Late onset depression: Association with clinical and imaging indicators of prodromal Parkinson's disease

Hiba Kazmi

Institute of Neurology, University College London

Submitted to the University College London, UK for the degree of Doctor of Philosophy

2019

Acknowledgements

Firstly, I would like to thank my supervisors Anette Schrag and Zuzana Walker for their guidance and support in the completion of this study and thesis. I have learnt a lot from you both.

I would like to thank Prof Jan Booij (Department of Nuclear Medicine, University of Amsterdam) for his time and expert advice with regards to the DAT SPECTs and to Sofie Adriaanse for both helping and hosting me during my trip to the Netherlands.

I am grateful to each of the participants that took part in this study, members of the research teams who helped with assessing patients and the various avenues through which recruitment was achieved. In particular, thank you to the Camden and Islington IAPT service, who were the main source of recruitment for the study.

Thank you to Carole Sudre (UCL Institute of Neurology, Dementia Research Centre), for processing and providing the volumetric data of the MRI scans and for her advice with regards to methodology and analysis of the volumetric data. I would like to thank Faraan Khan for his help as a secondary rater for the visual MRI ratings, to Sudhakar Praharaju (Essex Partnership NHS trust) for training me on rating the MR scans and to Kareem Khan for his help as a secondary rater for the UPDRS ratings. An additional thank you to Thomas Wagner and Daniel McCool (Department of Nuclear Medicine, The Royal Free Hospital) for their support with regards to obtaining the DAT SPECTs.

Finally, thank you to my family for all their support and in particular to my husband Danyal, for always believing in me.

Declaration

I, Hiba Kazmi confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Abstract

The pathology of late onset depression (LOD) is unclear, with vascular and structural abnormalities reported through the use of magnetic resonance imaging (MRI). Parkinson's disease (PD) can be preceded by LOD several years before overt motor symptoms. Use of the Dopamine Transporter Single Photo Emission Computerised Tomography (DAT SPECT) has been approved by the Food and Drugs Administration (FDA) for imaging pre synaptic dopaminergic dysfunction; aiding diagnosis in disorders such as PD. We investigated; a) presence of early clinical features associated with PD in patients with LOD (pLOD), b) dopaminergic functioning in pLOD, c) structural and vascular pathology associated with LOD. This thesis also compared visual and semi quantitative analysis (SQA) methods when rating DAT SPECTs. When compared with healthy controls (HC) (n=30) overall, pLOD (n=36) scored significantly higher on symptoms of apathy, REM sleep behaviour disorder (RBD), sleep symptoms associated with PD and autonomic and motor dysfunction. Seven pLOD were visually rated with abnormal uptake in comparison to one HC, also confirmed by the SQA which additionally found that overall, pLOD (n=29 with a DAT SPECT) had significantly lower binding in the caudate nuclei (CN). The pLOD with visually abnormal SPECTs had significantly lower clinical scores relating to olfactory dysfunction, cognition and autonomic dysfunction compared to pLOD with normal SPECTs. There were no significant group differences in structural or vascular pathology. There were significant correlations between age and left and right hippocampi (HIPP) and amygdala (AMY) and global white matter (WM) and grey matter (GM) volumes in pLOD (n=28 with an MRI). Overall, in controls (n=25), age and gender correlated with global WM. Severity of depression was not associated with regional

volumes. An audit of the correct use of clinical indications for the DAT SPECT at an NHS trust confirmed correct use in all cases (n=74) and a comparison of visual and SQA methods when rating DAT SPECTS in clinical cases also confirmed very good agreement between methods.

Impact statement

Depression after the age of 55 years: the role of dopamine, olfactory dysfunction, REM sleep behaviour disorder and age

Depression is the third leading contributor to the global burden of disease (World health organization 2004) affecting an estimated 350 million people worldwide. More than half of older adults with depression have their first episode of depression (known as LOD) after the age of 60 years (Fiske et al 2009) with pLOD at a higher risk of completed suicide (Bellini and Matteucci, 2001). Whilst depression in later life is commonly associated with lifestyle and social changes, comorbid illness' and bereavement, LOD is thought to be more likely to be due to *underlying organic brain disease.*

LOD has been reported prior to the diagnosis of another later life disorder; PD, which is a progressive disease of the nervous system typically affecting those aged over 65 years with a prevalence of approximately one per cent. PD is the second most common neurodegenerative disorder however the aetiology remains inconclusive.

The current study investigated the symptoms and brain pathology associated with PD in pLOD through the use of DAT SPECT imaging which is a technique which allows to image the binding and pattern of the DAT (dopamine is a neurotransmitter associated with mood and movement amongst other behaviours) available in the brain and MRI to investigate structural changes in the brain. Our research found that pLOD had significantly lower dopamine binding when compared to healthy control subjects. The pattern of dopamine was reflective of that

seen commonly in patients with PD and related neurodegenerative disorders. Additionally, these pLOD with abnormal patterns of uptake had significantly lower scores when clinically measuring sense of smell, cognitive abilities and autonomic dysfunction (i.e. urinary dysfunction and constipation). Overall in pLOD, there was an overlap of symptoms commonly associated with depression in general but also in PD. The pLOD had significantly more symptoms relating to REM sleep behaviour disorder; RBD (characterised by “acting out dreams”); a feature not usually associated with LOD but found in those with PD and related neurodegenerative diseases. Using MRI, there were significant associations between age and HIPP and AMY volumes (not found in control subjects). Depressed mood (and stressful events) increase the effect of age on the brain structure (Soares et al., 2014) with suggestions that current and lifetime major depressive disorder (MDD) has a negative impact on the neurodegenerative process of PD (Chagas 2017).

This exploratory pilot study hopes to promote further research into LOD and long term follow up into assessing clinical progression. Our results hope to aid further understanding into the associated symptomology of LOD; raise aware of LOD as a subtype of depression and also differentiate between those with LOD and a dopaminergic dysfunction.

Better understanding of these two later life diseases which have a global impact both socially and financially will help improve quality of life for patients and carers and improve treatment strategies and diagnosis at the initial stages.

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Abbreviations

AD Alzheimer's disease

AMY- Amygdala

AT- Akinesia Time

AU- Abnormal (PUT and CN SBRs) uptake

BaMoS- Bayesian Model Selection

BDNF- Brain derived neurotrophic factor

BRAIN- Bradykinesia Akinesia Incoordination

CN- Caudate nuclei

CVD- Cardiovascular disease

DA- Dopamine

DLB- Dementia with Lewy bodies

DAT SPECT- Dopamine Transporter Single Photo Emission Computerized Tomography

DICOM- Digital Imaging and Communications in Medicine

DSM 5 SMD - Diagnostic and statistical manual of Mental Disorders 5: Severity Measure for Depression

EMA-European medicines agency

EOD- Early onset depression

ET- Essential tremor

FAB- Frontal Assessment Battery

FDA- Food and drugs administration

FLAIR- Flair attenuated aversion recovery

GIF- Geodesic Information Flows

GM- Grey matter

HADS-D- Hospital Anxiety and Depression Scale-Depression

HADS-A- Hospital Anxiety and Depression Scale-Anxiety

HC – Healthy controls

HIPP- Hippocampi

HVA- Homovanillic acid

IF- Incidental finding

KS- Kinsesia score

LARS- Lille Apathy Rating Scale

LBD- Lewy body disorders

L/R – Left/Right

LL- White matter lesion load

L-DOPA- Levodopa

LLD- Late life depression

LOD -Late onset depression

MDD- Major depressive disorder

MDS- UPDRS- Movement Disorder Society Unified Parkinson's Disease Rating Scale

M-EDL- Motor Aspects of Experiences of Daily Living

MoCA -Montreal Cognitive Assessment

MPS- Mild Parkinsonian Signs

MRI- Magnetic resonance imaging

MSA- Multiple system atrophy

nM-EDL- Non Motor Aspects of Experiences of Daily Living

NMS- Non motor symptoms

NMP- Nuclear medicine physicians

NU- Normal (PUT and CN SBRs) uptake

PD- Parkinson’s disease

PD screening- Parkinson’s disease screening scale

PDSS- Parkinson’s disease Sleep Scale

PET- Positron Emission Tomography

pLOD - Patients with late onset depression

PS- Parkinsonian syndromes

PSP- Progressive supranuclear palsy

PUT- Putamen

RBD- REM sleep behaviour disorder

RBD1Q- The REM Sleep Behaviour Disorder single questionnaire screen

RBDSQ- The REM sleep Behaviour Disorder screening questionnaire

SBRs- Striatal binding ratios

SCOPA-AUT- Scales for Outcomes in Parkinson's disease- Autonomic

SD- Standard deviation

SERT- Serotonin transporter

SN- Substantia nigra

SNM- Society of Nuclear medicine

SNRIs- Serotonin and norepinephrine reuptake inhibitors

SPECT – Single Positron Emission Computerised Tomography

SSRIs- Selective serotonin reuptake inhibitors

SQA- Semi quantitative analysis

UPSIT- University of Pennsylvania Smell Identification Test

VR- Visual ratings

WM- White matter

WMH- White matter hyper intensities

WML- White matter lesions

1.5 /3 T- 1.5 tesla/3 tesla

[^{123}I] FP-CIT- [^{123}I] -labeled N-delta-(fluoropropyl)-2beta-carbomethoxy-3beta-(4-iodophenyl) tropane

^{123}I-β-CIT- [^{123}I] 2β-carboxymethoxy-3β-(4-iodophenyl) Tropane 5-HT- Serotonin

Chapter 1. **Main Introduction**

PD is characterised by the presence of clinical motor symptoms which are thought to occur when at least 50% of dopaminergic neurones in the substantia nigra (SN) have already been depleted (Fearnley and Lees, 1991). At least ten years (Schrag et al., 2015) prior to the emergence of motor features, non-motor symptoms (NMS) can present during the prodromal phase of PD, affecting autonomic, sleep and sensory domains. Neuropsychiatric symptoms such as depression and anxiety have also been reported four to six years prior to PD diagnosis (Gonera et al., 1997) and additionally a direct association between depression and subsequent PD over a two decade follow up has been reported (Gustafsson et al., 2015).

Whilst the role of dopamine (DA) in motor features (and other NMS) of PD is acknowledged, research into dopaminergic activity in LOD in those without PD through the use of imaging is limited. LOD is defined by **first onset** of depression presenting **only** after the age of 55 years.

Using DAT SPECT, this pilot study investigated the dopaminergic uptake in the nigrostriatal pathway in those pLOD. NMS and subtle motor features associated with PD were explored. Additionally, volumes of GM and WM and structural pathology associated with LOD such as reduced subcortical volumes, enlarged ventricles and white matter lesions (WML) in the brain were investigated through the use of MRI.

1.1 Overview of Parkinson's disease

PD is a progressive disease of the nervous system and as a movement disorder, is primarily characterised by a triad of motor symptoms; rigidity, bradykinesia and tremor. It typically affects those aged 65+ although can also be found earlier in life, with a prevalence of approximately one per cent. PD is the second most common neurodegenerative disorder and whilst the elderly population of the world increases, the aetiology of PD remains inconclusive. As well as the classic motor symptoms, patients are also affected by a variety of brainstem affected NMS relating to mood (depression, apathy, psychosis), sleep disturbances (RBD) autonomic dysfunction (bladder problems, constipation) and olfactory deficits (anosmia) (Postuma et al., 2012b, Ross et al., 2012, Hawkes, 2008). Experienced by virtually every patient, often it is these NMS which are ignored when they are largely treatable causing significant negative impact on quality of life (Chaudhuri et al., 2006). NMS have found to be more common in the history of PD patients and can present years before PD diagnosis (Gonera et al., 1997, Schrag et al., 2015).

1.1.1 Depression in PD

Thirty to forty per cent of PD patients suffer from a depressive disorder (Tandberg et al., 1996), with 17% of PD patients experiencing major depressive disorder and 13% being diagnosed with dysthymia (Reijnders et al., 2008). Ehrt et al. (2006) reported that depression associated with PD is a milder form of depression in comparison to those depressed without a neurological disorder.

Research has also reported the presence of symptoms of depression and anxiety in pre diagnostic PD. Gonera et al. (1997)'s retrospective

study of 60 patients with PD and age matched controls reported pre PD patients had more central nervous system, cardiovascular, musculoskeletal and psychological symptoms four to six years before a clinical PD diagnosis. Later research by Nilsson et al. (2001) reported patients with an affective disorder had a significantly higher risk (odds ratio 2.2) of being diagnosed with PD in comparison to control groups. Schuurman et al. (2002)'s retrospective study reported that pLOD had a positive association between depression and subsequent risk of PD with a large Hazard Ratio effect of 3.27, a finding also supported by Shiba et al. (2000). Gustafsson et al. (2015) also reported a direct association between depression and consequent PD. More recent studies include a meta-analysis by Wang et al. (2018) who reported that depression was associated with a 2.2-fold increase in the incidence of PD and Schrag et al. (2019) reporting that amongst other pre-diagnostic symptoms of PD, 18% of patients versus 10% of controls had been diagnosed with depression (after the age of 50) within five years of PD diagnosis Antidepressant therapy has also been reported to be associated with a higher risk of PD two years later (Alonso et al., 2009).

Pathology

Alongside psychosocial issues, various changes in the brain involving cerebrovascular disease, toxic stress, inflammation, genetic factors and monoaminergic disturbances (Aarsland et al., 2011) have been suggested to contribute to depressive symptoms in PD.

The progression of Lewy Body inclusions can provide some explanation for consequent non-motor and motor symptoms of PD, including depression (Table 1-1).

Lewy body proteins are found in the SN where they are thought to deplete the neurotransmitter DA, causing Parkinsonian symptoms.

The Braak staging hypothesis initially described the progression of Alzheimer's disease (AD) (Braak and Braak, 1991) and according to the six stages in PD (Braak et al., 2003), Lewy body inclusions reach the SN in stage 4, fitting in with the clinical evidence of motor symptoms and the development of prodromal NMS (anosmia, sleep disorder and constipation). Tsopelas et al. (2011) found evidence of subcortical Lewy bodies in those with late life depression (LLD) as well as evidence of neuronal loss of subcortical structures such as the SN. Evidence of the Braak stages has not been found in all patients and emotional disturbances only occur in the last stage of the Braak stage hypothesis, suggesting depression as a later rather than prodromal symptom of PD. However, it has still proved useful in part explaining the pathology of PD and related disorders.

Whilst serotonergic, noradrenergic and cholinergic neurotransmitter systems are recognised in PD (Gallagher and Schrag, 2012) (and in depression), the focus of the current study will be dopaminergic activity.

Table 1-1 Braak stages: the development of features of Parkinson's disease (PD)

Braak stage	Area of brain affected by Lewy bodies	Symptoms
1-2 "Pre-motor"	Brainstem	Autonomic and olfactory disturbances
3-4	Brainstem and cortical	Sleep and motor disturbances
5-6	Brainstem and cortical	Emotional and cognitive disturbances

1.1.2 Use of DAT SPECT in depression in PD

DA is a neurotransmitter commonly associated with mood, reward motivated behaviour and movement via the following four primary pathways; mesocortical and mesolimbic, tuberoinfundibular and the nigrostriatal. The association between a dopaminergic deficit in the nigrostriatal pathway and consequent movement dysfunction in PD is well established; symptoms of PD are thought to present upon a 50% loss of the nigral DA neurons; (Fearnley and Lees, 1991) or until there is a depletion of at least 80% of striatal DA (Stoessl, 2007).

Dopaminergic activity in the nigrostriatal pathway has been explored via Positron Emission Tomography (PET) and SPECT imaging techniques by assessing decarboxylase activity, DA receptors (DA 1; D_1 and DA 2 ; D_2) and the DA transporter (DAT) (Camardese et al., 2014). PET imaging using L-3,4-dihydroxy-6-[^{18}F]-fluorophenylalanine (^{18}F-DOPA) was the first neuroimaging technique validated for the assessment of presynaptic dopaminergic integrity and provided imaging evidence of striatal denervation in Parkinson's disease by measuring decarboxylase activity and DA turnover (Shen et al., 2012). With regards to imaging the DAT, the DAT protein is expressed exclusively on the membrane of the presynaptic dopaminergic terminal and involved in the reuptake of DA (Booij et al., 2007). Binding of DAT in the striatum is thought to reflect the number of dopaminergic neurones in the SN, with significantly higher levels of DAT expression in the SN pars compacta (Storch et al., 2004). PET tracers (such as [^{11}C]RTI-32) and SPECT tracers are now available for measuring the DAT (Shen et al., 2012).

SPECT tracers for measuring the DAT include the [^{99m}Tc]technetium [2-[[2-[[[3-(4-chlorophenyl)-8-methyl-8-azabicyclo [3.2.1]oct-2-yl]-methyl](2-mercaptoethyl)amino]-ethyl]amino]ethane-thiolato(3-)-N2,N2′,S2,S2′]oxo-[1R-(exo-exo)] (**^{99m}Tc-TRODAT-1),** the [**^{123}I**] 2β-carboxymethoxy-3β-(4-iodophenyl) Tropane (**^{123}I-β-CIT**) and the **^{123}I** -labeled N-delta-(fluoropropyl)-2beta-carbomethoxy-3beta-(4-iodophenyl) tropane (**[^{123}I] FP-CIT**). In particular the [^{123}I] FP-CIT has a high binding affinity to the DAT and faster kinetics than the ^{123}I-β-CIT tracer. [^{123}I] FP-CIT SPECT is also approved by the FDA and widely used for imaging clinically uncertain movement disorders such as PD (Bajaj et al., 2013).

When exploring dopaminergic activity in depression in PD using [^{11}C]RTI-32 PET, it was reported that depressed PD patients had lower DA and noradrenaline transporter binding in the limbic system and locus coeruleus compared to non-depressed PD patients (Remy et al., 2005). When using SPECT, alongside motor symptoms, depressive symptoms in PD have also been reported to correlate with left putamen (PUT) DAT availability (Weintraub et al., 2005) and correlate negatively with DAT uptake in the right caudate nuclei (CN); with multiple regression analysis showing that the depression score explained a significant amount of the uptake (Vriend et al., 2014). It can be suggested that DA has a role in depression in PD as well as alongside its established association with motor dysfunction in PD but to our knowledge, there has been no research investigating dopaminergic activity in LOD in pre diagnostic PD. Alongside changes in noradrenergic and dopaminergic activity, increased serotonin (5 HT) and acetylcholine deficits in those with depression and PD has also been reported (Boileau et al., 2008, Bohnen et al., 2007, Aarsland et al., 2011).

1.2 Overview of LLD

Depression is the third leading contributor to the global burden of disease (World health organization 2004) affecting an estimated 350 million people worldwide and is a mental health disorder experienced by approximately one in six people at some point in their lives. Depression can cause a wide variety of emotional and physical symptoms at different degrees affecting mood, appetite, cognition and daily living. It can be experienced at any age with research suggesting women have a prevalence rate up to twice that of men (Bebbington, 1996). There are several types of depression as classified by the Diagnostic and Statistical Manual of Mental Disorders V (DSM 5) criteria (Table 1-2), differing in symptoms and duration. The underlying basis of depression and LLD is likely multifactorial and involving psychological, biological and social risk factors (Fiske et al., 2009). However, as mentioned previously, abnormalities in the serotonergic, noradrenergic and dopaminergic transmitter systems are all considered important.

LLD

LLD is depression that occurs in patients later in life. In the over 65s, prevalence of major depression is two per cent and prevalence of depressive symptoms is between 10-15 % (Variend and Gopal, 2008). Depression in later life is associated with bereavement, social isolation, sleep disturbances, polypharmacy and physical illnesses/co morbidities (Cole and Dendukuri, 2003). Despite depression being a well-known result of later life diseases such as PD, the diagnosis of psychiatric disorders in PD is difficult especially because there is an overlap of symptoms found in both neurodegenerative disorders and depression

(Weintraub et al., 2005). Symptoms include psychomotor changes, fatigue, insomnia and apathy.

Table 1-2 Types of depression as classified by DSM 5 criteria

Type	Short description
Major depressive disorder (MDD)	5 persisting symptoms for 2 weeks or more e.g. depressed mood, loss of interest, change in weight, feelings of worthlessness, insomnia/hypersomnia, recurrent thoughts of death
Persistent depression disorder (Dysthymia)	2-4 persisting symptoms for 2 weeks or more
Other types	
Disruptive and mood dysregulation disorder	
Premenstrual dysphoric disorder	
Substance/Medicine induced disorder	
Unspecified depressive disorder	
Bipolar and related disorders	

LOD

LLD can be categorised into LOD and early onset depression (EOD). EOD and LOD vary not only in onset of time but also pathology and symptomology (Krishnan et al., 1995). LOD is characterised by onset of depression for the first time post 55 years of age with no previous history of significant depression. EOD refers to depression also occurring later in life but with onset *prior* to the age of 55 years. More than half of older adults with depression have their first episode of depression after 60 (Fiske et al 2009) and 3/4 of patients with a major depressive disorder referred to old age psychiatrists have LOD (Baldwin and O'Brien, 2002). It has been difficult to ascertain the overall prevalence and incidence of LOD (Variend and Gopal, 2008). Haigh et al. (2018)'s update on depression older adults reported that incidence rates of first onset in older age are generally lower than rates for younger or middle aged adults with studies reporting a 1.2% one year incidence rate for individuals over the age of 55 years (Eaton et al., 2007, Grant et al., 2009). LOD is thought to occur in approximately 30% of patients with LLD (Mackin et al., 2014, Janssen et al., 2006). It should be noted that reported prevalence and incidence dates for LOD are likely to be inclusive of all types of LOD (and not just due to organic pathology as will be explored in the current study).

Although some research has reported no differences between EOD and LOD phenomenology (Brodaty et al., 2001), there is evidence reporting some important differences between these two types of LLD (Schweitzer et al., 2002). Common LOD symptoms include psychomotor changes, anhedonia (Rapp et al., 2005) and greater cognitive impairment (discussed further below) in comparison to those with EOD (Naismith et al., 2003). On the other hand, patients with EOD more likely to have more suicidal thoughts and feelings of guilt and

worthlessness (Variend and Gopal, 2008, Reinhard et al., 2000, Janssen et al., 2006).

Rapp et al. (2005) specifically reported those with LOD had deficits in attention and executive functioning and those with EOD presented with deficits in episodic memory. Elderkin-Thompson et al. (2003) reported severity of cognitive deficits increases with depression severity regardless of age or education in LOD although Gallassi et al. (2006) reported that cognitive deficits in LOD can present even following recovery suggestive that deficits are not the result of depressed mood. In addition to cognitive impairment being more likely in those with LOD, alongside symptoms of depression, research has also reported associations between LOD and consequent dementia (Schweitzer et al., 2002) with research reporting that 40% per cent of LOD patients develop dementia (Alexopoulos et al., 1993).

Comorbid illnesses, higher chance of cognitive impairments and patients being older and frail can lead to difficulties in diagnosis with those with LOD at risk of being under-recognized (Mahapatra, 2015, Variend and Gopal, 2008). Furthermore, pLOD have also been associated with poorer outcome (including possible development of dementia) and whilst suicidal thoughts have been associated with those with EOD, Bellini and Matteucci (2001) reported that pLOD were at a higher risk of completed suicide, putting these patients at risk (Baldwin et al., 2004, Variend and Gopal, 2008).

1.2.1 Aetiology of LOD

The aetiology and presentation of depression in general is heterogeneous with the involvement of social, psychological and biological factors (genetic, neurochemical; including neurotransmitters and the brain derived neurotrophic factor; BDNF) and pathological

changes) (Kalia, 2005). In particular the monoamine hypothesis of depression, developed in the 1960's and 1970's in general suggests that the depletion of neurotransmitters 5HT, noradrenaline and DA in the central nervous system may in part form the pathophysiological basis of depression (Loonen and Ivanova, 2016). The effects of antidepressant drugs (Kalia, 2005) has furthered understanding of the role of neurotransmitters in depression with selective serotonin reuptake inhibitors (SSRIs) and selective norepinephrine reuptake inhibitors (SNRIs) targeting increasing serotonin and norepinephrine activity and alleviating symptoms of depression.

As the focus of this study, the role of the neurotransmitter DA in depression is discussed further below.

1.2.2 Pathology

DA

The effect of specific drugs on DA activity have also long been reported and provided understanding into the behaviour (mood) related to the activation or inactivation of DA. Examples include the effect of cocaine which increases DA levels and incites elevation of mood and the effect of reserpine which blocks DA receptors and consequently induces a depressed mood (D'Aquila et al., 2000, Jimerson, 1987). Other research investigating the role of DA has also included research into the nigrostriatal pathway (more commonly associated with movement) and mood. Bowden et al. (1997)'s post mortem study of depressed patients with completed suicides reported reduced DA in the CN (and nucleus accumbens). Additionally, Remy et al. (2005) reported a higher decrease in DAT binding in those patients with PD and depression compared to patients with just PD. Investigating metabolites of DA has also furthered understanding into the role of DA and associations with

depression with polymorphisms in genes involved in dopamine metabolism linked to major depression (Opmeer et al., 2010). In addition, reduced levels of homovanillic acid (HVA) which is a major metabolite of DA have been reported in those with depression (Bowers et al., 1969, Camardese et al., 2014, Lambert et al., 2000). The role of the BDNF and specifically DA has also been discussed by (Dailly et al., 2004) with research reporting that the BDNF controls the expression of the DA 3 (D3) gene (Sokoloff et al., 2002).

LOD is also thought to be heterogeneous (Van den Berg et al., 2001, Variend and Gopal, 2008, Fiske et al., 2009) and as a reaction to severe life stress **or** associated with a degenerative process. Severe life stress includes bereavement, illness and caregiving and research reporting depression as a prodrome to dementia is indicative of the degenerative nature of LOD (Alexopoulos et al., 1993, Fiske et al., 2009). The current study *excludes* those experiencing depression clearly related to a recent severe life stress or illness.

The pathophysiological basis for LOD remains unclear. LOD is thought to be more likely to be due to underlying organic brain disease than earlier onset depression (Gallagher et al., 2010, Kaimal and Nair, 2005) with suggestions for the underlying pathology including structural pathology (investigating volumes of areas of specific areas of the brain) and vascular and neurodegenerative pathology, as discussed below. On the other hand those with EOD have been reported to be more likely to have a family history of depression (Heun et al., 2001) suggestive of a genetic component.

