for example, was not studied despite being a core feature of early disease (Baig et al., 2015b). Secondly, the PD group included in the study was medicated making the direct comparison with the RBD group difficult. Lastly, potential genetic confounds were not explored.

5.1.2 Genetic risk factors

Siblings of PD patients with late onset disease have an increased risk of developing PD. Their risk ratio for developing PD is estimated at between 3.6 and 6.7 (Marder et al., 2003; Sveinbjörnsdóttir et al., 2000). There are a number of rare genetic variants that can account for this increased risk. The commonest of these is a mutation in the gene coding for the leucine-rich repeat kinase, LRRK2. The G2019S LRRK2 mutation occurs in 1% of sporadic and 4% of familial PD cases worldwide. The lifelong penetrance of the gene is between 32 and 74%, depending on the ethic background of the cases analysed (Goldwurm et al., 2007; Healy et al., 2008). In the single study of its kind, LRRK2 mutations in Ashkenazi Jews were not associated with symptoms of RBD (Saunders-Pullman et al., 2015).

Single heterozygous mutations in the Glucocerebrosidase (GBA) gene are also an important genetic risk factor for PD (Sidransky et al., 2009). Whilst homozygous

mutations in the gene are associated with Gaucher disease, a lysosomal storage disorder, heterozygous mutations seem to predispose to neurodegeneration (Sidransky et al., 2009). Depending on the methodology and the ethnic background of the populations studied, GBA mutations may be found in 2-23% of subjects with a clinically defined alpha-synucleinopathy (Sidransky et al., 2009; Mata et al., 2008; Tsuang et al., 2012; Goker-Alpan et al., 2006). In a British population, single heterozygous mutations in the GBA gene occur in 4% of patients with late-onset PD, compared to 1% of controls (Neumann et al., 2009).

Importantly, an increased prevalence of GBA mutations has also been demonstrated in patients with RBD (Gan-Or et al., 2015). In a study of 265 subjects with RBD and 2240 healthy controls, GBA mutation carriers had a odds ratio of 6.24 for RBD. This equated to 10.2% of the RBD subjects and 1.8% of the controls found to be carrying the mutation. In a smaller study, even though GBA-carriers were not found to have a increased prevalence of RBD symptoms at baseline (McNeill et al., 2012), the prevalence increased significantly with longitudinal follow-up, along with a deterioration in the motor scores and other markers of neurodegeneration (Beavan et al., 2015). This supports the view that RBD heralds the onset of clinically-defined PD.

5.1.3 Aims

It is possible that the development of early parkinsonian features in patients with RBD may simply represent the enrichment for genetic risk factors for PD. Therefore, the aims of the study presented in this chapter were as follows:

1. To assess the prevalence LRRK2 and GBA mutations in the RBD patients within the OPDC cohort; and

2. To clinically phenotype patients with RBD whilst minimising potential genetic

confounds.

5.2 Methods

5.2.1 Subjects

The study was undertaken with the understanding and written consent of each subject, with the approval of the local NHS committee, and in compliance with national legislation and the Declaration of Helsinki.

Seventy-four patients with RBD (64 men, age 64.6 $\pm$ 9.6 years, mean disease duration 2.9 $\pm$ 2.4 years) were consecutively recruited from the sleep disorders clinics at the John Radcliffe Hospital, Oxford and Papworth Hospital, Cambridge. The diagnosis of RBD was made on the basis of polysomnographic evidence according to standard International Classification of Sleep Disorders-II criteria by a consultant specialising in sleep disorders. RBD was defined as an increase in tonic or phasic chin EMG activity during REM sleep and, either history of elaborate motor activity associated with dream content, or the presence of dream-enactment behaviour during REM sleep during polysomnographic video recordings (Lapierre and Montplaisir, 1992). Patients were excluded if RBD was judged by their clinical team to be secondary to medication use, or was associated with other neurological conditions, including narcolepsy, Parkinson's disease, dementia or multiple system atrophy. RBD symptom duration was calculated as the time from the patient's-defined symptom onset; RBD diagnosis duration was taken from the date of the diagnostic polysomnogram (PSG).

Eighty-nine drug naïve patients with idiopathic PD (78 men, age 67.0 $\pm$ 9.5 years, mean disease duration 0.8 $\pm$ 0.8 years) and 146 healthy controls (126 men, age 66.1 $\pm$ 10.1 years) were frequency age- and gender-matched to the RBD group from the Oxford Parkinson's Disease Centre (OPDC) patient cohort. Patients with

PD were eligible for study inclusion if they met the UK Parkinson's Disease Society Brain bank criteria for the diagnosis of idiopathic Parkinson's disease (Hughes et al., 1992), as judged by a neurologist. Patients with secondary parkinsonism due to head trauma or medication use, or features of atypical parkinsonism syndromes, were excluded. Healthy controls were recruited from the spouses and friends of patients taking part in the study.

5.2.2 Subject evaluation

A study movement disorders neurologist and a trained research nurse carried out comprehensive evaluation of each participant. The following measures of interest were included:

1. **Patient demographics**, including age, gender and enumerated family history. A comprehensive past medical history was taken. For patients with PD, disease duration was defined as the time from when the diagnosis was made by a neurologist.

2. **Motor features of PD**, including part III of the Movement Disorders Society (MDS) revised Unified Parkinson's Disease Rating Scale (UPDRS) (Goetz et al., 2008). Hand dexterity, motor speed and coordination were assessed using the Purdue Pegboard Test; the total number of pegs inserted using each hand individually and then using both hands over 90 seconds were used as the outcome measures. The Purdue Pegboard assembly test score was also used as an outcome (Desrosiers et al., 1995). The Flamingo test (the ability of the patient to balance on one leg for 30s) was performed to assess postural stability.

3. **Non-motor features of PD**. Olfaction was assessed using the Sniffin' Sticks odour identification test. Participants were presented with 16 felt-tip pens

scented with 16 common odours and asked to identify each one from a choice of four (maximum score of 16). Cognition was assessed using the Mini-Mental State examination (MMSE) and the Montreal Cognitive Assessment (MoCA). Scores on both tests were normalized for years of education (calculated as the number of years at school plus years of further education). Cognitive impairment was defined according to the MMSE and MoCA screening cut-offs ($<26/30$) (Hu et al., 2014). For phonemic and semantic fluency, the total number of words generated beginning with F, A and S, and animal and boys' names category respectively over 60 seconds each was counted. The Beck Depression Inventory (BDI-II) (Beck et al., 1996) and the Leeds Anxiety and Depression Scale (LADS) (Snaith et al., 1976) were used to evaluate depression and anxiety. A postural drop in blood pressure was calculated as the difference in the systolic blood pressure measured with the patient lying on the examination couch for three minutes, and again two minutes after standing. In relation to constipation, participants were asked about their bowel frequency and laxative use, and defined as constipated if they reported an average bowel frequency of less than one per day, or were taking laxatives.

5.2.3 Genetic testing

All participants were screened for G2019S and R1441C mutations in the LRRK2 gene and N370S and L444P mutations in the GBA gene. DNA was extracted from whole blood using a Qiagen Autopure automated system and quantified using a Nanodrop (Churchill Hospital Genetics Laboratory, Oxford). Polymerase Chain Reaction (PCR) was performed using MegaMix Blue (Microzone) containing a

recombinant Taq polymerase. Primer sequences are as follows: G2019S: 5′-TTTAAGGGACAAAGTGAGCAC-3′ and 5′-ACTCTGTTTTCCTTTTGACTC-3′; R1441C: 5′-AAGGCATGAAGATGGGAAAG-3′ and 5′-TGATGGTTTTCCGAAGTTTTG-3′; N370S: 5′-GCCTTTGTCCTTACCCTC*G-3′ and 5′-GACAAAGTTACGCACCCAA-3′; L444P: 5′-GGAGGACCCAATTGGGTGCGT-3′ and 5′-ACGCTGTCTTCAGCCCACTTC-3′ (* indicates a mismatch that was introduced into the forward primer to create a restriction site). The PCR products for G2019S, R1441C, N370S and L444P were digested with SfcI (BfmI), BstUI, XhoI and NciI (BcnI), respectively, and resolved by agarose gel electrophoresis.

5.2.4 Statistical analysis

Analysis was computed using Statistical Package for the Social Sciences version 22 statistical software (SPSS, Chicago, IL. USA). For ordinal data, the χ^2 test was used. For continuous data, assessment of normality was performed using the Shapiro-Wilk test. Differences between the group means of normally distributed variables (age, Purdue Pegboard Test total and phonemic fluency scores, and orthostatic systolic hypotension) were assessed with one-way analysis of variance. For the remaining continuous variables (MMSE, MoCA, LADS, BDI-II, semantic fluency, smell and UPDRS III), group differences were assessed with the Kruskal-Wallis test. As PD versus control comparisons are the subject of many previous studies, only the RBD versus control and RBD versus PD comparisons have been considered here. In all cases post hoc significance was calculated and corrected using the Bonferroni method to reduce the probability of a type I error due to multiple significance testing. Odds ratios were calculated using logistic regression. Spearman

rank based method was used to calculate correlations between the motor and non-motor scores in the RBD group. The correlation metrix was created in R (R Development Core Team, 2010). Significance was defined as $P<0.05$.

5.2.5 Candidate's contribution to the study

The candidate designed the study. The RBD clinical data was collected by the candidate. The HC and PD data was collected by four study neurologists, including the candidate. The candidate performed all statistical analyses, interpreted the results and wrote the manuscript on which this chapter is based.

5.3 Results

5.3.1 Genetic risk factors for PD

Of the 309 subjects included in the study, 44 (14.2%) were found to have a genetic risk factor for PD and were excluded from further analyses. Five subjects tested positive for one of the alleles of interest; four subjects (three RBD (4.1%) and one PD (1.1%) tested positive for the GBA N370 mutation and one PD subject tested positive for the LRRK2 G2019S mutation. A further 39 subjects (12.6%) were found to have a family history of PD, including 6 healthy controls (4.1%), 21 PD (23.6%) and 12 RBD (16.2%) patients. For the remaining 265 participants, the three groups did not differ significantly in age (p=0.4) and gender (p=0.7).

5.3.2 Motor characteristics

Table 5.1 summarises the motor and non-motor characteristics in the three groups. Patients with RBD were less likely to successfully perform the Flamingo test (P=0.004) and had a significantly higher total UPDRS part III score than healthy controls (P<0.001). Whilst patients with RBD had similar scores to healthy controls on the Purdue Pegboard test, their performance on the assembly task was significantly impaired (P<0.001). Compared to patients with RBD, patients with PD had a significantly higher total UPDRS part III score (P<0.001) and were more impaired on the Purdue Pegboard task (P<0.001). No significant differences between these two groups were found on Purdue Pegboard assembly task (P=0.2), the Flamingo test (0.6) and the Get-up-and-go test (P=0.08).

5.3.3 Non-motor characteristics

Participants with RBD demonstrated impairment in all non-motor domains. Compared to healthy controls, the RBD group was more impaired on cognitive testing, with both MMSE and MoCA scores significantly lower (P<0.001 and P=002, respectively). Similarly, both the semantic and phonemic fluency scores were significantly lower in the RBD group (P<0.001). Participants with RBD had a significantly lower score on the Sniffin' smell test (P<0.001) and were also much more likely to suffer from constipation than healthy controls (54.2% vs 31.4%, P=0.01). Similarly, orthostatic systolic hypotension was more pronounced in the RBD group (P=0.007). Patient with RBD were more likely to suffer psychiatric symptoms with both the Beck depression inventory and the LADS anxiety score higher in this group (P<0.001). To ensure that the depression results were driven by the question pertaining to sleep, Beck

Table 5.1: Overview of motor and non-motor characteristics

Healthy controls (n=140)	Healthy controls (n=140)	RBD (n=59)	Drug naïve PD (n=66)	RBD versus controls P-value	RBD versus PD P-value
Motor					
UPDRS III, score	1.6 (2.4)	4.0 (4.2)	25.5 (12.1)	<0.001	<0.001
Purdue Pegboard, score	36.5 (6.1)	38.9 (8.7)	28.0 (6.3)	0.05	<0.001
Purdue Pegboard assembly, score	24.2 (6.6)	21.3 (6.4)	18.5 (5.6)	<0.001	0.2
Flamingo, %	73.4	49.1	39.4	0.004	0.6
Get-up-and-go, seconds	8.4 (1.7)	9.6 (3.9)	9.5 (2.2)	0.07	0.8
Non-Motor					
MMSE, score	28.5 (1.8)	27.6 (1.9)	27.9 (1.9)	<0.001	0.6
MoCA, score	26.5 (2.6)	25.1 (3.4)	25.0 (2.6)	0.002	0.8
Semantic fluency, score	40.1 (7.9)	34.8 (7.4)	34.5 (10.2)	<0.001	1
Phonemic fluency, score	45.1 (12.1)	36.1 (15.2)	41.6 (15.5)	<0.001	0.05
Sniffin' sticks, score	11.6 (2.6)	8.9 (3.0)	7.5 (3.0)	<0.001	0.046
Orthostatic systolic hypotension, mmHg	1.3 (11.2)	6.9 (14.0)	6.3 (13.1)	0.007	0.7
Constipation, %	31.4	54.2	44.6	0.01	0.6
BDI-II, score	4.3 (4.9)	10.5 (9.5)	8.3 (5.6)	<0.001	0.1
LADS (depression), score	2.3 (2.1)	4.6 (3.6)	3.3 (2.8)	<0.001	0.2
LADS (anxiety), score	1.8 (2.0)	4.1 (3.5)	2.8 (2.5)	<0.001	0.1

depression inventory scores were recalculated omitting this question. The scores remained significantly higher in the RBD group (P=0.002).

Of all the non-motor domains tested, only the performance in the Sniffin' sticks smell revealed a significant difference between the PD and RBD groups (P=0.046).

Using predefined cut-offs for clinically significant scores on the relevant questionnaires **(Table 5.2)**, participants with RBD were almost three-and-a-half times more likely to show evidence of cognitive impairment than healthy controls. Both olfactory dysfunction and depression was more than seven times more common in this group, with anxiety almost nine times more common. Autonomic dysfunction, as manifested by constipation and orthostatic hypotension, was also much more common in the RBD group.

Table 5.2: Increased risk of non-motor symptoms in patients with RBD compared to age and sex matched healthy controls.

