Clinical and neuroimaging markers of prodromal neurodegeneration in rapid eye movement sleep behaviour disorder



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A thesis submitted for the degree of
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Abstract

Patients with rapid eye movement sleep behaviour disorder (RBD) have a high long-term risk of developing Parkinson's disease or a related synucleinopathy. Their individual trajectories are enormously varied, however, causing considerable prognostic uncertainty. This thesis examined aspects of the clinical and neuroimaging phenotype of RBD patients which might shed light on the underlying neurodegenerative process and thereby contribute to future risk stratification.

We found that across a range of clinical prodromal features, RBD patients were at least as impaired as those with established Parkinson's and that neuropsychiatric disorders were particularly prominent in RBD patients. Genetic, environmental and demographic factors differed between PD and RBD groups. In a multi-centre longitudinal analysis, the outcomes of simple in-clinic tests were shown to substantially affect the risk of phenoconversion.

Using ioflupane SPECT, we found that 43% of RBD patients already had evidence of dopaminergic deficit, and that this declines by an estimated 7% per year. Neuromelanin-sensitive and susceptibility weighted MRI of the substantia nigra revealed signal changes in RBD patients consistent with early neurodegeneration. A combination of these novel MRI markers accurately distinguished PD patients from controls, suggesting an ability to capture the hallmark pathological process.

We found that apathy was common and under-recognised in RBD patients and dissociable from other neuropsychiatric features. SPECT neuroimaging data revealed that apathy was correlated with serotonin levels in the dorsal raphe nucleus, but not with dopaminergic availability. We used an eye-tracking paradigm to show that reward sensitivity is blunted in RBD patients, but that this relates to dopaminergic decline rather than clinical apathy.

Overall, our data indicate that RBD patients have extensive evidence of underlying neurodegeneration, which manifests as a clinical phenotype with unique characteristics, suggesting that RBD may represent the prodromal phase of a particular synucleinopathy subtype. Multimodal assessment with in-clinic measures, neuroimaging and biometric data has the potential to facilitate risk stratification on an individual basis and will be important in selecting high-risk RBD patients for neuroprotective clinical trials.

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Foreword

The Oxford Parkinson's Disease Centre Discovery Cohort

The majority of the data presented in this thesis were acquired from the Discovery cohort at the Oxford Parkinson's Disease Centre. This is a community-ascertained cohort established in 2010 to study the natural history of Parkinson's disease. More than 1000 patients with newly diagnosed PD were recruited between 2010 and 2015 from hospital clinics in the Thames Valley region. From 2013 to the present, patients with idiopathic rapid eye movement sleep behaviour (RBD) have also been recruited, from sleep clinics at the John Radcliffe Hospital (Oxford), the Royal Papworth Hospital and Sheffield Teaching Hospitals, in order to study the prodromal phase of parkinsonian disorders. Recruitment of RBD patients is ongoing; at the time of writing, more than 200 are under active follow-up. In addition, the cohort comprises more than 300 healthy control participants. Cohort participants undergo clinical assessments every 18 months. In addition to these core assessments, all eligible patients are invited to take part in the MRI sub-study, which is ongoing, as well as certain other investigations as they arise, such as the DaT SPECT imaging and pupil tracking studies described here.

My role in the OPDC

As a Clinical Research Fellow from 2016 to 2019, I have been part of a team of clinicians characterising the cohort in research clinics. This role includes the initial assessment of RBD patients newly recruited to the study as well as longitudinal assessments of existing cohort participants. I have been responsible for the acquisition of new MRI data, performing scans on a weekly basis. In addition, I have managed all aspects of the DaT SPECT imaging study and recruited participants to the apathy and eye-tracking sub studies. I performed all of the analyses presented in this thesis, other than those specifically acknowledged.

Data included in this thesis

Chapter 2 contains data collected from the Discovery Cohort since its inception in 2010. The neuroimaging data presented in chapters 4-6 was all collected prospectively from Discovery Cohort participants during the course of this DPhil, between 2016 and 2019. All of the data from RBD patients in chapters 7 and 8 was also collected prospectively from Discovery Cohort

participants between 2016-2018. The PD and control data in chapters 7 and 8 was acquired previously from a separate research cohort and was kindly provided by Dr Kinan Muhammed and Prof Masud Husain for the purposes of comparison.

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Previously published work

Parts of this thesis have been previously published or submitted in the papers listed on this page. All material reproduced from these publications was authored by me and agreed by the co-authors.

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Chapter 6

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List of abbreviations

AHI	Apnoea hypopnoea index
ANOVA	Analysis of variance
ASN	Alphasynuclein
AUC	Area under the curve
BMI	Body mass index
BDI	Beck depression inventory
BGN	Basal ganglia network
CI	Confidence interval
CR	Contrast ratio
CSF	Cerebrospinal fluid
DA	Dopamine agonist
DaT	Dopamine active transporter
DBP	Diastolic blood pressure
DBS	Deep brain stimulation
DLB	Dementia with Lewy bodies
DNH	Dorsal nigral hyperintensity
DOPA	Dihydroxyphenylalanine
DRN	Dorsal raphe nucleus
DTI	Diffusion tensor imaging
DWI	Diffusion weighted imaging
EEG	Electroencephalography
EMG	Electromyography
EOG	Electro-oculography
ESS	Epworth sleepiness scale
FC	Functional connectivity
FLAIR	Fluid attenuated inversion recovery
GBA	Glucocerebrosidase
HADS	Hospital anxiety and depression scale
HR	Hazard ratio
ICA	Independent component analysis
ICSD	International classification of sleep disorders
IQCODE	Informant questionnaire on cognitive decline

IRBDSG	International RBD study group
LADS	Leeds anxiety and depression scale
LARS	Lille apathy rating scale
LC	Locus coeruleus
LR	Likelihood ratio
LRRK2	Leucine rich repeat kinase 2
MCI	Mild cognitive impairment
MDS	Movement Disorders Society
MMSE	Mini mental state examination
MNI	Montreal Neurological Institute
MoCA	Montreal cognitive assessment
MPRAGE	Magnetization-prepared rapid acquisition with gradient echo
MRI	Magnetic resonance imaging
MSA	Multiple system atrophy
N1	Nigrosome 1
NM	Neuromelanin
NM-MRI	Neuromelanin-sensitive magnetic resonance imaging
OPDC	Oxford Parkinson's Disease Centre
OR	Odds ratio
OSA	Obstructive sleep apnoea
PET	Positron emission tomography
PD	Parkinson's disease
PDRP	Parkinson's disease related pattern
PPV	Positive predictive value
PSG	Polysomnography
QOL	Quality of life
RBD	Rapid eye movement sleep behaviour disorder
RBDSQ	Rapid eye movement sleep behaviour disorder screening questionnaire
REM	Rapid eye movement
ROC	Receiver operating characteristic
ROI	Region of interest
RS	Reward sensitivity
Rs-fMRI	Resting state functional magnetic resonance imaging
RSWA	Rapid eye movement sleep without atonia

SBP	Systolic blood pressure
SD	Standard deviation
SLD	Sublaterodorsal nucleus
SN	Substantia nigra
SNc	Substantia nigra pars compacta
SPECT	Single photon emission computed tomography
SPECT/CT	Single photon emission computed tomography with computed tomography-based attenuation correction
SPSS	Statistical package for the social sciences
SSRI	Serotonin selective reuptake inhibitor
SUR	Specific uptake ratio
SWI	Susceptibility weighted imaging
TCS	Transcranial sonography
UPDRS	Unified Parkinson's disease rating scale
VTA	Ventral tegmental area

Chapter 1. Introduction, aims and objectives

The prodromal phase of Parkinson's disease

Parkinson's Disease is currently diagnosed when the cardinal motor sign of bradykinesia appears alongside rigidity, tremor or postural instability[1]. These features are caused by degeneration of dopaminergic neurons in the substantia nigra pars compacta and consequent denervation of the dorsal striatum[2]. The pathological hallmark of the disease is the presence of Lewy bodies within these structures, containing intracellular aggregations of misfolded alpha-synuclein. The anatomical origin of the disease process, however, is likely to be further afield, perhaps even outside of the brain[3,4]. Braak's hypothesis suggests that neurodegeneration is initiated in the lower brainstem and anterior olfactory structures before ascending to the basal ganglia and cortical areas in a characteristic sequence. In keeping with this, non-motor symptoms such as hyposmia, autonomic failure and sleep disturbances commonly emerge many years before motor parkinsonism (figure 1)[5].

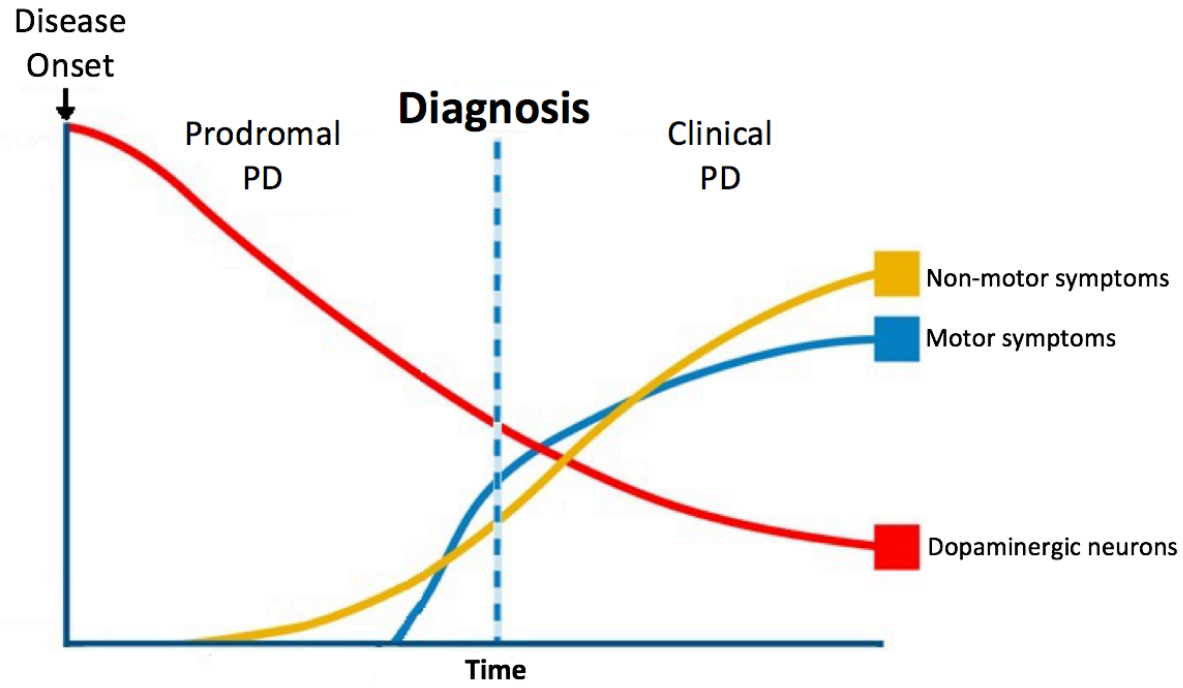


Figure 1. Schematic representation of the pathological progression of Parkinson's disease

It is now recognised that the point at which a clinical diagnosis of PD is made occurs late in the course of this degenerative process. Post-mortem and neuroimaging studies suggest that degeneration within the substantia nigra may commence up to 7 years prior to diagnosis[6,7], and that more than 50% of nigral dopaminergic neurons and up to 80% of nigrostriatal synaptic activity have been lost by the time the motor phenotype emerges[8,9]. Furthermore, prodromal symptoms such as hyposmia, constipation and depression can emerge long before dopaminergic decline becomes established, decades earlier than motoric disease in some cases (figure 2)[10–12]. In the face of such longstanding and irreversible neurodegeneration it may be too late to substantially alter disease course with neuroprotective agents by the time PD is diagnosed. Attention has therefore focused on the identification and prognostication of disease during the pre-motor phase of PD, in the hope that this period may offer a window of opportunity for therapeutic intervention.

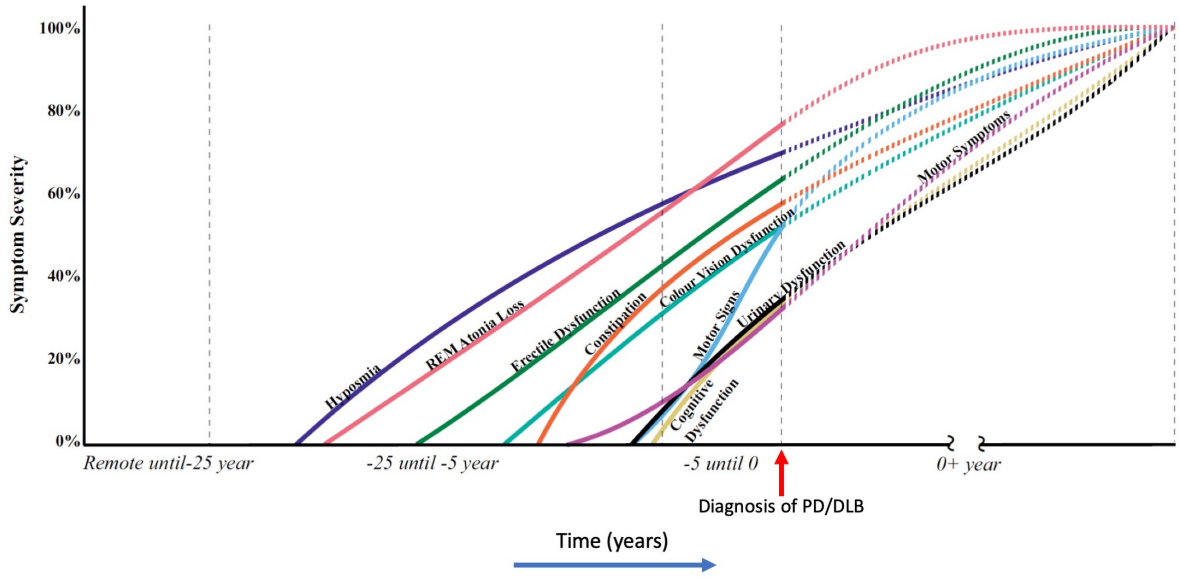


Figure 2. This graph, adapted from the study of Fereshtehnejad *et al*.[12] illustrates the progression of clinical features during prodromal PD, based on longitudinal data from actual patients. The development of symptoms such as hyposmia and REM sleep behaviour disorder many years before PD becomes clinically evident attests to the long period of neurodegeneration that has already occurred by the time a diagnosis of PD can be made.

Challenges in studying pre-clinical and prodromal disease

In recent years the terms *pre-clinical* and *prodromal* have been used to describe the period between the onset of neurodegeneration and the development of diagnostic motor signs, with *pre-clinical* referring to the asymptomatic phase and *prodromal* describing the emergence of non-motor symptoms as the disease progresses[13]. Just as the natural history of established Parkinson's encompasses a broad spectrum of disease phenotypes, so the rate of progression through the pre-clinical and prodromal phases is likely to be highly variable. Identifying, investigating and drawing conclusions from patients in these stages presents a number of important challenges.

When selecting populations in whom to investigate pre-clinical and prodromal disease, a balance must be struck between cohorts that are truly representative of sporadic disease as a whole and cohorts that are sufficiently enriched for at-risk individuals. Population-wide screening is the only way to capture the full spectrum of sporadic disease in its pre-clinical and prodromal phases, though the scale required by such studies limits the scope of investigations that can be performed. The PREDICT-PD cohort adopted this approach and from an initial study population of 1323 captured only 7 incident PD cases during a 3-year follow-up period[14]. Whilst this provides valuable epidemiological data, such cohorts are not suitable for specialist investigations such as neuroimaging, nor interventional studies.

Narrowing down population cohorts by selecting for known risk markers can to some extent overcome this problem, albeit with the potential introduction of bias towards a particular subtype of disease. Amongst the most consistent symptoms of prodromal disease is hyposmia: around 80% of people with Parkinson's have impaired olfaction and, in line with Braak's hypothesis, it is usually one of the earliest prodromal features to emerge[15]. Two prospective cohort studies have reported odds ratios of around 6 for the development of Parkinson's over a 3 to 4-year period in hyposmic individuals compared to those with normal olfaction[16,17]. The high sensitivity of hyposmia as a marker for the whole spectrum of Parkinson's phenotypes minimises bias in such prodromal cohorts. However, hyposmia is common in the population and relatively non-specific; its low predictive value means that large cohorts are still required in order to capture incident Parkinson's. One study that has

successfully used hyposmia to stratify a very large population cohort is the Parkinson's Associated Risk Study (PARS), which used olfactory impairment to select individuals for subsequent neuroimaging. This sequential approach overcomes some of the limitations of olfaction and neuroimaging as individual markers. The PARS study is discussed further in chapters 3 and 4.

Asymptomatic carriers of mutations that cause rare, monogenic forms of Parkinson's present the only opportunity to study a highly enriched cohort in the pre-clinical (rather than prodromal) phase. The most common monogenic cause of Parkinson's is mutation of the autosomal dominant Leucine Rich Repeat Kinase (LRRK2) gene[18], and families harbouring LRRK2 mutations have been the subject of many studies. However, the incomplete penetrance of this mutation, ranging from 24 – 74% by age 80 in various study populations[19–21], means that long term follow-up is still necessary to confirm true pre-clinical subjects. There is also evidence that the characteristics of the prodromal phase in genetic forms of Parkinson's vary according to the gene concerned, calling into question the generalizability of these findings to sporadic disease[22–24]. Heterozygous carriers of genes causing recessive forms of Parkinson's have also been studied, but the risk of Parkinson's in these subjects is low and the form of Parkinson's seen in homozygotes exhibits a different pattern of neurodegeneration to sporadic disease and is therefore even less representative[25,26]. The size of genetic cohorts is also a limitation as most of these mutations are rare and identifying asymptomatic carriers presents logistical and ethical challenges.

REM sleep behaviour disorder – a unique window into prodromal disease

Cohorts of patients with Rapid eye movement sleep behaviour disorder (RBD) present a unique combination of enrichment for future converters and generalisability, and as such RBD has emerged in recent years as the most promising marker of prodromal Parkinson's[27–29]. RBD is present in 30-50% of patients with Parkinson's disease and up to 90% of those with Dementia with Lewy Bodies (DLB) or Multiple System Atrophy (MSA)[30,31]. In a substantial proportion of these patients its onset occurs prior to motor symptoms[5]. Crucially, when seen in isolation, it is a highly specific marker of future synucleinopathy: long-term cohort studies indicate that more than 80% of people who develop isolated RBD will go on to develop an

alpha-synuclein related neurodegenerative disorder[32,33]. RBD is therefore common, representative of a large proportion of sporadic disease, and has a positive predictive value which exceeds even that of LRRK2 mutation carriers. For these reasons, patients with RBD have become the most widely studied prodromal population.

What is RBD? Definition and Diagnosis

Normal sleep architecture comprises discrete cycles of rapid eye movement (REM) and non-REM sleep stages, with the proportion of REM sleep increasing as sleep duration progresses. The REM phase of sleep is characterised by loss of skeletal muscle tone, an active electroencephalogram, narrative dreaming and rapid eye movements[34].

Rapid eye movement sleep behaviour disorder (RBD) is a parasomnia in which the normal muscle atonia of REM sleep is lost and abnormal behaviours such as vocalisation and dream enactment occur[35]. Although a history of abnormal sleep behaviours can be highly suggestive of RBD in the appropriate context, formal diagnosis requires polysomnography (PSG). This uses a combination of electroencephalography (EEG) and electromyography (EMG) to identify the cardinal polysomnographic feature of REM sleep without atonia (RSWA).

RSWA consists of excessive tone and/or phasic muscle twitching during REM sleep, typically recorded in the mentalis and flexor digitorum superficialis muscles[36]. Diagnosis of RBD according to the International Classification of Sleep Disorders (ICSD-3) requires the presence of RSWA alongside abnormal REM behaviours documented either by video recording at PSG or from the clinical history[37]. The significance of RSWA alone, without any overt behavioural component, is uncertain, although there is some evidence that it may be a precursor to RBD[38].

A characteristic feature of RBD is that the associated dreams are often unpleasant or violent in content[39]. A common theme involves the dreamer defending themselves against attack, with corresponding dream enactment behaviours that typically involve hitting or kicking out with shouting or screaming. If patients are woken during an event, they will typically regain full awareness rapidly and recall their unpleasant dream content. Unlike non-REM parasomnias, behaviours are almost always confined to the bed, although violent actions may

result in patients falling from the bed or striking bed partners, potentially causing significant physical harm. The threat or presence of such sleep related injuries are often the trigger for referral to medical services.

When RBD occurs in isolation, it is variously referred to as 'primary', 'isolated' or 'idiopathic', and when it occurs in association with an identifiable cause, it is considered secondary. In addition to its association with alpha-synuclein-mediated disease, secondary RBD can result from a range of other neurological disorders, including narcolepsy and structural brainstem lesions[40,41]. It can also arise as a side effect of certain medications, with selective serotonin reuptake inhibitors (SSRIs) the most commonly implicated drugs[42]. Because of the prognostic implications of an 'idiopathic' RBD diagnosis, it is important to exclude these secondary causes.

The pathogenesis of RBD

A complex and relatively poorly understood circuit of brainstem neuronal pathways underlies the regulation of REM sleep and the associated muscle atonia. The precise malfunction in RBD has not been fully elucidated, but data from animal studies suggest that the pontine sub-laterodorsal (SLD) nucleus (also referred to as the subcoeruleus nucleus) plays an important role[43]. Glutamatergic neurons from this small cluster are thought to inhibit spinal motor neurons by direct projections to inhibitory interneurons in the ventral horn of the spinal cord, and also through indirect connections via GABA- and glycinergic neurons in the ventral medulla[44]. Lesions to SLD neurons in rats cause disinhibition of spinal motor neurons and increased muscle activity during REM sleep[45].

Human evidence for this mechanism is more circumstantial, but radiological data and observed pathology in neurodegenerative disorders also implicate lower brainstem centres. In particular, both structural and inflammatory lesions in this region can cause RBD[40], and it is one of the earliest brain areas to be affected by Lewy body pathology in alpha-synucleinopathies[3], consistent with the emergence of RBD during prodromal disease. The presence of brainstem Lewy bodies in RBD has been confirmed by post-mortem studies and, although in vivo neuroimaging cannot resolve the SLD nucleus itself, loss of signal intensity in

the nearby neuromelanin-containing locus coeruleus has been described using high field strength MRI in RBD patients[10,46].

The Epidemiology of RBD

Determining the true prevalence of RBD in the population is complicated by several factors. Under-recognition, under-reporting and limited access to polysomnography almost certainly mean that the number of confirmed diagnoses represents a small proportion of prevalent cases. Furthermore, patients presenting to sleep clinics may constitute the most severe phenotypic cases and therefore not represent the true spectrum of disease. The specialist nature of the investigations required to confirm the diagnosis preclude widespread population screening, and, although questionnaire-based assessments exist with the sensitivity to pick up true cases, these have a high false positive rate in population settings due to RBD mimics[47,48]. Despite these limitations, a number of studies have used video polysomnography in conjunction with screening questionnaires to assess community-based cohorts and these have indicated a prevalence of around 1-2% in individuals over 60 years of age[38,49].

Age is the biggest risk factor for idiopathic RBD[35]. Indeed, when RBD is rarely seen in those under 50 years, it is more likely to be secondary to factors such as antidepressant treatment or part of a more complex parasomnia disorder[50]. Male sex is the second major risk factor, although the size of this effect may be exaggerated by reporting bias[38,51]. Between 70-90% of patients diagnosed with idiopathic RBD by polysomnography are men with a lower proportion in RBD secondary to Parkinsonian disorders[35]. It has been suggested that since men with RBD are more likely to exhibit violent sleep behaviours than women, their over-representation in sleep clinics may reflect a higher rate of sleep related injury[39].

Conversion of RBD to overt degenerative disorders

The delayed emergence of a Parkinsonian disorder in patients with idiopathic RBD was first reported by Schenck and colleagues in 1996, a decade after that group first described RBD itself[52,53]. They observed that out of 29 male patients diagnosed with idiopathic RBD in their sleep clinic, 11 (38%) went on to develop Parkinson's disease, after an average latency of 3.7

years from RBD diagnosis and 12.7 years from RBD symptom onset. A 16-year update was published in 2013, by which time 81% of patients with available follow up had converted to a neurodegenerative disorder[11]. PD was still the most common diagnosis amongst converters, accounting for 62% of cases, but cases of DLB (14%) and MSA (10%) were also observed. The mean interval between RBD symptom onset of more than 14 years highlights just how long the prodromal phase lasted in these individuals.

These pioneering findings were replicated in two larger cohorts from Barcelona and Montreal[54,55], and as the follow up duration of such studies has increased, so too has the cumulative risk of pheno-conversion observed. Projections of disease-free survival as low as 4% are reported at 25 years after RBD onset, though the number of cases followed for this long remains small[10].

The very high rates of long-term pheno-conversion in RBD have led some to suggest that all patients with iRBD have an underlying synucleinopathy and will convert to an overt parkinsonian disorder if they survive for long enough[56]. However, there remain some patients who have survived more than 20 years from RBD diagnosis without clinical progression, and in those who do progress the timing and evolution of the clinical phenotype is highly variable.[11] RBD patients therefore face a number of prognostic uncertainties, despite the clear long-term risk.

Key questions in RBD research

Before turning to the specific objectives of this thesis, it is worth reviewing the state of the field overall in RBD research by considering some of the key questions facing patients with RBD. These are highlighted in Figure 3, which outlines the diverse clinical outcomes that potentially face patients with RBD. In the following section, I will discuss the extent to which each of these questions remain to be addressed.

Is it really idiopathic RBD?

The first aspect to consider is the importance of accurately diagnosing 'idiopathic' RBD. Given that RBD can occur as a secondary phenomenon, it is possible that this accounts for some cases of long-term non-converters. For example, RBD can result from structural or vascular

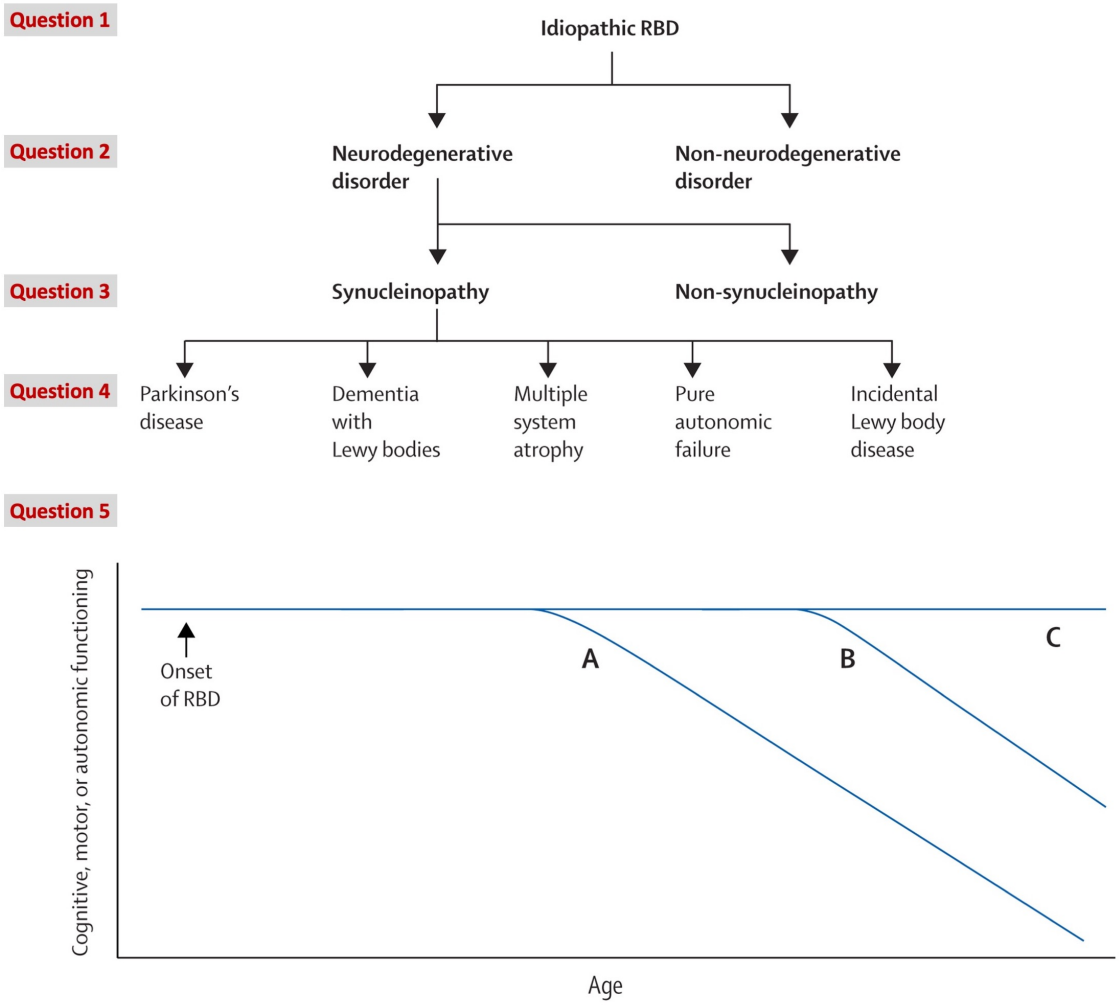


Figure 3. Key questions facing patients with RBD. Question 1: is RBD truly idiopathic or is there an unidentified secondary cause? Question 2: is the underlying cause of RBD neurodegenerative? Question 3: is the degenerative disorder a synucleinopathy? Question 4: what type of synucleinopathy will the patient develop in future? Question 5: will the patient convert to an overt clinical synucleinopathy in the near-term (A), after many years (B) or never (C)? (image adapted from Boeve, 2010[57])

lesions[40], and since it is not routine clinical practice to perform brain imaging in all cases when diagnosing RBD, it is possible that some secondary cases may be inadvertently included in idiopathic RBD cohorts, perhaps accounting for some long-term non-converters. Similarly, even with polysomnography it is possible for occasional errors or false positives to occur, which could lead to RBD mimics being misdiagnosed[39,58]. The now-recognised implications of

an idiopathic RBD diagnosis mean that the diagnostic work-up may need to become more thorough in future to address these issues.

Is there underlying neurodegeneration?

Assuming a correct diagnosis of 'idiopathic' RBD is made, is it the case that every individual has a neurodegenerative phenotype? One recent study has attempted to gain insight regarding this question from studying long-term non-converters. Iranzo and colleagues examined a group of 20 patients who had been followed up in excess of 10 years from their RBD diagnosis without pheno-converting, to explore whether they had a less 'neurodegenerative' phenotype[56]. They found that these individuals exhibited a range of prodromal markers, including clinical (constipation, hyposmia, mild motor signs), histological (alphasynuclein deposits in the colon or submandibular gland) and neuroimaging (DaT SPECT or nigral ultrasound) features, that were more severe than those seen in a matched control group. It was reported that all 20 patients had at least one such prodromal marker and the authors concluded that despite the long conversion-free follow-up, an underlying degenerative phenotype was evident. The data, however, are far from conclusive enough to firmly establish this. Prodromal signs such as constipation, mild motor parkinsonism and hyposmia are common in the general population and do not necessarily indicate neurodegeneration. Even neuroimaging biomarkers such as nigral ultrasound may be positive in healthy control subjects[59]. Furthermore, only 9 of the 20 patients (45%) met the Movement Disorders Society criteria for probable prodromal PD[60], and three patients had a probability of less than 10%. These probabilities can and will change with time[61], but such low values after 10 years of follow up is suggestive of a benign course in these individuals.

Are degenerative cases always alphasynucleinopathies?

This is an important question, not just because of the prognostic implications but also because there are many neuroprotective treatments currently under development which specifically target alpha-synuclein. Many commentators have suggested that these agents may be most effective if delivered during prodromal disease[62], but their success in RBD cohorts will depend on the presence of underlying synuclein-mediated degeneration.

Although the majority of patients who convert from RBD to an overt neurodegenerative disorder display a clinical phenotype consistent with underlying synuclein-mediated disease,

this is not always the case. Of note, in Schenck's original description of the delayed emergence of neurodegenerative disorders in RBD patients, a number of cases exhibited non-synucleinopathy syndromes, including Alzheimer's disease and vascular dementia[53]. Other cohorts have considered even mild cognitive impairment to be a neurodegenerative outcome, which, though in the presence of RBD is perhaps suggestive of emerging DLB, is certainly not specific for underlying synucleinopathy[32]. It is possible that dual or multiple pathologies coexist in some individuals, however, and therefore the presence of an Alzheimer's phenotype does not exclude synuclein pathology as the cause for RBD[63,64]. Indeed, these coexisting pathologies may explain some of the phenotypic variation observed in synucleinopathies.

Taking a more direct approach to underlying pathophysiological mechanisms in RBD, Boeve and colleagues have examined brain pathology in cases of RBD at autopsy. In a large series of patients, alpha-synuclein pathology was found in 98% of individuals with polysomnographically proven RBD, a figure that is often cited as evidence of synucleinopathy in RBD[5,41]. Although just one case was classed as idiopathic and others had clinical Parkinsonian disorders, it was noted that in half of these cases the RBD diagnosis preceded the development of the Parkinsonian syndrome. However, the fact that most patients by definition had secondary RBD at the time of autopsy makes it difficult to conclude that alphasynuclein pathology was the initial cause of the RBD, even if the diagnosis was made at an earlier stage. And since all but one cases had what was clinically a synucleinopathy phenotype, it is unsurprising that the corresponding pathology was observed in most cases. There may also be an element of reverse survivor bias in autopsy studies such as this, given that individuals with RBD who have a more benign course, and do not pheno-convert, would be less likely to reach the point of autopsy during the study period.

Very little histopathological evidence exists in cases of 'still-idiopathic' RBD. Two individual case reports have observed Lewy bodies in post-mortem analysis, but in one of these they were not accompanied by overt neurodegeneration[10,65,66]. Given the fact that Lewy bodies can be observed incidentally in otherwise healthy individuals[67], this evidence is not conclusive that alphasynuclein-mediated degeneration is present in all idiopathic RBD.

What will the future phenotype be?

The range of future phenotypes observed in RBD patients is broad, even within those who develop a synucleinopathy phenotype. Although conversion to MSA and pure autonomic failure is relatively rare, Parkinson's disease and DLB are seen with similar frequency[68]. The distinction between these two clinical syndromes is not always clear cut, but the question of whether an individual will develop cognitive impairment early in their disease course is one that has significant implications for the patient and their relatives. It also highlights the importance of not restricting biomarker research to measures of early motoric disease or nigro-striatal decline, since these will not distinguish between the two synucleinopathy subtypes[69]. Ultimately, it is likely that accurate prediction models will incorporate a range of biomarkers, measuring a spectrum of cognitive, motor, neuroimaging and molecular signatures of disease[70].

The varying trajectories of RBD patients also calls into question the generalisability of Braak's hypothesis of pathological spread, and the study of disease progression in RBD patients may therefore provide important insights into pathological mechanisms of disease propagation in Lewy body disorders[71].

What is the latency between RBD onset and pheno-conversion?

Perhaps the most critical question in current RBD research is that of *when* an individual with RBD will go on to develop an established neurodegenerative disorder, given the extremely wide variation in latencies to pheno-conversion that are observed[57,62]. As well as the significant prognostic implications for the individuals themselves, this is a crucial question for clinical trial development. Although one could argue that the high long-term conversion rates in RBD mean that all patients should ultimately be offered neuroprotective treatments, establishing the efficacy of such interventions will depend on enriching RBD cohorts for near-term converters[62]. The most meaningful outcome measure in a clinical trial will be conversion to overt neurodegenerative disorder, and achieving sufficient numbers with this outcome over the course of a 2-3 year trial period would require an unfeasibly large study population in unselected RBD patients.

Much RBD work has therefore focused on identifying biomarkers that can stratify RBD patients according to risk of near-term conversion. Amongst the spectrum of clinical

prodromal markers that are reported to occur in prodromal disease, the presence of olfactory impairment, abnormal colour vision and subtle motor dysfunction have been shown to increase risk of pheno-conversion in RBD patients[72]. However, other prodromal signs such as depression, anxiety, daytime sleepiness and certain features of autonomic dysfunction do not clearly confer an elevated risk[60,62]. This may in part be due to a lack of power in existing studies, since RBD cohort studies are a relatively recent development and long-term data regarding pheno-converters is lacking. However, even those clinical markers that do influence risk have a relatively modest effect, largely because they are mostly common in the general population and their association with synucleinopathies is weak compared to the high baseline risk that already exists in RBD patients.

More specific biomarkers of risk are therefore needed before lead times can be accurately predicted on an individual basis. Given the variable rate of progression seen across the spectrum of synucleinopathies, which presumably also applies to prodromal disease, markers that detect change in disease progression over time, which can be performed at multiple time points, would be particularly useful.

Neuroimaging research is one of the most promising avenues in this regard, and is a major focus of this thesis. Although many imaging modalities in current clinical use have limited value in the assessment of Parkinsonian disorders, a range of techniques probing the structure and function of neural pathways specific to synuclein-mediated degeneration are emerging as promising tools for the characterisation of prodromal patients[69,73]. A detailed review of these is presented in chapter 3.

Ultimately, risk stratification is likely to involve a multi-step process, with simple, inexpensive but sensitive measures used to screen large populations, followed by more specialist investigations in those with an elevated risk[70,74]. This approach has already been shown to be of value in identifying RBD patients from the general population[48], and as the number of patients diagnosed with idiopathic RBD grows, similar procedures are likely to be employed in order to direct investigations such as neuroimaging to those at greatest risk.

The unique clinical phenotype of RBD

Despite the high proportion of patients with idiopathic RBD that will go on to develop a parkinsonian disorder, these individuals represent a minority of the total PD caseload. Although up to 50% of prevalent PD patients have concomitant RBD, it is thought that only 20% of cases developed the sleep disorder prior to their motoric disease[5]. This figure is higher in MSA[75], but it remains the case that overall the majority of patients with synucleinopathies do not develop RBD during their prodrome. The question arises, therefore, of whether patients who develop RBD prior to their overt synucleinopathy syndrome are representative of prodromal disease as a whole, or whether they represent a subtype of disease with particular clinical phenotype.

There is some evidence that RBD cohorts do have unique demographic and clinical characteristics. Firstly, a male to female ratio of around 5:1 is typically seen in idiopathic RBD, compared with 2:1 in Parkinson's[39,68,76,77]. Although there is debate about whether RBD is truly distributed in this pattern or simply more likely to be reported in men, it is undoubtedly the case that our knowledge of RBD from the existing literature is based on cohorts in which men are over-represented when compared to synucleinopathies overall. Secondly, the risk factors for the development of RBD are not the same as those for other Lewy body disorders. A multi-centre case-control study demonstrated that whilst head injury and pesticide exposure were common risk factors for RBD and PD, others differed[77]. Smoking was more common in RBD patients, despite its association with reduced risk in PD, and caffeine intake was no different between RBD cases and controls, in contrast to the known inverse relationship between caffeine intake and PD risk[78]. A population-based study of over 12,000 Chinese adults suggested some additional risk factors unique to RBD, including cardiovascular risk factors and lower socioeconomic status, although it should be noted that the scale of this study precluded confirmation of RBD by polysomnography[51].

Thirdly, the genetic risk factors seen in PD/DLB only partially overlap with RBD, with the protective effect of the MAPT-H2 haplotype seen in PD being absent in RBD[79], while PD-causing mutations in the LRRK2 gene[80] or the APOE 4 allele associated with DLB and PD dementia[81] seem to have no role in RBD. Mutations in the GBA gene, however, are strongly

associated with RBD as in PD, and may also accelerate the conversion of RBD to defined neurodegenerative synucleinopathy[82].

These risk factor differences may in part reflect the range of future possible phenotypes observed in RBD, meaning that risk factors for dementia are likely to be present alongside those for Parkinson's. Within the spectrum of PD itself, however, there is increasing awareness of the varied subtypes of disease that occur[83]. As follow-up data accumulates on RBD patients who have pheno-converted to PD, there is also mounting evidence that PD patients who develop RBD in the prodromal phase may represent a subtype with a worse motor phenotype and more severe non-motor symptoms such as depression, autonomic failure and cognitive impairment[33,84,85]. Given that many of these features are recognised to occur during prodromal disease, it follows that this particular disease subtype may be apparent even before motoric disease appears. These factors have important implications not only for individual prognosis, but also in the interpretation of future clinical trials, where the natural history of disease subtypes must be clearly established in order to accurately compare the outcomes of treated cohorts.

Identifying the particular clinical syndrome that makes up RBD should not be viewed purely in terms of prognostic implications, however. It may also facilitate immediate treatment and improvement in quality of life, particularly as many non-motor symptoms can be as responsive to treatment as motoric disease. Furthermore, exploring the mechanisms that underly the phenotypic characteristics observed in RBD patients might have broader implications for our understanding of the mechanisms of phenotypic heterogeneity seen in synuclein-mediated diseases.

Apathy in prodromal synucleinopathies

Neuropsychiatric features in general have been a focus of RBD research for several reasons. Because of the high proportion of RBD patients who go on to develop cognitive impairment, patterns of early cognitive dysfunction and their underlying mechanisms have been assessed for their ability to inform regarding future disease trajectory[86–88]. Depression is another prodromal feature that has attracted attention, due to the frequency with which it is reported

to occur in the years leading up to a PD diagnosis, the high rates of depression observed in RBD cohorts and the interactions between certain antidepressant medication and RBD symptomatology[5,42,60,62].

One neuropsychiatric condition that is understudied by comparison, in both prodromal and clinically manifest synucleinopathies, is apathy[89]. Apathy is a disorder characterised by a pathological lack of motivation, which can affect both cognition and behaviour[90]. Apathy has received less recognition than other neuropsychiatric disorders, which may in part be because its frequent coexistence with depression and cognitive impairment has impeded consideration of apathy as a distinct syndrome[91]. However, awareness of the importance of apathy and its negative impact has increased considerably in recent years.

As with depression and cognitive complaints, apathy is frequently noted by PD patients to develop in the prodromal period[92]. RBD patients commonly report apathy when asked directly, but it has never been fully characterised in these individuals[93]. It is not known, therefore, whether apathy truly exists in prodromal patients, or whether its self-reporting could be accounted for by components of other neuropsychiatric disorders, or indeed by factors such as fatigue and sedative medication, which are common in RBD.

Interestingly, in established PD patients the presence of apathy appears to be associated with a worse subtype of disease, with more rapid progression, higher rates of cognitive impairment and reduced quality of life[94–98]. This pattern bears similarities to the more severe PD phenotype that is observed in patients with concomitant RBD[33,35], raising the possibility that RBD and apathy may form part of a common synucleinopathy subtype, potentially with a mechanistic link between the two disorders.

As understanding of apathy has increased, the complexity of the underlying mechanisms has also become apparent. There are many components to goal-directed behaviour, including the registration of external stimuli, evaluation of potential rewards, generation of behavioural options, estimation of the effort required to execute action, weighing up of the cost-benefit ratio, and initiation of action[99]. Dysfunction in any of these processes could potentially

contribute to the expression of apathy, and it is not surprising, therefore, that a wide range of neural pathways and transmitters have been implicated in its pathogenesis.

One mechanism that is of particular relevance to parkinsonian apathy is dopamine depletion. Dopamine plays an important role in the evaluation of rewards, and there is evidence that impairment of reward sensitivity may be an important factor in PD patients with apathy[100,101]. Reductions in metabolism within the ventral striatum, a key dopaminergic region involved in reward processing, have been shown to predict the development of apathy in PD patients undergoing deep brain stimulation[102]. Profound apathy has also been reported following lesions to the basal ganglia, which can be ameliorated with pharmacological dopaminergic stimulation[103]. Numerous neuroimaging studies have implicated dopaminergic pathways in the pathogenesis of apathy, with fronto-striatal limbic circuits believed to be key mediators[104].

There is also evidence, however, that dopamine depletion alone cannot fully explain clinical apathy. Many PD patients with apathy do not respond to dopaminergic medications, and apathy also occurs frequently in other neurodegenerative disorders, such as Alzheimer's disease and frontotemporal dementia, which are not characterised by dopamine deficiency[105–107].

Another neurotransmitter that has emerged recently as a potentially important factor in parkinsonian apathy is serotonin. One neuroimaging study in early PD, using positron emission tomography with separate ligands for serotonin and dopamine transporters, found that serotonin deficiency rather than dopaminergic decline was associated with apathy[108]. This is perhaps surprising, since serotonin has traditionally been thought of as a mediator of responses to aversive rather than rewarding stimuli[109], and medications that enhance serotonergic transmission have previously been reported to exacerbate apathy[110]. There is, however, some evidence that serotonin may play a role in effort sensitivity, and deficiency may therefore contribute apathy via a mechanism distinct from reward evaluation[90].

Evidence regarding the neurochemical basis of parkinsonian apathy extends to other neurotransmitters too. Degenerative changes in noradrenergic system have been implicated[111], and the improvement in apathy following treatment with rivastigmine suggests a possible cholinergic contribution[112].

These data emphasise the complexity of mechanisms underlying apathy. Multiple cognitive and behavioural processes are involved and the dominant mechanisms may vary not only between different neurodegenerative disorders, but also according to disease stage[113]. Uncovering the specific components that contribute to apathy in prodromal disease might therefore not only explain the syndrome, but may also contribute to understanding of disease progression and role of different neurotransmitters as disease advances.

Overview of objectives and questions to be addressed in this thesis

In the introduction above, I have given an overview of the current state of RBD research and discussed the key questions and uncertainties that remain to be addressed by the field in general. The following section now outlines the specific objectives of this thesis and how they fit into this overall picture. Chapters 2-8 fall into three broad experimental themes: part 1 explores the clinical phenotype of RBD; part 2 focuses on neuroimaging of the key brainstem structures involved in early parkinsonian degeneration; and part 3 characterises the mechanisms of apathy in RBD, one of the most common but least understood prodromal features.

Part 1: The clinical phenotype of RBD

What is the clinical phenotype of RBD?

Given the range of symptoms that commonly occur in prodromal disease, significant insight into an individual's underlying disease status and future trajectory could potentially be gained from studying these clinical prodromal features. To that end, in chapter 2 I characterised in detail the clinical phenotype of a large cohort of RBD patients at the point of recruitment to our longitudinal study. I assessed the extent to which prodromal parkinsonian features are evident by rating a wide range of motor and non-motor characteristics that are reported to emerge during the prodromal phase of parkinsonian disorders. I also examined demographic and environmental factors as well as the prevalence of common variants in two genes associated with PD. By comparing these clinical and genetic measures with a group of healthy controls and a group of newly-diagnosed PD patients, I examined whether RBD patients exhibit an intermediate phenotype, consistent with an earlier stage of PD, or whether there are certain features that mark out RBD as a unique clinical syndrome.

Can simple clinical measures inform regarding risk of pheno-conversion?

Using our baseline clinical data, I began to explore factors that may affect an individual RBD patient's probability of underlying synucleinopathy and risk of near-term pheno-conversion. Using hyposmia as a surrogate marker of neurodegeneration, I assessed characteristics that

may affect the timing of RBD presentation and consequently an individual's prodromal stage. I also evaluated the potential of the Movement Disorders Society Research Criteria for Prodromal PD as a stratification tool in RBD cohorts and considered some of the strengths and weaknesses of this approach.

Finally, I described the results of a collaborative study co-ordinated by the International RBD Study Group, which combines our baseline and follow-up data with that of 23 other RBD cohorts worldwide in order to calculate the power of clinical markers to predict pheno-conversion to an overt synucleinopathy.

Part 2: Imaging nigro-striatal degeneration in RBD

Though clinical phenotyping may enable a degree of risk stratification in RBD patients, it is unlikely to be specific enough on its own to confirm the presence of underlying synucleinopathy. Part 2 therefore explores the potential of neuroimaging to gain additional insight into neurodegenerative status and disease trajectory in RBD.

What can neuroimaging contribute to risk-stratification in prodromal disease?

In chapter 3, I reviewed the existing literature on neuroimaging in RBD and other pre-clinical and prodromal populations. I focused primarily on studies that measure nigro-striatal decline, either directly or indirectly, in order to identify parkinsonian degeneration and characterise pheno-conversion risk. I discuss some of the key challenges in prodromal imaging research and identify the most promising techniques for future development.

What is the extent and pattern of dopaminergic decline in RBD?

In chapter 4, I used dopamine transporter (DaT) SPECT imaging to assess the integrity of dopaminergic synapses in the basal ganglia in RBD patients. The value of this imaging technique is well-established in parkinsonian disorders when motoric disease is present, but its role in prodromal disease is less certain. I evaluated the different patterns of abnormality that occur in RBD patients and assessed whether abnormal imaging can be predicted by

clinical phenotypic characteristics. I compared two methods of SPECT registration, one based on the DaT signal alone and a second using structural MRI in parallel to define regions of interest. Finally, I compared the DAT SPECT imaging in RBD patients with findings from patients with established PD in order to estimate the average trajectory of dopaminergic decline between prodromal and clinically manifest disease.

Can MRI of the brainstem identify prodromal degeneration?

In chapters 5 and 6 I investigated the ability of two different MRI techniques to quantify neurodegeneration in the brainstem. Firstly, I used a neuromelanin-sensitive MRI sequence to calculate the volume and signal intensity of both the substantia nigra and locus coeruleus. Both of these structures are susceptible to early degeneration in parkinsonian disorders and contain neuromelanin, which is detectible as a bright signal with these modified T1-weighted sequences. I assessed how well these measures can quantify the known brainstem depigmentation that occurs in patients with PD, and then examined the degree to which this is evident in patients with RBD. I explored how changes in neuromelanin-sensitive MRI measures relate to dopaminergic decline and clinical characteristics, considering its potential as a disease progression marker.

Chapter 6 explores an alternative approach to MR imaging of the substantia nigra, using susceptibility-weighted imaging to measure changes in iron deposition. This technique has been shown to be effective in discriminating PD patients from controls using a simple, binary visual assessment of an area of high signal corresponding to nigrosome-1[114]. As well as investigating the potential of this binary visual assessment in RBD patients, I also explored methods of objectively quantifying SWI changes, both in the nigrosome-1 and more broadly in the substantia nigra. To facilitate this, I used anatomical templates of the substantia nigra defined from the neuromelanin-sensitive imaging characterised in chapter 5. Findings from these SWI analyses were compared to SPECT measures and clinical characteristics. Finally, I investigated the potential of multi-modal substantia nigra assessment by combining measures from SWI and neuromelanin-sensitive MRI.

Part 3: Mechanisms of apathy and reward insensitivity in RBD

One of the striking findings from part 1 was the high rate of apathy reported by RBD patients using a single screening question. Given the role of monoaminergic systems in motivation and reward, this neuropsychiatric feature might be an important marker of the underlying neurodegenerative process. In part 3, I sought to characterise apathy in detail and explore potential underlying mechanisms using some of the neuroimaging data from part 2 as well as an eye-tracking paradigm that measures sensitivity to reward.

How extensive is apathy in patients with RBD?

Though apathy is recognised as a prominent feature of established parkinsonian disorders and is frequently reported to emerge during prodromal disease, it has never been assessed in detail in patients with RBD. In chapter 7, I first characterised the prevalence and severity of apathy in RBD using the Lille Apathy Rating Scale, comparing outcomes with PD patients and healthy controls. I explored the extent to which apathy is dissociable from other neuropsychiatric conditions that commonly occur in RBD patients, and the accuracy of the commonly-used screening tool employed in chapter 2.

Is apathy in RBD related to dopaminergic or serotonergic decline?

In the second part of chapter 7, I went on to explore the neurochemical basis of apathy in RBD through further analysis of the SPECT data acquired in chapter 4. This study took advantage of the fact that ioflupane SPECT binds non-specifically to monoaminergic transporters and can therefore be used quantify both dopaminergic and serotonergic decline. Both of these neurotransmitters have been proposed to be key mediators of parkinsonian apathy; here I explored their relative associations with apathy in prodromal disease. To do this I used co-registration of SPECT and structural MRI in order to quantify the serotonergic signal in the dorsal raphe nucleus as well as other small brainstem regions that cannot be defined on the basis of their SPECT signal alone.

Is reward sensitivity blunted in RBD patients, and is this a marker of dopaminergic decline?

One mechanism shown to be of relevance to apathy in established PD is blunting of sensitivity to rewarding stimuli. In chapter 8 I examined whether this mechanism is evident in patients with RBD, using an eye-tracking task that measures pupil responses to stimuli of varying levels of reward. In PD patients, dopamine appears to be an important modulator of reward sensitivity. I therefore explored the hypothesis that impaired reward sensitivity might be an early marker of dopaminergic decline in RBD patients by examining the relationship between pupillary reward responses and dopaminergic SPECT signal in the basal ganglia.

Chapter 2: The prodromal phenotype of RBD: factors influencing prognostic risk

Introduction

The aim of this chapter is to characterise in detail the clinical phenotype of patients with REM sleep behaviour, by comprehensively assessing the baseline demographic, clinical, and genetic characteristics of a large, prospective cohort of RBD patients recruited from multiple UK sleep centres following polysomnographic confirmation of their diagnosis.

With this analysis, we aimed to address some of the key questions outlined in chapter 1. We sought to establish the extent to which evidence of prodromal disease characteristics is already apparent in RBD patients. To that end, the study was designed to compare their phenotype with a large cohort of healthy control subjects and individuals with early Parkinson's disease, with the latter group assessed prior to treatment initiation in order to avoid the potentially confounding effects of medication. Amongst the questions to be addressed was whether RBD patients, in keeping with their prodromal status, display an intermediate parkinsonian phenotype between that of PD patients and controls, or whether they have distinct characteristics that mark them out as an individual disease subtype. We also sought to explore some of the factors that might affect future risk of pheno-conversion, as well as the variation in lead times previously reported.

Part of this variation in latency from RBD diagnosis to conversion may be a result of differences in the time at which patients present to sleep services, such that patients presenting later may be at a more advanced prodromal stage. We sought to explore the effect of common comorbidities that may influence this. One such comorbidity is depression, since the use of antidepressants can exacerbate RBD symptoms and may therefore unmask the condition at an earlier stage[42]. Respiratory sleep disorders may conceivably exert a similar effect. Concomitant obstructive sleep apnoea (OSA) affects 34-61% of RBD patients[115–117], and it is common for RBD to be diagnosed following an initial referral for suspected OSA[39].