Vascular changes

With research proposing that pLOD acquire age related neurodegenerative diseases such as AD or vascular dementia (Schweitzer et al., 2002), vascular changes have been considered in the pathology of LOD, as reported by Van den Berg et al. (2001). The “Vascular Depression” hypothesis coined by Alexopoulos et al. (1997) suggests that cerebrovascular ischaemic damage may predispose and promote depressive symptoms (and executive dysfunction). To build on this hypothesis and provide evidence of pathological (vascular) changes, Krishnan et al. (1997) proposed the MRI defined vascular depression hypothesis and proposed evidence of white matter hyper intensities (WMH) as the basis of vascular depression through the use of T2 weighted MRI sequences (Taylor et al., 2013). Subsequent research has combined the vascular depression and MRI defined vascular depression hypotheses and investigated WMH in patients presenting with depression and investigated associations between vascular changes, LLD and cognition. Godin et al. (2008) reported that LLD and WML are strongly related in patients over the age of 65 (but with a lifetime history of depression) who were found with significantly higher WML load. Furthermore, depression free participants but with a higher WMH load at baseline were at a higher risk of developing depression at the four year follow up (odds ratio 1.3).

Research specific to LOD includes another MRI study between patients with EOD and pLOD (first episode of depression after the age of 60 years old) (Figiel et al., 1991). Figiel et al. (1991) reported 60% of pLOD presented with large deep WMH compared to 11% of patients with EOD who were age matched (with first depression onset before the age of 60 years) through the use of T2 sequences. O'Brien et al. (1996)’s use of T2 weighted sequences further reported that deep

white matter lesions (DWML) were most common in patients with first time depression onset after the age of 55 and with 50% of these patients with evidence of severe DWML. Despite this supportive research, due to a lack of robust findings between cerebrovascular disease and LLD in all studies, (Aizenstein et al., 2011, Tsopelas et al., 2011) the vascular depression hypothesis is not universally accepted. However, it has still proved useful in part in providing evidence of pathology associated with depression in later life and in research investigating depression as a prodrome to dementia (discussed later).

Other pathology: subcortical regions and ventricular volumes

Additionally, differences in the volumes of subcortical regions and ventricular regions have also been reported as pathology associated with LLD. Kempton et al. (2011)'s meta-analysis of structural neuroimaging studies reported that patients with MDD (regardless of age) were associated with smaller volumes of the basal ganglia and HIPP amongst other regions through the use of MRI and computed tomography (CT).

Specific to LOD, Greenwald et al. (1997) reported that pLOD (first onset of depression >60 years) had significantly more left medial temporal and left CN atrophy than EOD patients of a similar age. Egger et al. (2008) also reported a significant decrease of volume in the right AMY in pLOD (first depression onset after 60), when using voxel based morphometry. The role of the HIPP has been associated with depression in general (Egan et al., 2003, Kalia, 2005) and reduced HIPP volumes also reported in pLOD. Sivakumar et al. (2015)'s volumetric analysis of subregions of the HIPP using T1 MRI in 25 patients with first time depression onset after the age of 50 reported a significant reduction of volume in the posterior HIPP in comparison to controls. Depression severity also negatively correlated with volumes

of the posterior HIPP regions. In age corrected analysis, Hickie et al. (2005) reported reduced HIPP volumes in 17 pLOD with first onset of depression after the age of 50 years in comparison to controls. Those with EOD and a subtype of melancholic depression were also reported with reduced total and right HIPP volumes however significant differences between controls were found only with pLOD and not between those with EOD and HC; possibly providing evidence of differences in pathology within LLD.

Other pathological changes include reports of pLOD with greater ventricular size than those with EOD (Alexopoulos and Chester, 1992). Dahabra et al. (1998) also reported larger third and lateral ventricles and increased ventricular brain ratio (through the use of T1 and T2 MRI) in12 pLOD (first onset of depression post 55 years of age) in comparison to 11 age matched patients with EOD. In the same study, Dahabra et al. (1998) further reported greater severity of WML in pLOD when compared with EOD.

1.2.3 LOD and neurodegenerative disorders

Increasingly a link between LOD and neurodegenerative disorders is being suggested with studies suggesting that up to 40% of pLOD develop dementia (including AD, vascular disease and dementia with Lewy bodies (DLB) (Schweitzer et al., 2002, Fiske et al., 2009, Alexopoulos, 2005), but with no data existing for later development of related neurodegenerative diseases such as PD or LBD (Lewy body disease). However the risk of having had depression prior to the onset of symptoms is similar in AD and PD/LBD and their incidence in the age group 55-70 is similar (~0.3% (Perez et al., 2010, von Campenhausen et al., 2005, Kawas et al., 2000).

Within imaging research, associations between depression and later dementia have been reported by Schweitzer et al. (2002)'s review which suggested that pLOD are more likely to have cognitive impairment and DWML, but with LOD not hypothesised to be a prodrome to a particular type of dementia. In line with the findings from Schweitzer et al. (2002)'s review, associations between WMH, depression and different types of dementia have been found and will be discussed next.

O'Brien et al. (1996) also reported that DWML were also significantly more common those with AD (in comparison to heathy controls) suggestive of the role of WML in dementia and potential associations between depression and dementia due to shared pathology (WML). Nebes et al. (2001) reported that adults with LLD carrying the apolipoprotein E gene (ApoeE) (a susceptibility gene for AD) were reported with significant associations between depressive symptoms and presence of hyper intensities in DWL, with a similar risk between those with first time depression onset post 45 years of age.

Further evidence of shared pathology between LOD and neurodegenerative diseases comes from Barber et al. (1999) who reported total deep hyperintensities scores (WMH and basal ganglia hyperintensities) in those with vascular, DLB and AD, with higher total deep hyperintensity scores in the patients with vascular dementia. Barber et al. (1999) further reported that in all patients with dementia, frontal WMH were associated with higher depression scores. The reported findings of WMH in those with depression and associations between WMH and depression scores (as reported by Barber et al) are perhaps supportive of vascular depression as one of the explanations for LOD.

The neurodegenerative nature of LOD can be hypothesised with reports of hippocampal [also associated with AD and cognitive impairment; Teipel et al. (2006), Mackin et al. (2014)] and vascular changes and research investigating LOD as a prodrome to dementia (Schweitzer et al., 2002). Other pathology associated with AD includes global, parietal and medial temporal atrophy and will be explored in the participants in the current study.

Additionally, as also suggested by Schweitzer et al. (2002) the role of serotonin, noradrenaline and dopamine are just as relevant in the phenomena of dementia (as in PD) as they are in depression providing further links in the overlap of chemical mechanisms of these later life disorders.

1.2.4 DAT imaging: in depression

Within imaging research in depression, the role of DA (by assessing the binding of the DAT) has also been reported, although to a lesser extent than in PD. Camardese et al. (2014)'s review on DAT imaging in depression reported mixed results with a range of study samples and tracers used; some of which will be discussed below

The majority of studies investigating depression using the SPECT tracer ^{99m}Tc-TRODAT-1 (the most specific radio ligand for the DAT; Dresel et al. (1998), reported greater DAT availability in patients with major depression (Brunswick et al., 2003, Amsterdam et al., 2012) when measuring DAT in the striatum For example, Brunswick et al. (2003)'s sample of 15 (drug free) patients with depression (major, bipolar or non-specified) with a mean age of 40 years old (SD 12.0) reported significantly higher DAT uptake in the right anterior and

posterior PUT and the left CN when compared with 46 age and gender matched controls. Similarly Amsterdam et al. (2012) measured DAT binding in 39 patients who were a mean age of 42 years old (SD 11.1) experiencing unipolar or a bipolar episode and reported that patients had significant greater binding of the striatal regions (PUT and CN) with no differences in binding between the types of depression; suggestive that DAT activity is not specific to a type of depression.

However studies using the SPECT ^{123}I-β-CIT radioligand (which has a binding affinity to both the DAT and the 5HT transporter; SERT) in patients with depression and controls have reported conflicting results (with the relationship between tracers, DAT and SERT discussed later). Neumeister et al. (2001) reported a reduction in DAT binding in the left striatum in patients with symptoms of depression with a seasonal affective disorder. On the other hand, Laasonen-Balk et al. (1999) reported significantly higher DAT uptake in both sides of the striatum in 15 drug free patients with major depression in comparison to controls. Patients were a mean age of 35.2 years of age and overall experiencing a moderate level of depression. Although DAT activity is mainly associated with the striatum and serotonergic activity mainly associated with the midbrain (Laruelle et al., 1993, Piccini, 2003), it should be noted that with the ^{123}I-β -CIT tracer some loss could be attributed to SERT loss. The differences in uptake between studies using the ^{123}I-β -CIT tracer could perhaps be attributed to the different types of depression investigated.

In line with the findings from Neumeister et al. (2001), Meyer et al. (2001) 's study which used [^{11}C]RTI-32, a positron emission tracer (PET) tracer also reported significantly lower DAT binding the striatum in nine patients with depression (an average age of 37 years SD 10.0) when compared with 23 HC. However, the [^{11}C]RTI-32 tracer also has

an affinity to SERT and noradrenaline (NAT) and the potential loss observed could be attributed to loss of SERT or NAT terminals instead of exclusively DAT.

In the current study, we will explore the DAT activity in those with LOD using [^{123}I] FP-CIT SPECT imaging and to our knowledge, only one study has used this tracer in those with depression. Sarchiapone et al. (2006) investigated DAT binding in 11 patients with major depression who had anhedonia as a predominant feature and nine HC. Although this study investigated DAT binding in patients with depression, patients in the study were a mean age of 41.1 (SD 11.9) and with patients over 60 years of age excluded from the study. In line with earlier studies (Meyer et al., 2001, Neumeister et al., 2001), Sarchiapone et al. (2006) reported **reduced** left and right DAT binding in the striatum in patients with depression. Both Sarchiapone et al. (2006) and Meyer et al. (2001) attributed reduced binding of the DAT to a reduced number of DATs per neuron due to a downregulation secondary to lower DA concentrations and impairment of dopaminergic transmission. Meyer et al further suggested a similar explanation in studies reporting an *increase* in binding ratios in those with depression; an increase in ratios was due to a temporary upregulation to compensate for the reduced DATs.

The [^{123}I] FP-CIT tracer has a higher selectivity for the DAT (Piccini, 2003) than the ^{123}I-β-CIT and with moderate affinity to SERT (Booij and Kemp, 2008) . The ^{123}I-β-CIT and [^{123}I] FP-CIT's affinity to SERT (and the DAT) has been investigated by exploring the effects of SSRIs on binding ratios in the striatum, occipital lobe and other SERT rich areas (including the midbrain, hypothalamus and lungs). In particular, the use of antidepressant medications such as SSRIs and SNRIs have reported to decrease SERT ratios and *increase* DAT striatal binding

ratios (SBRs) by ten per cent (Booij and Kemp, 2008). The relationship between SERT and DAT when assessing DAT binding in the striatum will be discussed further below.

Role of SERT

The 5-HT system can be investigated by measuring SERT densities using tracers such as the ^{123}I-β-CIT (which has a high binding affinity to SERT as well as to DAT). A vivo human study by Kugaya et al (2003) reported the inhibition of SERT by (chronic treatment of) citalopram (an SSRI) and elevation of the DAT. The authors suggested that the increase might be caused by interaction between 5-HT and DA systems. Additionally, in a study by de Win et al. (2005) where citalopram was also administered, the authors reported increased ^{123}I-β-CIT binding in the striatum in comparison to the striatum of those that had a placebo. Earlier work by Scheffel et al. (1994) suggested that SSRIs potentially cause displacement of radiotracer from peripheral and central SERTs leading to an increased availability for binding to DATs. In line with this, de Win et al. (2005) hypothesised that the combination of increased tracer availability (due to peripheral SERT blockage; due to citalopram and consequent decreased SERT availability (due to central SERT blockage) in the brain might have led to increased availability of the tracer for binding to **DATs** and in particular in the striatum, which is DAT rich.

In particular Booij et al. (2007)'s study using the [^{123}I]-FP-CIT tracer (as will be used in the current study) also reported an increase in SBRs (specific to striatal-occipital lobe ratios; again, as will be used in the current study) in the striatum after intake of paroxetine (another SSRI). As with the earlier studies discussed using the ^{123}I-β-CIT tracer, Booij et al. (2007) suggested that paroxetine blocks the binding of SERT to

the tracer in the occipital cortex, increasing availability for the DAT and thus increased DAT SBRs.

It is apparent that either a relationship between serotonergic and dopaminergic systems or alternatively the suppression of SERT by SSRIs results in an increase in availability for the tracer to bind to the DATs (Booij et al., 2007, Kugaya et al., 2003, de Win et al., 2005, Scheffel et al., 1994). It should be noted that this ten per cent increase (also relevant to the use of SNRIs) is thought not to affect the visual interpretation of SPECT images, but should be controlled for when using SQA (Booij and Kemp, 2008). This will be discussed further in the methodology section of this thesis in “Chapter 4: DAT SPECT in LOD” where we investigate dopaminergic function in our study sample.

It can be suggested that the current role of DAT binding in depression is conflicting with discrepancies between the studies due to a number of factors. For example, varying (in some cases very small) sample sizes, the use of medication (and not adjusting for the effects of relevant medications) and different ages of onset and types of depression. Aside from clinical characteristics, studies have also used a range of different tracers (with affinities to other neurotransmitters alongside DAT, as discussed earlier) and imaging techniques, making it difficult to directly compare results. Camardese et al. (2014) discussed a trend of results based on the use of tracers noting that in 99mTc-TRODAT-1, a greater availability of DAT has more frequently been reported in depressed patients whilst for the [^{123}I]-FP-CIT, a lower DAT availability has been detected and additionally an inverse correlation between depressive symptoms and DAT levels in patients with PD. These discrepancies may be specific to the different affinities

of the tracers, however the use of different subtypes of depression cannot be excluded as a confounder for the differing results.

With discrepancies between the results reported by previous literature and with no literature available on the DAT activity specific to LOD, the following thesis will investigate DAT SBRs using [^{123}I]-FP-CIT SPECT in pLOD.

1.3 Study aims

The current study aims to use clinical assessments, DAT SPECT and structural MR imaging to explore whether pLOD present with features associated with prodromal PD.

Chapter 3 "Clinical correlates of LOD"

Objective 1: To compare non-motor and motor features in pLOD with those in HC, using clinical assessments. Whilst this is an exploratory study, we hypothesise that in comparison to HC, pLOD will present with features associated with prodromal PD including symptoms of apathy, autonomic dysfunction and subtle motor abnormalities. This is based on research reporting an overlap of symptoms such as apathy, cognitive deficits and psychomotor retardation present in LOD (Weintraub et al., 2005, Rapp et al., 2005, Gonda et al., 2015, Naismith et al., 2003) and also associated with PD (Darweesh et al., 2017, Chaudhuri and Schapira, 2009).

Chapter 4 "DAT SPECT in LOD"

Objective 1: To visually compare dopaminergic uptake in pLOD and HC. Additionally to compare the SBRs in pLOD with HC. We hypothesise that pLOD will present with patterns of abnormal dopaminergic uptake commonly associated with disorders such as PD and DLB (Fearnley and Lees, 1991, Bajaj et al., 2013, Walker et al., 2004). We propose that a proportion of pLOD will develop PD or a related neurodegenerative disease as we assume that at least 50% of LOD is neurodegenerative. This is based on previous follow-up studies suggesting 40% develop dementia (including AD, vascular disease and DLB) with no data available on PD (Alexopoulos, 2005, Schweitzer et al., 2002, Fiske et al., 2009). However, the associations between

depression and AD or PD are comparable (Shiba et al., 2000, Ishihara and Brayne, 2006, Ownby et al., 2006) and in the age group of 55-70 the incidence of PD/LBD and of AD is similar (both ~0.3%; (von Campenhausen et al., 2005, Perez et al., 2010, Kawas et al., 2000). Thus, we expect that after accounting for those with AD and vascular disease, approximately 25% of pLOD develop PD or dementia due to lewy body disease (LBD).

Objective 2: To assess non-motor and motor features in pLOD rated with abnormal DAT SPECTs. We hypothesise that pLOD with a dopaminergic deficit will present with more prodromal non motor and motor features associated with PD and DLB such as anosmia and REM sleep behavior disorder; with current research reporting those patients with anosmia or REM sleep behavior disorder **and** lower striatal SBRs are at a higher risk for PD (Berendse and Ponsen, 2009, Iranzo et al., 2010, Jennings et al., 2017).

Chapter 5 "MRI in LOD"

Objective 1: To compare visual assessments of global, parietal and medial temporal atrophy and the presence of WML in pLOD with HC. We hypothesise that pLOD in comparison to HC will present with greater atrophy and WML with reports of increased WML in pLOD in comparison to those with EOD (Salloway et al., 1996, Hickie et al., 2005). Atrophy in the brain is also widely reported in AD; another neurodegenerative disease. The reported pathology has led studies to suggest that pLOD have either underlying AD or vascular dementia (Schweitzer et al., 2002).

Objective 2: To compare the subcortical GM volumes in pLOD with HC. Regions include; 3rd and 4th ventricles and left and right; HIPP, AMY, CN and PUT. Additionally, to compare LL and global GM and global WM. We hypothesise that pLOD with present with increased presence of LL in comparison to HC and greater ventricular volumes, as reported previously (Dahabra et al., 1998, Hickie et al., 2005). In addition we hypothesise that pLOD will present with decreased subcortical volumes in line with previous research reporting associations between LOD and reduced volumes in regions including the HIPP, CN, PUT (Kempton et al., 2011) and the AMY (Egger et al., 2008).

Chapter 2. **Main study methodology**

The methods section below covers the methodology relating to the **results** of the following Chapters;

- Chapter 3: Clinical correlates of LOD
- Chapter 4: DAT SPECT in LOD
- Chapter 5: MRI in LOD

The **pLOD** that were recruited into the study met the inclusion and exclusion criteria listed below.

2.1 Inclusion and exclusion criteria

The pLOD recruited into the study were those with an existing/active or previous diagnosis of LOD provided onset was after the age of 55 with disease duration ≤10 years. LOD was defined by patients with an active or previous diagnosis of either major depression, mixed anxiety-depression, minor depressive disorder or dysthymia (as per the diagnostic and statistical manual of Mental Disorders V (DSM-V) criteria (American Psychiatric Association., 2013) **and/or** a score of 8 or more on the Patient health questionnaire (PHQ-9) by a primary care service for at least six months.

The pLOD did not need to be experiencing symptoms of depression at the time of the study day in order to take part provided they had experienced depression for the first time after the age of 55 and for ≤10 years.

On the day of the assessments, symptoms of depression and anxiety were assessed (alongside the other non-motor and motor evaluations)

for the purpose of the analysis (and not to officially assess patient progression). Participants were asked to complete DSM 5's Severity Measure for Depression; SMD (adapted from the PHQ-9) which assesses symptoms of depression. Participants also completed the Hospital Anxiety and Depression Scale (HADS) which measures symptoms of depression and also has a separate scale for symptoms of anxiety. As mentioned above, patients were **not** required to have an abnormal score **on the day** in order to participate in the study and the DSM 5 SMD and HADS scores were used for exploratory purposes and for comparison with the other assessments scores.

Exclusion criteria

- Existing diagnosis of manifest neurodegenerative disease, including dementia and PD
- Previous history of significant depression requiring input from secondary care that is less than 20 years prior to current episode
- A diagnosis of post-traumatic stress disorder, bipolar affective disorder/ and/or recurrent hospital admissions
- Patients experiencing bereavement
- Medication-induced depression or newly untreated systemic disorders which could the cause of symptoms of depression
- Patients with a life threatening physical illness and less than a 3 year life expectancy.

HC without evidence of depression based on previous history, neurodegenerative disease or family history (first degree relative) of Parkinson's disease, were matched to pLOD as closely as possible for age and gender.

Sample size power

This is an exploratory pilot study **primarily investigating dopaminergic functioning pLOD**. However, we assume that at least 50% of LOD is neurodegenerative. This is based on previous follow-up studies suggesting 40% develop dementia (including AD, vascular disease and DLB) with no data available on PD (Alexopoulos, 2005, Schweitzer et al., 2002, Fiske et al., 2009). However, the associations between depression and AD or PD are comparable (Shiba et al., 2000, Ishihara and Brayne, 2006, Ownby et al., 2006) and in the age group of 55-70 the incidence of PD/LBD and of AD is similar (both ~0.3%; (von Campenhausen et al., 2005, Perez et al., 2010, Kawas et al., 2000). Thus, we expect that after accounting for those with AD and vascular disease, approximately 25% of pLOD develop PD or dementia due to LBD i.e. a dopaminergic dysfunction related pathology.

With an estimated prevalence of incidental PD/LBD of 0.1% in this age group in controls, post hoc power analysis revealed a power of 79.5% at a significance level of 5% based on recruitment of 29 pLOD and 25 HC with a DAT SPECT.

2.2 Recruitment

The pLOD and HC were recruited over a 27-month period.

The pLOD group

The group of pLOD were recruited from old-age psychiatry services, GP surgeries, improving access to psychological therapies (IAPT service), old age voluntary organisations (Age UK, U3A) advertising within the Royal Free Hospital in London and the Princess Alexandra Hospital in Essex and local newspapers in London.

Figure 2-1 visually shows the contribution of these recruitment pathways that were responsive to recruiting pLOD for the study.

As pLOD did not need to be experiencing symptoms of depression at inclusion of the study (provided first depression onset had been after the age of 55 and for ≤10 years), 30 of the pLOD that were recruited were experiencing **current/active symptoms** of LOD and six pLOD **had previously experienced** LOD.

IAPT services and GP surgeries

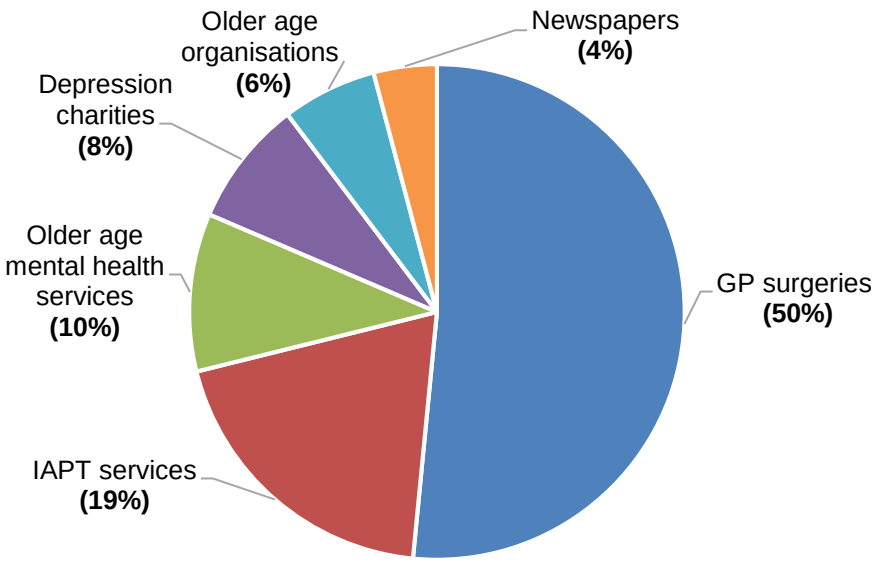
Nineteen per cent (Figure 2-1) of the IAPT services were responsive to recruiting for the study however they were the main source of recruitment for the study with Camden and Islington's IAPT service (iCope) recruiting 68% of pLOD. GP surgeries were the second biggest recruiter for the study, recruiting 16% of pLOD. Figure 2-2 displays a breakdown of the recruitment numbers from IAPT and GP surgeries.

HC

HC recruited were spouses of recruited patients (from the current study and other related studies taking place at the Princess Alexandra Hospital), or recruited from old age voluntary organizations, the Royal Free Hospital and Princess Alexandra Hospital or friends of other controls who had been recruited into the study.

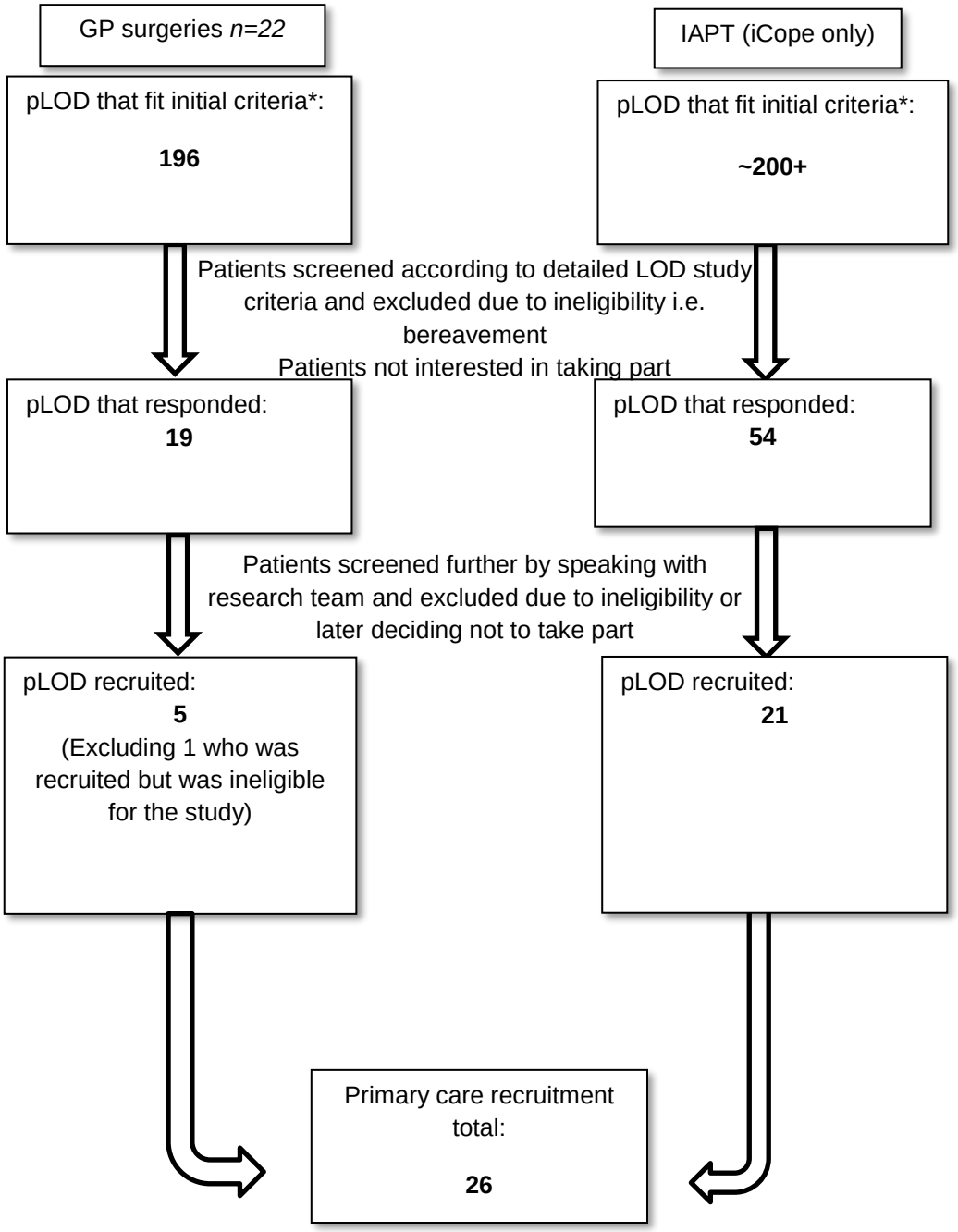
Total recruitment figures are shown in Figure 2-3.