	Odds ratio (95% Confidence interval)
Cognitive impairment[a]	3.49 (1.85 – 6.68)
Hyposmia[b]	7.53 (3.77 – 15.07)
Depression[c]	7.50 (3.03 – 18.57)
Anxiety[d]	8.89 (3.49 – 22.67)
Constipation	2.59 (1.39 – 4.83)
Orthostatic hypotension[e]	3.01 (1.15 – 7.86)

Defined as: [a]MoCA <26 (Hu et al., 2014); [b]Sniffin' Sticks (Hummel et al., 2007); [c]BDI-II >13 (Beck et al., 1996); [d]LADS Anxiety score >7 (Snaith et al., 1976); [e]Systolic blood pressure drop $\geq$ 20mmHg

5.3.4 Correlation between motor and non-motor symptoms

Several of the motor and non-motor scores in the RBD group were correlated to one another and could be divided into three broad clusters **(Figure 5.1)**. The first cluster revealed a strong positive correlation between the measures

of anxiety and depression. The Leeds anxiety and depression scores showed a weak (rho = 0.18, p=0.02 and rho = 0.32; p=0.007), but statistically significant, positive correlation with a prolonged Get Up & Go time. In the second cluster, the UPDRS part III score was correlated with performance on the Get Up & Go and the Purdue pegboard assembly task. The UPDRS part III score was inversely correlated to performance on cognitive tasks, such as the MoCA (rho = -0.34, p=0.03), phonemic (rho = -0.27, p=0.03) and semantic fluency (rho = -0.33, p=0.01). Cluster three revealed a positive correlation between the performance on the cognitive tasks and the Purdue pegboard assembly task. Olfaction was correlated with the MoCA (rho = 0.45, p=0.001), and phonemic (rho = 0.30, p=0.02) and semantic fluency (rho = 0.36, p=0.004).

5.4 Discussion

In the largest study of its kind to date, we present an extensive and detailed description of motor and non-motor characteristics associated with idiopathic RBD in comparison to age- and sex-matched patients with PD and healthy controls. Our study provides support for previous studies in showing the presence of early parkinsonian features in patients with RBD. For the first time, we demonstrate that whilst RBD and early, non-medicated PD clearly differ on diagnostic and fine motor tests, the groups are indistinguishable on non-motor tests. Hence, we establish a direct comparison with the early stages of motoric PD without confounds of dopaminergic medications. Importantly, we demonstrate that common genetic risk factors for PD are not enriched in the RBD cohort and, therefore, the clinical characteristics are likely to be representative of the prodromal stages of sporadic PD.

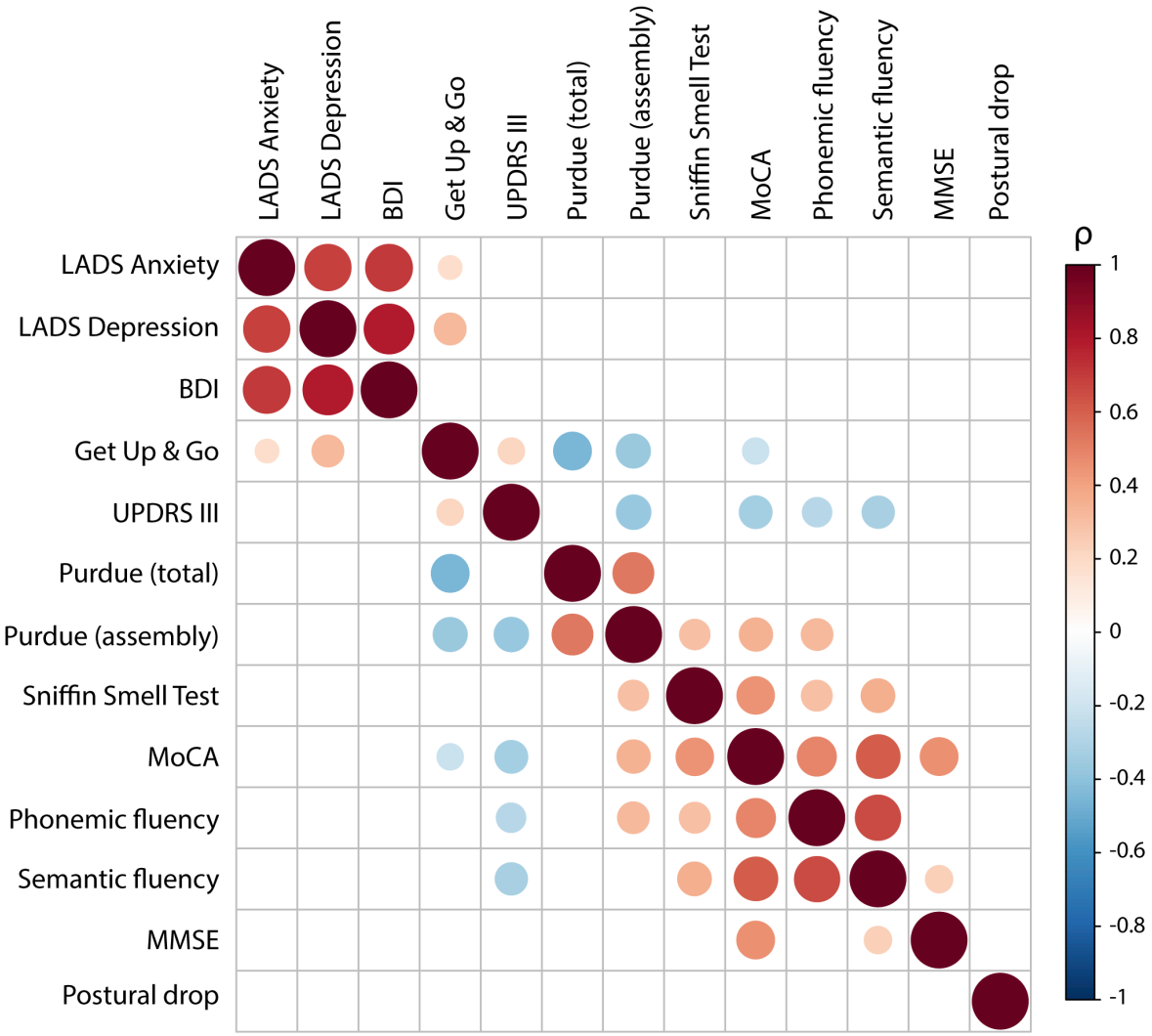

Figure 5.1: Correlation between motor and non-motor symptoms.

Correlation between motor and non-motor symptoms in RBD subjects. Only correlations with a $p>0.05$ are visualised.

5.4.1 RBD and genetic risk factors

Whilst carriers of heterozygous mutations in these genes may form a promising cohort for the study of prodromal PD, they may not be fully representative of sporadic PD. In the case of GBA, patients tend to present with an earlier age of onset and a higher prevalence of cognitive impairment (Neumann et

al., 2009; Healy et al., 2008). In our study, only three of the RBD patients (3/74, 4.0%) tested positive for a mutation in the GBA gene. Whilst the sample numbers are too small to estimate the true prevalence of these mutations, their frequency does not seem to be significantly increased in RBD, compared to that in a British PD population (Neumann et al., 2009). Therefore, patients from this cohort who eventually go on to develop PD are more likely to be representative of sporadic disease.

5.4.2 RBD and motor symptoms

In line with previous findings (Postuma and Montplaisir, 2006; Postuma et al., 2009b), we comprehensively show that RBD is associated with a number of motor and non-motor characteristics often associated with prodromal PD. As before, we show evidence of motor impairment, resulting in a higher UPDRS III score, and poorer performance on the Flamingo balance test, when compared to the healthy control group. Whilst the latter would suggest impairment in postural stability, this was not reflected in a statistically significant difference in the postural instability/gait disorder subscore of the UPDRS III. This, however, may simply represent the relatively low sensitivity of the UPDRS III for postural instability (Smithson et al., 1998). Unlike previous studies, we did not observe a significant difference between patients with RBD and healthy controls in performance on the Purdue pegboard test. This is rather surprising as performance on this test has been shown to correlate well with the severity (Müller et al., 2000) and the degree of nigrostriatal dopaminergic dysfunction (Vingerhoets et al., 1997) in established PD. However, the mean duration of RBD was significantly shorter in our cohort, when compared to that published by Postuma et al. (Postuma et al.,

2009b) (mean difference 2.8 years, $p<0.0001$). It is, therefore, possible that motor impairment sufficient enough to affect the pegboard test had not yet developed (Postuma et al., 2012). This would be supported by the milder degree of motor impairment experienced in the RBD compared to PD groups (see **Table 5.1**).

5.4.3 RBD and non-motor symptoms

Whilst the extent of the motor impairment in the RBD group was subtle, the effect on the non-motor characteristics was much more marked. As well as replicating findings showing lower scores on the MMSE and a higher frequency of constipation and hyposmia (Postuma and Montplaisir, 2006; Postuma et al., 2009b), for the first time, we show impairment in both semantic and phonemic frequency. Despite excluding cases where the symptoms of RBD were thought to be related to the use of anti-depressants, we found significantly higher scores on anxiety and depression screening questionnaires in the RBD group compared to controls. Using clinically relevant cut-offs on the screening questionnaires, patients with RBD were more likely to have cognitive impairment, hyposmia, constipation, depression and anxiety. In the case of PD, it has been well shown that non-motor symptoms have the greatest impact on the health-related quality of life of patients (Martinez-Martin et al., 2011). As this is also likely to be the case in RBD, it is important that these symptoms are screened for and treated, as appropriate in RBD individuals.

5.4.4 Comparison of RBD and early PD

Interestingly, there were no significant differences between the RBD and PD groups in any of the non-motor domains. The evidence for a wide spectrum of non-motor symptoms in early PD is well established (Khoo et al., 2013), with many of these predating the diagnosis of PD by a number of years (Lang, 2011). The evidence for the presence of hyposmia (Ross et al., 2006; Ross et al., 2008), constipation (Gaig and Tolosa, 2009; Abbott et al., 2001; Abbott et al., 2003) and depression (Gaig and Tolosa, 2009; Shiba et al., 2000) in the premotor stage of PD is the strongest. Along with the structures believed to be responsible for RBD (Boeve, 2010), the likely sites of causative pathology leading to these symptoms (Lang, 2011) correspond to Braak stages 1 and 2 on the pathophysiological classification of PD (Braak et al., 2003). Therefore, the presence of these non-motor symptoms in the RBD and early PD groups perfectly fits with RBD, at least in some cases, signifying the prodromal stage of idiopathic PD.

5.4.5 Heterogeneity within the RBD phenotype

In line with previous reports (Postuma and Montplaisir, 2006; Postuma et al., 2009b), we demonstrated significant correlations between a number of motor and non-motor features. We observed a positive correlation between measures of anxiety and depression and a prolonged Get Up & Go test. A similar result has recently been reported in elderly women with type 2 diabetes mellitus (de Souza Moreira et al., 2016). The authors speculate that this correlation may be attributable to a greater fear of falling. Unsurprisingly, we found a correlation between the motor tasks, and that the cognitive tasks correlated with one another. Interestingly, there was also a correlation

between gross motor performance and cognitive function. This is in line with previous findings that cognitive decline tracks motor progression in PD (Riggeal et al., 2007).

5.4.6 Limitations and future directions

There are some limitations to our study. Due to practical constraints, the PD and healthy control group did not undergo polysomnographic examination to exclude the presence of RBD. As the prevalence of RBD in the general population is estimated at approximately 0.5%, it is unlikely that the inclusion of RBD patients in the healthy control group would have had a significant impact on our overall results. The PD patients were not divided according to the presence of symptoms of RBD to allow comparisons to be made to a group representative of drug-naïve PD as a whole. Moreover, the breath of this study required the use of a number of screening questionnaires for conditions such as depression and cognitive impairment. Whilst these questionnaires can give an insight into the prevalence of certain patient-reported symptoms, further assessment is required to allow a clinical diagnosis to be made.

5.4.7 Conclusion

In this study we have presented evidence for motor and non-motor impairment in patients with RBD. As we did not observe a significantly increased frequency of mutations associated with a risk of PD, it is likely that these characteristics are associated with the prodromal stages of idiopathic PD. Longitudinal follow-up is currently underway to test whether any of the baseline findings are prognostic of future risk of PD.

Chapter 6

Patterns of working memory impairment in idiopathic REM sleep behaviour disorder

The study presented in this chapter has been published as a paper.

M Rolinski, N Zokaei, F Baig, K Giehl, T Quinnell, Z Zaiwalla, C E Mackay, M Husain, M T M Hu. "Visual short-term memory deficits in REM sleep behaviour disorder mirror those in Parkinson's disease" ***Brain***, 139(1):47–53, 2016

6.1 Introduction

In the previous chapter I have demonstrated that patients with RBD exhibit features of early PD, even when genetic risk factors are minimised. Therefore, at first look, patients with RBD and carriers of genetic risk factors, such as a GBA mutation, may manifest very similar parkinsonian features. However, as already discussed, PD associated with GBA mutations tends to have a distinct clinical phenotype. In this chapter I will present an analysis utilising a deeper clinical phenotyping method with a view of elucidating differences between idiopathic and genetic disease.

6.1.1 Short-term memory

A few studies have reported cognitive deficits in RBD, including modest or no impairments on short-term or working memory tests that measure 'span' or number of items that individuals can retain (Massicotte-Marquez et al., 2008; Fantini et al., 2011). However, traditional span measures rely on a binary response: either something is remembered correctly or it is not. But just because an individual fails to recall an item does not necessarily mean that it was completely lost from memory. Recently, an alternative theoretical and empirical approach has been developed to investigate the resolution or precision with which items are retained. Rather than simply asking whether an item is remembered or not (for a review see Ma et al., 2014), this approach provides a more sensitive measure of visual short-term memory (VSTM) performance than span, including in patients with Parkinson's disease (Zokaei et al., 2015).

Importantly, tasks that measure precision of recall also provide a means to dissect out sources of error contributing to the pattern of performance using modern statistical techniques (Bays et al., 2009). While it is known that many types of brain disorder can be associated with VSTM deficits, this might be due to different underlying mechanisms in different groups. In a recent study employing the same paradigm as used here, dissociable signature deficits associated with glucocerebrosidase (GBA) mutations the highest known genetic risk factor for developing Parkinson's disease and idiopathic Parkinson's disease were reported (Zokaei et al., 2014). Specifically, while GBA-positive individuals showed increased misbinding errors (reflecting interference between items stored in memory), cases with idiopathic Parkinson's disease demonstrated increased random errors (guesses). GBA-positive cases with Parkinson's disease showed both types of error. Crucially, there was no evidence that GBA-positive individuals without Parkinson's disease have the same type of memory impairment as those with Parkinson's disease.

6.1.2 Aims

The aims of this study were twofold:

1. To assess VSTM in subjects with RBD; and
2. Ascertain whether any deficit in their memory mirror the pattern observed in patients with established, idiopathic GBA-negative Parkinson's disease.

6.2 Methods

6.2.1 Subjects

Twenty-one patients with RBD were recruited from sleep clinics at the John Radcliffe Hospital, Oxford and Papworth Hospital, Cambridge. The diagnosis of RBD was made on the basis of clinical and polysomnographic evidence, according to standard International Classification of Sleep Disorders-II criteria (Lapierre and Montplaisir, 1992). RBD was defined as an increase in tonic or phasic chin EMG activity during REM sleep and either history of elaborate motor activity associated with dream content, or the characteristic behavioural manifestations occurring in REM sleep during polysomnographic recordings. Patients were excluded if their RBD was judged by their clinical team to be secondary to medication use or associated with other neurological conditions, including Parkinson's disease and narcolepsy. Sixteen of the patients were receiving treatment for their RBD; fifteen patients were taking clonazepam, seven were taking melatonin, and six patients were taking both medications.