A factor that may interact with obstructive sleep apnoea is body mass index, since high BMI is associated with OSA and is therefore an indirect risk factor for presentation to sleep services. There is also some evidence that BMI may have a direct effect on an individual's risk of Parkinson's disease. This is a somewhat controversial topic, and existing studies have varyingly demonstrated positive, negative and absent correlations between BMI and PD risk[118–120]. However, one of the largest and most robust recent investigations reported strong evidence of an inverse association, suggesting that high BMI is associated with a reduced risk[121]. A large population-based study has reported a similar association with risk of RBD itself[51]. The relationship between BMI and neurodegenerative risk has not been explored in RBD patients; given these interactions with both PD risk and OSA, we hypothesised that RBD patients with high BMI may display a lower-risk clinical phenotype.

Another factor that may contribute to regional variations amongst RBD cohorts is genetic risk. Recent evidence suggests that the genetic profile of RBD is not the same as for PD in general. Mutations in the Leucine Rich Receptor Kinase (LRRK2) gene are the most common cause of familial PD[122], but patients with LRRK2-associated PD have lower rates of RBD than seen in sporadic PD[123], and LRRK2 mutations were not detected in a large Spanish cohort of idiopathic RBD[24]. Mutations in the glucocerebrosidase gene (GBA), on the other hand, appear to be associated with a more severe non-motor phenotype in established PD[22,124], and have a high prevalence in idiopathic RBD[125]. The rates of such genetic risk factors show substantial variation amongst different ethnic groups[126] and these findings therefore require validation in geographically distinct populations.

This study was designed as a cross-sectional analysis of the baseline characteristics of RBD patients, assessed at the point of first recruitment to our longitudinal cohort. Although we include preliminary findings regarding pheno-conversion outcomes after short duration follow-up, we do not currently have enough longitudinal data to look in detail at predictors of near-term conversion. As an interim measure, we used hyposmia as a surrogate marker of neurodegenerative risk status. Hyposmia is a sensitive marker of Parkinson's disease and though lacking specificity in the general population, this is less of a problem in RBD cohorts[127]. Impaired olfaction is also a very early prodromal marker, and it is reasonable therefore to

consider that individuals without this may represent a group at lower risk of near-term conversion[128].

A more comprehensive method of risk stratification that we also sought to assess is the recently developed Movement Disorder Society (MDS) Criteria for Prodromal Parkinson's disease[60]. This method takes the likelihood ratios for future PD conferred by a number of background risk factors and early neurodegenerative signs and combines them into a probability score, with a suggested threshold of 80% indicating probable prodromal PD. Application of these criteria in population and prodromal cohorts has yielded promising results[48,129], but they require further validation in prospective cohort studies.

Most of the existing data regarding longitudinal outcomes in RBD patients comes from a limited number of relatively small cohort studies with long-duration follow-up[11,55,72]. In response to these pioneering studies, the number of RBD cohorts under investigation worldwide has expanded dramatically in recent years. As with our study, however, it will be many years before these young cohorts are able to assess predictors of pheno-conversion individually. To address this issue, a collaborative study was developed by the International RBD Study Group (IRBDSG) combining the baseline findings and follow-up outcomes from 24 centres worldwide, including the RBD patients described in this chapter. This not only enabled a very large study population to be compiled with the statistical power to accurately assess factors that affect long-term outcomes, but it also reduces the effect of centre-specific biases that may affect the outcomes of single-centre studies. The key findings from this study are reported here alongside the baseline data from our cohort.

Methods

Subjects

Patients with idiopathic RBD were recruited from sleep disorders clinics at three centres: The John Radcliffe Hospital, Oxford; Papworth Hospital, Cambridge; and Sheffield Teaching Hospital. The diagnosis of RBD was made on the basis of polysomnographic evidence according to International Classification of Sleep Disorders criteria[37]. Individuals with concomitant OSA were only included if the two conditions were unequivocally distinguishable by polysomnography (PSG). In uncertain cases, the diagnosis of RBD was either confirmed by repeat PSG with the use of continuous positive airway pressure (CPAP), or the individuals were excluded from the study.

Healthy controls and PD patients (diagnosed according to UK PD Brain Bank Criteria[130]) were selected from the Discovery Cohort of the Oxford Parkinson's Disease Centre (OPDC), a community ascertained cohort recruited from the Thames Valley region. In order to avoid the potential confounding effects of anti-parkinsonian medication, and to establish a comparison with the PD population closest to the prodromal phase, we only included patients with early, untreated PD. Full details of our clinical protocol are described elsewhere[131]. The study was approved by the local research ethics committee and informed, written consent was given by all participants.

Subject evaluation

A comprehensive, structured medical history was taken from all participants including co-morbidities, demographic information, environmental and occupational exposures, medications and family history. Motor features were assessed using: part III of the Movement Disorders Society (MDS) revised Unified Parkinson's Disease Rating Scale (UPDRS)[132]; the Purdue Pegboard Test[133]; the Flamingo test (the ability of the patient to balance on one leg for 30 seconds) and the timed 'Get-up-and-go' test[131]. Olfaction was assessed using the "Sniffin' Sticks" odour identification test[134]. Cognition was assessed using the Mini-Mental State examination (MMSE)[135] and the Montreal Cognitive Assessment (MoCA)[136], with scores adjusted for years of education. Mild Cognitive impairment was defined according to the

MoCA diagnostic cut-off (<24/30)[131]. For phonemic fluency, the total number of words generated beginning with F, A and S over 60 seconds was recorded. For semantic fluency, the number of animals and boys' names each generated in 60 seconds was counted. Both fluency scores were adjusted for age. The Beck Depression Inventory (BDI-II)[137] and the Leeds Anxiety and Depression Scale (LADS)[138] were used to evaluate depression and anxiety respectively. Subjective non motor symptoms were assessed using UPDRS part I. EQ-5D was used as a standardized self-report measure of health status[139]. Cardiovascular risk factors were defined as: history of cardiovascular disease (angina, myocardial infarction, stroke or TIA); current smoker; hypertension; hypercholesterolaemia; obesity (BMI>30kg/m^2); diabetes.

For dichotomized analyses, cut-offs for abnormal were defined as: UPDRS III score >4; MoCA <24; Sniffin' Sticks score <10; BDI-II >13; Leeds Anxiety Score >6; Constipation: less than 1 bowel movement per day or use of laxatives; Orthostatic drop in systolic blood pressure >20 mmHg.

Genetic testing

Genetic testing was kindly performed by Samuel Evetts in the OPDC lab, who provided details of the assay for this paragraph. Participants were screened for G2019S and R1441C mutations in the LRRK2 gene and N370S and L444P mutations in the GBA gene. For the LRRK2 screening, results were available for 289 controls, 136 RBD patients and 114 PD patients. For the GBA screening, results were available for 283 controls, 116 RBD and 106 PD patients. DNA was extracted from whole blood using a Qiagen Autopure automated system. Polymerase Chain Reaction (PCR) was performed using MegaMix Blue (Microzone) containing a recombinant *Taq* polymerase. Primer sequences were 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.

Statistical analysis

Between-groups comparisons of clinical features were made using a linear regression model for continuous variables and a logistic regression model for dichotomous variables. As the groups were not precisely age and gender matched, we included age and gender as covariates in the model to control for any effect of these (except in the case of genetic data, where we included gender but not age). Statistical significance is presented as absolute p-values, uncorrected for multiple comparisons. Borderline p-values should therefore be interpreted with caution due to the possibility of a type I error.

Risk stratification of RBD patients versus controls

We selected 3 risk factors that we hypothesized might help identify RBD cases where imminent phenoconversion to a neurodegenerative disorder is more likely. These were as follows: non-use (high risk) versus use (low risk) of antidepressants; presence (low risk) or absence (high risk) of obesity (BMI>30kg/m^2); and age above (high risk) versus below (low risk) 60 years. We then looked at the ability of these measures to predict the presence of hyposmia (Sniffin Sticks score <10^{th} centile adjusted for age and gender[134]), a surrogate marker of early neurodegeneration. Hyposmia was selected as the outcome because it is a common, early prodromal symptom (corresponding to stage 1 of the Braak hypothesis[3]) and, unlike motor or cognitive performance, it is unlikely to be influenced by obesity or depression themselves. To compare how each risk factor modified the overall risk of hyposmia between RBD cases and controls, we ran a series of multivariable logistic regression models to derive the odds ratio, with interaction terms for each risk factor and case status (RBD versus control). (This logistic regression and interaction analysis was kindly performed by Michael Lawton, Senior Research Associate, OPDC).

MDS criteria for prodromal Parkinson's disease

The probability of prodromal Parkinson's disease was calculated for each participant at their baseline assessment using the method described by Berg et al[60]. We used the following risk markers: sex, pesticide exposure, solvent exposure, caffeine use, smoking history, family history of PD, presence of gene mutation (GBA or LRRK2). The following prodromal markers were available for inclusion: RBD screening questionnaire (RBDSQ)[47], sub-threshold

parkinsonism (using UPDRS and Purdue Pegboard scores), olfactory loss, constipation, excessive daytime somnolence (measured by the Epworth Sleepiness Scale[140]), postural hypotension, urinary dysfunction and depression/anxiety. PSG-proven RBD data was available for the RBD participants only, where it was used instead of the RBDSQ. The likelihood ratio for motor impairment was included in the calculation for all participants, including those with Parkinson's. In cases where data was missing or ambiguous, a likelihood ratio of 1 was used.

Multi-centre study of longitudinal outcomes in RBD

In order to provide a definitive assessment of longitudinal outcomes and risk markers in RBD, data from this study were combined with centres from the International RBD Study Group in a large, multi-centre study. The study design was developed between investigators from all centres and the protocol is described in detail elsewhere[68]. The study included RBD patients with baseline assessment and at least one follow-up visit. 120 of the RBD patients described in this chapter met these criteria and were included in the analysis, making our cohort the third largest of the 24 centres involved. The primary data analysis was performed by Ronald Postuma, the principal investigator of the largest participating centre, with additional input from all centres.

Results

	Controls N=296	RBD N=171	PD N=119		P value
Age Mean (SD)	64.9 (10.2)	64.7 (9.0)	66.9 (9.1)	RBD vs Control: RBD vs PD: PD vs Control:	0.79 0.06 0.06
Sex (% male)	49.0	88.3	70.6	RBD vs Control: RBD vs PD: PD vs Control:	**<0.001** **<0.001** **<0.001**
BMI Mean, kg/m^2 (SD)	27.4 (4.93)	29.1 (5.91)	26.1 (3.73)	RBD vs Control: RBD vs PD: PD vs Control:	**0.003** **<0.001** **0.02**
Pesticide Exposure (%)	37.2	43.9	33.9	RBD vs Control: RBD vs PD: PD vs Control:	0.27 0.10 0.44
Solvent Exposure (%)	11.1	22.2	18.6	RBD vs Control: RBD vs PD: PD vs Control:	**0.05** 0.93 0.10
Caffeine Intake	4.71 (2.24)	5.39 (2.70)	5.00 (2.87)	RBD vs Control: RBD vs PD: PD vs Control:	0.08 0.41 0.47
Head Injury (%)	18.2	32.2	22.9	RBD vs Control: RBD vs PD: PD vs Control:	**0.02** 0.24 0.41
Smoking History (%)	43.2	63.2	40.2	RBD vs Control: RBD vs PD: PD vs Control:	**0.002** **<0.001** 0.31
Obesity (%)	23.3	36.8	16.0	RBD vs Control: RBD vs PD: PD vs Control:	**0.002** **<0.001** 0.16
Education (yrs)	15.1 (3.45)	13.7 (3.39)	15.1 (3.86)	RBD vs Control: RBD vs PD: PD vs Control:	**<0.001** **<0.001** 0.64
Antidepressant use (%)	11.5	32.2	12.6	RBD vs Control: RBD vs PD: PD vs Control:	**<0.001** **<0.001** 0.25
GBA Mutation (%)	0.40	2.6	0.90	RBD vs Control: RBD vs PD: PD vs Control:	**0.05** 0.31 0.40
LRRK2 Mutation (%)	0	0	0.90	RBD vs Control: RBD vs PD: PD vs Control:	n/a 1.0 0.99
Total number of cardiovascular risk factors, Mean (SD)	1.01 (1.11)	1.55 (1.44)	1.08 (1.14)	RBD vs Control: RBD vs PD: PD vs Control:	**<0.001** **<0.001** 0.98

Table 1. Demographic and background risk factors

A total of 171 patients with idiopathic RBD (mean symptom duration 7.07 years, standard deviation [SD] 6.30), 296 healthy controls and 119 subjects with early, untreated Parkinson's Disease (mean time since diagnosis 0.78 years, SD 0.78) were included in the study.

Demographics and background risk factors

Key demographic, environmental and genetic risk factors are shown in table 1. Compared to controls, RBD subjects had significantly higher rates of self-reported head injury and solvent exposure, both known risk factors for PD. Obesity and smoking were significantly more common in RBD subjects than controls or PD patients and the total number of cardiovascular risk factors was also higher in patients with RBD. The RBD group had a significantly higher rate of antidepressant use than controls or PD patients and a significantly shorter duration of formal education.

	Controls N = 296	RBD N = 171	PD N = 119	Significance (p values)
Motor	Values are mean (SD) unless otherwise stated			
UPDRS III, score	1.74 (2.74)	4.79 (5.97)	25.7 (11.1)	RBD vs Controls: **<0.001** RBD vs PD: **<0.001** PD vs Controls: **<0.001**
Purdue pegboard, score	37.5 (6.80)	36.8 (8.04)	28.6 (6.49)	RBD vs Controls: 0.69 RBD vs PD: **<0.001** PD vs Controls: **<0.001**
Flamingo, %	71.0	53.9	55.3	RBD vs Controls: **<0.001** RBD vs PD: 0.13 PD vs Controls: **0.002**
Get up and go, time (seconds)	8.51 (1.73)	9.49 (3.17)	9.56 (2.24)	RBD vs Controls: **<0.001** RBD vs PD: 0.67 PD vs Controls: **<0.001**

Table 2. Motor scores in controls, RBD patients and PD patients.

Genetic risk variants

GBA mutations were detected in 3 out of 116 (2.6%) RBD subjects for whom DNA analysis was available, compared to 1 out of 283 (0.4%) controls and 1 out of 106 (0.9%) PD. All GBA mutations detected were the N370S genotype. The difference in GBA mutation frequency between RBD patients and controls was of borderline significance (p=0.05). None of the 136

RBD or 289 control patients tested had either of the LRRK2 mutations G2019S or R1441C. One out of 114 PD patients tested had the G2019S mutation.

	Controls N = 296	RBD N = 171	PD N = 119	Significance (p values)
Non-Motor				
MMSE, score	28.3 (1.89)	27.3 (2.10)	27.6 (2.29)	RBD vs Controls: **<0.001** RBD vs PD: 0.13 PD vs Controls: **0.002**
MoCA, score	26.7 (2.67)	25.1 (2.92)	25.2 (3.32)	RBD vs Controls: **<0.001** RBD vs PD: 0.64 PD vs Controls: **<0.001**
Mild Cognitive Impairment (MoCA <24) %	12.5	24.7	27.6	RBD vs Controls: **0.01** RBD vs PD: 0.57 PD vs Controls: **0.003**
Semantic fluency, score	12.0 (3.33)	9.78 (3.35)	10.5 (3.34)	RBD vs Controls: **<0.001** RBD vs PD: 0.12 PD vs Controls: **<0.001**
Phonemic fluency, score	12.8 (3.72)	10.4 (4.00)	11.5 (4.01)	RBD vs Controls: **<0.001** RBD vs PD: **0.04** PD vs Controls: **0.004**
Sniffin' sticks, score	12.1 (2.28)	8.13 (3.26)	7.46 (2.88)	RBD vs Controls: **<0.001** RBD vs PD: 0.06 PD vs Controls: **<0.001**
Orthostatic systolic blood pressure drop, mmHg	0.09 (12.3)	5.33 (13.6)	3.75 (13.5)	RBD vs Controls: **<0.001** RBD vs PD: 0.27 PD vs Controls: **0.03**
Postural Light-headedness %	12.2	42.3	27.1	RBD vs Controls: **<0.001** RBD vs PD: **0.01** PD vs Controls: **0.005**
Constipation, %	34.7	47.3	39.8	RBD vs Controls: **0.002** RBD vs PD: 0.08 PD vs Controls: 0.34
Beck Depression Inventory Score	4.85 (5.02)	10.25 (9.59)	7.55 (5.88)	RBD vs Controls: **<0.001** RBD vs PD: **0.001** PD vs Controls: **<0.001**
Leeds Anxiety Score	2.12 (2.38)	4.29 (3.84)	2.77 (2.98)	RBD vs Controls: **<0.001** RBD vs PD: **<0.001** PD vs Controls: **0.007**
Apathy (UPDRS part I, % >0)	Not measured	29.8	16.2	RBD vs PD **0.02**
Quality of Life EQ5D % score	84.5 (10.6)	74.0 (19.7)	76.0 (14.4)	RBD vs Controls: **<0.001** RBD vs PD: 0.28 PD vs Controls: **<0.001**
UPDRS I, total score	Not measured	9.49 (6.45)	6.95 (4.53)	RBD vs PD **<0.001**

Table 3. Clinical non-motor features in controls, RBD patients and PD patients.

Early motor impairment in RBD

The key motor features assessed in this study are summarized in table 2. Evidence of early motor impairment in RBD is demonstrated by significant differences between RBD patients and controls in UPDRS-III scores, Get-up-and-go times and successful completion of the flamingo task. While the UPDRS-III scores in RBD patients reflected an intermediate phenotype between controls and PD patients, RBD and PD patients were equally impaired on the Flamingo and Get-up-and-go tasks. There was little difference on the Purdue pegboard test between RBD and control subjects, who both performed better than PD cases.

Non-motor parkinsonian features in RBD

Patients with RBD showed impairment in a wide range of Parkinsonian non-motor characteristics (table 3). They performed significantly worse than controls in the MMSE, MoCA, semantic and phonemic fluency tests, as well as in measures of olfaction, constipation and orthostatic hypotension. In all of these tests, RBD subjects were at least as impaired as PD subjects. RBD subjects were more likely to report symptoms of postural light-headedness than either controls or PD patients.

Mood disorders were significantly worse in RBD subjects than in controls or PD patients. Beck Depression Inventory and Leeds Anxiety Scale scores indicated a higher level of depression and anxiety in RBD subjects compared to controls or PD patients, and RBD subjects were almost twice as likely as PD patients to report apathy.

When asked to report their overall quality of life using the EQ5D score, the reduction seen in RBD compared to control subjects was as large as that seen in patients with established PD. Overall self-reporting of non-motor symptoms in the UPDRS part I revealed significantly worse symptom scores in RBD subjects than in those with PD.

Table 4 shows the odds ratios for various parkinsonian features in RBD subjects compared to controls, adjusted for age and gender differences. As expected, RBD patients showed increased odds ratios, varying from just over double in the case of cognitive impairment (OR 2.04, 95% CI 1.19-3.49) to hyposmia which showed around fourteen fold relative odds (OR 13.8, 95% CI 8.13-23.4).

	Odds ratio, RBD vs Controls (95% CI) adjusted for age and gender
Motor impairment	6.46 (3.64 – 11.5)
Cognitive impairment	2.04 (1.19 – 3.49)
Hyposmia	13.8 (8.13 – 23.4)
Depression	6.93 (3.54 – 13.5)
Anxiety	6.45 (3.06 – 13.6)
Constipation	2.07 (1.34 – 3.21)
Orthostatic hypotension	4.34 (2.12 – 8.88)

Table 4. Increased risk of parkinsonian features in RBD vs Controls

The effect of antidepressants and obesity

Within the RBD patient group hyposmia was less severe in those taking antidepressant medication. Mean Sniffin Sticks score was 9.69 in those taking antidepressants compared to 7.36 in those not ($p<0.001$). RBD subjects taking antidepressants were also significantly younger (59.9 years vs 67.0 years, $p<0.001$). Importantly, these differences were not seen in the control group when comparing those taking and not taking antidepressants ($p=0.63$ and $p=0.39$ for the differences in Sniffin Sticks scores and age respectively). A formal interaction test between patient group and antidepressant use revealed strong evidence of an interaction for both Sniffin Sticks ($p=<0.001$) and age ($p=0.022$).

A similar effect on olfaction was seen relating to BMI. RBD patients who were not obese ($BMI<30kg/m^2$) had significantly worse Sniffin Sticks scores (7.48 vs 9.18, $p=0.001$) than obese RBD subjects. This effect was not seen in the control group (non-obese vs obese Sniffin scores 12.0 vs 12.1, $p=0.99$) and a strong interaction was present between patient group and BMI for the Sniffin Sticks outcome ($p=0.003$), suggesting that the effect of BMI on olfaction is specific to RBD.

The combination of subject status for obesity, antidepressant use and age conferred an additive effect on the risk of impaired olfaction. Table 5 presents the odds ratios for hyposmia comparing RBD patients with controls depending on the presence or absence of antidepressant use, obesity and age. In RBD patients considered low risk for all three variables the difference in risk compared to controls was consistent with chance (Odds ratio 3.39, 95%

CI 0.78 – 14.8). RBD patients in the high risk category for all three variables had markedly increased risk (odds ratio 45.5, 95% CI 21.1 – 98.0, p<0.001).

Not on antidepressants	*BMI < 30*	*Age > 60*	*Odds ratio (95 % CI) for hyposmia*, RBD vs. Control*
No	No	No	3.39 (0.78, 14.8)
Yes	No	No	5.63 (1.57, 20.2)
No	No	Yes	7.42 (1.91, 28.8)
Yes	No	Yes	12.3 (4.45, 34.0)
No	Yes	No	12.5 (2.62, 60.0)
Yes	Yes	No	20.8 (6.79, 63.8)
No	Yes	Yes	27.4 (6.56, 114.6)
Yes	Yes	Yes	45.5 (21.1, 98.0)

Table 5. Effect of combined markers on risk of hyposmia in RBD patients

MDS criteria for prodromal Parkinson's

Table 6 shows the probability of prodromal Parkinson's Disease for the Control, RBD and early PD groups. Median values of absolute probability were 92.8% for RBD patients, 0.48% for controls and 52.2% for patients with early PD. Using the suggested MDS cut-off of >80% for a diagnosis of probable prodromal PD, 74% (95% CI 66-80%) of RBD patients fulfilled the criteria compared to 0.3% (95% CI 0.009-2%) of controls and 21.8% (95% CI 14.8-30.4%) of early PD patients. With a lower threshold of >50%, 92.4% (95% CI 87.4-95.6%) of RBD patients met criteria compared to 1.4% (95% CI 0.4-3.4%) of controls and 51.3% (95% CI 41.9-60.5%) of early PD patients.

Conversion to defined neurodegenerative disease

The mean duration of follow up for the RBD cohort at the time of analysis was 2.1 years (SD 1.25). Of the 171 patients with RBD recruited to the study, 16 were subsequently diagnosed with a defined neurodegenerative disorder. The diagnosis was PD in 9 subjects; DLB in one; MSA in two; dementia without parkinsonism in 3 subjects, and pure autonomic failure in one. Neurodegenerative diagnoses were made after a mean latency of 5.6 years (SD 2.45) from PSG confirmation of RBD, and 9.8 years (SD 3.17) from RBD symptom onset. Of the 10 patients

who converted to PD or DLB, 8 met the MDS criteria for probable prodromal Parkinson's at baseline.

	Controls n=296	RBD N=171	PD N=119	Converted from RBD to PD or DLB at follow up (N=10)
Observed median probability of prodromal PD	0.48%	92.8%	52.2%	96.3%
>80% probability	0.3%	73.7%	21.8%	80.0%
>50% probability	1.40%	92.4%	51.3%	100%

Table 6. MDS Criteria for Prodromal Parkinson's at baseline according to PD, RBD or control status, and for RBD converters to PD/DLB

IRBDSG multicentre study of outcomes in RBD

The full results of this study are described elsewhere[68]; some key findings are summarised here. 1280 RBD patients were included in total. 82% were male, mean age at baseline was 66.3 years and the mean follow-up duration was 3.6 years.

The overall risk of pheno-conversion was 6.25% per year, with a cumulative risk of 60% after 10 years. The overall Kaplan-Meier plot of conversion-free survival is shown in figure 1. Conversion diagnoses were PD in 52% of cases, DLB in 43.5% and MSA in 4.5%.

Baseline markers that significantly increased the risk of pheno conversion were: quantitative motor testing (HR = 3.16), UPDRS III score (HR = 3.03), olfactory deficit (HR = 2.62) mild cognitive impairment (HR = 1.91-2.37), erectile dysfunction (HR = 2.13), subjective motor symptoms (HR = 2.11), abnormal DAT SPECT imaging (HR = 1.98), colour vision abnormalities (HR = 1.69), constipation (HR = 1.67), REM atonia loss (HR = 1.54) and age (HR = 1.54). Combining prodromal markers using the MDS prodromal criteria resulted in greater risk stratification than any individual marker (HR for those with >80% probability = 5.37).

The only phenotypic feature that distinguished between those who converted to Parkinson's disease and those who developed DLB was cognitive score at baseline.

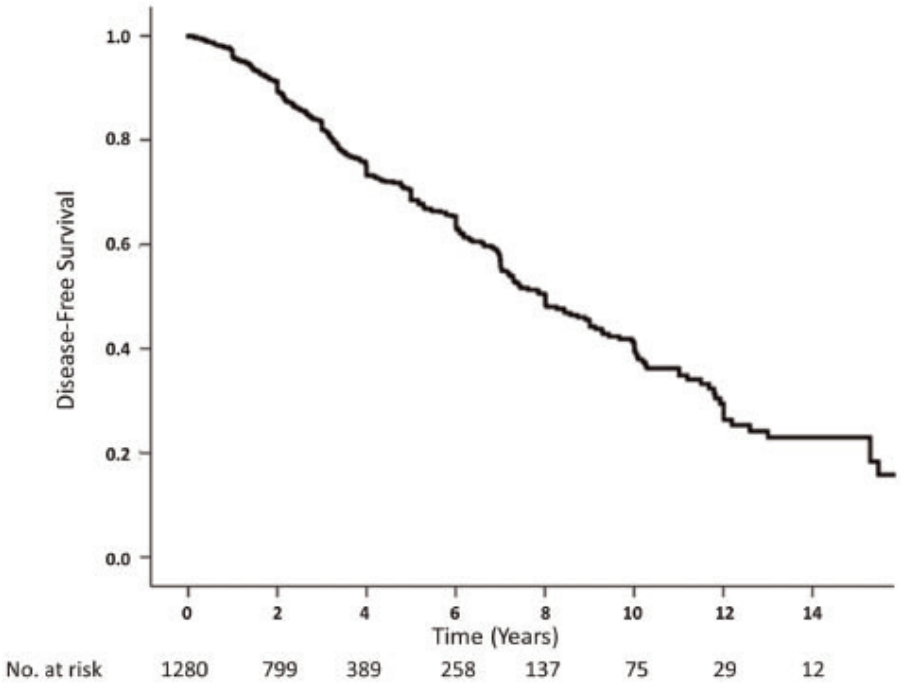


Figure 1. Kaplan-Meier plot of conversion-free survival in RBD patients calculated from the IRBDSG multi-centre study (reproduced with permission from Postuma et al, 2019[68])

Discussion

Phenotypic characteristics of RBD

In the largest study to date comparing the clinical phenotype of patients with RBD to that of PD patients and healthy controls, we have demonstrated evidence of motor, autonomic, mood and cognitive impairment in patients with RBD. In every non-motor feature, RBD subjects are at least as impaired as patients with early PD and in measures of depression, anxiety and apathy RBD patients score worse than those with established Parkinson's. This may explain the finding that RBD subjects rate their quality of life (QOL) as low as patients with early PD. We have previously shown that in patients with established PD, the presence of RBD is associated with lower QOL[141]. Our data suggest that this effect appears during the

prodromal phase and highlight the importance of recognising and actively managing non-motor symptoms.

The fact that RBD patients do not exhibit an intermediate non-motor phenotype between controls and PD patients could be considered an unexpected finding considering their earlier disease stage compared to patients with established PD. However, there are a number of possible explanations for this. Firstly, we have compared RBD patients with PD patients only, whereas the future trajectory of RBD also includes DLB and MSA patients, which by definition exhibit a more diffuse non-motor phenotype. However, this is unlikely to explain the pattern alone, since only a small percentage of RBD patients will develop MSA[68], and apart from cognition there was no difference in the ability of non-motor features to differentiate between future PD and DLB in the combined IRBDSG analysis. Second, it is possible that because we only included PD patients who were untreated, our PD cohort may have been biased towards those with a milder phenotype, since patients with more severe disease would be more likely to have initiated medication prior to recruitment.
There is also mounting evidence, however, that within the spectrum of Parkinson's disease, RBD is a marker of a more diffuse-malignant phenotype, with worse motor and non-motor disease[33,35,142]. Our data suggest that this particular subtype of disease is already emerging during the prodromal phase. The fact that PD patients who progress from idiopathic RBD tend to develop the akinetic-rigid/postural instability-gait difficulty (PIGD) subtype of disease may also explain why our RBD and early PD patients are equally impaired on the flamingo test (a measure of postural instability) and the get-up-and-go test (a measure of gait) despite RBD patients having substantially lower overall UPDRS III scores.

Part of the variation in conversion rates reported in longitudinal studies can be explained by the fact that some studies consider MCI as a pheno-conversion outcome[10], whereas others do not[11,62]. We have demonstrated that cognition is significantly impaired in RBD, with 23.7% scoring in the MCI range of the MOCA compared to 12.5% of controls. Whilst this may indicate evidence of early neurodegeneration in RBD patients, it cannot be considered a specific marker of synucleinopathy since MCI is common in the general population, affecting >25% of older adults in several population studies[143]. We suggest therefore that MCI alone should not

be considered a neurodegenerative endpoint, despite its association with progression to Dementia with Lewy Bodies.

Demographic, environmental and genetic risk factors

Amongst demographic and environmental variables, we found that smoking and history of head injury were more common in RBD patients than controls and duration of formal education was shorter, replicating the findings of a large multicentre study of risk factors for RBD[77]. We also found that exposure to chemical solvents, but not pesticides, was more common in RBD and that the total number of cardiovascular risk factors was higher compared to controls. The findings with respect to head injury and solvent exposure are consistent with their known status as risk factors for Parkinson's. The higher prevalence of smoking, on the other hand, is in conflict with the protective effect observed in relation to PD[60]. The explanation for this is not clear; it remains uncertain whether there is indeed a real effect of smoking and vascular risk on the development of RBD or whether these differences are a result of selection bias in RBD cohorts.

Our findings support recent studies of the association between RBD and mutations in the LRRK2 and GBA genes. Taking our data alongside the only other published study of LRRK2 in idiopathic RBD[24], no mutations have been found in a combined total of 261 RBD patients, substantially less than the prevalence of around 3% seen in sporadic PD[19]. This provides further evidence that LRRK2-PD is associated with a lower incidence of RBD in the prodromal phase of the disease. In contrast, we found a higher prevalence of two common GBA mutations in RBD compared with controls. Although this result was of borderline statistical significance, it is in keeping with recent evidence linking GBA mutations with RBD in both PD and non-PD GBA carriers[22,125,144].

We have shown that in patients with idiopathic RBD, lower body mass index (BMI) is associated with worse hyposmia, a common feature of prodromal PD corresponding to stage 1 of the Braak pathological staging system[3]. Importantly, this association is not seen in the control group, suggesting that the effect is specifically related to RBD. This potentially provides support to the evidence from a recent Mendelian randomisation study linking

increased BMI to a reduced risk of developing PD[121], although this association has not been a consistent finding[118–120]. Another potential explanation is the link between higher BMI and respiratory sleep disorders. OSA is a common condition that frequently coexists with RBD[115–117], and it is not uncommon for patients to be diagnosed with RBD following presentation to the sleep clinic with suspected OSA[39]. It is possible that the presence of even mild OSA or other sleep-disordered breathing may prompt earlier referral to a sleep centre and consequent diagnosis of RBD at an earlier prodromal stage than in those with RBD alone. Bias towards the referral of obese individuals may also underlie the significantly higher rate of obesity observed between RBD and control participants.

A similar effect is seen with antidepressant medications, use of which has been reported elsewhere as associated with a substantially reduced risk of conversion from RBD to neurodegenerative disease[62]. Antidepressants are known to exacerbate RBD[42] and their use may therefore also lead to earlier polysomnographic examination. In keeping with this hypothesis, RBD patients taking antidepressants are younger and have less hyposmia and orthostatic hypotension than those not taking antidepressants, an effect that is not seen in the control group. It is important to note that we are not suggesting that these patients have RBD purely as a side effect of their medication; patients in whom this was suspected were not eligible for inclusion. Rather, we suggest that the antidepressants exacerbate the co-existing sleep disorder, in keeping with evidence reported from other RBD cohorts[42].

Combining obesity and antidepressant status with age, we demonstrate that RBD patients considered low risk in all three measures have an odds ratio of 3.39 (95% CI 0.78 – 14.8) compared to controls for impaired olfaction, a difference that is consistent with chance. In those considered high risk in all three categories the odds ratio is 45.5 (95% CI 21.1-98). Longitudinal follow-up will establish whether these ‘high risk’ patients are more likely to convert to a neurodegenerative disorder, but the differences are striking and suggest that these simple demographic data can contribute significantly to risk stratification.

Evaluation of the MDS criteria for prodromal Parkinson's

The finding that 73.7% of patients with RBD fulfil the MDS criteria for probable prodromal PD is in line with longitudinal studies demonstrating a similar long-term risk of conversion to neurodegenerative disease[62]. However, this figure is largely accounted for by the polysomnographic (PSG) diagnosis of RBD itself, as excluding the likelihood ratio relating to this and using the RBDSQ instead would result in just 12% of RBD patients fulfilling the criteria. Whilst this reflects the importance of PSG-confirmed RBD as a prodromal marker, it also highlights the reliance of these criteria on specialist investigations in order to obtain high sensitivity. This is further illustrated by the result in our early PD cohort, where patients did not undergo PSG or other invasive investigations. Using simple clinical measures only, just 21.3% of these patients met the criteria (NB although these patients by definition are not prodromal, the diagnosis of PD is recent (median time since diagnosis 0.56 years) and the risk factors incorporated in the MDS criteria will not reduce with time, so one can assume that probability scores would have been the same or lower in the prodromal phase). If, on the other hand, all of these patients had had positive neuroimaging with DAT SPECT, the sensitivity of the MDS criteria would improve to 87%.

The low number of control subjects fulfilling the criteria (0.3%) makes this a potentially useful tool for recruitment of prodromal patients to clinical trials, where a low false positive rate may take precedence over high sensitivity. The sensitivity of the criteria could be increased by using a threshold of 50% instead of 80%, as others have suggested[129]. In this instance 98.6% of our healthy controls would still fall below the threshold, but 51.3% of patients with early PD would now meet criteria using simple clinical measures alone, and 92.4% of RBD subjects. Importantly, 100% of the RBD patients in our cohort who converted to PD or DLB would have met the 50% threshold at baseline.

Longitudinal outcomes from the multi-centre IRBDSG study

The pheno-conversion data from the IRBDSG collaborative study, which includes 120 of the patients described in this chapter, provide valuable evidence of the long-term prognosis of RBD, as well as highlighting some limitations of the currently available data. The overall conversion rate of 6.25% per year is lower than previously reported[145]. The reasons for this

are unclear, but the scale and geographical diversity of the study make this likely to be the most accurate estimation. The 10-year conversion rate of 60% confirms how early in the prodromal period RBD tends to occur, implying considerable potential for neuroprotection in these individuals.

However, this long average latency to conversion raises significant problems for the design of clinical neuroprotective trials in RBD. Postuma *et al* calculate that for a 2-year trial to have 80% power to detect a 30% reduction in pheno-conversion risk, 959 RBD patients would be needed in each arm of a placebo-controlled study[68]. Selecting patients on the basis of the prodromal markers which best stratify risk would reduce the number needed, but the effect is relatively modest due to the high prevalence of these markers in RBD cohorts. For example, selecting on the basis of MDS criteria positivity would reduce the required sample size by 18% and using hyposmia would result in a 28% reduction.

None of the markers evaluated in the IRBDSG study were able to 'rule out' near term conversion. Even patients testing negative for the most strongly predictive marker, MDS criteria, had a conversion risk of 5% at 3 years, compared to 17.9% overall. Interestingly, dopaminergic imaging had only a modest stratification benefit, performing less well in this regard than several of the simple clinic-based tests.

On one hand, the relatively limited stratification potential of these prodromal markers is disappointing. Underlying factors contributing to this include the wide variation in the likely rate of progression through prodromal stages that occurs, as well as the high overall risk in RBD patients which leads to a ceiling effect on hazard ratios. However, most of the markers tested are fairly crude measures of prodromal characteristics and the positive results even from these mean that the development of superior biomarkers, such as more sensitive, technology-based measures of motor function or more sophisticated brain imaging techniques, should be encouraged.

Limitations of this study

Some limitations of our data should be noted. Interpretation of the demographic differences is somewhat limited by the fact that the RBD cohort was recruited from sleep centres throughout the UK, whereas the PD and control participants were recruited only from the Thames Valley region. Although our findings are consistent with two other large studies evaluating environmental risk factors for RBD[51,145], it is possible that in our study the differences simply reflect confounding by geographical variation. The large number of participants included in this study meant that it was not feasible to undertake polysomnography on the control or PD subjects. However, the primary comparison was between RBD and controls, and RBD is rare in the general population. If a small number of RBD patients were inadvertently present in the control group, the effect would be small and would be likely to reduce the differences observed between RBD and control subjects rather than exaggerate them.

Concluding remarks

In conclusion, our study has demonstrated extensive evidence of neurodegeneration in a large RBD cohort that is in keeping with the prodromal phase of alpha-synucleinopathies. Our data suggest that simple clinical measures can be used to risk-stratify these patients. Work to further refine the stratification model with novel neuroimaging, molecular and technology-based is likely to improve our ability to identify those at highest risk of near-term pheno-conversion, which will be crucial in selecting patients for clinical trials of neuroprotective agents.

Chapter 3: Lessons from neuroimaging in pre-motor Parkinson's.

Introduction

The data described in chapter 2 illustrate that risk stratification of RBD cohorts can be achieved with simple in-clinic tests of prodromal phenotypic characteristics. Whilst these findings have important implications for widespread screening of potential prodromal populations, they still lack the precision required to address many of the key questions outlined in chapter 1. This is inevitable given the fact that these features, such as hyposmia and constipation for example, lack specificity for underlying synuclein-mediated degeneration. This problem is exacerbated in RBD patients compared with other prodromal cohorts because the pre-test probability of underlying neurodegeneration is so high, limiting the additional stratification benefit of other clinical markers.

For this reason, investigators have sought biomarkers with greater specificity for the underlying disease process. Neuroimaging has been at the forefront of this search, focusing largely on aspects of nigrostriatal degeneration, the common pathophysiological hallmark found across the range of synucleinopathy phenotypes that RBD patients are at risk of[146]. Development of an imaging biomarker in pre-clinical or prodromal Parkinson's is a significant challenge given that such a test has so far failed to supplant clinical diagnosis in established Parkinsonian disorders. However, recent expansion in the number of prodromal cohorts under investigation and the development of novel imaging techniques make this a rapidly advancing field with great near-term potential.

Before reviewing the approaches that have been taken to image prodromal cohorts, it is worth considering the particular motivations for doing so, as the characteristics of the ideal imaging test depends on the question being asked. A 'diagnostic' imaging biomarker, for example would aim to identify cases of prodromal disease with a high degree of accuracy, for example by confirming the presence of ongoing nigro-striatal degeneration. In this scenario, separation of cases from controls with high sensitivity and specificity would be the desired

outcome. Comparison with patients who have established synucleinopathies is less relevant, though demonstrating a consistently abnormal test in these patients is still important. If a low-risk neuroprotective treatment were available already, this type of test might be all that were needed before initiating treatment, though some might argue that in the case of RBD even this would not be necessary given the magnitude of the long-term risk. However, such a diagnostic test would not necessarily inform about the timing of progression.

This timing issue is crucial for tests that seek to stratify risk of near-term conversion, a key goal in prodromal cohorts where the variation in latency is wide. As discussed in chapter 1, enriching prodromal cohorts for near-term converters is an important consideration in the design of neuroprotective clinical trials[62]. Even if a disease-modifying therapy were available, targeting this to individuals at highest risk of near-term conversion would be desirable as the risk-benefit ratio might be considered too high in those who are likely to remain disease free for many years.

A risk-stratification test would have different characteristics to a diagnostic test. Here the target would be to separate a prodromal cohort into a high-risk minority and a low-risk majority. For example, given an annual pheno-conversion rate of 6% in RBD cohorts, an imaging test that perfectly predicted 3-year conversion would be abnormal in less than 20% of RBD patients at baseline. Separation of RBD patients from controls is less important in this scenario, though it would be desirable to demonstrate that the low-risk patients appeared more like healthy controls and the high-risk patients more like those with established synucleinopathies.

The change over time of a progression marker might simply be a binary process where a marker becomes positive shortly before pheno-conversion, though potentially more useful would be a marker that could track the gradually progressive neuronal decline as it occurs, since this would potentially inform regarding progression rate as well as prodromal stage. Either way, change over time is a highly desirable characteristic and requires longitudinal investigation to establish.

Though most imaging approaches described in this chapter focus on either confirming the presence of prodromal disease or stratifying near-term risk, there are other reasons to image prodromal patients. Characterising phenotypic variation is another important goal, both in

terms of predicting the future disease subtype and understanding the mechanisms of features that already exist in prodromal disease. For example, clinical features such as cognitive impairment, depression and apathy are common in prodromal and manifest disease, and these are likely to have underlying mechanisms that are distinct from those that define pheno-conversion[86,87,147,148]. Focusing imaging away from the nigrostriatal system towards cortical changes, and towards neurotransmitters other than dopamine, may be important in characterising the aetiology of these features, which may in turn lead to distinct treatment approaches.

There are some important considerations in imaging biomarkers that are common to all of these approaches. Longitudinal follow-up is crucial in the validation of any biomarker, and in most prodromal cohorts this will take many years to establish. In the absence of this, meaningful correlations with established prodromal markers may provide important interim evidence. Reproducibility is a major challenge in neuroimaging research and efforts to improve data-sharing and multi-site collaboration will be key in overcoming this. Generalisability is a further consideration, for example imaging the olfactory bulb may be relevant to hyposmic subjects[149], just as imaging the subcoeruleus may be to RBD patients[46,150], but the ideal imaging test would capture the whole spectrum of prodromal disease. Finally, any test that is to be used on the scale required for a condition as prevalent as prodromal Parkinson's needs to be accessible, cost effective and ideally repeatable in serial assessment.

The following review (which is also published elsewhere[73]) aims to capture the current state of the field in prodromal imaging research. The focus is predominantly on those studies with diagnostic and prognostic potential for prodromal Parkinson's in general, rather than studies devoted to mechanisms of pathogenesis, phenotypic variation or cohort-specific factors, though these will be covered briefly when relevant. Although there is particular emphasis on RBD, as the most widely studied prodromal population, investigations in other prodromal cohorts will also be covered. The generalisability from one population to another is considered, as well as the ways in which differing results from distinct prodromal or risk cohorts can inform about the utility of a particular test and the underlying mechanisms of disease that it seeks to describe.

The wide range of imaging modalities that has been investigated can be broadly divided into three approaches: 1) those that target dopaminergic function in the basal ganglia 2) those that directly image the substantia nigra and 3) those assessing perturbations of disease-related brain networks.

Dopaminergic radiotracer imaging

Assessment of the integrity of dopaminergic circuits in the basal ganglia can be made using a range of radio-labelled ligands, detected with single photon emission computed tomography (SPECT) or positron emission tomography (PET)[151]. The most frequently labelled structure with SPECT is the presynaptic dopamine transporter (DAT), which is responsible for re-uptake of dopamine from the synaptic cleft by the pre-synaptic nerve terminal. Visualisation of this structure with ^{123}I-FP-CIT (ioflupane) SPECT is currently the only imaging method to be approved by the Food and Drug Administration (FDA, United States) and the National Institute for Health and Care Excellence (NICE, United Kingdom) for the differentiation of parkinsonian syndromes from other causes of tremor. In this regard it can separate cases of parkinsonism from essential tremor with sensitivity of 95% and specificity of 93%[152], and consistently demonstrates greater specificity than clinical examination in diagnostically uncertain cases[153,154].

PET has been used with various radio-labelled ligands to interrogate aspects of dopaminergic transmission in the basal ganglia[151,155]. The presynaptic dopamine transporter (DAT) can be targeted with compounds such as ^{18}F-FP-CIT, ^{11}C-methylphenidate (^{11}C-MP) or ^{18}F-FE-PE2I[156]. The intracellular vesicular monoamine transporter (VMAT2) is responsible for re-packaging dopamine into vesicles, and is commonly labelled with ^{11}C-dihydrotetrabenazine (^{11}C-DTBZ) or 18F-fluoropropyl-dihydrotetrabenazine (18F-FP-DTBZ). ^{18}F-DOPA is taken up by neurons in the same manner as L-DOPA, where it is a substrate for dopa decarboxylase; imaging this compound therefore reflects the brain's capacity to take up, process and store dopamine. Finally, ligands such as 11C-raclopride or 18F-fallypride can be used to measure binding to the

post-synaptic dopamine D2 and D3 receptor, as can the SPECT compound ^{123}I-IBZM (iodobenzamide).

The presynaptic DAT signal is depressed in the posterior putamen early in the course of Parkinson's disease and demonstrates characteristic asymmetry in the pattern of signal loss[69]. The level of striatal DAT binding in PD correlates with contralateral clinical measures of bradykinesia and rigidity[157], as well as with the degree of dopaminergic cell loss in the substantia nigra post-mortem[158]. It is sensitive to longitudinal changes, demonstrating a significantly faster rate of decline in patients with Parkinson's than in healthy controls[159,160], although this must be interpreted with caution in the context of treatment with medications that might themselves lead to downregulation of DAT levels[151]. This potential confounding effect of medication is avoided when studying pre-motor Parkinson's, and the fact that substantial nigro-striatal cell loss occurs before the onset of motor symptoms makes dopaminergic radiotracer imaging an attractive modality to evaluate preclinical and prodromal disease. A major disadvantage of both PET and SPECT, however, is the exposure of subjects to ionising radiation. This is a particular limitation in younger subjects and in serial longitudinal studies where the cumulative risk of repeated scans may be unacceptable.

Dopaminergic imaging in hyposmic cohorts

Several studies using SPECT have demonstrated pathologically reduced DAT signal in a proportion of people with idiopathic hyposmia[161–163]. In one cohort comprising relatives of Parkinson's patients, 18% of hyposmic individuals had an abnormal DAT SPECT scan, and the rate of signal loss on serial imaging was significantly higher than in subjects with normal olfaction[161]. Of the seven hyposmic relatives with an abnormal baseline scan, the four with the lowest DAT binding developed Parkinson's within 2 years.

By far the largest study to evaluate DAT SPECT in prodromal Parkinson's is the Parkinson's Associate Risk Syndrome Study (PARS), which also selected patients on the basis of olfactory impairment[162]. 11% of 203 hyposmic subjects had abnormal DAT imaging, compared to 1% of 100 normosmic controls. The combination of hyposmia with two other known Parkinson's risk factors – male sex and constipation – increased the abnormal scan rate to >40%, as high

as that seen in RBD[164]. After follow-up period of 4 years, 19 (10%) of the hyposmic group had converted to Parkinson's disease[74]. The additional stratification conferred by DAT SPECT was substantial: 67% of the hyposmic individuals with a DAT deficit had converted to Parkinson's, compared to 9% of those with intermediate DAT findings and just 3% of those with a normal scan. This translates to a relative risk of 17 for a DAT deficit in hyposmic patients, demonstrating substantial enrichment for a population-ascertained cohort. Given the low overall incidence of PD in this group, however, identification of clinical features that can further risk stratify subjects prior to imaging will be important to make this approach more cost-effective.

Dopaminergic imaging in REM Sleep Behaviour Disorder (RBD) cohorts

Early studies using PET and SPECT to image presynaptic and vesicular dopamine transporters in RBD confirmed that a deficit is detectable in these patients[165,166], and subsequent larger cohorts comparing RBD subjects to healthy controls have demonstrated that around 40-50% of RBD patients have abnormal DAT imaging[164,167–169]. The DAT deficit seen in RBD is less severe than in established Parkinson's[170,171], suggesting that dopaminergic imaging may have the potential to quantify progression through the prodromal phase. The presence of subtle motor abnormalities in RBD subjects is more predictive of a DAT deficit than hyposmia[172,173], consistent with longitudinal studies in RBD showing that motor signs occur late in the prodromal phase whereas olfaction is lost early[128]. Reduced F-DOPA uptake in the putamen has also been reported in a single study of 11 RBD patients with concurrent major depressive disorder[174].

In one of the largest studies using DAT SPECT, Iranzo and colleagues found pathologically reduced ^{123}I-FP-CIT binding in 17 out of 43 individuals with RBD[164]. After 2.5 years, 6 of these subjects had developed an alpha-synuclein related neurodegenerative disorder (PD in 4 cases, DLB in one and MSA in one). However, two subjects with a normal SPECT scan also pheno-converted, while 65% of those with an abnormal scan remained disease free. A follow-up study with serial imaging in 20 RBD subjects suggests that the rate of decline in DAT signal may be a predictor of imminent conversion[175] (figure 1). Over 3 years, the DAT signal declined by an average of 6% per year in RBD subjects, compared to around 3% per year in controls. In

the three subjects who converted to Parkinson's during follow-up, the rate of decline was 10%, and these three subjects had the lowest DAT signal at baseline assessment.

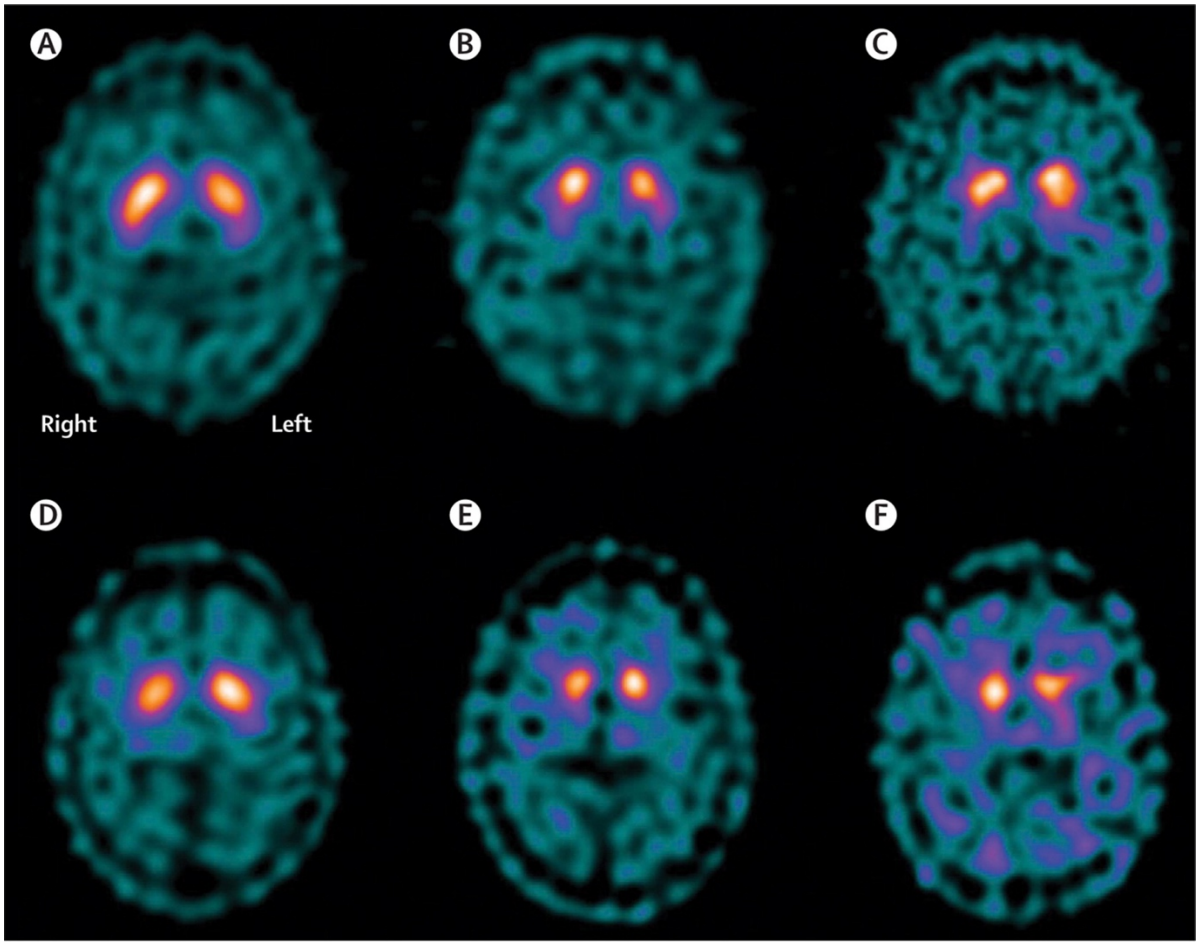


Figure 1. Serial SPECT images from two patients (A-C and D-F) with RBD, showing labelling of the basal ganglia with ^{123}I-FP-CIT. Images are at baseline (A and D), 1.5 years (B and E) and 3 years (C and F). A greater signal decline over time is seen in the patient who developed Parkinson's (D-E) than in the patient who did not (A-C). (Iranzo et al 2011[175], reused with permission)

The latest update from this group was published in 2017 and reported the 5-year outcomes of some 87 RBD patients[169]. The proportion of patients with a baseline DAT deficit was higher at 58%, and the predictive accuracy of an abnormal scan for pheno-conversion at 5 years revealed 75% sensitivity, 51% specificity, 44% positive predictive value and 80% negative predictive value. We can draw some conclusions from these data about disease progression. Firstly, DAT SPECT abnormalities must be an early prodromal sign, since they are observed in 58% of RBD patients yet only 6% per year are expected to pheno-convert. Secondly, the rate

of progression in dopaminergic decline must be highly variable to account for the proportion of RBD patients who remain conversion free at 5 years despite an abnormal scan, alongside those who convert despite normal baseline imaging.