Figure 2-1 Groups that were responsive to recruiting for the study



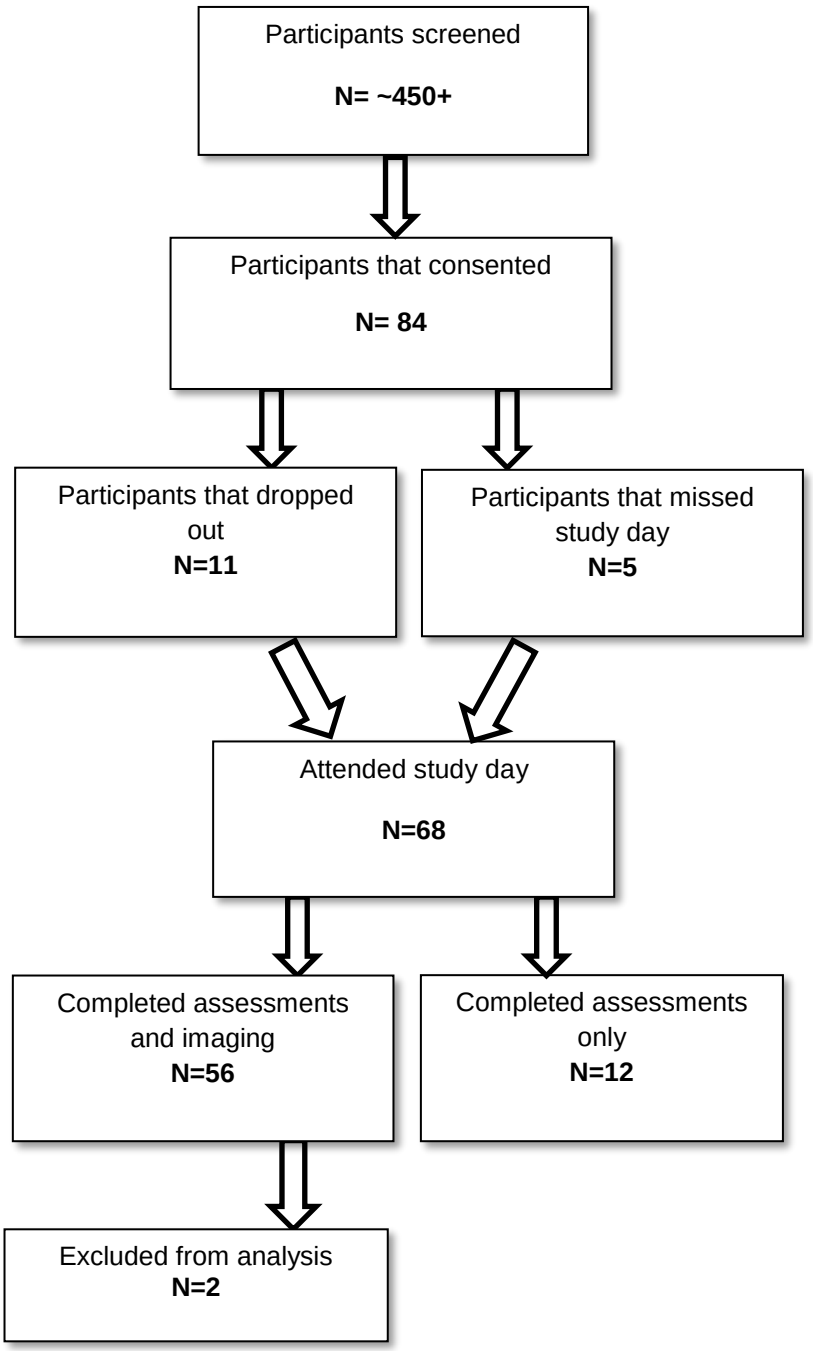
IAPT=The Improving Access to Psychological Therapies

Figure 2-2 iCope and GP surgeries recruitment process



*Experiencing depression for less than 10 years and over 55 years of age
pLOD= patients with late onset depression

Figure 2-3 LOD study total recruitment flowchart



2.3 Study procedure

Participants were invited to spend a “Study day” at either the Royal Free Hospital in London or the Princess Alexandra Hospital in Essex depending on which hospital was most easily accessible to participants. Written informed consent was obtained from all participants before the study day. Participants were invited to complete the full study day which consisted of completing the clinical assessments included in Chapter 3 “Clinical correlates of LOD”; (Table 3-1 ; Page 63). Where relevant (clinician led), the clinical assessments were conducted on **all** participants by **the same** researcher (HK). Additionally, participants were invited to have a DAT SPECT and a MRI scan.

Participants were given the choice of completing the MRI and DAT SPECT and/or the clinical assessments based on the feedback from participants during the recruitment process. Many pLOD in particular suffered from claustrophobia and anxiety and did not wish to take part in the brain imaging aspect of the study.

For those participants that did complete the assessments and the scans, the DAT SPECT and an MRI was conducted on the same day of completing the clinical assessments (if visiting the Royal Free hospital) and for those visiting the Princess Alexandra hospital, on the same day as the DAT SPECT.

Participants were reimbursed for their travel and food costs for the day, and given £25 as a thank you for their time.

After taking part in a study day, those participants who had a DAT SPECT and MRI received a courtesy call or email the next day by the study researcher (HK).

Confidentiality

All patients from IAPT had previously registered an interest to take part in research and had consented to be contacted or had been informed of the study by their psychologist. Any patients that were not interested in the study or expressed a desire to not take part in any research at all were not contacted again. All patients from GP surgeries were contacted via the surgery and patient data was not handled by the study team until patient consent.

Participants excluded from the study

Two recruited pLOD were excluded from the analysis; one pLOD did not meet the study criteria due to an onset of depression **before** the age of 55. The second pLOD was suspected to have an undiagnosed neurodegenerative disease following completion of the assessments; they were unable to complete the Montreal Cognitive Assessment (MoCA) and had great difficulty in completing the other assessments. This patient was referred to a memory clinic for further follow up.

Incidental findings (IF)

One MRI IF was regarded as potentially serious for feedback (categorised under vascular malformation) using the biobank criteria (See Appendix A for full biobank criteria). A report by the neuro radiologist report stated; "*…imaging characteristics in keeping with a cavernoma.*" Following this report, the participant was contacted by the study team and referred to a specialist for follow up. The referral resulted in follow up MRI scans periodically to assess any progression of the IF; follow up information relayed back to the study researcher (HK) by the participant in question at the one year follow up.

Chapter 3. **Clinical correlates of LOD**

3.1. Clinical correlates introduction

It has been reported that pLOD are more likely to present with cognitive impairments, apathy (Weintraub et al., 2005) and psychomotor changes (Rapp et al., 2005), when compared with those with EOD. These clinical motor and non-motor related correlates of LOD often overlap with the symptomology present in other later life diseases such as PD and dementia. In the current chapter, clinical evaluation assessed the following domains also affected in PD: cognition, sleep, mood, hyposmia (reduced sense of smell) and parkinsonian signs and autonomic dysfunction.

3.2 Objective

Objective 1: To compare non-motor and motor features in pLOD with those in HC, using clinical assessments. Whilst this is an exploratory study, we hypothesise that in comparison to HC, pLOD will present with features associated with prodromal PD including symptoms of apathy, autonomic dysfunction and subtle motor abnormalities. This is based on research reporting an overlap of symptoms such as apathy, cognitive deficits and psychomotor retardation present in pLOD (Weintraub et al., 2005, Rapp et al., 2005, Gonda et al., 2015, Naismith et al., 2003) and also associated with PD (Darweesh et al., 2017, Chaudhuri and Schapira, 2009).

3.3 Methods

Patients

Sixty six participants; 36 pLOD and 30 HC were recruited into the study and completed the clinical assessments in Table 3-1. The pLOD met the inclusion and exclusion criteria required for the study as detailed in Chapter 2 “Main study methodology”.

Clinical assessments procedure

Demographic information and the clinical assessments were administered around the DAT SPECT and MRI appointments, for those participants that also completed the imaging. The clinical assessments (collectively) took approximately two hours to complete and were completed in the order shown in Table 3-1.

Assessments not completed by participants

The Frontal Assessment Battery (FAB) was not completed by three pLOD and the Parkinson’s Disease Sleep Scale (PDSS) by one HC, due to participants being unable to complete the assessments due to tiredness. The Bradykinesia Akinesia Incoordination *(*BRAIN) test was not completed by 21 participants; 13 pLOD and eight HC due to problems with accessing the online test. One HC was excluded from the Movement Disorder Society Unified Parkinson’s Disease Rating Scale motor examination subscale **(**MDS UPDRS ME) as they could not complete the full examination due to a leg injury.

Analysis of clinical assessment scores

Table 3-1 provides a list of all the clinical assessments (completed by participants. Although this is an exploratory study and cut offs specific to those pLOD have not previously been published, to provide some indication of what scores were within normality, where available, cut off scores for the assessments from the literature were used. Below is an overview of the clinical assessments used.

Montreal Cognitive Assessment (MoCA)

The MoCA was developed as short (10 minute) cognitive screening to detect mild cognitive impairment, validated by assessing 94 patients with MCI (determined by other psychometric measures), 93 patients with AD and 90 HC (Nasreddine et al., 2005).

In the current study, we have used a cut off score of 23 (with a lower score, indicating higher severity) with Luis et al. (2009) reporting 96% sensitivity and 95% specificity in detecting MCI when using a cut off of 23. Domains measured in the MoCA include; visuospatial abilities, executive functions, fluency, attention, concentration, working memory, language and orientation.

Frontal Assessment Battery (FAB)

This short battery was developed by Dubois et al. (2000) to assess frontal lobe functions and consists of six subsets exploring conceptualization, mental flexibility, motor programming, sensitivity to interference, inhibitory control, and environmental autonomy. The battery was developed to be of use in ascertaining vascular dementias and parkinsonian disorders (including screening for executive dysfunction in PD; Lima et al 2008). Patients with frontal lobe dysfunction were compared with 42 normal control subjects and 89.1%

of cases were correctly identified in a discriminant analysis of patients and controls. To differentiate between frontal dysexecutive type dementias and AD type dementia, a cut off score of 12 was suggested by the authors.

Lille Apathy Rating Scale (LARS)

The LARS was developed by Sockeel et al. (2006) and is a structured standardised interview to assess apathy and reported by the authors to be sensitive in distinguishing between apathy and depression. The scale consists of 9 domains (corresponding to clinical manifestations of apathy) and was validated by 159 patients with probable PD and 58 HC. Probable PD was defined by the authors as patients with PD and dementia, patients with PD with severe symptoms and patients with PD and early in the course of the disease.

A cut off of -21 (which was used in the current study to denote symptoms of (mild) apathy) was considered as mildly apathetic by the authors with a higher score indicating a greater degree of apathy.

Diagnostic and statistical manual of Mental Disorders 5: severity measure for depression (DSM 5 SMD)

The DSM 5 SMD (American Psychiatric Association., 2013) is a self-rated scale for assessing the severity of depressive symptoms in adults. The scale asks patients to rate the severity of their depression during the last seven days. The measure was adapted from the patient health questionnaire (PHQ-9) and can be used in research and evaluation to enhance clinical decision making (and not the sole basis for a clinical diagnosis). The PHQ9 does not differentiate between symptoms of anxiety and depression (unlike the HADS, below). Cut offs for the scale included; 0-4; no symptoms, 5-9; mild depression, 10-

14; moderate depression, 15-19; moderately severe depression and scores between 20-27 as severe depression.

Hospital Anxiety and Depression Scale (HADS)

The HADS was developed by Zigmond and Snaith (1983) and is widely used to measure anxiety and depression separately. However it does not include all the diagnostic criteria of DSM IV/V such as questions on appetite, sleep and self-harm/suicidal thoughts (Stern 2014), of which the DSM 5 SMD addresses. The anxiety and depression scales are rated separately, with a score of seven and under indicating non cases, scores of 8-10 as mild symptoms, 11-14 as moderate and 15-21 as severe (Stern, 2014).

REM Sleep Behavior Disorder Single-Question Screen (RBD1Q)

The RBD1Q was developed by Postuma et al. (2012a) as a short yes/no response scale with the authors suggesting that current screening tools too long for large scale epidemiological surveys. This one question based screening tool queries the classic dream enactment behaviour of RBD. Participants validating the scale were 242 patients with RBD (confirmed by a polysomnogram) and 242 HC. The single question screen had a sensitivity of 93.8% and specificity of 87.2% to ascertain patients with symptoms indicative of RBD.

The REM sleep Behaviour Disorder screening questionnaire (RBDSQ)

The RBDSQ was developed by Stiasny-Kolster et al. (2007) which is a self-rating questionnaire covering clinical features of RBD. The questionnaire was validated by its use on 54 patients with confirmed RBD (polysomygraphically) and 160 control participants. Using a cut off score of 5, there was a reported sensitivity of 0.96 ad specificity of 0.56

by the authors. This cut off was also used in the current study to denote abnormality. No correlation between the scores of the questionnaire the duration of RBD were found which the authors suggest is indicative of the questionnaire's ability to detect mild RBD cases.

Parkinson's disease Sleep Scale (PDSS)

The PDSS, developed by Chaudhuri et al. (2002) was developed to quantify sleep problems in PD. The scale covers 15 commonly reported symptoms associated with sleep disturbance by 800 patients with PD. The scale was validated by use in 143 patients with PD ranging from newly diagnosed to advanced stage compared with 137 age matched HC. PD patients with advanced disease had impaired scores in comparison to early disease patients with significant differences in scores between patients with PD and HC.

Parkinson's disease screening scale (PD screening)

This nine scale questionnaire was developed by Tanner CM (1990) to assess motor features associated with PD. In the current study it will be used alongside the other motor assessments below to assess symptoms associated with motor dysfunctions (often associated with PD).

Movement Disorder Society Unified Parkinson's Disease Rating Scale (MDS UPDRS)

The MDS UPDRS is a revision of the original UPDRS scale and measures progression of non-motor and motor symptoms associated with PD (Goetz et al., 2008). The scale consists of four parts; Part I: Non motor experiences of daily living (nM-EDL), Part II: Motor experiences of daily living (M-EDL), Part III; motor examination (ME)

and Part IV; motor complications. The MDS UPDRS ME subscale (Part III) has been used to assess mild parkinsonian signs (MPS) as discussed below.

MPS

The MDS UPRS ME (above) does not have a cut off threshold for abnormality. However it is useful for calculating MPS which are signs of subthreshold Parkinsonism in individuals who do not fit established criteria for PD however are exhibiting signs associated with PD. These MPS include gait and balance changes, rigidity, bradykinesia, and tremor (Louis and Bennett, 2007, Louis et al., 2003). Originally calculated using the original UPDRS (Louis et al., 2003), in the current study we calculated MPS using the guidelines provided by (Berg et al., 2012) as replicated in Noyce et al. (2017) and as follows; MPS were a sum of all the MDS UPDRS motor examination items (excluding action and postural tremor) and a score of ≥6 indicated subthreshold parkinsonism to help assess the presence of any subtle motor abnormalities associated with PD in the pLOD in the current study.

Bradykinesia Akinesia Incoordination Test *(BRAIN)*

The BRAIN test is an online computer key board tapping task (developed by Noyce et al. (2014b); https://www.braintaptest.com/. This test measures upper limb function by assessing four different components; Kinesia score (KS; number of taps in 30 seconds), Akinesia time (AT; mean dwell time on each key in milliseconds), Incoordination scores (IS: variance of travelling time between key presses) and dysmetria scores (accuracy of key presses). For the current study only KS and AT scores were investigated as they were reported with the highest sensitivity in discrimination between patients with PD and controls (Noyce et al., 2014b).

In the current study, the left and right hand scores for KS and AT were averaged to give one score for KS and AT per participants, as described by the authors, when comparing with the results of the other assessment scores. The authors also reported no significant differences between whether participants were left or right handed in KS and AT scores.

Scales for Outcomes in Parkinson's disease- Autonomic (SCOPA-AUT)

Visser et al. (2004) developed the SCOPA-AUT to assess autonomic symptoms in patients with PD. The questionnaire was evaluated in 140 patients with PD and 100 controls. The scale measures the following regions; gastrointestinal, urinary, cardiovascular, thermoregulatory, pupillomotor, and sexual dysfunction. The authors reported that autonomic problems (excluding sexual dysfunction) increased significantly with increasing disease severity. The scale has been reported by the authors to discriminate between PD patients and controls and between controls and PD groups of mild, moderate and severe disease stages.

University of Pennsylvania Smell Identification Test (UPSIT)

The UPSIT was developed by Doty et al. (1984) as a clinically useful and reliable quantitative measure of olfactory function and consists of 40 scratch and sniff booklets. Adult population cut offs of normality are sensitive to gender and with percentile values based on age and gender also available to identify where patients have scored in comparison to a database of 1819 men and 2109 women.

The UPSIT provides general adult cut offs for indications of mild, moderate and severe hyposmia and anosmia; with a lower score indicative of a higher degree of severity.

To account for the effects of age and gender, in the current study participants that scored ≤15th percentile of male and female norms provided by Doty et al. (1984) were categorised with “definite hyposmia”, as used in previous literature (Noyce et al., 2014a, Siderowf et al., 2007)

Table 3-1 Clinical assessments completed by participants

Assessment	Abbreviations	Cut off denoting an abnormality
Demographics; Smoking habits, weight, caffeine and alcohol intake, occupation, past medical and medication history		
Montreal Cognitive Assessment	MoCA	<23
Diagnostic and statistical manual of Mental Disorders 5: severity measure for depression	DSM 5 SMD	≥5
Hospital Anxiety and Depression Scale:	HADS	
Depression subscale	HADS-D	≥8
Anxiety subscale	HADS-A	≥8
Frontal Assessment Battery	FAB	<12
Parkinson's disease screening scale	PD screening	-
Movement Disorder Society Unified Parkinson's Disease Rating Scale includes:	MDS- UPDRS	-
Motor Aspects of Experiences of Daily Living	M-EDL	
Non Motor Aspects of Experiences of Daily Living	nM-EDL	-
Motor examination	ME	-
Mild parkinsonian signs	MPS	
Lille Apathy Rating Scale		>6
REM Sleep Behavior Disorder Single-Question Screen	LARS	-21
The REM sleep Behaviour Disorder screening questionnaire	RBD1Q	"yes"
Parkinson's disease Sleep Scale	RBDSQ	≥5
BRadykinesia Akinesia Incoordination Test	PDSS	-
Akinesia Time	BRAIN	-
Kinsesia score	AT	--
Scales for Outcomes in Parkinson's disease-Autonomic	KS	
University of Pennsylvania Smell Identification Test	SCOPA-AUT	-
"Definite hyposmia"	UPSIT	≤15th percentile

3.3.1 Statistical analysis

The primary analysis was a comparison of clinical assessment scores between HC and pLOD. Histograms and QQ plots were used to check the distribution of variables. For comparison of continuous data, independent sample t tests for normally distributed data and Mann-Whitney U tests for non-normally distributed data were used. Chi-Square tests and Fisher's Exact tests were used for comparison of categorical data. Pearson's r or Spearman's rho correlations were used to assess for associations between symptoms of depression (using DSM 5 SMD scores), anxiety (using the HADS-A scores) and other clinical scores in **pLOD only**. Partial correlations were used to adjust for age and gender.

Significance for the study was set at $p<0.05$. However to correct for multiple testing, a value of $p<0.002$ using Bonferroni correction was also provided in the relevant results tables.

MDS UPDRS ME

HK conducted 65 out of the 66 UPDRS motor examinations. One ME was conducted by a researcher also working on the study. A selection of the UPDRS motor examinations (which were video recorded) were also rated by a secondary rater (KK) who was blinded to the participant groups. Intra class correlation (ICC) compared scores on 61 out of 66 cases between HK and KK on all aspects of the motor examination excluding rigidity which could not be scored from a recording.

Medications affecting clinical scores

Associations between antidepressants (in particular SSRIs and SNRIs) and RBD have been reported (Postuma et al., 2013). Anti-hypertensives and neuroleptics may also influence motor scores and

some features measured in the SCOPA-AUT. Therefore to account for the effect of medications on motor, RBD and hypotension/autonomic dysfunction related clinical scores, secondary analysis was run with participants on the relevant medications were excluded from the analysis. When assessing the SCOPA-AUT, two types of secondary analysis were run; the first excluding **all** patients on **relevant** medications and the second, not excluding patients on Pregablin, Olanzapine and Risperidone; with these medications thought to have a milder effect.

A full list of the relevant medications taken by participants are listed in Appendix B and which participants were excluded based on their medication.

One year follow up

To assess any progression in clinical symptoms after one year, change in scores of the clinical assessments (an increase or decrease) from baseline and the one year follow up were calculated using Formula 3.1 and applied separately to each clinical score. A Mann Whitney U test was used to compare mean change in scores between pLOD and HC.

Formula 3.1 Calculating change from baseline to follow up

$$Change = Clinical\ score\ at\ follow\ up - Clinical\ score\ at\ baseline$$

Spearman's rho correlation was used to assess associations between baseline scores with a change in scores.

All analyses were performed on IBM SPSS 24. Graphs were produced using Microsoft Excel 2016.

3.4 Results

There were no significant differences ($p<0.05$) between HC and pLOD in the demographic data collected (Table 3-2). HC were on average 70.6 years of age (SD 9.3) and pLOD an average age of 67.4 (SD 7.8) years. Thirty seven per cent of the HC group were male and 39% of the pLOD group were male. Both groups had a majority Caucasian population: HC; 97% and pLOD; 89%. Group differences in use of anti-hypertensives missed significance ($p=.052$).

The pLOD group

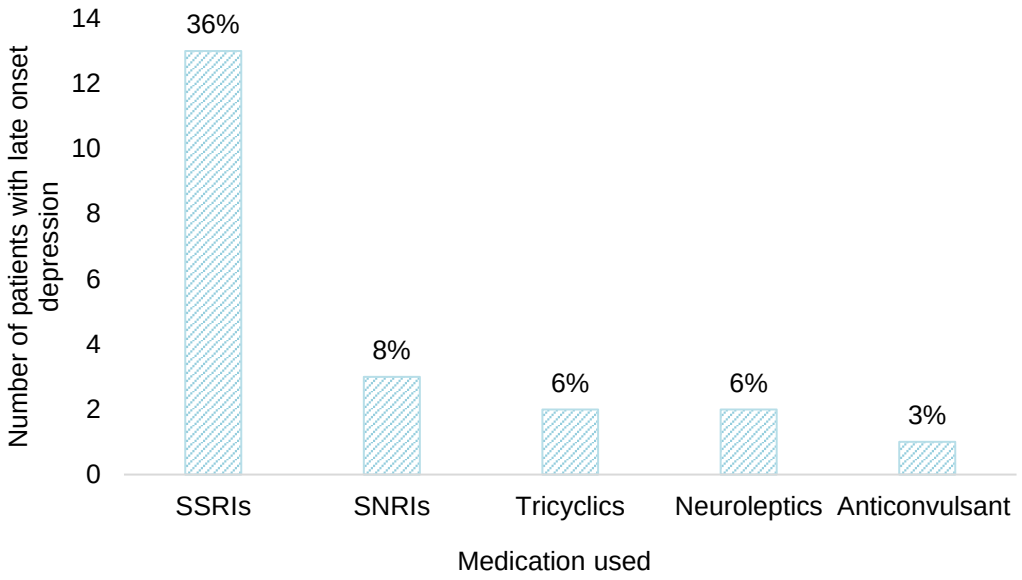
The study included 30 pLOD diagnosed with active symptoms of depression/anxiety and six patients with a previous experience of LOD. On average, pLOD had been experiencing symptoms of depression/anxiety for 3.3 years (SD 2.8). Seventeen pLOD were taking medication(s) for the treatment of symptoms associated with depression/anxiety. Medication included the following SSRIs; sertraline, fluoxetine, citalopram and escitalopram, the following SNRIs; paroxetine and venlafaxine, the following tricyclics; amitriptyline and nortriptyline, neuroleptics including; risperidone and olanzapine and an anticonvulsant; pregabalin (Figure 3-1).