Fifteen non-medicated and 11 medicated patients with Parkinson's disease, as well as 26 age-matched healthy individuals participated (see 6.1 for participants' demographics). Ethical approval was given by the Oxford University Research Ethics Committee. Patients with Parkinson's disease were recruited if they met the

Table 6.1: Demographic information on all patient groups and healthy controls.

	Healthy controls (n=26)	RBD patients (n=21)	PD patients (n=26) Non-medicated (n=15)	Medicated PD (n=11)
Age	66 (7)	66 (9)	65 (7)	67 (6)
Gender (M/F)	18/8	19/2	9/6	6/5
Years of education	14.7 (3.3)	14.6 (3.5)	15 (3.4)	14 (3)
MMSE	29 (1.1)	27.8 (1.5)	28.9 (0.9)	28.0 (1.3)
Years of diagnosis	n/a	2.7 (1.9)	0.66 (0.74)	2.7 (1.3)
Daily levodopa equivalent dose, mg	n/a	n/a	n/a	355 (152)
UPDRS III	n/a	n/a	13 (4)	15 (3)

Values are mean (SD). MMSE = Mini-Mental State Examination; n/a = not applicable; PD = Parkinson's disease; UPDRS = Unified Parkinson's Disease Rating Scale.

UK Parkinson's Disease Society Brain Bank criteria for the diagnosis of idiopathic Parkinson's disease (Hughes et al., 1992). All participants had normal or corrected-to-normal vision and normal colour vision. All patients with RBD and Parkinson's disease were screened for common GBA mutations and control participants had no neurological disease or family history of Gaucher's disease. All cases with Parkinson's disease and RBD reported here were confirmed GBA-negative.

6.2.2 Genetic testing

All patients, except for 7 PD patients that are discussed later, were screened for N370S and L444P mutations in the glucocerebrosidase (GBA) gene by extracting genomic DNA from blood samples using the AutoPure LS (QIAGEN). PCR reactions were carried out with AmpliTaq Gold DNA polymerase (Applied Biosystems). Primer sequences used for N370S were: 5′- GCCTTTGTCCTTACCCTC*G -3′ and 5′-GACAAAGTTACGCACCCAA-3′. A mismatch was engineered into the forward primer in order to create a XhoI restriction site in the PCR product from participants carrying the N370S mutation. For the L444P mutation primers used were: 5′-GGAGGACCCAATTGGGTGCGT-3′ and 5′- ACGCTGTCTTCAGCCCACTTC-3′. The resulting PCR products were digested with XhoI (NEB) for N370S and NciI (NEB) for L444P and resolved by agarose gel electrophoresis. Mutations were then confirmed by sequencing. Briefly, DNA was treated with an ExoSAP reaction

as follows: 1X SAP buffer, shrimp alkaline phosphatase (500 U; SAP, Promega), ExonucleaseI (2 U; NEB). Samples were incubated at 37C for 1 hour and then 80 C for 20 minutes. The sequencing reaction was performed according to BigDye Terminator v3.1 Cycle Sequencing protocol (Applied Biosystems). Following a clean up step, the sequencing read was performed on a 3700 DNA Analyser (Applied Biosystems) sequencing platform. GBA mutation in 7 PD cases was screened by sequencing exons 1 to 11 of the GBA gene using previously published protocol (Zokaei et al., 2014). After amplification by PCR, the product was run on 1% agarose gel with ethidium bromide and size-checked to ensure intronic sequences using the Dye Terminator Sequencing Kit (Applied Biosystems) on an ABI 3700xl genetic analyser.

6.2.3 Visual short-term memory task

The 4-item VSTM task was identical to that previously used by Zokaei et al. 2014 **(Figure 6.1A)**. In each trial, a sequence of four coloured bars of different orientation appeared on the screen centre and participants were asked to remember both the colour and orientation of the bars. At the end of each sequence, a randomly oriented probe bar of the same colour as one of the bars in the sequence was presented at screen centre. Participants were instructed to use a rotating dial to match the orientation of same coloured bar in the sequence. They clicked on the dial to confirm their selected orientation. Stimuli presented in any of the serial positions within the sequence were probed with equal probability and participants did not know beforehand which item would later be probed.

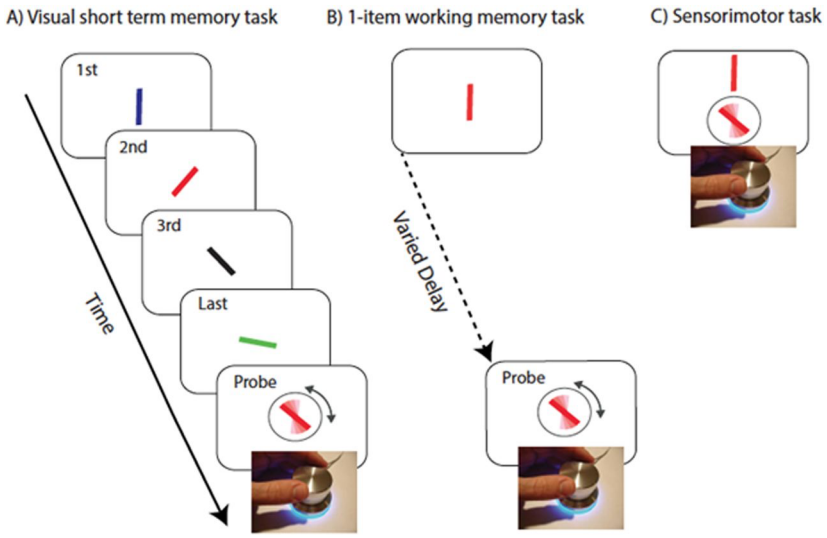

Figure 6.1: Task to measure precision of recall. (A) A sequence of four coloured oriented bars were presented sequentially. Any of the bars could be probed by colour of the response stimuli and participants were asked to adjust the orientation of the probed bar to the orientation of the bar with same colour (red in this example). (B) One-item working memory task. A rotating dial is used to orient the probe bar (surrounded by circle) to match the orientation of the probed bar presented following a delay. The maintenance period preceding the probe was randomly chosen from one of the following delays: 500, 1500, 2500 and 3500 ms. (C) Sensorimotor task. A rotating dial is used to orient the probe bar to match the orientation of the target bar presented above the probe and continuously on view.

6.2.4 Control tasks

Poor performance in the VSTM task might be attributed to factors other than the ability to maintain multiple items. To ensure various issues were not a concern for subsequent interpretation, three control tasks were administered: (i) pre-cueing: an identical design to the 4-item VSTM task but with 100% informative cues which tell the participant which colour will be probed; (ii) one-item VSTM with variable delays **(Figure 6.1B)** to match durations between the probed item and appearance of the probe in VSTM task; (iii) sensorimotor task **(Figure 6.1C)**: participants simply match the orientation of a continuously presented bar using the response dial.

These tasks are identical to those previously used by Zokaei et al., 2014 and aim

to control for deficits in attentional filtering, temporal decay of information and difficulties with dexterity in using the dial, respectively.

All tasks were presented on a laptop (32' x 19') at ∼52 cm, in random order across participants. For each of the experimental VSTM, pre-cueing and 1-item VSTM tasks healthy controls completed 100–200 trials, patients with RBD completed 100 trials and patients with Parkinson's disease completed 50–200 trials depending on their availability. All patients with Parkinson's disease and RBD as well as 17 healthy controls completed 20 trials of the sensorimotor control task.

6.2.5 Analysis

Recall precision was used as an overall measure of performance, calculated simply as the reciprocal of the circular standard deviation of response error (difference in response and target angle), with less variability corresponding to a more precise memory. To identify mechanisms underlying VSTM impairments associated with RBD and Parkinson's disease, we fitted a probabilistic model that dissociates different sources of error in memory (Bays et al., 2009). In tasks similar to the one employed here, several sources of error can contribute to impaired performance (Ma et al., 2014). Error can arise due to (i) increased variability in memory for the orientation of the probed (target) item; (ii) increase in random responses; or (iii) systematic interference by other items retained in VSTM – these are responses centred on other, non-probed items in the memory array ('misbinding' errors). Figure 6.2 is a schematic of the types of error associated with tasks similar to the one used here. Error can arise due to increased variability in memory for the orientation **(Figure 6.2A)**, increased misbinding errors **(Figure 6.2B)** or increased random responses **(Figure 6.2C)**. Maximum a posteriori probabilities for three sources of error were estimated using the MemToolbox (Suchow et al., 2013) (memtoolbox.org).

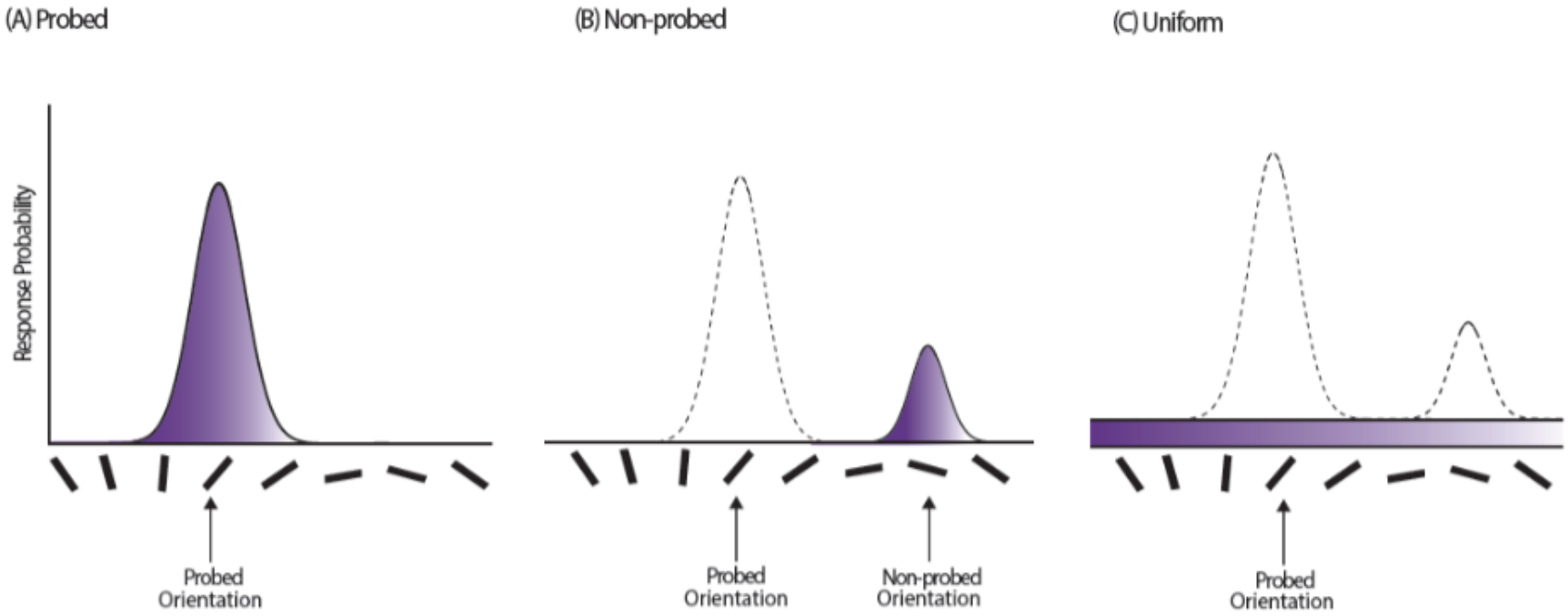

Figure 6.2: Three possible sources of error that can corrupt in visual short-term memory. A) A Von Mises (circular Gaussian) distribution with concentration parameter , centred on the probed value, captures variability in memory for the probed orientation, with the area under the distribution (shaded) being proportional to the probability of responding to the probe. Effectively this is the noisiness of retaining the orientation of the probed item. B) Von Mises distribution with concentration parameter κ, centred on one of the non-probed items, i.e. one of the other bars shown in the memory array. The area under the distribution corresponds to the proportion of non-probed responses or misbinding errors. C) Uniform distribution of error corresponding to random error. Hence this distribution is constant across all possible orientations, regardless of whether they were actually presented in the memory array. The area under this distribution corresponds to the proportion of random responses.

6.2.6 Candidate's contribution to the study

The candidate designed the study, in conjunction with Dr Zokaei. The candidate collected the majority of the data, with additional input from Drs Zokaei and Baig, and Ms Giehl. In collaboration with Dr Zokaei, the candidate performed all analyses, interpreted the results and wrote the manuscript on which this chapter is based.

6.3 Results

There was no significant difference in age and years of education between the three groups. However, both patients with RBD and Parkinson's disease scored significantly worse than healthy controls on the Mini-Mental State Examination (MMSE) (Mann-Whitney U = 131, P = 0.002 and U = 232.5, P = 0.042, respectively), although, on average, both groups scored higher than 27 (a cut-off for mild cognitive impairment) on this measure. Furthermore, there was no significant correlation between MMSE and any of the measures of interest reported below. Specifically there was no significant correlation between MMSE and proportion of random responses either within each patient group or the combined group of participants.

6.3.1 VSTM impairments in cases with RBD and Parkinson's disease

There was no difference in overall VSTM performance between non-medicated and medicated patients with Parkinson's disease and hence these two groups were collapsed for analysis (n = 26). ANOVA with serial position of probe as within subject factor and group as between subject factor yielded a main effect of group

[F(2,70) = 6.3, P = 0.003]; compared to healthy participants, overall performance was significantly worse (i.e. less precise) in patients with RBD [t(42.4) = 2.3, P = 0.025] as well as cases with Parkinson's disease [t(40.7) = 3.2 P = 0.003, Figure 6.3B]. Moreover, there was a significant effect of serial position on recall precision, showing the well-known effect of recency [degrees of freedom were corrected using Greenhouse-Geisser estimate; F(1.53,107) = 66, P $<$ 0.001].