When comparing these RBD data to the findings of the PARS study in hyposmic patients[74], there are some surprising findings. Firstly, the PPV of 44% for an abnormal DAT scan is lower than that seen in hyposmic individuals (67%), despite RBD conferring a much higher risk of conversion than hyposmia. The likely explanation for this highlights one of the difficulties in DAT SPECT studies, which is that defining what constitutes an abnormal scan is very difficult in prodromal disease, when findings are mild or borderline and without the corroboration of clinical parkinsonism. Iranzo's study probably set a relatively low threshold for defining abnormal, resulting in a high proportion of patients with abnormal scans and a relatively low predictive value. The PARS study, on the other hand, included an 'intermediate abnormality' group, meaning that those included in the 'definite abnormal' group were likely to have more severe deficits. This emphasises the importance of having standardised means of classifying scans in order to enable comparisons to be made, but this is not straightforward in DAT SPECT imaging, where subtle differences in the pattern of signal uptake may be as important as absolute uptake values.

The comparison between these two studies also highlights the effect of the pre-test probability on the additional stratification risk that a test may confer. Whilst an abnormal DAT SPECT increased the risk of pheno-conversion 17-fold in hyposmic patients, the positive likelihood ratio reported in Iranzo's RBD study was just 1.54, with a 95% confidence interval 1.02-2.32. This reveals a particular difficulty of biomarker application in RBD cohorts: the baseline risk is so high that moderately informative tests may still confer only a limited additional stratification benefit.

An interesting finding from a recent meta-analysis of dopaminergic imaging in RBD and manifest Parkinson's disease, is that PD patients with concomitant RBD have more severe dopaminergic deficits than those without[176]. In some comparisons, patients with idiopathic RBD had deficits as great as PD patients without RBD, yet not as severe as PD patients with RBD. This mirrors the pattern of clinical findings seen in chapter 2, and suggests that the

unexpectedly severe degenerative phenotype observed in RBD patients compared to PD is not simply accounted for by patients in the prodromal phase of atypical parkinsonian disorders, but rather that even within typical PD the presence of RBD may be a marker of more aggressive disease.

Dopaminergic imaging in genetic cohorts

The use of dopaminergic imaging as a pre-clinical biomarker in asymptomatic LRRK2 carriers has yielded some elegant results. In a cross-sectional study of a large Spanish cohort, DAT SPECT revealed pathologically reduced striatal uptake in 43.7% of 32 asymptomatic carriers[177]. This figure closely matches the observed penetrance of the mutation and it will be interesting to see from longitudinal follow-up whether these individuals are indeed those who go on to develop clinical disease.

The use of three different PET ligands in a study of two North American kindreds revealed a pattern of progressive neurodegeneration in asymptomatic LRRK2 carriers that provides insight into the mechanism of pathogenesis at different pre-motor stages[178]. Out of six asymptomatic carriers, all had normal F-DOPA uptake; four had abnormal DAT signal measured by ^{11}C-MP, and two of these subjects had abnormal VMAT2 levels, measured by ^{11}C-DTBZ. At follow-up after 2.5 years one of these two subjects had converted to clinical Parkinson's, and this was accompanied by the development of abnormal F-DOPA uptake[179]. This intriguing result mirrors the changes seen in early Parkinson's, where compensatory mechanisms downregulate presynaptic DAT in the already reduced number of presynaptic terminals, and upregulate dopa decarboxylase[180]. It follows that reduction of DAT might be the most sensitive marker of prodromal disease in the striatum, whilst loss of the ability to synthesise and store dopamine may be the critical factor that precipitates clinical motoric disease. Despite the histopathological differences that can occur between LRRK2-related and sporadic Parkinson's[181], affected relatives in this kindred had PET imaging findings that were indistinguishable from those seen in sporadic disease. Larger studies in other prodromal cohorts using this multiple PET ligand approach would be valuable in determining its capacity to delineate prodromal Parkinson's and predict conversion more generally.

Subtle deficits on dopaminergic radiotracer imaging have been demonstrated in asymptomatic heterozygotes carrying one copy of the *parkin* mutation[182,183] - a rare cause of recessive familial Parkinson's - and it has been suggested that there may be an increased risk of Parkinson's in these subjects. However, despite the presence of mild motor signs in some cases, the imaging and clinical deficits do not appear to deteriorate significantly with time[184]. Furthermore, the pattern of striatal signal loss in homozygous individuals with parkinsonism is quite different from sporadic disease, with less sparing of the caudate nuclei[185]. A similar story is reported with *PINK1* heterozygotes, another gene causing recessive Parkinson's; despite moderate group-level reductions in F-DOPA uptake and subtle motor signs, the risk of developing overt Parkinson's appears to be low[185]. These findings, as well as the scarcity of such subjects, make recessive heterozygotes less promising as pre-clinical cohorts.

Mutations in the glucocerebrosidase (GBA) gene, which cause the autosomal recessive lysosomal storage disorder Gaucher's disease, are one of the most prevalent genetic risk factors for Parkinson's disease[186]. Heterozygous GBA mutations occur in around 4-7% of sporadic cases and are 5 times more common in PD patients than in controls[187]. However, despite evidence of a more severe non-motor prodrome in these patients[23], the only study to conduct F-DOPA PET in non-PD heterozygous carriers found no evidence of signal loss in the striatum[188]. There was, however, reduced striatal F-DOPA uptake in a small proportion of Gaucher's patients without Parkinsonism, potentially revealing a pre-parkinsonian disease state. On this evidence GBA heterozygotes in isolation are unlikely to be a useful pre-clinical cohort, but given the high frequency of GBA mutations the presence of these could potentially contribute to the risk stratification of population cohorts.

Imaging of the substantia nigra

Direct visualisation of the substantia nigra with neuroimaging has obvious appeal as a potential diagnostic approach given the specificity of degeneration in this area to parkinsonian disorders. Quantification of nigral tissue using cross-sectional imaging has been limited by the spatial resolution of these methods and the lack of sensitivity to detect subtle

signal change in this area, though recent advances in MRI methodology have shown promise in overcoming these hurdles. Transcranial ultrasound on the other hand has been used for many years to image this key pathological area, and may have a role to play in pre-clinical risk stratification.

Transcranial sonography (TCS)

Transcranial ultrasound across the temporal bone can be used to directly visualise midbrain structures and qualitatively assess their echogenic properties. The key abnormality detected by this technique is increased echogenicity of the substantia nigra, which is consistently found in up to 90% of patients with Parkinson's disease[189–192] (figure 2). The origin of this signal is not fully understood but it has been shown to correlate with iron deposition[193] and microglia activation[194] in the substantia nigra. Hyperechogenicity does not appear to reflect the dynamics of the neurodegenerative process as the signal can be detected at an early age, does not change with time in established disease and does not correlate with objective measures of disease severity[189,195,196]. The diagnostic value of TCS is limited by the presence of an abnormal signal in around 10% of healthy subjects[197] and it does not accurately differentiate PD from other causes of parkinsonism[198]. Furthermore, insufficient bone windows in 10-20% of Caucasian individuals and up to 60% of people of Asian origin are an additional drawback[59]. Despite these limitations, it is a safe, inexpensive and non-invasive test which makes it a good candidate for screening large unselected cohorts.

TCS in population studies

In a study using TCS in a population-ascertained cohort of 1847 older persons, 17% were found to have abnormal echogenicity of the substantia nigra[199]. After 5 years, 21 individuals had developed Parkinson's, of whom 78% had an abnormal baseline signal, making the risk of Parkinson's in those with an abnormal test 20 times higher than in those without[200]. The high prevalence of hyperechogenicity in the population and relatively low conversion rates limited the positive predictive value of the test to only 6.5% at 5 years, but this study nevertheless demonstrates the ability of TCS to substantially enrich cohorts for at-risk individuals.

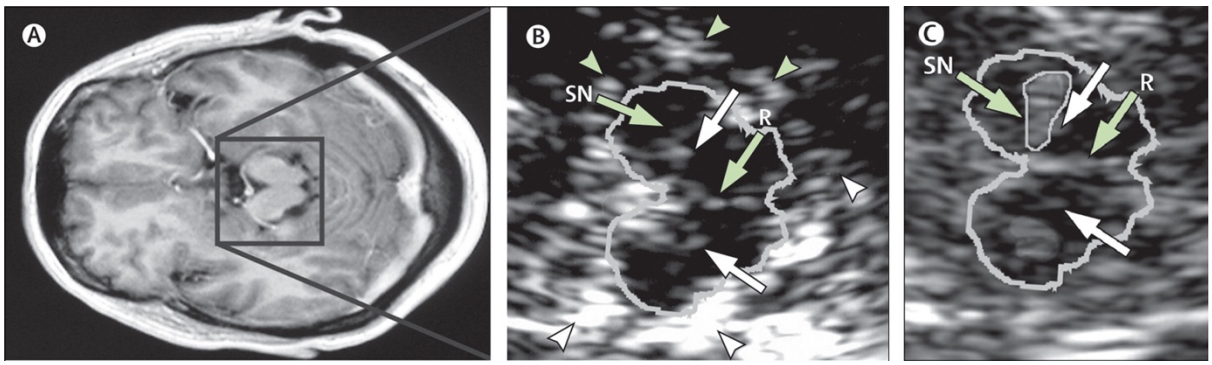


Figure 2. Transcranial ultrasound (TCS) of the midbrain. A: axial T1-weighted MRI at the level of the midbrain demonstrating the approximate area imaged by TCS in B and C. B: TCS of the midbrain (outlined) in an individual with normal appearances of the substantia nigra (SN). Arrowheads indicate signal from the surrounding basal cisterns. R = raphe. C: TCS from an individual with hyperechogenicity of the substantia nigra (SN, encircled within the midbrain outline). White arrows indicate the position of the red nuclei. (Reproduced with permission, Berg et al 2008[201])

TCS in REM sleep behaviour disorder (RBD)

Hyperechogenicity of the substantia nigra has been demonstrated in 35-50% of patients with idiopathic RBD, in keeping with the recognised prodromal risk status in these individuals[202–205]. As with established Parkinson's, the signal does not change with time in RBD and its ability to predict pheno-conversion in longitudinal studies is disappointing[205]. Several studies have found no correlation in RBD subjects between substantia nigra hyperechogenicity and dopaminergic dysfunction measured by DAT or PET[172,206–208]. These results further suggest that the TCS finding may be a binary susceptibility marker in RBD rather than a quantifiable measure of neurodegeneration. The combination of TCS and DAT SPECT was shown to be 100% sensitive for conversion from RBD to a parkinsonian disorder in one study[206]. Whilst this might provide some reassurance to those with normal imaging, the result is difficult to interpret given the lack of concordance between the two imaging modalities.

Interestingly, if the signal is truly static it ought to provide an estimate of the number of prodromal subjects in a given cohort, by comparing the rates of hyperechogenicity with those seen in Parkinson's and controls in the same centre. For example, Iwanami and colleagues[203] found an abnormal TCS signal in 14 out of 34 RBD subjects, as well as in 53% of Parkinson's patients and 10% of healthy controls. If the signal does not change over time, one could

calculate that that the RBD cohort may be comprised of 25 individuals with prodromal Parkinson's and 9 without.

TCS in genetic cohorts

A number of studies have investigated TCS in asymptomatic LRRK2 carriers, the results of which highlight the complexity of genetic susceptibility factors in Parkinson's. Substantia nigra hyperechogenicity is reported in up to 90% of asymptomatic carriers[177,209–211], a figure that exceeds the penetrance of the mutation. Furthermore, non-carrier relatives have markedly increased rates of hyperechogenicity compared to unrelated control subjects[211]. These observations suggest the sonographic findings are the result of a more widespread genetic risk profile and are unlikely to be a useful predictor of future pheno-conversion in this population. Again, the limitations in generalizability using genetically predisposed cohorts are illustrated by such studies.

MRI of the substantia nigra

Whilst MRI is commonly used in clinical practice as an adjunctive test in the differential diagnosis of parkinsonian syndromes, reliable identification of the specific neurodegenerative signature of idiopathic PD has not been possible with routine imaging sequences[69,212]. However, the capacity to resolve the key brainstem structures and identify relevant pathological changes has improved with the advent of high field strength MRI scanners and sequences targeting specific molecular processes. Some of the most promising results come from direct imaging of the substantia nigra.

Diffusion tensor imaging (DTI) has been used to assess the integrity of nigrostriatal fibres in Parkinson's in a number of studies. Reduced fractional anisotropy in the substantia nigra in PD patients compared to controls is frequently reported[213] and reached significance in a recent meta-analysis[214]. However, there is substantial variation in results amongst DTI studies and as yet a consistent biomarker has not emerged that can accurately categorise individual patients[215]. Evidence in prodromal conditions is limited to a small number of studies in RBD, which have yielded similarly mixed results. Group level changes in diffusion parameters have been reported in the substantia nigra[149], but this is not a consistent finding[87,216]. Much of the

variation between studies is a result of methodological differences and it remains to be seen whether DTI can reliably detect early nigral degeneration once this technique is further refined.

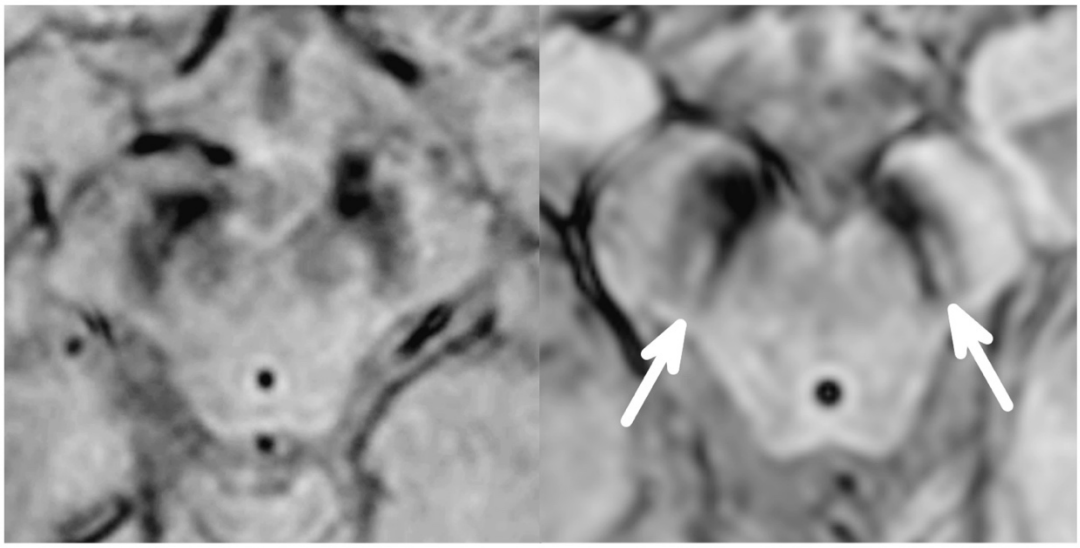


Figure 3. Clinical high resolution 3D-T2*/SWI MRI of the midbrain showing the normal appearance of the substantia nigra (right image) with high intensity signal in the dorsolateral region, and absence of this feature (left image) in a patient with Parkinson's. (Reproduced with permission, Schwarz et al 2014[217])

More promising has been the finding that high field strength MRI with T2*-weighted sequences reveals a characteristic high signal area within the dorsolateral substantia nigra that is present in healthy controls and lost in patients with Parkinson's. A study comparing *in vivo* 7-Tesla MRI with post mortem MRI and histopathology found that this signal corresponds to the anatomical location of nigrosome 1, the nigral area that sustains maximal loss of dopaminergic neurons in Parkinson's[218]. A follow-up study using clinical 3T MRI with a susceptibility weighted (SWI) sequence replicated this finding in a retrospective evaluation of 9 PD patients and 81 controls, and a prospective analysis of 10 PD patients and 9 controls[217]. The authors coined the term 'swallow tail sign' in reference to the characteristic appearance of the presumed nigrosome 1 in healthy controls (figure 3). In blinded assessments of this sign by two independent neuroradiologists, accuracy against the gold standard clinical diagnosis was 96% for the retrospective series, with sensitivity of 100% and specificity of 95%. In the prospective study 8 of 9 controls and 8 of 10 PD patients were correctly classified. A subsequent study replicated the classification accuracy of this sign in PD and extended the

finding to MSA and PSP, where it was 100% sensitive[219]. A limitation of this technique at present is that in 5-10% of cases the images are of insufficient quality for diagnostic assessment, usually due to motion artefacts.

Three small studies have reported on this sign in RBD patients, where rates of the absent swallow tail sign have ranged from 60-77%[220–222]. Whilst lower than the rate seen in established PD, this is perhaps surprisingly high. This may indicate that the sign develops early in the prodromal phase, or that it indicates a susceptibility trait in the same manner as TCS hyperechogenicity. One of the three RBD studies reported some limited follow-up data, finding that in 5 RBD patients who developed parkinsonism within 18 months of imaging, all had loss of the swallow tail sign at baseline[220]. Further longitudinal studies in larger and more diverse prodromal cohorts are required to build on these encouraging data. It remains to be determined whether the signal can stratify patients according to their prodromal stage, but even if it is a trait marker, the low positivity observed in controls would make its predictive value greater than that of TCS.

Another disease-specific target of MRI that has emerged recently is neuromelanin, a pigment expressed in high quantities in the substantia nigra and locus coeruleus[223]. Neuromelanin is responsible for the for the dark colour of these structures and may contribute to their susceptibility to alpha-synuclein mediated neurodegeneration. It is an iron chelator, which makes it visible to MR imaging due to the complex's paramagnetic properties. Modified T1-weighted sequences that are sensitive to neuromelanin display high signal intensity in areas where it is expressed and differences in this signal are observed between Parkinson's patients and controls[224]. A recent study used an automated segmentation protocol with neuromelanin-sensitive images to make an objective assessment of focal atrophy in the substantia nigra[225]; this technique separated PD patients from controls with an AUC of 0.93-0.94. Evidence that nigral neuromelanin-sensitive MRI might be of value in quantifying disease progression in Parkinson's disease is suggested by the fact that significant differences are observed between the nigra contralateral and ipsilateral to the clinically affected side, as well as correlations with motoric disease severity and dopaminergic SPECT imaging[226,227].

In the context of prodromal disease, neuromelanin-sensitive sequences have been used in RBD patients, where loss of neuromelanin in the coeruleus/subcoeruleus complex is observed[46]. Just one published study has investigated nigral neuromelanin depigmentation in RBD patients, which reported a significant reduction in neuromelanin volume and signal intensity compared to controls[228]. The combination of these two imaging parameters separated the groups with an AUC of 0.88. Such a strong discrimination accuracy is perhaps unexpected in a measure of nigral integrity, since one would expect a significant proportion of RBD patients to be many years from conversion and therefore to have minimal nigral degeneration. It also implies a greater difference than that observed with dopaminergic imaging, whereas one might expect the earliest changes to occur at the nigro-striatal synapses. A PD group was not included in this study, and it therefore the comparative overlap RBD patients show with controls and PD patients remains an important question to be addressed.

Imaging brain network activity

Many of the imaging techniques described so far either directly or indirectly measure changes in the structural integrity of brain regions involved in disease pathogenesis and as such they reveal a degree of irreversible neurodegeneration. Disruptions of normal brain network dynamics may instead reflect early changes in synaptic function caused by the accumulation of abnormal alpha-synuclein. The possibility that such functional changes precede neuronal death makes them of particular interest in pre-clinical diagnostics. Furthermore, changes in functional brain organisation can potentially reveal patterns that are more disease specific than structural abnormalities and may therefore be more predictive of future phenotype in prodromal cohorts[229]. Various imaging modalities have been applied to investigate network-level changes, measuring either metabolic activity, regional cerebral blood flow or changes in blood oxygenation. Most studies record these parameters in the resting state, avoiding the potential confounding factors in task-related imaging and therefore increasing their replicability and potential for translation into the clinical arena.

Imaging networks through metabolism and perfusion

PET is commonly used to measure metabolic activity in the brain with the ligand ^{18}F-fluorodeoxyglucose (FDG). This gives a readout of cellular glucose uptake, which is physiologically most correlated to afferent synaptic activity and neuronal connectivity. Patterns of brain perfusion have been investigated in pre-motor Parkinson's primarily with SPECT, using ^{99m}Tc-Ethylene Cysteinate Dimer (ECD) as a marker of regional cerebral blood flow (rCBF). These two characteristics are not analogous, particularly in the context of medications that have vasoactive properties[229]; nevertheless, consistent patterns of brain activity have been demonstrated using both modalities. More recently, the development of arterial spin labelled (ASL) MRI has enabled brain perfusion to be imaged non-invasively and without exposure to ionising radiation. ASL is limited by a relatively poor signal to noise ratio and its clinical potential remains somewhat uncertain, but it has demonstrated similar network patterns to PET and SPECT in established PD[230,231].

The identification of a spatial covariance pattern specific to Parkinson's disease has been one of the most promising applications of network imaging techniques. This Parkinson's Disease Related Pattern (PDRP) is detected using a principal component analysis (PCA)-based method known as the Scaled Subprofile Model (SSM). The approach, which is described in detail elsewhere[232], involves the extraction of biologically relevant signals from pooled control and patient data using a whole brain multivariate analysis. The process is entirely data driven and as such it does not depend on *a priori* hypotheses regarding specific brain areas. The PDRP is characterised by increased activity in the pallido-thalamic and pontine regions with relative cortical reductions in the premotor, supplementary motor and parietal association areas[233]. It is highly reproducible within subjects and between populations[234,235] and in established Parkinson's demonstrates progression over time and correlation with clinical severity measures[236,237]. All of these factors make it a potentially valuable biomarker in clinical neuroprotective trials. Other network patterns have also been described in Parkinson's using this approach, which appear to reflect particular phenotypic features such as tremor and cognitive impairment[238,239].

Brain perfusion and metabolism in prodromal cohorts

REM sleep behaviour disorder has been the focus of most of the studies to investigate metabolic and perfusion abnormalities in prodromal disease. Group level assessments of regional blood flow in RBD patients compared to controls have generated somewhat inconsistent results, but tend to show increased flow in subcortical regions, including the pons and putamen, and decreases in cortical areas[240,241]. Clinical measures of cognition, olfaction and colour vision have been reported to correlate with decreased perfusion in the hippocampus, anterior parahippocampal gyrus and visual cortex respectively, suggesting that these perfusion measures have clinical relevance in prodromal disease[242,243]. Decreases in rCBF over time have also been found with serial imaging of RBD patients, perhaps indicating a biomarker sensitive to progressive neurodegeneration[244]. A reproducible finding in RBD patients is increased perfusion in the hippocampus compared to controls, which is supported by the demonstration of a corresponding increase in metabolic activity in this region using FDG PET[243,245,246]. In one of the few longitudinal experiments to assess the predictive value of ECD SPECT, a larger increase in hippocampal perfusion was observed in 10 RBD patients who subsequently pheno-converted to parkinsonian disorders compared to 10 RBD patients who did not[245].

Tang and colleagues investigated PDRP expression in a pseudo pre-motor situation by measuring the clinically unaffected hemisphere of early Parkinson's patients with unilateral signs[247]. They found increased PDRP expression in the 'asymptomatic' hemisphere, which preceded the development of clinical disease in the contralateral limbs by 2 years. However, interpretation of this finding is limited by the fact that the PDRP signal was always symmetrical, which suggests that its expression on the 'unaffected' side may be attributable to contralateral basal ganglia degeneration.

Evidence that PDRP expression may be able to quantify the transition from prodromal to clinical Parkinson's is presented by Wu et al in a comparison of the network between RBD, Parkinson's and control subjects using FDG PET[248]. They found the PDRP to be expressed at intermediate levels in RBD subjects, significantly higher than in healthy controls but lower than patients with moderate PD. However, the increase in expression from RBD subjects to

early stage Parkinson's did not reach significance. The authors also identified a distinct network in RBD subjects that was not present in controls and was expressed at lower levels in PD patients. They propose that this RBD related pattern is a marker of the prodromal phase of Parkinson's that decreases with the emergence of clinical disease, though since they did not exclude the presence of RBD in their PD cohort the specific relationship of this pattern to RBD itself cannot be assessed. Further investigation with longitudinal follow-up will no doubt resolve this question.

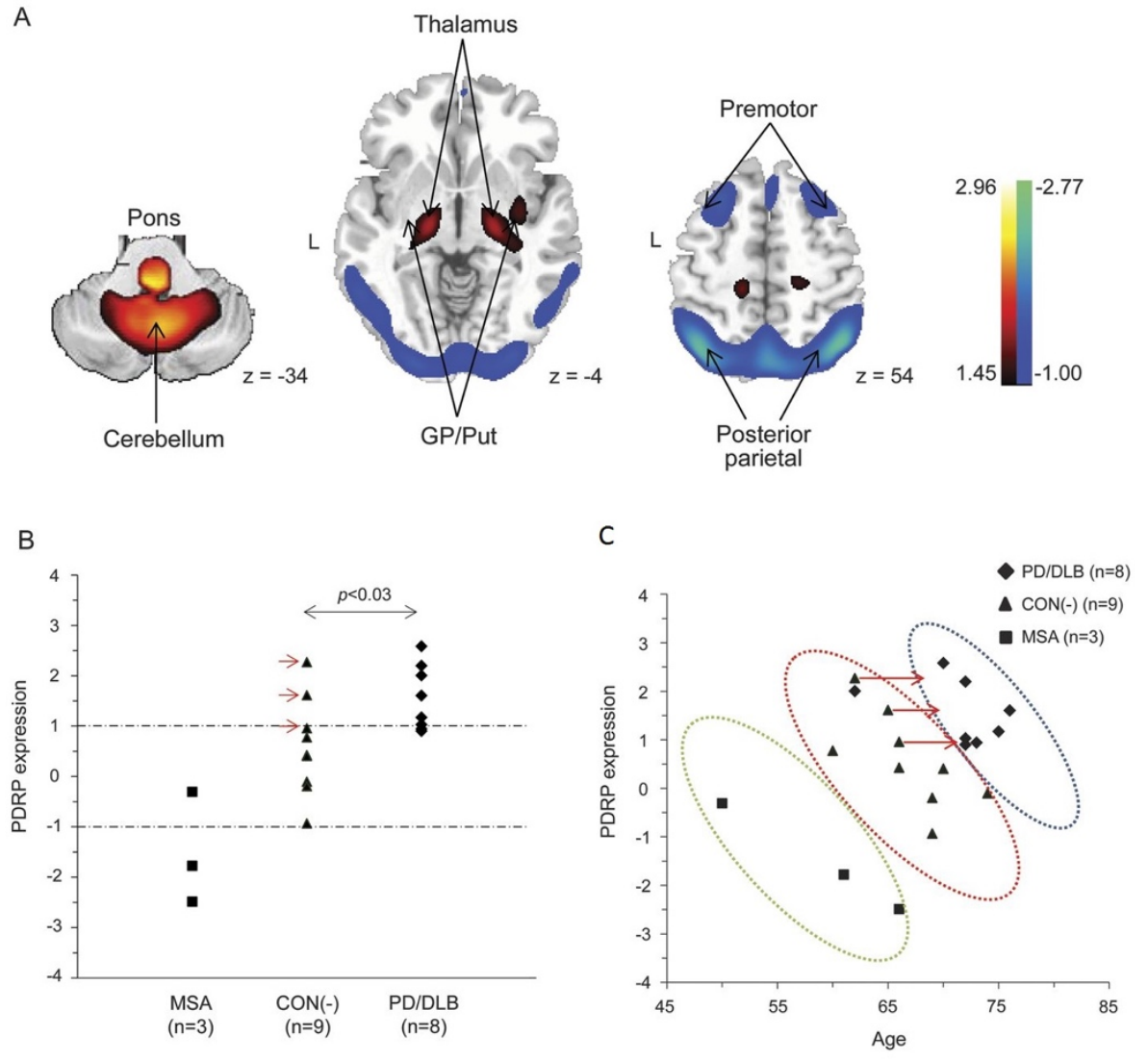


Figure 4. A: representation of the Parkinson's Disease Related spatial covariance pattern (PDRP) derived from FDG-PET imaging, with increased metabolic activity denoted in red-yellow and decreased metabolic activity in blue-green. B: baseline PDRP expression levels in 20 individuals with RBD, divided into those who remained disease free (CON(-)) and those went on to develop MSA or PD/DLB. C: A combination of PDRP expression and age was able to separate the three groups into distinct clusters. (Holtbernd et al 2014[249], reused with permission)

One benefit of the PDRP as a biomarker is that it can differentiate idiopathic PD and DLB from other parkinsonian disorders such as MSA and PSP[250], something that is not always possible with presynaptic dopaminergic radiotracer imaging[151]. This is important in the context of pre-motor disease as MSA in particular can exhibit a similar prodromal phenotype to Parkinson's disease. An intriguing recent study in RBD with considerable longitudinal follow up suggests that this distinction may be possible prior to the emergence of motoric disease[249]. Firstly, the presence of PDRP expression was confirmed in two RBD cohorts, using FDG PET and ECD SPECT respectively. The latter cohort was evaluated clinically after a 4.6-year mean follow-up period, during which 8 patients converted to either PD or dementia with Lewy Bodies (DLB). A logistic regression analysis combining baseline PDRP expression and age separated converters and non-converters into two distinct clusters. Further analysis of 3 RBD subjects who subsequently developed MSA showed that these formed a separate cluster using this model, which was largely accounted for by reduced PDRP expression compared to both non-converters and PD/DLB converters (figure 4). The fact that converters to PD and DLB could not themselves be differentiated is unsurprising since these disorders likely represent a spectrum of common disease rather than truly distinct syndromes. It seems increasingly likely that pre-motor diagnosis will require a combination of both clinical and imaging biomarkers; this study provides encouraging evidence that the PDRP might contribute to such a model.

Functional MRI (fMRI) networks

Fluctuations in the resting brain's activity can be inferred from changes in the blood oxygen level dependent (BOLD) signal detected with appropriate T2*-sensitive MRI sequences[251]. Approaches to network characterisation generally involve either temporal correlation of this activity with a pre-defined region of interest or a data-driven approach, for example with independent component analysis (ICA)[252]. The functional connectivity networks that emerge closely mirror patterns of activation observed in task-based fMRI experiments[253], suggesting that physiologically relevant interactions between brain regions can be probed in the resting state. Advantages of imaging brain connectivity with MRI are that such scans are short, non-invasive, free from ionising radiation and easy to acquire using widely-available hardware. Resting state functional MRI (rs-fMRI) can reveal perturbations of functional connectivity in neurodegenerative disorders in the absence of structural changes[254], in some cases decades

before the onset of clinical disease[255], suggesting that these methods are sensitive to early pathogenic processes.

Group level differences in functional connectivity (FC) between patients with established Parkinson's disease and controls have been observed in various brain regions, and the demonstration of correlations between FC and clinical measures is encouraging for the development of a quantifiable biomarker[256]. One study has even reported a correlation between reduced FC in the sensorimotor network and reduced CSF alpha-synuclein levels, potentially indicating a more direct representation of the pathological process, since PD is associated with reduced total CSF alpha-synuclein compared to controls[257,258].

Disturbances of connectivity within the basal ganglia are a potentially disease-specific characteristic and these have been demonstrated in Parkinson's disease. Hacker *et al* used a seed-based approach to assess connectivity between the striatum and brainstem, and as well as showing reduced FC in Parkinson's patients they demonstrated a connectivity gradient across the striatum that mirrors the known susceptibility of different striatal regions to PD-related neurodegeneration[259]. More recently, our group used an ICA-based approach to define a basal ganglia network (BGN) from a large group of control participants[260]. Measurements of FC within this network differentiated Parkinson's patients from controls with 100% sensitivity and 89.5% specificity. This pattern of reduced connectivity in the BGN is robust to methodological variations[261] and is not seen in Alzheimer's neurodegeneration[262]. Importantly, the reduction in FC seen in Parkinson's patients was reversed with levodopa, mirroring the clinical feature of levodopa responsiveness which forms an important part of PD diagnosis.

Functional connectivity in RBD

Only two studies have assessed rs-fMRI in patients with REM sleep behaviour disorder. Using the same ICA-derived template described above, we recently investigated functional connectivity in the basal ganglia network in 26 individuals with RBD compared with Parkinson's patients and healthy control participants[263]. Interestingly, the reduction in BGN connectivity seen in RBD was of a similar magnitude to that in established Parkinson's, despite

evidence of less striatal denervation in a subset RBD patients who also underwent DAT SPECT (figure 5). Longitudinal follow-up is underway to assess the predictive value of the BGN signal for future pheno-conversion but this evidence suggests that connectivity disruptions may be a very early sign of pre-motor neurodegeneration. In contrast with our data, Ellmore and colleagues demonstrated an intermediate phenotype in RBD patients using a seed-based analysis to look at connectivity with the substantia nigra (SN)[264]. The pattern was not always consistent, but correlations between left SN and putamen, and between right SN and superior occipital gyrus, were reduced in RBD patients compared to controls but not to the extent seen in PD patients. Both of these approaches require replication in other cohorts, as well as longitudinal investigations to assess the change in these signals with time.

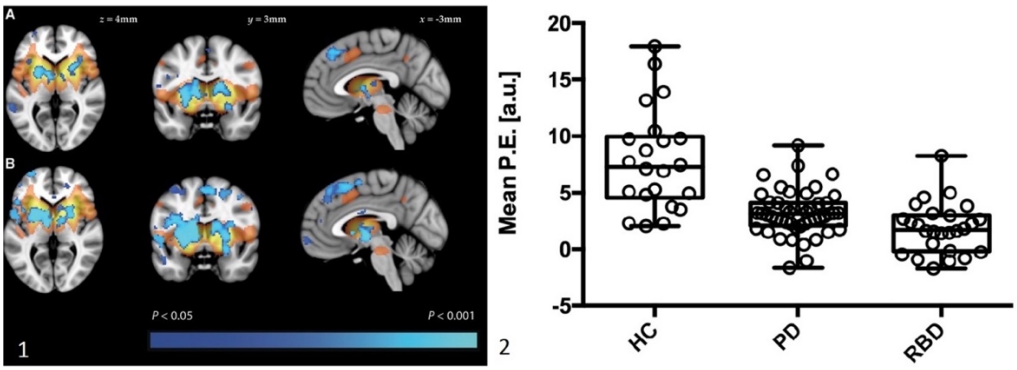


Figure 5. Resting state MRI in individuals with RBD, PD and healthy controls. Panel 1 (left): resting state functional connectivity in PD (A) and RBD (B). The basal ganglia network template derived from a separate group of control participants is shown in red-yellow. Areas where functional connectivity was significantly reduced compared to controls are shown in blue. Panel 2 (right): extracted mean parameter estimates from the clusters with significantly different connectivity in the PD versus control and RBD versus control comparisons. Box plots represent the group mean and quartiles with whiskers denoting minimum and maximum values. (Rolinski et al 2016[263])

Functional connectivity in genetic cohorts

Studies in asymptomatic LRRK2 mutation carriers have also revealed pre-symptomatic changes in brain network activity. Two task-based fMRI studies have demonstrated increases in effective connectivity between basal ganglia and cortical regions including the premotor cortex[265,266], perhaps suggestive of mechanisms compensating for early neurodegeneration. Increased task-related premotor cortex activity has also been reported in heterozygous

carriers of the PINK1 and *parkin* mutations[267]. Resting state connectivity using a seed-based approach in 37 asymptomatic LRRK2 carriers revealed a shift in the pattern of connectivity between the right inferior parietal cortex (IPC) and the putamen[268]. Compared to controls, the LRRK2 carriers had decreased connectivity between right IPC and dorsoposterior putamen and increased connectivity between right IPC and ventro-anterior putamen, in keeping with early degeneration in the posterior regions and compensation anteriorly. Similar group-level cortico-striatal connectivity reductions have been seen in a separate LRRK2 cohort, which also reported correlations between FC and subcortical grey-matter volumes[269].

Whilst these resting state fMRI studies support the idea that connectivity changes precede the development of motor Parkinson's, some limitations of the technique should be noted. The signal is rather sensitive to motion artefacts and shows test-retest variability within subjects. At present the reproducibility of findings amongst different cohorts remains to be established and it is uncertain whether the promising group-level results will translate into biomarkers that can be used on an individual basis.

Concluding remarks and Future directions

The considerable volume of work dedicated to neuroimaging in pre-clinical and prodromal Parkinson's in recent years has generated a number of promising early biomarkers. In addition to results from imaging modalities that are well characterised in established Parkinson's, novel techniques analysing brain networks and nigral structure give particular cause for optimism. Developments in MRI biomarkers raise the tantalising possibility of a widely available, non-invasive diagnostic test for prodromal disease in the near future.

Despite this promise, some important obstacles remain to be overcome. Reproducibility is a major issue and several of the methodologies described in this review require further replication in distinct populations; efforts to improve data-sharing and multi-site collaboration will be key in overcoming this. Translation of group level differences into meaningful individual results is also a challenge, and is limited by the relatively small sample

size in many studies. Few of the studies discussed include longitudinal follow-up, something that will be crucial both to establish the change in imaging signals over time as well as to validate putative biomarkers against clinical outcomes.

All of the imaging methods studied thus far give a somewhat indirect readout of disease status rather than a specific quantification of the underlying pathological process. This issue has led to problems in the study of other neurodegenerative diseases, particularly in the context of clinical trial design[270], and given the fallibility of clinical diagnosis in up to 20% of Parkinson's disease cases[130] this is an important consideration. The development of a method to image alpha-synuclein directly would address this, but there remain significant methodological obstacles to doing so. Compared to beta-amyloid, which has been successfully imaged with PET in Alzheimer's disease, alpha-synuclein is far less abundant and is predominantly intracellular[271]. If these issues are overcome, however, *in vivo* visualisation of the key pathological protein in Parkinson's could prove an important marker of disease progression and response to alpha-synuclein targeted therapies.

The feasibility and reproducibility of imaging approaches are likely to be enhanced by automated registration, segmentation and signal quantification methods. Many of the techniques described above rely on subjective raters for these steps, which is time consuming and introduces bias. In the following three chapters, we explore three of the most promising prodromal imaging techniques in RBD patients and investigate robust, automated methods of quantification that may be suitable for inclusion in multimodal risk stratification models.

Chapter 4: Imaging prodromal dopaminergic decline in RBD

Introduction

As described in chapter 3, the characteristic patterns of dopaminergic decline that occur in parkinsonian disorders can be captured by functional neuroimaging techniques that label components involved in dopaminergic transmission. The method most widely used in clinical practice uses single photon emission computed tomography (SPECT) to detect the radioligand ^{123}I-FP-CIT (ioflupane). We have seen that dopamine transporter (DaT) SPECT imaging can play an important role in the diagnosis of Parkinsonian disorders, where it accurately differentiates these from non-degenerative conditions such as dystonic tremor[152]. However, there is also evidence that this modality can be used to track the progressive decline in dopaminergic synapses as disease progresses[159]. Given the extent to which this degeneration becomes established in prodromal disease[272], DaT SPECT is a natural choice for the investigation of this phase.

In this chapter, we sought to use DaT SPECT imaging to characterise in detail the dopaminergic abnormalities that occur in patients with RBD, describing the varying patterns of decline as well as exploring different methods of signal quantification. We explored the relationship between dopaminergic imaging and the clinical data from chapter 1, and used comparisons with SPECT imaging from established PD patients to shed light on the trajectory of decline from prodromal to manifest synucleinopathy.

Challenges of dopaminergic imaging in prodromal disease

The limited data accumulated thus far regarding DaT SPECT in prodromal disease paints a mixed picture of its likely usefulness. In population studies, where the pre-test probability of developing PD is low, DaT SPECT has the capacity to substantially improve risk stratification, but such studies are costly and relatively inefficient[74]. Conversely, studies in RBD are more feasible due to the greater enrichment for prodromal cases, but the high baseline risk means that the additional benefit of DaT imaging is limited[169]. In a recent multicentre study of

predictors of conversion in RBD, DaT SPECT performed less well than simple in-clinic markers such as quantitative motor testing[68].

This may in part be explained by the relative timing of these prodromal measures. The best predictors of near-term conversion will be those that emerge close to the conversion point, such as mild motor Parkinsonism[12,68]. Abnormalities that develop early in prodromal disease, or that show considerable variation in this timing, will have less predictive value. These early markers of neurodegeneration are arguably more important in the long term as they may identify disease at a time when neuroprotective potential is greatest, but their utility in the context of short-duration clinical trials is currently limited. Despite these limitations, longitudinal RBD studies show that DaT SPECT does confer some stratification advantage and until a superior imaging technique is validated, it remains a gold standard for assessing nigro-striatal integrity[164,169].

If a single cross-sectional measure of nigro-striatal integrity is limited in its ability to predict an individual's disease course, serial imaging ought to be more informative[175]. Practical considerations limit the application of repeated SPECT scanning, but serial measurements are an important aim of novel MRI-based neuroimaging techniques. Interpreting the rate of decline depends on an understanding of the average trajectory of this decline in Parkinsonian disorders. Relatively few studies have examined this, and most serial imaging data involves just two time points separated by a relatively short interval[159,273,274]. Another, indirect way to estimate the average decline is to compare individuals at different time points, where their respective average time from pheno-conversion can be estimated. To examine this, we have included DaT SPECT imaging from a small number of patients with established PD alongside our RBD imaging cohort.

Quantification of dopaminergic imaging

Whilst quantifying the dopaminergic SPECT signal with specific uptake ratios is straightforward, using this objective data to reliably identify abnormalities presents certain problems, which are particularly relevant in RBD patients. The range of different patterns of abnormalities that can develop, and the limited extent of the deficits observed at this early

prodromal stage, mean that subtle changes in the pattern of signal uptake can be more important than the absolute uptake ratios[69,176,263]. Distinguishing such borderline changes from the natural decline that occurs in healthy ageing is challenging and as a result there are no standardised values that can define abnormal. For this reason, in clinical practice DaT SPECT scans are typically reported in a qualitative, descriptive manner.

However, quantification remains important for research purposes, particularly when measuring the change in signal over time. The measurement of basal ganglia regions is usually achieved by registering individual SPECT images to a standard template using the SPECT signal itself to identify the location of the principal regions of interest in the caudate and putamen nuclei. This works well in these broad regions, where contrast with the surrounding tissues is high, but it cannot be used in smaller areas with relatively lower signal contrast. Furthermore, SPECT images provide little information regarding the anatomical structures underlying the dopaminergic signal. In this study, we sought to address these limitations by performing DaT SPECT and structural MRI in parallel and co-registering the images in standard space. This allows regions of interest to be defined in the basal ganglia based on their true anatomical location, and it enables the signal to be probed further by subdivision of the nuclei into smaller regions. This method of co-registration also enables the signal to be sampled from smaller nuclei outside of the basal ganglia, which cannot be defined on the basis of their SPECT signal alone (this will be explored further in Chapter 7).

In order to maximise the accuracy of the SPECT signal extracted from our regions of interest, it is important to consider the way in which SPECT images are reconstructed. Attenuation correction is required to account for the signal attenuation that occurs as the gamma radiation traverses the tissues between the signal origin (e.g. the basal ganglia) and the gamma camera[275,276]. Traditional reconstruction techniques treat the tissues of the head as homogenous, whereas in fact the attenuation due to brain is considerably different from that due to bone. In this study, we use CT-based attenuation correction to account for this. The importance is illustrated by figure 1, showing the DaT SPECT scans from two individuals with RBD, superimposed on their CT imaging. It is clear from this example that the thickness of the skull varies considerably between individuals, which will cause differences in the signal attenuation. CT-based attenuation correction accounts for this by using information from the

CT scan to estimate the attenuation due to the actual tissue types present[275]. This is particularly important where the signal of interest originates in brain regions that are surrounded by a large amount of bone, such as lower brainstem regions.

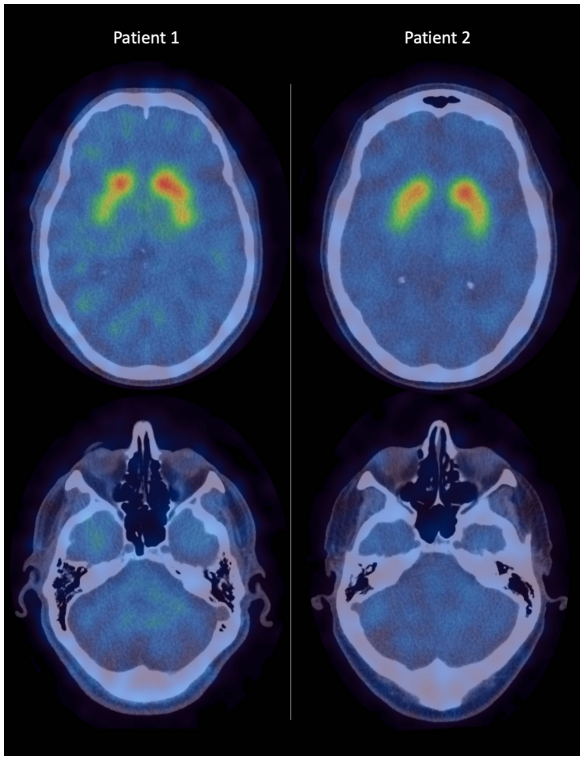


Figure 1. Example images from two RBD patients showing DaT SPECT images overlaid on CT images. Differences in skull thickness and anatomy illustrate the importance of accurate attenuation correction.

Aims

In summary, this chapter aimed to characterise in detail the dopaminergic abnormalities present in RBD patients and their relationship to clinical prodromal markers. We sought to maximise the potential of our SPECT imaging by using CT-based attenuation correction techniques and exploring methods of SPECT quantification using co-registration with MRI. By comparing SPECT imaging in RBD and PD patients, we aimed to estimate the rate of dopaminergic decline that occurs between prodromal and manifest parkinsonian disorders. Finally, the SPECT data acquired here were also designed to provide a 'gold standard' imaging assessment against which to measure the novel imaging techniques explored in the following chapters.

Methods

Participants & clinical data

Forty-nine patients with RBD were recruited from the cohort described in chapter 2. All RBD participants underwent brain imaging with ^{123}I-ioflupane (DaT) SPECT/CT. Forty-five of the RBD patients underwent MRI in parallel, from which the T1 structural sequences were used in the analysis described in this chapter. Nine patients with established Parkinson's disease were also recruited from the Discovery cohort of the Oxford Parkinson's Disease Centre and were scanned with DaT SPECT/CT using the same protocol, for comparison. All patients were examined on the day of MRI using the Movement Disorders Society Unified Parkinson's Disease Rating Scale, part III (UPDRS III)[132]. Other clinical variables were recorded at separate clinic visits according to the protocol described in chapter 2[277].

SPECT/CT acquisition and reconstruction

DaT SPECT/CT scans were acquired according to the standard clinical protocol at the Department of Nuclear Medicine, Churchill Hospital, Oxford. Potassium iodide 120mg was administered one hour prior to, and 24 hours after, injection of ^{123}I-ioflupane to block thyroid uptake. Subjects were injected with 185 MBq +/- 10% of ^{123}I-ioflupane (provided as DaTscan^{TM} injection, GE Healthcare). SPECT/CT images were acquired three hours post injection on a Discovery 670 gamma camera (GE Healthcare, Haifa). SPECT acquisition parameters: 120 projections, 30 seconds per projection, 128 x 128 matrix. CT parameters: 16 slice, helical acquisition, 120 KV, 40 mA, noise index 30. The SPECT/CT data was reconstructed using HERMES Hybrid Recon (HERMES Medical Solutions, AB Stockholm) OSEM, 15 iterations, 4 subsets with attenuation correction from CT, collimator resolution recovery, and Monte Carlo scatter correction.

MRI acquisition

MRI scanning was performed at the Oxford Centre for Clinical Magnetic Resonance Research (OCMR) using a 3T Siemens Trio (Erlangen, Germany) with a 12-channel head coil. High-resolution T1-weighted images were acquired (MPRAGE, TE/TR = 4.7 ms/2040 ms; 192 axial slices; resolution 1 × 1 × 1 mm3; 6 minutes) and used for registration.

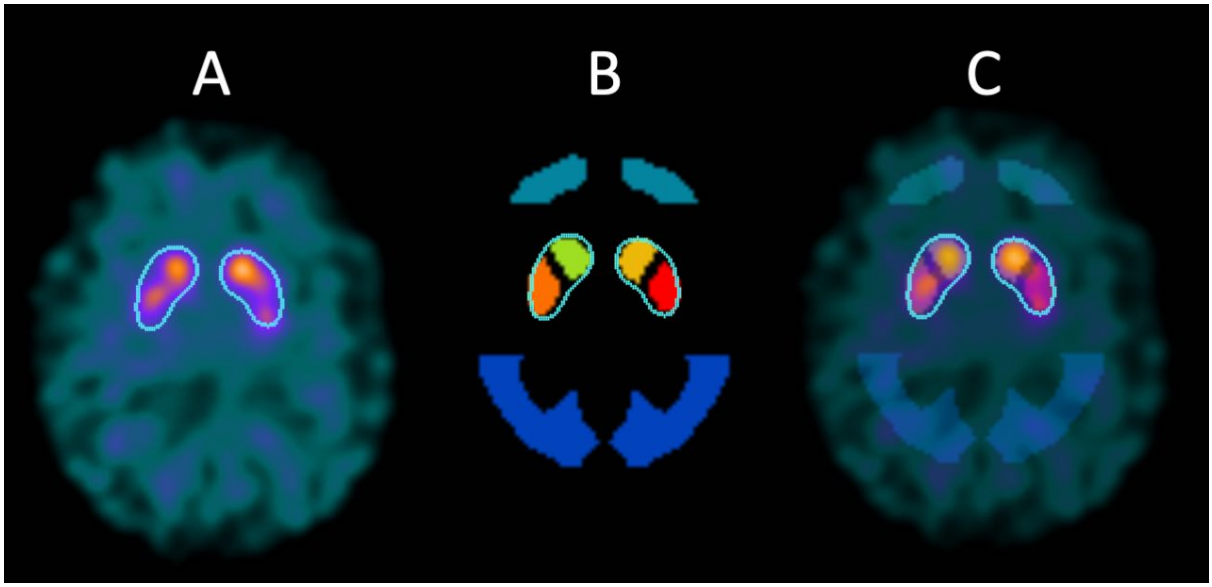


Figure 2. Registration and templates used in DaT SPECT analysis with Hermes software. A: an individual's DaT SPECT image registered to the standard template. B: regions of interest used to quantify the SPECT signal in the caudate (yellow, green), putamen (red, orange) and background reference region (dark blue). The anterior light blue region was not used. C: template ROIs overlaid on the DaT SPECT image from A.

DAT SPECT/CT analysis

SPECT/CT data were analysed using BRASS software (HERMES Medical Solutions, AB Stockholm). Reconstructed images for each patient were registered to a standard template including ROIs for the caudate and putamen on each side (figure 2). This registration process is based on the SPECT signal itself rather than any corroborating anatomical information. Uptake ratios were calculated for these ROIs using a standard occipital reference region. From these four ratios, the following additional variables were calculated: mean caudate, mean putamen, mean whole striatum (all 4 regions), lower putamen, and caudate:putamen ratio on each side. Percentage asymmetry was calculated for whole striatum and putamen respectively as follows:

$$\% \text{ asymmetry} = (\text{abs}(\text{left}[\text{ROI}] - \text{right}[\text{ROI}]) \times 100) / \text{mean}[\text{ROI}]$$

Additionally, for the RBD participants, each DaT SPECT/CT scan was reported descriptively in a standard clinical manner by an experienced nuclear medicine radiologist (Dr Kevin Bradley,

Head of Nuclear Medicine, Radiology Department, Churchill Hospital) who was blinded to all clinical and MRI data except for the participants' age, gender and presence of RBD. The descriptive reports were then categorised into one of 5 patterns: normal; borderline; asymmetric putamen signal loss (typical PD pattern); generalised striatal signal loss (atypical parkinsonism pattern); and asymmetric caudate signal loss. An example of each category is shown in figure 3. The full reports of all 49 RBD DaT SPECT/CT scans are provided in appendix 1. For dichotomised analyses of normal versus abnormal scans, borderline scans were included in the normal category, with the three types of abnormal scan combined together as a single abnormal group.