Table 3-2 Participant demographics, by group

	HC (n=30)	pLOD (n=36)		p
Age in years, mean (SD)	70.6 (9.3)	67.4 (7.8)	1.544	.128
Male, n (%)	11 (37)	14 (39)		.528
<12 years education, n (%)	7 (23)	10 (28)		.451
Active LOD, n (%)	-	30 (83)		
Duration of LOD in years, mean (SD)	-	3.3 (2.8)		
Family member with depression, n (%)	4 (13)	5 (19)		
SSRIs use, n (%)	-	13 (36)		
SNRIs use, n (%)	-	3 (8)		
Tricyclics use, n (%)	-	2 (6)		
Neuroleptics use, n (%)	-	2 (6)		
Other medications, n (%)	-	1 (3)		
Hand tremor, n (%)	1	4 (11)		.240
Q risk score, mean (SD)	19.3 (11.6)	18.6 (11)	479.0	.923
Anti-hypertensive use, n (%)	6 (20)	15 (42)		.052
Ethnicity, n (%)			1.615	.446
Caucasian, n (%)	29 (97)	32 (89)		
Other, n (%)	1 (3)	4 (11)		
A current smoker, n (%)	0 (0)	3 (8)		.156
Ex-smoker, n (%)	12 (40)	17 (47)		.368
Weekly Caffeine intake, mean cups (SD)	17.3 (12.1)	20.3 (12.2)	-.914	.364
Weekly Alcohol intake, mean units (SD)	4.9 (8.2)	5.2 (7.1)	516.0	.754
Imaging				
Completed DAT SPECT, n (%)	25 (83)	29 (81)		
Completed MRI, n (%)	20 (67)	28 (78)		

Independent samples t test, Mann Whitney U test and Chi square or Fisher's exact
Ethnicity "other" includes; African American, Hispanic and Asian as on the University of Pennsylvania Smell Identification Test (UPSIT), Q risk score= measuring cardiovascular disease, DAT SPECT= Dopamine Transporter Single Photo Emission Computerized Tomography, MRI= Magnetic resonance imaging. HC=healthy control, pLOD= patients with late onset depression

Figure 3-1 Medication reportedly used for the treatment of symptoms associated with depression/anxiety



SSRIs=selective serotonin reuptake inhibitors, SNRIs= serotonin and norepinephrine reuptake inhibitors

3.4.1 Part one: Clinical scores

NMS

Symptoms of depression and anxiety

Group differences between the clinical scores assessing non-motor symptoms were analysed. There were significant group differences in symptoms of anxiety and depression, with pLOD on average, scoring significantly higher than HC on the following scales; HADS depression and ($p<.001$), HADS anxiety (HADS-A) ($p<.001$) and the adapted DSM 5 SMD ($p=.023$).

The mean DSM 5 SMD score in pLOD was 9.1 (SD 6.3) with scores ranging from 0 to 22. The pLOD group included 12 patients with evidence of no depressive symptoms, eight with mild symptoms, seven with moderate symptoms, eight with moderately severe symptoms and one with severe depression on the day of the assessments, based on DSM 5 SMD scoring. For HC, mean scores were 1.2 (SD 1.0), ranging from 0-7. Twenty eight HC had evidence of no symptoms of depression and 2 HC with mild symptoms.

Mean anxiety scores on the day of assessments were 9.6 (SD 5.1) and scores ranged from 0-21. Eight pLOD scored with presence of no anxiety symptoms, ten with mild, six with moderate and seven with severe symptoms based on HADS-A scores. For HC, mean anxiety scores on the day were 2.8 (SD 2.8), ranging from 0-12. Twenty nine HC scored with no symptoms of anxiety and one HC scored with moderate symptoms of anxiety.

Other NMS

There were also significant group differences in apathy, measured by the LARS (p=.044) and in the PDSS, a scale which measures PD related sleep behaviour (PDSS; p<.001). Additionally, there were significant group differences in the scores of the RBDSQ (p=.001 which measures symptoms associated with RBD and with on average, more pLOD also had a score of “yes” on the RBD1Q (p=.050) which uses only one questionnaire to ascertain potential RBD. On average pLOD also scored higher in comparison to HC on the SCOPA AUT (p<.001), which measures symptoms related to autonomic dysfunction. There were no significant group differences on the MoCA, FAB or UPSIT.

Motor related symptomology

Group means of motor related assessments in HC and pLOD were also analysed as shown in Table 3-4.

MDS UPDRS

There were significant group differences on the MDS UPDRS scale, with the total mean score higher for the pLOD group; 19.2 (SD 12.7) in comparison to 6.1 (SD 5.6) for the HC (p<.001). On average, pLOD also scored significantly higher on all the MDS UPDRS subscales, which include the nM-EDL (p<.001); measuring non motor symptoms, the ML EDL subscale (p=.007); measuring motor experiences and the ME score (p=.003).

MDS UPDRS ME

The ME scores (by HK) ranged from 0- 12 with the majority of HC with a score of 0 (n=20) in comparison to pLOD (n=11). The MPS was calculated to ascertain subtle motor symptoms associated with PD such as bradykinesia, rigidity and gait and was calculated using the ME

score, with participants with an abnormal score shown in Table 3-4 and Figure 3-2. Those with an abnormal MPS score were suggestive of symptoms associated with subthreshold Parkinsonism and in particular, pLOD scored abnormally on the following items of the MDS UPDRS ME; rigidity, speech, facial expression, finger tapping, toe tapping, leg agility, pronation supination and posture and gait.

Inter rater reliability

ICC between HK and KK was at 0.76 (96% CI 0.59 to 0.85). This was excluding four cases could not be recorded and one case had not been rated by HK.

There were also significant group differences in the PD screening scores (p=.012).

The BRAIN test was completed by 23 pLOD and 22 HC. In these participants there were significant group differences in the mean KS scores of the left and right hand (p=.010) which measured number of key stroke per minute with pLOD with fewer strokes than HC and mean AT scores of the left and right hand (p=.025) which measures the cumulative time keys are depressed with pLOD with a longer time than HC

Figure 3-2 shows a visual comparison of the number of participants by group, who scored past normal cut off thresholds on the non-motor and motor assessments.

Medications affecting clinical scores

To account for the effect of medications on motor, RBD and hypotension/autonomic dysfunction related clinical scores, participants

on the relevant medications were excluded from the analysis with the results shown in Table 3-5. A full list of the relevant medications taken by participants are listed in Appendix B and which participants were excluded based on their medication.

After excluding participants on the relevant medications (Table 3-5), significant group differences remained in clinical scores measured by the MDS UPDRS ME (p=.004), the RBDSQ (p=.015) and the SCOPA-AUT (p=.002). Group differences in the RBD1Q missed significance (p=.075).

Table 3-3 Mean scores of non-motor assessments completed by participants

Assessment	HC (n=30*)	pLOD (n=36*)		*p*
	Mean (SD) Median			
MoCA	26.5 (2.7) 26.0	26.0 (3.6) 26.5	.667	.507
FAB	17.0 (1.3) 17.5	17.1 (1.1) 17.0	-.194	.847
LARS	-27.0 (4.7) -28.0	-23.3 (9.6) -25.0	-2.067	**.044**
HADS-D	1.2. (1.0) 1.0	7.1 (4.6) 6.0	-7.463	**<.001**
HADS-A	2.8 (2.8) 2.0	9.6 (5.1) 10.0	-6.836	**<.001**
DSM 5 SMD	1.4 (1.9) 0.5	9.1 (6.3) 8.0	-2.470	**.023**
RBD1Q “yes” n (%)	2 (6.7) -	10 (27.8) -		**.030**
RBDSQ	2.1 (2.1) 2.0	4.3 (3.2) 3.5	-3.411	**.001**
PDSS	127.6 (13.1) 130.0	98.1 (24.4) 98.2	6.221	**<.001**
UPSIT	29.5 (6.6) 31.0	30.7 (5.7) 33.0	-.803	.425
SCOPA-AUT	7.7 (4.9) 7.0	14.9 (8.7) 13.5	-4.087	**<.001**

Independent samples t test, Fisher’s exact. Significant ($p<0.05$) p values are in **bold**. A significant p value at a Bonferroni adjustment would be $p<0.002$, calculated by 0.05 / number of clinical assessments

*Three pLOD did not complete the FAB and one HC did not complete the PDSS.

Abbreviations: MoCA= Montreal Cognitive Assessment, FAB=Frontal Assessment Battery, LARS= Lille Apathy Rating Scale, HADS = Hospital Anxiety and Depression Scale; D=depression scale, A=anxiety scale, DSM 5 SMD= Diagnostic and statistical manual of Mental Disorders severity measure of depression, RBD1Q= REM Sleep Behavior Disorder Single-Question Screen, RBDQ= The REM sleep Behaviour Disorder screening questionnaire, PDSS= Parkinson’s disease Sleep Scale, UPSIT= University of Pennsylvania Smell Identification Test, SCOPA-AUT= Scales for Outcomes in Parkinson's disease- Autonomic

Table 3-4 Mean scores of motor assessments completed by participants

Assessment	HC (n=30*)	pLOD (n=36)		*p*
	Mean (SD) Median			
MDS-UPDRS Total	6.1 (5.7) 5.0	19.2 (12.7) 18.0	-5.540	**<.001**
nM-EDL	4.1 (3.5) 4.0	13.4 (8.6) 12.5	-5.948	**<.001**
M-EDL	1.3 (2.0) 0.5	3.5 (3.9) 2.0	-2.794	**.007**
ME	0.7 (1.34)	2.3 (2.7)	-3.116	**.003**
MPS n (%)	0 (0) .00	3 (8) 1.0		.156
BRAIN KS	58.7 (8.7) 61.0	52.5 (10.1) 53.8	2.195	**.034**
BRAIN AT	98.6 (49.4) 83.0	126.3 (59.2) 109.5	139.5	**.010**
PD screening questionnaire	0.8 (1.2) 0.0	1.8 (1.9) 1.0	-2.602	**.012**

Independent samples t test, Fisher's exact and Mann Whitney U test. Significant ($p<0.05$) p values are in **bold**. A significant p value at a Bonferroni adjustment would be $p<0.002$, calculated by 0.05 / number of clinical assessments
*13 pLOD and eight HC did not complete the BRAIN test. One HC was excluded from the MDS UPDRS ME as they could not complete the full examination
Abbreviations: MDS- UPDRS= Movement Disorder Society Unified Parkinson's Disease Rating scale; ML EDL= Motor Aspects of Experiences of Daily Living, nM-EDL= Non Motor Aspects of Experiences of Daily Living, ME=motor examination, MPS= Mild parkinsonian signs, BRAIN test= BRadykinesia Akinesia Incoordination Test; KS= kinesia score, AT; akinesia time, PD screening= Parkinson's disease screening

Table 3-5 Mean clinical scores when excluding participants on medications[1] which may affect clinical scores

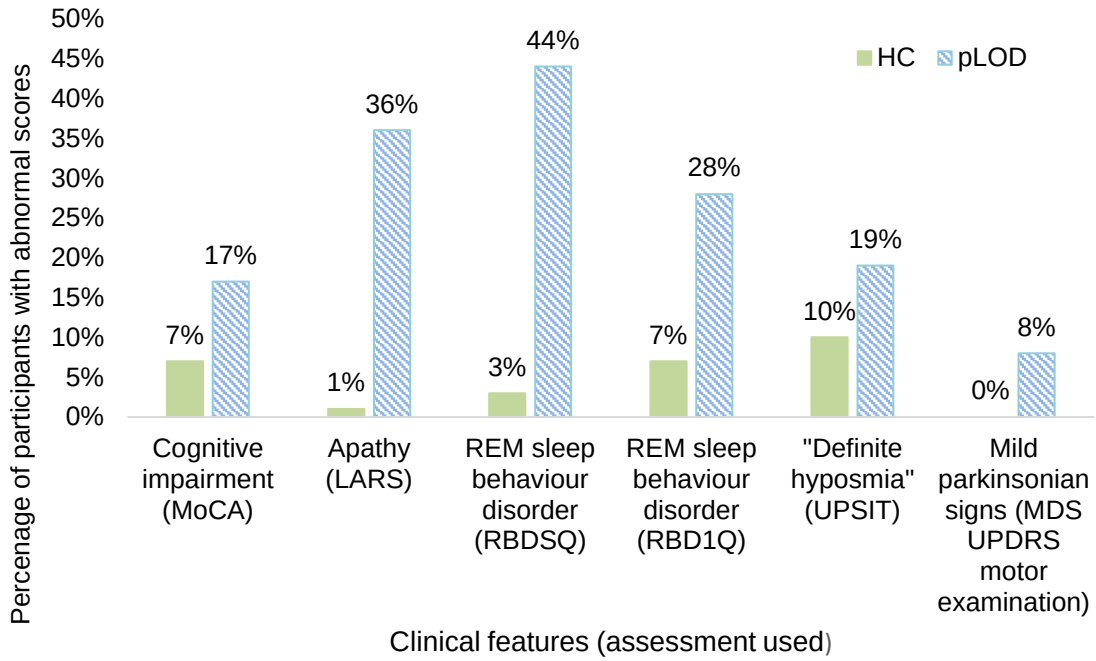
Assessment	HC	pLOD		*p*
	Mean (SD) Median			
	n=24	n=33		
MDS-UPDRS	0.7 (1.5)	1.9 (2.5)	229.0	**.004**
	0.0	1.0		
	n=30	n=19		
RBDSQ	2.07 (2.1)	4.1 (3.1)	168.0	**.015**
	2.0	3.0		
RBD1Q “yes” n	2	5		.075
	n=24	n=26		
SCOPA-AUT	7.6 (5.1)	14.5 (7.9)	153.9	**.002**
	7.0	12.0		
	n=24	n=28		
SCOPA-AUT[2]	7.6 (5.1)	14.3 (7.7)	161.0	**.001**
	7.0	12.0		

Mann Whitney U test and Fisher’s exact test between groups. Significant p values ($p<0.05$) are in **bold**

[1] Medications listed in Appendix B

[2]Two patients using Olanzapine, Risperidone and Pregabalin were included

Figure 3-2 Percentage of patients with LOD (pLOD) and healthy controls (HC) with abnormal (past abnormal cut off threshold values) non-motor and motor assessment scores



Associations between demographics, clinical scores and symptoms of depression and anxiety

Any associations between demographics, the other clinical scores and symptoms of depression (DSM 5 SMD and HADS-D scores) and anxiety (HADS-A) were investigated in pLOD and shown in Table 3-6.

After the adjustment for age and gender, scores for apathy (LARS), frontal lobe dysfunction (FAB) non-motor and motor abnormalities (MDS UPDRS scales; nM-EDL, M-EDL, MDS UPDRS total) and PD related sleep symptoms (PDSS) were significantly associated with symptoms of depression (DSM 5 SMD), as shown in Table 3-7.

In addition to the significant correlations between the clinical scores mentioned above, the MDS UPRS ME also correlated with **HADS-D scores.** The PDSS however just missed significance (p=.070) with HADS-D scores.

Symptoms of anxiety (HADS-A) also significantly correlated with the FAB, the MDS UPDRS total scores and the nM-EDL subscale. Sleep symptoms associated with PD (PDSS) missed significance.

Controlling for relevant medications

Participants that were on medications that could potentially affect RBD, SCOPA AUT and MDS UPDRS ME scores were excluded and with the results between clinical scores and symptoms of depression and anxiety in these pLOD shown in Table 3-8.

When controlling for age and gender, only HADS-D scores significantly correlated with SCOPA-AUT scores after excluding pLOD on the relevant medications (Table 3-8).

Table 3-6 Associations between demographics, clinical scores and DSM 5 SMD, HADS-D, HADS-A scores in patients with late onset depression (pLOD)

	Correlations *r, (p)*		
	DSM 5 SMD scores	HADS-D scores	HADS-A scores
Age	-.48 **(.002)**	-.20 (.243)	-.23 (.107)
Gender	.08 (.640)	-.13 (.442)	.04 (.797)
Duration of LOD	.05 (.733)	.-04 (.804)	.05 (.738)
Clinical Assessment			
MoCA Total	.11 (.504)	-.21 (.214)	.16 (.503)
FAB total	-.13 (.448)	-.36 **(.040)**	-.20 (.257)
LARS	.36 **(.029)**	.52 **(.001)**	.10 (.550)
PD screening questionnaire	.28 (.090)	.46 **(.004)**	.28 (.097)
MDS UPDRS M-EDL	.53 **(.001)**	.50 **(.002)**	.42 **(.009)**
MDS UPDRS nM-EDL	.68 **(<.001)**	.47 **(.003)**	.65 **(<.001)**
MDS UPDRS ME	.27 (.110)	.38 **(.021)**	.16 (.349)
MDS UPDRS Total	.67 **(<.001)**	.56 **(<.001)**	.61 **(<.001)**
BRAIN AT	.20 (.361)	.27 (.206)	.47 **(.022)**
BRAIN KS	-.08 (.717)	-.32 (.132)	.04 (.857)
RBD1Q	.15 (.368)	-.15 (.373)	.10 (.539)
RBDSQ	.33 **(.045)**	.14 (.407)	.44 **(.007)**
PDSS	-.42 **(.010)**	-.23 (.161)	-.39 **(.016)**
SCOPA- AUT	.40 **(<.014)**	.50 **(.002)**	.27 (.100)
UPSIT	.13 (.426)	-.17 (.318)	.10 (.529)

Pearson's r and Spearman's rho correlations. Significant ($p<0.05$) p values are in **bold**.

Abbreviations: MoCA= Montreal Cognitive Assessment, FAB=Frontal Assessment Battery, LARS= Lille Apathy Rating Scale, HADS = Hospital Anxiety and Depression Scale; D=depression scale, A=anxiety scale, DSM 5 SMD=Diagnostic and statistical manual of Mental Disorders severity measure of depression, RBD1Q= REM Sleep Behaviour Disorder single questionnaire, RBDSQ= The REM sleep Behaviour Disorder screening questionnaire, PDSS= Parkinson's disease Sleep Scale, UPSIT= University of Pennsylvania Smell Identification Test, SCOPA-AUT= Scales for Outcomes in Parkinson's disease- Autonomic, MDS- UPDRS= Movement Disorder Society Unified Parkinson's Disease Rating scale; ML EDL= Motor Aspects of Experiences of Daily Living, nM-EDL= Non Motor Aspects of Experiences of Daily Living, UPDRS ME= Unified Parkinson's Disease rating scale motor examination, BRAIN test= BRadykinesia Akinesia Incoordination Test; KS= kinesia score, AT; akinesia time, PD screening= Parkinson's Disease screening

Table 3-7 Associations between clinical scores and DSM 5 SMD, HADS-D, HADS-A scores in patients with late onset depression (pLOD), when controlling for age and gender

	Correlations *r, (p)*		
Clinical Assessment	DSM 5 SMD scores	HADS-D scores	HADS-A scores
MoCA Total	.08 (.362)	.04 (.434)	.09 (.360)
FAB total	-.54 **(.010)**	**-.60 (.004)**	-.53 **(.012)**
LARS	.60 **(.004)**	**.60 (.004)**	.37 (.062)
PD screening questionnaire	.31 (.103)	.31 (.099)	.32 (.096)
MDS UPDRS M-EDL	.58 **(.005)**	**.41 (.045)**	.29 (.122)
MDS UPDRS nM-EDL	.79 **(<.001)**	**.69 (.001)**	.75 **(<.001)**
MDS UPDRS	.29 (.115)	**.52 (.013)**	.21 (.196)
MDS UPDRS Total	.76 **(<.001)**	**.69 (.001)**	.63 **(.003)**
BRAIN AT	.13 (.555)	.11 (.625)	-.04 (.853)
BRAIN KS	-.13 (.291)	-.21 (.194)	.04 (.432)
RBD1Q	.15 (.267)	-.23 (.171)	.02 (.458)
RBDSQ	.15 (.266)	.12 (.312)	.32 (.093)
PDSS	**-.40 (.048)**	-.36 (.070)	-.37 (.065)
SCOPA- AUT	.20 (.208)	.21 (.196)	.12 (.315)
UPSIT	.01 (.472)	.04 (.428)	.07 (.382)

Partial correlations with age and gender included as a covariates. Significant ($p<0.05$) p values are in **bold**.

Abbreviations: MoCA= Montreal Cognitive Assessment, FAB=Frontal Assessment Battery, LARS= Lille Apathy Rating Scale, HADS = Hospital Anxiety and Depression Scale; D=depression scale, A=anxiety scale, DSM 5 SMD=Diagnostic and statistical manual of Mental Disorders severity measure of depression, RBD1Q= REM Sleep Behaviour Disorder single questionnaire, RBDSQ= The REM sleep Behaviour Disorder screening questionnaire, PDSS= Parkinson's disease Sleep Scale, UPSIT= University of Pennsylvania Smell Identification Test, SCOPA-AUT= Scales for Outcomes in Parkinson's disease- Autonomic, MDS- UPDRS= Movement Disorder Society Unified Parkinson's Disease Rating scale; ML EDL= Motor Aspects of Experiences of Daily Living, nM-EDL= Non Motor Aspects of Experiences of Daily Living, UPDRS ME Motor examination= Unified Parkinson's Disease rating scale motor examination, BRAIN test= BRadykinesia Akinesia Incoordination Test; KS= kinesia score, AT; akinesia time, PD screening= Parkinson's Disease screening

Table 3-8 Associations between clinical scores and symptoms of depression and depression excluding patients on relevant medications[1]

	Correlations *r, (p)*		
	DSM 5 SMD scores	HADS-D scores	HADS-A scores
MDS UPDRS ME	.29 (.060)	.26 (.077)	.10 (.296)
RBD1Q	-.04 (.443)	-25 (.168)	.02 (.474)
RBDSQ	.19 (.238)	.13 (.307)	.39 (.060)
SCOPA- AUT	,25 (,116)	**.40 (.030)**	.16 (.221)
SCOPA- AUT[2]	.25 (.106)	**.34 (.044)**	.16 (.216)

Partial correlations controlling for age and gender

[1]Full list available in Appendix B

[2]Two patients using Olanzapine, Risperidone and Pregabalin included

3.4.2 Part two: One year follow up

Participants were invited back after one year for a follow up of the clinical assessments. Of the 36 pLOD, 13 patients took part in the one year follow up and of the 30 HC, nine took part. Fifteen pLOD and 19 HC were still within one year of being first seen and therefore were not eligible at the time for a follow up. Participants that were eligible to be seen but did not take part was due to not being contactable, being unable to secure a time to be seen or moving away.

Of the participants that attended the follow up, there were significant differences only in age, with pLOD on average older than HC (p=.019), as shown in Table 3-9. The pLOD were **not** taking any new medication for the treatment of their symptoms of anxiety and depression since their last assessment. Mean differences in the clinical scores from baseline and the one year follow up were investigated in pLOD and HC (Table 3-10). There were significant differences between the mean differences in scores of the HADS-A and HADS-D with HC increasing in scores and pLOD with a decrease in scores related to symptoms of depression and anxiety. However, pLOD on average scored significantly higher than HC in the one year follow up RBDSQ and SCOPA-AUT as HC decreased in both. There were no significant correlations between baseline scores and a change in scores in pLOD. Significant correlations in HC are shown in Table 3-11.

Table 3-9 Participant demographics at a one year follow up, by group

	HC (n=9)	pLOD (n=13)		p
Mean (SD) age in years	63.1 (4.5)	69.6 (7.4)	-2.54	**.019**
Male n (%)	3 (33.3)	4 (30.8)		.628
Medication use n (%)				
SSRIs	-	4 (30)		
SNRIs	-	1 (8)		
Tricyclics	-	1 (8)		
Neuroleptics	-	0		
Other	-	1 (8)		
New medication for depression/anxiety n (%)	-	0		
Antihypertensive use n (%)	1	0		
New antihypertensive use n (%)	0	0		
Weekly Caffeine intake (cups)	12.6 (7.4)	19.0 (27.5)	48.50	.799
Weekly Alcohol intake (units)	6.4 (6.67)	7.7 (7.6)	50.50	.913
Imaging n (%)				
Completed DAT SPECT	9 (100)	13 (100)		
Abnormal DAT SPECT visual OR SQA	0	3 (23)		
Normal DAT SPECT SQA	9 (100)	10 (77)		
Completed MRI	9 (100)	13 (100)		

Independent samples t test, Mann Whitney U test and Fisher's exact. Significant p values ($p<0.05$) **in bold.**
DAT SPECT= Dopamine Transporter Single Photo Emission Computerized Tomography, MRI= Magnetic resonance imaging. HC=healthy control, pLOD= patients with late onset depression

Table 3-10 Mean difference of change in clinical assessments scores from baseline to the one year follow up

Assessments	HC (n=9*)	pLOD (n=13)*		p
	Mean (SD)			
MoCA Total	1.1 (3.1)	2.2 (2.6)	51.00	.647
FAB total	-0.3 (1.4)	0.0 (0.9)	44.00	.710
DSM 5 SMD	0.1 (1.5)	-1.4 (4.8)	33.50	.096
HADS-D	0.7 (1.6)	-1.1 (2.2)	25.50	**.025**
HADS-A	1.2 (1.9)	-2.1 (2.2)	13.00	**.001**
LARS	-3.8 (5.9)	0.1 (4.4)	34.50	.110
PD screening	-0.4 (0.7)	-0.1 (1.3)	44.50	.357
MDS UPDRS M-EDL	0.0 (2.5)	1.4 (4.1)	56.00	.896
MDS UPDRS nM-EDL	0.3 (2.1)	0.8 (5.1)	55.00	.845
MDS UPDRS ME	0.1 (0.6)	1.0 (2.2)	48.00	.459
MDS UPDRS Total	0.8 (0.5)	3.2 (8.1)	53.50	.744
BRAIN AT	4.0 (6.6)	21.8 (26.4)	6.00	.257
BRAIN KS	3.0 (2.7)	-1.0 (8.4)	10.50	.762
RBDSQ	-1.1 (1.8)	1.2 (2.9)	28.00	**.043**
PDSS	-11.0 (32.8)	-2.8 (23.8)	51.50	.972
SCOPA- AUT	-1.4 (4.4)	3.5 (4.4)	25.50	**.025**
UPSIT	-1.8 (2.9)	-1.4 (5.2)	50.50	.601

Mann Whitney U test of mean difference between groups. Significant ($p<0.05$) p values in **bold**.