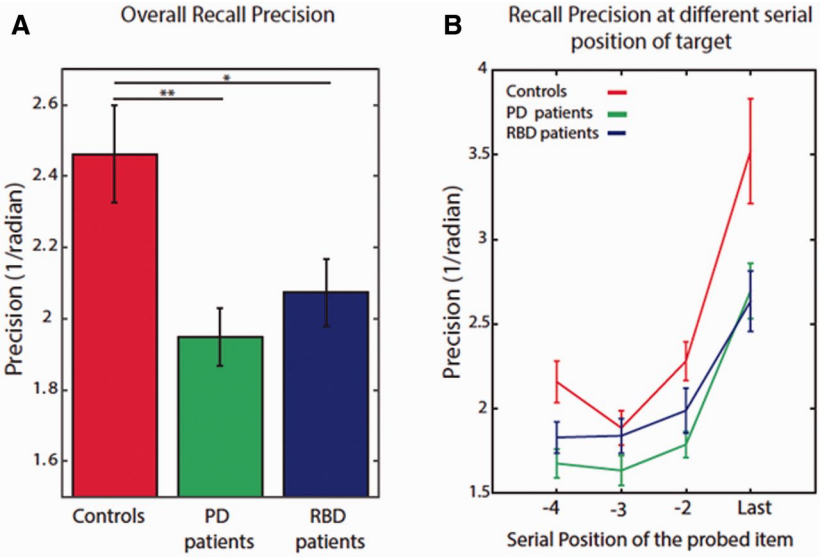

Figure 6.3: Performance in the VSTM task. (A) Overall recall precision in both patient groups was significantly worse compared to healthy controls. (B) This occurred at all serial positions of the probed item. PD = Parkinsons disease.

6.3.2 Sources of error in VSTM

Although both patient groups performed worse than healthy participants, the overall VSTM performance is not informative of the source of error, or the pattern of deficit, in these disorders. To quantify the possible sources of error, we next applied a statistical mixture model of responses error. The results demonstrated no effect of group on variability in memory for the probed orientation **(Figure 6.4A)** or on proportion of misbinding errors **(Figure 6.4B)**. However, there was a significant effect on proportion of random responses [Welch's adjusted F ratio:

F(2,36) = 8.8 P = 0.001] **(Figure 6.4C)**. Compared to healthy controls, both patients with RBD and Parkinson's disease made significantly more random responses [t(33.9) = 3.7, P = 0.001 and t(23.8), P = 0.024, respectively]. This was accompanied by a significant main effect of group on proportion of responses to the probed item [F(2,72) = 5.2, P = 0.008]. There was no significant difference in all model estimates, between patients with Parkinson's disease and RBD.

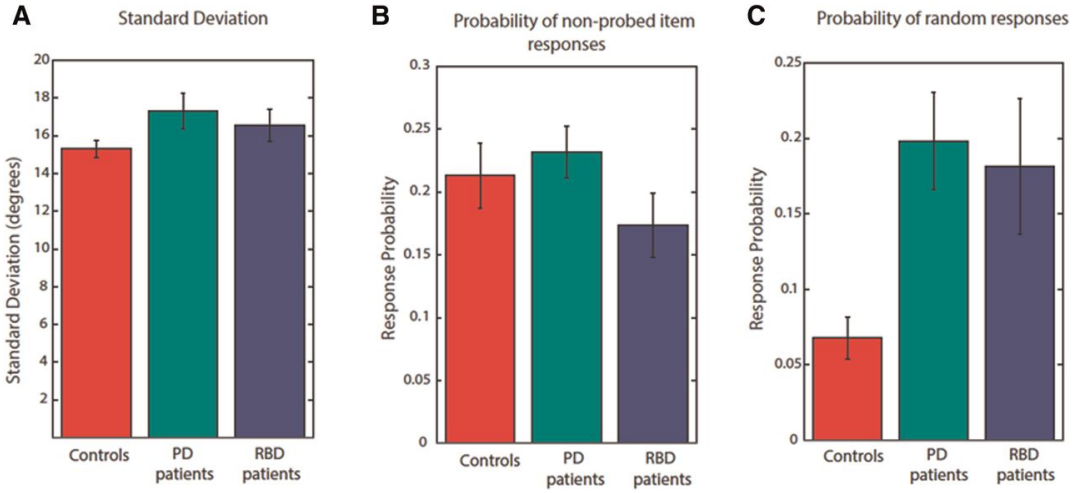

Figure 6.4: Model estimates for different sources of error in VSTM performance. (A) Concentration parameter (κ) did not differ significantly between patient groups and controls. (B) Probability of non-probed responses (misbinding errors) did not differ between groups. (C) Probability of random responses was significantly higher in both patients with Parkinson's disease (PD) and RBD compared to controls.

6.3.3 Performance in control tasks

There were no significant differences in performance on the sensorimotor control task between the three groups (Fig. 1C). This is important in excluding impaired dexterity as a confounding factor in the 4-item VSTM task results, particularly for the Parkinson's disease group. There were also no significant differences between the patient groups and healthy controls in the pre-cueing and 1-item (Fig. 1B) VSTM tasks. Any significant effect observed in the 4-item VSTM task therefore

cannot be attributed simply to deficits in attending to items presented sequentially or in temporal decay of information. These findings also make it unlikely that excessive sleepiness in RBD is the reason for the deficit observed on the 4-item VSTM task.

6.4 Discussion

The findings presented here demonstrate for the first time, to the best of our knowledge, that patients with RBD, and therefore at high risk for developing Parkinson's disease, show deficits in VSTM identical to those observed in Parkinson's disease. Specifically, deficits in recall precision in both patient groups are due to random corruption of memory, suggesting that they share the same underlying impairment in memory. The results of control experiments show that this is independent of sensorimotor deficits, difficulties in attending to different serial positions in a sequence or temporal decay of information, which makes it unlikely that excessive sleepiness in RBD is the reason for the deficit observed on the 4-item VSTM task.

Importantly, the paradigm used here allowed us to analyse the sources of error in performance (Ma et al., 2014). Recall error can firstly arise due to increase in variability in memory for the probed feature, that is, how well the probed feature is reproduced. Secondly, participants may make random responses, guessing the orientation of the probed bar, possibly due to failures at encoding or retrieval. Lastly, VSTM precision may be affected by misbinding errors. Unlike random responses, misbinding errors have been linked to hippocampal and medial temporal lobe pathology, including Alzheimer's disease (Parra et al., 2009; Pertzov et al., 2013) and mutations in GBA (Zokaei et al., 2014).

6.4.1 Impaired VSTM in PD and RBD

Individuals with Parkinson's disease made significantly more random responses than controls, but importantly not significantly more misbinding errors, replicating and strengthening previous findings using this task (Zokaei et al., 2014). The same pattern of results was also present in patients with RBD. Although the precise mechanism underlying this type of error is yet to be established, it might be due to increased noise within neuronal networks involved in encoding or maintaining information. This could potentially arise due to cholinergic disruption in Parkinson's disease (Kehagia et al., 2010; Hasselmo and Sarter, 2011) with associated fluctuations in attention leading to encoding or retrieval failure and therefore guessing on some trials (Hasselmo and Sarter, 2011). Increased random responses could also be a consequence of dopaminergic dysfunction, associated with lower neural signal-to-noise ratio (Sawaguchi and Goldman-Rakic, 1991; Kroener et al., 2009). Indeed, improvements in VSTM performance on this task have now been reported in patients with Parkinson's disease treated with dopaminergic drugs (Zokaei et al., 2015).

Dysfunction within both cholinergic and dopaminergic systems has now been reported in RBD before the onset of clinically defined neurodegenerative disease. Single-photon emission computed tomography (SPECT) has demonstrated decreased ^{123}I-FP-CIT uptake in the striatum of cases with RBD, with 40% of patients having an abnormal scan (Selikhova et al., 2009). Similarly, decreased ^{11}C-dihydrotetrabenazine (^{11}C-DTBZ) striatal binding on PET scanning points to loss of dopaminergic neurons in RBD (Ferini-Strambi et al., 2004). Although evidence for cholinergic dysfunction is scarcer, one PET study has revealed reduced acetylcholinesterase activity in RBD (Valerio et al., 2013).

A number of previous studies have reported cognitive impairment in RBD (Ferini-Strambi et al., 2004; Massicotte-Marquez et al., 2008; Terzaghi et al., 2008),

which has been shown to be progressive (Fantini et al., 2011). Moreover, as shown in chapter 4, the presence of RBD in established Parkinson's disease is associated with increased frequency of cognitive impairment, and greater risk of dementia (Postuma et al., 2012). These pioneering studies have assessed several cognitive domains in RBD, but the mechanisms underlying the observed impairments and their relationship to idiopathic Parkinson's disease remain poorly understood. The more focused approach used here implicates a similar mechanism underlying VSTM deficits in Parkinson's disease and RBD. Importantly, as patients with RBD were not impaired in any of the control experiments, sleep disturbances in these patients is unlikely to explain the pattern of results. It is possible that increased random responses on the VSTM task might reflect a general cognitive decline in both patients with Parkinson's disease and RBD. However, we did not find any significant correlation with MMSE scores.

6.4.2 Idiopathic versus genetic disease

The results presented here in RBD are in contrast to those found in individuals with GBA mutations, who carry the strongest genetic risk factor for developing Parkinson's disease (Neumann et al., 2009; Sidransky et al., 2009). On the paradigm used here, it has previously been shown that asymptomatic GBA-positive individuals are more likely to make misbinding errors than controls (Zokaei et al., 2014). Their pattern of deficit is different to that of cases with Parkinson's disease who show increased random corruption of VSTM. Patients with Parkinson's disease who are also GBA-positive appear to have a double hit, showing both increased misbinding and random corruption of VSTM (Zokaei et al., 2014). Although Parkinson's disease is a heterogeneous condition and there might not be a typical' phenotype (Selikhova et al., 2009), our results suggest that RBD is more representative of prodromal stages of idiopathic Parkinson's disease than GBA because both RBD and

Parkinson's disease are associated with the same type of VSTM deficit. Hence RBD might be a better candidate disorder for clinical trials of novel disease-modifying interventions in Parkinson's disease, potentially using the pattern of impairment identified here as a cognitive marker for incipient Parkinson's disease in this group. Longitudinal studies are required to assess the feasibility of such an approach.

6.4.3 Conclusion

Our results demonstrate that it is possible to detect the signature of memory impairment associated with Parkinson's disease in individuals with REM sleep behaviour disorder, a condition associated with a high risk of developing Parkinson's disease. The pattern of visual short-term memory deficit potentially provides a cognitive marker of prodromal Parkinson's disease that might be useful in tracking disease progression and for disease-modifying intervention trials.

Chapter 7

Neuroimaging of idiopathic REM sleep behaviour disorder

The study presented in this chapter has been published as a paper.

M Rolinski, L Griffanti, P Piccini, A A Roussakis, K Szewczyk-Krolikowski, R A L Menke, T Quinnell, Z Zaiwalla, J C Klein, C E Mackay, M T M Hu. "Basal ganglia dysfunction in idiopathic REM sleep behaviour disorder parallels that in early Parkinson's disease" ***Brain***, *139(8):2224–2234, 2016*

7.1 Introduction

In the first experimental chapter I demonstrated a promising method for looking at early basal ganglia disfunction in early established PD. In the chapters that followed, I introduced RBD as a robust marker of prodromal PD. Here, in the final experimental chapter, I will present an analysis designed to explore the potential of resting state fMRI in prodromal PD.

7.1.1 Aims

The aims of the study are three-fold fold:

1. To establish whether basal ganglia dysfunction in patients with RBD is de-

tectable using rs-fMRI;

2. To directly compare the imaging signature seen in RBD to patients with established PD; and
3. To explore the relationship between resting state functional magnetic resonance imaging basal ganglia network dysfunction and loss of dopaminergic neurons assessed with dopamine transporter single photon emission computerised tomography.

7.2 Methods

7.2.1 Subjects

MRI

The study was undertaken with the understanding and written consent of each subject, with the approval of the local NHS committee, and in compliance with national legislation and the Declaration of Helsinki.

Twenty-six patients with RBD (22 males, age 67.0 $\pm$ 7.7 years, symptom duration 7.0 $\pm$ 3.6 years, disease duration 2.4 $\pm$ 2.1 years) were consecutively recruited from the sleep disorders clinics at the John Radcliffe Hospital, Oxford and Papworth Hospital, Cambridge. The diagnosis of RBD was made on the basis of polysomnographic evidence according to standard International Classification of Sleep Disorders-II criteria by a consultant specializing in sleep disorders (Lapierre and Montplaisir, 1992). RBD was defined as an increase in tonic or phasic chin EMG activity during REM sleep and, either history of elaborate motor activity associated with dream content, or the presence of behavioural manifestations occurring during REM sleep during polysomnographic recordings (Lapierre and Montplaisir, 1992). Patients were excluded if RBD was judged by their clinical

team to be secondary to medication use, or was associated with other neurological conditions, including narcolepsy, Parkinson's disease, dementia or multiple system atrophy. RBD symptom duration was calculated as the time from the patient's defined symptom onset; RBD diagnosis duration was taken from the date of the diagnostic polysomnogram. Seventeen of the patients were receiving treatment for their RBD; thirteen were taking clonazepam, seven were taking melatonin, and three patients were taking both medications.

Forty-eight age- and gender-matched patients with a clinical diagnosis of idiopathic Parkinson's disease according to the UK Parkinson's disease Society Brain Bank criteria (Hughes et al., 1992) [31 males, age 67.0 $\pm$ 7.7 years, disease duration 1.8 $\pm$ 1.5 years, Unified Parkinson's Disease Rating Scale (UPDRS) III 26.4 $\pm$ 12.3, Hoehn and Yahr 1-2, all but five patients were on dopaminergic therapy] and 23 healthy control subjects were recruited from the Oxford Parkinson's Disease Centre patient cohort. Further clinical characteristics across the RBD, Parkinson's disease and control groups are summarized in Table 1, and were compared using Kruskal-Wallis test with a post hoc Dunn's test. Twenty-eight patients with Parkinson's disease and 11 healthy control subjects overlapped with those included in our previous study (Szewczyk-Krolikowski et al., 2014). Patients on dopaminergic medications were scanned after at least a 12 h withdrawal, in a clinically defined 'OFF' state. The control subjects had no evidence of significant neurological or psychiatric illness during structured interview and formal neurological examination with a trained movement disorders neurologist.

SPECT

Eight RBD patients had one single single photon emission computerized tomography (SPECT) scan with ^{123}I-ioflupane (six males; age 68.5 $\pm$ 6.8; disease duration from diagnosis 5.3 $\pm$ 3.0; disease duration from onset; 6.3 $\pm$ 3.2, **Table 7.3**). For one

Table 7.1: Comparison of clinical characteristics in RBD, Parkinson's disease and control groups.

Variable	RBD (n=26)	PD (n=48)	Controls (n=23)	P-value[a] RBD vs. PD vs. Controls	P-value[b] RBD vs. PD	P-value[b] RBD vs. Controls
UPDRS III	3.3 (3.5)	26.4 (12.3)	0.7 (1.1)	<0.001	<0.001	0.067
BDI	9.1 (8.6)	7.7 (4.6)	4.9 (5.6)	0.035	0.40	0.020
Leeds Depression	3.9 (3.6)	3.7 (3.0)	2.9 (3.0)	0.47	0.44	0.17
Leeds Anxiety	2.9 (2.3)	2.6 (2.4)	1.9 (2.7)	0.12	0.27	0.022
MoCA[c]	25.3 (2.9)	27.4 (2.3)	28.2 (1.4)	<0.001	<0.001	<0.001
MMSE	27.3 (1.7)	28.5 (1.5)	29.3 (1.0)	<0.001	<0.001	<0.001
Phonemic fluency[d]	10.9 (4.7)	12.9 (3.8)	15.0 (3.0)	0.006	0.046	<0.001
Semantic fluency[d]	9.8 (3.1)	11.3 (2.9)	13.2 (3.0)	0.003	0.048	<0.001

PD = Parkinson's disease; BDI = Becks Depression Inventory; MoCA = Montreal Cognitive Assessment; MMSE = Mini-Mental State Examination. [a]Kruskal-Wallis; [b]Dunn's test for pairwise comparisons; [c]adjusted for education years; [d]fluencies are age adjusted. Data shown are mean (SD).