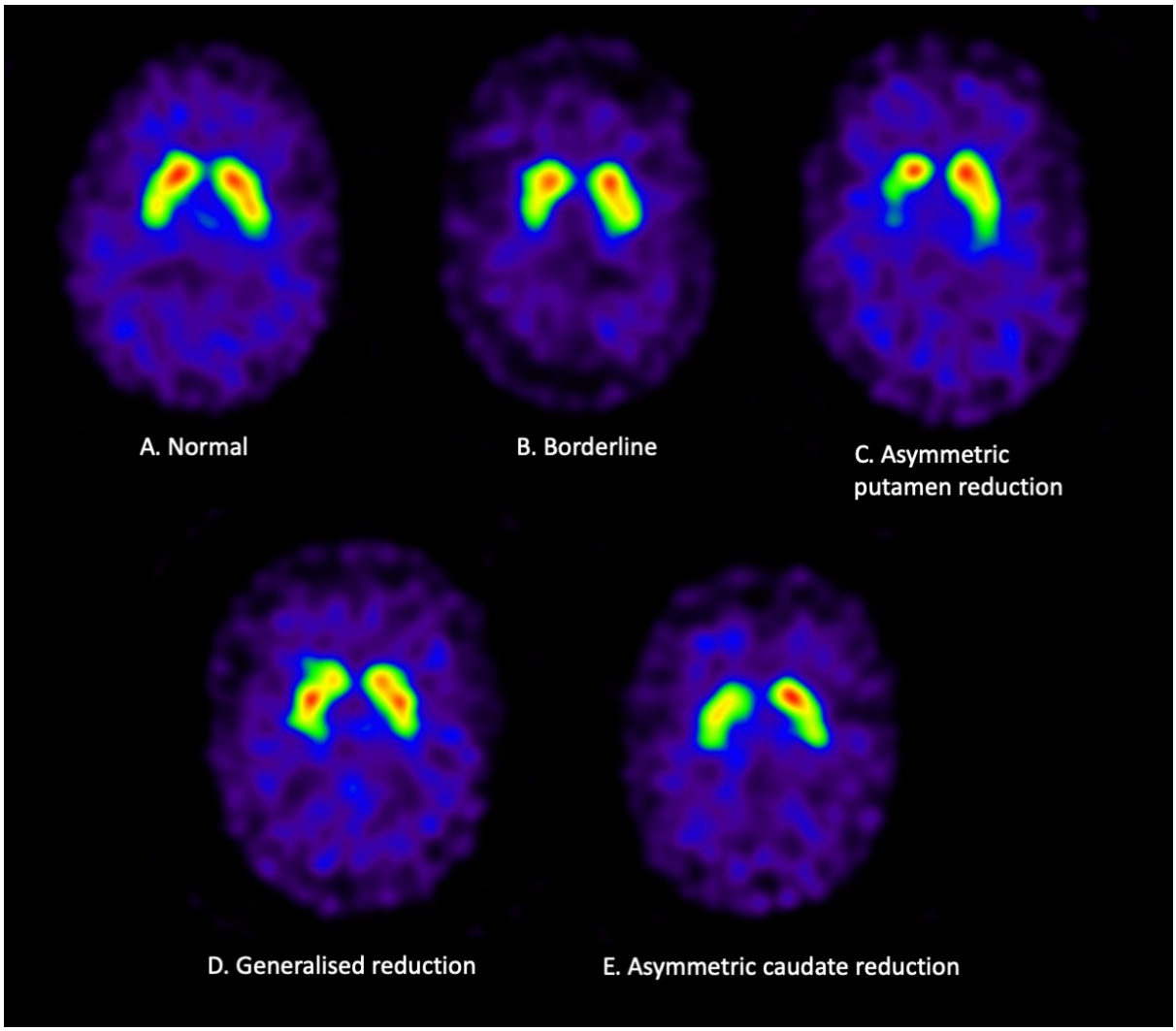


Figure 3. Example DaT SPECT/CT images from 5 different participants illustrating the range of patterns of abnormality seen.

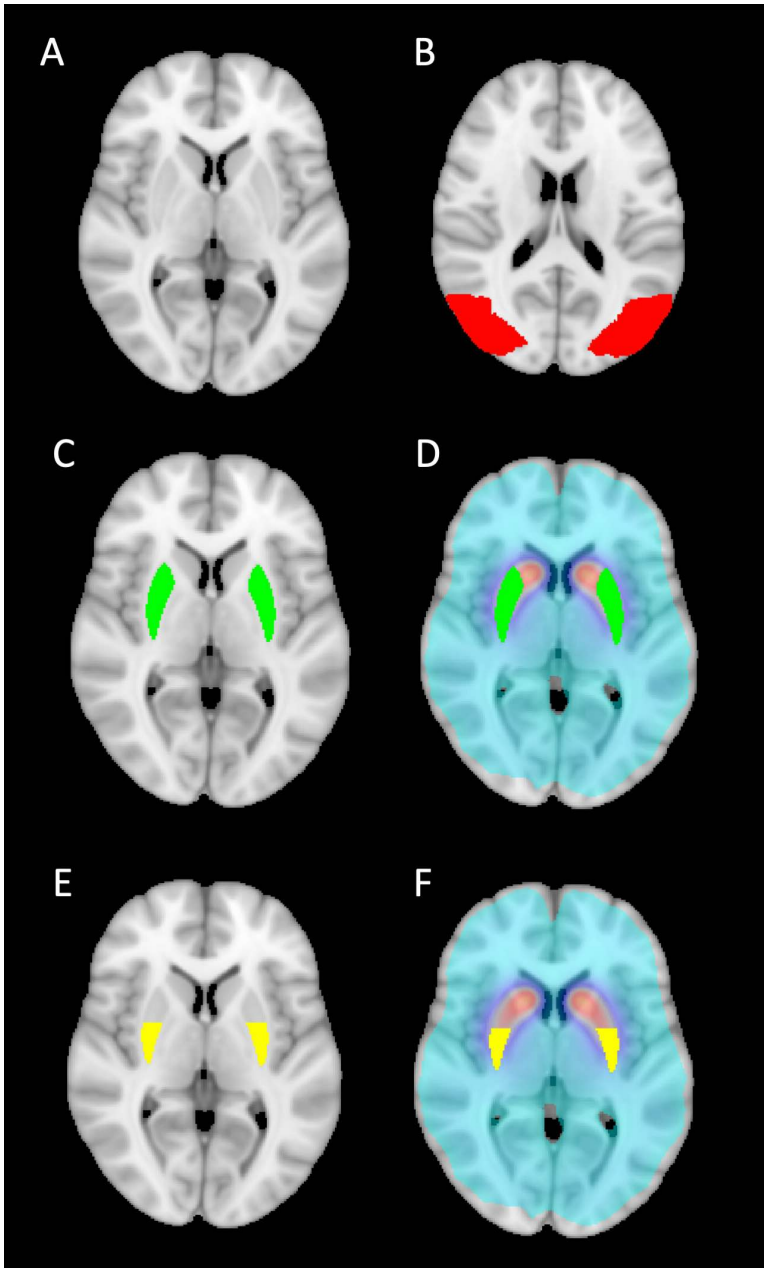


Figure 4. Regions of interest for combined DaT SPECT/CT and MRI analysis. A: MNI standard space T1 template. B: ROI for the superior lateral occipital cortex, used as a reference region. C,E: ROIs for the whole putamen (C, green) and posterior putamen (E, yellow) based on the Harvard-Oxford subcortical atlas. D,F: Putamen ROIs overlaid on the group average DaT SPECT image.

DAT SPECT/CT & MRI combined analysis

Registration of SPECT imaging to standard space was kindly performed by Dr Johannes Klein (Senior Clinical Research Fellow, OPDC) as follows: reconstructed ^{123}I-ioflupane SPECT scans were rigid-registered to each individual's structural MRI using mutual information as

similarity measure. Structural MR images were transformed into 1mm^3 standard Montreal Neurological Institute (MNI152) space with FMRIB's non-linear image registration tool (FNIRT)[278]. Using the parameters determined from MRI, individual SPECT scans were then transformed into MNI152 space. An average SPECT image of all 45 RBD subjects in standard space was also created for illustration (figure 4D & 4F)

Regions of interest (ROI) for the combined SPECT-MRI analysis were all derived from the Harvard-Oxford Cortical atlas and the Harvard-Oxford subcortical atlas[278]. The superior lateral occipital cortex was used as a reference region (figure 4B). This was chosen because it is low in monoamine transporters, distant from any signal from the regions of interest studied, and had full MRI and SPECT coverage in all participants. To derive the putamen ROI, the Harvard-Oxford template for this region was thresholded at 50% and binarized (Figure 4C) to provide an ROI corresponding tightly to the anatomical location of the putamen. Figure 4D shows this ROI template superimposed on the standard space average SPECT image.

Because typical Parkinsonian neurodegeneration affects the posterior putamen first before proceeding anteriorly[69], the SPECT signal from the posterior part of the putamen may conceivably be a more sensitive marker of early disease than the putamen as a whole. For this reason, a posterior putamen ROI was created by taking the whole putamen region (Figure 4C) and retaining only the voxels posterior to the MNI co-ordinate of zero in the y plane (Figure 4E and 4F).

Average intensities for the voxels within the ROIs were then calculated in standard space for each subject's images using the '*fslmeants*' command. Specific uptake ratios were calculated by dividing the mean intensity of the region of interest by the mean intensity of the reference region. Asymmetry indices were calculated in the same manner as the SPECT-only analysis.

Statistical analysis

Statistical analyses were performed using SPSS. Linear regression was used to compare imaging with clinical variables, as well as for comparisons of variables between dichotomised patient groups, with age included as a covariate where appropriate to control for the known decline in basal ganglia dopamine transporters in normal ageing. The ability of variables to discriminate between groups was calculated using a receiver operating characteristic curve. Results are presented without correction for multiple comparisons.

Results

Descriptive/categorical DaT SPECT/CT outcomes

Baseline demographic variables for all included participants are shown in table 1, with RBD patients dichotomised according to normal or abnormal DaT SPECT outcomes. Overall, 21 out of 49 (43%) patients had definitely abnormal DaT SPECT imaging according to the radiologist report (appendix 1). There was no difference in the age (p=0.43), gender (p=0.38) or time since RBD diagnosis (p=0.96) between RBD patients with normal versus abnormal DaT SPECT imaging.

	RBD normal (inc. borderline)	RBD abnormal	PD
Number of cases	28	21	9
Age (years)	65.5 (8.42)	63.8 (6.36)	67.4 (10.9)
Gender (% male)	100	96	78
Years since diagnosis	2.58 (2.28)	2.54 (2.32)	6.0 (0.88)

Table 1. Baseline characteristics of the DaT SPECT/CT study population, with RBD patients dichotomised according to DaT SPECT/CT outcome. Diagnosis was recorded as the point of PSG confirmation in RBD patients, and clinician confirmation in PD patients.

Table 2 shows the full breakdown of RBD patients according to DaT scan outcome, with average scores for a range of clinical motor and non-motor prodromal parkinsonian signs presented for each group of patients.

Of the 21 patients with definitely abnormal imaging, 12 (57%) had asymmetric putamen signal loss in a pattern consistent with typical early Parkinson's disease. Six (29%) had more generalised signal loss across the striatum bilaterally, a pattern sometimes seen in atypical Parkinsonian disorders such as Dementia with Lewy Bodies and Multiple System Atrophy. Three individuals (14%) had asymmetric signal loss anteriorly, predominantly affecting the caudate nucleus unilaterally. The significance of this is uncertain. Such a pattern can occur with infarction of the caudate, though none of the 3 patients had evidence of this on

separately acquired FLAIR MRI images. It is possible that it represents another atypical early presentation of Lewy Body degeneration.

Interestingly, clinical variables were broadly the same across patients with all categories of DaT SPECT outcomes. None of the abnormal patient groups scored significantly worse than patients with normal imaging in any of the variables tested.

	Normal	Borderline	Putamen asymmetry	Generalised reduction	Caudate asymmetry
Number of cases	22	6	12	6	3
Age	64.0 (8.46)	71.3 (5.55)	62.8 (6.09)	66.7 (7.40)	60.7 (2.12)
UPDRS III	5.27 (3.01)	7.00 (2.90)	5.58 (3.75)	5.33 (4.50)	1.67 (1.53)
Purdue Pegboard	32.3 (6.20)	30.3 (1.21)	33.5 (4.03)	30.8 (7.08)	36.7 (2.52)
MoCA	26.0 (2.69)	26.7 (2.42)	26.1 (2.15)	24.9 (3.45)	26.7 (2.52)
BDI	13.4 (10.28)	4.8 (6.77)	9.5 (6.79)	7.2 (8.13)	7.7 (6.11)
Orthostatic SBP drop	1.9 (11.93)	15.8 (14.9)	0.3 (11.10)	16.4 (13.20)	13.5 (4.95)
Sniffin Sticks score	8.0 (3.65)	7.3 (2.58)	8.4 (3.60)	5.4 (2.30)	No data
MDS probability	82.6 (15.62)	90.4 (15.04)	83.9 (15.36)	88.4 (15.44)	72.8 (21.20)

Table 2. Categorical DaT SPECT/CT outcomes in RBD patients and clinical scores for each group

Even when those with abnormal scans were grouped together and compared against those with normal or borderline scans, there were no differences in any of the variables tested (figure 5).

Region of interest DaT SPECT/CT outcomes

Signal values from the SPECT-defined regions and the MRI-defined regions were compared for their ability to distinguish RBD patients with overall normal SPECT scans (as defined by the descriptive reports) from those with subjectively abnormal dopaminergic imaging. The results of this analysis are shown in table 3.

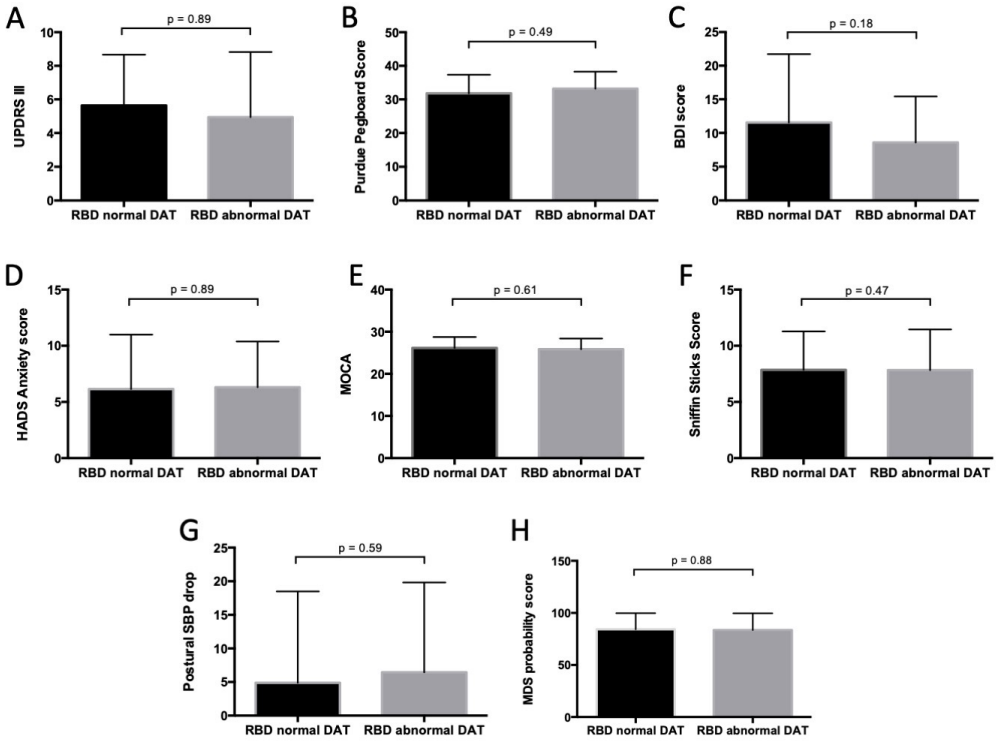


Figure 5. Clinical variables in RBD patients according to dichotomised DaT SPECT/CT outcome

SPECT defined regions of interest			
	AUC Normal vs abnormal DaT SPECT/CT	AUC 95% CI	p value
Mean putamen	0.866	0.760 – 0.972	**<0.001**
Lower putamen	0.892	0.795 – 0.989	**<0.001**
Mean whole striatum	0.869	0.763 – 0.975	**<0.001**
Mean caudate	0.825	0.697 – 0.953	**<0.001**
% putamen asymmetry	0.694	0.543 – 0.845	**0.021**
% whole striatum asymmetry	0.702	0.542 – 0.863	**0.016**
Mean Caudate:putamen ratio	0.681	0.520 – 0.843	**0.031**
MRI defined regions of interest			
	AUC Normal vs abnormal DaT SPECT/CT	AUC 95% CI	p value
Mean putamen	0.821	0.695 – 0.947	**<0.001**
Lower putamen	0.811	0.681 – 0.940	**<0.001**
% putamen asymmetry	0.626	0.446 – 0.805	0.16
Mean posterior putamen	0.782	0.640 – 0.924	**0.002**
Lower posterior putamen	0.819	0.694 – 0.944	**<0.001**
% posterior putamen asymmetry	0.630	0.462 – 0.798	0.14

Table 3. Comparison of the discriminatory ability of DaT SPECT signal values from SPECT-only and combined MRI-SPECT defined ROIs.

When examining the variables derived from the SPECT-defined regions, good separation accuracy for mean (bilateral) values was seen for the putamen and whole striatum. A slightly greater accuracy was seen for the putamen only when comparing the lower of the two sides, presumably reflecting the asymmetric onset of many cases of early disease. Asymmetry indices and caudate:putamen ratios performed less well at identifying abnormal cases. A similar pattern of results was seen for the MRI-defined regions, though here the asymmetry indices were unable to separate normal from abnormal cases. Mean and lower putamen values performed better, but were not as accurate as the equivalent SPECT-defined variable. Interestingly, subdividing the putamen into a posterior region did not improve the classification accuracy.

Next, various ROI measures were correlated against clinical markers of prodromal Parkinsonism, the results of which are shown in table 4.

	Mean striatum (SPECT)	Mean putamen (SPECT)	Lower putamen (SPECT)	Mean putamen (MRI)	Lower putamen (MRI)	Mean posterior putamen (MRI)	Lower posterior putamen (MRI)
Age	**r = -0.35 p = 0.014**	**r = -0.30 p = 0.04**	r = -0.21 p = 0.15	r = -0.18 p = 0.24	r = -0.22 p = 0.15	r = -0.27 p = 0.07	r = -0.20 p = 0.19
UPDRS III	β = -0.07 p = 0.66	β = -0.09 p = 0.60	β = -0.06 p = 0.73	β = -0.14 p = 0.43	β = -0.12 p = 0.48	β = -0.14 p = 0.44	β = -0.14 p = 0.42
Purdue Pegboard	β = -0.08 p = 0.58	β = -0.05 p = 0.74	β = -0.08 p = 0.60	β = -0.23 p = 0.13	β = -0.17 p = 0.26	β = -0.19 p = 0.21	β = -0.09 p = 0.57
MoCA	β = 0.04 p = 0.76	β = 0.03 p = 0.83	β = 0.03 p = 0.83	β = -0.01 p = 0.93	β = 0.01 p = 0.94	β = 0.02 p = 0.87	β = 0.06 p = 0.70
BDI	β = 0.21 p = 0.14	β = 0.21 p = 0.16	β = 0.23 p = 0.12	β = 0.26 p = 0.08	β = 0.24 p = 0.11	β = 0.18 p = 0.23	β = 0.15 p = 0.35
Orthostatic SBP drop	β = -0.18 p = 0.22	β = -0.16 p = 0.29	β = -0.19 p = 0.20	β = -0.11 p = 0.50	β = -0.11 p = 0.49	β = -0.05 p = 0.74	β = -0.08 p = 0.60
Sniffin Sticks score	β = 0.01 p = 0.95	β = -0.03 p = 0.84	β = 0.01 p = 0.94	β = 0.11 p = 0.54	β = 0.09 p = 0.61	β = 0.14 p = 0.42	β = 0.11 p = 0.55
MDS probability	r = -0.14 p = 0.33	r = -0.17 p = 0.24	r = -0.18 p = 0.21	r = -0.06 p = 0.69	r = -0.08 p = 0.61	r = -0.10 p = 0.53	r = -0.09 p = 0.56

Table 4. Correlations (calculated as regression coefficient) between DaT SPECT variables and clinical measures of prodromal parkinsonian features.

As with the dichotomised analyses, no significant relationships were observed between clinical and imaging variables, other than age. This makes it difficult to compare amongst the different imaging variables, but overall, despite the lack of significant results, correlations tended to be stronger with SPECT-derived variables than with MRI-derived variables.

Comparison between RBD and PD SPECT imaging: estimating rate of decline

MRI data was not available for PD patients in this study, therefore only SPECT-derived regions were recorded. Figure 6 and table 5 present a comparison of the SPECT ROI variables between RBD patients, with normal and abnormal SPECT imaging, and PD patients. As expected, the most pronounced changes were observed in the putamen, where the mean signal in PD patients was just 31.5% of that in RBD patients with normal imaging. A more modest reduction to 77.3% was observed in RBD patients with subjectively abnormal imaging.

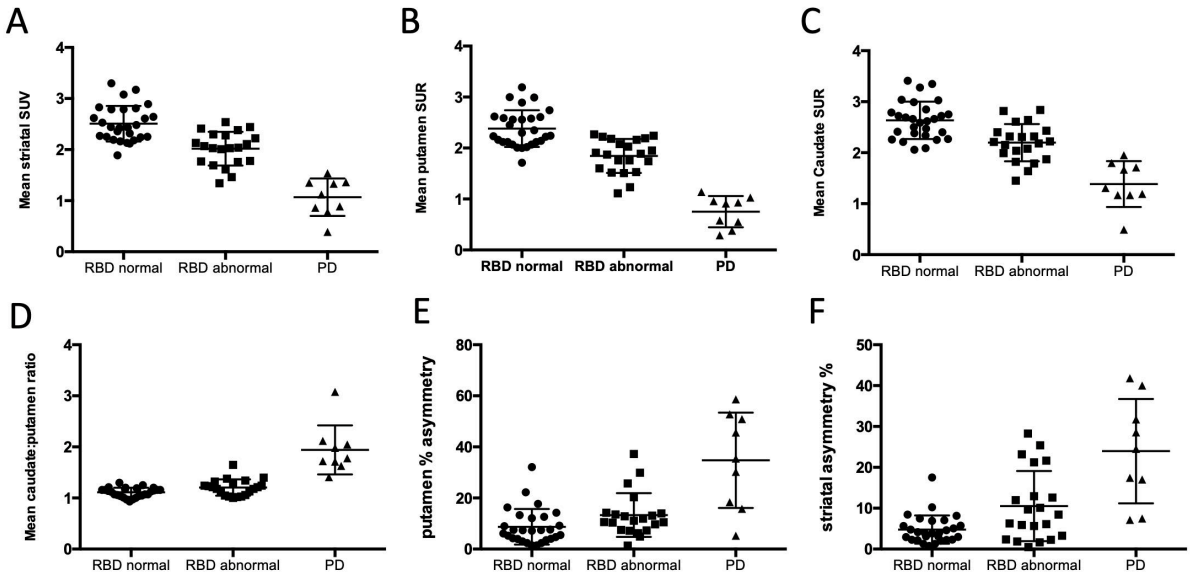


Figure 6. DaT SPECT/CT variables compared between RBD patients with normal and abnormal imaging and PD patients.

Using the data from our RBD and PD patients, along with longitudinal data previously published in RBD patients, it is possible to estimate the rate of dopaminergic decline. In a large, longitudinal study of RBD patients who had baseline DaT SPECT imaging, the median time between imaging and conversion to Parkinsonian disorder was 6.5 years in those RBD

patients with abnormal SPECT imaging[169]. The PD patients included in this study have a mean disease duration of 6.0 years since diagnosis. We can therefore estimate that these PD patients are on average 12.5 years further into their disease course than our RBD patients with abnormal SPECT imaging. Given that we know the dopaminergic signal in the putamen of both groups (table 5), we can calculate the rate of decline as follows:

Dopamine level (RBD_abnormal_DAT) x $Y^{12.5}$ = dopamine level (PD)

Where Y is the annual decline in dopamine signal

1.84 x $Y^{12.5}$ = 0.75

$Y^{12.5}$ = 0.408

Y = 0.93

	RBD Normal DaT SPECT	RBD Abnormal DaT SPECT	PD
Caudate	2.63 (0.36)	2.20 (0.37)	1.38 (0.45)
% of normal		83.7	52.5
Putamen	2.38 (0.36)	1.84 (0.34)	0.75 (0.31)
% of normal		77.3	31.5
Whole striatum	2.51 (0.35)	2.01 (0.33)	1.07 (0.37)
% of normal		83.7	42.6
Caudate:putamen ratio	1.11 (0.09)	1.20 (0.16)	1.94 (0.48)
% of normal		108.1	174.8

Table 5. Comparison of DaT SPECT/CT measures in RBD patients and PD patients. For RBD patients with abnormal imaging, and PD patients, figures are given for the mean percentage of the value in RBD patients with normal imaging.

Therefore, the estimated decline in dopaminergic signal from the putamen is 7% per year. This trajectory is plotted in figure 6. According to this estimate, the average time to conversion for RBD patients as a whole (green line) would be around 8.5 years. The estimated trajectory also suggests an average latency to conversion of at least 10 years in those RBD patients with normal dopaminergic imaging, compared to the 6.5 years in those with

abnormal imaging. Finally, the trajectory predicts that phenoconversion occurs on average when the dopaminergic signal in the putamen reaches a specific uptake ratio of 1.15.

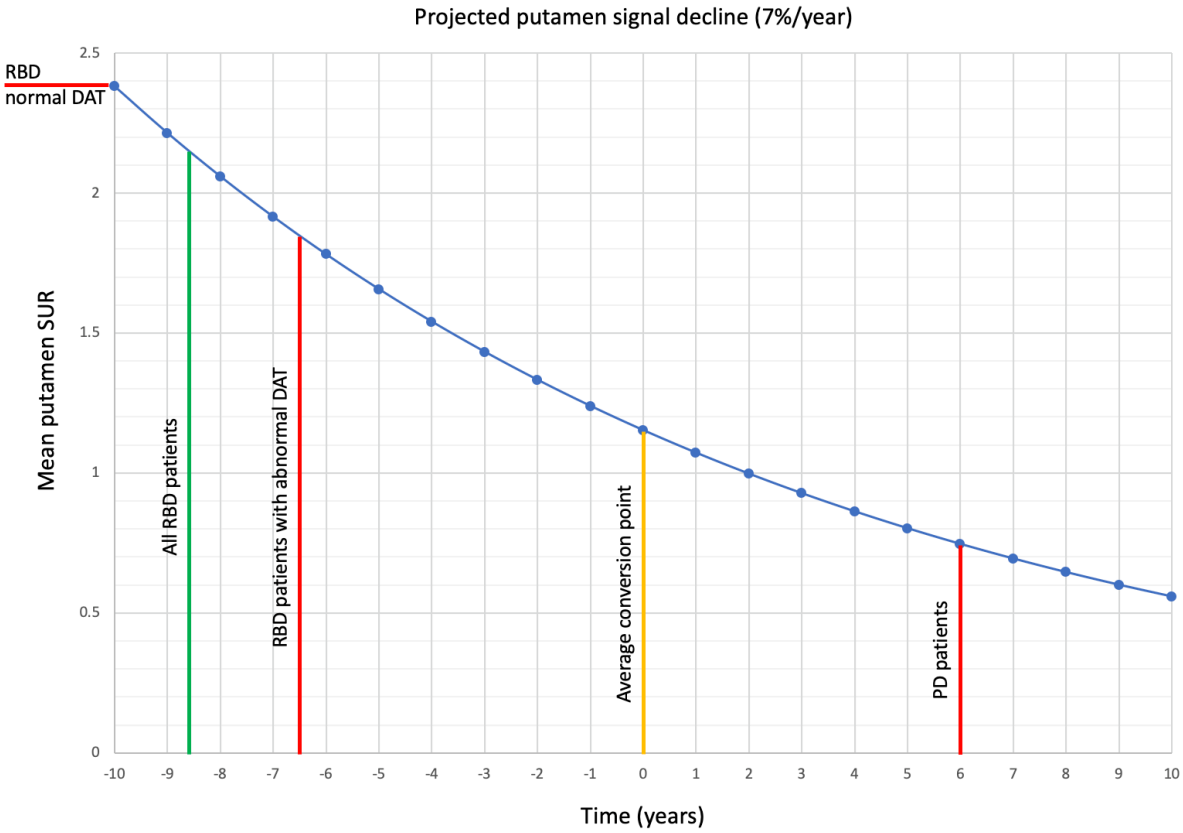


Figure 6. Estimated trajectory of dopaminergic decline in the putamen during prodromal and clinically manifest PD, extrapolated from data in RBD and PD patients.

Discussion

Summary of findings

In one of the largest studies to date examining dopaminergic SPECT in RBD patients, using the latest CT-based attenuation correction techniques, we have found evidence of dopaminergic dysfunction in almost half of RBD patients. Descriptive analysis of the imaging data shows that abnormal scans exhibit a range of different patterns of abnormality, potentially corresponding to the diversity of future disease phenotypes that RBD patients are known to develop. As expected, dopaminergic decline is most evident in the putamen, with signal decline in this region the best predictor of overall scan abnormality in RBD patients as well as showing the greatest reduction in patients with established Parkinson's disease. The fact that the lower putamen side predicted abnormality slightly better than the mean of left and right is in keeping with the asymmetric onset of disease that is expected to occur in the majority of cases.

Extrapolating the putamen signal between RBD and PD patients gives an estimated rate of decline of 7% per year in those with evidence of established dopaminergic dysfunction.

In line with other cross-sectional studies[206,279], we found no significant correlations between the level of dopaminergic decline and any of the clinical markers of known prodromal Parkinsonian features, and as such we remain unable to predict DaT SPECT abnormality using these simple clinic-based tests.

Overall, the results of signal quantification using MRI-based segmentation were broadly similar to those using SPECT-based segmentation. MRI-based quantification was no better at classifying abnormal scans, nor at correlating with clinical prodromal markers.

Dopaminergic dysfunction is an early prodromal sign

The high rate of abnormal scans observed in our population is similar to that reported in other RBD cohorts and as such is reassuring that the RBD population sampled for this imaging study is representative of RBD in general[169,280]. The fact that 43% of RBD patients have an abnormal scan, yet only around 6% of patients are expected to convert to an overt synucleinopathy per year implies that dopaminergic dysfunction as measured by DaT SPECT is a relatively early

prodromal sign[68]. Even if it performed perfectly at predicting those who will convert first, it would take around 6 years at this rate for all of those with abnormal scans to do so.

This may in part be explained by the fact that measuring the level of pre-synaptic dopamine transporters would potentially detect pre-degenerative adaptive changes in nigro-striatal synapses. As dopamine transmission begins to fall as a result of neuronal dysfunction, compensatory downregulation of these transporters occurs in order to preserve synaptic dopamine levels[151,180]. This adaptive change would be reflected in reduced ioflupane binding, meaning the signal change is not accounted for entirely by cell degeneration.

Data from the largest longitudinal study of DaT SPECT in RBD patients support the notion that dopamine dysfunction occurs early[169]. Over a 5-year follow-up period, Iranzo and colleagues reported that DaT SPECT abnormality predicts pheno-conversion with a specificity of just 50%, attesting to the high proportion of RBD patients who remain disease-free for many years despite evidence of dopaminergic deficit.

Estimated rate of dopamine decline is 7% per year

By extrapolating the putamen signal level between our RBD patients with abnormal dopaminergic imaging and our PD patients, and using pheno-conversion latency data from Iranzo's longitudinal study, we are able to estimate that once neurodegeneration is established in this region, it proceeds at around 7% per year on average. Few studies have examined long-term dopaminergic decline with serial DaT SPECT, but there are some data that corroborate our estimate. A recent longitudinal study from the Parkinson's Progressive Marker's Initiative (PPMI) reported a decline of around 8.3% per year over 4 years in patients with established PD[273]. A similar rate of decline has also been reported using an alternative nuclear imaging approach, with F-Dopa PET revealing an 8.1% annual loss in putamen Dopa decarboxylase activity in PD patients[281]. The only study to have examined RBD patients with serial DaT SPECT reported an average putamen decline over a 3-year period of around 17% in all RBD patients[175]. The assumption that degeneration proceeds at a constant exponential rate during prodromal and manifest disease is likely to be an oversimplification, and there is some evidence that the rate of decline is greater in the years immediately before and after pheno-conversion[175,282]. However, it provides a useful estimate of the likely disease trajectory for the group as a whole.

A 7% rate of decline would imply an average putamen specific uptake ratio of 1.15 at the point of conversion, and would suggest that our RBD group as a whole (who have a mean ratio of 2.15) are on average 8.5 years from conversion. This latency is remarkably similar to the actual median latency to conversion of 8.0 years in unselected RBD patients reported by the International RBD Study Group[68].

It is important to note that all of these estimates are of average progression rates, yet we know that the actual rate of progression can vary substantially from patient to patient[83,273]. Whilst the low specificity of cross-sectional DaT SPECT in predicting conversion implies slow progression in some RBD patients with DaT abnormality, the 5-year sensitivity of 75% implies that one in four converters had a significantly more rapid rate of dopaminergic decline[169].

The early onset of dopamine decline and its variable rate of progression may explain why the predictive value of a single DaT scan is limited in RBD patients. It may also explain why RBD patients with abnormal DaT imaging do not score worse on any clinical markers of prodromal features. As shown in figure 6, RBD patients with and without abnormal imaging are actually quite close to each other in terms of average prodromal stage. Both groups have many years to phenoconversion, and over that period even small differences in the rate of progression will lead to substantial overlap in their respective latencies to conversion. Some RBD patients with normal DaT imaging might therefore be closer to conversion than those with abnormal imaging. It is worth noting also that the emergence of certain prodromal markers, such as hyposmia, may occur earlier than both RBD itself and dopaminergic decline[12]. Such floor effects may also contribute to the lack of differences observed between the two groups of RBD patients.

Some caveats of this analysis should be noted, in addition to aforementioned assumption of a constant rate of exponential decline. We have extrapolated between RBD patients and PD patients, assuming that they are on the same disease trajectory. However, in reality RBD patients may convert not just to PD but also to DLB or MSA, with potentially differing rates of disease progression[68,69]. Furthermore, not all PD patients progress to PD from idiopathic RBD, and there is some evidence that those who do not may have a milder neuroimaging phenotype, with less aggressive dopaminergic decline (see chapter 9 for further

discussion)[33,84]. It is probably the case, therefore, that actual progression rates amongst RBD patients vary quite considerably.

Patterns of dopaminergic abnormality in RBD

Another assumption made in the analyses above is the accuracy of classification between normal and abnormal DaT SPECT imaging. In practice, dichotomising in this way is difficult in RBD patients for a number of reasons. As we have seen, there are several different patterns of abnormality that can occur in RBD patients. Whilst the highest number of abnormal scans were in a typical PD-like pattern, these accounted for only 57% of abnormal scans. The other 43% had more diffuse disease, or more caudate predominance. This variation may reflect the range of future disease trajectories seen in RBD. As many patients will convert to DLB as PD, and patients with DLB tend to have more caudate involvement than PD patients[69]. The range of patterns seen makes objective quantification of the abnormality difficult. Certain metrics that are accurate in differentiating PD patients from controls, such as the caudate:putamen ratio and left-right asymmetry index[283,284], are less useful in RBD patients, as many will not present with the characteristic unilateral putamen involvement seen in typical PD.

Additionally, the high proportion of borderline or mildly affected cases expected in prodromal disease, and the lack of a clinical motoric correlate of the imaging findings, make defining abnormal more problematic than in patients presenting with Parkinsonism, the scenario in which DaT SPECT was validated. We have already seen in the comparison between studies of hyposmic patients and studies of patients with RBD that different definitions of abnormal based on scan quantification can lead to superficially unexpected findings: though DaT SPECT abnormality appears to have greater predictive value in hyposmic patients, this is likely due to a more stringent definition of abnormal in that study[74,169].

The benefits of MRI-based segmentation

We have examined here the co-registration of structural MRI and DaT SPECT imaging to facilitate the segmentation of regions of interest, the idea being that this would improve the anatomical accuracy of the regions from which we sampled the SPECT signal. It also enabled us to subdivide the putamen into a posterior region, which would be expected to be more

sensitive to earlier dopaminergic decline. In practice, however, there was little difference between the MRI-defined and SPECT-defined parameters, and if anything, the SPECT-defined values performed better. There are several potential reasons for this. The registration steps involved in manipulating the SPECT images into standard space may have introduced error. The MRI regions could also be considered spuriously precise, given the comparatively low resolution of the SPECT signal itself[285]. It could also be argued that the precise anatomical origin of the signal is actually less important than the signal itself, and that therefore regions defined from the signal are more meaningful.

For analyses of the caudate and putamen, therefore, there would seem little advantage in using this segmentation method. However, a major advantage of this co-registration is that it enables regions outside of the basal ganglia to be examined. Such regions may be considerably smaller and have less signal contrast with surrounding areas. In this context, anatomical localisation is likely to be more useful than signal-defined segmentation. Such methods will be used to explore monoaminergic transmission more widely in chapter 7.

Concluding remarks

We have seen how variation in the rate of progression and the pattern of abnormality can limit the potential of a single DaT SPECT scan, however accurately the abnormality can be quantified at that point in time. This is reflected in the fact that DAT SPECT has a lower predictive value for pheno-conversion than some simple clinic-based tests[68]. Serial imaging is therefore likely to provide a more accurate estimation of an individual's disease trajectory. Although data from a small number of cases has suggested that serial DAT SPECT imaging can indeed inform regarding risk stratification[175], practical considerations limit the widespread application of this: DaT SPECT imaging is costly, time-consuming, and involves radiation exposure. These limitations, combined with the lack of clinical predictors of dopaminergic decline in prodromal disease, indicate a need for alternative neuroimaging biomarkers. The development of MRI techniques that accurately quantify nigro-striatal degeneration would have the potential to overcome many of the limitations of SPECT. Two of the most promising MRI biomarkers will be explored in the following two chapters.

Chapter 5. Imaging the substantia nigra and locus coeruleus with neuromelanin-sensitive MRI

Introduction

Whilst dopamine transporter imaging can give an assessment of synaptic integrity at the nigro-striatal junction, it is to some extent an indirect measure of nigral degeneration. Imaging the progressive loss of cell bodies that occurs during prodromal disease in the substantia nigra (SN) itself would be an attractive alternative strategy. Although prodromal cell loss in the SN is extensive, it is by no means the earliest structure to be involved in disease. The characteristic caudo-rostral pattern of pathological progression seen in Parkinson's, and the particular susceptibility of neurons in the locus coeruleus (LC) to neurodegeneration, mean it is conceivable that changes in this nucleus may be detectible even earlier than those in the nigra[3,286]. The ability of MRI to quantify these structural changes has in the past been limited by inadequate resolution of these small nuclei and a lack of contrast with surrounding areas. However, sequences sensitive to neuromelanin at high field strength have the potential to overcome these.

In this chapter, we use neuromelanin-sensitive MRI (NM-MRI) to quantify the extent of brainstem depigmentation that occurs in patients with RBD, comparing this with PD patients and healthy controls. We look at how neuromelanin decline relates to the dopaminergic deficits measured in chapter 4 and consider its potential as a disease progression marker in prodromal patients.

Background

Neuromelanin is an insoluble pigment molecule that is abundant within neuronal cell bodies in both the SN and LC[287]. It first appears in early childhood and continues to accumulate linearly throughout adult life to the point where it takes up almost half of the cytosolic volume[288,289]. The structure and function of neuromelanin are incompletely understood, but it is known to have a strong affinity for iron, which is sequestered by neuromelanin in a redox

inactive form[290,291]. It also binds neurotoxins such as paraquat with high affinity[292]. It has been suggested, therefore, that the chelating effect of neuromelanin protects neurons from toxicity and oxidative stress[223].

In Parkinson's disease, dysregulation of this process may contribute to the selective vulnerability of the SN and LC. When cells in these nuclei die, neuromelanin is released and toxic compounds dissociate; activated microglia phagocytose the neuromelanin molecules leading to localised inflammation and oxidative stress[293,294]. The neurodegeneration that occurs as a consequence of this leads to a decline in the number of neuromelanin-containing neurons and a reduction in their pigmentation level (figure 1)[3,272], and it is this change that neuromelanin-sensitive MRI (NM-MRI) seeks to measure.

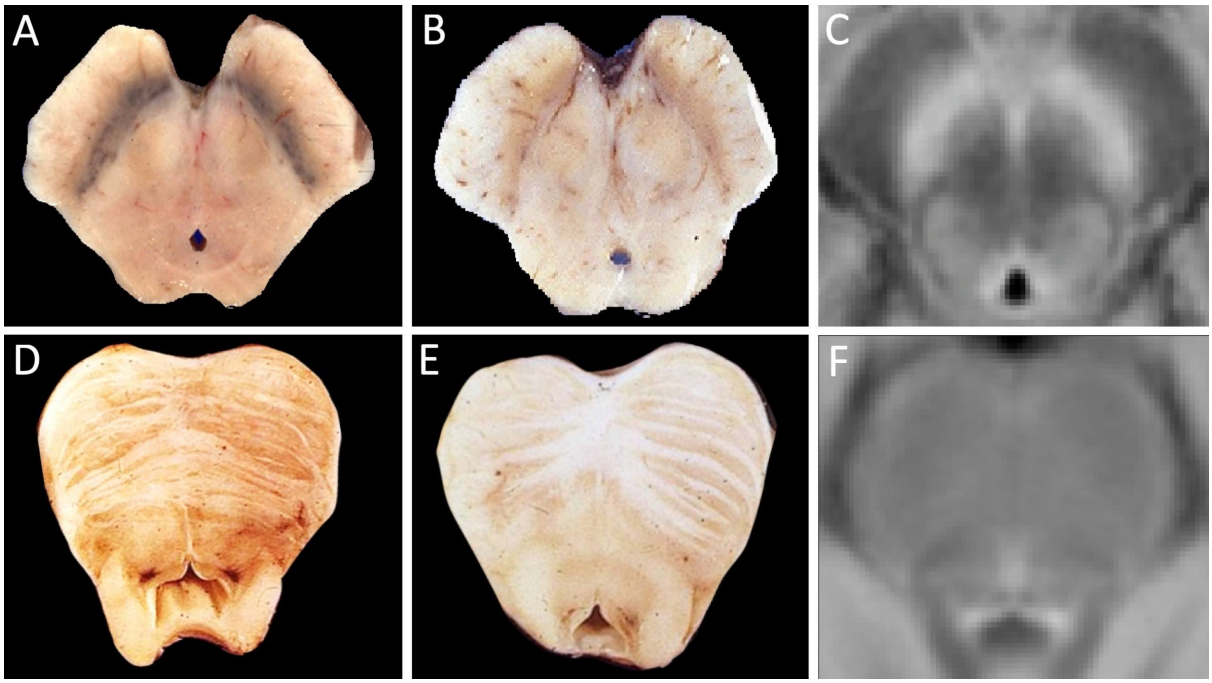


Figure 1. Post-mortem sections from a subject without Parkinson's disease showing neuromelanin pigment in the nigra (A) and locus coeruleus (D). Sections from a patient with Parkinson's show loss of pigmentation in these regions (B,E). Neuromelanin sensitive MRI demonstrates high signal intensity in regions corresponding to the nigral (C) and locus coeruleus (F) pigmentation.

The paramagnetic properties of the neuromelanin molecule make it detectible with MRI using modified T1 sequences[295–297]. Metal-bound neuromelanin causes shortening of T1 as well as

magnetisation transfer effects, which give areas containing neuromelanin contrast with surrounding areas[224]. By applying a specific magnetisation transfer pulse to a T1-weighted sequence, this contrast can be maximised so that neuromelanin-containing regions appear hyperintense against a low intensity background (Figure 1C and 1F)[295].

Approaches to imaging neuromelanin

A number of different quantification methods have been proposed to measure the integrity of neuromelanin-containing structures using this technique, but all are based on this contrast with the surrounding tissue[227,296,298–300]. Volume measurements can be made by isolating voxels in the region of interest that exceed a certain threshold above the mean background signal intensity (typically 3 or 4 standard deviations above) and multiplying by the voxel size[227,296,298]. Alternatively, signal intensity measurements can be made by measuring the signal level of a specific region of interest relative to the background signal[224,300].

Regions for the structures of interest and background areas can be defined by manual segmentation of individual subject images[298], but this approach is laborious and somewhat subjective. Automated segmentation and quantification methods using standardised regions of interest would therefore potentially have significant advantages that might facilitate reproducibility and generalisability.

Most studies of NM-MRI in established Parkinson's disease have focused on the substantia nigra rather than the locus coeruleus, because of its greater disease-specificity and its direct mechanistic link with the clinical motor syndrome. The presence of nigral NM-MRI abnormalities in Parkinson's patients has been widely replicated, and the demonstration of correlations with disease severity measures and functional dopaminergic decline suggest that it is a promising marker of disease progression[226,227]. A similar decline in nigral NM-MRI signal has also been reported in patients with MSA[301]. Only one published study has evaluated the technique in prodromal disease[228]. It should be interpreted with caution due to the small numbers of participants and the lack of a Parkinson's disease comparator group, but nevertheless differences between RBD patients and controls were demonstrated.

Although there are some variations in the methodology used in these studies to segment the substantia nigra, they have all examined the structure as a whole, calculating measures of global nigral integrity. We know from histopathological studies, however, that the nigra is not uniformly affected by neurodegeneration in parkinsonian disorders[272]. Rather, there is selective vulnerability of neurons in the lateral tiers. As discussed in chapter 4, prodromal imaging abnormalities are expected to be more subtle than those in established disease and therefore harder to detect. It is possible that by focusing analysis on the nigral subregion most vulnerable to early pathology, sensitivity to detect these borderline changes may be increased.

Significant signal reductions in the locus coeruleus in Parkinson's patients have also been demonstrated in several studies using NM-MRI, though the clinical implications of this are less clear[296,300]. One study has suggested that LC abnormalities may be a marker of RBD in PD patients[150], while another has demonstrated a link with autonomic disturbances[148]. In patients with idiopathic RBD, reductions in the NM-MRI LC signal have been shown to relate to the level of polysomnographic REM sleep without atonia, a marker of RBD severity[46]. These findings may imply that the observed changes in the LC are a general marker of medullo-pontine degeneration, acting as a surrogate for disease in the nearby nuclei that regulate REM sleep.

Summary and aims

This chapter represents the first study to examine NM-MRI of the SN and LC together in patients with RBD, and the first to compare NM-MRI in either structure across RBD patients, healthy control subjects and patients with established Parkinson's disease. With these comparisons we sought to shed light on the stage-dependent pattern of brainstem abnormalities in early Parkinson's disease. We hypothesised that NM-MRI measures in RBD patients would be at an intermediate level, between those of control and Parkinson's subjects, reflecting more limited degeneration in the LC and SN. We also hypothesised that due to the selective vulnerability of cells in the postero-lateral nigra, a focused analysis of NM-MRI signal in this region would be superior to whole-nigra measures in the detection of early degenerative changes.

We also sought to investigate the relationship between NM-MRI measures and the dopaminergic SPECT/CT findings described in chapter 4. We hypothesised that nigral NM-MRI measures in particular would be closely related to dopamine transporter levels in the basal ganglia, as a result of the anatomical connectivity between these two structures, and may therefore offer an alternative method of measuring nigro-striatal integrity in prodromal disease.

Methods

Subjects

Forty-four patients with RBD, 29 patients with Parkinson's Disease and 30 healthy control subjects were recruited from the Discovery Cohort of the Oxford Parkinson's Disease Centre, described in chapter 2 and elsewhere[277]. All participants underwent MRI, including the neuromelanin-sensitive sequence and structural T1-weighted imaging. 42 of the RBD patients were also scanned with ^{123}I-ioflupane (DaT) SPECT. Control and Parkinson's participants were not scanned with SPECT.

Image Acquisition

MRI scanning was performed at the Oxford Centre for Clinical Magnetic Resonance Research (OCMR) using a 3T Siemens Trio (Erlangen, Germany) with a 12-channel head coil. The neuromelanin-sensitive images were obtained using a modified T1-weighted fast-spin echo sequence with a magnetization transfer pulse (TE/TR = 17/1400 ms; 24 axial slices; in-plane resolution 0.78 × 0.78 mm^2; slice thickness = 2.4 mm (2 mm, distance factor 20%); 3 averages; 3:41 minutes). If needed, the number of slices was reduced to remain within the specific absorption rate (SAR) limits without altering other acquisition parameters. Axial slices were positioned perpendicular to the brainstem. High-resolution T1-weighted images were acquired (MPRAGE, TE/TR = 4.7 ms/2040 ms; 192 axial slices; resolution 1 × 1 × 1 mm^3; 6 minutes) and used for registration.

DAT SPECT/CT imaging was acquired and processed as described in Chapter 4.

MRI processing and registration

Quantitative measurements for SN and LC were derived from NM-MRI images with an automated approach using tools from the FMRIB Software Library (FSL)[278]. NM-MRI images were first corrected for bias field and registered to MNI standard space in a two-stage process: linear registration (FMRIB Linear Image Registration Tool, FLIRT) was used to align NM-MRI and T1-weighted images; linear and non-linear registration (FMRIB Non-linear Image Registration Tool, FNIRT) was used to register T1 images to MNI standard space, and the same transformation matrix was then applied to NM-MRI images to register these in MNI standard space. Corresponding inverse transformations were also calculated.

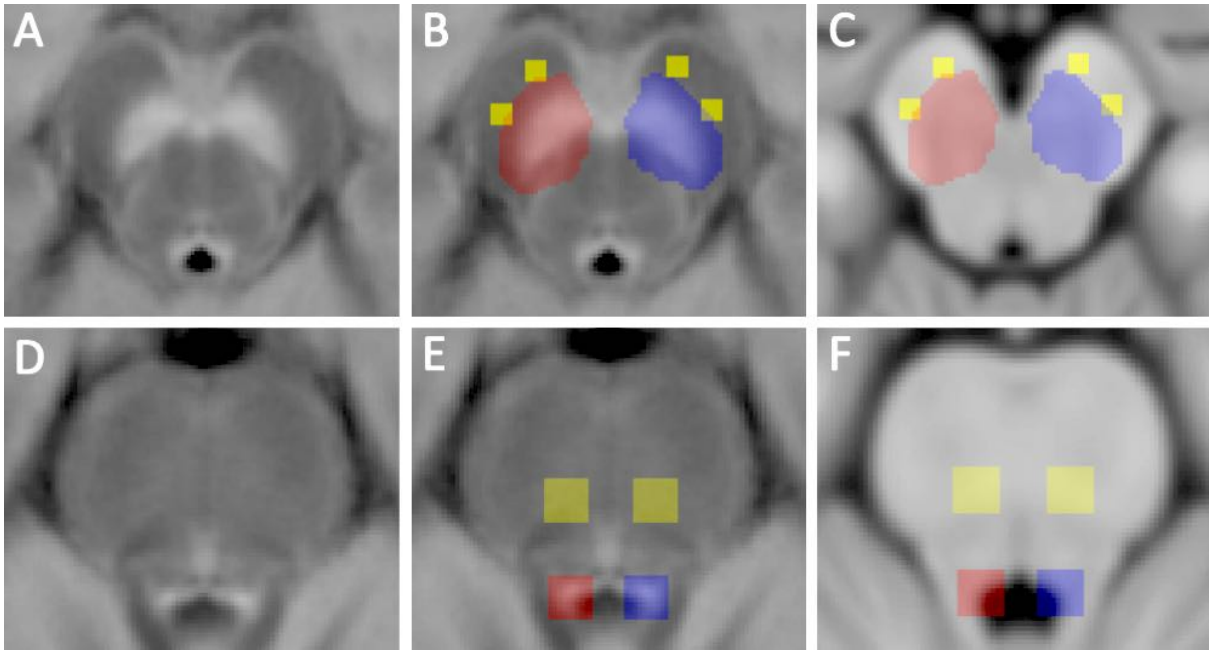


Figure 2. Standard space templates used to define regions of interest. A,D: Average neuromelanin images of 20 RBD patients and 20 controls at the level of the nigra (A) and locus coeruleus (D). B,C,E,F: Search regions for the nigra and locus coeruleus (right = red, left = blue) and reference regions (yellow) superimposed on the average neuromelanin images (B,E) and MNI standard T1 template (C,F).

Segmentation of regions of interest

Twenty RBD patients and twenty age-matched healthy controls were randomly selected to build a study-specific NM-MRI template in MNI standard space. This was used with the

standard T1 MNI brain template to define search and reference regions for the SN and LC (Figure 2).

Search regions were drawn to include the high signal voxels of the SN and LC respectively.

For the SN search region, the border was drawn manually in 0.5mm^3 standard space, on 20 consecutive axial slices (corresponding to a cranio-caudal length of 10mm). The antero-medial border was defined using the T1 template so as not to include any of the high signal voxels from outside the brainstem in this region (figure 2C). The remaining border within the brainstem parenchyma was drawn to include a margin of low intensity, non-nigral tissue so as to ensure complete coverage of the nigra (figure 2B). The LC search region was drawn as a series of 10x10 voxel (5x5mm) squares on 22 consecutive axial slices (11mm) covering the bright signal corresponding to the anatomical location of the LC (figure 2E-F).

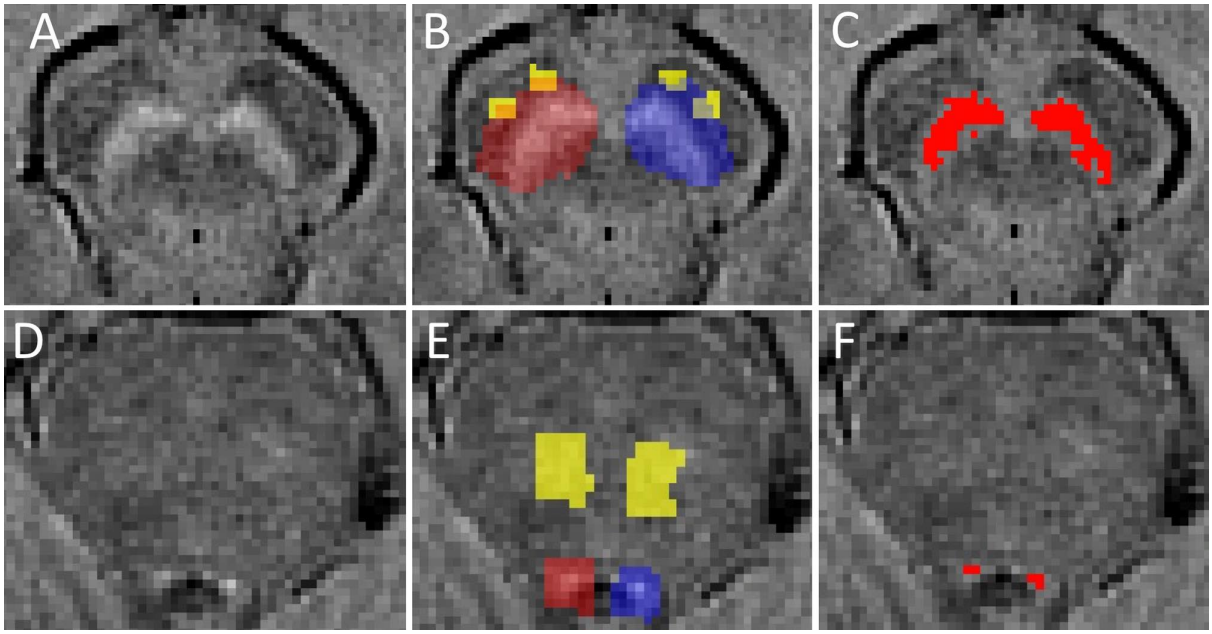


Figure 3. Example of an individual subject NM-MRI at the level of the nigra (A) and locus coeruleus (D). B,E: The search regions from figure 2 have been registered in the subject space images. C,F: Voxels exceeding the calculated threshold define the volume of the substantia nigra (C) and locus coeruleus (F).