*Seven pLOD and five HC did not complete the BRAIN test, one pLOD did not complete the FAB

Abbreviations: MoCA= Montreal Cognitive Assessment, FAB=Frontal Assessment Battery, LARS= Lille Apathy Rating Scale, HADS = Hospital Anxiety and Depression Scale; D=depression scale, A=anxiety scale, DSM 5 SMD=Diagnostic and statistical manual of Mental Disorders severity measure of depression, RBD1Q= REM Sleep Behavior Disorder Single-Question Screen, RBDSQ= The REM sleep Behaviour Disorder screening questionnaire, PDSS= Parkinson's disease Sleep Scale, UPSIT= University of Pennsylvania Smell Identification Test, SCOPA-AUT= Scales for Outcomes in Parkinson's disease- Autonomic, MDS- UPDRS= Movement Disorder Society Unified Parkinson's Disease Rating scale; ML EDL= Motor Aspects of Experiences of Daily Living, nM-EDL= Non Motor Aspects of Experiences of Daily Living, UPDRS ME= Unified Parkinson's Disease rating scale motor examination, BRAIN test= BRadykinesia Akinesia Incoordination Test; KS= kinesia score, AT; akinesia time, PD screening= Parkinson's Disease screening

Table 3-11 Associations between clinical baseline scores and the change in scores

Assessments	HC (n=9*)	pLOD (n=13*)
	r, (p)	
MoCA Total	-.70 **(.034)**	-.51 (.071)
FAB total	.96 **(<.001)**	.41 (.201)
DSM 5 SMD	-.09 (.806)	.07 (.809)
HADS-D	**.73 (.024)**	**.**04 (.887)
HADS-A	-.41 (.268)	-.37 (204)
LARS	**-.78 (.013)**	-.34 (248)
PD screening questionnaire	**-.84 (.004)**	-.44 (.132)
MDS UPDRS M-EDL	-.39 (.269)	-.16 (.585)
MDS UPDRS nM-EDL	-.36 (.332)	-14 (.629)
MDS UPDRS ME	-.49 (.176)	-.09 (.763)
MDS UPDRS Total	-.21 (.579)	-.17 (.572)
BRAIN AT L/R hand mean	-.80 (.200)	.429 (.397)
BRAIN KS L/R hand mean	.00 (1.00)	-.25 (.623)
RBDSQ	-.59 (.092)	-.23 (.442)
PDSS	-.14 (.728)	-.04 (.873)
SCOPA- AUT	-.63 (.068)	-.09 (.756)
UPSIT	-.60 (.083)	-.08 (.780)

Spearman's rho correlations. Significant ($p<0.05$) p values are in **bold**.
*Seven pLOD and five HC did not complete the BRAIN test
Abbreviations: MoCA= Montreal Cognitive Assessment, FAB=Frontal Assessment Battery, LARS= Lille Apathy Rating Scale, HADS = Hospital Anxiety and Depression Scale; D=depression scale, A=anxiety scale, DSM 5 SMD=Diagnostic and statistical manual of Mental Disorders severity measure of depression, RBD1Q= REM Sleep Behavior Disorder Single-Question Screen, RBDSQ= The REM sleep Behaviour Disorder screening questionnaire, PDSS= Parkinson's disease Sleep Scale, UPSIT= University of Pennsylvania Smell Identification Test, SCOPA-AUT= Scales for Outcomes in Parkinson's disease- Autonomic, MDS- UPDRS= Movement Disorder Society Unified Parkinson's Disease Rating scale; ML EDL= Motor Aspects of Experiences of Daily Living, nM-EDL= Non Motor Aspects of Experiences of Daily Living, UPDRS ME= Unified Parkinson's Disease rating scale motor examination, BRAIN test= BRadykinesia Akinesia Incoordination Test; KS= kinesia score, AT; akinesia time; Left (L) or right hand (R), PD screening= Parkinson's Disease screening

3.5 Discussion

In the current study, on average, pLOD scored significantly higher than HC on the following features; subtle motor abnormalities, autonomic dysfunction, apathy, sleep dysfunction associated with PD and RBD. There were *no* significant mean group differences in mean cognition, hyposmia or frontal lobe dysfunction scores. After controlling for the effects of age and gender, symptoms of depression (measured by the DSM 5 SMD) were associated with scores measuring apathy, frontal lobe dysfunction and motor and non-motor features as measured by the MDS UPDRS total score. A one year follow up found on average pLOD significantly increased in clinical scores associated with **RBD and autonomic dysfunction** when compared with HC.

On the day of the assessments, pLOD on average scored with mild symptoms of depression (DSM 5 SMD score of 9.1 SD 6.3) and anxiety (HADS-A; 9.6 SD 5.1). The mean DSM 5 SMD and HADS-A scores of the pLOD group in *general* are supportive of previous reports of the presence of both anxiety and depression in affective disorders in the elderly (Andreescu et al., 2008). Sertraline, citalopram and venlafaxine were the most common antidepressants taken by 36.1% of pLOD and in line with research suggesting that SSRIs and SNRIs should be first in the line of antidepressant treatment and the safest to treat depressive symptoms in the elderly (Rodda et al., 2011). The number of pLOD taking anti-hypertensives in comparison to HC just missed significance (p=.052) with research reporting cardiovascular comorbidity in those pLOD (Rapp et al., 2005, Variend and Gopal, 2008).

Symptoms of depression and anxiety can also be present as feature of prodromal PD and also after diagnosis, with 30- 40% of patients

(Tandberg et al., 1996) with PD diagnosed with symptoms of depression. Alongside symptoms of depression and anxiety, there is also an overlap between other symptoms present in depression in general and in LOD that are also associated in the course of PD and will be discussed next.

Other NMS

Apathy

Thirty six per cent of pLOD scored abnormally on the LARS compared with one per cent of HC (using the cut off for mild apathy). On average, both pLOD and HC scored within normality on the LARS (cut off of -21 for mild symptoms) although pLOD on average scored significantly higher than HC (p=.044). Apathy scores were significantly associated with symptoms of depression; supportive of the clinical profile of depression in general and LOD, with research reporting symptoms of apathy and anhedonia more likely in pLOD (Weintraub et al., 2005, Rapp et al., 2005) than patients with EOD. Apathy is also a NMS reported in PD and can occur with or without depression (Chaudhuri and Schapira, 2009).

Cognitive impairment and frontal lobe dysfunction

Seventeen per cent of pLOD scored below the cut off for normality for cognition (using the MoCA) (compared with seven per cent of HC) with previous research linking depression with a decline in cognitive abilities (Gonda et al., 2015) and reports of greater cognitive impairment in pLOD (Naismith et al., 2003). However, there were no significant group differences between MoCA scores. Additionally, MoCA scores were not significantly associated with DSM 5 SMD scores indicating that depression severity alone is unlikely to account for the cognitive

impairment as severity of cognitive deficits increases with depression severity (Elderkin-Thompson et al., 2003).

Cognitive impairment has been reported in PD with up to 80% of patients with advanced PD affected by dementia (Aarsland et al., 2003, Chaudhuri and Schapira, 2009) and subtle cognitive changes may be present in early PD (Darweesh et al., 2017). In addition to an overlap of cognitive impairment present in LOD and neurodegenerative disorders, research has further suggested LOD as prodrome to dementia (Schweitzer et al., 2002).

In the current study both groups scored within normality on the FAB with no HC or pLOD with a score below 12 (indicative of dementia of AD type; Dubois et al. (2000). FAB scores were however associated with symptoms of depression and anxiety. Executive dysfunction has also been reported specifically in those pLOD who have cognitive impairment (Rapp et al., 2005). Patients with PD can also present with frontal lobe dysfunction scoring significantly lower on the FAB in comparison to controls (Lima et al., 2008). This includes the prodromal phase (Darweesh et al., 2017).

Sleep disruptions

When assessing sleep dysfunction associated with PD (using the PDSS), the pLOD in the current study on average scored 98.1 (SD 24.4) and significantly lower than HC (who had a mean score of 127.6, SD 13.1). Additionally, in the current study, 44% cent of pLOD scored abnormally on the RBDSQ (in comparison to three percent of HC) and 28% scored abnormally on the single questionnaire RBDSQ scale (in comparison to seven percent of HC) on both scales. Sleep disruptions such as early morning awakenings and insomnia have been reported in those with LLD (Maggi et al., 1998, Mallon L, 2000, Kay and

Dzierzewski, 2015). RBD, which is characterised by loss of normal atonia of REM sleep with patients consequently “acting out” the content of their dreams, however is not commonly associated with LLD or LOD. Antidepressants, in particular SSRIs and SNRIs, are known triggers for RBD (Postuma et al., 2013) but are now thought to unmask rather than cause RBD. In addition, the differences between pLOD and HC clinical scores measuring RBD persisted when those taking antidepressants were excluded (RBDSQ; p=.015).

Sleep disruption is also common throughout the course of PD, including RBD which has been reported as one of the earliest markers of consequent PD preceding up to a decade before clinical motor symptoms and diagnosis (Chaudhuri and Schapira, 2009). Chaudhuri et al. (2002) reported that on average, PD patients scored 101.1 (SD 21.7) on the PDSS with a lower score indicative of an increase in symptoms and the control group scoring 120.7 (SD 21.0). The large standard deviation is indicative of variability in scores, also reported in the results of the current study. It is evident (in the current study) that more pLOD than HC tended to experience sleep disruptions also associated with diagnosed and pre diagnostic PD. Nevertheless, as insomnia is one of the key criteria for depression and correlated with depression severity, this is difficult to interpret.

Autonomic dysfunction

There were significant group differences in scores of the SCOPA-AUT. HC scored with an average score of 7.7 (SD 4.9) and pLOD scored much higher; 14.9 (SD 8.7). Symptoms of autonomic dysfunction have also been reported in those with depression but not specific to those with LLD or LOD. Most PD patients suffer from constipation (Magerkurth et al., 2005) and as with RBD, people with constipation are at a higher risk of developing PD even predating PD by over a

decade (Adams-Carr et al., 2016). Visser et al. (2004) reported that patients with PD on average scored 18.8 (SD 8.5) and controls scored 8.8 (SD 5.4) on the SCOPA-AUT. With the results of the current study, it is evident that the mean SCOPA-AUT scores for pLOD appear to fall in line with the higher scores found in patients with PD with the scores of HC in the current study similar to those reported in Visser et al. (2004).

Hyposmia

On average, pLOD tended to have a lower mean score on the UPSIT however this was not a significant difference. When using a cut off score denoting “definite hyposmia”, 19.4% of pLOD scored as abnormal in comparison to ten percent of HC. Alongside RBD and constipation, olfactory dysfunction is one of the earliest non-motor features of PD (Doty, 2012) and over 70% of PD patients experience abnormal smell loss (Hawkes et al., 1997).

Motor symptoms

There were group differences in all measures of motor function and on average, pLOD scored with significant higher scores (indicative of severity) on the motor (and non-motor) subscales of the MDS UPDRS). Eight per cent of pLOD demonstrated MPS (compared with no HC) with common MPS such as bradykinesia, rigidity and gait/posture. Furthermore, there were significant group differences in the scores of the PD screening questionnaire with on average, pLOD scoring higher than HC ($p=.012$). There were also significant group differences on the BRAIN KS ($p=.034$) and AT scores ($p=.019$) with worse scores in pLOD compared to controls indicating slowness of movement with pLOD on average presenting with few strokes per minute and a longer time on depressed keys when completing the online test which was

devised as a test for upper limb motor dysfunction. An average of both hands were calculated for KS and AT scores (as in the original paper by Noyce et al. (2014b) which reported no significant differences in handedness. Symptoms of depression and anxiety were found to significantly correlate with motor (and non-motor) symptoms as assessed by the MDS UPDRS and the BRAIN AT scales. These results are supportive of motor features/slowness of movement in pLOD with psychomotor changes already reported in pLOD (Rapp et al., 2005). Whilst motor abnormalities have already been associated with pLOD and perhaps not suggestive of PD and a process of neurodegeneration, pLOD also scored significantly higher than HC on the PD screening questionnaire which specifically measured motor features associated with PD and the MDS UPDRS scales which measures symptoms associated with PD.

PD is characterised by the presence of clinical motor features and there has been research reporting both the presence of motor and non-motor features in the prodromal phase of PD. Noyce et al. (2014b) who developed the online version of the original BRAIN test) reported those at high risk of PD had significantly lower tapping speed from those at low risk and also a poorer sense of smell and evidence of RBD. Additionally, Postuma et al. (2012b) assessed motor dysfunction in RBD patients (as an “at risk” PD group) and found patients had voice/ face akinesia, rigidity, gait abnormalities and limb bradykinesia between nine to four years before PD diagnosis. In line with this previous research, pLOD in the current study have also demonstrated presence of a mixture of motor and non-motor features with patients scoring significantly higher than HC on scales associated with RBD and subtle motor dysfunction. Anxiety has also been suggested as a pre-motor risk factor for PD alongside depression onset and is also

significantly linked to the development of motor symptoms (Shiba et al., 2000).

It is evident that there are number of non-motor and motor features found in pLOD in the current study. Features relating to sleep, apathy and psychomotor retardation have been previously reported in pLOD with these symptoms perhaps as a result of LOD and associated with depression severity. The presence of these NMS and motor symptoms have also been discussed with regards to prodromal PD due to the overlap in symptoms. However, the underlying explanations for these symptoms in both these later life disorders should need to be considered. As mentioned earlier, the use of antidepressants (amongst other medications) cannot be ruled out with reported effects of SSRIs on autonomic functioning, Parkinsonism and RBD; all three features which have been found in the pLOD in the current study. The aetiology of depression is multifactorial with the involvement of biochemical and cerebrovascular pathology amongst other factors. Vascular factors (such as WML) have been reported in those with LOD (in comparison to patients with EOD) and vascular disease can also cause symptoms of Parkinsonism. Additionally, the role of DA (which has also been associated with symptoms of depression and those with depression but also widely acknowledged in PD) should also be considered in the pathology of the symptoms found in the current chapter.

One year follow up

Whilst number of participants eligible for follow up were very small, when assessing differences in group mean clinical scores at baseline and the one year follow up, there were significant differences between pLOD and HC with pLOD scoring higher on symptoms of RBD (p=.023) and autonomic dysfunction (p=.045) a year later. There were no

significant correlations however, between baseline scores and a change in scores at follow up in any of clinical scores in pLOD.

With regards to RBD, autonomic dysfunction and consequent PD, retrospective studies have reported a lag time of five to seven years before PD diagnosis in those with constipation (Darweesh et al., 2017, Schrag et al., 2015). Postuma et al. (2012b) discussed a reported mean latency of 13 years to PD disease diagnosis in those with RBD (Schenck et al., 1996, Iranzo et al., 2006, Postuma et al., 2009). With regards to hyposmia and consequent PD, Ponsen et al. (2004) reported that onset of PD was confirmed in ten per cent of non PD patients that had hyposmia but were relatives of PD patients. These relatives also had significantly lower SBRs when using [^{123}I] β-CIT imaging. Ross et al. (2012) also reported a higher incidence of PD in patients with hyposmia during a four year follow up as did a more recent study by (Jennings et al., 2017) also investigating hyposmia and lower DAT activity.

The results of the study show that pLOD scored significantly more than HC in symptoms of apathy, slowness of movement and anxiety; features that are found in LOD and also in neurodegenerative disorders, suggesting an overlap of symptoms between later life disorders. Additionally, pLOD also scored significantly higher than HC in symptoms related to autonomic dysfunction and motor and sleep symptoms commonly associated with PD. There were no significant group differences in cognition scores, hyposmia or frontal lobe dysfunction.

Limitations

Whilst there are established reports of differences in pathology and symptomology between LOD and EOD, not all research is supportive of differences between these two types of LLD (Brodaty et al., 2001). The current study only investigated symptomology in pLOD, with no comparison with an EOD group. Suggested future research would be to additionally include an EOD group to assess whether clinical differences lie only between pLOD and HC or also between LOD and EOD; further supporting the role of LOD as a separate later life depression.

Multiple comparisons

The p value for the current study was set at $p<0.05$. However, to account for multiple testing due to a large number of assessments (and thus a chance for significant findings more likely), a Bonferroni correction was also included in the relevant non-motor and motor symptom group comparisons. Taking into account the higher value, significant group differences remained in symptoms of anxiety and depression but more importantly in measures of RBD and sleep dysfunction (RBDSQ, PDSS), autonomic dysfunction (SCOPA-AUT) but also in total MDS UPDRS scores.

Of particular interest, symptoms of RBD were also found in pLOD. Furthermore, early stage follow up identified that more pLOD increased in symptoms associated with RBD and autonomic dysfunction, with similar findings reported in studies assessing “at risk” PD groups (Postuma et al., 2012b). The current study provides a clinical profile of LOD and suggests greater incidence of RBD in pLOD, with progression of RBD symptoms a year later. However with symptoms of depression, psychomotor retardation, cognitive impairments and sleep disturbances

found in in the pLOD in the current study (and also previously reported), it is difficult to ascertain whether we are measuring symptoms of LOD or a process of neurodegeneration due to the overlap of symptoms between LOD and PD. To gain a clearer understanding of these symptoms and the relationship between LOD and PD, a long term follow up, a larger sample size and other methods of investigation (i.e. imaging) of patients to assess progression is necessary. Although it can be suggested that pLOD in the current study have demonstrated symptoms specific to PD (suggestive of a neurodegenerative process rather than symptoms of depression), with symptoms of anosmia and RBD in comparison to HCs, however further investigation (i.e. polysomnography to confirm pathological RBD and without the use of antidepressants) is required. The next chapters of this thesis will investigate the imaging correlates of LOD; assessing dopaminergic binding using DAT SPECT and structural and vascular pathology associated with LOD using MRI.

Chapter 4. **DAT SPECT in LOD**

4.1 DAT SPECT introduction

DAT availability in patients with depression has been investigated through the use of different ligands and imaging techniques and mixed results have been reported (Camardese et al., 2014). Specific to studies using [^{123}I] FP-CIT SPECT, Sarchiapone et al. (2006) reported significantly lower DAT binding in patients with depression (aged 18-59 years) in comparison to controls. More associations between symptoms of depression and DAT binding have been evaluated through research into Parkinsonian syndromes (PS) such as PD (Chagas et al., 2013). Specifically, research has reported negative correlations between symptoms of depression (and anxiety and total affect scores) with DAT availability in established PD (Weintraub et al., 2005); with correlations between higher effect scores and diminished uptake in the left anterior PUT. Vriend et al. (2014) as also reported negative correlations with DAT uptake in the right CN. However to our knowledge, research into dopaminergic activity in the nigrostriatal pathway in those with only LOD using the SPECT tracer [^{123}I] FP-CIT is unknown.

Guidelines for visually rating DAT SPECT images focus on DAT uptake in the PUT and CN, using findings from research in those with PS and largely PD to categorise different patterns of uptake. Patients with PD lose approximately 50% of their nigral dopaminergic neurones before clinical presentation and research has found associations between DAT deficit in the PUT and CN and early and mild motor symptoms in those with PD (Marek et al, 1996; Tissingh et al, 1998). DAT SPECT

images using [^{123}I] FP-CIT have shown high uptake in the striatal region in HC and reduced uptake in the posterior PUT (in earlier PD subjects) with the anterior PUT and whole striatum (including the CN) being affected as the disease progresses (GEHealthcare, 2012). In other PS such as DLB, typically a balanced loss of PUT and CN in both left and right striatum has been observed, with more loss in the CN in those with DLB in comparison to those with PD (Walker et al., 2004).

4.2 Objectives

Objective 1: To visually compare dopaminergic uptake in pLOD and HC. Additionally to compare the SBRs in pLOD with HC. We hypothesise that pLOD will present with patterns of abnormal dopaminergic uptake commonly associated with disorders such as PD and DLB (Fearnley and Lees, 1991, Bajaj et al., 2013, Walker et al., 2004). We propose that a proportion of pLOD will develop PD or a related neurodegenerative disease as we assume that at least 50% of LOD is neurodegenerative. This is based on previous follow-up studies suggesting 40% develop dementia (including AD, vascular disease and DLB) with no data available on PD (Alexopoulos, 2005, Schweitzer et al., 2002, Fiske et al., 2009). However, the associations between depression and AD or PD are comparable (Shiba et al., 2000, Ishihara and Brayne, 2006, Ownby et al., 2006) and in the age group of 55-70 the incidence of PD/LBD and of AD is similar (both ~0.3%; (von Campenhausen et al., 2005, Perez et al., 2010, Kawas et al., 2000). Thus, we expect that after accounting for those with AD and vascular disease, approximately 25% of pLOD develop PD or dementia due to LBD.

Objective 2: To assess non-motor and motor features in pLOD rated with abnormal DAT SPECTs. We hypothesise that pLOD with a dopaminergic deficit will present with more prodromal non motor and motor features associated with PD and DLB such as anosmia and REM sleep behavior disorder; with current research reporting those patients with anosmia or REM sleep behavior disorder **and** lower striatal SBRs are at a higher risk for PD (Berendse and Ponsen, 2009, Iranzo et al., 2010, Jennings et al., 2017)

4.3 Methods

Patients

The same 66 participants included in Chapter 3: “Clinical correlates of LOD” were also invited to have a DAT SPECT. Out of the 66 participants, 54 participants also had the DAT SPECT (and completed the clinical assessments), which included 25 HC and 29 pLOD.

Assessments not completed by participants

The FAB was not completed by three pLOD and the PDSS by one HC, due to participants being too tired to complete the assessments. The BRAIN test was not completed by 19 participants; 12 pLOD and seven HC due to problems with accessing the online test. One HC was excluded from the MDS UPDRS ME as they could not complete the full examination due to a leg injury.

DAT SPECT

Full exclusion criteria for the use of DaTscan ([^{123}I] FP-CIT) is listed in the GE healthcare DaTscan prescribing information (http://us.datscan.com/) and includes the following exclusion criteria relevant for this study population;

Patients with significant cerebrovascular disease, as indicated by major confluent lesions or strategic lesions on a MRI scan were excluded, due to potential effect of vascular lesions on the pattern of DAT uptake.

Patients with renal and Hepatic Impairment:

The effect of renal or hepatic impairment on DaTscan imaging has not been established. The kidney excretes DaTscan; patients with severe renal impairment may have increased radiation exposure and altered DaTscan images.

Use of drugs

As recommended by the GE healthcare DaTscan prescribing information (http://us.datscan.com/), the following drugs with high binding affinity to the DAT which may potentially interfere with interpretation of the images were excluded: amoxapine, amphetamine, amoxapine, amphetamine, benztropine, bupropion, buspirone, cocaine, mazindol, methamphetamine, methylphenidate, norephedrine, phentermine, phenylpropanolamine and selegiline and sertraline. Other SSRIs paroxetine and citalopram were also stated to potentially interfere with the interpretation of the images and are discussed in further detail below.

Antidepressants

As suggested by the DaTscan prescribing information, antidepressant medication may potentially interfere with the interpretation of the images. Studies have controlled for the effects of antidepressant medication by recruiting drug naive patients or including those drug free for a certain period prior to the scan (Neumeister et al., 2001, Sarchiapone et al., 2006). For the current study, we did not exclude patients on antidepressant medication in line with our ethics approval and instead recalculated the SBRs in those using SSRIs or SNRIs on the basis of previous research into DAT drug interactions, as explained below.

In Booij and Kemp (2008)'s review of the potential effects of drugs in imaging DAT using [^{123}I] FP-CIT, it was reported that the SSRI sertraline, significantly influences the quantification of [^{123}I] FP-CIT (Owens et al., 2001, Tatsumi et al., 1997), increasing striatal to occipital ratios by ten percent. Additionally, paroxetine was also reported to increase SBRs by approximately ten percent (Booij et al., 2007) and Grosset et al. (2014)'s safety analysis reported that citalopram could increase SBRs by 18% (Ziebell et al., 2010).

Furthermore, it was reported that use of the SNRI venlafaxine, significantly increased striatal ratios by ten percent when using the [^{123}I] β-CIT radiotracer (Shang et al., 2007). Therefore Booij and Kemp (2008) suggested that the potential ten percent increase of DAT binding ratios could be made for other SNRIs and SSRIs, when using [^{123}I] FP-CIT imaging. Tricyclic antidepressants such as amitriptyline and imipramine have reported to not be potent to the DAT (Tatsumi et al., 1997).

Regarding the visual interpretation of the scans and the use of SSRIs and SNRIs, the ten percent increase on the SBRs is too small to influence the visual interpretation of the ligand and the characteristic uptake pattern of normal or abnormal scans. The effect of tricyclics on DAT imaging in humans has not been evaluated however it is unlikely that tricyclics will significantly increase the DAT binding enough to hinder the visual interpretation of the scans (Booij and Kemp, 2008).

The methods used to recalculate SBRs to account for antidepressant medication are detailed in the SQA section of this chapter.

4.3.1 DAT SPECT methodology

4.3.1.1 Image acquisition

Participants had the DAT SPECT on the same day as the assessments at either the Royal Free Hospital in London (n=38), or the Princess Alexandra Hospital in Essex (n=16), depending on which hospital was most easily accessible for participants. Both hospitals used the same DAT SPECT scanner to perform the scans; a Siemens Symbia Dual Headed Scanner, using the [^{123}I] FP-CIT (DaTscan, GE Healthcare) SPECT tracer (http://us.datscan.com/).

4.3.1.2 DAT SPECT procedure

After completing the safety questions, participants were required to take the thyroid blockade medication (in the form of a liquid or tablet) to safeguard against unnecessary uptake of radioiodine in the thyroid. An hour after the medication a single intravenous [^{123}I] FP- CIT injection (DaTscan) was administered with a volume of 2.5ml and radioactivity between 111-185 MBq. Three to four hours after the injection, participants had the SPECT scan on a Siemens Symbia Dual Headed Scanner (with a high resolution collimator) which lasted approximately 25 minutes and measured the presynaptic uptake of the dopamine transporter in the striatum. SPECT was acquired in a 128*128 matrix obtaining 120 frames over 360°. No phantom studies were obtained for the current study as both hospitals have previous experience with [^{123}I] FP-CIT SPECT imaging and undergone previous phantom studies.

Aftercare

Participants scanned at the Princess Alexander Hospital were given additional thyroid blockade medication to take home and advised to take it 24 hours after initial ingestion as an extra safety precaution as per their standard protocol.

All participants received a courtesy call or email the next day, by the study researcher (HK).

4.3.1.3 Visual analysis: Ratings

The images were visually assessed for normal and abnormal dopaminergic uptake by HK, using GE's guidelines, shown in Figure 4-1 (adapted from the GE reporting guide; GEHealthcare (2013). To ensure that the ratings were not influenced by any clinical information when rating the scans, HK was blinded to the participant group (HC or pLOD) and any participant information. All participant scans had an anonymised ID from a premade list and this was the only information available to the rater (i.e LOD001). GE's DAT SPECT ratings were categorised into normal, abnormal type 1, abnormal type 2, abnormal type 3 and striatal loss (shown and described in Figure 4-1).