RBD patient from this subgroup, MRI data were unavailable for technical reasons. Ten separately recruited age- and sex-matched patients with a clinical diagnosis of idiopathic Parkinson's disease according to the UK Parkinson's disease Society Brain Bank criteria (six males, age 68.6 $\pm$ 6.1; disease duration from diagnosis 0.4 $\pm$ 0.6; disease duration from onset; 1.5 $\pm$ 0.6) had a SPECT scan with ^{123}I-ioflupane similarly to the group of RBDs. All Parkinson's disease patients who undertook SPECT scan with ^{123}I-ioflupane had early unilateral disease (Hoehn and Yahr = 1.0). In addition, a group of 10 separately recruited healthy volunteers (five males, 60.5 $\pm$ 8.9) were recruited as healthy controls. All participants of the SPECT arm of the study were not taking any dopaminergic or serotonergic medication.

7.2.2 Data acquisition

MRI

Data acquisition was performed at the Oxford Centre for Clinical Magnetic Resonance Research (OCMR) using a 3 T Trio Siemens MRI scanner equipped with a 12-channel coil. T1-weighted images were obtained using a 3D magnetization prepared-rapid acquisition gradient echo (MPRAGE) sequence (192 axial slices, flip angle 8 degrees, 1 $\times$ 1 $\times$ 1 mm3 voxel size, echo time/repetition time/inversion

time = 4.7 ms/2040 ms/900 ms) for volumetric and registration purposes. Resting state functional MRI was acquired using gradient echo planar imaging (EPI) (repetition time = 2000 ms, echo time = 28 ms, flip angle = 89 degrees, resolution = 3 $\times$ 3 $\times$ 3.5 mm). 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 their eyes open.

SPECT

Prior to the administration of ^{123}I-ioflupane, thyroid gland blockade was performed by oral administration of potassium iodide 60 mg twice daily starting 24 h prior to the SPECT scan day, and for three consecutive days in total, in accordance with the clinical protocol of Imperial College Healthcare NHS Trust's Nuclear Medicine Department. SPECT data acquisition was performed at the Charing Cross Hospital, using a Symbia^{TM} SPECT-CT scanner (Siemens). Patients were scanned in a supine position using dedicated head restraint to minimize movement.

SPECT images were acquired 3 h after intravenous bolus injection of ^{123}I-ioflupane. SPECT images were obtained continuously while participants were at rest for $\sim$45 min (acquisition parameters: 128 views with 128 $\times$ 128 matrix and 1.45 zoom with 30 s per view in step-and-shoot mode; 15% energy window centred on the 159 keV photopeak of ^{123}I; 2 million total counts). The mean activity dose of ^{123}I-ioflupane was 185 MBq (provided as DaTscan^{TM} injection, GE Healthcare). Tomographic imaging data were reconstructed using the OSEM algorithm incorporating corrections for attenuation, scatter and resolution using Hybrid Recon^{TM} software (HERMES Medical Solutions, Sweden). Reconstructed images were smoothed using a 3D Gaussian filter (full-width at half-maximum = 0.70 cm). SPECT imaging of patients with RBD was performed within 8 $\pm$ 5.6

months apart from magnetic resonance scanning.

7.2.3 Data analysis

MRI

Analyses were performed using tools from the FMRIB Software Library (FSL) (Jenkinson et al., 2012). Voxel-based morphometry analyses of the T1-MPRAGE data were carried out using FSL-VBM (Douaud et al., 2009), testing for reduction of grey matter concentrations in Parkinson's disease and RBD patients compared to controls. We used the recommended FSL pipeline, including segmentation with FAST, non-linear registration with FNIRT and construction of a study-specific standard space template.

Resting state analysis was performed using probabilistic independent component analysis (ICA) as implemented in the Multivariate Exploratory Linear Optimized Decomposition into Independent Component FSL tool (MELODIC) (Beckmann and Smith, 2004). Individual pre-statistical processing consisted of motion correction, brain extraction, unwarping using fieldmap data, spatial smoothing using Gaussian kernel of full-width at half-maximum of 6 mm, and high-pass temporal filtering of 150 s. To account for the effect of motion, non-neural physiology, scanner artefacts and other confounds, we used FIX, an ICA-based denoising approach (Griffanti et al., 2014; Salimi-Khorshidi et al., 2014). Once preprocessed, data were linearly registered to the corresponding structural image using FLIRT (Jenkinson et al., 2002), and registered to Montreal Neurological Institute (MNI) space using non-linear registration.

A previously developed template of resting state networks generated from 80 healthy elderly participants was used (Szewczyk-Krolikowski et al., 2014). It included the BGN and 21 residual noise components that were not fully removed

by FIX and were identified as residual noise based on the identification of standard noise components (Beckmann, 2012) and location of signal peaks in non-grey matter areas (e.g. white matter, CSF, skull), were also included as nuisance covariates. The dual regression approach (Filippini et al., 2009) was used to identify individual temporal dynamics and the associated spatial maps of the resting state networks. Statistical comparisons were performed using permutation-based non-parametric inference within the framework of the GLM using Randomise (v2.1). Results were considered significant for $P < 0.05$, after correction for multiple comparisons (family-wise error) using the threshold-free cluster enhancement (TFCE) approach (Smith and Nichols, 2009), which enhances sensitivity to spatially distributed effects. The design included linear regressors for age and sex.

A post hoc analysis was performed to further characterize the connectivity changes within the BGN between the study groups. For each participant, parameter estimates representing the connectivity of a given voxels with the time-course of the whole network, were averaged within a binary mask containing only significant clusters from the voxel-wise analysis. A receiver operating characteristic (ROC) curve was generated to assess the separation between the two groups. Last, to assess the intra-network connectivity within individual parts of the basal ganglia, subcortical masks were created from the Harvard-Oxford Subcortical Atlas (Mazziotta et al., 2001). The generated masks were used to mean parameter estimates from subject-specific BGN spatial maps, from the following regions of interest: caudate, pallidum and the posterior and anterior putamen, bilaterally. The boundary between the anterior and posterior putamen was taken to be the posterior aspect of the fornix on the axial plane.

SPECT

^{123}I-ioflupane SPECT data were analysed using the BRASS software (HERMES medical solutions, Sweden) following a semi-quantitative approach (Filippi et al., 2005). Each individual's reconstructed image was automatically registered to a predefined template, provided with the software. Following automatic alignment, all scans were inspected visually and manually to fit to the predefined template where necessary. Uptake ratios of^{123}I-ioflupane were calculated for each striatum, caudate, putamen, anterior and posterior putamen relative to the non-specific uptake measured in the occipital cortex. The uptake is defined as the specific binding ratio [(striatal countsbackground counts)/background counts]. The specific DaT binding as reflected by ^{123}I-ioflupane uptake values was calculated for both hemispheres. The average binding for region of interest was calculated per individual as the mean uptake value for both hemispheres.

We tested for differences in tracer uptake between Parkinson's disease, RBD and control groups using the Kruskal-Wallis test. Post hoc Dunn's tests were performed to identify differences between (i) Parkinson's disease and controls; (ii) Parkinson's disease and RBD; and (iii) RBD and controls. All tests used a threshold of $P < 0.05$ one-tailed. Applying methodology similar to that used in the Parkinson Associated Risk Syndrome Study (Jennings et al., 2014), we determined the percentage of expected ^{123}I-ioflupane tracer uptake in the lowest putamen of each RBD and Parkinson's individual by comparing to the mean of the lowest putamen in the 10 control subjects. Individual subjects were categorized as having dopamine transporter (DaT) deficit ($\leq$65% expected lowest putamen ^{123}I-ioflupane binding), intermediate (65-80% expected lowest putamen ^{123}I-ioflupane binding), or no DaT deficit (>80% expected lowest putamen ^{123}I-ioflupane binding).

7.2.4 Correlation analysis: MRI and SPECT

We tested for significant correlation between regional ^{123}I-ioflupane tracer uptake, and BGN parameter estimates for the whole BGN network, and for the individual regions studied, that is caudate nucleus, whole putamen, anterior and posterior putamen, using Spearman's rank correlation. Due to the low number of subjects receiving SPECT and the exploratory nature of the DaT analysis, we did not apply correction for multiple comparisons.

7.2.5 Candidate's contribution to the study

The candidate designed the study. The majority of the MRI data was collected by the candidate, with additional input from Drs Griffanti, Szewczyk-Krolikowski and Klein. SPECT data was collected and analysed by Dr Roussakis and Prof. Piccini. The candidate performed all MRI and statistical analyses, interpreted the results and wrote the manuscript on which this chapter is based.

7.3 Results

7.3.1 Voxel-based morphometry

Voxel-based morphometry analysis did not yield any significant grey matter differences between the three groups, including within cortical or brainstem subregions. Hence, voxel-wise grey matter masks were not included as covariates in the functional MRI analysis.

7.3.2 Resting state network analysis

The mean relative (time point-to-time point) and absolute head motion during functional MRI acquisition did not differ significantly between the three groups

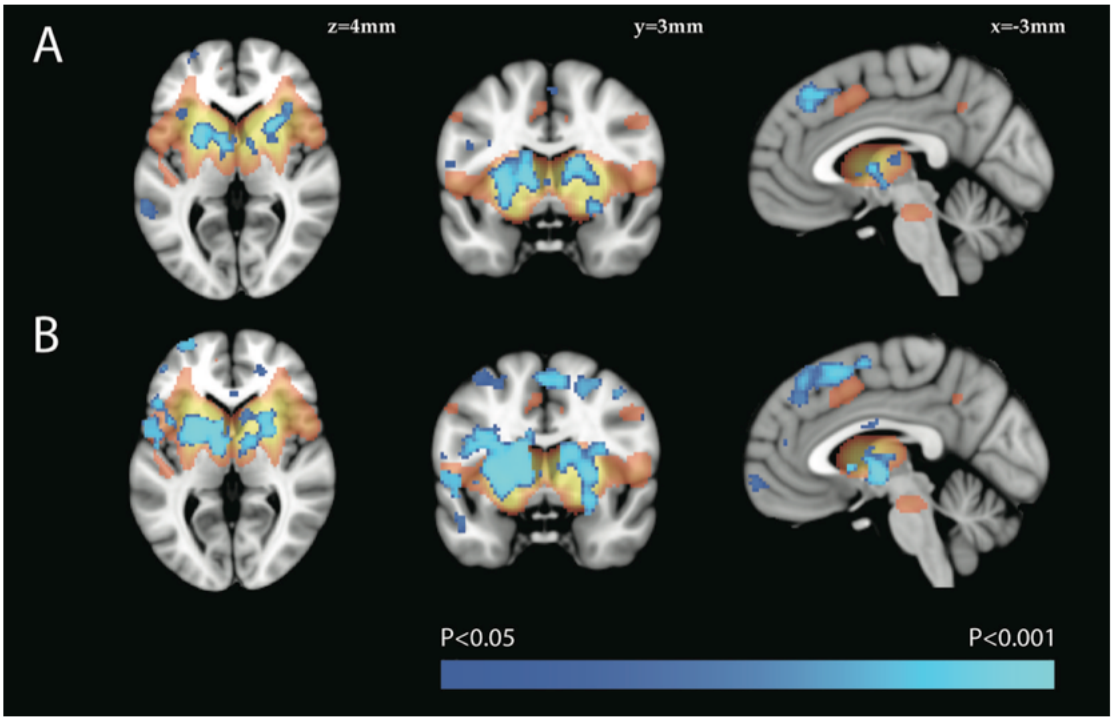

Figure 7.1: Results of resting state fMRI analysis. Group difference maps illustrate clusters of significantly reduced connectivity (blue) patients with A. PD and B. RBD, when compared to healthy controls. Clusters are thresholded at $P<0.05$ after FWE correction. A map of the basal ganglia network (BGN) in shown in orange (thresholded at $Z<2.6$).

[$F(2,94) = 2.93$, $P = 0.06$ and $F(2,94) = 1.58$, $P = 0.2$, respectively].

Significantly reduced coactivation within the BGN was found in patients with Parkinson's disease and RBD, when compared to healthy controls **(Figure 7.1)**. In both cases, significant clusters were found within the basal ganglia, as well as frontal regions, such as the cingulate and paracingulate gyri, the frontal orbital cortices and the inferior and middle frontal gyri **(Table 7.2)**. Voxel-wise comparison did not reveal any statistically significant differences when patients with RBD were compared to patients with established Parkinson's disease.

Individual mean parameter estimates were extracted from the significant clusters. In the case of Parkinson's disease, the mean parameter estimate differentiated the disease group from the healthy controls with a sensitivity and specificity

Table 7.2: Regions showing significantly lower basal ganglia network activity in patients with Parkinson's disease and RBD, compared to healthy controls.

Cluster location	Cluster size (voxels)	Most significant voxel (MNI coordinates: {x, y, z})
Parkinson's disease		
L putamen	1583	–24, 4, 0
R paracingulate gyrus	1493	4, 26, 42
R putamen	1127	24, 12, 8
L inferior temporal gyrus	324	–58, –52, –12
R putamen	216	28, 0, –10
L inferior frontal gyrus	133	–50, 10, 12
L frontal pole	105	48, 26, 28
RBD		
L putamen (extending into R putamen)	11639	–24, 6, 0
R frontal orbital cortex	703	50, 28, –12
L frontal orbital cortex	455	–26, 18, –12
R middle frontal gyrus	133	42, 16, 36
R cingulate gyrus	66	16, –38, 32
L middle temporal gyrus	36	–54, –16, –16
L middle temporal gyrus	16	–62, –46, 0

L = left; R = right. $P < 0.05$ FWE corrected, cluster $\geq$10 voxels.

of 95.8% [95% confidence interval (CI) 85.6–99.5] and 73.9% (95% CI 51.6–89.8), respectively. The area under the curve (AUC) was 0.90 (95% CI 0.83–0.98). The RBD cases could be differentiated from the healthy controls with a sensitivity of 96.2 (95% CI 80.4–99.9) and specificity of 78.3 (95% CI 56.3–92.5). The AUC was 0.92 (95% CI 0.85–1.00). The distribution of individual mean parameter estimates extracted from the clusters that showed significant difference in both comparisons is illustrated in **Figure 7.2**.