The reference region for the substantia nigra was drawn as two 5x5 voxel squares on each side within the low intensity voxels of the cerebral peduncles on the same axial slices as the

SN search regions. Overlap with the margin of the search region was permitted as long as there was no overlap with the high signal voxels of the nigra itself. The reference region for the LC was drawn as two 10x10 voxel squares in the low intensity voxels of the pontine tegmentum on the same axial slices as the LC search regions.

Volume and within-volume measurements

For each subject, voxels representing the SN and LC were isolated using the following automated segmentation process. Firstly, the standard space search and reference ROIs defined above for the SN and LC were registered to the individual NM-MRI images using the inverse transformation matrix obtained from the registration described above (figure 3B, 3E). The mean and standard deviation (SD) of the signal intensity of the reference regions was then calculated. For the SN, a threshold of 3 SD above the reference mean was then applied to the voxels within search region. Voxels exceeding the threshold were isolated, and the largest contiguous cluster of these voxels on each side was taken as the SN (figure 3C).

A similar method was used for the LC, with two differences: 1) a higher threshold of 4 SD above the reference mean was used to isolate the LC voxels. 2) because the LC is smaller and more diffuse on NM-MRI than the SN, the requirement for a contiguous cluster was not applied, meaning any voxels within the search region that exceeded the threshold were included.

The thresholds of 3 SD and 4 SD for the SN and LC respectively have been shown to be the optimal thresholds for isolating the high signal voxels that correspond to these structures, and are the standard thresholds used for this methodology[227,296].

Using the voxels defined in single-subject space as SN or LC, the following measures were then derived:

- SN/LC volumes were calculated as the number of voxels in the SN/LC multiplied by the voxel size.

- SN/LC peak intensity was calculated as the maximum voxel intensity within the SN/LC volume (vol_{max}) normalised to the average intensity in the reference region (ref_{mean}): Peak = (vol_{max} / ref_{mean}) x 100

- SN/LC contrast ratio (CR) was used as a measure of the average intensity within the SN/LC voxels. This was calculated as the difference between the mean intensity of the SN/LC volume (vol_{mean}) and the mean intensity of the respective reference region ref_{mean}, normalised to the reference mean:

  $CR = (vol_{mean}\text{-}ref_{mean}) / ref_{mean}$

The calculation of these measures was performed in collaboration with Dr Ludovica Griffanti (Clinical Research Fellow, OPDC), who kindly provided a script combining the various registration and analysis steps performed.

The above measures were defined for left and right search regions separately. For each measure, the mean of left and right was calculated. As well as the mean value, the lower value of the two sides was also used in analysis to account for asymmetric disease onset.

An alternative approach: template-based intensity measures

The method described above has been widely used to characterise the substantia nigra in PD. By analysing only those voxels exceeding a certain signal threshold, the technique aims to isolate the remaining, NM-containing nigral neurons for analysis. Measuring the reduction in number of such voxels can therefore give an indication of the reduction in volume of NM-containing tissue. However, quantifying the signal intensity in these isolated voxels will only describe the integrity of these remaining neurons, whereas nigral regions below the threshold, where neurodegeneration may be at its most advanced, are excluded from analysis. Measuring signal intensity in this way may therefore be insensitive to early degenerative changes. An alternative would be to measure the signal intensity of the entire area where the 'normal' nigra should be.

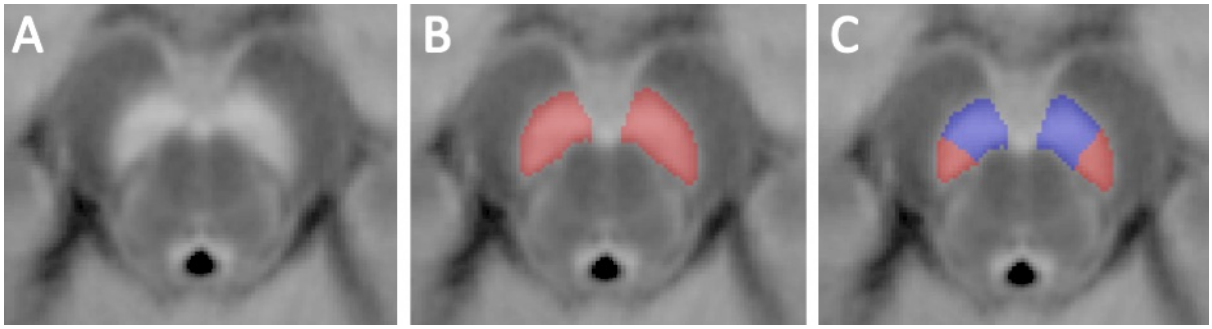


Figure 4. Group average NM-MRI image (A) with superimposed region of interest corresponding to the average location of hyperintense nigral voxels (B). The nigral ROIs were subdivided into anterior (blue) and posterior (red) regions (C).

To do this, we used our study-specific template (fig 4A) to define regions for the 'normal' substantia nigra. Firstly, the voxels within the SN search region (fig 2, red & blue regions) were isolated from this template. A threshold was then applied such that the surviving voxels best corresponded to the location of the bright SN voxels by visual assessment. These voxels were then binarized to create an average SN template in standard space (fig 4B).

The individual subject NM-MRI images registered to MNI standard space were then normalised to their background by dividing the whole image by the mean signal intensity from the SN reference region. The normalised standard space images were then merged into a single 4D file. The mean signal intensity within the region of the average SN template was then calculated using the *fslmeants* command.

One advantage of this method is that further subdivision of the regions of interest in standard space is straightforward, enabling us to focus our analysis on the nigral region most vulnerable to neurodegeneration. To that end, the average SN template was split into anterior and posterior divisions perpendicular to its long axis in axial sections (figure 4C). The mean signal intensity for these anterior and posterior SN sections was then calculated for each participant as above.

This average template method was used to define regions only for the SN, and not for the LC. This is because the LC is smaller and more diffuse, and therefore defining its normal average location on MRI is less certain. Furthermore, unlike the SN, where there is good evidence for regional differences in susceptibility to disease, we did not wish to subdivide the LC region.

DaT SPECT/CT analysis

Data from DaT SPECT/CT imaging was analysed as described in chapter 4. Dichotomous categories were defined using the descriptive reports. Signal intensity values were calculated using the SPECT-defined registration and ROI segmentation (Hermes).

Clinical Data

Assessments of clinical phenotypic characteristics were acquired as described in chapters 2 and 4. For analyses where the PD patients were split according to the presence or absence of RBD, the RBD screening questionnaire was used to identify RBD[47]. As is standard, a threshold of 6 was used to positively identify cases, higher than the threshold of 5 used in population screening, because all PD patients by definition score 1 point for question 13 (presence of a neurological disorder)[302].

Statistical Methods

Comparisons between groups of subjects for the NM-MRI variables described above were performed using linear regression. Age was included as a covariate because the three groups were not perfectly age-matched and the level of neuromelanin in the brainstem is known to vary with age[223]. To compare which variables best separated two groups of patients, a receiver operator characteristic (ROC) analysis was used. Comparisons between the sides ipsilateral and contralateral to the most clinically affected side in PD patients were made using a paired samples t-test. Correlations between NM-MRI variables and DaT SPECT/CT measures, and between imaging variables and clinical scores, were calculated using linear regression, with age included as a covariate, and expressed as standardised coefficients. P-values are given unadjusted for multiple comparisons. All statistical analyses were performed using SPSS (IBM).

Results

Baseline characteristics and effect of age

The baseline characteristics of the participants included in the study are shown in table 1. The three groups were incompletely age- and sex-matched. Patients with RBD were significantly younger than control participants (t-test, p=0.02), but there were no significant differences in age between controls and PD, nor between RBD and PD participants.

	Controls	RBD	PD
Number of cases	30	44	29
Age (years)	69.4 (6.98)	65.1 (7.64)	66.1 (8.40)
Gender (% male)	83	98	59
Years since diagnosis		2.56 (2.33)	5.2 (2.19)
UPDRS III	2.6 (2.09)	5.3 (3.41)	33.5 (13.17)

Table 1. Baseline characteristics of the NM-MRI study participants.

We first sought to investigate the relationship between age and our neuromelanin MRI measures. Correlation coefficients are shown in table 2. The most pertinent results are those of control participants, where the relationship between age and brain neuromelanin content is not confounded by neurodegeneration. There were no significant correlations between any substantia nigra NM-MRI measure and age in any of the participant groups, nor across all subjects. In the locus coeruleus there were consistently negative correlations between age and NM-MRI measures. In control participants this reached significance only for the LC contrast ratio, but a borderline result was seen for LC volume. Across all patients there were significant negative correlations between age and LC volume, and between age and peak LC signal intensity. The latter correlation was also significant in RBD patients and of borderline significance in PD patients. Although these results should be interpreted with caution due to the borderline significance levels (none of these findings survive correction for multiple

comparisons), the trend is in keeping with histological studies showing that neuromelanin levels in the LC decline after the age of 50 in healthy individuals, whereas levels in the SN do not[303,304].

NM-MRI Measure (mean of left & right)	Correlation with age			
	Across all patients	Controls	RBD	PD
SN volume	r = -0.78 p = 0.44	r = 0.03 p = 0.88	r = -0.17 p = 0.27	r = -0.20 p = 0.30
SN peak intensity (volume)	r = -0.11 p = 0.28	r = -0.28 p = 0.14	r = -0.03 p = 0.83	r = -0.15 p = 0.42
SN contrast ratio (volume)	r = 0.03 p = 0.73	r = -0.22 p = 0.25	r = 0.06 p = 0.73	r = -0.20 p = 0.31
SN mean intensity (template)	r = 0.00 p = 0.98	r = -0.07 p = 0.73	r = -0.03 p = 0.83	r = -0.06 p = 0.76
Anterior SN mean intensity (template)	r = -0.01 p = 0.93	r = -0.05 p = 0.81	r = -0.01 p = 0.96	r = -0.10 p = 0.60
Posterior SN mean intensity (template)	r = 0.01 p = 0.90	r = -0.08 p = 0.69	r = -0.07 p = 0.64	r = 0.02 p = 0.92
LC volume	**r = -0.22** **p = 0.026**	r = -0.35 p = 0.06	r = -0.28 p = 0.07	r = -0.22 p = 0.25
LC peak intensity	**r = -0.29** **p = 0.003**	r = -0.24 p = 0.21	**r = -0.41** **p = 0.006**	r = -0.36 p = 0.06
LC contrast ratio	r = -0.18 p = 0.06	**r = -0.36** **p = 0.048**	r = -0.20 p = 0.19	r = -0.14 p = 0.47

Table 2. Correlations of NM-MRI variables with age

Group differences in NM-MRI measures: substantia nigra

The average group values for the various NM-MRI measures are shown in table 3. Parkinson's disease patients had significantly reduced SN volumes compared to control participants. Amongst intensity measures within the SN volume (table 3a), only the peak intensity on the lower side was significantly reduced in PD patients compared to controls. Intensity measures

Table 3a. Measures based on SN volumes, group mean (SD)					
	Controls	RBD	PD		P value
SN volume, mean L-R	285.0 (51.88)	253.2 (59.98)	249.2 (63.74)	Control vs PD: Control vs RBD: PD vs RBD:	**0.016** **0.015** 0.86
SN volume, lower side	274.0 (53.86)	243.1 (60.29)	232.9 (63.95)	Control vs PD: Control vs RBD: PD vs RBD:	**0.006** **0.018** 0.56
SN peak intensity, mean	146.7 (4.65)	146.7 (5.29)	145.1 (2.89)	Control vs PD: Control vs RBD: PD vs RBD:	0.07 0.78 0.16
SN peak intensity, lower	145.0 (4.16)	144.8 (4.87)	142.9 (2.71)	Control vs PD: Control vs RBD: PD vs RBD:	**0.018** 0.70 0.06
SN contrast ratio, mean	0.255 (0.017)	0.257 (0.023)	0.248 (0.016)	Control vs PD: Control vs RBD: PD vs RBD:	0.10 0.83 0.06
SN contrast ratio, lower	0.252 (0.017)	0.254 (0.022)	0.245 (0.016)	Control vs PD: Control vs RBD: PD vs RBD:	0.11 0.78 0.054
Table 3b. Measures based on SN average template region, group mean (SD)					
	Controls	RBD	PD		P value
SN intensity, mean	1.181 (0.020)	1.173 (0.023)	1.168 (0.019)	Control vs PD: Control vs RBD: PD vs RBD:	**0.008** 0.10 0.31
Anterior SN intensity, mean	1.188 (0.023)	1.181 (0.025)	1.178 (0.020)	Control vs PD: Control vs RBD: PD vs RBD:	0.07 0.20 0.69
Anterior SN intensity, lower	1.177 (0.023)	1.176 (0.026)	1.177 (0.022)	Control vs PD: Control vs RBD: PD vs RBD:	0.80 0.85 0.83
Posterior SN intensity, mean	1.172 (0.022)	1.162 (0.023)	1.152 (0.023)	Control vs PD: Control vs RBD: PD vs RBD:	**0.001** **0.047** 0.07
Posterior SN intensity, lower	1.165 (0.022)	1.154 (0.024)	1.145 (0.023)	Control vs PD: Control vs RBD: PD vs RBD:	**0.002** **0.047** 0.10

Table 3. Group differences in NM-MRI measures of the substantia nigra

in the average SN template (table 3b) showed greater relative reductions in PD patients. When the SN template region was divided into anterior and posterior segments, only the signal intensity in the posterior region was significantly reduced.

RBD patients also had significant reductions in SN volumes compared to control participants (figure 5A). Amongst intensity measures, only the posterior nigral template region showed

significant a significant reduction in intensity in RBD patients (figure 5B). There were no significant differences between PD and RBD patients in any of the SN neuromelanin measures.

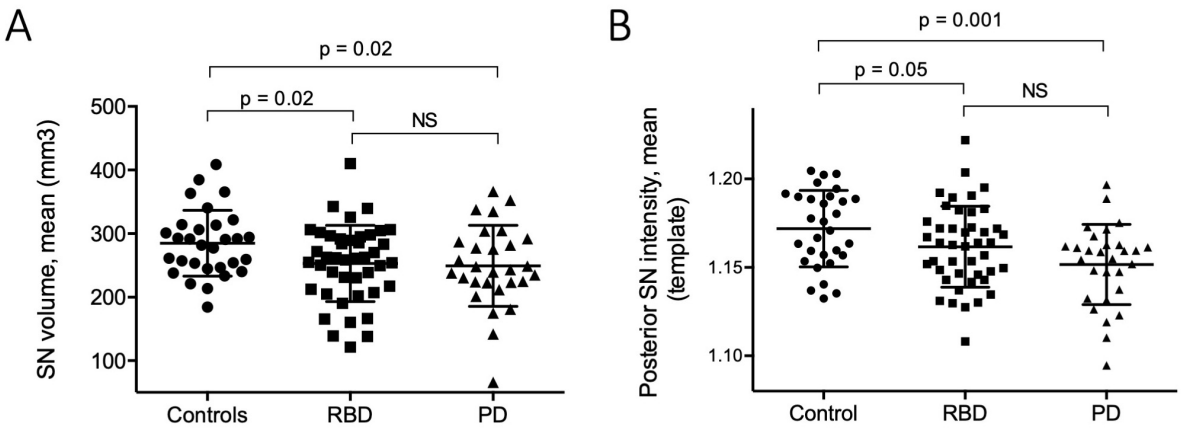


Figure 5. NM-MRI measurements of SN volume (A) and posterior SN signal intensity (B)

	Controls	RBD	PD		P value
LC volume, mean L-R	13.4 (6.01)	10.4 (7.31)	11.1 (7.28)	Control vs PD: Control vs RBD: PD vs RBD:	0.09 **0.012** 0.61
LC volume, lower side	8.9 (5.58)	6.3 (5.51)	7.3 (5.99)	Control vs PD: Control vs RBD: PD vs RBD:	0.18 **0.008** 0.40
LC peak intensity, mean	139.3 (4.07)	138.3 (15.20)	136.3 (4.68)	Control vs PD: Control vs RBD: PD vs RBD:	**0.003** 0.23 0.58
LC peak intensity, lower	135.9 (4.37)	133.7 (7.21)	133.0 (4.15)	Control vs PD: Control vs RBD: PD vs RBD:	**0.003** **0.038** 0.73
LC contrast ratio, mean	0.310 (0.036)	0.306 (0.065)	0.297 (0.041)	Control vs PD: Control vs RBD: PD vs RBD:	0.10 0.36 0.56
LC contrast ratio, lower	0.293 (0.060)	0.277 (0.092)	0.276 (0.079)	Control vs PD: Control vs RBD: PD vs RBD:	0.21 0.26 0.98

Table 4. Group differences in NM-MRI measures of the locus coeruleus

Group differences in NM-MRI measures: locus coeruleus

The pattern of results with respect to the locus coeruleus was different from that in the SN (table 4). Significant reductions in LC volume compared to control values were seen in RBD patients, but not in PD patients. Conversely, the average peak intensity of the LC was reduced in PD patients but not RBD patients, though the reduction in RBD patients was significant when looking at the lower of the two sides. Again, there were no significant differences between RBD and PD patients in any of the LC neuromelanin measures.

Table 5a. SN volume-based measures				
	AUC PD vs Controls	p value	AUC RBD vs Controls	p value
SN volume, mean L-R	0.677	**0.02**	0.622	0.08
SN volume, lower side	0.689	**0.013**	0.627	0.07
SN peak intensity, mean	0.609	0.15	0.477	0.74
SN peak intensity, lower	0.666	**0.03**	0.494	0.94
SN contrast ratio, mean	0.652	**0.045**	0.512	0.86
SN contrast ratio, lower	0.643	0.06	0.509	0.90
Table 5b. SN template-based measures				
	AUC PD vs Controls	p value	AUC RBD vs Controls	p value
SN intensity, mean	0.713	**0.005**	0.623	0.08
Anterior SN intensity, mean	0.669	**0.026**	0.621	0.08
Anterior SN intensity, lower	0.515	0.844	0.495	0.95
Posterior SN intensity, mean	0.730	**0.002**	0.618	0.09
Posterior SN intensity, lower	0.734	**0.002**	0.619	0.09
Table 5c. LC volume-based measures				
	AUC PD vs Controls	p value	AUC RBD vs Controls	p value
LC volume, mean L-R	0.621	0.11	0.645	**0.037**
LC volume, lower side	0.594	0.22	0.659	**0.022**
LC peak intensity, mean	0.694	**0.01**	0.694	**0.005**
LC peak intensity, lower	0.702	**0.008**	0.679	**0.010**
LC contrast ratio, mean	0.595	0.21	0.548	0.486
LC contrast ratio, lower	0.600	0.19	0.558	0.404

Table 5. Discrimination accuracy of NM-MRI measures

Discrimination accuracy

An alternative way of considering the group differences in NM-MRI measures between groups is to look at the discrimination accuracy. Values for the area under the curve (AUC) are shown in table 5. Because no significant group differences were found between PD and RBD patients, the test was only applied to the control-RBD and control-PD comparisons respectively.

The measure that best discriminated PD patients from controls was the signal intensity in the posterior SN template region. None of the SN measures separated RBD patients from controls with better than chance accuracy.

In the locus coeruleus, only LC peak intensity measures separated PD patients from controls, whereas both LC volume and LC peak intensity measures showed significant discrimination between RBD patients and controls. The most accurate separation was seen with LC peak intensity measures.

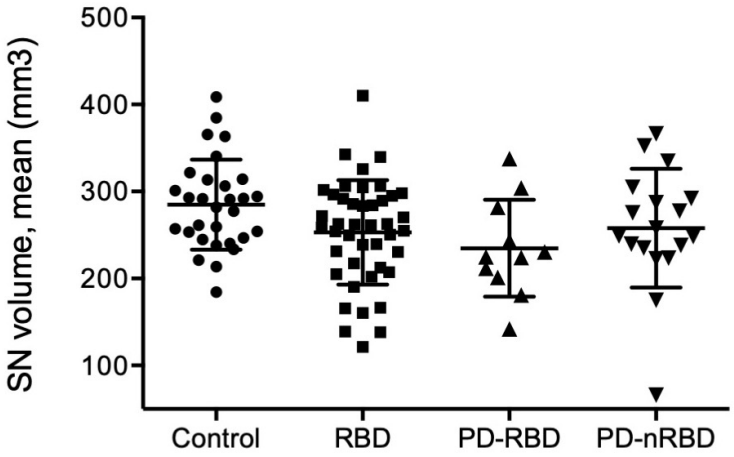


Figure 6. SN volumes across groups, with PD patients split according to the presence or absence of RBD

Effect of RBD within PD patients

There is evidence from dopamine transporter imaging that PD patients with concomitant RBD have a more severe neuroimaging phenotype than PD patients without[176]. This may have an effect on the comparison between PD patients as a whole and idiopathic RBD patients, since RBD patients who convert will progress to the more severe PD-RBD category. For this reason,

we conducted a post-hoc analysis, splitting the PD group into those with and without concomitant RBD (based on the RBD screening questionnaire). The number of patients in each group was low (PD-RBD, n=11; PD-nonRBD, n=18) making statistical analysis underpowered. As such, there was no difference in any of the NM-MRI measures between PD-RBD and PD-nonRBD patients. However, in the SN volume measurements (figure 6) there was a trend towards lower values in PD-RBD patients.

	Contralateral to most affected side, mean (SD)	Ipsilateral to most affected side, mean (SD)	p value
SN volume	240.1 (65.27)	267.8 (64.97)	**<0.001**
SN peak intensity (volume)	144.2 (3.59)	146.3 (4.47)	0.07
SN contrast ratio (volume)	0.245 (0.0174)	0.250 (0.0172)	**0.004**
Anterior SN mean intensity (template)	1.180 (0.0242)	1.184 (0.0224)	0.052
Posterior SN mean intensity (template)	1.146 (0.0222)	1.159 (0.0242)	**<0.001**
LC volume	11.6 (8.77)	11.7 (9.01)	0.95
LC peak intensity	137.1 (6.36)	135.7 (5.76)	0.36
LC contrast ratio	0.298 (0.0663)	0.292 (0.0641)	0.74

Table 6. Comparison of NM-MRI measures between sides contralateral and ipsilateral to most clinically affected side in PD patients

Relationship between NM-MRI and clinical disease severity measures

In PD patients, an internal disease severity comparison can be made due to the asymmetric nature of disease, since degeneration will be more advanced in the nigra contralateral to the most clinically affected side of the body. Table 6 shows the comparison of NM-MRI measures between the contralateral and ipsilateral hemispheres in the PD patient group (three PD patients in whom disease onset was reported as not clearly asymmetric are excluded from this analysis).

SN volume, contrast ratio within the SN volume, and signal intensity within the posterior SN were all significantly reduced in the contralateral nigra compared to ipsilateral. The difference

with respect to the SN peak intensity and anterior SN intensity were of borderline significance. None of the LC measures were different between contralateral and ipsilateral LC.

NM-MRI Measure (mean of left & right)	Correlation with UPDRS III		
	Controls	RBD	PD
SN volume	β = -0.19 p = 0.31	β = -0.06 p = 0.72	β = -0.26 p = 0.20
SN peak intensity (volume)	β = -0.15 p = 0.42	β = -0.13 p = 0.46	β = 0.26 p = 0.19
SN contrast ratio (volume)	β = -0.10 p = 0.61	β = 0.00 p = 1.00	β = 0.18 p = 0.39
SN mean intensity (template)	β = -0.15 p = 0.45	β = -0.03 p = 0.86	β = -0.25 p = 0.23
Anterior SN mean intensity (template)	β = -0.11 p = 0.59	β = -0.02 p = 0.93	β = -0.15 p = 0.47
Posterior SN mean intensity (template)	β = -0.19 p = 0.35	β = -0.05 p = 0.77	β = -0.33 p = 0.11
LC volume	β = -0.29 p = 0.11	β = -0.20 p = 0.25	**β = -0.39** **p = 0.049**
LC peak intensity	β = 0.25 p = 0.19	β = -0.01 p = 0.95	**β = -0.41** **p = 0.026**
LC contrast ratio	**β = 0.48** **p = 0.006**	β = -0.12 p = 0.50	**β = -0.39** **p = 0.048**

Table 7. Correlations (calculated as regression coefficients) between NM-MRI measures and clinical motor parkinsonism, measured by UPDRS III

Table 7 shows the correlations between motor disease severity (UPDRS III) and NM-MRI measures. The results with respect to PD patients are the most meaningful, since control and RBD participants by definition do not have significant motor disease. Interestingly, none of the SN measures correlated with motor severity, whereas significant negative correlations were seen between UPDRS III scores and all three of the LC measures, suggesting that reductions in neuromelanin level in the LC are associated with increasing motor severity.

As we have noted in previous chapters, disease severity measures or markers of prodromal stage are lacking in RBD patients. We did, however, investigate the correlation between NM-

MRI measures and a number of clinical prodromal signs. No significant relationships were observed between any NM-MRI measure and the following: hyposmia (Sniffin sticks score), quantitative motor impairment (Purdue Pegboard score), cognition (MoCA score), orthostatic hypotension (postural systolic or diastolic blood pressure drop), depression (BDI score), anxiety (HADS anxiety score), composite prodromal risk score (MDS probability of prodromal PD).

NM-MRI Measure (mean of left & right)	Correlation with DaT SPECT/CT	
	Whole striatum	Putamen
SN volume	β = 0.14 p = 0.42	β = 0.18 p = 0.28
SN peak intensity (volume)	β = 0.33 p = 0.053	β = 0.26 p = 0.13
SN contrast ratio (volume)	β = 0.22 p = 0.22	β = 0.13 p = 0.46
SN mean intensity (template)	β = 0.20 p = 0.24	β = 0.18 p = 0.29
Anterior SN mean intensity (template)	β = 0.08 p = 0.64	β = 0.09 p = 0.61
Posterior SN mean intensity (template)	**β = 0.37** **p = 0.025**	β = 0.31 p = 0.06
LC volume	β = 0.08 p = 0.64	β = 0.14 p = 0.40
LC peak intensity	β = 0.18 p = 0.26	β = 0.16 p = 0.29
LC contrast ratio	β = 0.16 p = 0.33	β = 0.17 p = 0.30

Table 8. Correlations between NM-MRI measures and DaT SPECT/CT uptake ratios in the whole striatum and putamen, respectively, in RBD patients.

Relationship between NM-MRI and DaT SPECT/CT measures in RBD patients

The lack of a reliable clinical measure of prodromal disease stage makes the comparison between NM-MRI measures and DaT SPECT/CT imaging particularly relevant. Correlations between measures from these two modalities are shown in table 8, using SPECT/CT values from the whole striatum and putamen, respectively. The only significant correlation was

between NM-MRI signal intensity in the posterior SN and DaT SPECT/CT signal across the whole striatum. There was also a borderline significant correlation between striatal DaT SPECT/CT values and peak signal intensity in the SN. However, these findings should be interpreted with caution due to the multiple comparisons performed.

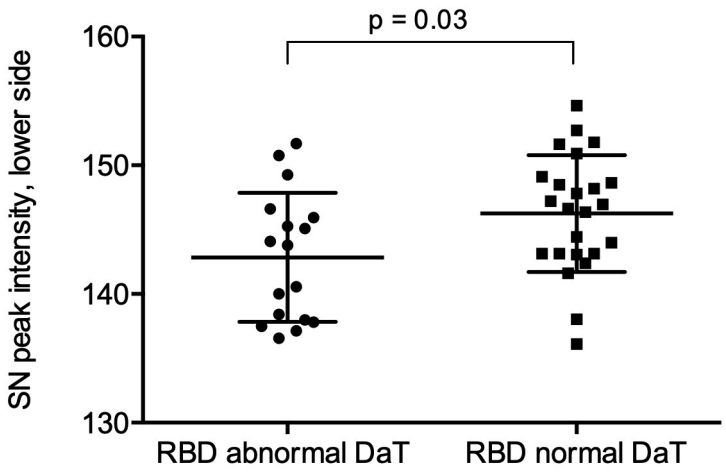


Figure 7. The only significant difference in NM-MRI measures between RBD patients with normal versus abnormal DaT SPECT/CT outcomes was SN peak intensity on the lower side.

Finally, we looked at differences in NM-MRI measures between RBD patients with normal versus abnormal DaT SPECT/CT outcomes according to the clinical radiologist's descriptive report. The only measure that differed between these groups was the peak SN intensity on the lower side, which was significantly lower in RBD patients with abnormal DaT SPECT/CT imaging (figure 7).

Discussion

SN Volume measurements

In the first study to compare NM-MRI across RBD, PD and control subjects, we found significant SN volume reductions in both RBD and PD patients compared to healthy controls. Rather than showing an intermediate imaging phenotype, as might be expected in prodromal disease, the extent of volume loss in RBD was comparable to that seen in established disease. This is a recurring pattern in RBD studies: we have already seen in chapter 2 that across a range of clinical measures RBD patients are at least as impaired as those with PD, and a similar pattern was also observed in another recent imaging study measuring functional connectivity in the basal ganglia[263].

One explanation for this pattern would be a floor effect in the degree to which nigral decline can be quantified. This would be supported by a study showing substantial overlap in nigral NM-MRI measures between patients with de novo PD and those imaged 2-5 years after diagnosis[305]. However, it would also be in keeping with the presence of a more severe neurodegenerative subtype of parkinsonism in patients who progress from idiopathic RBD. This pattern is observed in dopamine transporter imaging, where PD patients with concomitant RBD have more severe dopaminergic deficits[176]. It is also suggested to some extent by the trend seen in our data towards lower SN volumes in PD patients with concomitant RBD than in those without.

The accuracy of SN volume measurements in discriminating PD patients from controls was lower than that observed in other studies[225,298]. This may in part be due to differences in the MRI sequence or to the simpler, automated segmentation methodology that we have used. It may also be due to differences in the clinical characteristic of the participants in our study. However, whilst the AUC values are not high enough for this to be a useful diagnostic test on its own, we have shown that NM-MRI volume reductions in the SN are reproducible using an automated quantification method.

SN signal intensity measurements

In our analysis, signal intensity values from the average SN template regions generally performed better than those from the extracted SN volumes in separating PD patients from controls. It is possible that these measures inform regarding different aspects of the degenerative process, with template-based measures more indicative of early degeneration, and volume-based measures giving a more focussed assessment of the remaining nigral neurons. It is clear from our data, however, that focusing on the posterior segment of the nigra does increase the sensitivity to detect disease-related changes. Not only was this the measure that best separated PD patients from controls, but it was also the only intensity measure that was significantly reduced in RBD patients. The posterior nigral intensity measure also showed more of a stepwise decline across the three groups than the SN volume measure, with a greater difference between RBD and PD patients. It is possible, therefore, that there is less of a floor effect with this intensity metric.

LC volume measurements

Interestingly, NM volumes in the locus coeruleus were only reduced compared to controls in the RBD group, and not in PD patients. This may indicate that depigmentation in this region is more closely related to the presence of RBD itself than to disease stage. There is anatomical plausibility to this, since the LC is adjacent to the subcoeruleus complex, which is involved in the regulation of REM sleep and strongly implicated in the pathophysiology of RBD[43]. It is also consistent with two recent studies of NM-MRI in the locus coeruleus: one showing that the signal is lower in PD patients with concomitant RBD than in those without, and another in RBD patients demonstrating a relationship between the NM signal intensity and the severity of REM sleep without atonia[46,150].

Relationship between NM-MRI and clinical measures

We observed somewhat conflicting patterns from the two methods used to assess the relationship of NM-MRI measures to disease severity in PD patients. When comparing the nigra contralateral and ipsilateral to the most affected side, nigral measures were lower in the contralateral side, an expected finding given the role of the SN in motor disease. Scores of motor severity using UPDRS III, however, showed significant correlations with locus

coeruleus measures but not with the SN. The LC is not directly implicated in the pathophysiology of motor parkinsonism, but one possible explanation may again be the confounding effect of RBD itself. PD patients who progress from idiopathic RBD tend to exhibit a more severe motor phenotype than those without RBD[33,84], and it may be that the presence of RBD in these patients explains the reduced LC signal alongside more severe motor disease. Consistent with this hypothesis, PD patients in our study with concomitant RBD had a higher average motor scores than those without, though this did not reach significance, perhaps due to the low numbers in each group (mean UPDRS III, 38 vs 31, p = 0.18).

Relationship between NM-MRI and DaT SPECT/CT

Although correlation analyses between the two imaging modalities were generally negative, it is encouraging that the one MRI measure that did correlate with dopaminergic SPECT/CT signal – the posterior nigral NM signal intensity – was also the measure that best separated PD patients from controls. The consistency of these findings suggests that this is likely to be the NM-MRI measure that best represents early nigrostriatal degeneration.

The relatively poor agreement between MRI and SPECT/CT does not necessarily indicate that MRI is inferior, as we have already seen that SPECT/CT itself is far from perfect as a marker of prodromal staging[169]. Furthermore, these distinct imaging modalities may describe different aspects of the degenerative process. Histological evidence shows that loss of tyrosine hydroxylase staining (and by implication dopaminergic function) in the nigra is more advanced than loss of neuromelanin pigmented cell bodies at all stages of PD[306]. This may mean that NM-MRI reductions are a later prodromal sign than DaT SPECT/CT and therefore a better predictor of near-term conversion. Longitudinal follow-up of our cohort is required to answer this, but even if NM-MRI does not outperform DaT SPECT/CT, there remains the possibility that a combination of the two imaging modalities might be more informative than either in isolation.

A note on multiple comparisons

The exploratory nature of the study described in this chapter means that a large number of different variables were calculated to assess the neuromelanin contrast of the SN and LC.

Although certain approaches, such as analysis of the posterior nigra, were hypothesis driven, others were designed to evaluate the various quantification metrics that have been published by other groups to determine which related best to clinical characteristics. We did not correct for multiple comparisons as this was felt to be overly conservative in this context, and therefore the borderline-significant findings presented should be interpreted with caution due to the possibility of type 1 error.

Concluding remarks

This study provides convincing evidence that neuromelanin-sensitive MRI can detect early degenerative changes in the substantia nigra and locus coeruleus that emerge during prodromal disease. These abnormalities appear to be most pronounced when analysis is focused on the sub-region of the substantia nigra most susceptible to disease.

A key question to be addressed in future studies is the extent to which change over time can be reliably quantified using NM-MRI, and whether the rate of change in serial imaging is superior to a single test in terms of its prognostic value. The non-invasive nature of MRI and the ease with which NM imaging can be performed are major advantages that will facilitate this longitudinal imaging. Ultimately, long-term outcome data from the RBD patients in this study will be required to fully appraise the various measures investigated.

Chapter 6: Susceptibility-Weighted MRI of the Substantia Nigra in RBD

Introduction

The data presented in chapter 5 demonstrate how an MRI sequence sensitive to neuromelanin allows imaging to be targeted at the pars compacta of the substantia nigra, the region most affected by alpha-synuclein disease. In the following chapter, we explore how certain aspects of nigral anatomy and constitution can be resolved yet further using susceptibility-weighted imaging (SWI), an alternative MRI sequence that has emerged in the last few years as an important tool in PD biomarker development.

Background

Within the substantia nigra pars compacta (SNc) dopaminergic neurons are clustered into five distinct subgroups, which are known as nigrosomes 1-5[307]. Histological analysis of the nigra in cases of PD indicate that degeneration in these nigrosomes is more pronounced than in other nigral areas, making them potentially more sensitive markers of the disease[218]. Amongst the five nigrosomes, the largest and most vulnerable is nigrosome 1, situated in the dorso-lateral nigra[308]. This structure in particular has been a target of SWI.

Susceptibility weighted imaging is an MRI modality based on T2*-weighted gradient-echo sequences[309]. SWI is derived by multiplying the magnitude and phase images together to produce images with contrast sensitivity to compounds such as blood, iron and calcium. It is the contrast due to iron that makes SWI of particular interest in visualising nigrosomes and the changes that occur in them due to parkinsonian neurodegeneration.

In healthy individuals, nigrosome-1 appears on SWI as an ovoid region of high signal intensity against a hypointense background (figure 1A-B)[222]. This surrounding low signal is presumed to be due to iron deposition in the substantia nigra, which accumulates in healthy ageing but

is more pronounced in PD[218]. The reason for the high SWI signal in nigrosome 1 has not been fully determined, but it may be that the redox-inactive iron sequestered by neuromelanin in the nigrosomes exhibits a different MRI susceptibility compared to unbound ferric iron in the surrounding tissues[310].

Crucially for biomarker discovery, this area of high signal intensity representing nigrosome 1 is lost in patients with Parkinson's disease[217,219,311]. Again, the mechanism of this has not been fully elucidated, but is likely to relate to the loss of nigrosomal neuromelanin-containing cells and with them their iron-sequestering capacity, leading to an increase in unbound iron deposition that renders the SWI signal hypointense and therefore indistinguishable from the surrounding region (figure 1C-D).

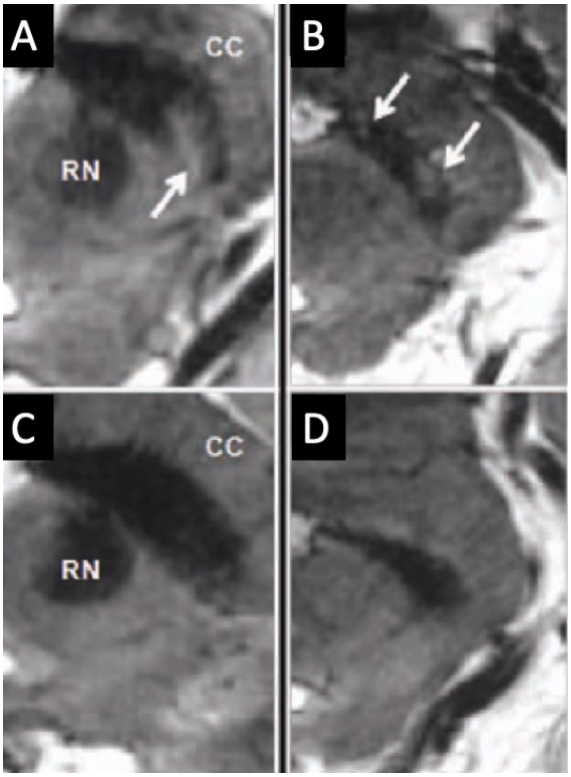


Figure 1. SWI images obtained at 7-Tesla field strength illustrating the normal appearance of nigrosome 1 (A-B, white arrows) and its absence in PD (C-D). CC, cerebral crus; RN red nucleus. Reproduced with permission[311]

Existing data

Initial studies of nigrosome imaging were performed using 7-Tesla MRI, and at this field strength it is possible to resolve all 5 nigrosomes, albeit with varying degrees of precision[307]. Nigrosome 1, however, can also be visualised at 3-Tesla, which has enabled more widespread investigation of the technique. A meta-analysis of these 3T studies has demonstrated that the absence of the nigrosome 1 signal, termed the dorsal nigral hyperintensity (DNH), can distinguish PD patients from controls with 94.6% sensitivity and 94.4% specificity[114]. Preliminary investigations of the technique in limited numbers of RBD patients suggest that the DNH is absent in a smaller proportion of patients than is the case in PD, suggesting a potential role in the characterisation of disease progression[220–222].

Most studies examining SWI of nigrosome-1 have used subjective, binary visual ratings to measure the presence or absence of the DNH[217]. Whilst there are some advantages to a method that straightforwardly dichotomises individuals into normal or abnormal, these subjective ratings are potentially subject to bias, and a binary measure is limited in its sensitivity to change over time. Furthermore, it could be problematic in borderline or uncertain cases, which are likely to be common in prodromal disease. The value of SWI as a prodromal progression marker could therefore potentially be enhanced by an objective method of quantifying the DNH signal which reflected the gradually progressive degeneration that occurs in the substantia nigra. This has been attempted using 7-Tesla MRI in PD patients, and the intensity of the nigrosome-1 region was observed to correlate with motor severity[307]. However, this study involved manual outlining of the nigrosome-1 signal on each individual patient's images, which is time-consuming and prone to bias when compared to automated segmentation.

SWI beyond nigrosome-1

As well as focusing on the nigrosomes themselves, it is interesting to consider the different characteristics that are described by neuromelanin-sensitive and susceptibility-weighted MRI in the nigral region as a whole. These can be illustrated by the average images shown in figure 2. It can be seen that bright NM-MRI signal gives an approximation of the structure of the NM-containing SNc, and can therefore quantify the extent to which this remains intact in PD.

The low SWI signal, on the other hand, extends well beyond the anatomical boundary of the SNc into the pars reticularis and even into the white matter of the cerebral peduncles. Rather than indicate structural aberrations, this may instead give an indication of the progressive iron deposition that occurs as degeneration proceeds. These explanations are as yet unproven, but the different patterns described by the two modalities suggest that they convey distinct aspects of the disease process, raising the possibility that the combination of the two sequences may be more informative than either alone when evaluating the nigra.

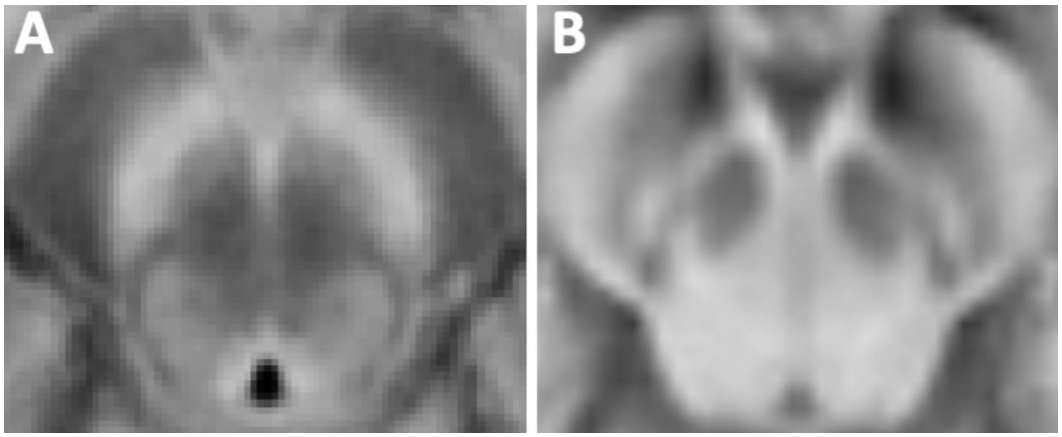


Figure 2. Template images created from healthy control subjects using neuromelanin-sensitive MRI (A) and susceptibility-weighted imaging (B)

The different characteristics of the two modalities can also potentially be exploited for methodological purposes, taking advantage of the structural information provided by NM-MRI to refine the analysis of SWI[312]. To this end, we proposed using NM-MRI to define the anatomical boundaries of the normal SNc in our SWI images, enabling us to explore SWI changes not just in nigrosome-1 but throughout the SNc. Because this method uses the same average NM-MRI templates used in chapter 5 to quantify individuals' nigral NM-MRI signal intensity, it also gives us the opportunity to directly compare the signal characteristics of the two modalities from exactly the same nigral regions.

Summary & aims

This study was designed to explore various methods of measuring substantia nigra integrity using SWI. We first sought to build on promising existing literature by assessing the binary dorsal nigral hyperintensity (DNH) in a much larger cohort of RBD patients, confirming the absence of this marker in a proportion of individuals. Comparing the outcomes of this with our DaT SPECT/CT imaging, we hypothesised that those who had lost the DNH would exhibit reduced striatal dopaminergic signal, and that SWI could therefore be used as an alternative method of measuring nigro-striatal decline.

Our second aim was to develop novel, automated methods of quantifying the nigral SWI signal that might have greater sensitivity to longitudinal change in future assessments. We used templates from our neuromelanin-sensitive imaging to try to maximise the anatomical accuracy of these nigral measurements.

Finally, we sought to explore the comparisons between these SWI metrics and the neuromelanin-sensitive MRI measures from chapter 5. We hypothesised that because these two modalities measure distinct properties of the substantia nigra, there would be important differences in the pattern of results from each. Following on from this, we sought to combine SWI and NM-MRI measures in a single classification model, predicting that this would perform better than each modality alone in distinguishing PD patients from controls.

Methods

Subjects

Data were available for a total of 105 participants from the OPDC Discovery cohort. The initial study population comprised 46 patients with RBD, 27 patients with Parkinson's disease and 32 healthy control subjects.

All participants underwent imaging with susceptibility weighted MRI in the same session as neuromelanin-sensitive imaging (chapter 5). 42 of the RBD patients were also scanned in parallel with ^{123}I-ioflupane SPECT/CT. Participants were examined on the day of imaging by a neurologist using the Movement Disorders Society Unified Parkinson's Disease Rating Scale (MDS-UPDRS) part III[132]. Other clinical variables were obtained at separate clinic visits (see chapter 2 for details).

MRI acquisition and processing

T2*-weighted images were acquired on a 3T Siemens Trio (Erlangen, Germany) using a 12 channel receive-only head coil (gradient-recalled echo, 256 matrix, voxel size = 0.86x0.86x1.50 mm^3, TE/TR = 20 ms/27 ms, flip angle=15^o; 5 minutes). Preprocessing of the SWI images was kindly performed by Dr Johannes Klein (Senior Clinical Research Fellow, OPDC) as follows: phase images were high-pass filtered using a 50x50 window in Fourier space to remove macroscopic phase artefacts. This window size was selected empirically to suppress artefacts in the midbrain caused by nearby aerated structures. Paramagnetic phase components only were taken to the 4th power and then multiplied with the magnitude images to give the final susceptibility-weighted images.

T1-weighted structural MRI and neuromelanin-sensitive MRI were acquired as described in chapter 5.

SPECT/CT acquisition and analysis

SPECT/CT acquisition, processing and analysis was performed as described in chapter 4.

MRI analysis: binary visual ratings

For the subjective ratings of the dorsal nigral hyperintensity (DNH), SWI images from all 105 subjects were first anonymised and compiled into a single 4D file in random order by an investigator (Dr Johannes Klein) independent from the two raters. Assessments were then performed by two separate raters based on the method described by *Schwarz et al*[217]. Rater 1 was a clinical neurologist with expertise in neuroimaging (Dr Thomas Barber). Rater 2 was a research scientist specialising in brain MRI analysis (Dr Ludovica Griffanti). Briefly: for each subject the DNH was scored as present, absent or uncertain for the left and right substantia nigra respectively (figure 3, A-B). Using this data the scans were classified into two categories as follows: 1) bilateral presence of the DNH, or unilateral presence with the contralateral side uncertain, was considered normal and is referred to as 'DNH present'; 2) definite absence of the DNH unilaterally or bilaterally was considered abnormal and is referred to as 'DNH absent'. In cases where one rater classed the DNH as uncertain bilaterally and the other rater gave a definite classification, the definite classification was used. Cases where both raters considered the images uncertain bilaterally were classed as 'non-diagnostic'. Where there was disagreement in outcome between the two raters and one rater's scoring included 'uncertain' for one side, a consensus rating was achieved by overruling the uncertain score (e.g. if left/right was scored by one rater as 'present/absent' (i.e. abnormal) and by the other rater as 'present/uncertain' (i.e. normal), the final rating used would be 'present/absent').

Region of interest analyses

For the quantification of SWI signal intensity in nigral regions, SWI images were first registered to Montreal Neurological Institute (MNI) standard space via T1-weighted images. Registered SWI images were then normalised to the background image intensity for each individual, calculated as the mean intensity of a region of interest in the brainstem parenchyma remote from the substantia nigra (figure 4). A study-specific SWI template image was created using the mean values from the 32 healthy control subjects (figure 3C). Using this template as a guide to the normal average location of the DNH, a region of interest was drawn manually in the area of the DNH on each side (figure 3D, figure 6A-B). For the whole SN, anterior SN and posterior SN analysis, the regions of interest were defined using the same neuromelanin average template described in chapter 5. Figure 6C-D shows these regions overlaid on the

study-specific SWI template. The mean intensity within each ROI was then extracted from the individual subject normalised, standard-space images. Values presented are the mean of left and right ROIs for each subject.

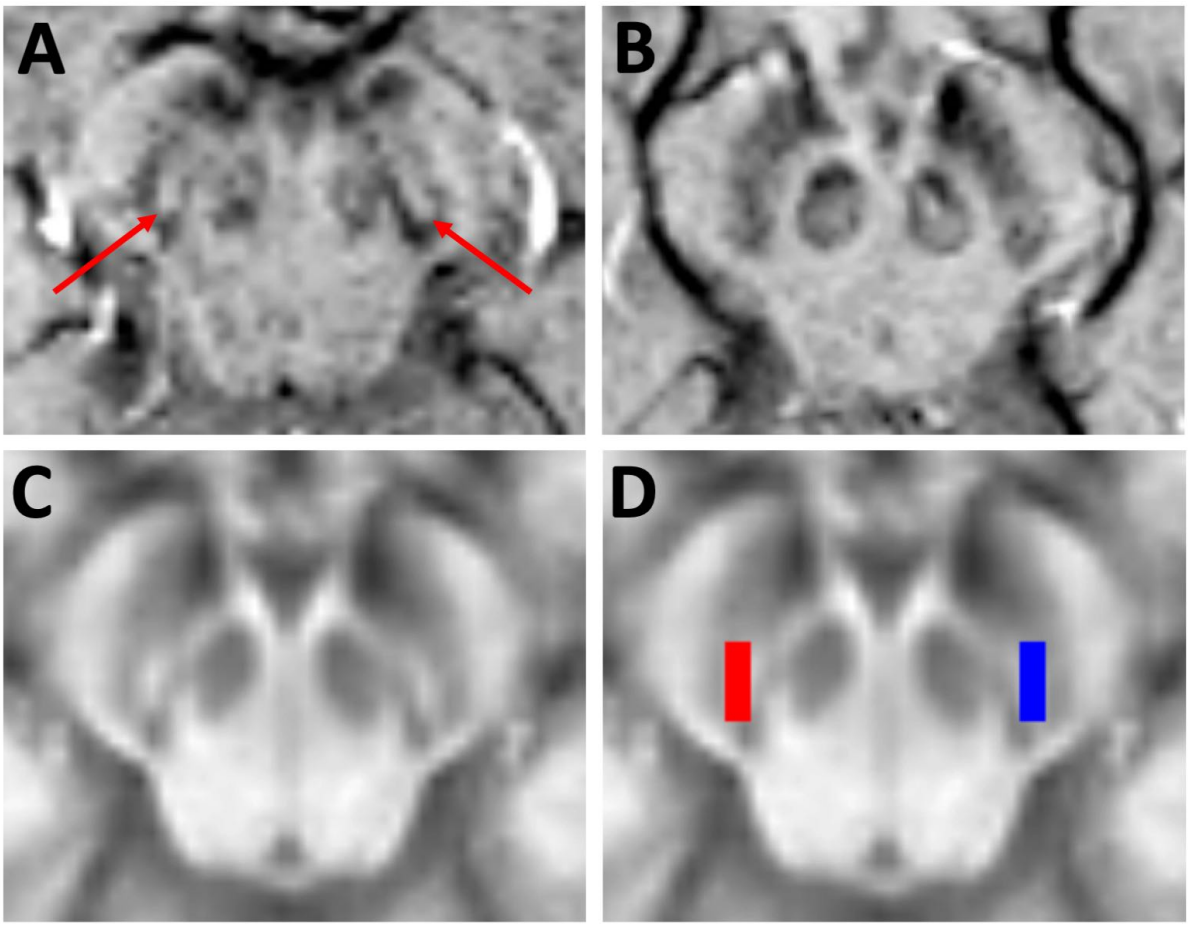


Figure 3. A-B: Example SWI images at the level of the substantia nigra from two individual patients, showing the normal appearance of the DNH (A, red arrows) and its absence in PD (B). C: study-specific template created from the average images of the 32 control participants, showing the average location of the DNH. D: nigrosome ROI for the automated quantification of DNH signal.

Statistical methods

Group comparisons and correlations between variables were performed using linear regression as described in chapter 5. Due to the changes in brain iron deposition that occur with age and their potential confounding effect on the SWI signal, group comparisons and correlation analyses were adjusted for age. Comparison between contralateral and ipsilateral values within RBD patients was performed using a paired samples t-test.

For the ROC analysis in which multiple MRI variables are combined, the variables were first entered into a binary logistic regression model with the dichotomous group state as the dependent variable. The predicted probabilities from this model were then used as the test variable in the ROC analysis to discriminate the two groups.

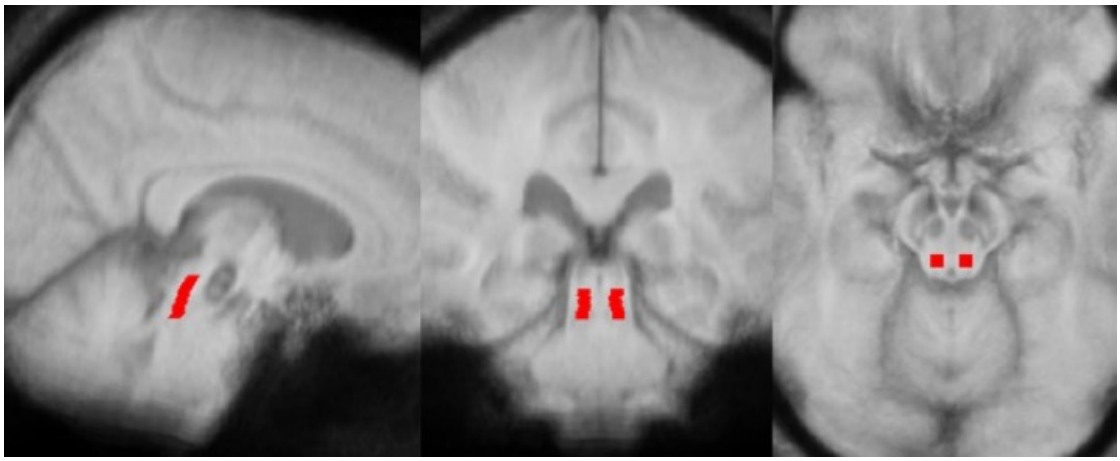


Figure 4. The reference ROI used to calculate the average background signal intensity is shown in red.