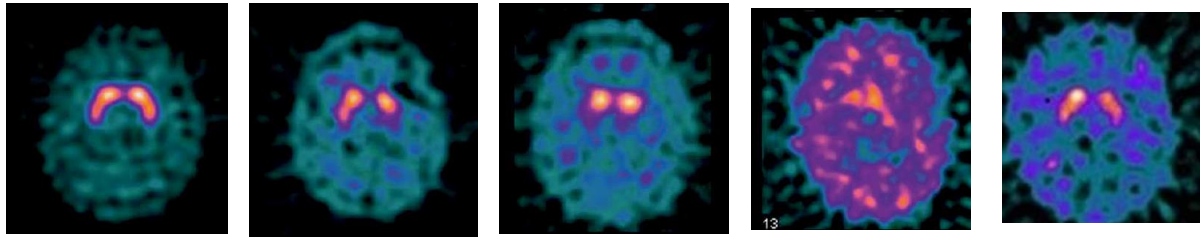
HK rated the scans with guidance from GE's guidelines and training from Dr Zuzana Walker (Institute of Psychiatry, University College London). The colour scale in which the scans were rated in was GE colour, Rainbow, Cool, as shown in Figure 4-1.

As this was an exploratory study and assessing for any/subtle dysfunction in dopaminergic uptake, an additional rating of equivocal DAT SPECT was used. Equivocal has been used in clinical settings

(Pencharz D R, 2014) when visually there is uncertainty around uptake in the DAT SPECT. In this study, it was included in the cases where the dopaminergic uptake was slightly reduced and not decreased enough to fit within the abnormal DAT SPECT types 1, 2, 3 and striatal loss which are patterns of uptake usually seen in diagnosed PD or DLB. A slight reduction could be attributed to natural changes and where visually the pattern of uptake has not degraded significantly, causing uncertainty around the uptake.

Further to specifying the type of abnormal uptake, participants were also dichotomised into ratings of normal (including equivocal) and abnormal (inclusive of abnormal type 1, type 2, type 3 and striatal loss) to analyse the **total number** of DAT SPECTs rated with normal and abnormal uptake.

Figure 4-1 GE healthcare DaTSCAN visual reporting guidelines (excluding equivocal)



Normal | Abnormal Type 1 | Abnormal Type 2 | Abnormal Type 3 | Striatal loss

Abnormal types	
Abnormal Type 1	Asymmetric activity e.g. activity in the region of the putamen of one hemisphere is absent or greatly reduced with respect to the other.
Abnormal Type 2	Activity is absent in the putamen of both hemispheres and confined to the caudate nuclei.
Abnormal Type 3	Activity absent in the putamen of both hemispheres and greatly reduced in one or both caudate nuclei.
Striatal loss	Balance striatal loss to the caudate nuclei and putamen with high levels of back ground activity.

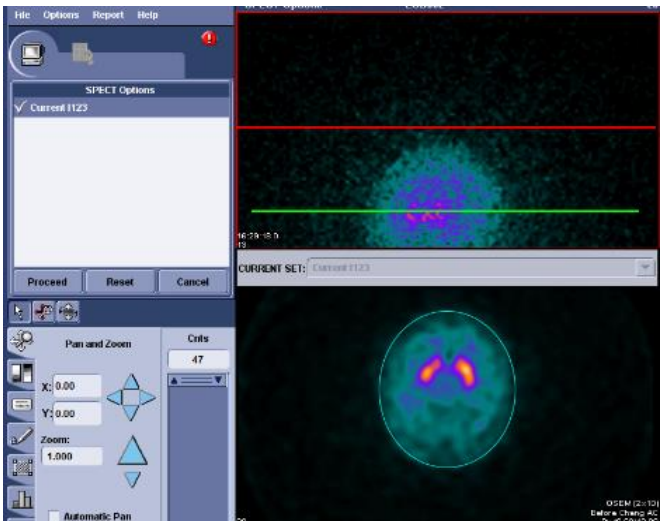
Images provided courtesy of Birmingham City Hospital, UK and descriptions by GEHealthcare (2013)

Image processing

The Nuclear imaging departments at both hospitals provided the raw data of the scans in a digital imaging and communications in medicine (DICOM) format. To prepare the scans for the visual analysis, using GE DATQUANT (GEHealthcare, 2012), I applied image reconstruction with OSEM 2 x 10 with a Butterworth filter 0.6 cut off. Chang's correction attenuation was also applied (Figure 4-2) individually to each scan, with attenuation correction recommended by the European association of nuclear medicine (EANM) guidelines for SPECT imaging (Darcourt et al., 2010).

Reconstructing all the scans on DaTQUANT enabled uniform comparison between images when rating visually, but also with the DaTQUANT control database.

Figure 4-2 Example of interface when applying Chang's attenuation correction in DaTQUANT



4.3.2 Analysis of DAT SPECT results

4.3.2.1 DAT SPECT images

The primary analysis for the DAT SPECT was the visual assessment.

4.3.2.2 SQA

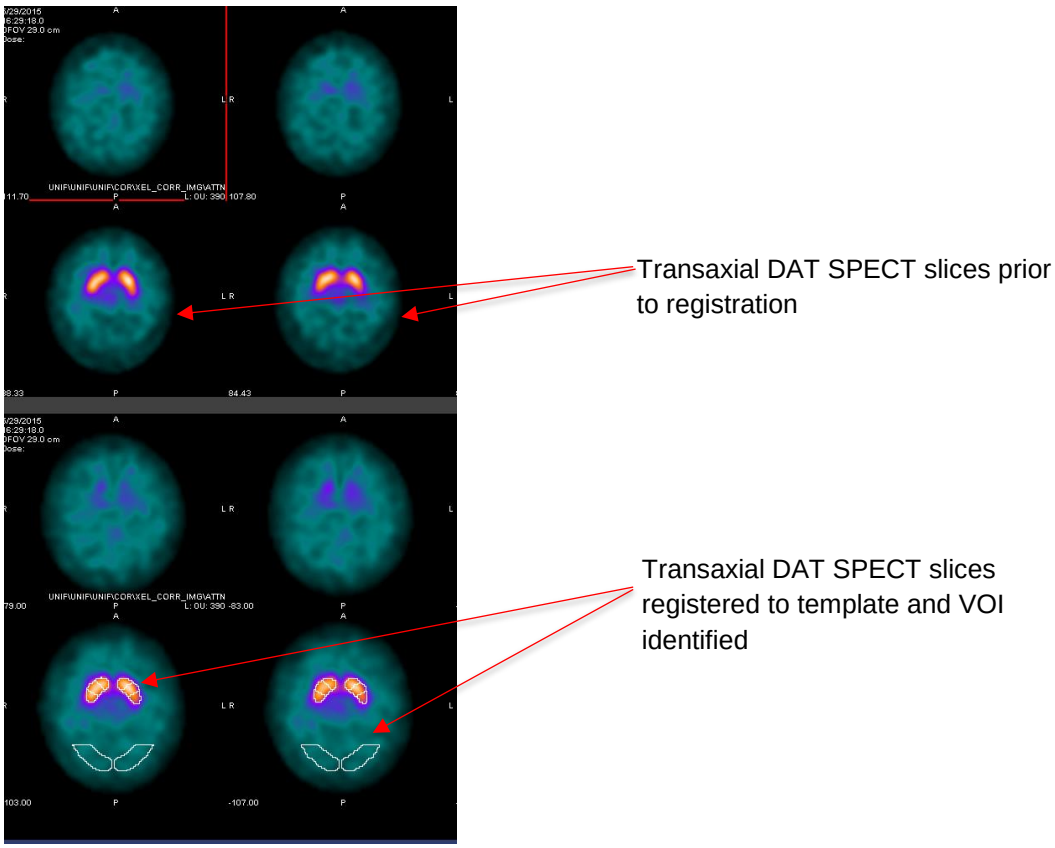
In addition, SPECT scans were analysed semi quantitatively, specifically looking at the SBRs of the PUT and CN.This secondary form of analysis was conducted using a software provided by the manufacturer (GE healthcare) of the [^{123}I] FP- CIT SPECT tracer to assess whether the DAT SPECTs had normal or abnormal dopaminergic uptake in striatal regions (DaTQUANT) in comparison to a control database. Potential advantages of the application include earlier detection of findings seen in Parkinson's disease, detection of subtle deficits in uptake (GEHealthcare, 2012) and as an aid to visual interpretation; increasing diagnostic accuracy (Djang D, 2004).

To briefly explain the quantification methods used in DaTQUANT (GEHealthcare, 2012);

Each DAT SPECT is automatically registered to a template defined in the Montreal Neurological Institute (MNI) space, which is created from a set of participants from the European [^{123}I] FP-CIT SPECT normal database (ENCDAT project). Further details; (Evans A C, 1993, Tossici-Bolt, 2011) and (http://packages.bic.mni.mcgill.ca/tgz/mni-models_icbm152-lin-1.0.tar.gz).

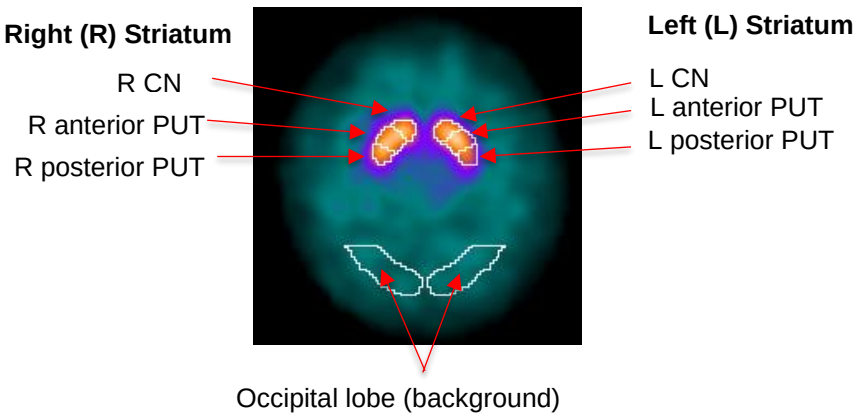
Template registration (Figure 4-3) is followed by interrogation of the uptake in the striatal regions using a volume of interest (VOI) atlas.

Figure 4-3 Example of the interface on DaTQUANT during template registration



The regions used in the VOI include the occipital lobe (background; B) and the left and right striatum (Figure 4-4). Each side of the striatum consists of the PUT (including the anterior and posterior) and the CN (GEHealthcare, 2012). For the study, the SQA analysed specifically the PUT and CN regions. Left (L) and right (R) regions of the PUT and CN were calculated separately by computing striatal binding ratios (SBRs) for each side using the B counts, as proposed by (Booij J, 2001) and as shown in Formula 4.1; (GEHealthcare, 2012).

Figure 4-4 Regions of striatal DAT uptake and participant image as shown on GE DaTQUANT.



Formula 4.1 Calculating the left and right putamen (PUT) and caudate nuclei (CN) striatal binding ratios (SBRs) using the background/ occipital lobe (B)

$$(Left\ or\ Right)\ PUT\ SBR = \frac{(Left\ or\ Right)\ PUT\ counts}{B\ counts}$$

$$(Left\ or\ right)\ CN\ SBR = \frac{(Left\ or\ right)\ CN\ counts}{B\ counts}$$

Antidepressant use

Once the PUT and CN SBRs were obtained from DATQUANT (as in Formula 4.1), the SBRs had to be recalculated in those pLOD taking SSRIs and SNRIs to account for the ten percent increase in SBRs caused by the antidepressant use (Booij and Kemp, 2008) as explained earlier. L and R PUT and CN SBRs were recalculated separately using Formula 4.2, in only those pLOD taking SSRIs and SNRIs.

Formula 4.2: Accounting for a 10% effect of antidepressant medication on SBRs

$$Left\ or\ right\ SBR * 0.9 = adjusted\ SBR$$

SBR= PUT or CN striatal binding ratio

Analysis of the SBRs

Once the relevant SBRs were adjusted for antidepressant use, the lowest SBRs were also calculated for additional analysis. Lowest SBRs were calculated by using the lowest SBR value of the R and L PUT and CN (separately for both areas) in each participant.

Comparing with DaTQUANT's control database

In order to explore which participants had abnormal uptake/DAT SPECTs, the calculated (and for the relevant pLOD; recalculated due to antidepressant use) SBRs were compared with the SBRs from a control database (provided on DaTQUANT) using two approaches (Formula 4.3 and 4.4). The database of controls was obtained from the Parkinson's Progression Markers Initiative (PPMI) database (Marek et al., 2011). Characteristics of the control database were: healthy control subjects who do not have a first degree blood relative with Parkinson's, an average age of 60.3 with 73 (61%) males and 45 (39%) females. Participant ages from the **current** study sample ranged from 56 years of age to 79 years.

As described next, in both the approaches, participants with abnormal uptake were classified as those with PUT and CN SBRs one standard deviation (SD) below the mean SBRs of **age matched** control participants from DaTQUANT's control database. A cut off of two SDs has been applied in similar studies exploring SBRs in participants with prodromal clinical PD features (Meles et al 2017; Sommer et al 2004), however as this is an exploratory study and we are examining any/subtle dopaminergic deficit, a one SD cut off was considered more appropriate.

SQA Approach one: Dichotomised ratings

This approach was used to assess the **total number** of participants with abnormal uptake (AU) and normal uptake (NU). The side (L or R) or region (PUT and CN) in which there was AU or NU was not specified.

Participants with PUT or CN SBRs one SD below the mean PUT and CN SBRs, of the same side (L or R) of controls of the same age in the control database were grouped into those with AU for their age. Participants with ALL PUT or CN SBRs above or equal to the mean PUT and CN SBRs, of the same side (L or R) of controls of the same age in the control database were grouped into those with NU for their age. (Formula 4.3).

Formula 4.3: Calculating participants with abnormal and normal uptake

$$Abnormal\ uptake = \\ Participant\ left\ or\ right\ SBR < left\ or\ right\ age\ matched\ control\ \ SBR - 1\ SD$$

$$Normal\ uptake = \\ Participant\ left\ or\ right\ SBR \geq left\ or\ right\ age\ matched\ control\ \ SBR - 1\ SD$$

SBRs= putamen and/or caudate nuclei striatal binding ratios

SQA Approach two: Region (PUT and CN) and side specific (left or right) ratings

This approach provides a breakdown of the findings from Approach one, specifically observing whether participants had abnormal or normal uptake in the L and R PUT and CN (Formula 4.4). As with Approach one, participants were categorised as abnormal if they had SBRs one SD below the SBRs of the same side and region of the means of age matched controls from DaTQUANT's control database.

Formula 4.4: Calculating and specifying region and side specific ratings of normal and abnormal uptake

$$Side\ specific\ abnormal\ \boldsymbol{PUT}\ uptake = left\ or\ right\ SBRs < left\ or\ right\ age\ matched\ control\ PUT\ SBR - 1\ SD$$

$$Side\ specific\ abnormal\ \boldsymbol{CN}\ uptake = left\ or\ right\ SBRs < left\ or\ right\ age\ matched\ control\ CN\ SBR - 1\ SD$$

PUT= putamen, SBR= striatal binding ratio, SD= standard deviation, CN= caudate nuclei

4.3.3 Statistical Analysis

The main analysis was a comparison between pLOD and HC, using the ratings of the visual assessments and of the semi quantitative assessments. Histograms and Q-Q plots were used to visually assess the distribution of data. Differences between categories were compared using Chi-square tests or the Fisher's exact for small numbers and independent sample t tests. In SBRs and relevant clinical scores with significant group differences, the effects of age and gender were analysed using analysis of covariance (ANCOVA).

The same clinical assessments described in Chapter 3; "Clinical correlates of LOD" were completed by participants as they were hypothesised to be relevant the SBRs. Mean scores of the clinical assessments were compared between groups and cut off values (where available from the literature) were used to identity which participants scored abnormally on each scale (cut offs as described in Chapter 3 "Clinical correlates of LOD").

Scatter plots were created for a visual inspection of the spread of L and R PUT) and CN SBRs and relevant clinical assessment scores.

Bar charts were used as visual representation of the number of clinical assessments that participants visually rated with an abnormal DAT SPECT also scored abnormally on. Pearson's r and Spearman's rho correlations were used to look for associations between relevant clinical assessment scores and SBRs.

In participants with abnormal DAT SPECTs, for the UPSIT, general adult gender specific (not age specific) cut offs were used to assess participants with mild, moderate, severe hyposmia and anosmia (Table 4-1; Doty et al., 1984). Additional cut offs to denote what was defined

as (age and gender specific) “definite hyposmia” in the current study were participants that scored ≤15th percentile of norms, as described in Chapter 3 “Clinical correlates of LOD”.

Table 4-1 General adult olfactory diagnosis (gender not age specific)

Test score	Olfactory diagnosis
00-05	Probably malingering
06-18	Total anosmia
19-25	Severe microsmia
26-29	Moderate microsmia (males)
26-30	Moderate microsmia (Females)
30-33	Mild mircorsmia (Males)
31-34	Mild microsmia (Females)
34-40	Normosmia (Males)
35-40	Normosmia (Females)

Medication use

As described in Chapter 3 “Clinical correlates of LOD”, in a secondary analysis, participants on medications which may affect clinical RBD, MDS UPDRS ME and SCOPA-AUT scores were excluded to assess any differences in clinical scores upon exclusion. All full list of the medications and participants excluded is available in Appendix B.

Comparison between visual ratings (VR) and the SQA

Cohen’s Kappa was used to test interrater agreement between HK and the dichotomized SQA ratings of abnormal and normal DAT SPECTs.

Significance for the study was set at $p<0.05$. However to correct for multiple testing, a value of $p<0.002$ using Bonferroni correction was also provided in the relevant results tables.

4.4 DAT SPECT results

There were no significant group differences in participant demographics. HC had an average age of 69.7 (SD 9.0) years and pLOD had an average age of 67.7 (SD 7.5) years. Thirty-six per cent of HC and 45% of pLOD were male. Thirteen pLOD were taking SSRI or SNRI antidepressant medication and their SBRs were recalculated, as described in Formula 4.2 (Table 4-2).

SBRs

There was a significant group difference in lowest CN SBRs (p=.035) and R CN SBRs (p=.019).

When adjusting for age and gender using ANCOVA, the group difference in lowest CN SBRs remained significant (age; p=.019, gender; p=.025) which was also the case for R CN SBRs (age; p=.043, gender; p=.011).

Clinical assessments

The pLOD and HC who had a DAT SPECT significantly differed in symptoms of depression and anxiety; DSM 5 SMD (p<0.001); HADS depression (HADS-D) (p<0.001) and HADS anxiety (HADS-A) (p=<0.001).

There were also significant group differences on non-motor and motor symptoms measured by the MDS UPDRS sub scales; MDS UPDRS Total (p=.001), ML-EDL (p=.037), nM-EDL (p=0.001), motor ME (p<0.001) and on the PD screening questionnaire (p=.016).

Significant group differences were also observed in assessments measuring REM sleep behavior disorder (RBDSQ; p=.001) and symptoms of autonomic dysfunction, measured by the SCOPA AUT (p=.001), as shown in Table 4-3.

Medication use

Significant group differences remained in the MDS UPDRS ME, RBDSQ and SCOPA-AUT scores when participants on the relevant medications were excluded (Table 4-4). Group differences in the RBD1Q missed significance (p=.077) when excluding participants on

the relevant medications. A full list of the medications is available in Appendix B.

Table 4-2 Mean group demographics and left (L) and right (R) putamen (PUT) and caudate nuclei (CN) striatal binding ratios (SBRs)

	HC (n=25)	pLOD (n=29)		*p*
	Mean (SD)			
Average Age	69.7 (9.0)	67.7 (7.5)	0.90	.370
Male n (%)	9 (36)	13 (45)		.352
Duration of depression/anxiety	-	3.4 (3.1)		
Antidepressant use n (%)	0 (0)	13 (45)		**<.001**
Lowest PUT SBR	2.39 (0.40)	2.28 (0.54)	0.81	.418
Lowest CN SBR	2.64 (0.43)	2.36 (0.49)	2.16	**.035**
PUT (L) SBR	2.43 (0.39)	2.31 (0.54)	0.86	.390
PUT (R) SBR	2.48 (0.44)	2.36 (0.54)	0.91	.367
CN (L) SBR	2.70 (0.42)	2.46 (0.50)	1.85	.069
CN (R) SBR	2.73 (0.45)	2.42 (0.49)	2.41	**.019**

Fisher's exact and independent sample t tests between groups. Significant p values ($p<0.05$) in **bold**
Abbreviations: PUT= putamen, CN=caudate nuclei, SBR=striatal binding ratios, L=left side, R=right side

Table 4-3 Mean clinical assessment scores

Clinical assessments	HC (n=25*)	pLOD (n=29*)		*p*
	Mean (SD)			
MoCA	26.8 (2.6)	25.2 (3.6)	1.90	.062
FAB	16.8 (1.4)	17.2 (1.1)	-1.12	.266
MDS UPDRS Total	6.3 (6.1)	20.3 (13.2)	-5.11	**<.001**
MDS UPDRS ML-EDL	1.4 (2.2)	3.6 (4.2)	-2.44	**.020**
MDS UPDRS nM- EDL	4.1 (3.7)	14.0 (8.7)	-5.56	**<.001**
MDS UPDRS ME	0.8 (1.4)	2.8 (2.7)	-3.36	**.002**
LARS	-26.3 (4.9)	-22.2 (10.2)	-1.94	.058
BRAIN KS	57.9 (8.4)	51.8 (11.2)	1.82	.077
BRAIN AT	101.8 (53.6)	125.1 (67.2)	98.50	.072
PD Screening questionnaire	0.9 (1.3)	2.0 (2.0)	-2.49	**.016**
HADS-D	3.9 (2.4)	17.3 (8.8)	-6.86	**<.001**
HADS-A	2.7 (2.2)	9.9 (5.3)	-6.66	**<.001**
DSM 5 SMD	1.3 (2.0)	9.9 (5.3)	-6.24	**<.001**
RBDSQ	2.2 (2.2)	4.8 (3.3)	-3.44	**.001**
RBD1Q n (%)	2 (8.0)	10 (34.5)		**.045**
PDSS	127.67 (13.1)	95.3 (25.0)	6.04	**<.001**
UPSIT	29.3 (7.0)	30.5 (6.2)	-0.65	.519
SCOPA-AUT	7.7 (5.3)	14.5 (8.6)	-3.53	**.001**

Fisher's exact and independent sample t tests, Mann Whitney U between groups. Significant p values ($p<0.05$) in **bold**. A significant p value at Bonferroni adjustment would be $p<0.002$, calculated by 0.05 / number of clinical assessments

*Three pLOD did not complete the FAB, one HC the PDSS and MDS UPDRS ME, 12 pLOD and seven HC the BRAIN test.

Abbreviations: MoCA= Montreal Cognitive Assessment, FAB=Frontal Assessment Battery, MDS- UPDRS= Movement Disorder Society Unified Parkinson's Disease Rating scale; ML EDL= Motor Aspects of Experiences of Daily Living, nM-EDL= Non Motor Aspects of Experiences of Daily Living, UPDRS Motor examination= Unified Parkinson's Disease rating scale , LARS= Lille Apathy Rating Scale, BRAIN test= BRadykinesia Akinesia Incoordination Test; KS= kinesia score, AT; akinesia time; PD screening= Parkinson's Disease scale, HADS = Hospital Anxiety and Depression Scale; D=depression scale, A=anxiety scale, DSM 5 SMD=DSM 5 criteria Severity Measure of Depression, RBDSQ= REM Sleep Behaviour Disorder single questionnaire, RBDQ= The REM sleep Behaviour Disorder screening questionnaire, PDSS= Parkinson's disease Sleep Scale, UPSIT= University of Pennsylvania Smell Identification Test, SCOPA-AUT= Scales for Outcomes in Parkinson's disease-Autonomic.

Table 4-4 Mean clinical scores when excluding participants on relevant medications

Clinical assessments	HC	pLOD		*p*
	Mean (SD)			
	n=20	n=26		
MDS UPDRS ME	0.8 (1.57)	2.5 (2.6)	124.00	**.002**
	n=25	n=14		
RBDSQ	2.2 (2.17)	4.6 (3.4)	102.00	**.033**
RBD1Q n (%)	2 (8.3)	5 (35.7)		**.077**
	n=20	n=22		
SCOPA-AUT	7.6 (5.5)	13.9 (7.5)	110.00	**.005**
	n=20	n=24		
SCOPA-AUT[2]	7.6 (5.5)	13.6 (7.3)	118.00	**.004**

Mann Whitney U test and Fisher's exact test. Significant p values ($p<0.05$) in **bold**.
[2]Two patients taking Olanzapine, Pregabalin and Risperidone were included

4.4.1 Part One: DAT SPECT visual analysis

Visual ratings by HK

Examples of participant scans and the types of ratings found by HK are shown in Figure 4-5.

Seven pLOD were visually rated with abnormal DAT SPECTs by HK which included; two patients rated with abnormal type 1 (activity in the region of the PUT of one hemisphere is absent or greatly reduced with respect to the other), two rated with type 2 (activity absent in the PUT of both hemispheres and confined to the caudate nuclei) and threerated with striatal loss (balanced striatal loss to the CN and PUT and an increase in background activity). Twenty-two pLOD were rated with normal scans including 2 rated as equivocal (visually normal but with some doubt). One HC was rated with an abnormal scan with striatal loss (balanced striatal loss to the CN and PUT). Twenty-four controls were rated with normal scans including three rated as equivocal.

There were no DAT SPECTs visually rated with abnormal type 3 uptake (activity absent in the PUT of both hemispheres and greatly reduced in one or both CN).

There was a significant difference between the number of abnormal scans between groups (pLOD and HC); p= 0.042 (Table 4-5).