To control for laterality we compared the parameter estimates extracted from the BGN within the areas that showed significant differences between Parkinson's disease and controls (i) between Parkinson's disease subjects with unilateral versus bilateral signs on the UPDRS III; and (ii) between Parkinson's disease subjects with a higher UPDRS III scores for the left side and Parkinson's disease subjects with higher UPDRS III scores for the right side. No significant differences were found in either case. To further investigate the influence of laterality of symptoms with functional connectivity we correlated the parameter estimates extracted from the BGN with the contralateral UPDRS III score. No significant correlation was found.

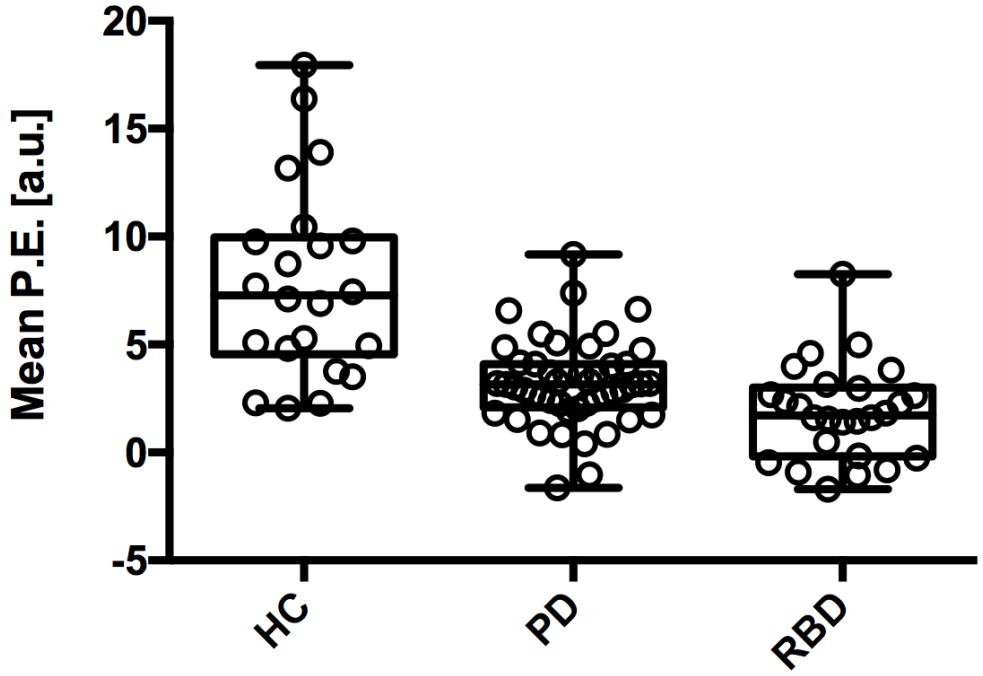

Figure 7.2: Mean parameter estimates extracted from significant clusters that appeared in both the healthy controls versus Parkinson's disease and healthy controls versus RBD comparisons. Each boxplot represents (from bottom to top) quartile 1, median, and quartile 3, with whiskers representing the minimum and maximum mean parameter estimate (P.E.) values for the group.

7.3.3 Anatomical regions of interest

The mean parameter estimates extracted from anatomical regions within the basal ganglia are shown in **Figure 7.3**. Both the Parkinson's disease and RBD groups had significantly lower parameter estimate values within the caudate, pallidum, and the anterior and posterior putamen, when compared to the healthy control group. There were no statistically significant differences between the RBD and Parkinson's disease groups.

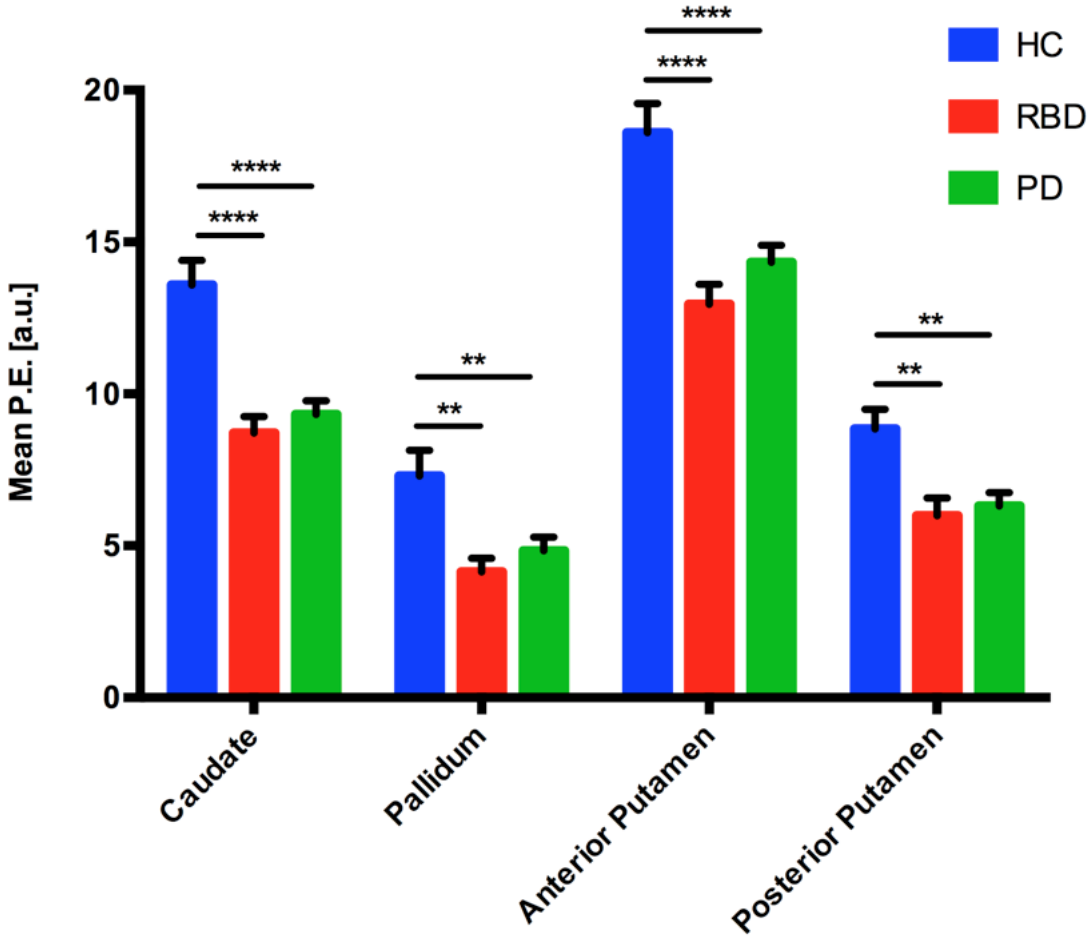

Figure 7.3: Mean parameter estimates extracted from anatomical regions. The mean parameter estimate (P.E.) values were significantly lower in both the Parkinson's disease and RBD groups, when compared to the healthy control group, in all four areas tested. There was no significant difference in any of the regions when RBD patients were compared to those with established Parkinson's disease. The bars represent the group mean and the standard error of the mean. P-values corrected using Dunnett's multiple comparison test. *P $<$ 0.05; **P $<$ 0.01; ***P $<$ 0.001; ****P $<$ 0.0001.

7.3.4 SPECT data

The clinical characteristics and mean uptake values from of the ^{123}I-ioflupane SPECT study are summarized in **Tables 7.3** and **7.4**.

Parkinson's disease patients showed reduced ^{123}I-ioflupane uptake in all five regions of interest compared to control subjects (P $<$ 0.01). RBD patients showed

Table 7.3: Clinical characteristics of SPECT participants.

	RBD patients	**Healthy controls**	**Parkinson's disease patients**
Number of subjects	8	10	10
Sex ratio (male:female)	6M:2F	5M:5F	6M:4F
Age at time of scan (years)	68.5 ± 6.8	60.5 ± 8.9	68.6 ± 6.1
MMSE	28.4 ± 1.3	29.7 ± 0.7	28.5 ± 1.1
Hoehn and Yahr stage	n/a	n/a	1 ± 0
Disease duration from onset (years)	6.3 ± 3.2	n/a	1.5 ± 0.6
Disease duration from diagnosis (years)	5.3 ± 3.0	n/a	0.4 ± 0.6

Table 7.4: Uptake values of ^{123}I-ioflupane SPECT.

	RBD patients	**Healthy controls**	**Parkinson's disease patients**
Striatum	2.93 ± 0.45	3.26 ± 0.30	2.15 ± 0.52***†
Caudate	3.19 ± 0.70	3.43 ± 0.43	2.47 ± 0.53**†
Putamen	2.69 ± 0.39	3.10 ± 0.29	1.86 ± 0.54***†
Anterior putamen	3.03 ± 0.46	3.50 ± 0.33	2.20 ± 0.63***
Posterior putamen	2.32 ± 0.44	2.67 ± 0.32	1.30 ± 0.44***†

*P $<$ 0.05, **P $<$ 0.01, ***P $<$ 0.001. Comparison to *controls, or †RBD at P $<$ 0.05

a trend towards reduced ^{123}I-ioflupane uptake compared to normal controls that failed to reach significance in all five regions of interest. Finally, Parkinson's disease patients showed reduced ^{123}I-ioflupane uptake compared to RBD patients in the striatum (P $<$ 0.05), caudate (P $<$ 0.05), putamen (P $<$ 0.05), and posterior putamen (P $<$ 0.05). **Figure 7.4** shows individual level ^{123}I-ioflupane DaT binding in the putamen with the lowest uptake (right or left) for healthy controls, Parkinson's disease and RBD subjects. Eight of ten Parkinson's disease subjects and one out of eight RBD subjects were categorized as having DaT deficit ($\leq$65% expected lowest putamen ^{123}I-ioflupane binding), with one out of ten Parkinson's disease and two of eight RBD subjects categorized as having intermediate DaT deficit (65-80% expected lowest putamen ^{123}I-ioflupane binding). The mean uptake value of ^{123}I-ioflupane for the RBD group in the putamen was 13.2% lower than the

mean value of the normal controls, and 30.8% higher than the mean value of the Parkinson's disease patients.

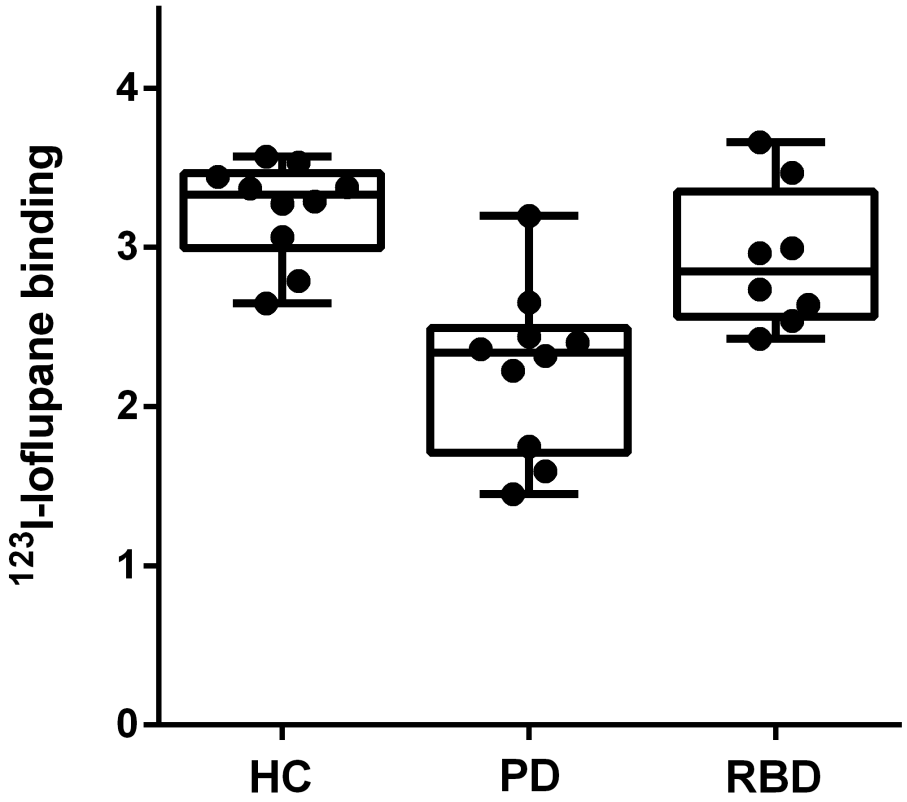

Figure 7.4: ^{123}Iioflupane binding in the lowest putamen of control, Parkinson's disease and RBD subjects. Each boxplot represents (from bottom to top) quartile 1, median, and quartile 3, with whiskers representing the minimum and maximum ^{123}Iioflupane binding for the group. HC = healthy controls; PD = Parkinson's disease.

7.3.5 Correlation analysis: MRI and SPECT

Both MRI and SPECT data were available for seven of eight subjects. We did not detect correlation between regional ^{123}I-ioflupane tracer uptake, and BGN parameter estimates for any of the striatal subregions, or the striatum as a whole.

7.4 Discussion

In this study, we explore the potential of resting state functional MRI to quantify basal ganglia dysfunction in patients with RBD and to identify an imaging signature of Parkinson's disease before the onset of motor disease. To this end, we performed voxel-wise and region of interest analyses of the BGN, directly comparing the results to healthy controls and patients with established, clinically defined, Parkinson's disease. Additionally, we explore the relationship of basal ganglia dysfunction quantified with resting state functional MRI with dopaminergic state as assessed by ^{123}I-ioflupane SPECT.

7.4.1 Impaired BGN connectivity in RBD

Our data show that widespread aberrant connectivity within the BGN is detectable using resting state functional MRI in patients with RBD who do not manifest significant motor impairment. These changes are most prominent within the basal ganglia themselves, with further extra-striatal changes observed predominantly in the frontal lobes. Moreover, having replicated our previous results in early Parkinson's disease (Szewczyk-Krolikowski et al., 2014) in a larger group, we show that BGN connectivity in patients with RBD directly mirrors that observed in established Parkinson's disease, that is, the Parkinson's disease and RBD groups show a comparable decline in BGN function relative to controls.

Dopaminergic transmission, however, differs between these two groups. In keeping with previously reported data (Eidelberg et al., 1990), our SPECT analysis demonstrated an intermediate dopaminergic phenotype in some RBD patients. While reduction of dopaminergic terminals in the striatum failed to reach significance at a group level in RBD relative to controls, three of eight RBD compared to nine of ten Parkinson's disease subjects were categorized as having dopaminergic

deficits based on putamen ^{123}I-ioflupane uptake.