Results

	Controls N = 30	RBD patients N = 42	PD patients N = 25	p-value
Age, years, mean (SD)	69.1 (8.15)	64.8 (8.03)	64.8 (10.12)	Con vs RBD: **0.03** Con vs PD: 0.08 RBD vs PD: 0.97
Sex (% male)	80%	98%	60%	Con vs RBD: **0.01** Con vs PD: 0.10 RBD vs PD: **<0.001**
Disease duration, years, mean (SD)	n/a	2.5 (2.44)	5.2 (2.31)	n/a
UPDRS III score, mean (SD)	2.7 (2.07)	5.6 (3.68)	31.9 (14.28)	Con vs RBD: **<0.001** Con vs PD: **<0.001** RBD vs PD: **<0.001**

Table 1. Baseline characteristics of the study population

Eight out of 105 participants (4 RBD, 2 PD, 2 Controls) had SWI images that were of inadequate quality for DNH assessment due to image artefacts, and were excluded from all analyses.

Baseline demographic and clinical variables for the 97 included participants are shown in table 1.

Visual DNH assessment

One included scan (an RBD patient) was considered non-diagnostic by both raters in manual visual assessment of the DNH. Of the remaining 96 scans, there was concordance between the two raters in 89 (93%). In the 7 scans in which there was initial disagreement, a consensus rating was achieved in 2, leaving 5 cases that could not be resolved. These 5 cases with inter-rater disagreement comprised 4 control subjects and one RBD patient. The results of manual DNH assessments for the 91 subjects with consensus ratings are shown in table 2.

	Controls	RBD patients	PD patients
Number with DNH present (% of patient group)	24 (92.3%)	29 (72.5%)	1 (4.0%)
Number with DNH absent (% of patient group)	2 (7.7%)	11 (27.5%)	24 (96.0%)

Table 2. Results of binary manual assessments of the dorsal nigral hyperintensity

Excellent discrimination accuracy was observed between healthy controls and patients with Parkinson's disease, with the absence of DNH achieving a sensitivity of 96%, specificity of 92%, positive predictive value of 92% and negative predictive value of 96%. Amongst RBD patients with rateable images, 11 out of 40 (27.5%) had absence of the DNH, whilst 29 (72.5%) had DNH present. The rate of DNH absence in RBD patients was significantly higher than controls (27.5% vs 7.7%, $p = 0.048$) and significantly lower than PD patients (27.5% vs 96.0%, $p < 0.001$). Unilateral abnormalities were more common in RBD than PD patients, perhaps suggesting an intermediate pattern of abnormality. Of the 11 RBD patients with DNH absence, 7 (64%) had retained DNH presence on one side, whereas In PD patients with DNH absence, only 2 out of 24 cases (8%) had unilateral DNH presence.

RBD Patients n = 36	DaT SPECT/CT Normal	DaT SPECT/CT Abnormal	DaT SPECT/CT Borderline
DNH present	17	6	2
DNH absent	2	8	1

Table 3. Comparison of DNH assessments and DaT SPECT/CT outcomes in RBD patients

Comparison of visual DNH ratings and DaT SPECT/CT imaging

Of the 42 RBD patients with parallel SWI and DaT SPECT/CT imaging, 36 had diagnostic SWI imaging with consensus DNH ratings. Nineteen (53%) of these patients had normal DaT SPECT/CT imaging as rated by the blinded radiologist, whilst 14 (39%) had abnormal imaging and 3 (8%) were classed as borderline. The results of the SWI DNH assessments according to these categorical DaT SPECT/CT outcomes are shown in table 3. There was good agreement between imaging modalities for the 19 RBD patients with normal dopaminergic imaging: 17 (89%) of these individuals had retained presence of the DNH. There was less concordance in the 14 RBD patients with abnormal dopaminergic imaging: 6 (43%) of these patients had DNH present, whilst 8 (57%) had loss of the DNH. One of the three RBD patients with borderline dopaminergic imaging had loss of the DNH and the other two had DNH present.

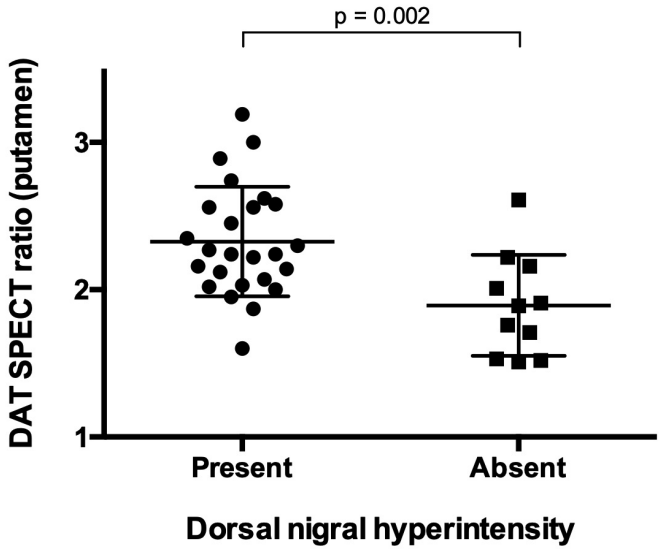


Figure 5. Mean putamen DaT SPECT uptake ratios in RBD patients grouped according to presence or absence of the dorsal nigral hyperintensity.

Figure 5 shows DaT SPECT/CT ratios extracted from the putamen (mean of left and right) of RBD patients grouped according to presence or absence of DNH on SWI imaging. Those with absence of the DNH had significantly lower DaT SPECT/CT signal in the putamen compared to those with the DNH present (mean putamen uptake ratios 1.89 vs 2.33, p = 0.002).

In the 7 RBD patients with unilateral loss of the DNH, dopaminergic signal in the putamen ipsilateral to the DNH loss was significantly lower than in the contralateral putamen (mean putamen ratios 1.72 ipsilateral vs 1.87 contralateral, p = 0.01).

Automated quantification of DNH signal intensity

Correlations between age and SWI signal in the regions of interest studied are shown in table 4. Although significant correlations were observed in the nigrosome-1 and posterior nigra regions, these were not present within either the control or PD groups individually and are therefore likely to be caused by group differences, in particular within the RBD patients, rather than a real effect of age on SWI signal. Furthermore, the correlation is in the opposite direction to that expected, with higher nigral SWI signal (and therefore lower iron deposition) associated with increasing age.

Correlation with age	All subjects N = 96	Controls N = 30	RBD N = 41	PD N = 25
Whole SN intensity (SWI)	r = 0.10 p = 0.32	r = -0.06 p = 0.74	r = -0.15 p = 0.34	r = 0.27 p = 0.19
Anterior SN intensity (SWI)	r = 0.04 p = 0.70	r = -0.13 p = 0.50	r = -0.23 p = 0.15	r = 0.28 p = 0.17
Posterior SN intensity (SWI)	**r = 0.20** **p = 0.046**	r = 0.08 p = 0.66	r = 0.08 p = 0.64	r = 0.24 p = 0.25
Nigrosome-1 intensity (SWI)	**r = 0.33** **p = 0.001**	r = 0.10 p = 0.59	**r = 0.38** **p = 0.02**	r = 0.34 p = 0.10

Table 4. Correlations between SWI variables and age

The normalised mean intensity values extracted from the nigrosome-1 region, as well as from the neuromelanin template defined nigral regions are shown in figure 6, grouped according to patient category and excluding only the 8 subjects with artefact-degraded images (N.B. the

5 subjects with inter-rater discordance and the 1 subject with non-diagnostic imaging by manual rating are included).

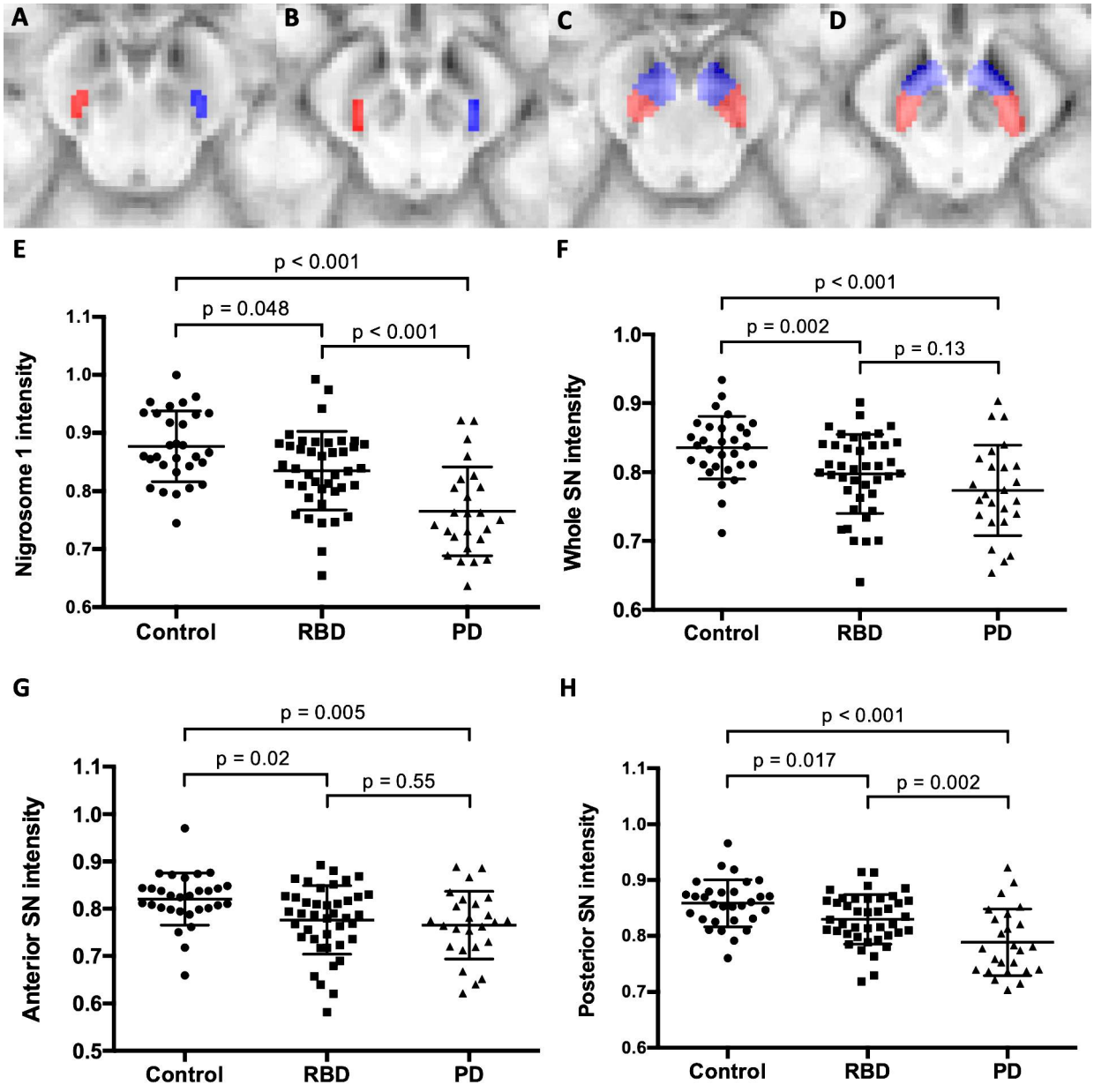


Figure 6. Normalised SWI intensity measures from nigral ROIs. A-B: nigrosome ROI, values displayed in E. C-D: ROIs for the anterior (blue, displayed in G), posterior (red, displayed in H) and whole nigra (mean red & blue, displayed in F)

In all regions examined, both RBD and PD patient groups had significant reductions in SWI signal intensity compared to controls. In addition, the signal intensity in the nigrosome-1 and posterior SN regions was significantly lower in PD patients than RBD patients (figure 6E & 6H). In a receiver operating characteristic analysis, the signal from the nigrosome-1 region performed best at discriminating PD patients from controls, with and area under the curve of 0.86 (table 5). As expected, there was less separation between RBD patients and controls, but all four ROIs still separated these two groups with better than chance accuracy.

	AUC PD vs Controls	p value	AUC RBD vs Controls	p value
Nigrosome-1 intensity	0.863	**< 0.001**	0.649	**0.033**
Whole SN intensity	0.787	**< 0.001**	0.696	**0.005**
Anterior SN intensity	0.743	**0.002**	0.686	**0.008**
Posterior SN intensity	0.828	**< 0.001**	0.680	**0.010**

Table 5. Discriminatory accuracy of SWI measures

Relationship between SWI outcomes and clinical variables

Table 6 shows the scores across a range of prodromal markers in RBD patients, grouped according to the presence or absence of DNH. Amongst individual markers, none were more severely affected in RBD patients who had lost DNH. However, when markers were combined using the MDS research criteria for prodromal Parkinson's disease, RBD patients with DNH absence had a significantly higher probability of prodromal Parkinson's than those with DNH presence, suggesting that DNH loss in RBD is associated with an increased risk of future Parkinson's disease.

Across all four of the quantified nigral regions, there were no significant correlations with motor severity, nor with any other clinical variable examined in any of the patient groups (data not displayed).

	RBD DNH present	RBD DNH absent	p value
Age (years)	63.6 (8.48)	65.9 (6.07)	0.41
Motor severity (UPDRS III score)	5.1 (3.57)	5.7 (3.74)	0.99
Quantitative motor testing (Purdue Pegboard score)	32.1 (5.61)	34.0 (3.23)	0.26
Olfaction (Sniffin Sticks score)	8.2 (3.51)	6.6 (3.86)	0.51
Cognition (MoCA score)	25.4 (3.18)	26.0 (2.28)	0.50
Postural systolic blood pressure change	-7.6 (13.86)	-1.4 (14.09)	0.07
Depression (BDI score)	10.7 (9.73)	6.3 (4.59)	0.29
Anxiety (HADS score)	7.1 (4.64)	3.6 (3.93)	0.08
Apathy severity (LARS)	-19.8 (7.52)	-23.6 (4.57)	0.22
Epworth sleepiness score	7.4 (4.35)	5.8 (5.69)	0.70
MDS prodromal PD probability, %	82.2 (19.6)	95.2 (9.19)	**0.01**

Table 6. Comparison of clinical variables in RBD patients according to DNH presence (Abbreviations: MDS, Movement Disorders Society; UPDRS, Unified Parkinson's Disease Rating Scale; MOCA, Montreal Cognitive Assessment; HADS, Hospital Anxiety and Depression Scale; LARS, Lille Apathy Rating Scale.)

	RBD DNH present	RBD DNH absent	p value
SN volume	263.7 (45.74)	248.5 (85.76)	0.59
SN peak intensity (volume)	148.9 (5.03)	144.7 (3.51)	**0.011**
SN contrast ratio (volume)	0.263 (0.0219)	0.249 (0.0237)	**0.049**
SN mean intensity (template)	1.179 (0.0221)	1.164 (0.0218)	0.06
Anterior SN mean intensity (template)	1.186 (0.0262)	1.174 (0.0244)	0.20
Posterior SN mean intensity (template)	1.169 (0.0203)	1.149 (0.0214)	**0.011**

Table 7. Comparisons of neuromelanin-sensitive MRI measures in RBD patients according to presence of DNH in binary visual assessment.

Comparison of SWI measures with neuromelanin-sensitive MRI and DaT SPECT/CT

Table 7 shows the substantia nigra measures calculated from neuromelanin-sensitive MRI (NM-MRI) compared between RBD patients with vs without the DNH present on visual assessment of their SWI images (see chapter 5 for details of the NM-MRI measures). Peak NM-MRI intensity and contrast ratio within the SN volume, and mean intensity in the posterior nigra template, were significantly lower in RBD patients who had lost the DNH signal in SWI.

SWI vs NM-MRI ROI intensity	All subjects N = 85	Controls N = 23	RBD N = 39	PD N = 23
Whole SN	**β = 0.28 p = 0.010**	β = 0.38 p = 0.08	β = 0.27 p = 0.11	β = 0.02 p = 0.93
Anterior SN	**β = 0.32 p = 0.003**	**β = 0.43 p = 0.045**	**β = 0.36 p = 0.034**	β = 0.11 p = 0.63
Posterior SN	β = 0.19 p = 0.09	β = 0.16 p = 0.47	β = 0.11 p = 0.52	β = -0.06 p = 0.79

Table 8. Correlations between NM-MRI and SWI measures from the same standard space regions of interest

Correlations between the NM-MRI and SWI signal intensities from the same nigral regions of interest are described in table 8. Interestingly, correlations were strongest anteriorly in the SN, and within the healthy control group reached significance in only this region. A significant correlation in this region was also observed in RBD patients, but not in PD patients, suggesting a divergence between the signal from the two MRI modalities as disease becomes established.

Finally, direct correlations between SWI intensity measures and DaT SPECT signal were examined in RBD patients. None of these reached statistical significance (data not displayed).

Discriminatory accuracy of combined NM-MRI and SWI measures

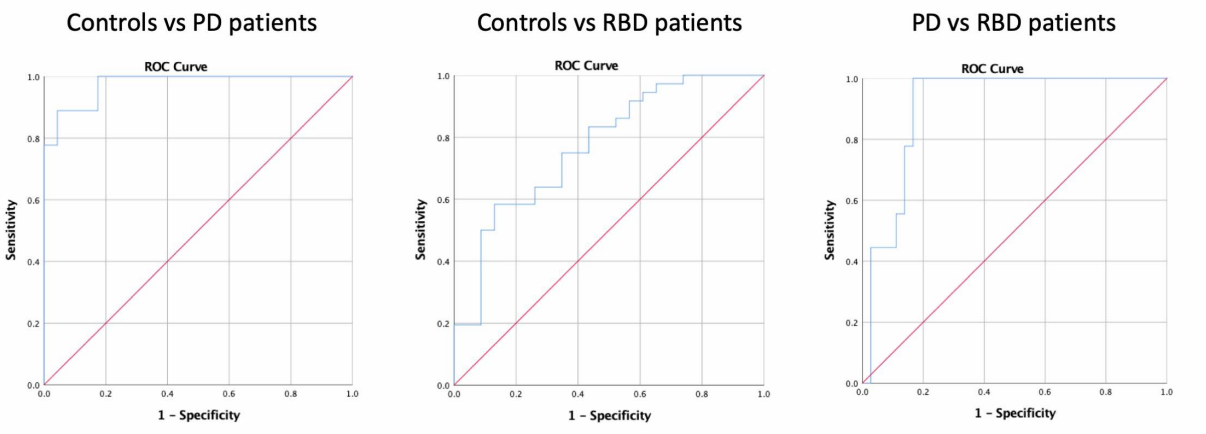
Because the NM-MRI and SWI measures may describe different aspects of brainstem degeneration, we looked at the discriminatory ability of these measures in combination using

a logistic regression model. First, we selected all NM and SWI measures that were significantly different between controls and PD patients. If two or more of such measures within the same modality had substantial overlap in the region of interest from which they were derived (e.g. the nigrosome-1 and posterior nigra regions in SWI), only the measure with the best individual discriminative accuracy was included and others were excluded. The following variables were therefore included in the model:

NM-MRI measures: SN volume
posterior SN mean intensity
SN peak intensity
LC peak intensity

SWI measures: Nigrosome-1 mean intensity
Anterior SN mean intensity

The combination of these variables was able to distinguish PD patients from controls with an AUC of 0.976, a near-perfect classification accuracy and substantially more accurate than any measure individually (figure 7). This same model classified RBD patients from controls with an AUC of 0.772, and PD patients from RBD patients with an AUC of 0.907.



AUC	0.976
95% CI	0.930 – 1.0
p value	< 0.001

AUC	0.772
95% CI	0.649 – 0.895
p value	< 0.001

AUC	0.907
95% CI	0.821 – 0.993
p value	< 0.001

Figure 7. Discriminatory accuracy of combined SWI and neuromelanin measures.

Discussion

Visual assessment of nigrosome-1 in RBD

Our study demonstrates that around a quarter of patients with idiopathic RBD have loss of the dorsal nigral hyperintensity (DNH) on SWI imaging, a pattern of abnormality that occurs in established Parkinson's disease and indicates degeneration affecting nigrosome-1[218]. This provides important *in vivo* evidence of prodromal disease in the substantia nigra and raises the possibility that SWI may be of value in the risk-stratification in RBD patients. The observation that RBD patients with loss of the DNH have reduced dopamine transporter binding in the putamen compared to those with the DNH present is important evidence that this SWI sign is indicative of nigro-striatal integrity. This is supported by the finding of a higher probability of prodromal PD, calculated using the MDS research criteria[60], in patients with DNH loss.

There was partial discrepancy in findings between the dichotomised outcomes of SWI and DaT SPCECT/CT. Whilst we observed DNH loss in only 28% of our RBD patients, the overall rate of DaT SPECT/CT abnormality was higher at 39%, a figure broadly in line with other studies indicating that 40-50% of RBD patients have evidence of a dopaminergic deficit[206,280]. Interestingly, 89% of those with normal dopaminergic imaging also had normal SWI imaging, whereas only 57% of RBD patients with a dopaminergic deficit had absence of the DNH.

A potential explanation for this would be that DNH loss may occur at a later stage of nigro-striatal degeneration than dopamine transporter deficit. The retained presence of the DNH alongside abnormal dopaminergic imaging would be in keeping with the proposed 'dying-back' pattern of nigro-striatal axonal degeneration, in which synaptic dysfunction precedes loss of the nigral cell bodies[313]. The hypothesis that DNH loss is a later prodromal sign that DaT abnormality remains to be tested by long-term follow up of the patients in this study. However, if this were the case, it would have important implications for the risk-stratification of RBD patients, with perhaps greater potential for the enrichment of near-term converters using SWI. This would be advantageous in the development of prodromal cohorts for neuroprotective clinical trials in which conversion to an overt synucleinopathy were the primary outcome measure.

It is notable that the degree of discordance in outcomes that we observed between SWI and DaT SPECT/CT is greater than has been reported previously[220,222]. This is mainly due to the proportion of our RBD patients with DNH present who had abnormal DaT SPECT/CT outcomes. In contrast, the high positive predictive value of DNH loss is a consistent finding across all studies: when our data is combined with these two previous studies, more than 90% of RBD patients with loss of DNH have had abnormal dopaminergic imaging. This makes DNH a potentially useful stratification tool for clinical trial recruitment, where a high positive predictive value for underlying disease would be more important than a high sensitivity.

Study	Number of RBD patients with adequate imaging	Mean age of RBD patients, years	Mean RBD duration, years	Proportion with absent DNH on SWI	Proportion of abnormal cases with unilateral DNH absence
De Marzi et al, 2016[6]	13	68.9	5.3	10/13 (77%)	1/10 (10%)
Frosini et al, 2017[7]	16	69.3	6.3	9/16 (60%)	2/9 (22%)
Bae et al, 2018[8]	18	70.5	5.9	11/18 (61%)	2/11 (18%)
This study	40	64.5	2.5	11/40 (28%)	7/11 (64%)

Table 9. Comparison of outcomes in RBD studies of binary DNH assessment using SWI

Comparison of our SWI outcomes with three smaller studies that have previously investigated loss of the DNH in RBD patients also reveals some important differences, with possible implications for prodromal disease staging. All three studies reported higher rates of DNH loss than we have observed, as well as a higher proportion of bilateral abnormalities (table 9)[220–222]. Methodological variations including differences in imaging protocol, rating strategy and rater experience may have influenced outcomes, and it is possible that the effect of these is greater in RBD patients than in controls or PD patients, where the presence of borderline cases may increase the ambiguity of binary assessment. In our cohort, RBD patients were

more likely to have asymmetric DNH findings than PD patients and in this context an 'uncertain' rating on one side makes a crucial difference in the binary outcome, since a rating of 'normal/uncertain' is scored as normal, whereas 'normal/absent' is considered abnormal.

However, it is also possible that our patients are on average at an earlier prodromal stage. The long and variable latency to phenoconversion in RBD patients means that the small cohorts described previously could be easily influenced by a few individuals who are near to conversion. The study of Bae *et al*, for example, reported DNH absence in 11 out of 18 RBD patients[220]. However, they also observed conversion to a neurodegenerative disorder in 5 out of 18 RBD patients (28%) after an average 18-month follow-up period. This is much higher than would be expected from the overall average conversion rate of 6% per year in RBD[68], and suggests that their RBD patients were at a relatively advanced prodromal stage at the time of imaging.

In the absence of longitudinal data, we cannot yet say if our patients were at an earlier stage when imaged; although they were younger and had a shorter RBD disease duration than other studies, neither of these are reliable indicators of prodromal staging.
If it were the case, however, that more advanced prodromal cases had higher rates of DNH loss, it would suggest that this marker could develop as patients progress through the stage of prodromal disease after RBD diagnosis. Such sensitivity to disease progression would be an important characteristic, and in contrast to substantia nigra ultrasound, another binary risk marker, which does not show change over time[205].

Quantification of SWI/N1 signal

Despite the promise of nigral SWI as a biomarker, it is limited in its observer rating system by a degree of subjectivity, inter-rater discrepancy, and a binary outcome measure that lacks quantification and sensitivity to change over time. Though this method performs well enough to accurately discriminate Parkinson's patients from controls, a binary outcome may be more problematic in prodromal patients where there are expected to be many more borderline cases. To address this, we used a simple, automated method of quantifying the SWI signal

from the nigrosome-1 region as well as nigral regions of interest derived from neuromelanin-sensitive imaging.

The overall pattern of results obtained using this method matched that of the binary ratings, with a stepwise decline in signal intensities across all regions from controls to RBD patients and from RBD patients to PD patients. The region that performed best in separating PD patients from controls was the nigrosome-1 region, attesting to the particular vulnerability of this region to parkinsonian degeneration. Although this discrimination was slightly less accurate than manual ratings, importantly it included subjects with non-diagnostic manual ratings. A possible explanation for this is that automated signal quantification will not distinguish between high signal from nigrosome-1 and high signal caused by artefacts. Imperfect registration may also lead to a limited amount of bright signal from outside the nigra being included in the nigrosome ROI inadvertently in some cases.

As with the binary observer ratings, RBD patients were more similar to controls than to PD patients, but significantly different from both. Interestingly, however, the nigrosome-1 region was the least sensitive SWI measure in discriminating RBD patients from controls, with SWI signal across the whole SN performing best in this regard.

Despite the superior discrimination between groups achieved with SWI compared to neuromelanin-sensitive imaging, direct correlations between quantified SWI measures and clinical variables were not observed. The fact that we did not find a correlation between motor severity and DNH signal intensity in PD patients may be accounted for by a floor effect. The majority had bilateral complete loss of the DNH and it is likely that the low signal remaining in the dorsolateral nigra (figure 1C-D) is insensitive to further change. In RBD patients, the limitations of clinical scores in quantifying disease progression mean that correlations with imaging variables are often negative[279]. A lack of correlation between the quantified DNH signal and DaT SPECT values, on the other hand, was disappointing.
It therefore remains to be seen with longitudinal follow-up whether these objective measurements, and their change over time, outperform binary ratings in delineating disease stage. However, the intermediate spread of values observed in RBD patients in this cross-

sectional assessment is promising, and suggests that further investigation of this technique is warranted.

Comparison of SWI with neuromelanin-sensitive imaging

NM-MRI Intensity measures in the NM-defined nigral volume as well as the NM nigral template were significantly reduced in those patients who had lost the DNH on SWI imaging. It is encouraging that these two distinct MRI modalities, each seeking to measure different aspects of nigral integrity, show a degree of concordance, particularly in patients with RBD. These data suggest that loss of nigrosome-1 is accompanied by generalised nigral neuromelanin depletion, which would be in keeping with the DNH signal being lost as the iron-sequestering capacity of the nigra declines and increasing amounts of ferric iron are deposited in the tissues.

Interestingly, NM-MRI and SWI intensity measures obtained from the same regions of interest showed limited correlation with each other. In control and RBD participants only the anterior nigra showed such a relationship, with a positive correlation consistent with the notion that high neuromelanin levels correspond to low iron deposition (and therefore higher SWI signal intensity). The fact that this association is not present in PD patients, however, implies a more complex picture in the context of established neurodegeneration.

Our data support the idea that combining variables from different MRI modalities could potentially provide a more thorough assessment of disease status than individual measures. When we combined NM-MRI and SWI measures from the nigra and locus coeruleus, we were able to achieve substantially greater group classification accuracy than with any measure individually and near perfect discrimination between PD patients and controls.

This is an encouraging finding and highlights another important aspect of MRI: the large volume of data that can be acquired in a relatively short scanning session. The sequences required for our combined analysis (NM-MRI, SWI and T1) take around 20 minutes in total to acquire, making multimodal assessment feasible even in frail patients. The further addition of data from other sequences, such as diffusion-weighted imaging or resting state MRI, could increase the accuracy of the assessment even further[228,314]. Prediction and classification models based on large, multimodal data sets in this way are likely to be important tools in

future, and will be greatly facilitated by the quick and objective methods of signal automation that we have explored in our analysis.

Summary and concluding remarks

SWI imaging of the substantia nigra is a promising biomarker of parkinsonian neurodegeneration. In combination with neuromelanin-sensitive MRI, SWI can discriminate PD patients from controls with near perfect accuracy. RBD patients exhibit an intermediate state of abnormality between PD patients and healthy controls, and RBD patients with loss of DNH on SWI have significant dopaminergic deficits and evidence of neuromelanin depletion compared to those without. Further longitudinal analysis is planned, which will determine whether the sequential imaging signature of prodromal Parkinson's is indeed loss of DAT signal followed by DNH loss, and imminent conversion to Parkinson's disease. If so, this would demonstrate that MRI, in particular SWI, has great potential for the enrichment of near-term phenoconvertors and could emerge as an important tool for prodromal cohorts and future neuroprotective trials.

Chapter 7. Apathy in RBD and its underlying mechanisms

Introduction

The clinical syndrome of apathy

Apathy is a common and debilitating feature of Parkinson's Disease (PD) and Dementia with Lewy Bodies (DLB), which impacts on the quality of life of both patients and carers[89,315,316]. Characterised by pathological lack of motivation, it manifests as a reduction in goal-directed thoughts and behaviours, with or without emotional blunting[317]. Many components of apathy are now recognised, including deficits in self-awareness and emotional responses as well as action initiation and intellectual curiosity[318]. It is not equivalent to anhedonia, as patients with apathy are still able enjoy activities if they are prompted to act. Although it frequently coexists with other neuropsychiatric conditions such as depression and cognitive impairment, apathy is increasingly considered to be a distinct clinical syndrome[319]

As with many non-motor features of PD and DLB, apathy is often reported retrospectively to occur before the onset of motor symptoms[92]. Despite this, the syndrome has never been formally assessed in the prodromal period. Whilst we and others have found that RBD patients do frequently report apathy[93,277], the accuracy of the single question self-report measures used to determine this is uncertain, and it is likely that such simple screening questions will fail to capture the full spectrum of the condition.

Our own study of the clinical phenotype of RBD described in chapter 2 demonstrated that depression, anxiety and cognitive impairment are particularly common, suggesting that RBD might represent the prodromal phase of a subtype of alphasynucleinopathy with a more severe neuropsychiatric phenotype. Apathy might therefore be of particular relevance to RBD patients. A thorough assessment of the extent of apathy in RBD using a comprehensive, validated rating scale is therefore required in order to confirm the extent of the problem and its interaction with other neuropsychiatric conditions.

As well as furthering understanding of the clinical phenotype of RBD, recognising apathy if it occurs might lead to interventions with specific psychological and pharmacological therapies to improve quality of life. Little evidence currently exists to guide treatment approaches specific to apathy, and this may in part be due to an underappreciation of the scale of the problem[320]. Another reason to characterize apathy in RBD patients is its potential role in risk-stratification models. Non-motor features including depression and cognitive impairment have been shown to increase an individual's risk of conversion to PD/DLB[60,68], but the predictive capacity of apathy in this regard is unknown. Accurately establishing the presence and severity of apathy in RBD is therefore an important objective for many reasons.

In the first part of this chapter, we investigate the frequency and severity of apathy in a large cohort of RBD patients using the Lille Apathy Rating Scale (LARS). This comprehensive assessment tool consists of a structured clinical interview evaluating sub-domains of apathy as well as an overall measure of apathy severity[318]. Unlike some other apathy ratings, it does not rely on subjective reports of care-givers and therefore has high inter-rater consistency and excellent accuracy compared to clinical judgement[321]. It can effectively distinguish apathy from depression and has been validated in established Parkinson's with and without dementia[318]. In addition, we sought to explore the overlap between apathy, cognition and depression, and also to assess the screening accuracy of a commonly used apathy rating question from the UPDRS part I[132].

The neurochemical basis of apathy in parkinsonian disorders

As recognition of apathy as a disabling component of parkinsonian disorders has increased, so have efforts to explain the underlying mechanism leading to the syndrome. Much of the initial focus has been on the role of dopamine deficiency, given the importance of dopaminergic decline in the pathophysiology of PD and the well-established contribution of dopaminergic projections in the processing of reward and motivation for goal directed behaviour[100,272]. In established Parkinson's disease, evidence that dopamine depletion underlies apathy is largely circumstantial. It is recognised that withdrawal of dopaminergic medication is associated with increased levels of apathy, particularly in the context of deep brain stimulation, where dose reductions can be substantial and abrupt[322]. Dopamine

receptor agonists are able to ameliorate apathy in this situation[323], and excessive stimulation of the dopaminergic system in this way is associated with impulse control disorders, which can be considered at the other end of the motivational spectrum from apathy[324]. Direct evidence linking dopamine depletion to apathy comes from a small number of neuroimaging studies showing greater dopaminergic deficits in apathetic patients, but it is possible that this is simply a surrogate marker of a more diffuse disease subtype rather than a causal mechanism[325,326].

Recognition of PD as a multisystem disorder in recent years has turned attention towards other neurotransmitter systems and some of the most compelling recent studies to explore mechanisms of parkinsonian apathy have implicated non-dopaminergic transmission in its pathogenesis. The importance of serotoninergic depletion in particular has been suggested by both functional and structural neuroimaging studies[108,327]. Of note, these investigations focused on newly-diagnosed PD patients, whereas much of the evidence for dopamine's role in the pathogenesis of apathy comes from patients at more advanced stages. It is conceivable, therefore, that the mechanisms underlying apathy vary according to disease stage, with serotonergic deficits potentially an earlier contributing factor.

This hypothesis would be consistent with the typical pattern of disease progression observed in PD. The brain's principal source of serotonergic projections is the dorsal raphe nucleus (DRN), from which neurons project extensively to forebrain limbic regions implicated in the pathogenesis of apathy[328]. The characteristic caudal to rostral spread of brainstem pathology in Parkinson's Disease means that when the DRN is affected, this tends to occur before disease affects the nigro-striatal dopaminergic system[3]. It is conceivable, therefore, that pathological changes here may lead to neuropsychiatric impairment, with corresponding serotonergic imaging deficits, during the prodromal phase of Parkinson's disease.

As described in chapter 4, the compound ^{123}I-ioflupane is commonly used in SPECT imaging in the investigation of Parkinsonian disorders in order to label presynaptic dopamine transporters in the striatum[69]. However, the ligand in fact binds relatively non-specifically to monoaminergic transporters and so its imaging signal reflects the predominant transporter type in any given region[329,330]. This property has made it possible to assess serotonergic signal

in the raphe nuclei, where very few dopamine transporters exist, alongside dopamine transporter binding in the basal ganglia[331]. Figure 1 illustrates this concept using example SPECT images from two of our RBD patients, overlaid on a standard structural MRI template. It can be seen that despite similar levels of dopaminergic signal in the striatum (axial images, right), a marked difference in signal in the brainstem is apparent (sagittal images, left). This signal is in part attributable to ioflupane binding to serotonin transporters in the DRN, and illustrates the dissociation between signal that can be seen between different monoaminergic regions.

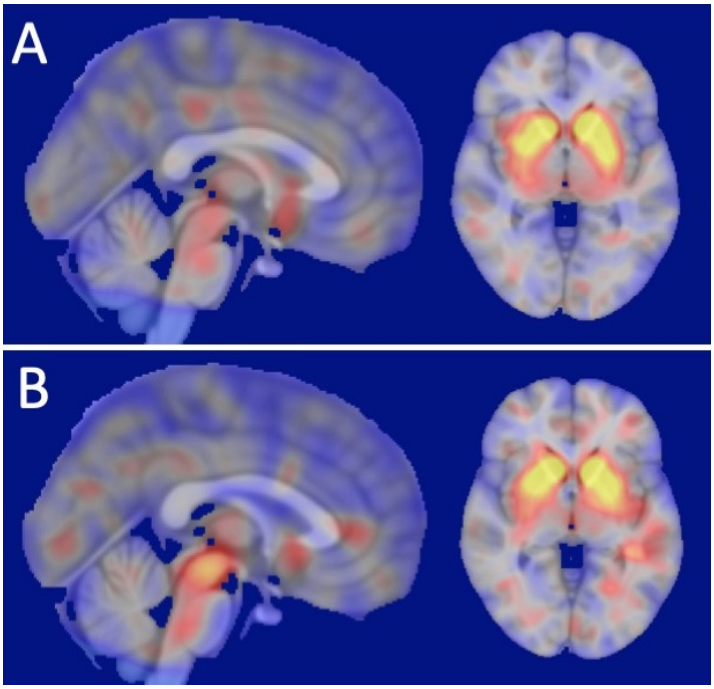


Figure 1. Example ioflupane SPECT images from two RBD patients showing signal peaks in the brainstem (sagittal images, left) and striatum (axial images, right). Note the marked difference in brainstem signal despite similar signal in the striatum.

In the second part of this chapter, we use the ioflupane SPECT imaging described in chapter 4 to investigate the neurochemical basis of apathy in a subgroup of RBD patients. By examining both dopaminergic and serotonergic regions and exploring a range of neuropsychiatric features, we sought to compare the associations of dopaminergic and serotonergic loss to the expression of apathy in RBD patients.

Methods

Subjects

Eighty-eight patients with RBD were recruited from the Oxford Parkinson's Disease Centre Discovery cohort, as described previously. Sixty-five Parkinson's patients were recruited separately from neurology clinics in the Oxfordshire area. Thirty-three control participants were recruited from a volunteer database and screened to exclude those with neurological conditions. All RBD diagnoses were made by polysomnography according to International Classification of Sleep Disorders criteria[37]. RBD patients were examined by a neurologist to exclude the presence of PD and other neurological disorders. Patients with dementia (identified using a combination of cognitive testing (MOCA[136]), informant ratings (using the IQCODE[332]), and clinical judgement) were not included. The study was approved by the Oxfordshire Research Ethics Committee and all participants provided informed, written consent.

Clinical assessments

Apathy was assessed using the Lille Apathy Rating Scale (LARS)[318]. The scale includes 33 items, divided into nine domains. Global scores range from −36 to +36, with a higher score representing a greater degree of apathy. We used the following cut-off values for categorical classification as indicated in the validation of the scale: no apathy $\leq$ -22; mild apathy -21 to -17; moderate to severe apathy $\geq$ -16. PD and RBD participants also completed the Movement Disorders Society Unified Parkinson's Disease Rating Scale (MDS-UPDRS)[132], including the motor assessment (part III) and the screening questions for apathy (part I, question 1.5) and depression (part I, question 1.3)

Other clinical variables were quantified using the methods described in chapter 2. In dichotomised analyses, the following cut-offs were used: Depression, BDI score >13; Cognitive impairment, MOCA score <24; excessive daytime sleepiness, ESS score >10.

Data from polysomnography was taken from the diagnostic studies and was therefore not collected at the same time as clinical assessments.

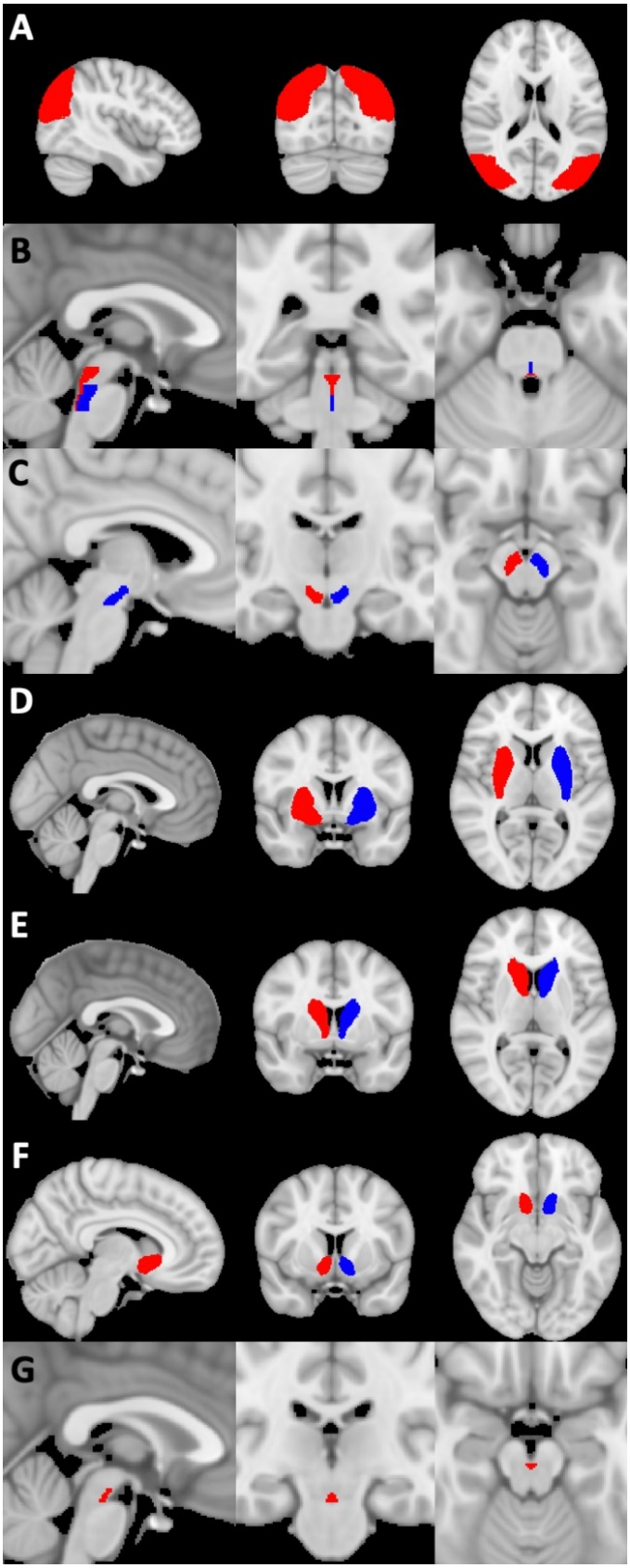


Figure 2. Regions of interest used for the calculation of SPECT specific uptake ratios. A: background reference region; B: dorsal raphe nucleus (red) and median raphe nucleus (blue); C: substantia nigra; D: putamen; E: caudate; F: nucleus accumbens; G: ventral tegmental area.

SPECT/CT and MRI analysis

A subgroup of 43 of the RBD patients underwent ioflupane SPECT imaging with structural MRI in parallel, using the acquisition protocol described in chapter 4. Controls and PD patients in this study did not undergo neuroimaging.

SPECT images were registered to MNI152 standard space via structural MR images as described in chapter 4. All regions of interest were defined in MNI152 space (figure 2). Regions of interest for the dorsal raphe nucleus, median raphe nucleus and ventral tegmental area were obtained from the Harvard Ascending Arousal Network Atlas[333]. Regions for the caudate, putamen and accumbens nuclei were derived from the Harvard-Oxford subcortical structural atlas. A substantia nigra region was drawn using an in-house template derived from neuromelanin-sensitive MRI (chapter 5). A background reference region in the superior lateral occipital cortex was defined using the Harvard-Oxford cortical structural atlas (chapter 4). Normalised specific uptake ratios for each region of interest were calculated as the mean SPECT/CT value of voxels within that region divided by the mean SPECT/CT value of voxels within the reference region.

Statistical analysis

Between groups comparisons for continuous variables were made using linear regression and for dichotomous variables using logistic regression, with gender (as a categorical variable), age, BDI scores and MOCA scores (as continuous variables) included as covariates where indicated to control for any between group differences which may also impact on apathy ratings. Comparisons of LARS scores within patient groups where other variables were not adjusted for were made using the Mann-Whitney U-test or Kruskal-Wallis test as appropriate, and the Chi-square test was used for categorical outcomes. Pearson correlation coefficients were used to assess relationships between two continuous variables. Sensitivity, specificity and positive predictive value of the UPDRS part I screening questions were calculated using standard formulae, with a UPDRS I response > 0 considered as a positive screening result. Only RBD and PD cases but not controls had a UPDRS assessment. Subgroups for the sensitivity analyses were generated using a frequency matching process. Linear regression was used to compare the relative effects of apathy, depression and cognitive impairment on

dorsal raphe ^{123}I-ioflupane signal. Statistical significance was considered as $p<0.05$, with Bonferroni thresholds for multiple comparisons indicated where applicable.

Results

Baseline characteristics

Clinical assessments were completed in 88 patients with RBD, 65 age-matched Parkinson's patients and 33 healthy controls. RBD patients had a mean duration of 8.5 years (SD 6.7) since symptom onset and 3.0 years (SD 2.5) since polysomnographic diagnosis. PD patients had mean disease duration since diagnosis of 4.4 years (SD 4.0) and mean total UPDRS of 41.8 (SD 17.5). 10 PD patients were drug naïve, 47 were receiving treatment with L-dopa, 26 with a dopaminergic agonist and 30 were taking other PD medications (monoamine oxidase inhibitors, catechol-O-methyl transferase inhibitors or amantadine).

	Control N=33	RBD N=88	PD N=65	P-value RBD vs Control	P-value RBD vs PD
Age	68.4 (8.94)	66.9 (7.62)	66.4 (5.65)	0.31	0.70
% male	45.5	94.3	72.3	**<0.001**	**0.001**
UPDRS	n/a	5.25 (4.02)	41.8 (17.5)	n/a	**<0.001**
MOCA	28.3 (1.44)	24.9 (3.08)	27.6 (2.30)	**<0.001**	**<0.001**
BDI	4.91 (4.91)	9.40 (7.68)	10.2 (7.01)	**0.01**	0.48
Total LARS	-29.1 (4.33)	-21.0 (6.00)	-23.3 (6.85)	**<0.001**	0.10
LARS Intellectual curiosity	-3.30 (0.63)	-2.37 (0.96)	-2.29 (1.07)	**<0.001**	0.41
LARS Emotion	-2.62 (1.03)	-1.98 (1.13)	-2.32 (1.25)	0.10	0.19
LARS Action initiation	-3.76 (0.47)	-2.77 (1.00)	-3.25 (0.89)	**<0.001**	**0.005**
LARS Self-awareness	-3.12 (1.32)	-2.03 (1.52)	-2.97 (1.32)	**0.002**	**<0.001**
Mild apathy (≥-21 on LARS)	3.0%	45.5%	30.8%	**0.008**	0.23
Moderate to Severe apathy (≥-16 on LARS)	3.0%	15.9%	18.5%	0.22	0.40

Table 1. Baseline demographics and neuropsychiatric features

Clinical assessments

The results of clinical assessments are shown in Table 1. Comparisons between groups for all assessment scores are adjusted for age and gender. In keeping with previous reports, measures of cognition and depression were significantly worse in RBD patients compared to controls. Apathy classed as mild or worse was present in 45.5% (95% CI 34.8, 56.4%) of RBD subjects, compared to 30.8% (95% CI 19.9, 43.4%) of PD patients and only 3.2% (95% CI 0.1, 16.7%) of control participants. Moderate to severe apathy was present in 15.9% (95% CI 9.0, 25.2) of RBD patients, 18.5% (95% CI 9.9, 30.0) of PD patients and 3.2% (95% CI 0.1, 16.7%) of control subjects. Mean total LARS scores in RBD patients were significantly worse than in controls, and comparable to those in PD patients. Breakdown of the LARS scores revealed that RBD patients were impaired across all subdomains compared to controls except the one that seeks to measure emotional apathy where their worse score was consistent with chance variability. Compared to PD patients, action initiation and self-awareness LARS scores were actually worse in RBD cases.

Using LARS score $\geq$-21 as indicative of apathy, the sensitivity of the UPDRS I apathy screening question in RBD patients was only 33% (95% CI 19, 49%), specificity 85% (95% CI 72, 94%), positive predictive value 65% (95% CI 41, 85%), negative predictive value 60% (95% CI 48, 72%) positive likelihood ratio 2.2 and negative likelihood ratio 0.79. The sensitivity for moderate to severe apathy (LARS score $\geq$-16) was slightly improved at 50% (95% CI 23, 77%) but with wide confidence intervals due to relatively small numbers. The sensitivity of the UPDRS screening question for depression in RBD (defined as BDI >13), in comparison was 80% (95% CI 63, 98).

Effect of potential confounders

The extent of apathy in RBD patients was not explained by excessive daytime sleepiness. There was no significant correlation between LARS scores and Epworth Sleepiness Scale (ESS) scores in RBD patients as a whole (r=0.10, p=0.34) nor in apathetic RBD patients (r=0.14, p=0.40). Furthermore, there was no evidence for apathy occurring as a result of disturbed sleep itself, since LARS scores did not correlate with polysomnographic measures of sleep

quality (total sleep time: r=-0.04, p=0.76; sleep efficiency: r=0.16, p=0.19; % slow wave sleep: r=0.13, p=0.27).

40 RBD patients (45%) were taking clonazepam at the time of testing as treatment for RBD symptoms. Sedation commonly occurs as a side effect of clonazepam and may conceivably contribute to symptoms of apathy. Although RBD patients taking clonazepam had higher total LARS scores (mean -19.8) than those not taking clonazepam (mean -22.0), this was consistent with chance (p=0.07). Furthermore, RBD patients not taking clonazepam were still significantly more apathetic than healthy controls (p<0.001), and showed comparable levels of apathy to PD patients (p=0.12), meaning that apathy in RBD cannot be fully explained as a side effect of clonazepam.

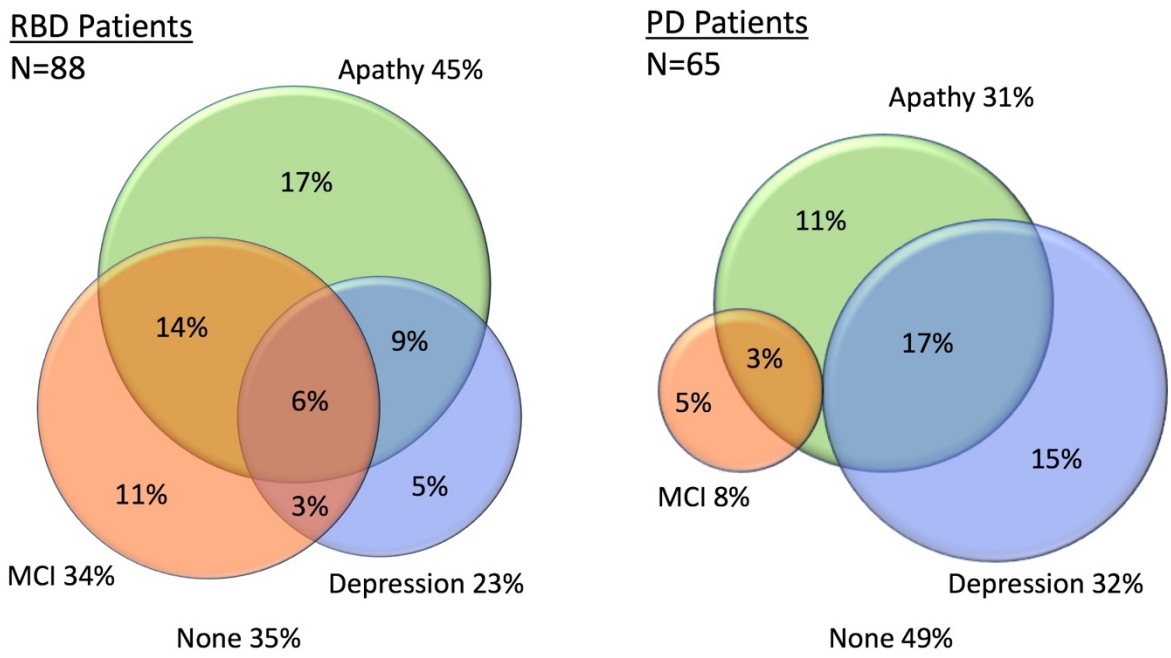


Figure 3. Overlap between apathy, depression and cognitive impairment in RBD (left panel) and PD (right panel) patients. Apathy is defined as LARS score ≥-21; Cognitive Impairment as MOCA <24; Depression as BDI >13.

Dissociation from depression and cognitive impairment

The overlap between apathy, depression and cognitive impairment in RBD and PD patients is shown in Figure 3. Although these features frequently coexisted, there was clear dissociation between them. 33% of RBD patients had one condition alone and crucially two thirds of those with apathy were not depressed. PD patients in this sample actually had less cognitive impairment, but the dissociation between apathy and depression was similar to that seen in RBD.

To explore the relationship between depression and apathy further, we examined the correlation between LARS and BDI scores in the three patients groups. In control participants there was no correlation (r=0.0, p=0.99) whereas moderate correlations were present in RBD (r=0.29, p=0.007) and PD (r=0.46, p<0.001) patients. Amongst apathetic RBD patients, however, there was no significant correlation between the severity of depression and apathy (r=0.10, p=0.53). Moreover, RBD patients with depression were not significantly more apathetic than non-depressed RBD patients (mean LARS scores -19.1 vs -21.6, p=0.12). When individuals with depression (BDI >13) were excluded in a sensitivity analysis, RBD patients remained significantly more apathetic than controls (Table 2).

	Control N=31	RBD N=66	PD N=43	p-value RBD vs Control	p-value RBD vs PD
Age	69.0 (8.88)	67.2 (7.63)	66.6 (4.46)	0.27	0.66
% male	45.2	92.4	72.1	<0.001	0.007
MOCA	28.3 (1.47)	26.5 (2.98)	28.0 (2.25)	**<0.001**	**<0.001**
LARS	-29.0 (4.44)	-21.64 (5.75)	-25.0 (5.63)	**<0.001**	**0.01**
Mild apathy (%)	3.2	39.4	20.9	**0.03**	0.15
Moderate to severe apathy (%)	3.2	9.1	11.6	0.77	0.39

Table 2. Comparison of apathy severity between groups excluding patients with depression. Group comparisons are adjusted for age and gender.