Figure 4-5 Examples of DAT SPECTs from the study and their visual ratings by HK
A; Normal, B; Equivocal, C; Abnormal type 1, D; Abnormal type 2, E; Balanced striatal loss

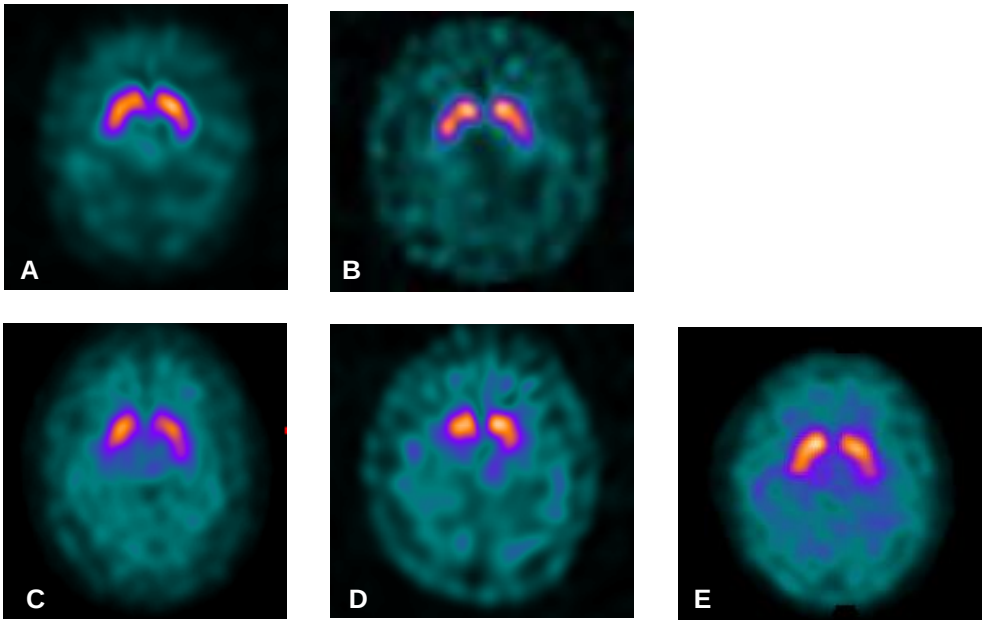


Table 4-5 HK visual ratings of the DAT SPECTs in patients with LOD (pLOD) and healthy controls (HC)

Rating	HC (n=25)	pLOD (n=29)	p
	n (%) of participants		
Normal	21 (84)	20 (69)	
Equivocal	3 (12)	2 (7)	
Abnormal Type 1	0 (0)	2 (7)	
Abnormal Type 2	0 (0)	2 (7)	
Abnormal Type 3	0 (0)	0 (0)	
Striatal Loss	1 (4)	3 (10)	
Total Abnormal	1 (4)	7 (24)	**0.042**

Fisher's exact t test between groups. Significant p values (<0.05) in **bold**
n= number of participants, total abnormal includes all types (1, 2, 3 and striatal loss)

4.4.2 Part Two: DAT SPECT SQA

4.4.2.1 Dichotomised ratings

Using the dichotomised ratings (Formula 4.3), 11 pLOD were rated with abnormal DAT SPECTs and 18 were rated with normal DAT SPECTs. Three HC had abnormal and 22 HC had normal DAT SPECTs. There was a significant (p=.030) group difference in the number of abnormal scans. (Table 4-6).

4.4.2.2 Region and side specific ratings

Using the region and side specific SQA (Formula 3.4), the majority of participants had normal L PUT (pLOD; n= 23, HC=22) and normal R PUT (pLOD; n= 23, HC=22) SBRs. Six pLOD were rated with abnormal SBRs in both the Land R PUT in comparison to three HC as shown in Table 4-7 (p=.316).

The majority of participants also had normal L and R CN SBRs. However, there was a significant ($p<0.05$) group difference in R CN SBRs with only one HC with abnormal SBRs and nine pLOD with abnormal SBRs (p=.011). In addition, pLOD (n=eight) tended to have abnormal L CN SBRs more often than HC (n=two) (p=.065), as shown in Table 4-8.

Figure 4-7 and Figure 4-6 demonstrate a visual representation of Formula 4.4 and show the spread of L and R PUT and CN SBRS in pLOD and HC and to show the variation by age in comparison to age matched mean SBRs from DATQUANT's control database. Participants below the green line had SBRs below the control database.

Abnormal ratings of DAT SPECTs when SBRs have not been adjusted for medication use are included in Appendix C.

Table 4-6 Dichotomised ratings of putamen and caudate nuclei striatal binding ratios

Rating	HC n (%)	pLOD n (%)	p
Normal	22 (88)	18 (62)	
Abnormal	3 (12)	11 (38)	**.030**

Fisher's exact test between groups. Significant p values (p<0.05) in **bold**.
pLOD= patients with late onset depression, HC= healthy controls, n=number of participants

Table 4-7 Side specific ratings of **putamen** striatal binding ratios

	Rating	HC n (%)	pLOD n (%)	p
Putamen left	Normal	22 (88)	23 (79)	
	Abnormal	3 (12)	6 (21)	.316
Putamen right	Normal	22 (88)	23 (79)	
	Abnormal	3 (12)	6 (21)	.316

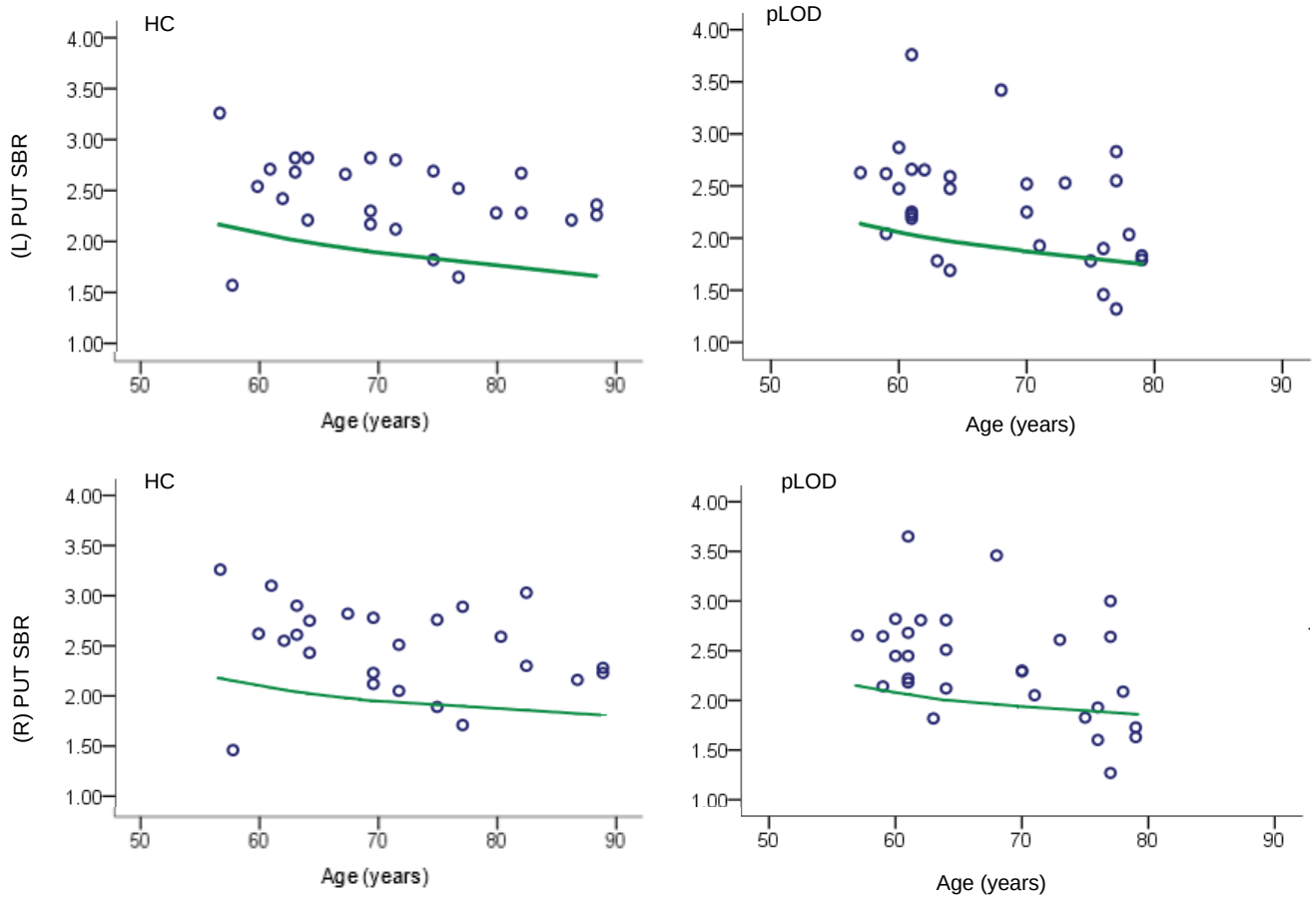
Fisher's exact test between groups
pLOD= patients with late onset depression, HC= healthy controls, n=number of participants

Table 4-8 Side specific ratings of **caudate nuclei** striatal binding ratios

	Rating	HC n (%)	pLOD n (%)	p
Caudate left	Normal	23 (92)	21 (72)	
	Abnormal	2 (8)	8 (28)	.065
Caudate right	Normal	24 (96)	20 (79)	
	Abnormal	1 (4)	9 (31)	**.011**

Fisher's exact test between groups. Significant p values (p<0.05) in **bold**.
pLOD= patients with late onset depression, HC= healthy controls, n=number of participants

Figure 4-6 Participant (pLOD n=29, HC n=25) left (L) and right (R) putamen (PUT) striatal binding ratios (SBRs) by age compared with mean PUT SBRs from DaTQUANT's control database
(Formula 4.4). Participants under the green line are abnormal

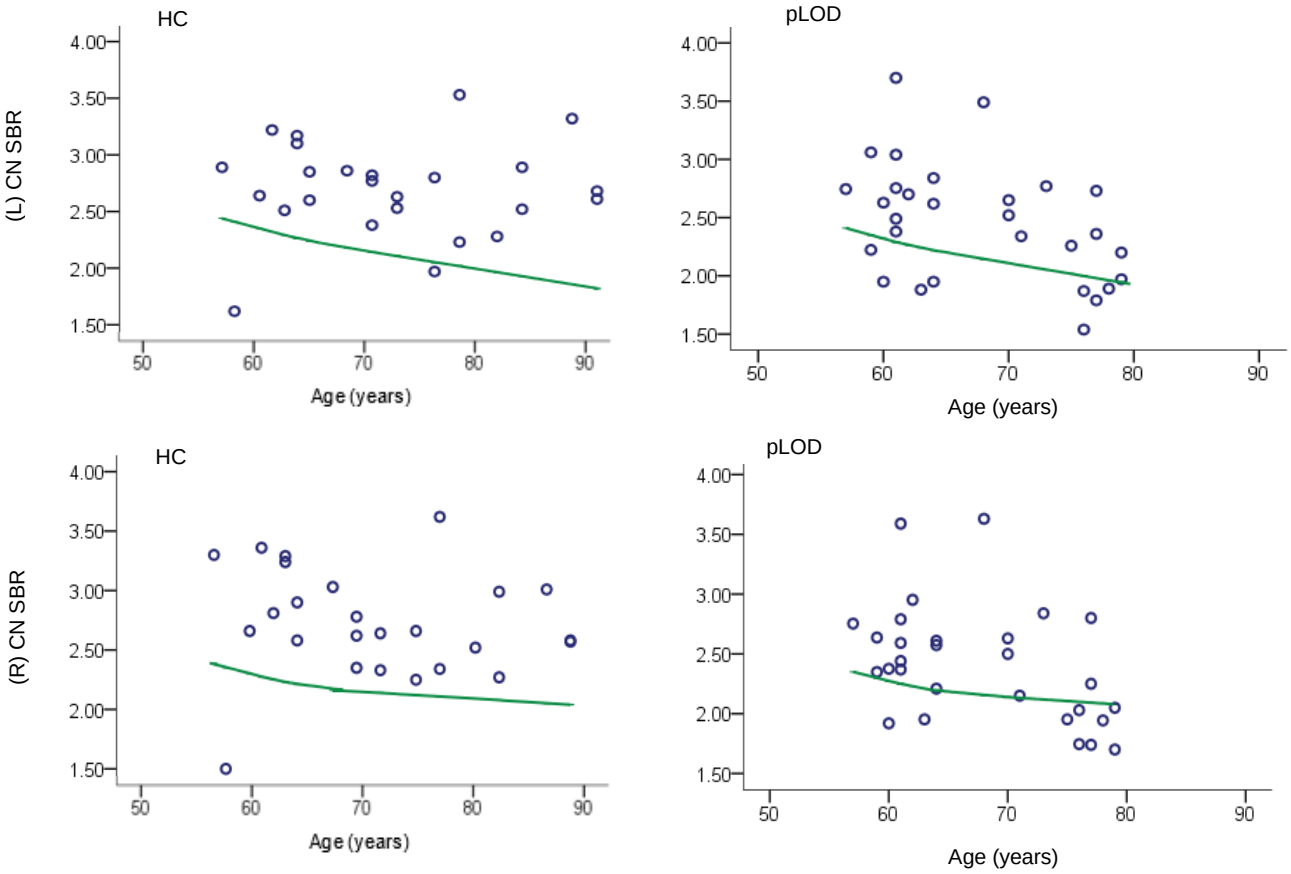


Participant putamen (PUT) striatal binding ratios (SBRs)

---- PUT SBRs from DaTQUANT's control database (-one standard deviation)

HC= healthy controls, pLOD= patients with late onset depression

Figure 4-7 Participant (pLOD n=29, HC n=25) left (L) and right (R) caudate nuclei (CN) striatal binding ratios (SBRs) by age compared with mean CN SBRs from DaTQUANT's control database (Formula 4.4). Participants under the green line are abnormal



Participant caudate nuclei (CN) striatal binding ratios (SBRs)
CNSBRs from DaTQUANT's control database (-one standard deviation)
HC= healthy controls, pLOD= patients with late onset depression

4.4.3 Part Three: Demographics, clinical scores and DAT SPECTs

Associations between demographics, clinical scores and SBRs

Associations between demographic, clinical scores and PUT and CN SBRs in HC and pLOD were investigated. For the correlations, the lowest SBRs in the PUT and CN were used.

Correlations between demographics and clinical assessments with SBRs (not controlling for age and gender) are shown in Table 4-9 and Table 4-11.

Controlling for age and gender

After controlling for age and gender, there were no significant associations between the scores of any of the clinical assessments and lowest PUT SBRs in HC. An association between RBDSQ scores and lowest PUT SBRs missed significance (r=.50, p=.058), as shown in Table 4-10.

In pLOD, there were significant associations between lowest PUT SBRs and PD screening questionnaires scores (r=.55, p=.048) and scores of the motor experiences (M-EDL) subscale of the MDS UPDRS (r=.64, p=.018) as shown in Table 4-12.

These same scales were also significantly associated with CN SBRs; PD screening questionnaire (r=.62, p=.023), M-EDL (r=.64, p=.017).

Medication use

When controlling for the relevant medications, the results did not change (shown in Appendix D).

Table 4-9 Associations between clinical assessments and lowest putamen (PUT) striatal binding ratios (SBRs) by participant group

	HC (n=25*)	pLOD (n=29*)
Clinical Assessment	Correlation with PUT SBR *r, (p)*	
Age	-.27 (.176)	**-.45 (.015)**
Gender	**.42 (.036)**	**.52 (.004)**
Duration of depression/anxiety	-	-.25 (.191)
MoCA Total	.16 (.553)	.33 (.080)
FAB total	.21 (.322)	.17 (.411)
LARS	.12 (.555)	-.04 (.853)
DSM 5 SMD	.33 (.107)	.29 (.123)
HADS-D	.24 (.242)	.08 (.664)
HADS-A	.01 (.975)	.12 (.533)
PD screening questionnaire	.06 (.781)	.14 (.470)
MDS UPDRS nM-EDL	.23 (.275)	.11 (.561)
MDS UPDRS M-EDL	.07 (.747)	.19 (.333)
MDS UPDRS ME	-.25 (.227)	.09 (.646)
MDS UPDRS Total	.10 (.629)	.15 (.432)
BRAIN AT	.04 (.890)	-.02 (.946)
BRAIN KS	.08 (.755)	.27 (.296)
RBDSQ	**.46 (.019)**	-.16 (.399)
PDSS	-.18 (.390)	.01 (.960)
SCOPA- AUT	**.42 (.039)**	-.08 (.675)
UPSIT	**.41 (.044)**	**.53 (.003)**

Pearson's r and Spearman's rho correlations. Significant p values (<0.05) in **bold**
A significant p value at Bonferroni adjustment would be p<0.002, calculated by 0.05 / number of clinical assessments
*The FAB was not completed by three pLOD, the PDSS and MDS UPDRS ME not completed by one HC, the BRAIN test was not completed by 12 pLOD and seven HC.
Abbreviations: MoCA= Montreal Cognitive Assessment, FAB=Frontal Assessment Battery, MDS- UPDRS= Movement Disorder Society Unified Parkinson's Disease Rating scale; ML EDL= Motor Aspects of Experiences of Daily Living, nM-EDL= Non Motor Aspects of Experiences of Daily Living, UPDRS Motor examination= Unified Parkinson's Disease rating scale , LARS= Lille Apathy Rating Scale, BRAIN test= BRadykinesia Akinesia Incoordination Test; KS= kinesia score, AT; akinesia time; L/R=Left/Right hand, PD screening= Parkinson's Disease scale, HADS = Hospital Anxiety and Depression Scale; A=anxiety scale, D=depression, DSM 5 SMD=DSM 5 criteria Severity Measure of Depression, RBDSQ= REM Sleep Behaviour Disorder single questionnaire, RBDQ= The REM sleep Behaviour Disorder screening questionnaire, PDSS= Parkinson's disease Sleep Scale, UPSIT= University of Pennsylvania Smell Identification Test, SCOPA-AUT= Scales for Outcomes in Parkinson's disease- Autonomic.

Table 4-10 Associations between clinical assessments and lowest putamen (PUT) striatal binding ratios (SBRs) by participant group when controlling for age and gender

	HC (n=25*)	pLOD (n=29*)
Clinical Assessment	Correlation with PUT SBR *r, (p)*	
MoCA Total	.23 (.418)	.21 (.483)
FAB total	-.05 (.867)	.02 (.949)
LARS	.09 (.746)	-.12 (.691)
DSM 5 SMD	.03 (.928)	.19 (.532)
HADS-D	.32 (.126)	.06 (.742)
HADS-A	-.22 (.423)	-.01 (.987)
PD screening questionnaire	-.04 (.895)	**.55 (.048)**
MDS UPDRS nM-EDL	.05 (.870)	.13 (.660)
MDS UPDRS M-EDL	.03 (.927)	**.64 (.018)**
MDS UPDRS ME	-.16 (.581)	.41 (.161)
MDS UPDRS Total	-.00 (.989)	.43 (.148)
BRAIN AT	.17 (.535)	.41 (.168)
BRAIN KS	-.21 (.448)	.05 (.866)
RBDSQ	.50 (.058)	-.37 (.214)
PDSS	.09 (.736)	-.23 (.457)
SCOPA- AUT	.26 (.349)	-.10 (.740)
UPSIT	-.06 (.830)	.44 (.130)

Partial correlations controlling for age and gender. Significant p values ($p<0.05$) in **bold**

A significant p value at Bonferroni adjustment would be $p<0.002$, calculated by 0.05 / number of clinical assessments

*The FAB was not completed by three pLOD, the PDSS and MDS UPDRS ME not completed by one HC, the BRAIN test was not completed by 12 pLOD and seven HC.

Abbreviations: MoCA= Montreal Cognitive Assessment, FAB=Frontal Assessment Battery, MDS- UPDRS= Movement Disorder Society Unified Parkinson's Disease Rating scale; ML EDL= Motor Aspects of Experiences of Daily Living, nM-EDL= Non Motor Aspects of Experiences of Daily Living, UPDRS Motor examination= Unified Parkinson's Disease rating scale , LARS= Lille Apathy Rating Scale, BRAIN test= BRadykinesia Akinesia Incoordination Test; KS= kinesia score, AT; akinesia time; L/R=Left/Right hand, PD screening= Parkinson's Disease scale, HADS = Hospital Anxiety and Depression Scale; A=anxiety scale, D=depression, DSM 5 SMD=DSM 5 criteria Severity Measure of Depression, RBDSQ= REM Sleep Behaviour Disorder single questionnaire, RBDQ= The REM sleep Behaviour Disorder screening questionnaire, PDSS= Parkinson's disease Sleep Scale, UPSIT= University of Pennsylvania Smell Identification Test, SCOPA-AUT= Scales for Outcomes in Parkinson's disease- Autonomic.

Table 4-11 Associations between clinical assessments and lowest caudate nuclei (CN) striatal binding ratios (SBRs) by participant group

	HC (n=25*)	pLOD (n=29*)
Clinical Assessment	Correlation with CN SBR *r,(p)*	
Age	-.04 (.863)	**-.45 (.015)**
Gender	1.24 (.255)	**.57 (.001)**
Duration of depression/anxiety	-	-.19 (.321)
MoCA Total	.04 (.869)	**.52 (.004)**
FAB total	.02 (.914)	.28 (.159)
LARS	-.06 (.776)	.02 (.935)
DSM 5 SMD	.32 (.120)	.25 (.186)
HADS-D	.09 (.664)	.01 (.926)
HADS-A	-.13 (.547)	.20 (.292)
PD screening questionnaire	.16 (.457)	.02 (.916)
MDS UPDRS nM-EDL	.29 (.154)	.17 (.372)
MDS UPDRS M-EDL	.34 (.101)	.20 (.292)
MDS UPDRS ME	-.22 (.283)	-.02 (.934)
MDS UPDRS Total	.24 (.241)	.18 (.365)
BRAIN AT L/R hand	.06 (.806)	-.16 (552)
BRAIN KS L/R hand	-.06 (.802)	.36(.159)
RBDSQ	.25 (.232)	-.04(.839)
PDSS	-.22 (.308)	-.12(.540)
SCOPA- AUT	**.45 (.023)**	-.14(.462)
UPSIT	.04 (.835)	**.59(.001)**

Pearson's r and Spearman's rho correlations. Significant p values ($p<0.05$) in **bold**
A significant p value at Bonferroni adjustment would be $p<0.002$, calculated by 0.05 / number of clinical assessments
*The FAB was not completed by three pLOD, the PDSS and MDS UPDRS ME not completed by one HC, the BRAIN test was not completed by 12 pLOD and seven HC.
Abbreviations: MoCA= Montreal Cognitive Assessment, FAB=Frontal Assessment Battery, MDS- UPDRS= Movement Disorder Society Unified Parkinson's Disease Rating scale; ML EDL= Motor Aspects of Experiences of Daily Living, nM-EDL= Non Motor Aspects of Experiences of Daily Living, UPDRS Motor examination= Unified Parkinson's Disease rating scale , LARS= Lille Apathy Rating Scale, BRAIN test= BRadykinesia Akinesia Incoordination Test; KS= kinesia score, AT; akinesia time, L/R=Left/Right hand ,PD screening= Parkinson's Disease scale, HADS = Hospital Anxiety and Depression Scale; A=anxiety, D=depression, DSM 5 SMD=DSM 5 criteria Severity Measure of Depression, RBDSQ= REM Sleep Behaviour Disorder single questionnaire, RBDQ= The REM sleep Behaviour Disorder screening questionnaire, PDSS= Parkinson's disease Sleep Scale, UPSIT= University of Pennsylvania Smell Identification Test, SCOPA-AUT= Scales for Outcomes in Parkinson's disease- Autonomic.

Table 4-12 Associations between clinical assessments and lowest caudate nuclei (CN) striatal binding ratios (SBRs) by participant group when controlling for age and gender

	HC (n=25*)	pLOD (n=29*)
Clinical Assessment	Correlation with CN SBR *r,(p)*	
MoCA Total	1.68 (.549)	.46 (.118)
FAB total	-.04 (.882)	.04 (.905)
LARS	-.02 (.949)	.02 (.955)
DSM 5 SMD	.19 (.487)	.27 (.374)
HADS-D	.10 (.637)	-.01 (.945)
HADS-A	-.18 (.517)	.17 (.571)
PD screening questionnaire	.24 (.396)	**.62 (.023)**
MDS UPDRS nM-EDL	.21 (.453)	.22 (.481)
MDS UPDRS M-EDL	.29 (.300)	.**64 (.017)**
MDS UPDRS ME	-.23 (.409)	.14 (.160)
MDS UPDRS Total	.14 (.607)	.47 (.102)
BRAIN AT L/R hand	.02 (.955)	.18 (.559)
BRAIN KS L/R hand	-.11 (.700)	.18 (.558)
RBDSQ	.41 (.129)	-.26 (.390)
PDSS	-.19 (.499)	-.44 (.135)
SCOPA- AUT	.33 (.230)	.08 (.807)
UPSIT	-.29 (.304)	.35 (.241)

Partial correlations controlling for age and gender. Significant p values ($p<0.05$) in **bold**

A significant p value at Bonferroni adjustment would be $p<0.002$, calculated by 0.05 / number of clinical assessments

*The FAB was not completed by three pLOD, the PDSS and MDS UPDRS ME not completed by one HC, the BRAIN test was not completed by 12 pLOD and seven HC.

Abbreviations: MoCA= Montreal Cognitive Assessment, FAB=Frontal Assessment Battery, MDS- UPDRS= Movement Disorder Society Unified Parkinson's Disease Rating scale; ML EDL= Motor Aspects of Experiences of Daily Living, nM-EDL= Non Motor Aspects of Experiences of Daily Living, UPDRS Motor examination= Unified Parkinson's Disease rating scale , LARS= Lille Apathy Rating Scale, BRAIN test= BRadykinesia Akinesia Incoordination Test; KS= kinesia score, AT; akinesia time, L/R=Left/Right hand ,PD screening= Parkinson's Disease scale, HADS = Hospital Anxiety and Depression Scale; A=anxiety, D=depression DSM 5 SMD=DSM 5 criteria Severity Measure of Depression, RBDSQ= REM Sleep Behaviour Disorder single questionnaire, RBDQ= The REM sleep Behaviour Disorder screening questionnaire, PDSS= Parkinson's disease Sleep Scale, UPSIT= University of Pennsylvania Smell Identification Test, SCOPA-AUT= Scales for Outcomes in Parkinson's disease-Autonomic.