7.4.2 RBD and prodromal PD

Our results fit with the hypothesis that, in many, RBD may represent the prodromal stage of Parkinson's disease, with an estimated period of 10-15 years of progressive neuronal loss before the onset of the core motor symptoms (Hawkes, 2008). In line with evidence from radiotracer imaging studies, we found significantly decreased functional connectivity affecting the caudate, putamen and globus pallidus, bilaterally. Previous SPECT scans have demonstrated decreased ^{123}I-FP-CIT uptake in the striatum of patients with idiopathic RBD, with 40% of patients classified as having a clinically abnormal scan (Eisensehr et al., 2003; Iranzo et al., 2010). Similarly, decreased ^{11}C-dihydrotetrabenazine (^{11}C-DTBZ) striatal binding on PET scanning suggests loss of dopaminergic neurons in patients with RBD (Albin et al., 2000). Supporting the concept of a BGN dysfunction in RBD, a previous PET study has established an expression of the metabolic Parkinson's disease-related spatial covariance pattern in RBD using ^{18}FDG-PET (Holtbernd et al., 2014).

In the only previously published study of resting state connectivity in RBD, Ellmore and colleagues (Ellmore et al., 2013) reported on seed-based nigrostriatal and nigrocortical connectivity in 10 RBD patients, 11 Parkinson's disease patients and 10 healthy controls. The authors reported altered connectivity between the left substantia nigra and the left putamen, and the right substantia nigra and the right cuneus/precuneus/superior occipital gyrus. In all cases, the connectivity between these structures was significantly different in patients with RBD compared to both the Parkinson's disease and healthy control groups. However, there was not always a difference between Parkinson's disease and healthy controls, creating uncertainty on the relationship between these seed-based measures and nigrofugal pathway

dysfunction. In contrast, our study used a data-driven approach to investigate the basal ganglia functional network as a whole. Unlike the previous seed-based study, we found no significant BGN differences between the RBD and Parkinson's disease groups, whether comparing the groups on a voxel-wise or region of interest basis.

The apparent floor effect we have observed across the basal ganglia functional network in Parkinson's disease and RBD subjects compared to the intermediate dopaminergic phenotype seen in RBD with ^{123}I-ioflupane uptake may be best understood by appreciating what these different imaging modalities measure. Resting state functional MRI uses resting blood oxygen level-dependent signal to identify brain regions showing a strong temporal coherence (coactivation) in low frequency fluctuations (typically $<$0.1 Hz). These regions are defined as resting state networks, and reflect the intrinsic properties of brain organization (Filippini et al., 2009). Increased default mode network (DMN) coactivation in APOE ϵ4 carriers at higher risk of future dementia has been demonstrated decades before any clinical, structural or neurophysiological correlate of neurodegeneration in young healthy adult carriers (Filippini et al., 2009). These changes were unexplained by differences in memory performance, brain morphology or resting cerebral blood flow.

7.4.3 Symptoms of PD and rs-fMRI

In our prodromal PD subjects, the observed changes in BGN connectivity may occur years or even decades before the onset of clinical PD symptoms, let alone significant motor impairment leading to a Parkinson's diagnosis. As shown in chapter 5, RBD subjects frequently manifest subtle features of motor impairment prior to their Parkinson's disease diagnosis. This might suggest that RBD and Parkinson's disease are not discrete clinical entities, but in fact manifestations of the same condition at different time points, with a detectable resting state

functional MRI correlate very early in the disease evolution. Although longitudinal clinical and neuroimaging follow-up of the study groups is currently underway to formally assess this, our results would suggest that there is no increase in desynchronisation within the BGN as individuals move from the premotor to the motor stage of Parkinson's disease. This is consistent with findings in chapter 3 that basal ganglia connectivity does not correlate with the severity of motor impairment in established Parkinson's disease.

In contrast, dopaminergic function estimated with ^{123}I-ioflupane SPECT or ^{18}F-Fluorodopa PET is directly related to proportion of surviving substantia nigra dopaminergic neurons and related dopaminergic nerve terminal density, with its strongest clinical correlate being contralateral rigidity and bradykinesia (Leenders et al., 1990). Our finding of an intermediate dopaminergic phenotype in RBD compared to Parkinson's disease may simply reflect the relative temporal progression seen with these imaging modalities, with functional coherence being affected many years prior to the onset of dopaminergic neuronal degeneration. Furthermore, significant motor symptoms generally emerge only after 50-70% of dopaminergic nerve terminals have been irreversibly lost (Fearnley and Lees, 1991), while compensatory or reactive changes in functional brain networks measured with resting state functional MRI will inevitably predate this by several years. Longitudinal studies will also help address the interesting question of whether the transition from RBD to Parkinson's disease might be marked by changes in the functional coherence of resting state networks other than the BGN, such as the default mode network, which may be of particular relevance given the higher cognitive burden when early Parkinson's disease is associated with concomitant RBD, as seen in chapter 4.

7.4.4 Extrastriatal abnormalities

We also detected reduced connectivity outside the basal ganglia, including a number of frontal areas, such as the cingulate, paracingulate and middle frontal gyri in Parkinson's disease and RBD subjects compared to controls. Functional connections between the basal ganglia and these frontal areas are known to be associated with executive function (Gordon et al., 2015). Although executive dysfunction was not formally assessed in this study, global measures of cognitive function (Montreal Cognitive Assessment, Mini-Mental State Examination) and verbal fluency were reduced in RBD compared to controls, and in RBD compared to Parkinson's disease subjects **(Table 7.1)**. Interestingly, voxel-wise comparison did not reveal any statistically significant differences in these frontal areas when patients with RBD were compared to patients with established Parkinson's disease, despite the observed clinical differences in global cognition and verbal fluency. Executive dysfunction is known to be common in early Parkinson's disease (Dirnberger and Jahanshahi, 2013) and has also been shown to be associated with RBD (Massicotte-Marquez et al., 2005). Our imaging findings would support this work.

7.4.5 Rs-fMRI as a biomarker

Connectivity within the basal ganglia network differentiated patients with RBD from healthy controls with a sensitivity and specificity of 96.2% and 78.3%, respectively. While useful in itself, the greatest utility for this approach would be to facilitate the diagnosis of prodromal Parkinson's disease, expressed as BGN network dysfunction in these subjects. However, the utility of BGN dysfunction as an imaging marker for the detection of prodromal Parkinson's disease will only be addressed through careful longitudinal assessment of a larger RBD cohort, which is currently underway.

We did not detect a significant correlation between BGN dysfunction and radiotracer uptake in the seven participants in whom both data were available, which may simply reflect a lack of statistical power. Despite best efforts, we were unable to perform SPECT scans in a larger RBD subgroup within the time constraints for this study, as participants were frequently unwilling to travel the longer distances incurred. A previous longitudinal study with serial ^{123}I-FP-CIT SPECT revealed significant decline in tracer uptake in patients with RBD, consistent with progressive nigrostriatal dopaminergic dysfunction (Iranzo et al., 2011). Importantly, it was those patients with the lowest tracer uptake at baseline that developed Parkinson's disease within the 3-year follow-up period. However, these results hold on a group level only, and due to considerable overlap of uptake values between RBD and controls, the predictive value of a single SPECT scan is limited. In contrast, resting state functional MRI analysis of BGN network dysfunction in our study yielded a sensitivity of 96.2% and specificity of 78.3%, indicating its potential as an indicator of early basal ganglia dysfunction. Moreover, compared to radiotracer imaging, resting state functional MRI does not carry an ionizing radiation burden; it is also cheaper and more readily accessible.

7.4.6 Limitations and future directions

The advanced imaging techniques included in this study are currently research tools. Further independent validation and correlation with clinical outcomes will be necessary before they may be considered for true diagnostic use. Longitudinal clinical and MRI follow-up of our cohort, as well as acquisition of locally-acquired SPECT data, are currently underway to allow us to assess the potential for resting state functional MRI to predict the onset of Parkinson's disease, and to investigate its relationship with dopaminergic dysfunction.

In our study, voxel-based morphometry analysis did not yield any significant

grey matter differences between the three groups, including within cortex or the brainstem subregions, which could account for the differences in functional connectivity. Whilst previous studies have reported grey matter abnormalities associated with RBD (Ellmore et al., 2010; Scherfler et al., 2011; Hanyu et al., 2012), subjects in these studies have generally had a longer reported RBD disease duration (9.2 years, Scherfler et al., 2011) than the mean of 2.4 years in our relatively early cohort, which may have influenced results. Our findings mirror those in early Parkinson's disease, where the use of structural compared to functional imaging has been somewhat disappointing (Menke et al., 2014). One could therefore speculate that on the basis of our results, the imaging correlate of RBD progression to established motoric Parkinson's disease is the evolution from functional network reorganization, through mild cortical and subcortical atrophy, followed by significant midbrain dopaminergic cell loss.

The diagnosis of RBD was confirmed through stringent clinical and polysomnographic assessment, but logistical and technical constraints meant that, in control subjects, the presence of RBD could not be formally excluded using polysomnography. However, the prevalence of RBD in the general population is low (Kang et al., 2013), and accidental inclusion of such a subject would not impact negatively on our conclusions. The PD group was not screened for RBD as the study was not esigned and, therefore, not powered to assess the impact of concomitant RBD on the basal connectivity in PD patients. This is an interesting subject that could be tackled in future studies.

7.4.7 Conclusion

We have demonstrated resting state functional changes in the BGN of patients with RBD, and they mirror those of established Parkinson's disease. Our findings support the presence of early basal ganglia dysfunction in these patients even

before the onset of clinically relevant motor symptoms. Clinical and neuroimaging follow-up is necessary to assess the clinical utility of resting state functional MRI as an imaging biomarker to identify those most at risk of future conversion to the motor stages of Parkinson's disease. This emerging MRI technique has the potential to deliver individualized risk assessment using a multimodal approach combined with other clinical measures, and has important implications for future neuroprotective trials in this key prodromal group.

Chapter 8

Final Discussion and Conclusions

8.1 Summary of Objectives and Results

The overarching aim of the thesis was to assess the utility of a resting state fMRI-based biomarker in a group representative of prodromal Parkinson's disease. Details of the objectives and results of the experimental chapters were as follows.

8.1.1 Early Parkinson's disease and the resting brain (Chapter 3)

The focus of this chapter was to expand on previous work to develop a rs-fMRI biomarker in early motor PD and assess whether changes within the BGN are disease-specific. A priori anatomical confines were placed on the analysis with a view of making this methodology more translatable and reproducible.

Reassuringly, altered BGN activity was only observed in PD, with no significant changes seen in patients with AD. Measures of functional connectivity extracted from anatomical regions within the basal ganglia were significantly lower in subjects with PD, when compared to the two control groups. Consistent with previous radiotracer studies, the greatest drop in functional connectivity was observed in the posterior putamen. When the connectivity measures were combined into a single component score, rs-fMRI could be used to differentiate subjects with PD from subjects with AD and healthy controls with a diagnostic accuracy of 81%.

8.1.2 REM sleep behaviour disorder and concomitant Parkinson's disease (Chapter 4)

In this chapter the association between established PD and concomitant RBD were explored. In order to better understand the relationship between these two conditions, the prevalence of concomitant RBD and its impact on the motor and non-motor phenotypes of PD were assessed.

Overall, almost half of patients with established PD had symptoms suggestive of RBD. Interestingly, none of the 475 patients studied had been previously diagnosed with RBD. Although concomitant RBD did not seem to have an effect on the motor phenotype, non-motor symptoms were much more common and severe in this group. The coexistence of PD and RBD had a negative impact on health-related quality of life.

8.1.3 Clinical characteristics of idiopathic REM sleep behaviour disorder (Chapter 5)

Following on from the work described in the previous chapter, here, patients with idiopathic RBD were screened for motor and non-motor features of PD. In an attempt to minimise the potential confound of genetic risk factors, patients with a mutiation in either the GBA or LRRK2 genes, or a family history of PD, were excluded from the analysis.

The study supported previous research in showing the presence of early parkinsonan features in patients with RBD. For the first time, it was shown that whilst RBD and early, non-medicated PD clearly differ on diagnostic and fine motor tests, the groups are indistinguishable on non-motor tests. Hence, a direct comparison with the early stages of motoric PD without confounds of dopaminergic medications was established. Importantly, it was demonstrated that common genetic risk

factors for PD are not enriched in the RBD cohort.

8.1.4 Patterns of working memory impairment in idiopathic REM sleep behaviour disorder (Chapter 6)

The phenotype of patients with idiopathic RBD was explored further using a task designed to assess visual short-term memory (VSTM). It was shown that not only did patients with RBD have impaired VSTM, the pattern of impairment was similar to that seen in sporadic PD. This is in contrast to previous research which showed a different pattern of VSTM impairment in symptomatic and asymptomatic carriers of a GBA mutation.

8.1.5 Neuroimaging of idiopathic REM sleep behaviour disorder (Chapter 7)

Lastly, resting-state fMRI was used to explore the question of whether this method can be used to assess early basal ganglia dysfunction in patients with RBD. Abnormalities within the basal ganglia network were found in patients with RBD, the imaging signature matching that of patients with early PD. Rs-fMRI could differentiate subjects with RBD from healthy controls on the group level, as well as on an individual level with a sensitivity and specificity of 96.2% and 78.3%, respectively. In a sub-group analysis, abnormalities in the rs-fMRI basal ganglia network seemed to precede abnormalities on a SPECT scan.

8.2 RBD as prodromal alpha-synucleinopathy

The term idiopathic relates to a condition for which the cause is unknown. In the case of RBD, this no longer seems to be the case. Studies in chapters 4, 5

and 6 support the increasingly undeniable link between RBD and PD. Not only is RBD a common symptom in established early PD, but both motor and non-motor symptoms of PD can be observed in, so called, idiopathic RBD.

As discussed in chapter 4, the onset of RBD symptoms fits the generally accepted Braak staging of PD (Braak et al., 2003). As the alpha-synucleinopathy-related neuronal damage progresses rostrally, nuclei implemented in the pathophysiology of RBD are affected before sufficient damage to the nigrostriatal pathway occurs and the diagnosis of PD can be made. Therefore, by the time the motor symptoms occur, many will already have symptoms of RBD. The lack of RBD symptoms in some patients with PD is not surprising given the well-known heterogeneity of the disease (Lawton et al., 2015).

The relationship between concomitant RBD and PD needs to be explored further. A few relatively small studies have explored the impact of RBD on the longitudinal progression of PD symptoms. Work is already underway collecting longitudinal data on the patients in the OPDC cohort. This will allow the prospective assessment on the impact that the presence of RBD has on PD progression. As well as potentially helping clinicians prognosticate at the time the diagnosis of PD is first made, this data may also help to better select patients for recruitment in future drug trials.

As demonstrated in chapters 5 and 6, RBD is associated with the motor and non-motor symptoms attributable to PD. Therefore, the process that has led to the development of RBD is clearly not occurring in isolation. Although limited histopathological data is available, the data that is available suggests that the process leading to this plethora of symptoms is alpha-synucleinopathy (Iranzo et al., 2013).

Deeper clinical phenotyping of the RBD group was explored in chapter 6. At least as far as visual short term memory is concerned, the clinical phenotype

observed in patients with RBD is representative that of sporadic PD. Although more work requires to be done, data obtained so far suggests that results obtained from these subjects seem to be generalisable to the general prodromal PD population. Crucially, this implies that any diagnostic biomarkers developed in this group should be applicable to the general population.