In the PD group, patients with depression were significantly more apathetic than those without (mean LARS -19.9 vs -25.0, p=0.01). When excluding all patients with depression, RBD

patients were significantly more apathetic than PD patients (Table 2). These data suggest that whilst there is an association between apathy and depression, particularly in PD, apathy in RBD patients cannot be explained simply by the presence of depression.

There were weak correlations between LARS scores and MOCA scores in all the groups (control: r=0.20, p=0.27; RBD: r=-0.17, p=0.11; PD: r=-0.17, p=0.17), which were consistent with chance. There was a trend towards more severe apathy in RBD patients with cognitive impairment (MOCA <24) than in those without, but this was also consistent with random variability (mean LARS -19.5 vs -21.8, p=0.08).

	Control N=33	RBD N=88	PD N=65	P-value RBD vs Control	P-value RBD vs PD
Total LARS	-29.1 (4.33)	-21.0 (6.00)	-23.3 (6.85)	**0.002**	0.35
LARS Intellectual curiosity	-3.30 (0.63)	-2.37 (0.96)	-2.29 (1.07)	**0.01**	0.45
LARS Emotion	-2.62 (1.03)	-1.98 (1.13)	-2.32 (1.25)	0.44	0.57
LARS Action initiation	-3.76 (0.47)	-2.77 (1.00)	-3.25 (0.89)	**0.02**	**0.05**
LARS Self-awareness	-3.12 (1.32)	-2.03 (1.52)	-2.97 (1.32)	**0.02**	**0.02**
Mild apathy (≥-21 on LARS)	3.0%	45.5%	30.8%	**0.05**	0.41
Moderate to severe apathy (≥-16 on LARS)	3.0%	15.9%	18.5%	0.76	0.17

Table 3. Apathy scores with group comparisons adjusted for age, gender, BDI and MOCA scores

Despite this evidence of dissociation between apathy, depression and cognitive impairment, there remains some degree of overlap in their clinical features. In order to control for this, we repeated the comparisons between RBD vs PD and RBD vs Controls using a linear regression model that included BDI and MOCA scores as covariates in addition to age and gender. The results of this are shown in table 3. The same group differences in LARS total scores and subdomains were observed using this analysis. To further control for group differences, we

undertook additional sensitivity analyses using two subgroups of patients, the first with subjects matched for age and MOCA scores (table 4), and the second with subjects matched for gender (table 5). The same group differences in apathy scores remained, with RBD patients always significantly more apathetic than controls and of comparable apathy severity to PD patients.

	Control N=28	RBD N=34	PD N=54	p-value RBD vs Control	p-value RBD vs PD
Age	66.5 (7.93)	65.9 (6.51)	66.0 (5.53)	0.70	0.95
% male	42.9	94.1	77.8	**<0.001**	0.06
MOCA	28.3 (1.48)	27.9 (1.09)	28.2 (1.40)	0.45	0.48
BDI	4.71 (5.28)	10.0 (7.73)	10.5 (7.13)	**0.01**	0.75
LARS	-29.1 (4.34)	-22.0 (5.05)	-23.2 (7.22)	**<0.001**	0.47
Mild apathy (%)	3.6	41.2	31.5	**0.02**	0.53
Moderate to severe apathy (%)	3.6	11.8	20.4	0.40	0.24

Table 4. Groups matched for MOCA scores (group comparisons adjusted for age & gender)

	Control N=16	RBD N=88	PD N=50	P-value RBD vs Control	P-value RBD vs PD
Age	70.6 (9.00)	66.9 (7.62)	66.1 (5.78)	0.06	0.56
% male	94	94	94	0.94	0.93
MOCA	28.3 (1.25)	24.9 (3.08)	27.6 (1.95)	**<0.001**	**<0.001**
BDI	4.69 (4.72)	9.40 (7.68)	10.32 (7.06)	**0.03**	0.52
Total LARS	-29.0 (4.94)	-21.0 (6.00)	-22.6 (6.93)	**<0.001**	0.15
Mild apathy %	6.3	45.5	36.0	**0.02**	0.26
Moderate to severe apathy (%)	6.3	15.9	22.0	0.34	0.38

Table 5. Groups matched for gender (group comparisons adjusted for age)

Effect of dopaminergic medication

One potential confounding variable in the Parkinson's disease group is the use of dopaminergic medication. Dopamine receptor agonists in particular have been shown to reduce apathy and may therefore contribute to lower apathy ratings in the PD participants[323]. Table 6 shows the same variables as table 1, with Parkinson's patients divided into 3 groups according to treatment: drug naïve, those taking dopamine receptor agonists (with or without L-dopa) and those taking L-dopa without agonists. PD patients taking dopamine receptor agonists (DAs) were less apathetic than drug naïve patients and those taking L-dopa, though there was no significant effect of treatment type (Kruskal-Wallis test across PD patients by treatment type, p=0.21). However, PD patients taking DAs were significantly less apathetic than RBD patients (mean LARS -24.4 vs -21.0, p=0.003).

	Control N=33	RBD N=88	PD Drug naive N=10	PD Agonist +/-L-dopa N=26	PD L-dopa without DA N=26	p-value across 3 PD groups	p-value RBD vs PD patients not taking DA
Age	68.4 (8.94)	66.9 (7.62)	64.8 (7.19)	65.8 (5.70)	67.3 (5.10)	0.37	0.98
% male	45.5	94.3	50	69	81	0.18	**0.003**
MOCA	28.3 (1.44)	24.9 (3.08)	25.9 (3.14)	28.3 (1.93)	27.7 (2.09)	**0.04**	**<0.001**
BDI	4.91 (4.91)	9.40 (7.68)	11.1 (7.79)	9.0 (7.07)	11.0 (6.59)	0.44	0.29
Total LARS	-29.1 (4.33)	-21.0 (6.00)	-23.2 (7.61)	-24.4 (6.84)	-21.8 (6.50)	0.21	0.39
LARS Intellectual curiosity	-3.30 (0.63)	-2.37 (0.96)	-2.53 (1.24)	-2.37 (0.94)	-2.12 (1.12)	0.42	0.63
LARS Emotion	-2.62 (1.03)	-1.98 (1.13)	-1.85 (1.55)	-2.56 (1.40)	-2.17 (0.94)	0.10	0.79
LARS Action initiation	-3.76 (0.47)	-2.77 (1.00)	-3.25 (0.95)	-3.48 (0.66)	-3.00 (1.07)	0.28	0.23
LARS Self-awareness	-3.12 (1.32)	-2.03 (1.52)	-2.90 (1.29)	-2.88 (1.48)	-2.96 (1.25)	0.97	**0.006**
Mild apathy	3.0%	45.5%	30%	19%	42%	0.20	0.74
Severe apathy	3.0%	15.9%	20%	12%	27%	0.37	0.16

Table 6. Distribution of apathy scores in PD patients according to treatment type

To remove any effect of DAs on overall group apathy ratings, we performed a comparison between RBD and PD patients excluding those taking DAs (table 6). There remained no significant difference in overall LARS scores. With respect to the whole group comparison, the difference in action initiation subscores was no longer significant, but RBD patients remained significantly worse in the self-awareness subscore. We conclude that some of the apparent differences between RBD and PD may reflect dopaminergic therapy (making the PD group appear less apathetic), but even without the effect of DAs, RBD patients are at least as apathetic as PD patients.

Within the PD group, there was evidence of an association between apathy and disease severity: UPDRS motor scores (part III) were positively correlated with LARS scores ($r=0.29$, $p=0.02$) and this effect remained after adjusting for age ($p=0.02$). There was no such relationship in RBD patients ($r=0.03$, $p=0.75$), but since these patients do not have significant motor Parkinsonism, UPDRS III is not a sensitive measure of prodromal disease stage.

RBD neuroimaging subgroup

Clinical characteristics

The mean age of the RBD patients included in the neuroimaging subgroup was 65.1 years (SD 7.60) and 42 patients were male. The mean duration of RBD symptoms was 8.7 years (SD 7.34) and the mean time from polysomnographic diagnosis was 2.5 years (SD 2.27).

The mean LARS score of the RBD subgroup was -21.0 (SD 6.69) and 18 patients (42%) scored in the apathetic range of the LARS. Since apathy itself might be a marker of emerging Parkinsonism, we compared a number of other clinical variables according to apathy status to investigate whether apathetic patients had more extensive signs of prodromal disease (table 7). Postural hypotension was more severe in *non*-apathetic patients, but the significance of this did not reach the Bonferroni adjusted threshold of $p<0.006$. There was no difference in the degree of depression, anxiety, cognitive impairment, hyposmia, daytime sleepiness, or the rate of antidepressant use between apathetic and non-apathetic patients.

These data suggest that apathetic patients in the imaging subgroup were on average not at a more advanced prodromal stage than non-apathetic patients.

Variable	Non-apathetic patients N = 25	Apathetic patients N = 18	p-value
Age, mean (SD), years	66.5 (6.92)	63.1 (8.18)	0.15
Male, number (%)	24 (96%)	18 (100%)	0.39
Number treated with antidepressants, (% of group)	3 (12%)	5 (28%)	0.19
Depression, mean (SD), BDI score	8.6 (7.15)	10.2 (10.57)	0.57
Cognition, mean (SD), MOCA score	26.0 (2.72)	24.9 (3.06)	0.26
Orthostatic systolic hypotension, mean (SD), mmHg	-10.6 (11.56)	-2.1 (15.06)	**0.04**
Olfaction, mean (SD), Sniffin Sticks score	7.0 (3.71)	7.7 (3.30)	0.50
Motor impairment, mean (SD), MDS-UPDRS III score	5.2 (4.08)	3.5 (2.57)	0.14
Anxiety, mean (SD), HADS score, anxiety component	5.76 (4.25)	5.2 (4.52)	0.67
Daytime sleepiness, mean (SD), ESS score	7.2 (4.77)	5.9 (4.61)	0.41

Table 7. Comparison of clinical variables between apathetic and non-apathetic RBD patients in the neuroimaging subgroup

Neuroimaging data

Apathetic patients had significantly lower ^{123}I-ioflupane signal in the DRN than non-apathetic patients (mean 1.45 vs 1.70, p=0.005), whereas the ^{123}I-ioflupane signal did not differ in any other region of interest (table 8). Across all patients there was a significant negative correlation between apathy severity and ^{123}I-ioflupane signal in the DRN ($r = -0.55$, $p < 0.001$), indicating that greater apathy was associated with reduced serotonin signal (figure 4A). To exclude a possible confounding effect of antidepressant medication, we conducted a sensitivity analysis excluding the 8 participants treated with antidepressant medication. The same association between apathy and DRN signal was observed ($r = -0.51$, $p = 0.002$, figure 4B).

Region of interest specific uptake ratio	Non-apathetic patients N = 25	Apathetic patients N = 18	p-value
Dorsal Raphe nucleus, mean (SD)	1.70 (0.259)	1.45 (0.277)	**0.005**
Median Raphe nucleus, mean (SD)	1.79 (0.344)	1.71 (0.293)	0.42
Ventral Tegmental Area, mean (SD)	2.35 (0.536)	2.28 (0.581)	0.67
Right Accumbens nucleus, mean (SD)	3.74 (0.393)	3.94 (0.792)	0.34
Left Accumbens nucleus, mean (SD)	4.26 (0.467)	4.40 (0.676)	0.45
Right Caudate nucleus, mean (SD)	2.84 (0.383)	2.89 (0.417)	0.70
Left Caudate nucleus, mean (SD)	3.09 (0.377)	3.07 (0.389)	0.90
Right Putamen, mean (SD)	4.21 (0.381)	4.18 (0.686)	0.84
Left Putamen, mean (SD)	4.10 (0.462)	4.31 (0.693)	0.24
Right Substantia Nigra, mean (SD)	2.29 (0.394)	2.12 (0.545)	0.24
Left Substantia Nigra, mean (SD)	2.11 (0.353)	2.11 (0.539)	1.0

Table 8. Comparison of ioflupane SPECT signal between apathetic and non-apathetic RBD patients, across a range of regions of interest

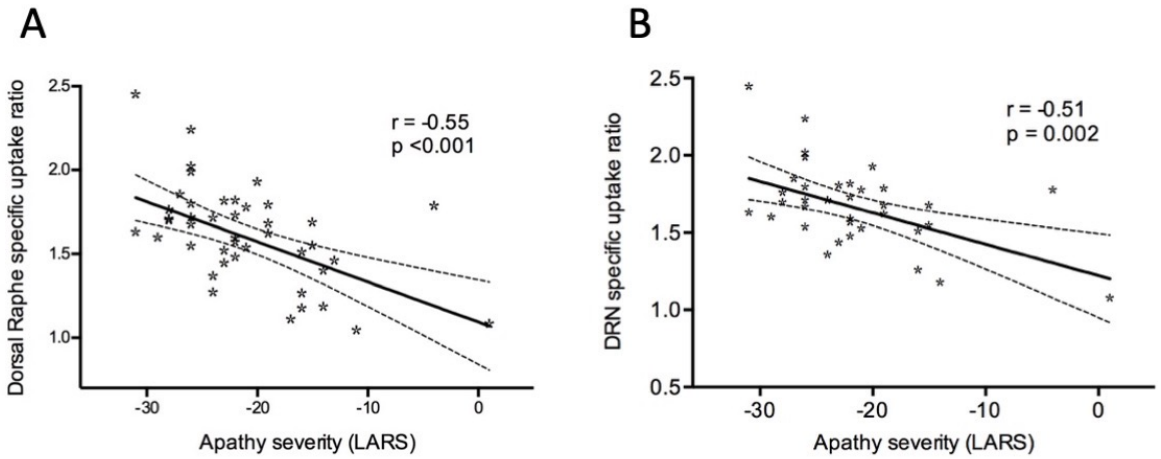


Figure 4. A: A significant correlation is demonstrated between apathy severity, measured by the Lille Apathy Rating Scale, and 123I-ioflupane SPECT/CT signal in the DRN. B: The same association is observed when excluding patients being treated with SSRI medication. Dashed lines indicate the 95% confidence interval of the best fit line

Adjusting for signal spill-over from surrounding regions

Figure 5A below shows a midline sagittal image of the group average ioflupane signal, overlaid on a standard space structural MRI image. It can be seen that the area of peak signal in the brainstem is situated largely in the midbrain, slightly anterior and rostral to the DRN. In order to ensure that the signal extracted from our DRN was not influenced by spill-over from this nearby peak, we calculated the mean signal from this region, shown in figure 5B. We then undertook a multiple regression analysis with apathy severity as the dependent variable and signal from this region as a covariate, along with the DRN region of interest.

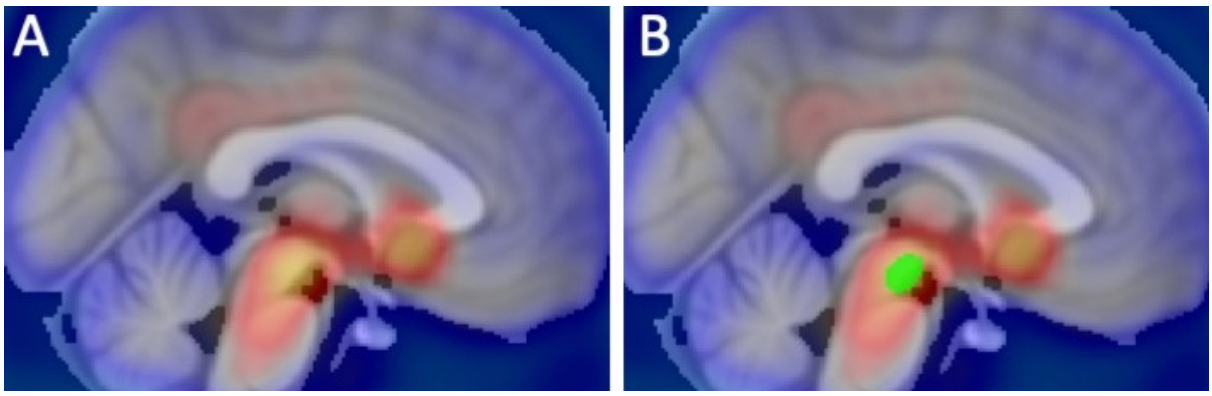


Figure 5. A: midline sagittal view of the average ioflupane signal across all participants B: region of interest representing the peak voxels from the area of high signal in the midbrain is shown in green.

There was no direct correlation between the midbrain peak region and apathy severity ($r = -0.02$, $p = 0.88$).

In the multiple regression analysis including the DRN and midbrain peak ROIs as covariates, the association between lower DRN signal and increased apathy severity was stronger (beta = -0.70, $p<0.001$) than seen with the simple correlation analysis. This is because the effect of the midbrain peak ROI in the regression analysis was in the opposite direction, with *increased* signal in this region predicting greater apathy severity (beta = 0.32, $p = 0.03$).

Given the univariate result and biological considerations, it is unlikely that preserved dopaminergic transmission leads to more severe apathy. However, the net result is that spill-over of signal from the midbrain to the DRN, albeit numerically small, leads to underestimation of the raphe serotonergic deficit. Thus, we can conclude that the association

seen between DRN signal and apathy severity is indeed not caused by spill-over from the adjacent high signal region in the midbrain.

Region of interest specific uptake ratio	Pearson correlation coefficient with apathy severity (LARS) across all patients (n=43)	p-value
Dorsal Raphe nucleus	-0.55	**<0.001**
Median Raphe nucleus	-0.25	0.11
Ventral Tegmental Area	-0.02	0.91
Right Accumbens nucleus	0.18	0.28
Left Accumbens nucleus	0.16	0.30
Right Caudate nucleus	0.12	0.46
Left Caudate nucleus	0.17	0.27
Right Putamen	0.07	0.66
Left Putamen	0.17	0.27
Right Substantia Nigra	-0.02	0.88
Left Substantia Nigra	-0.08	0.62

Table 9. Correlation between apathy severity and ioflupane SPECT/CT signal in regions of interest

Association between apathy and other monoaminergic regions

To investigate whether the association between apathy and DRN signal was explained by a general reduction in brain monoamine transporters in apathetic patients, we looked at the relationship between apathy and ^{123}I-ioflupane signal in the median raphe nucleus and a number of dopaminergic regions (table 9). LARS scores did not correlate significantly with ^{123}I-ioflupane signal in any of these regions. This specific association between apathy and signal in the DRN suggests that apathy is not simply a marker of generalised prodromal neurodegeneration.

Variable	Correlation with Dorsal Raphe Nucleus across all patients (n=43)	p-value
Apathy (LARS score)	-0.55	**<0.001**
Depression (BDI score)	-0.25	0.10
Anxiety (HADS score)	-0.07	0.66
Cognition (MOCA score)	0.25	0.11
Motor impairment (MDS-UPDRS III score)	0.19	0.22
Daytime sleepiness (ESS score)	-0.10	0.51
Orthostatic Systolic BP drop	-0.27	0.08
Olfaction (Sniffin Sticks score)	-0.08	0.65
Age	0.02	0.92

Table 10. Correlation between dorsal raphe SPECT/CT signal and clinical variables

Association between DRN signal and other neuropsychiatric features

We next assessed the degree to which the relationship between DRN serotonin signal and apathy was dissociable from other Parkinsonian symptoms. Of particular interest were comparisons with depression and cognitive impairment respectively, as these syndromes exhibit considerable phenotypic overlap with apathy. Table 10 shows correlations between signal in the DRN and secondary clinical measures of interest. None of these correlations reached significance (Bonferroni adjusted significance threshold, $p < 0.006$). Signal in the dorsal raphe did not correlate with age ($r = 0.02$, $p = 0.92$).

To further dissociate the closely-related neuropsychiatric features of apathy, depression and cognitive impairment, we used multiple linear regression, with dorsal raphe ^{123}I-ioflupane signal as the dependent variable and LARS, BDI and MOCA scores as predictors. Only LARS scores had a significant effect on dorsal raphe signal (beta = -0.50, $p = 0.001$), whereas BDI scores (beta = -0.17, $p = 0.20$) and MOCA scores (beta = -0.09, $p = 0.51$) did not. Thus apathy, but not depression or cognitive function, appeared to be significantly and specifically associated with ^{123}I-ioflupane signal in the DRN.

Discussion

Apathy is common and under-recognised in RBD

In the first study to quantify the extent and severity of apathy in RBD, we have shown that the condition is both common and under-recognised. Approximately half of all RBD patients in this cohort were affected by apathy, a prevalence similar to that seen in established Parkinson's disease in our sample. The presence of apathy in RBD was not explained by excessive daytime sleepiness, poor sleep quality or sedative medication. Amongst subdomains of apathy, all were impaired in RBD except emotional responses, suggesting that problems with motivation for action, intellectual curiosity and self-awareness, rather than affect, predominate in RBD patients.

Our data suggest that the single screening question for apathy in the UPDRS assessment has modest sensitivity, capturing only one third of all apathetic RBD patients and just half of those with moderate to severe apathy, and a relatively weak positive likelihood ratio. This is in contrast to the UPDRS screening question for depression, which was 80% sensitive in this study. There are a number of possible explanations for this. Firstly, the UPDRS question asks only about 'indifference to doing activities or being with people' and therefore may not capture other subdomains of apathy[132]. Secondly, apathy might be associated with reduced insight compared to depression as a result of impaired self-awareness, which would limit the sensitivity of self-report measures. Finally, it may be accounted for in part by the high rate of cognitive impairment in RBD, since studies of apathy in other populations have shown discordance between self-report and clinician-rated measures of apathy in cognitively impaired individuals[334].

Apathy is dissociable from other neuropsychiatric features

Although there is significant overlap between apathy, depression and cognitive impairment, these are clearly dissociable: only 33% of apathetic RBD patients were depressed and less than half had cognitive impairment. Furthermore, differences in the degree of apathy between patient groups could not be explained by differences in cognition and depression.

This evidence of apathy as a distinct neuropsychiatric syndrome highlights the importance of actively looking for apathy even when other neuropsychiatric features are absent. It also raises the possibility that where apathy coexists with depression and/or cognitive impairment, these are distinct manifestations of a common underlying neurodegenerative process rather than a single neuropsychiatric condition.

The fact that RBD patients were as apathetic as those with PD – in our sample – mirrors the findings presented in chapter 2, where we demonstrated that a wide range of non-motor features are at least as severe in RBD as they are in newly diagnosed PD. As discussed, this is not inconsistent with RBD patients being at an earlier neurodegenerative stage. Accumulating evidence suggests that PD patients who develop RBD first might represent a distinct subtype of disease with a more severe non-motor phenotype, and that many of these non-motor features emerge prior to the onset of motor parkinsonism[35,85]. In a separate study of established Parkinson's patients, apathy was shown to be significantly more common in PD patients with concomitant RBD than in those without[141]. The co-occurrence of RBD and other non-motor features might be a consequence of more widespread Lewy Body pathology in these patients, affecting brain regions involved in the regulation of sleep, cognition and mood in addition to motor control[335].

Apathy in RBD is associated with serotonin depletion

In our imaging sub-study, we demonstrate for the first time a relationship between serotonin loss in the dorsal raphe nucleus (DRN) and clinical apathy in patients with RBD. Serotonergic dysfunction is associated with apathy early in Parkinson's disease[108]; our data extend this important finding by revealing that this association emerges prior to the onset of clinical Parkinsonism. Importantly, neither depression, anxiety, nor cognition correlated with the DRN serotonin signal, further emphasising apathy as a distinct neuropsychiatric entity.

A key dissociation was also seen between DRN serotonergic signal and dopaminergic binding in the basal ganglia. The lack of correlation between dopaminergic signal and apathy not only implies that serotonergic degeneration might be a more important factor in the pathogenesis of apathy, but also suggests that the association is not explained simply by more advanced

prodromal neurodegeneration in apathetic patients. This assertion is further supported by the finding that apathetic patients did not have more severe clinical markers of evolving Parkinsonism than non-apathetic patients.

Whilst patients with idiopathic RBD can offer an unprecedented insight into the early stages of Parkinsonian disorders, they are a heterogeneous population. Around two thirds are likely to develop Parkinson's disease or DLB, while a smaller number will convert to Multiple System Atrophy[68]. In some cases, however, this may occur more than a decade from RBD onset, and a minority of individuals may never develop a neurodegenerative disorder[56]. The fact that an association between DRN serotonergic signal and apathy was observed despite this heterogeneity suggests that the role of serotonin in apathy might be a particular feature of patients with RBD. One possible explanation for this is the close anatomical proximity of the raphe nuclei to the locus coeruleus/subcoeruleus complex, an important player in the regulation of REM sleep. The arrival of ascending neurodegeneration in tandem in these neighbouring structures during prodromal disease would be consistent with Braak's hypothesis of Parkinson's pathology[3], and it may be that the extent to which this occurs underlies the correlation between raphe signal and apathy observed in RBD.

Apathy may be an idiosyncratic feature of RBD rather than a prodromal progression marker

The association between apathy and severity of motor disease in PD patients suggests that in established PD apathy may be related to disease progression. However, the dissociation between apathy and dopaminergic decline in RBD patients implies that this may not be the case in prodromal disease. Rather than being a harbinger of emerging parkinsonism, serotonin-dependent apathy may be an example of the way in which the differential involvement of neurotransmitter systems amongst patients, due to subtle variations in the anatomical pattern of disease, might explain some of the phenotypic variation observed in synucleinopathies. In patients with established Parkinson's disease, for example, it is known that motor signs relate to nigro-striatal dopamine loss[157], whilst noradrenergic pathways are implicated in the development of postural hypotension[336]. Our data suggest that patients

with a greater burden of disease in serotonergic pathways may represent a subtype prone to developing apathy.

Though no other studies have examined the relationship between serotonergic signalling and apathy in RBD, the possibility that disruption in these pathways affects a subtype of apathetic patients rather than being a general marker of neurodegeneration is supported by a recent study of patients with early Parkinson's, which used PET with multiple tracers to further dissociate the roles of serotonin and dopamine[108]. No differences were observed in serotonin signal between non-apathetic Parkinson's patients and controls, despite significantly reduced dopaminergic uptake in the Parkinson's disease group. Within Parkinson's patients, however, reduced serotonin signal was associated with the presence of apathy.

Apathy may not be the only consequence of raphe serotonergic degeneration. Using similar methodology to ours in a large Parkinson's disease cohort, a recent study also demonstrated that serotonin depletion affected a subgroup of patients and was dissociable from dopaminergic degeneration[331]. Apathy was not assessed, but an association was found between raphe signal and tremor severity. Given that the whole raphe complex was included in their region of interest, this result may reflect the role of other subpopulations of serotonergic neurons, including more caudal projections to the cerebellum and deep brain nuclei implicated in the pathogenesis of tremor.

It is unlikely that serotonin signalling alone underlies the complex clinical syndrome of apathy in Parkinsonian disorders. Exogenous dopamine (or dopamine receptor agonists) can not only improve the symptoms of apathy, but also modulate objective measures of reward sensitivity, a key component of motivation for action[101,323]. It is possible that the differential contributions of serotonin and dopamine loss to the expression of apathy in Parkinson's disease may vary according to disease stage, with mesolimbic dopaminergic pathways affected later than the raphe serotonergic system. This might have important implications for the treatment of apathy in patients with prodromal and early Parkinson's, where serotonergic agents could conceivably have greater efficacy. Interventional studies testing such hypotheses must be a near-term priority since little evidence exists to guide the specific treatment of apathy.

Limitations of the study

In the comparisons of clinical variables between control, RBD and PD participants, accurately matching across all three groups for age, gender, depression and cognition was not feasible given the way these variables are differentially distributed amongst the populations studied. RBD is diagnosed predominantly in males[68], and it is expected that measures of cognition and depression will be worse in RBD than controls[62,337]. The fact that our RBD group had lower MOCA scores than the PD group is perhaps more surprising, but it should be noted that RBD patients are as likely to develop Dementia with Lewy Bodies as Parkinson's, and the extent of cognitive impairment in RBD patients may reflect this[338]. We have controlled as much as possible for the group differences by including all variables in our regression model where appropriate, and by performing sensitivity analyses, but it is still possible that some of the variation in apathy is accounted for by differences in these other characteristics.

One of the main limitations of our imaging study is that the nuclei studied are small and therefore close to the resolution of our SPECT imaging. This emphasizes the importance of the CT-based attenuation correction that we employed for SPECT/CT processing, which, as discussed in detail in chapter 4, increases the precision with which signal from small brainstem structures can be measured. The accuracy of our brainstem nuclei measurements was further enhanced by the use of histologically derived regions of interest, giving us the best possible representations of their true anatomy.

A second limitation is the use of a relatively non-specific SPECT ligand, meaning that our assessment of the serotonergic system is limited to regions that do not contain significant numbers of dopamine transporters. We were therefore not able to demonstrate serotonergic correlates of apathy in the basal ganglia, for example, as others have done with more specific tracers[108].

Although the lack of a SPECT imaging control group could be considered a limitation, the primary aim of our study was to explore the neural basis for the phenotypic heterogeneity that occurs amongst RBD patients, rather than to show how they differ from control subjects. It is interesting to note, however, that control subjects rarely exhibit apathy despite having very similar levels of raphe serotonin transporters to Parkinson's patients[331]. One possible

explanation for this apparent discrepancy would be that individuals may have different levels of intrinsic reserve in the raphe serotonergic system, which might lead to different levels of clinical apathy only when the system is exposed to a neurodegenerative insult.

Summary and future directions

We have shown that apathy is common in RBD patients and under-recognised by a simple self-report screening question. It is dissociable from other neuropsychiatric features, in particular depression, which is present in less than half of apathetic RBD patients. These findings underline apathy as a major and distinct component of prodromal parkinsonian disorders. Our imaging findings demonstrate that apathy in RBD is not associated with dopaminergic decline, but is strongly related to serotonergic depletion in the dorsal raphe nucleus, suggesting that the mechanism of apathy in prodromal disease is dissociable from nigro-striatal disease progression. Future work exploring the mechanisms by which serotonin modulates motivation for goal-directed behaviour will be important in understanding how disruptions in this lead to apathy in RBD patients. Investigation of the potential of pharmacological modification of serotonergic transmission to alleviate apathy must also be a near-term priority.

Chapter 8: Reward insensitivity, clinical apathy and dopaminergic decline in RBD

Introduction

The capacity of subcortical neural networks to compensate for nigro-striatal neurodegeneration means that the progressive dysfunction and loss of these dopaminergic neurons during the prodromal phase of Parkinson's disease is accompanied by few overt clinical signs. Although a wide range of non-motor symptoms commonly develops in the years preceding motoric disease, the majority of these are not thought to be dopamine-dependent[277]. As we have described in chapter 4, RBD patients with abnormal dopaminergic imaging are no different from those with normal imaging across a range of clinic-based measures of prodromal features[279]. The lack of a specifically dopaminergic clinical phenotype during prodromal disease is a problem, however, because when measured, dopamine depletion is one of the strongest predictors of conversion to a clinically overt synucleinopathy[68].

Reward insensitivity in Parkinson's disease

One non-motor feature that has been linked to dopamine dysfunction in patients with established Parkinson's is the impairment of sensitivity to reward[339,340]. In PD patients, there is evidence that this reward insensitivity contributes to the expression of clinical apathy[101]. Although the evidence presented in chapter 7, alongside studies in newly-diagnosed PD patients[108], suggest that serotonergic mechanisms may play a more important role in apathy in prodromal and early manifest Parkinson's, it is possible that dopamine dysfunction is also involved through its role in regulating the evaluation of rewards.

Evidence of the role of midbrain dopaminergic projections in the brain's response to rewarding stimuli is well established[100]. Responses to reward can be modulated by exogenous dopamine in PD patients both with and without clinical apathy[101]. However, the fact that dopaminergic medications can ameliorate reward insensitivity does not necessarily mean

that dopamine depletion is the underlying cause; in fact, a direct relationship between reward sensitivity and dopamine availability in the brain has not previously been demonstrated. Furthermore, it is not known whether this potentially dopaminergic characteristic emerges during prodromal disease, at the earliest stages of nigrostriatal degeneration.

We have seen in previous chapters not only that patients with REM sleep behaviour disorder (RBD) have a high long-term risk of conversion to overt synucleinopathy, but also that around half already show evidence of dopaminergic dysfunction in the basal ganglia when imaged with ^{123}I-ioflupane single photon emission computed tomography (SPECT)[169]. RBD patients therefore offer an opportunity to investigate the relationship between reward sensitivity and dopamine depletion, in the absence of the potentially confounding effect of dopaminergic medications.

Measuring reward sensitivity

An individual's sensitivity to reward can be indexed objectively by measuring pupillary responses to auditory cues[340]. This technique is based on the observation that pupils dilate to rewarding stimuli and that the magnitude of the physiological response increases with the level of the potential reward on offer. Studies in healthy control subjects, as well as those with established Parkinson's disease, have shown this to be a useful method of quantifying reward sensitivity, which appears to be independent of autonomic dysfunction[101,340].

The potential role of other neurotransmitter systems

Another mechanism that may influence pupillary responses to rewarding stimuli is the brainstem noradrenergic system[341,342]. Projections from the locus coeruleus are involved in general arousal mechanisms and the response to salient stimuli, and there is evidence that activity in the locus coeruleus relates to pupil diameter[343]. The locus coeruleus is involved in early Parkinsonian degeneration to at least as great an extent as the substantia nigra and deficits in this system have been shown to relate to clinical non-motor phenotype[148,150,344]. Interactions are also present between noradrenergic and dopaminergic pathways[342,344], and it is conceivable therefore that the locus coeruleus may be involved not only in dysfunctional reward processing but also in the response to exogenous dopaminergic modulation. Markers

of degeneration in the locus coeruleus are specifically related to RBD, in patients with and without parkinsonism[46,150], but their association with pupillary responses has never been studied.

This study was designed to investigate the relative effects of dopaminergic and noradrenergic degeneration on pupillary responses to reward in patients with RBD. Dysfunction in these two systems was quantified using ioflupane (DaT) SPECT and neuromelanin-sensitive MRI, respectively. We hypothesised that the effects of these would be dissociable, with deficits in sensitivity to reward magnitude related specifically to dopamine availability rather than a consequence of generalised neurodegeneration. As such, we proposed that measurements of pupillary reward sensitivity might be a marker of prodromal dopaminergic decline.

Methods

Subjects

41 Patients with RBD were recruited prospectively from the Discovery cohort of the Oxford Parkinson's Disease Centre (Chapter 2). All RBD patients underwent pupil reward testing and DaT SPECT brain imaging. 35 of the RBD patients also underwent neuromelanin-sensitive MRI. The data in this chapter from patients with Parkinson's disease and healthy controls was collected separately and has been previously published. It was not acquired specifically for the present study, though the protocol used was identical. The data are used here for comparison of the ocular measures only. PD and control participants did not undergo neuroimaging. PD patients were tested in the 'off' state, with L-dopa having been withdrawn overnight.

Apathy was measured using the Lille Apathy Rating Scale (LARS, chapter 7).

Eye tracking paradigm

Eye tracking was assessed using an identical paradigm to that described by *Muhammed et al*[101] (Figure 1). Briefly, the method uses an infra-red device to measure saccadic velocity and changes in pupil diameter in response to an on-screen task. The task involves repeated trials

where the subject is required to make a saccadic eye movement in response to a visual cue. For each trial, the subject has the possibility of winning a monetary reward up to a specified maximum value. The actual reward received is a percentage of the maximum calculated according to reaction time such that faster performance results in a higher percentage of the maximum reward.

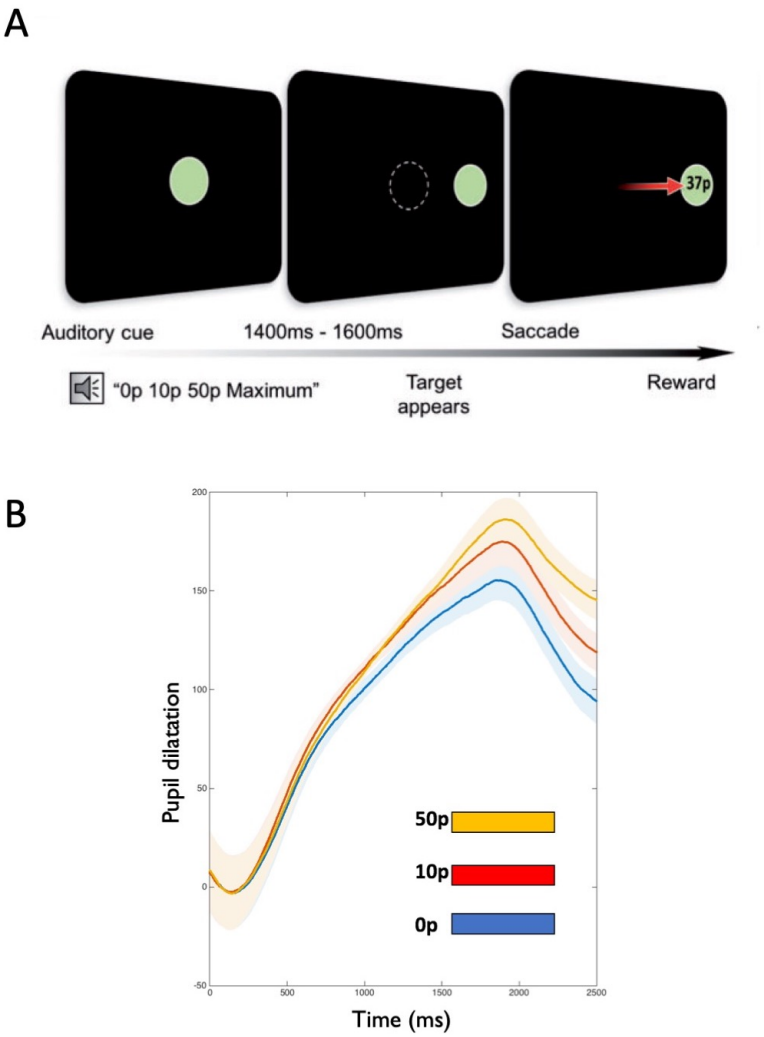


Figure 1. A: schematic representation of the eye tracking task (adapted from Muhammed *et al*)[101]. B: example pupil traces from a healthy control participant. Pupil dilation is plotted against time from auditory reward cue. The degree of pupil dilatation increases with the magnitude of the reward.

The subject first fixates on a disc in the centre of the screen, at which point baseline pupil size is measured. After 500 ms, the participant hears a recorded voice informing them of the

maximum reward available for that trial, which is either 0p, 10p or 50p. After a randomly variable delay of 1400-1600 ms, the central disc is replaced by a new target that appears randomly either to the left or right, at 11 degrees eccentricity, to which the subject must redirect their gaze. The percentage of the maximum reward obtained is determined by the reaction time for the saccadic movement, and displayed on the screen at the end of each trial. Importantly, this is continuously adjusted according to the individual's average reaction time over the previous 20 trials so that all participants received equal reward amounts.

Participants performed 270 trials in 5 blocks of 54, with 90 trials in total at each of the three reward levels.

Calculation of eye-tracking metrics

Processing of the raw eye-tracking data for the newly-acquired RBD data and calculation of the variables of interest was kindly performed by Dr Kinan Muhammed, using the same protocol as that used previously for the PD and control participants[101]. The following variables were provided for each participant, in addition to the average baseline pupil size:

- **Reaction time**: The time from target onset to completion of a saccade.
- **Peak saccadic velocity**: the maximum velocity during a saccade.
- **Pupil dilatation**: the proportional change from average baseline pupil size during the period 1400-2400 ms after the auditory reward cue.

For all three variables, the average value of trials at each of the three reward levels (0p, 10p and 50p) was calculated for each participant.

The single variable of 'reward sensitivity' was calculated as the difference in the average response at 50p to the average response at 0p.

Neuroimaging

SPECT imaging was acquired, processed and analysed as described in chapter 4. MRI-based registration was not used in the analysis of SPECT. Specific uptake ratios were calculated using Hermes software. Dichotomisation of RBD patients into those with normal or abnormal imaging was based on the descriptive reports by Dr Kevin Bradley, consultant radiologist. As previously, only definitely abnormal scans were classed as abnormal; those with borderline findings were included in the normal category.

Acquisition, processing and analysis of neuromelanin-sensitive MRI for the calculation of locus coeruleus metrics was performed as described in chapter 5.

Statistical methods

Two approaches were used to analyse the variation in pupil responses according to reward level. Firstly, a repeated-measures ANOVA was used with the three different reward levels (0p, 10p and 50p) as the within-subjects factor, and group categories as between-subjects factors. Secondly, linear regression was performed using the 'reward sensitivity' metric described above as the dependent variable, and either group category or variable of interest (e.g. DaT SPECT ratio) as independent predictor variables.

Baseline pupil size could potentially have a confounding effect on the analyses, since pupils that are smaller at baseline have a greater potential for proportional change. We therefore included baseline pupil size as a covariate in all between-groups analyses, as well as age and gender to control for any effect of these variables.

Results

Baseline characteristics

The baseline characteristics of the participants, and apathy severity scores are shown in table 1. The RBD group had a higher proportion of males than the control and PD groups, but the three groups were well matched for age. As discussed in chapter 7, RBD and PD patients were significantly more apathetic than control subjects (t-test RBD vs controls, $p < 0.001$; PD vs controls, $p < 0.001$), and there was no difference in overall apathy severity between RBD and PD patients ($p = 0.48$), nor between RBD patients with normal versus abnormal DAT SPECT imaging ($p = 0.94$).

Pupil responses

Average *baseline* pupil size (ANOVA, $p = 0.61$) and average pupil proportional change across all three reward levels (ANOVA, $p = 0.10$) did not differ significantly between the three groups

of participants, suggesting that resting autonomic tone at the pupillary muscles and general pupil responsiveness were no different between controls, RBD patients and PD patients.

	Controls N = 41	All RBD patients N = 41	RBD normal DAT N = 23	RBD abnormal DAT N = 18	PD patients N = 40
Age (years)	64.8 (10.25)	65.2 (7.71)	65.7 (8.87)	64.4 (6.09)	66.4 (5.91)
Sex (% male)	61	98	96	100	65
LARS score	-28.8 (4.06)	-21.3 (5.89)	-21.2 (5.23)	-21.3 (6.76)	-22.4 (8.13)

Table 1. Baseline characteristics of the included participants.

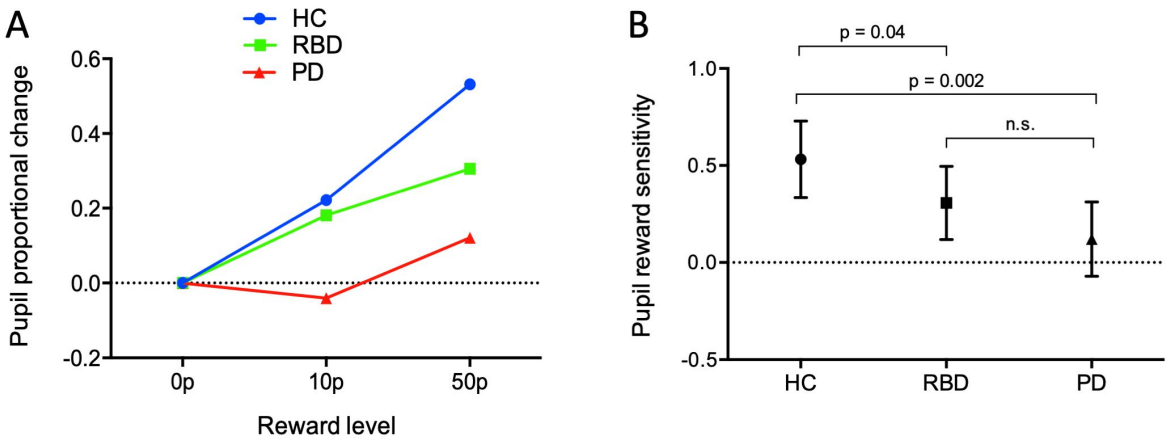


Figure 2. A: pupil dilation according to reward level in healthy controls, RBD and PD patients. B: reward sensitivity across the groups, calculated as the difference in pupil response to 50p and 0p.

Figure 2A illustrates the proportional pupil changes at each reward level across the three groups of participants, with each group normalised to the 0p level. Examination of each group separately in a repeated measures ANOVA, revealed a significant effect of reward level on pupil proportional change in the controls [$F(1.8,73.3) = 16.9$, $p < 0.001$] and RBD patients

[F(1.8,73.7) = 6.8, p = 0.003], but not in PD patients [F(1.5,56.6) = 2.5, p = 0.10]. After controlling for age, gender and average pupil size, there was a significant group by reward level interaction [F(3.6,209.9) = 4.5, p = 0.002).

Figure 2B demonstrates this using the reward sensitivity measure (the difference between pupil response at 0p and 50p levels). A stepwise decline in reward sensitivity is observed from controls to RBD patients and from RBD to PD patients. In PD patients, the 95% CI crosses zero, indicating that their pupils were no more responsive to 50p than to 0p. Pairwise comparisons between the groups (adjusting for age, gender and average pupil size) showed that reward sensitivity was significantly lower in RBD patients than controls and significantly lower in PD patients than controls but not significantly different between RBD and PD patients.

Effect of dopaminergic decline on reward sensitivity in RBD patients

Eighteen out of 41 RBD patients had DAT SPECT imaging that was considered abnormal by the reporting radiologist, and 23 had normal imaging. The latter category included 5 individuals with borderline findings that were not definitely abnormal. This expert assessment takes into account factors such as the pattern of distribution of the SPECT signal and the expected signal for the participant's age, as well as the absolute signal values. Nevertheless, as can be seen in Figure 3, patients with imaging classed as abnormal had significantly lower dopaminergic signal in the basal ganglia than those with normal imaging.

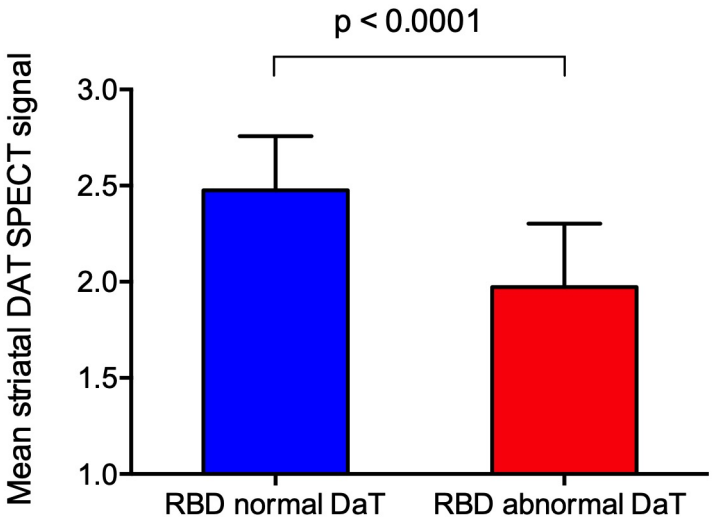


Figure 3. Mean striatal DAT SPECT signal in RBD patients classified according to normal or abnormal in blinded assessment by a radiology consultant.

We first analysed the effect of dopaminergic depletion on pupillary reward sensitivity in RBD patients using this dichotomised outcome. Figure 4 shows the same variables as Figure 2, but with the RBD patients split according to whether their DaT SPECT imaging was normal or abnormal.

In a repeated measures ANOVA within the RBD patients, there was a significant reward level by DaT SPECT group interaction [$F(1.9,69) = 4.4$, $p = 0.017$], indicating that the differential response to reward level is affected by DaT SPECT abnormality. Examination of each group of RBD patients separately, there was a significant effect of reward level in the normal DaT SPECT group [$F(1.8,39.2) = 10.0$, $p < 0.001$] but not in the RBD patients with abnormal DaT SPECT imaging [$F(2.0,33.6) = 0.2$, $p = 0.83$].

Using the reward sensitivity metric, RBD patients with abnormal DaT SPECT imaging had significantly lower reward sensitivity than those with normal imaging (Figure 4B). Whilst RBD patients with abnormal imaging were comparable to PD patients in showing no difference in pupil response between 0p and 50p, RBD patients with normal imaging were indistinguishable from healthy controls.

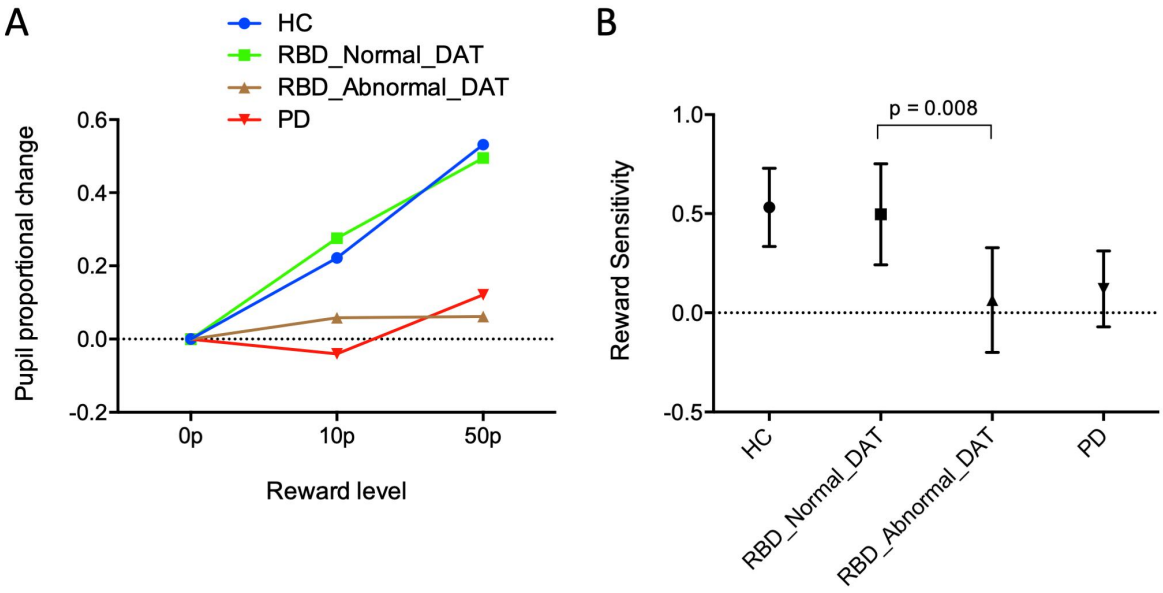


Figure 4. The same pupillary measures shown in figure 2, presented with RBD patients split according to outcome of DAT SPECT imaging.

In contrast, there was no difference in baseline pupil size (β = -0.12, p = 0.47) or average pupil responses (*across all reward levels*) between RBD patients with normal versus abnormal imaging (β = 0.20, p = 0.17). This implies that the group difference in reward sensitivity is not due to generally more responsive pupils in patients with normal imaging. Similarly, there was no difference in average reaction time (β = 0.07, p = 0.67) nor average peak saccadic velocity (β = 0.13, p = 0.43) between the two groups of RBD patients, indicating that their task performance was comparable.

We next assessed the effect of dopamine depletion on reward sensitivity by using the DaT SPECT specific uptake ratios as a continuous measure, instead of the subjective clinical reports. In the repeated measures design, there was a significant reward level by mean putamen signal interaction [F(1.9,69.7) = 4.1, p = 0.021], indicating that the differential pupil response to different reward levels was modulated by dopamine level in the putamen. A similar result was obtained using the mean DaT SPECT signal from the whole striatum [F(1.9,69.5) = 3.73, p = 0.030].

In the reward sensitivity analysis, there was a significant effect of the mean putamen signal (β = 0.40, p = 0.015), and the mean overall striatal signal (β = 0.41, p = 0.017) on reward sensitivity (Figure 5).

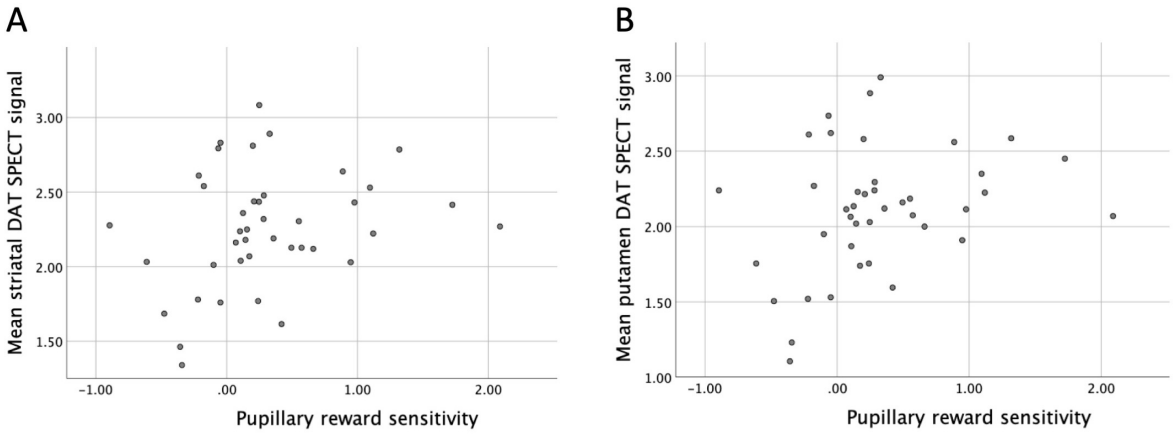


Figure 5. Scatter plots showing the correlation between pupillary reward sensitivity and DAT SPECT signal from the whole striatum (A) and putamen (B) in RBD patients

Relationship between apathy and reward sensitivity in RBD patients

Given that dopaminergic decline is associated with reward insensitivity (above) but not with clinical apathy (chapter 7) in RBD patients, it is perhaps unsurprising that we found no association between apathy and pupil reward sensitivity. There was no correlation between LARS scores and pupil reward sensitivity ($r = 0.02$, $p = 0.88$) and no difference in pupil reward sensitivity between patients who met the LARS threshold for clinical apathy and those who did not (mean pupil reward sensitivity in apathetic patients 0.32 vs non-apathetic patients 0.24, $p = 0.64$).

Effect of locus coeruleus integrity on pupil responses

None of the locus coeruleus measures used had a significant effect on pupil reward sensitivity in RBD patients. For brevity, only the regression analysis using the reward sensitivity metric are given here: effect of LC volume, $\beta = 0.08$, $p = 0.67$; effect of LC peak intensity, $\beta = 0.04$, $p = 0.83$; effect of LC contrast ratio, $\beta = -0.20$, $p = 0.32$.

There was also no effect of any LC measure on the average pupil response: effect of LC volume, $\beta = 0.04$, $p = 0.81$; effect of LC peak intensity, $\beta = -0.11$, $p = 0.55$; effect of LC contrast ratio, $\beta = -0.25$, $p = 0.16$.

These data suggest that measures of LC integrity not only have no effect on reward sensitivity, but they are also unrelated to general pupil responsiveness.

Discussion

Summary of findings

This study has demonstrated for the first time that pupillary responses to reward are impaired in some patients with REM sleep behaviour disorder, suggesting that this important component of parkinsonian apathy emerges during prodromal disease. A mechanistic role for dopamine in this is suggested by the observation that reward sensitivity is lost in RBD patients with evidence of dopaminergic decline on ^{123}I-ioflupane SPECT, but not in those with normal levels of dopamine transporter availability. Furthermore, pupillary responses are not related

to markers of neurodegeneration in the locus coeruleus. Taken together, these findings imply that pupillary reward sensitivity may be a specific marker of prodromal dopaminergic degeneration in parkinsonian disorders. The lack of a behavioural correlate of this suggests that this dopamine-dependent mechanism may be detectible whilst still clinically silent.

Dopamine depletion and reward insensitivity in prodromal disease

Whilst previous studies have shown that exogenous dopamine can improve reward sensitivity in Parkinson's patients, this is not necessarily indicative that the underlying deficit is caused by a lack of dopamine. For example, patients who receive dopamine agonists for other disorders that are not accompanied by dopaminergic degeneration can develop impulse control disorders that are thought to be caused by disordered reward processing[345–347]. By demonstrating a direct link between brain dopamine availability and reward responsiveness, our study provides important evidence implicating dopamine depletion in the mechanism of decreased reward sensitivity.

Study of this mechanism in patients with RBD not only sheds light on the prodromal phase of Parkinsonian disorders, but also removes the potential confounding effect of dopaminergic medications. Since these patients have never received such treatments, we are able to see the effects of dopaminergic neurodegeneration in its natural state. Furthermore, the degree of dopamine depletion is much less extensive in RBD patients than in those with established Parkinson's disease[176], which suggests that pupillary reward responses may be sensitive to even modest reductions in dopamine availability.

It should be noted that whilst we have measured dopamine depletion in the putamen in this study, we do not suggest that this region itself is the key mediator in reward appraisal. It may be the case that other dopaminergic areas, such as the nucleus accumbens and ventral tegmental area, have a more important mechanistic role, but that abnormalities in the putamen may be more sensitive measures of prodromal dopamine decline since this region is proportionally affected more in early disease[113,348,349].

The role of the locus coeruleus

Involvement of the locus coeruleus is a consistent feature of synucleinopathies and is linked to the presence and severity of RBD, perhaps as a consequence of parallel degeneration in the adjoining subcoeruleus complex, which has a role in the regulation of rapid eye movement sleep[46,150,350,351]. Given the link between pupil size and locus coeruleus activity[343], it is important to consider the influence of disease in this region on pupil responses. We did not observe any relationship between pupillary reward sensitivity measures and markers of locus coeruleus integrity using neuromelanin-sensitive MRI.

It may be that to some extent methodological limitations underly this result. For example, functional imaging using noradrenergic tracers may give a better indication of the integrity of this system than NM-MRI measures, which are somewhat indirect[148,351]. However, if the locus coeruleus was implicated in the pupil responses we observed, one might expect reward sensitivity to be more blunted in RBD patients than PD patients (or at least as much) given that LC degeneration is a particular feature of RBD. Instead, we observed an overall stepwise decline in reward sensitivity from controls to RBD patients to PD patients, a pattern that more closely mirrors that of dopamine decline[176].

These findings suggest that the effect of reward is not mediated simply by differential levels of arousal modulating pupil size. This is not to say that the noradrenergic system is not involved – there is evidence of substantial crosstalk between this and dopaminergic pathways[352] – but it implies that the specific effect of reward level is more likely to be a dopaminergic phenomenon. It also argues that the relationship between dopamine decline and reward sensitivity is not simply a consequence of generally more advanced degeneration in reward insensitive individuals.

Reward sensitivity and clinical apathy

Interestingly, the dopamine-related reward insensitivity that we have observed in RBD patients shows no correlation with clinical measures of apathy. This finding contributes to an emerging picture of reward-based decision making in Parkinson's disease in which apathy and dopaminergic mechanisms are partially dissociable[90,353–356]. In a recent study comparing apathetic and non-apathetic Parkinson's disease patients, both on and off dopaminergic

medication, Le Heron and colleagues demonstrated that although apathy and dopamine depletion were both associated with a reduction in willingness to exert effort for reward, the mechanisms were different[354]. Apathy resulted in the increased rejection of low value rewards, whilst dopamine increased responses to high rewards requiring high effort in both apathetic and non-apathetic patients. In a separate study, dopamine was again shown to increase effort-based decisions in Parkinson's patients despite the absence of clinical apathy[353]. As with our findings, this implies that dopamine-dependent mechanisms involved in motivational deficits may be subclinical and not sufficient to cause overt clinical apathy. Taken together, these studies suggest that whilst exogenous dopamine may have a generalised invigorating effect on patients, whatever their motivational state, distinct mechanisms may underlie clinical apathy.

The cognitive process involved in effort-based decisions and goal-directed behaviour can be thought of as a weighing of the balance between the potential reward on offer and the effort required to achieve this. It has been suggested that whilst sensitivity to the reward component may be predominantly dopamine driven, effort sensitivity may involve serotonergic pathways, with disruption in either mechanism potentially leading to a behavioural deficit[90]. The findings of Le Heron *et al.* would be consistent with a hypothesis that disturbances in the evaluation of effort are more closely linked to Parkinsonian apathy. If this were a serotonin-dependent phenomenon, it would be in keeping with recent evidence from functional neuroimaging studies suggesting that the effect of serotonin may predominate during early and prodromal disease[108,147]. It would be interesting to address this question with a study specifically measuring effort sensitivity in our patients. However, the fact that RBD patients with reward insensitivity performed the task with the same degree of vigour as those with normal reward sensitivity suggests that at this stage, they are able to exert a normal level of effort despite their reduced pupillary response to reward. This may in part explain why reward insensitive patients do not manifest clinical apathy.

Reward insensitivity as a potential prodromal risk marker

Despite the lack of a clear behavioural correlate of reduced reward sensitivity in RBD, the finding might have implications for risk stratification of prodromal patients as a marker of

dopaminergic decline. Numerous studies have demonstrated that up to half of patients with RBD have a measurable dopaminergic deficit in the basal ganglia, and there is clear evidence that these patients have a higher short-term risk of converting to an overt synucleinopathy[68,169]. However, these studies, as well as the data presented in chapter 4, have been notable for being unable to identify any clinical markers of this deficit during prodromal disease[164,175,279].

Part of the reason that clinical predictors of dopamine decline have remained elusive may be due to the limitations of the tools used to measure them. Most scales used to measure motor and non-motor Parkinsonian features have been transposed directly from those used in established Parkinson's disease, yet these traditional clinic-based measures may be insensitive to the subtle changes that occur in prodromal disease. Technology has emerged as a potential solution to this problem and techniques using computer and smartphone-based assessments are increasingly being introduced to measure motor and cognitive decline[357,358]. Measuring an individual's sensitivity to reward in this way using computer-based tasks is feasible and might prove to be a simple and practical way of gaining insight into an individual's dopaminergic status. Whilst it is unlikely to supplant neuroimaging as a definitive test, in combination with other tests of Parkinsonian features, measuring reward sensitivity could form an important part of a multimodal risk stratification model.

Concluding remarks

In summary, our data suggest that reward insensitivity, as indexed by pupil responses, emerges during prodromal disease in association with dopaminergic decline. Unlike in established PD, this does not relate to clinical apathy in RBD patients, suggesting that the aetiology of apathy may vary with disease stage and is not accounted for by disruption in reward evaluation pathways alone. Further studies exploring the interactions between effort- and reward- sensitivity in RBD, including how these can be modulated by dopaminergic and serotonergic pharmacological intervention, will be valuable in further elucidating the mechanisms underlying apathy in prodromal disease.

Chapter 9: General Discussion

Once considered to be a movement disorder presenting with bradykinesia, Parkinson's disease is now firmly recognised as a multisystem neurodegenerative disease of which motor impairment is just one, relatively late component. Related synucleinopathies such as dementia with Lewy bodies (DLB) and multiple system atrophy (MSA) similarly have a long prodromal period before the syndrome-defining clinical features emerge. Patients with REM sleep behaviour disorder offer a unique opportunity to study this phase of disease given the very high rates at which these overt synucleinopathies ultimately develop in RBD patients. In this thesis, I sought to characterise clinical and neuroimaging evidence of early neurodegeneration in RBD patients. In doing so, I aimed to further our understanding of the unique phenotype of this key prodromal population and evaluate biomarkers that might address some of the prognostic uncertainties facing RBD patients.

Summary of findings

The clinical prodromal phenotype of RBD

In chapter 2, we evaluated the baseline clinical, demographic and genetic characteristics of a large cohort of RBD patients at the point of recruitment to a longitudinal cohort study. We compared them to patients with early, untreated Parkinson's and healthy controls to examine the extent to which early neurodegenerative signs were evident, and to explore ways in which RBD patients diverge from the typical PD phenotype.

Across a wide range of parkinsonian motor and non-motor features, RBD patients were impaired relative to age-matched controls. Evidence of emerging synucleinopathy was suggested by the presence of mild motor parkinsonism, cognitive impairment, hyposmia, autonomic dysfunction and constipation. Interestingly, in all of these characteristics except motor function, RBD patients were at least as impaired as patients with manifest PD, rather than exhibiting the intermediate phenotype that might be expected from their prodromal

stage. In measures of depression, anxiety and apathy, RBD patients were more severely affected even than PD patients.

There were also some important differences in demographic characteristics. Obesity, smoking, cardiovascular risk factors and antidepressant use were all more common in RBD patients than in PD patients or controls, and the average duration of education was lower in RBD patients. There was some evidence of a different genetic background in RBD patients, as mutations in the glucocerebrosidase (GBA) gene were more common in RBD patients than controls and no mutations in the leucine rich repeat kinase 2 (LRRK2) gene were detected in RBD patients.

We next examined whether these demographic characteristics could inform regarding the risk of underlying neurodegeneration, using hyposmia as a surrogate marker of this. We found that antidepressant use and high BMI were associated with a substantially reduced risk of hyposmia in RBD patients, perhaps suggesting a less 'degenerative' phenotype. This is in line with recent evidence linking high BMI to a reduced risk of Parkinson's disease[121], though an alternative explanation could be that both obesity and antidepressant use can exacerbate sleep disorders, perhaps leading to earlier presentation to medical attention, consequently at an earlier prodromal stage.

To further explore the potential of clinical markers to inform regarding neurodegenerative risk, we applied the newly-developed Movement Disorders Society Research Criteria for Prodromal Parkinson's to all three groups of participants. This involves combining every risk factor and clinical marker known to increase the risk of PD into a single estimate of the probability of prodromal PD[60]. 74% of RBD patients met the threshold for probable prodromal Parkinson's, a figure roughly in line with the long-term pheno-conversion rates in RBD. The fact that less than 1% of control participants fulfilled these criteria indicates that they may result in a low false positive rate, but their limited sensitivity is evidenced by the fact that just 22% of PD patients met the threshold using in-clinic assessments alone.

In order to maximise the longitudinal data available to validate these risk stratification methods, the data from RBD patients described in chapter 2 was combined with that from 23 other RBD cohorts worldwide in a study conducted by the International RBD Study Group, led

by Professor Ron Postuma[68]. This multi-centre analysis of more than 1200 RBD patients revealed an average conversion rate of 6.25% per year across all RBD patients, lower than previous estimates. Using the MDS criteria described above resulted in the greatest stratification, with criteria positivity resulting in a hazard ratio for pheno-conversion of 5.37. Amongst individual markers, the largest increase in risk was conferred by the presence of mild motor impairment, followed by hyposmia and then mild cognitive impairment. At baseline assessment, only cognition differed between those who converted to PD and those who developed DLB.

These data show that emerging markers of a neurodegenerative phenotype are evident in RBD patients, and that in combination they have some risk stratification potential. None, however, are capable of confirming the presence of underlying synucleinopathy nor accurately predicting near-term pheno-conversion. In the next section, we explored the potential of neuroimaging techniques to provide a more specific assessment of some of the key brain structures involved in prodromal neurodegeneration.

Imaging brainstem degeneration in RBD

Chapter 3 reviewed some of the neuroimaging methods used to measure the extensive neurodegeneration that is known to occur during prodromal disease[73]. Abundant evidence from modalities including ultrasound, nuclear imaging and MRI indicates that disease related changes are detectible in prodromal cohorts, but the ability of these biomarkers to predict future outcomes is less certain. In many cases sufficient long-term data has not yet accumulated for them to be judged, but there are many challenges already apparent. One of the striking features of this review was how many imaging biomarkers are already abnormal in a high proportion of RBD patients. This suggests that positivity in these markers occurs early in prodromal disease rather than in the immediate lead-up to pheno-conversion and implies that their ability to capture the minority of patients who will convert in the 2-3 years following imaging may often be limited. A further challenge, and a priority for neuroimaging research, is to develop markers that are sensitive to change over time with the precision to track the slowly progressive decline in nigrostriatal integrity that will ultimately lead to conversion.

In Chapter 4, we documented the patterns of nigrostriatal synaptic dysfunction that occur in RBD patients using dopamine-transporter SPECT imaging. In line with previous studies, we observed abnormal imaging in nearly half of our RBD patients, further evidence that dopaminergic decline begins many years prior to the emergence of clinical parkinsonism. The patterns of abnormality were diverse, perhaps reflecting the range of future trajectories faced by RBD patients. In quantifying the DaT SPECT signal, we found that defining regions of interest based on the SPECT signal itself, rather than using structural MRI to define the anatomical structure from which the signal is presumed to originate, was a more accurate method of discriminating normal from abnormal scans. Consistent with a limitation of many prodromal neuroimaging studies, we were unable to identify any clinical measure that correlated with dopaminergic decline. Finally, by extrapolating between the extent of dopaminergic decline in RBD and PD patients, we estimated that this proceeds at an average rate of 7% per year, and may take more than 10 years to reach the threshold at which clinical parkinsonism emerges.

In chapters 5 and 6 we investigated the potential of two novel MRI techniques to characterise brainstem neurodegeneration in prodromal and manifest disease. Neuromelanin-sensitive imaging (NM-MRI) was the focus of the first experiments, in which we developed automated methods of quantifying signal change in the substantia nigra and locus coeruleus. Volume and signal reductions in the substantia nigra were observed in both RBD and PD patients compared to controls, though there was substantial overlap between the three groups. Whilst the reductions observed in PD patients were generally greater than those in RBD patients, none of these nigral measures were significantly different between RBD and PD patients, mirroring the pattern of impairment in clinical features observed in chapter 2. Neuromelanin signal intensity in the posterior nigra was the only measure that correlated with dopaminergic SPECT signal, suggesting this may be the most reliable NM-MRI indicator of striatal denervation.

Neuromelanin-sensitive MRI measures of locus coeruleus volume exhibited a somewhat different distribution between the three groups, with significant reductions in RBD patients but not PD patients, when compared to healthy controls. A possible explanation for this is that degeneration in the region of the locus coeruleus is a feature of RBD itself and therefore represents a marker of disease subtype rather than progression. Interestingly, in PD patients,

locus coeruleus measures correlated with motor disease severity, which would be consistent with this subtype of disease featuring a more severe motor phenotype.

The susceptibility-weighted imaging used in chapter 6 takes a different approach to quantifying degenerative changes in the substantia nigra. Rather than assessing its structural properties, this technique is sensitive to the changes in nigral iron deposition that occur in parkinsonian disorders. We first confirmed its utility in distinguishing PD patients from controls using a visual binary assessment of the discrete high signal region corresponding to nigrosome 1, which we found to be absent in 96% of PD patients and present in 92% of controls. In RBD patients this dorsal nigral hyperintensity (DNH) was absent in 28% of cases; these individuals had lower dopaminergic SPECT signal and higher MDS prodromal probability scores than RBD patients with retained DNH presence, evidence that this marker might identify patients with more advanced underlying neurodegeneration.

Following on from this, we explored methods of objectively quantifying the nigral SWI signal. As well as measuring the signal intensity within the region of nigrosome 1, we also used a nigral template derived from neuromelanin-sensitive MRI to define anatomical regions of interest. These SWI intensity measures separated the three groups of participants better than those from NM-MRI, displaying a stepwise decline from controls to RBD patients and from RBD to PD patients, with significant differences in all three pairwise comparisons. Finally, we combined a range of measures from NM-MRI and SWI into a single logistic regression model, which distinguished PD patients from controls with near perfect classification accuracy. This highlights the potential of these automated quantification methods in multimodal neuroimaging assessments.

Mechanisms of apathy in RBD

In chapter 7 we investigated the prevalence and severity of apathy in RBD, finding it to be both common and under-reported[359]. Following a similar pattern to the non-motor features quantified in chapter 2, RBD patients were at least as severely affected by apathy as those with established Parkinson's disease. The presence of apathy was not explained by sleep quality nor sedative medication and it was dissociable from other neuropsychiatric

symptoms. In particular, though most depressed RBD patients were also apathetic, the reverse was not true: less than half of RBD patients with apathy were depressed.

We then used the SPECT imaging acquired in chapter 4 to explore the neurochemical correlates of apathy in our RBD patients. Because ioflupane binds to both dopaminergic and serotonergic transporters, we were able to use the signal from these images to assess the integrity of both neurotransmitter systems, by focusing on regions where just one transporter type predominates. To achieve this, we employed co-registration of SPECT and structural MRI, which enabled regions to be defined for the raphe nucleus, as well as other relevant small brainstem nuclei, based on precise anatomical information.

In this study, apathy was found to correlate with the serotonergic signal in the dorsal raphe nucleus, whereas no correlations with dopaminergic regions were observed[147]. This intriguing finding supports recent studies in PD patients suggesting that serotonin depletion may be an important factor in the development of parkinsonian apathy, especially early in disease[108]. The proximity of the raphe nucleus to regions involved in the regulation of REM sleep may explain why apathy is so common in RBD patients. The fact that other clinical features, including depression, showed no association with serotonin transporter levels, is further evidence of apathy as a distinct entity with its own pathophysiological mechanisms.

One component of apathy shown to be of relevance in patients with established PD is blunting of sensitivity to reward[101]. The experiments performed in chapter 8 were designed to investigate whether this is also evident in RBD patients. Reward sensitivity was indexed using a pupil tracking paradigm where changes in pupil diameter in response to stimuli of varying reward levels were measured. We found that pupillary reward sensitivity in RBD patients was significantly reduced compared to matched controls, suggesting that reward insensitivity may be a feature of prodromal disease. Overall there was no difference in reward sensitivity between RBD and PD patients, but a revealing pattern emerged when dopaminergic SPECT imaging was used to stratify the RBD patients. Those with normal basal ganglia imaging had reward sensitivity levels comparable to control subjects, whereas RBD patients with abnormal scans had significantly reduced reward sensitivity by comparison, to a level that was indistinguishable from PD patients. In contrast, reward sensitivity was not related to SPECT signal from serotonergic regions. This is in keeping with dopamine's well-established role as

a key mediator of reward processing. Although our findings require replication, they also raise the possibility that objective measures of pupil responses to reward might be useful as a marker of prodromal dopaminergic decline.

In contrast to data from patients with established PD, the dopamine-related reward insensitivity that we observed in RBD patients bore no relation to clinical apathy. This somewhat unexpected finding highlights the complexity of the mechanisms underlying disordered motivation in parkinsonian diseases. It is likely that both dopaminergic and serotonergic pathways are important, and the relative dysfunction in these may well vary according to disease stage and subtype.

General comments

RBD as a distinct synucleinopathy subtype

A recurring theme of the varied investigations comprising this thesis is that RBD patients have evidence of more extensive disease than might be expected from their prodromal status. Their clinical phenotype is at least as severe as that of patients with established Parkinson's disease and in many of the neuroimaging markers assessed RBD and PD patients were equally affected compared to controls. A similar picture has been reported in other comparative studies, for example examining the functional connectivity of basal ganglia networks[263].

There are a number of possible explanations for such a pattern. The first is the potential floor effect in some markers, of which olfaction is one example. Though RBD can occur very early in prodromal disease, the loss of olfactory neurons and consequent hyposmia may occur at an even earlier stage[3,12]. Once olfaction has declined to the extent seen in many RBD patients, further quantifiable change over time may be limited.

A second factor is that RBD patients are a heterogenous population with a wide range of future disease trajectories. Importantly, they will convert not only to typical Parkinson's disease but in equal numbers to Dementia with Lewy bodies and to a lesser extent to Multiple System Atrophy[68]. The latter two conditions by definition have more predominant non-motor features than PD, and it could be that the emergence of these in the prodromal phase

accounts for their prominence in RBD patients. However, MSA is rare as an outcome and in the IRBDSG multi-centre study of pheno-conversion no non-motor features other than cognition were found to differ between those who went on to develop DLB versus PD[68].

It is possible, therefore, that RBD itself marks a subtype of synucleinopathy with a more diffuse and malignant phenotype, and that this is already apparent in the prodromal phase. Evidence for this is starting to emerge from longitudinal follow-up. In terms of motor disease, PD patients with concurrent RBD are more likely to have a postural instability-gait disturbance (PIGD) phenotype, with higher Hoehn and Yahr stage, more rapid progression, higher rates of motor complications such as gait freezing, and less response to dopaminergic medication[33–35,360]. In addition, more widespread disease is indicated by higher rates of cognitive impairment, orthostatic hypotension and constipation in PD-RBD patients[361,362]. Evidence of a more severe disease phenotype is not limited to clinical signs, with dopaminergic SPECT also showing greater decline in those with concomitant RBD[176]. Though phenotypic variation is less extensively documented in DLB, there is some evidence that the presence of RBD marks a more severe phenotype in this condition also[363].

Perhaps the most important effect of the RBD phenotype is on quality of life. It has been demonstrated that non-motor symptoms have as much of an effect on this as motoric disease in established PD, and that PD patients with concurrent RBD have lower quality of life measures than those without[302,364]. The extensive non-motor disease already apparent in RBD patients means that quality of life is already reduced by as much as in patients with newly-diagnosed PD[277]. Many of the symptoms observed in RBD patients are treatable, and RBD should perhaps therefore be considered as an established multisystem disorder in its own right, to help focus attention on managing symptoms and improving quality of life rather than simply waiting for motor disease to emerge.

The importance of disease subtypes, particularly in the context of clinical trial design, is increasingly recognised in established Parkinson's disease[365]. As these studies are introduced into the prodromal period, the same is likely to apply. Comparisons between RBD patients and PD patients with a relatively benign, tremor-dominant phenotype without RBD, are likely to be spurious, for example. There may also be implications for the selection of

neuroprotective treatments in RBD patients. Insights from monogenic forms of Parkinson's disease as well as genome wide association studies have led to the development of neuroprotective treatments that target specific molecular pathways[366–368]. The efficacy of these may depend on an individual's genetic background and, as we have seen in the case of GBA and LRRK2 mutations, there is evidence that RBD patients are not equivalent to PD in this regard. As our understanding of the genetic and other factors that underly disease subtypes expands, personalising treatment interventions in this way is likely to become an important consideration[369].

RBD and the pathological mechanisms of synucleinopathies

It is interesting to consider the wide range of disease trajectories seen in RBD in the context of the theories of pathological spread in Parkinson's disease. The most established of these is the pathological staging system described by Braak, in which synuclein pathology first appears in the peripheral tissues of the gut and olfactory bulb before progressing to lower brainstem centres (including those responsible for REM control) and then ascending to the midbrain nigrostriatal neurons, causing manifest PD to emerge at stage 3[3,286]. Further progression to cortical areas in stages 5 and 6 is proposed to account for the cognitive decline that occurs late in disease in typical cases of PD. As an extension of this theory, some have proposed that after an initial pathological event in the gut or olfactory bulb, perhaps as a result of contact with an environmental trigger, the pathologically misfolded alphasynuclein spreads in a prion-like manner, reaching the brain via the vagus nerve before ascending rostrally[370–373].

An alternative explanation for the pattern of pathology seen in parkinsonian disorders is the so-called threshold hypothesis, which posits that the disease process begins in parallel in every tissue in the body, but that local, tissue-specific factors affect the relative susceptibility of cells to degeneration[71]. Additional differences in the inherent functional reserve of different neuronal systems are proposed to contribute to the time course in which symptoms emerge. For example, it is suggested that a lack of reserve in gut tissues leads to constipation at a time when the nigro-striatal system is still able to compensate for progressive Lewy body disease, meaning that motor symptoms take much longer to emerge despite neurodegeneration progressing in parallel in gut and brain tissues.

Both of these theories would be consistent with variable disease progression rates, due to individual factors affecting the spread or accumulation of pathology. However, the threshold hypothesis has a degree of flexibility that means it may better account for the range of disease phenotypes seen, not only amongst RBD patients but also in established Parkinsonian disorders. If the fundamental pathological process has the potential to develop in parallel in any of the affected tissues, the timing by which this progresses to the threshold at which symptoms emerge could be affected by a number of individual factors. At the same time, other factors may selectively lower the threshold in certain tissues. For example, individual differences affecting the processing and degradation of misfolded proteins in certain tissues could accelerate the formation of Lewy Bodies[374]. On the other hand, co-morbid pathology such as amyloid or tau deposition in cortical areas could conceivably lower the threshold for the expression of cognitive dysfunction, making such individuals susceptible to cognitive impairment at an earlier stage than would be expected from a rigid interpretation of Braak's hypothesis[63,64]. One could further speculate, for example, that individuals who develop RBD but do not pheno-convert even after decades of follow-up may have particular susceptibility factors in the brain regions that regulate REM atonia, alongside protective factors elsewhere that mean the threshold for clinical disease is never reached during their lifetime in other tissues. If the susceptibility factors affecting the subcoeruleus complex were shared with neighbouring regions such as the raphe nucleus, this could explain why serotonin-dependent characteristics, including apathy, are particularly common in RBD patients.

Directions for future research

Refining clinical predictors with better quantification of prodromal motor signs

It is unsurprising that of all the prodromal markers studied to date, measures of motoric disease are the best predictor of near-term conversion to a parkinsonian disorder[68]. Given that it is this motor phenotype that defines conversion, it could be argued that mild motor signs are not so much a risk marker but the pheno-conversion itself emerging. Nevertheless,

they are likely to be an important component of any risk stratification process. It is interesting to note, therefore, that quantitative motor testing is a better predictor of conversion than the clinician-rated UPDRS III. The UPDRS III, which was designed to quantify disease severity in established PD, has obvious limitations in prodromal disease. The low scores observed in RBD patients (a mean of less than 5 points in our cohort, in a scale that extends to over 100) mean that it is not only likely to be insensitive to subtle changes in this range, but also that confounding factors such as co-morbidities will have a greater effect in obscuring true parkinsonian signs. Nevertheless, the quantitative motor testing methods used to demonstrate superior predictive capability, such as the Purdue Pegboard test, are fairly crude. Developing more sophisticated methods of quantifying prodromal motor signs is likely to be a fruitful avenue of future research.

Perhaps the most promising method of doing so is through the use of technology, with portable accelerometers emerging as an increasingly accessible tool for this purpose. One option is to use body-worn sensors in controlled laboratory conditions[375]. These might simply involve performing the same tests involved in the UPDRS III examination, but using sensors to more precisely quantify the motor output. Alternatively, such testing may focus on particular features such as gait and postural instability with specific outcomes in mind such as gait freezing or falls[376].

Smartphone technology has enabled this approach to be employed on a much larger scale, meaning that large amounts of data can be easily collected, including testing at multiple time points. Short, structured tests using smartphone applications combine this efficiency of data collection with some of the control of laboratory-based experiments. These simple tests show excellent discriminative accuracy in separating PD patients from controls and the fact that they can predict an individual's UPDRS III score with a high level of precision attests to their potential for monitoring disease severity and progression[357,358,377,378].

Taking the concept of testing in the home environment yet further is possible with free-living accelerometry devices, which can be attached to an individual for continuous motion recording over several days[379–381]. Such data sets are inevitably noisier than task-based

testing, but the advantage of recording in the natural environment may be that real-life outcomes such as falls, fatigue and freezing can be more accurately predicted.

Data in RBD is more preliminary, but good discriminatory accuracy has been demonstrated between RBD patients and controls using smartphone-based tests, an important proof of the concept that prodromal signs can be detected using this technology[382]. Important next steps will be to determine which particular features best identify motor parkinsonism in early PD, so that these features can be assessed in prodromal disease, and crucially to explore with longitudinal follow-up which are the characteristics that best predict pheno-conversion in RBD. Another potential use of technology-based assessments will be to seek measures that can accurately predict neuroimaging abnormalities, something that has not been possible with traditional clinical measures[279].

Challenges in neuroimaging biomarker development

Given the risk stratification potential of simple, clinical markers and the relatively disappointing results from prodromal neuroimaging achieved to date, the role of neuroimaging in RBD cohorts remains limited at present. This is despite the very promising results from a number of studies in characterising disease-related abnormalities[73]. The slow progress of prodromal imaging is due in part to the scale of study that is required to validate a prodromal imaging biomarker. With a conversion rate of just 6% per year, any study seeking to demonstrate a predictive advantage requires either a very large sample size or a long follow-up period in order to capture sufficient converters. An important way of accelerating biomarker assessment will be to develop collaborative, multi-centre studies investigating common techniques. This will not only enable large data sets to be accumulated in a shorter time, but it will also help to address another major limitation of many imaging studies – the issue of reproducibility.

In terms of methodological advances, the advent of high field strength MRI has the potential to substantially improve the characterisation of nigral pathology using the techniques described in this thesis. Both SWI and neuromelanin-sensitive MRI have been investigated at 7-Tesla and the increased resolution is an advantage when imaging such a small

structure[222,310,311,383]. This is likely to increase the reliability of visual assessments of nigrosome-1 as well as automated measures of neuromelanin loss, which are somewhat limited in our data by the relatively small number of voxels covered by the nigra.

In the absence of a single diagnostic biomarker, the use of multimodal imaging also warrants further investigation. As well as providing a direct comparison of different imaging tools, the combination of these may yield more diagnostic information than either alone. Within our nigral imaging measures, we were able to significantly increase the discriminatory accuracy by combining SWI and neuromelanin measures into a single model. Others have demonstrated that the diagnostic accuracy of substantia nigra MRI is increased in both PD and RBD patients when neuromelanin-sensitive imaging is combined with diffusion tensor images[228,314]. There is also some evidence that combining transcranial sonography and DAT SPECT in persons with RBD increases the sensitivity for future pheno-conversion[206].

As well as maximising diagnostic accuracy, multimodal imaging may have the potential to inform about disease staging and pathological mechanisms. An intriguing paper recently published combined imaging of parasympathetic gut innervation (^{11}C-Donepezil PET), sympathetic cardiac imaging (MIBG scintigraphy), neuromelanin-sensitive imaging of the locus coeruleus, noradrenergic locus coeruleus function (^{11}C-methylreboxetine PET) and nigrostriatal dopamine storage capacity (^{18}F-DOPA PET) in RBD patients, PD patients and control subjects[351]. Patients with PD were impaired across all modalities, whereas RBD patients showed a similar pattern of impairment in all except ^{18}F-DOPA PET, in which they more closely matched controls. The authors claim that this 'in vivo staging' effectively recapitulates the pattern of pathological spread described by Braak[286]. Such extensive investigations are unlikely to be possible on a wide scale, but this study illustrates the power of multimodal imaging.

One area with the potential to enhance neuroimaging analysis is the field of machine learning and artificial intelligence[70,384]. Every scan of an individual's brain contains many thousands of potential data points and the ability to process and analyse these big data sets in their entirety could potentially add significant insight. We have seen from the DaT SPECT imaging described in chapter 4 that multiple different patterns of abnormality can occur in prodromal disease

and that these patterns might be more informative than any single quantifiable metric. Artificial intelligence may offer a means of recognising and analysing the components of these patterns that contain the greatest risk stratification information, and which may inform regarding future phenotype as well as overall risk.

Synuclein-specific biomarkers

Lessons from clinical trials in Alzheimer's disease (AD) have highlighted the importance of confirming the presence of the molecular targets of putative treatments. Certain studies using monoclonal antibodies against amyloid in AD patients included a significant proportion of patients who were subsequently found to not have pathological levels of amyloid in the brain, and this has been cited as a potential reason for the failure of some trials[385]. None of the clinical or imaging biomarkers assessed in this thesis are specific for alpha-synuclein-mediated degeneration and, as discussed in the introduction, it is by no means certain that all RBD patients have underlying synucleinopathy. Although in manifest PD it could be argued that the clinical phenotype is more specific to the pathophysiological mechanism than is the case in AD, in prodromal disease it would be particularly useful to be able to confirm the presence of abnormal synuclein deposition, particularly in the context of the many treatments under development that are directed against alpha-synuclein[386–388].

The development of alpha-synuclein PET would be an ideal way of proving the presence of abnormal alpha-synuclein in the brain in vivo, but this is considerably more challenging than imaging amyloid in Alzheimer's disease, since Lewy bodies are intracellular and much less abundant[271]. CSF testing may provide an alternative method of measuring abnormal alpha-synuclein in the central nervous system and one particular method has shown considerable promise in this regard. Using a similar assay to that used to identify prion disease, real-time quaking-induced conversion (RT-QuIC) measures the ability of abnormally aggregated alpha-synuclein in an individual's CSF to induce similar aggregation in exogenous recombinant alpha-synuclein[389,390]. The attraction of this technique is that it measures not simply the presence of alpha-synuclein, but its presence in the pathological conformation. Preliminary results indicate that this assay can correctly identify 95% of Parkinson's patients and 92% of DLB patients[389]. Crucially in the context of trial design, it has a specificity of 100% when

compared to Alzheimer's disease patients and controls. This technique has not yet been explored in prodromal disease but will be an important avenue of future research.

A more indirect method of capturing the molecular signature of Lewy body disorders is through the detection of phosphorylated alpha-synuclein deposits in peripheral tissues. These deposits have been reported in a number of different tissues in PD patients[391], but perhaps the most promising data are those from skin biopsies, since these are easy to perform and relatively non-invasive. A sensitivity of 100% has been reported for the ability of alpha-synuclein deposits in the skin to identify Parkinson's disease in one study[392]. This figure has not always been replicated, but as with the CSF RT-QuIC assay, specificity appears to be consistently high[393]. One prodromal study of this technique found that only 55% of RBD patients test positive for dermal alpha-synuclein[394].

Synuclein deposits have also been reported from biopsies of the submandibular gland, which show an interesting pattern when compared across controls, RBD patients and PD patients that warrants further investigation[395]. Again, no positive cases were observed amongst the 26 controls, suggesting a very high specificity. However, whilst deposits were seen in 89% of RBD patients with successful biopsies, they were only observed in 67% of PD patients. This study has considerable limitations[396], but it would seem from these data that the test is not a fully sensitive measure of underlying synucleinopathy. However, the high positive rate in RBD patients is compelling. Furthermore, the authors report that synuclein deposits were seen more commonly in PD patients with concomitant RBD than those without, adding further weight to the notion that RBD marks a distinct synucleinopathy phenotype.

Longitudinal follow-up will be important in answering some of the questions raised by these findings, such as whether RBD patients testing positive for synuclein deposition have a higher risk of conversion and whether some of those who test negative will go on to develop these dermal synuclein deposits over time. Nevertheless, it is an attractive idea that such tests might identify patients most likely to benefit from alphasynuclein-directed treatments.

Multimodal biomarker platforms

It is likely that future biomarker development in prodromal PD will involve the combination of multiple individual markers, including trait, state and rate indicators[70]. We have seen how combining even small numbers of imaging parameters can substantially improve accuracy compared to individual markers alone. Future models can build on this by combining the widest range of multimodal markers, including clinical, genetic, imaging and fluid biomarkers as well as proteomic and metabolomic approaches. Model-free machine learning techniques have already shown promise in analysing such large and heterogenous data sets[384].

The MDS prodromal criteria have been an important step in developing multimodal criteria for prodromal disease, and although they were designed for use in population studies, they have demonstrated stratification value in RBD populations also[61,68]. A limitation of this approach that needs to be addressed is the interaction between individual markers, as the assumption of independence used in the model is an over-simplification. For example, DaT SPECT abnormality confers a 40-fold risk increase in the MDS criteria, based on data from population studies. This likelihood ratio (LR) is applied whether or not the LR for RBD also applies, yet in patients who test positive for RBD the relative risk is considerably lower because of their increased pre-test risk[169].

An alternative multimodal approach to risk stratification has recently been described using data from the Parkinson's Progressive Marker Initiative. Using a logistic regression model to combine olfactory function, family history, age and gender with a comprehensive genetic risk score, the authors were able to accurately classify typical PD patients from controls[397]. Perhaps more promising from a prodromal perspective was the finding that amongst a group of subjects without evidence of dopaminergic dysfunction (SWEDDs - individuals with parkinsonian signs but normal dopaminergic radiotracer imaging) the model correctly classified 80% of those who converted to Parkinson's disease within one year. This particular model was designed to include only inexpensive, widely available markers and could therefore be of value in screening large populations. It would be interesting to investigate the ability of this model to identify near-term RBD converters.

Exploring mechanisms of apathy in Lewy body disorders

Despite growing evidence of a role for serotonin in the expression of parkinsonian apathy, the mechanism underlying this is poorly understood. Experiments focusing on aspects other than reward will be important in elucidating this in RBD patients. In terms of the cognitive component of motivated behaviour, serotonin depletion has been linked to the response to aversive rather than rewarding stimuli, and it would be interesting to investigate the responses of RBD patients in this context[109]. On the behavioural side, motivated actions require evaluation and execution of effort[90,354]. Patients with early PD have been shown to have particular problems with auto-activation that relate to serotonin depletion[108,398], and there is also evidence of a link between willingness to exert effort and clinical apathy in PD[353,354]. Similar experiments in RBD patients may help resolve whether this link is also important mechanistically in prodromal disease.

Rather than existing as a single clinical entity, apathy is a phenotypic spectrum[398]; it may be the case that different subtypes of apathy are attributable to distinct mechanisms. A striking finding from chapter 7 is that the one component of apathy (as measured by the LARS) not impaired in RBD patients is emotional apathy. Emotional blunting and diminished emotional resonance are said to be expressions of a reward deficiency subtype of apathy that responds to dopaminergic treatment[398]. Preserved emotional responses in RBD patients may therefore explain to some extent the disconnect between overall apathy scores and reward sensitivity measures. Further elucidation of the specific mechanisms underlying subtypes of apathy, could be important in directing therapeutic intervention.

Unlike apathy, reward sensitivity, as indexed by pupillary responses, is strongly linked to dopamine availability in RBD patients. Although not the primary aim of our study, it is noteworthy that pupil reward sensitivity is, to our knowledge, the only biomarker that has been shown to correlate with prodromal dopaminergic decline. The potential role of this biomarker in risk stratification will therefore be important to consider with longitudinal follow-up. If pupillary reward sensitivity does indeed have predictive value, the development methods of simplifying its measurement, for example using smartphones or other portable

devices, would facilitate its widespread use and incorporation into multimodal biomarker platforms.

Interactions between the serotonergic and dopaminergic systems are widespread and complex[399]. Given that both are affected by Lewy body degeneration[3], it is likely that the contribution of each to the expression of parkinsonian apathy varies with disease stage. This may explain the compelling evidence of a link between dopamine and apathy in the context of deep brain stimulation, which is typically performed in relatively advanced patients[322,323]. It will be important, therefore, to repeat the experiments in chapter 7 in patients with well-established Parkinson's disease, to examine whether the same link between serotonin and apathy exists or whether dopaminergic depletion becomes more important as disease progresses. It will also be interesting to see if the serotonin-apathy link maintains its association with RBD within PD patients.

Finally, a natural next step to follow from our data would be to explore the effects of serotonergic medication on clinical apathy in early or prodromal disease. Ultimately the goal of this would be to improve symptomatic apathy in parkinsonian disorders without the risk of impulse control disorders that accompanies dopaminergic treatments. An important first step, however, will be to examine how serotonergic agents modulate the cognitive and behavioural components of apathy in the context of Lewy body disease, which may shed further light on the mechanisms underlying our findings[400].

Considerations in the diagnosis of pheno-conversion

Despite progress in biomarker research, diagnosis of parkinsonian disorders still relies on clinical opinion. Although this achieves an accuracy of up to 95% when compared to definitive histopathological confirmation at autopsy, the performance of expert opinion is lower in early disease when patients typically first present[401,402]. Even then, however, the decision is considered in the presence of suggestive symptoms that are significant enough for the individual to have sought medical attention. When following prodromal cases, on the other

hand, the emerging signs are often captured at much earlier timepoints, when symptoms may be trivial or even absent. The additional consideration of the range of possible conversion phenotypes in RBD patients means that we can expect diagnostic accuracy in this scenario to be lower still. This presents some unique practical and ethical challenges.

One particular question, given this uncertainty, is whether it is ethical to reveal the presence of clinical parkinsonism to individuals who are asymptomatic. This is not an uncommon scenario in RBD research clinics, as the early signs of parkinsonism can often go unnoticed by patients themselves for a considerable time. One could argue that to withhold this information denies the patient's autonomy to some extent. On the other hand, it may be the case that disclosing signs to an asymptomatic patient when the diagnosis is uncertain could cause unnecessary suffering. Since there are no disease modifying treatments currently available, and dopamine replacement is presumably not indicated in asymptomatic individuals, it could be argued that there is no benefit in disclosing the information. Guidance from our own ethics board has been to disclose information only when it will make a difference to the individual's clinical care, but it is clear that practice varies between centres[68].

Diagnostic uncertainty is also a significant issue for clinical neuroprotective trials. Where pheno-conversion is likely to be the primary end point, clarity and consistency of diagnosis is of paramount importance. Diagnostic errors, as well as inter-individual differences in practice between, for example, watchful waiting and making a diagnosis could be great enough to obscure a modest treatment effect. It will be important, therefore, to have a clear protocol for defining pheno-conversion. This could involve consensus diagnosis between two movement disorder specialists, as well as subsequent confirmation of the diagnosis after an interim period. Criteria for diagnosis such as those recently published by the Movement Disorders Society will also aid consistency in this scenario[403,404].

Concluding remarks

This thesis has demonstrated that a broad array of clinical and neuroimaging evidence of underlying neurodegeneration exists in patients with RBD. These signs are often more extensive than would be expected from a prodromal population and there are certain

features, including apathy, that are particularly prominent in RBD patients, suggesting that they may represent a particular phenotypic subtype of alpha-synucleinopathy. Longitudinal assessment of the imaging markers examined here, as well as the development of multimodal models combining them with genetic, molecular and technology-based biomarkers are key priorities for future research.

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Appendix 1 – Descriptive DaT SPECT/CT reports

The following table lists the descriptive DaT SPECT/CT reports provided by Dr Kevin Bradley, Consultant Radiologist and Head of Nuclear Medicine, Churchill Hospital, Oxford. The scans were reported by Dr Bradley blinded to all clinical details other than the patient's age, gender and the presence of RBD. Reports are presented in the order that the scans were performed, with all patient identifiable information removed. Each report was classified into one of 5 categorical outcomes by Thomas Barber – normal; borderline; asymmetric putamen reduction (typical PD pattern); generalised striatal reduction (atypical pattern); asymmetric caudate reduction. 51 participants were scheduled for SPECT imaging. One scan was lost to short notice cancellation and another was cancelled due to the participant having converted to PD. 49 scans were therefore successfully completed.

Scan number	Descriptive report	Categorical outcome
1	Abnormal DaTscan. There is marked asymmetry in specific uptake with a moderate reduction in specific uptake by the posterior aspect of the right lentiform nucleus which becomes more mild anteriorly with a further definite mild reduction in specific uptake by the left head of caudate. No definite reduction in specific uptake within the left basal ganglia, although the appearance is slightly more typical of an older patient and therefore it is suspicious that there is an early left sided specific reduction, particularly posteriorly. Comment: Asymmetric, definite right sided dopaminergic synaptic loss with possible early left sided dopaminergic synaptic loss, appearances are typical of parkinsonism.	Asymmetric putamen reduction
2	Although the contour of the specific uptake of DaTscan within both basal ganglia is relatively preserved there appears to be a mild generalised reduction in specific uptake throughout both lentiform nuclei and both heads of caudate, particularly for the patient's age. Comment: mild generalised reduction in specific uptake throughout the basal ganglia bilaterally.	Generalised reduction
3	There is asymmetric DaTscan uptake with a moderate reduction in specific uptake by the posterior aspect of the right lentiform nucleus which becomes more mild anteriorly. No other definite focal reduction in specific uptake of DaTscan although perhaps there is a superimposed, subtle generalised reduction in specific uptake for a person of this age. Comment: Definite right-sided dopaminergic synaptic loss with possible early left sided loss.	Asymmetric putamen reduction
4	There is no focal loss of specific DaTscan uptake within either lentiform nucleus or head of caudate but the overall uptake within the basal ganglia, relative to background, is slightly less than expected in a patient of this age.	Generalised reduction
5	There is some minor asymmetry in specific uptake within the basal ganglia but overall, specific uptake is within normal limits within both lentiform nuclei and both heads of caudate. Comment: no evidence of significant dopaminergic synaptic loss.	Normal
6	There is a moderate, generalised reduction in specific uptake throughout both lentiform nuclei and both heads of caudate. Comment: Generalised reduction in dopaminergic synapses, particularly for age. The pattern is not typical of that seen in idiopathic Parkinson's disease but is definitely abnormal and is a pattern more frequently seen in Lewy body dementia or occasionally Parkinson plus syndromes.	Generalised reduction
7	There is some minor asymmetry in specific uptake between the lentiform nuclei but overall uptake is within normal limits for age. No definite dopaminergic synaptic loss.	Borderline
8	There is some subtle asymmetry in specific uptake between the most posterior aspects of the lentiform nuclei, relatively reduced on the right but overall with good specific uptake throughout the basal ganglia. COMMENT: Possible early	Borderline

	dopaminergic synaptic loss posteriorly within the right lentiform nucleus but overall uptake is within normal limits for age.	
9	There is a minor asymmetry in specific uptake at the posterior limits of the lentiform nuclei, possibly slightly reduced on the right but with otherwise normal specific uptake more anteriorly within the lentiform nuclei and within the heads of caudate.	Borderline
10	Normal specific uptake throughout both lentiform nuclei and both heads of caudate. COMMENT: No evidence of dopaminergic synaptic loss.	Normal
11	Normal specific uptake both lentiform nuclei and both heads of caudate.	Normal
12	Mild generalised reduction in specific uptake throughout both basal ganglia for age but with no focal abnormality.	Generalised reduction
13	Normal specific uptake throughout both lentiform nuclei and both heads of caudate.	Normal
14	There is a mild, symmetric reduction in specific uptake throughout both basal ganglia. No focal abnormality.	Generalised reduction
15	The contour of specific uptake within basal ganglia is preserved but there is definite asymmetry in uptake which appears relatively reduced within the head of the right caudate nucleus with the remainder of uptake appearing within normal limits. This pattern of defect usually reflects a small focal vascular event within the right head of caudate.	Asymmetric caudate reduction
16	There is a subtle but definite asymmetry in specific uptake by the posterior aspect of the lentiform nuclei with an early reduction on the left.	Asymmetric putamen reduction
17	Normal specific uptake of DaTscan throughout both lentiform nuclei and both heads of caudate. Normal study.	Normal
18	There is a very subtle asymmetry in DaTscan uptake between the basal ganglia with a possible early reduction posteriorly within the right lentiform nucleus, slightly more than expected for age but with no other definite focal abnormality in uptake.	Borderline
19	There is a symmetric mild generalised reduction in specific uptake throughout both lentiform nuclei and both heads of caudate, particularly for age.	Generalised reduction
20	Normal specific uptake throughout both lentiform nuclei and both heads of caudate.	Normal
21	There is an early, relative reduction in specific uptake by the posterior aspect of the right lentiform nucleus. The remainder of uptake is within normal limits.	Asymmetric putamen reduction
22	There is possibly, subtle early relative reduction in specific uptake by the posterior aspect of the left lentiform nucleus although this is not completely definite at this age. The remainder of specific uptake is within normal limits.	Borderline
23	Normal specific uptake throughout both lentiform nuclei and both heads of caudate.	Normal
24	There is a relatively minor, but definite asymmetry in specific uptake by DaTscan with a relative defect in uptake involving the right head of caudate compared to the left. No other definite abnormality.	Asymmetric caudate reduction
25	There is minor asymmetry in specific uptake of DaTscan with a minor reduction in the left head of caudate compared to the right. There is also a possible relatively	Asymmetric caudate reduction

	subtle generalised decrease in specific uptake for a patient of this relatively young age.	
26	DaTscan normal. Good uptake.	Normal
27	Normal specific uptake throughout both lentiform nuclei and both heads of caudate. Normal study. No evidence of dopaminergic synaptic loss.	Normal
28	Definite asymmetry in specific DaTscan uptake throughout the left lentiform nucleus, most conspicuous posteriorly with a probable small defect in specific uptake at the posterior limit of the right lentiform nucleus. Uptake in the right head of caudate is preserved and is probably also satisfactory within the left head of caudate. Comment: Definite left sided probable early right sided dopaminergic synaptic loss in a pattern suggestive of Parkinson's disease.	Asymmetric putamen reduction
29	Normal specific uptake throughout both lentiform nuclei and both heads of caudate. CONCLUSION: No evidence of dopaminergic synaptic loss.	Normal
30	There is a relatively small but definite asymmetry in specific uptake between the posterior aspects of the lentiform nuclei with a small but definite moderate defect at the posterior aspect of the left lentiform nucleus. The remainder of specific uptake is within normal limits for age.	Asymmetric putamen reduction
31	Normal specific uptake throughout both lentiform nuclei and both heads of caudate.	Normal
32	Specific uptake within both lentiform nuclei and both heads of caudate is just within normal limits for age.	Normal
33	Abnormal DaTscan. There is a moderate reduction in specific uptake within the left lentiform nucleus, particularly posteriorly with a further mild reduction in specific uptake within the left head of caudate. There is a relatively subtle reduction in specific uptake by the most posterior aspect of the right lentiform nucleus but with relatively normal specific uptake within the right head of caudate. Findings are in keeping with Parkinsonism.	Asymmetric putamen reduction
34	Specific uptake within both lentiform nuclei and both heads of caudate is just within normal limits for age.	Normal
35	There is a possible, subtle generalised reduction in specific uptake throughout both basal ganglia, for age but no focal loss of specific uptake.	Borderline
36	Excellent, normal specific uptake of DaTscan throughout both lentiform nuclei and both heads of caudate.	Normal
37	Definite asymmetry in DaTscan uptake with a mild to moderate reduction within the posterior aspect of the right lentiform nucleus. Otherwise normal specific uptake within the left lentiform nucleus and both heads of caudate. COMMENT: Definite loss of dopaminergic synapses posteriorly within the right lentiform nucleus in keeping with relatively early Parkinsonism.	Asymmetric putamen reduction
38	Excellent normal specific uptake of DaTscan throughout both lentiform nuclei and both heads of caudate. Normal study.	Normal
39	Excellent normal specific uptake of DaTscan throughout both lentiform nuclei and both heads of caudate. Normal study.	Normal
40	Abnormal DaTscan. There is a moderate reduction in specific uptake throughout both lentiform nuclei with a further moderate reduction in specific uptake within the right head of caudate and a mild reduction in specific uptake in the left head of caudate. Comment: Definite bilateral dopaminergic synaptic loss greater on the right than the left side.	Asymmetric putamen reduction

41	Excellent normal specific uptake of DaTscan throughout both lentiform nuclei and both heads of caudate. Normal study.	Normal
42	No focal loss of specific uptake nor asymmetry but there is a borderline, very subtle, possible generalised loss in specific uptake throughout both basal ganglia although this may be in keeping with a prominent age-related loss of dopaminergic synapses.	Borderline
43	Abnormal DaTscan. There is a moderate reduction in specific uptake within the posterior aspect of both lentiform nuclei, possibly slightly more conspicuous on the left which becomes more mild anteriorly with a further probable subtle reduction in specific uptake within both heads of caudate. CONCLUSION: Bilateral dopaminergic synaptic loss in keeping with Parkinsonism.	Asymmetric putamen reduction
44	Normal specific uptake throughout both heads of lentiform nuclei and both heads of caudate. CONCLUSION: Normal study no evidence of dopaminergic synaptic loss.	Normal
45	Normal specific uptake throughout both heads of lentiform nuclei and both heads of caudate. CONCLUSION: Normal study no evidence of dopaminergic synaptic loss.	Normal
46	There is a mild to moderate defect in specific uptake involving the posterior aspect of the left lentiform nucleus with relatively preserved specific uptake anteriorly within the left lentiform nucleus and normal specific uptake within the right lentiform nucleus and both heads of caudate. CONCLUSION: Abnormal DaTscan with dopaminergic synaptic loss posteriorly within the left lentiform nucleus in keeping with Parkinsonism.	Asymmetric putamen reduction
47	Excellent normal specific uptake of DaTscan throughout both lentiform nuclei and both heads of caudate. Normal study.	Normal
48	Excellent normal specific uptake of DaTscan throughout both lentiform nuclei and both heads of caudate. Normal study.	Normal
49	Markedly abnormal study. There is a severe reduction in specific uptake by the posterior aspect of both lentiform nuclei which becomes more moderate anteriorly with a further moderate reduction in specific uptake by the right head of caudate and a mild reduction in specific uptake by the left head of caudate. COMMENT: Bilateral, asymmetric dopaminergic synaptic loss more advanced within the right basal ganglia and in keeping with Parkinsonism.	Asymmetric putamen reduction