8.3 Developing a neuroimaging biomarker

As discussed in the Introduction, there is real shortage of potential treatment for neurodegenerative disorders being translated from the benches of the basic scientists, passing through the sequential phases of clinical trials to be finally approved for clinical use (Cummings et al., 2014) . It is clear that the reason for this is multifaceted and it would be naive to assume that a single intervention could transform the whole process. However, it is likely that new and better biomarkers are likely to have a big impact, especially once the potential drug enters clinical trials (Baker, 2005).

For neuroimaging to fill the need for better biomarkers, it must be able to provide answer on the level of the individual subject and not just the group as a whole. Previous results (Szewczyk-Krolikowski et al., 2014) have demonstrated that rs-fMRI can be used to study basal ganglia function. When compared to healthy controls, the effect of PD on the basal ganglia network is so profound that rs-fMRI can be used to separate the groups with a good sensitivity and specificity. The results presented in chapter 3 suggest that gross abnormalities within the BGN are confined to diseases associated with basal ganglia dysfunction. Even when the analysis was constrained to specific parts of the basal ganglia selected on the basis of the pathophysiology of the disease, good group separation was achieved.

As will be discussed later, further research and development is clearly required.

However, the work presented here takes rs-fMRI a step further on the path of clinical biomarker development. This is required to transform the results of a research project to a clinically useful tool.

So far, we have explored the use of rs-fMRI in established PD, and RBD as a model of prodromal PD. However, The overall aim of this thesis was to contribute the development of a neuroimaging biomarker that may be applied in prodromal PD. In order to do this, rs-fMRI was used to study basal ganglia dysfunction in patients with idiopathic RBD.

The results depicted in chapter 7 show that rs-fMRI is sensitive enough to pick up early basal ganglia dysfunction in patients with RBD. The imaging signature obtained is almost identical to early PD. Although SPECT-based studies have previously identified early nigrostriatal denervation in patients with RBD (Iranzo et al., 2010; Iranzo et al., 2011), my results suggest that rs-fMRI changes precede significant impairment on SPECT. These results come from a small substudy and need to be validated on a much larger sample. Putting this work in context of drug development discussed above, rs-fMRI has the potential to be used to identify those at highest risk of developing PD. Not only could drugs be trialled at an earlier stage of the disease but also the diagnosis of PD could act as a hard endpoint.

8.4 Future Developments

Although the results presented as part of this thesis show a lot of promise, many questions remain unanswered and many steps must be taken before rs-fMRI can be considered a viable clinical biomarker. I will now discuss a number of options to take this work further.

8.4.1 Is RBD a subtype of PD?

Although the results I have presented would suggest that patients with RBD who go onto develop PD are representative of 'sporadic' PD, only longitudinal follow up of this patients will able to answer this question for sure. Because of recall bias and diagnostic uncertainty, is it impossible to accurately retrospectively ascertain what proportion of patients with established PD first had symptoms of RBD. Prospective follow up of patients with RBD is currently underway as part of the OPDC patient cohort. As a proportion of these patients develops PD, a direct comparison can be made to the PD group in the study.

8.4.2 Is there progression of rs-fMRI changes?

The relationship between the national history of PD and the associated rs-fMRI changes needs to be better characterised. Although the results in chapter 7 suggest that there is no measurable difference between the connectivity seen in the PD and the RBD groups, it is possible individual variability makes identifying differences difficult. Longitudinal clinical and imaging follow up will allow the variation of the signal with time to be studied. Most importantly, the longitudinal follow up of patients with RBD will allow us to answer whether rs-fMRI connectivity is associated with the risk of PD i.e. are the patients with the lowest connectivity score most likely to develop PD?

8.4.3 Can rs-fMRI be used in clinical practice?

As discussed before, a lot must happen before a research result is translated into clinical practice. Although the results in chapter 3 showed that there is a difference in basal gangliia connectivity in patients with PD and AD, these two groups are hardly difficult to distinguish clinically. Moreover, it is not clear whether the results

obtained in strict experimental conditions can be reproduced in clinical settings. The addition of a short resting state sequence to clinical scans could help to answer these questions. The viability of this sequence could be tested and the utility of rs-fMRI to distinguish PD from other neurological conditions, such as essential tremor or dystonic tremor, and other parkinsonian syndromes, such as multiple system atrophy or progressive supra nuclear palsy, could be tested.

8.5 Conclusions

In summary, the research presented as part of this DPhil thesis identified an appropriate group representative of prodromal PD and demonstrated that rs-fMRI may play an important role as PD biomarker. A clear link between PD and RBD was established. Not only is RBD a common and important symptom in early PD, patients with idiopathic RBD have both the motor and non-motor features associated with PD. Deeper phenotyping suggest that patients with RBD are likely to be generally representative of prodromal PD. Analysis of the basal ganglia network using rs-fMRI demonstrated marked abnormalities in patients with early PD, not seen in Alzheimer's disease, where basal ganglia function is preserved. Patients with idiopathic RBD were indistinguishable from those with PD on rs-fMRI despite obvious differences on dopamine transporter single photon emission computerised tomography. The research presented in this DPhil thesis showed that basal ganglia connectivity, as measured using rs-fMRI, is a promising biomarker for the detection of early basal ganglia network dysfunction, and may help to identify patients at risk of developing PD in the future.

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Appendix 1

	Question	Answer
English		
1.	I sometimes have very vivid dreams.	yes/no
2.	My dreams frequently have an aggressive or action-packed content.	yes/no
3.	The dream contents mostly match my nocturnal behaviour.	yes/no
4.	I know that my arms or legs move when I sleep.	yes/no
5.	It thereby happened that I (almost) hurt my bed partner or myself.	yes/no
6.	I have or had the following phenomena during my dreams:	
6.1.	speaking, shouting, swearing, laughing loudly	yes/no
6.2.	sudden limb movements, "fights"	yes/no
6.3.	gestures, complex movements, that are useless during sleep, e.g., to wave, to salute, to frighten mosquitoes, falls off the bed	yes/no
6.4.	things that fell down around the bed, e.g., bedside lamp, book, glasses	yes/no
7.	It happens that my movements awake me.	yes/no
8.	After awakening I mostly remember the content of my dreams well.	yes/no
9.	My sleep is frequently disturbed.	yes/no
10.	I have/had a disease of the nervous system (e.g., stroke, head trauma, parkinsonism, RLS, narcolepsy, depression, epilepsy, inflammatory disease of the brain), which?	yes/no

Figure 8.1: The REM Sleep Behaviour Screening Questionnaire. Reproduced, with permission, from Stiasny-Kolster et al., 2007

Appendix 2

		PD (n=219)	PD/RBD (n=176)	p-value
Age, y		68.2±9.2	67.9±9.4	0.4
Sex, % male		57.5	68.2	0.03
Disease duration, y		1.4±0.8	1.6±1.0	0.2
Delay to diagnosis, y		1.6±1.7	1.5±1.6	0.5
Taking PD medications	Any, %	90.0	93.4	0.2
	Levodopa, %	51.1	55.7	0.4
	Dopamine agonist, %	41.1	39.8	0.8
	LEDD, mg/d	319.3±185.3	343.6±185.2	0.2
Patients reporting improvement on medication, %		79.5	84.9	0.2
Smoking history, %		32.9	41.7	0.07

Supplementary Table 1. Demographic and medication data in subjects with Parkinson's disease (PD-nonRBD) and Parkinson's disease and probable REM sleep behavior disorder (PD-RBD), excluding participants on medications previously reported as causing symptoms of RBD.

		PD-nonRBD (n=219)	PD-RBD (n=176)	Adjusted difference in means* (PD-RBD – PD-nonRBD) (95% CI)	p-value*
Continuous measures					
UPDRS III	Total	26.8±11.6	27.2±10.4	0.11 (-2.07, 2.29)	0.9
	Tremor score	3.2±2.4	3.3±2.8	0.01 (-0.50, 0.53)	1.0
	PIDG score	1.1±1.2	1.2±1.2	0.06 (-0.16, 0.29)	0.6
	Hoehn & Yahr	1.8±0.5	1.9±0.5	0.08 (-0.54, 0.38)	0.7
Purdue Pegboard, s	Total	28.4±6.8	28.1±7.0	-0.13 (-1.40, 1.15)	0.8
	Assembly	16.9±6.0	17.0±6.0	0.23 (-0.89, 1.33)	0.7
Timed Up and Go, s		9.4±4.6	10.5±5.1	0.73 (-0.22, 1.68)	0.1
Binary measures				Adjusted odds ratio* (95% CI)	
UPDRS IV, %	Dyskinesia	4.1	4.5	0.95 (0.35, 2.58)	0.9
	Motor fluctuations	3.3	2.3	0.53 (0.15, 1.94)	0.3
UPDRS II, %	Speech	31.3	47.4	1.79 (1.17, 2.74)	0.007
	Saliva and drooling	46.3	59.3	1.54 (1.01, 2.33)	0.04
	Chewing and swallowing	17.0	27.2	1.70 (1.03, 2.78)	0.04
	Eating tasks	46.3	46.2	0.96 (0.64, 1.44)	0.8
	Dressing	53.2	63.0	1.41 (0.93, 2.14)	0.1
	Hygiene	35.9	43.9	1.39 (0.91, 2.10)	0.1
	Handwriting	56.9	62.4	1.15 (0.76, 1.75)	0.5
	Doing hobbies and other activities	57.8	64.2	1.24 (0.82, 1.88)	0.3
	Turning in bed	46.8	59.0	1.70 (1.12, 2.56)	0.012
	Tremor	79.8	79.8	0.97 (0.58, 1.61)	0.9
	Getting out of bed	64.7	70.5	1.32 (0.85, 2.07)	0.2
	Walking and balance	58.3	68.0	1.48 (0.97, 2.27)	0.07
	Freezing	10.1	20.2	2.24 (1.24, 4.02)	0.007
Flamingo, %		45.3	45.7	-0.04 (-0.50, 0.41)	0.9

Supplementary Table 2. Motor symptoms in subjects with Parkinson's disease (PD-nonRBD) and Parkinson's disease and probable REM sleep behavior disorder (PD-RBD), excluding participants on medications previously reported as causing symptoms of RBD.

*Adjusted for age, sex, disease duration and smoking history

		PD-nonRBD (n=219)	PD-RBD (n=176)	Adjusted difference in means* (PD-RBD – PD-nonRBD) (95% CI)	p-value*
Continuous measures					
Sniffin Smell Test		7.5±2.9	7.0±2.8	-0.40 (-0.98, 0.18)	0.2
Orthostatic systolic BP drop, mmHg		4.9±16.3	10.0±17.1	3.49 (1.14, 1.25)	0.002
Beck Depression Inventory		7.7±5.3	9.8±6.3	2.18 (0.95, 3.41)	<0.001
MMSE		27.5±2.1	27.1±2.4	-0.46 (-0.89, -0.03)	0.04**
MoCA		24.9±3.5	24.3±3.7	-0.43 (-1.12, 0.27)	0.2
Fluency	Phonemic	38.5±14.0	38.9±13.9	0.36 (-2.43, 3.15)	0.8
	Semantic	35.2±8.9	34.2±9.4	-0.82 (-2.53, 0.88)	0.3
Binary measures				Adjusted odds ratio* (95% CI)	
Epworth Sleepiness Scale >9, %		35.5	52.1	1.99 (1.27, 3.14)	0.003
<1 bowel motion/day, %		40.1	48.0	1.47 (0.97, 2.23)***	0.07***
QUIP, %	Gambling	1.4	0.6	0.44 (0.04, 4.35)	0.5
	Sex	3.3	4.6	1.17 (0.40, 3.42)	0.8
	Buying	3.8	4.1	0.95 (0.32, 2.75)	0.9
	Eating	4.7	6.3	1.34 (0.54, 3.33)	0.5
	Hobbyism	10.7	13.2	1.12 (0.57, 2.20)	0.7
	Punding	3.3	5.8	1.63 (0.60, 4.45)	0.3
	Walkabout	0.5	1.1	1.97 (0.17, 22.81)	0.6
	Medication Use	1.4	3.5	2.34 (0.56, 9.81)	0.2
UPDRS I, %	Cognitive impairment	28.9	44.3	1.89 (1.24, 2.88)	0.003
	Hallucinations	8.3	19.9	2.61 (1.41, 4.84)	0.002
	Depressed mood	15.1	22.7	1.83 (1.08, 3.08)	0.02
	Anxious mood	23.7	29.0	1.42 (0.90, 2.26)	0.1
	Apathy	11.9	19.3	1.83 (1.05, 3.21)	0.03
	Features of DDS	2.3	4.5	2.19 (0.69, 6.94)	0.2
	Sleep problems	63.8	83.1	2.95 (1.80, 4.84)	<0.001
	Daytime sleepiness	77.4	84.3	1.60 (0.94, 2.75)	0.09
	Pain	75.7	84.9	1.92 (1.13, 3.26)	0.02
	Urinary problems	59.6	70.3	1.72 (1.11, 2.65)	0.02
	Constipation	39.8	59.5	2.23 (1.47, 3.38)	<0.001
	Light headedness on standing	34.1	48.6	1.83 (1.21, 2.77)	0.004
	Fatigue	68.7	75.1	1.36 (0.86, 2.14)	0.2

Supplementary Table 3. Non-motor symptoms in subjects with Parkinson's disease (PD-nonRBD) and Parkinson's disease and probable REM sleep behavior disorder (PD-RBD), excluding participants on medications previously reported as causing symptoms of RBD.

*Adjusted for age, sex, disease duration and smoking history; **Adjusted for age, sex, disease duration, smoking history and years of education; ***Adjusted for age, sex, disease duration, smoking history and laxative use

		PD-nonRBD (n=219)	PD-RBD (n=176)	Adjusted difference in means* (PD-RBD – PD-nonRBD) (95% CI)	p-value*
EQ-5D	**Continuous measures**				
	Patient-reported health score	72.8	68.0	-4.77 (-8.17, -1.37)	0.006
	Binary measures			Adjusted odds ratio* (95% CI)	
	Problems with mobility, %	42.9	51.4	1.41 (0.94, 2.13)	0.1
	Problems with self-care, %	17.9	25.7	1.61 (0.98, 2.63)	0.06
	Problems with activities, %	37.4	51.4	1.77 (0.86, 2.14)	0.007
	Problems with pain, %	54.3	63.2	1.57 (1.03, 2.39)	0.04
	Problems with anxiety and depression, %	31.1	47.4	2.10 (1.38, 3.29)	0.001

Supplementary Table 4. The impact of health on the activities of daily living in subjects with Parkinson's disease (PD-nonRBD) and Parkinson's disease and probable REM sleep behavior disorder (PD-RBD), excluding participants on medications previously reported as causing symptoms of RBD.

*****Adjusted for age, sex, disease duration and smoking history