Clinical breakdown of participants with abnormal DAT SPECTs

Participants shown in Table 4-13 are those rated with AU on the DAT SPECTs using the visual ratings (VR) by HK (n=eight) and the SQA (n= 14), in order of the highest to lowest PUT SBRs found.

Having established associations of SBRs with clinical scores and demographic data in pLOD and HC, the clinical scores of those rated as having abnormal DAT SPECTs (Table 4-13), were explored in the participant breakdown, below. When describing the results the SQA (below), Formula 4.4 was used to specify the region (PUT and CN) and side (L or R) of abnormal uptake. In total three HC and 11 pLOD were rated with abnormal DAT SPECTs/uptake.

Participant breakdown:

Participant 1 was a 60-year-old male with a diagnosis of depression for two years with moderately severe symptoms (DSM 5 SMD) and moderate symptoms of depression using the HADS-D and mild symptoms of anxiety (HADS-A) on the day of the assessments. He scored just within the normal cut off score (23) for cognition on the MoCA with a score of 23. This participant scored with moderate microsmia on the UPSIT and within the 13th percentile when adjusted for age and gender. Using the SQA, he had abnormal uptake in the Land R **CN**.

Participants 2, 3 and 4 were rated with abnormal uptake when their SBRs were recalculated due to antidepressant use, indicative of low deviations from mean SBRs from age matched controls from DaTQUANT's control database. These patients were visually rated with **normal** uptake by HK.

Participant 2 was a 59-year-old female with a diagnosis of depression for two years with moderately severe symptoms (DSM 5 SMD) and mild symptoms of depression (HADS-D) and severe symptoms of anxiety (HADS-A) on the day of assessments. She further scored with symptoms of apathy (LARS) and scored below the cut off for RBD (using the RBDSQ). She did not score abnormally on the UPSIT (score within the 28th percentile) or any assessments measuring motor features, however reported waking up in the mornings with a tremor in their right hand. Using the SQA, abnormal uptake was found in the L **PUT** and **CN**. This participant had a third degree relative diagnosed with dementia.

Participant 3 was a 78-year-old female with a diagnosis of depression for a year with symptoms of mild depression (DSM 5 SMD) and no symptoms of anxiety (HADS-A) on the day of the assessments. She scored below the cut off for normal cognition using the MoCA and further scored with RBD (RBDSQ) and with mild microsmia (UPSIT) and within the 57th percentile when adjusted for age and gender. Using the SQA, this participant had abnormal uptake in the L and R **CN**. This participant had a second degree relative with PD.

Participant 4 was a 75-year-old male with a diagnosis of depression for ten years with symptoms of mild depression (DSM 5 SMD) and anxiety (HADS-A) on the day of assessments. He scored below the cut off for RBD using the RBDSQ normal cognition and just within the normal cut off score of 23 using the MoCA. Using the SQA, he had abnormal uptake in the L and R **PUT** and the R **CN**. This participant scored within normality on the UPSIT and with the 87th percentile when adjusted for age and gender.

Participant 5 and Participant 6 scored abnormally on the most clinical assessments in comparison to other participants rated with abnormal DAT SPECTs (n=14)**.**

Participant 5, a 63-year-old male had a diagnosis of depression for four years with symptoms of severe depression (DSM 5 SMD and HADS-D) and anxiety (HADS-A) on the day of the assessments. He scored below normal cut offs for cognition (MoCA), apathy (LARS), and RBD (both RBD questionnaires). Finally, this participant scored with severe microsmia (and within the 11th percentile when adjusted for age and gender) and also reported problems with taste (UPSIT questionnaire). Using the SQA, he had abnormal uptake in the L and R **PUT** and L and R **CN**.

Participant 6, a 79-year-old male had diagnosis of depression for two years, with symptoms of moderate depression (DSM 5 SMD), severe symptoms using the HADS-D and severe symptoms of anxiety (HADS-A) on the day of the assessments. This participant also scored below normal cut offs for cognition (MoCA) and apathy (LARS). Additionally, they also scored with MPS,scoring abnormally on facial, speech, posture, gait, global movement and finger tap items of the MDS UPDRS ME Scores on both the RBDSQ questionnaires were within normal range. However, on the UPSIT he scored with severe microsmia and in the 27th percentile when adjusted for age and gender. Using the SQA, this participant had abnormal uptake in the R **PUT** and **CN.**

Participant 7 was a 76-year-old male with a diagnosis of depression for two years and did not score with symptoms of depression (DSM 5 SMD) or anxiety (HADS-A) on the day of the assessments. However using the HADS-D scoring, this patient scored with severe symptoms of depression. He scored below the cut off for normality on cognition

(MoCA) and apathy (LARS). He scored with severe microsmia using the UPSIT and in the 23rd percentile when adjusted for age and gender. Using the SQA, this participant had abnormal uptake in the L and R **CN**.

Participant 8 was a 73-year-old male who was recruited as a HC. He also scored as anosmic using the UPSIT and within the 8th percentile when adjusted for age and gender. Using the SQA, uptake was abnormal in the L and R **PUT** and L **CN**. Visually, this participant was rated as **normal.**

Participant 9 was a 64-year-old male with a diagnosis of depression for ten years with mild symptoms of depression (DSM 5 SMD) and anxiety (HADS-A). This participant scored below the normal cut off for apathy (LARS). His scores indicated symptoms of RBD (scoring “yes” on the single RBD single questionnaire) and with moderate microsmia on the UPSIT and within the 15th percentile when adjusted for age and gender. Using the SQA, he had abnormal uptake in the L **PUT** and **CN.** This participant had a first degree relative diagnosed with dementia.

Participant 10 was recruited as a 74-year-old male who was recruited as a **HC** for the study. This participant scored with severe microsmia (UPSIT); in the 23rd percentile when adjusted for age and gender. Using the SQA, there was abnormal uptake in the L and R **PUT.** Visually, this participant was rated with an **equivocal** scan**.** Their UPDRS score (indicative of motor abnormalities was 0). This male had a first degree relative diagnosed with dementia.

Participant 11 was a 78-year-old male was a diagnosis of depression for a year, with no symptoms of depression (DSM 5 SMD) or anxiety (HADS-A) on the day of the assessments. He scored abnormally on both the RBD related questionnaires and with moderate microsmia on

the UPSIT and in the 32nd percentile when adjusted for age and gender. Using the SQA, he had abnormal uptake on the R **PUT** and **CN**.

Alongside Participant 5, Participants 12, 13 and 14 scored with abnormal uptake in both the L and R **PUT** and L and R **CN**, using the SQA.

Participant 12 was a 76-year-old male diagnosed with depression for six years with no symptoms of depression (DSM 5 SMD) or anxiety (HADS-A) on the day of assessments. This participant scored below the normal cut off for cognition (MoCA). He scored with anosmia on the UPSIT (in the 15th percentile when adjusted for age and gender).

Participant 13 was a male HC, aged 56 years of age and did not score abnormally on any of the clinical assessments. He scored as normosmic (one score away from mild microsmia) on the UPSIT and in the 56th percentile when adjusted for age and gender. This participant had a second degree relative with PD.

Participant 14 was a 77-year-old male, diagnosed with depression for two years, with no symptoms of depression (DSM 5 SMD) and mild symptoms of anxiety (HADS-A) on the day of the assessments This participant scored with MPS based on the following items of the MDS UPDRS ME; rigidity, finger tapping, pronation supination and posture. He also scored abnormally on both RBD questionnaires and as anosmic using the UPSIT; in the 10th percentile when adjusted for age and gender.

Group clinical assessment scores

Having provided a clinical breakdown by participant, the figures explained below provide a visual representation of **group** clinical assessment scores in those with visually and semi quantitatively abnormal DAT SPECTs (Table 4-13).

Figure 4-8 demonstrates how many participants with AU (Table 4-13) scored abnormally on the clinical assessments. The majority of pLOD also scored abnormally on symptoms of depression (63.6%; DSM 5 SMD), symptoms of anxiety (63.6%; HADS-A) with 54.5% of pLOD scoring abnormally on the RBDSQ in comparison to 0 of HC.

Eight two per cent of pLOD and 33% of HC scored with either RBD or definite hyposmia (Figure 4-9). Definite hyposmia was defined as participants with UPSIT score ≤ the 15th percentile of norms. Twenty seven per cent of pLOD and no HC scored abnormally on either of the RBD questionnaires and the UPSIT for definite hyposmia (Figure 4-10).

Figure 11 displays the breakdown of UPSIT ratings using the cut offs explained in Table 4-1 with both HC and pLOD rated with scores from normal to anosmic.

Table 4-13 Abnormal clinical scores in participants rated with an abnormal DAT SPECT by the visual ratings and SQA

Participants with abnormal SBRs (in order of highest- lowest PUT SBRs)

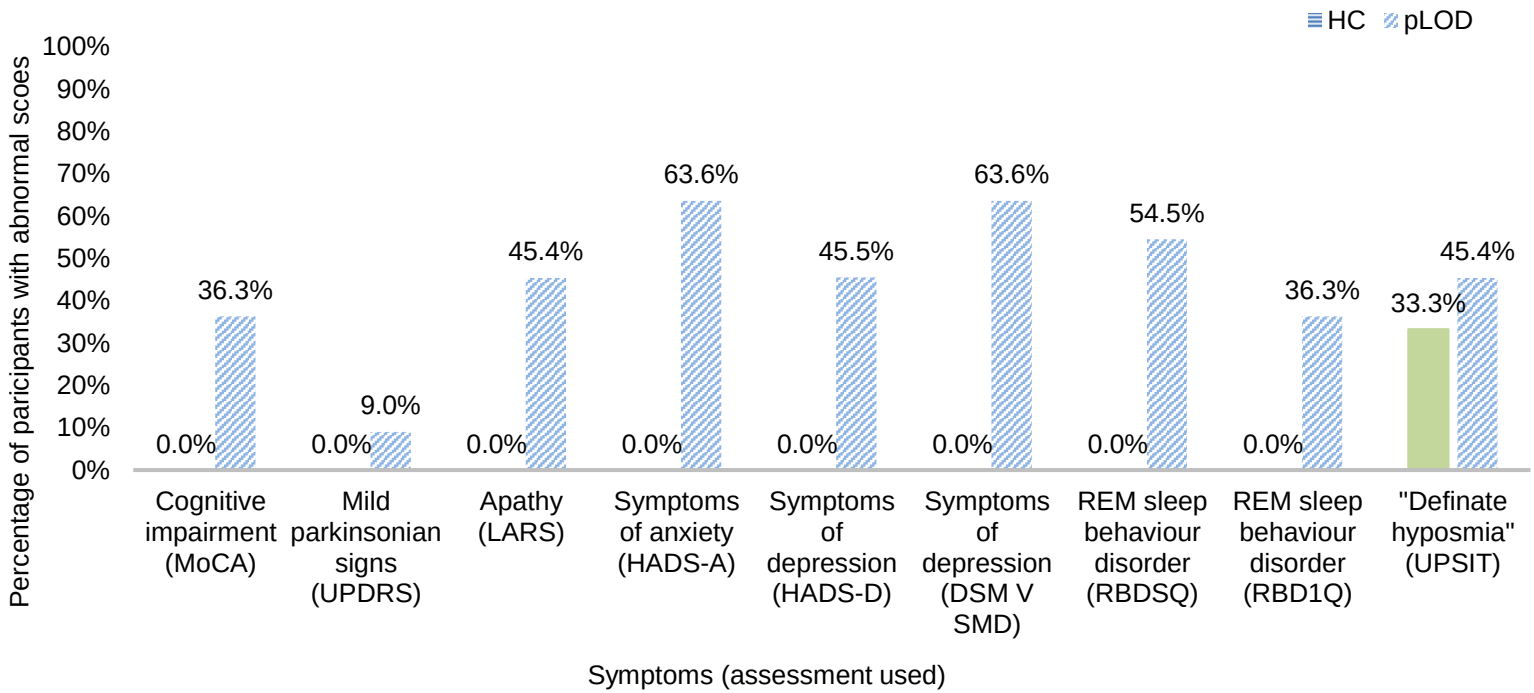
Participant group	1 year follow-up	Age (years)	Gender: Male	MoCA	FAB	MPS	LARS	HADS-A	HADS-D	DSM 5 SMD	*LOD Duration (years)*	Antidepressant use	RBDSQ	RBD1Q	UPSIT general adult rating	UPSIT age &gender percentile	DAT SPECT SQA rating	DAT SPECT visual rating
1		60									2				SM	13th	C	
2		59									2					28th	P/C	
3		78									1				MiM	57th	C	
4		75									10					57th	P/C	
5		63			X						4				SM	11th	P/C	
6		79									2				SM	27th	P/C	
7		76			X						2				SM	23rd	C	
8		73									-				A	8th	P/C	
9		64									10				MM	15th	P/C	
10		74									-				SM	23rd	P	
11		78									1				MM	32nd	P/C	
12		76									6				A	15th	P/C	
13		56									-					56th	P/C	
14		77									2				A	10th	P/C	

Key

X	Missing data
P/C	PUT/CN
E	Equivocal
	Healthy control
	Patient with LOD
	Normal
	Yes
	Abnormal
UPSIT	
MiM	Mild microsmia
MM	Moderate microsmia
SM	Severe microsmia
A	Anosmia

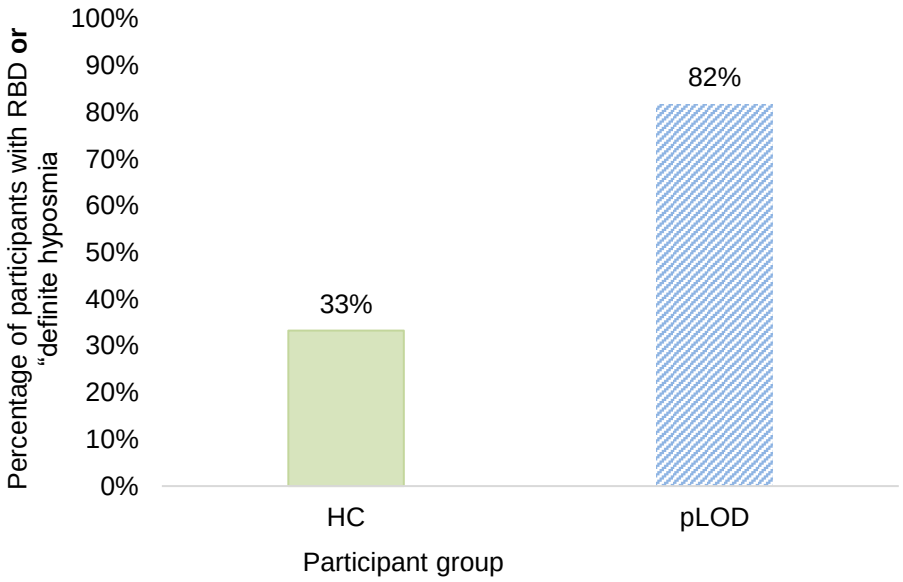
Cut offs in Table 3-1 and specific UPSIT cut offs in Table 4-1. Raw scores in Appendix G.
PUT= putamen, CN= caudate nuclei, SQA= semi quantitative analysis.

Figure 4-8 Participants with abnormal DAT SPECTs visually and using the SQA that had clinical scores past normal cut offs (as in Table 4-13) (HC n=3, pLOD n=11)



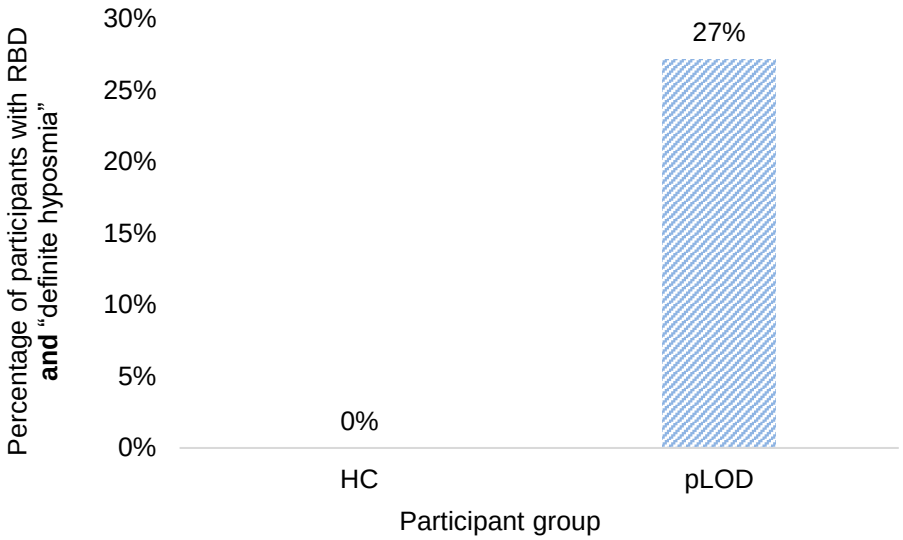
Abbreviations and cut off for abnormality HC=healthy controls, pLOD= patients with late onset depression, MoCA= Montreal Cognitive Assessment <23, FAB=Frontal Assessment Battery <12, MPS=Mild parkinsonian symptoms >6 , LARS= Lille Apathy Rating Scale -21, HADS-A = Hospital Anxiety and Depression Scale; anxiety scale ≥8 , DSM 5 SMD=DSM 5 criteria Severity Measure of Depression≥5, RBDSQ= REM Sleep Behaviour Disorder single questionnaire ≥5, RBD1Q= The REM sleep Behaviour Disorder screening questionnaire score of “yes”, UPSIT= University of Pennsylvania Smell Identification Test ≤15 percentile

Figure 4-9 Participants with abnormal DAT SPECTs visually and using the SQA that had abnormal scores on assessments measuring RBD **or** “definite hyposmia” (pLOD HC n=3, n=11)



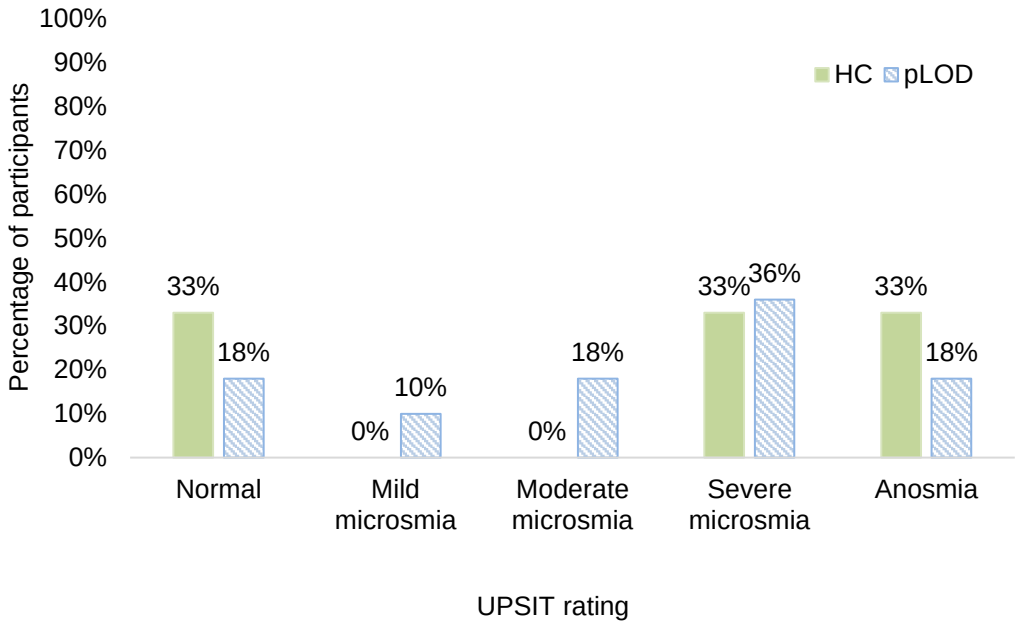
Abbreviations: RBD= REM sleep behavior disorder, HC= heathy control, pLOD= patient with late onset depression. Hyposmia was defined as participants ≤15 percentile, RBD cut off; RBDSQ ≥5, RBD1Q score of “yes”

Figure 4-10 Participants rated with abnormal DAT SPECTs visually and using the SQA that had abnormal scores on assessments measuring RBD **and** "definite hyposmia" (HC n=3, pLOD n=11)



Abbreviations: HC= heathy control, pLOD= patient with late onset depression, RBDSQ= REM Sleep Behaviour Disorder single questionnaire, UPSIT= University of Pennsylvania Smell Identification Test
Definite hyposmia was defined as participants ≤15 percentile, RBD cut off; RBDSQ ≥5, RBD1Q score of "yes"

Figure 4-11 UPSIT ratings (according to adult UPSIT rating criteria) in participants rated with abnormal DAT SPECTS (HC n=3, pLOD n=11)



UPSIT cut offs available in Table 4.1

Clinical scores in participants with **normal and abnormal** DAT SPECTs

Having explored clinical scores in HC and pLOD with AU, next the clinical scores of HC and pLOD with AU and NU were compared.

Clinical scores between HC and pLOD with abnormal DAT SPECTs

Clinical scores between only HC and pLOD with an abnormal SPECT (SQA) were compared. There were no significant group differences when comparing HC and pLOD with abnormal clinical scores (Table 4-14). However there were significant group differences in **mean scores** of the MDS UPDRS Total, HADS-D, HADS-A, DSM 5 SMD, PDSS and the SCOPA-AUT (Table 4-15).

Clinical scores within pLOD

Clinical scores between only pLOD with an abnormal SPECT and normal SPECT were analysed. When grouped by the VR, there were significant group differences within pLOD in the MoCA, FAB, MDS UPDRS, BRAIN KS and the UPSIT (Table 4-16).

There were also significant group differences in the pLOD with normal and abnormal DAT SPECTs on the MDS UPDRS ME. Specifically, there were significant differences in the following items of the scale; neck rigidity (p=.002) and lower extremity rigidity (p=.048) which was a mean score of the Land R lower extremity scores. A full breakdown of the means (SDs) and differences are in Appendix E.

When grouped by the SQA ratings, there were significant group differences within pLOD again in the **MoCA, UPSIT** and also the **SCOPA-AUT (**Table 4-17). There was also a significant difference in age between groups. Group differences remained significant in the MoCA, UPSIT and SCOPA-AUT even after controlling for age (ANCOVA).

Within HC

When comparing mean scores between HC with a normal and abnormal DAT SPECT rating (using the SQA), there were no significant group differences (Appendix F).

Table 4-14 Between groups: number of participants with abnormal clinical scores. Participants are grouped by abnormal DAT SPECTs by the semi quantitative analysis

	n (%)			
	HC (n=3)	pLOD (n=11*)		*p*
MoCA	0 (0)	4 (36.4)		.330
FAB	0 (0)	0 (0)		
MPS	0 (0)	1 (9.1)		.727
LARS	0 (0)	5 (45.5)		.231
HADS-A	0 (0)	0 (0)		
Mild	0 (0)	3 (27.2)		.154
Moderate	0 (0)	1 (9.1)		
Severe	0 (0)	2 (18.2)		
DSM 5 SMD total	0 (0)	7 (63.6)		.096
Mild	0 (0)	3 (27.2)		
Moderate	0 (0)	1 (9.1)		
Moderate- severe	0 (0)	2 (18.2)		
Severe	0 (0)	1 (9.1)		
RBDSQ	0 (0)	7 (63.6)		.096
RBD1Q	0 (0)	5 (45.5)		.231
UPSIT			15.00	.885
Normal	1 (33.3)	2 (18.2)		
Mild microsmia	0 (0)	1 (9.1)		
Moderate microsmia	0 (0)	2 (18.2)		
Severe microsmia	1 (33.3)	4 (36.3)		
Anosmia	1 (33.3)	2 (18.2)		
“Definite hyposmia”	1 (33.3)	5 (45.4)		.615

Chi square test, Fisher’s exact and Mann Whitney U test. Significant p values ($p<0.05$) in **bold.**

*Two pLOD excluded from the FAB.

Abbreviations and cut off: MoCA= Montreal Cognitive Assessment <23, FAB=Frontal Assessment Battery <12, MPS=Mild parkinsonian symptoms ≥6 , LARS= Lille Apathy Rating Scale -21, HADS-A = Hospital Anxiety and Depression Scale; anxiety scale ≥8 , DSM 5 SMD=DSM 5 criteria Severity Measure of Depression≥5, RBDSQ= REM Sleep Behaviour Disorder single questionnaire ≥5, RBDQ= The REM sleep Behaviour Disorder screening questionnaire, UPSIT= University of Pennsylvania Smell (cut offs listed in Table 4.1).

Table 4-15 Between groups: Mean clinical assessment scores by abnormal DAT SPECT SQA rating

	Abnormal DAT SPECT Mean (SD)			
Clinical assessments	HC (n=3)	pLOD (n=11*)		*p*
Age in years	68.3 (9.8)	71.4 (8.0)	-.57	.579
Gender: Male	3	9 (81.8)		.604
Education less than 12 years	1 (33.3)	5 (45.5)		.615
MoCA	28.3 (2.0)	23.3 (3.9)	2.06	.061
FAB	16.6 (1.5)	16.7 (1.5)	-.10	.917
MDS UPDRS Total	4.3 (4.9)	21.4 (12.5)	3.50	**.038**
MDS UPDRS ML-EDL	0.6 (1.1)	3.5 (3.2)	5.50	.088
MDS UPDRS nM- EDL	2.0 (1.0)	14.3 (9.0)	3.00	**.038**
MDS UPDRS ME	1.6 (2.8)	3.5 (2.3)	9.00	.291
LARS	-29.3 (4.5)	-22.5 (10.9)	-1.02	.325
BRAIN KS	59.5 (6.6)	47.2 (14.5)	5.50	.267
BRAIN AT	78.8 (29.2)	145.7 (99.9)	4.00	.183
PD Screening questionnaire	0.3 (0.5)	2.5 (2.1)	4.00	.060
HADS-D	1.0 (1.0)	8.2 (5.2)	0.50	**.005**
HADS-A	2.0 (2.0)	8.6 (4.8)	3.00	**.038**
DSM 5 SMD	0.6 (1.1)	8.3 (6.8)	2.00	**.022**
RBDSQ	1.6 (1.5)	5.4 (3.1)	5.00	.088
RBD1Q n (%)	0 (0)	5 (45.5)		.321
PDSS	130. 8 (15.6)	91.3 (24.2)	2.62	**.019**
UPSIT	24.0 (10.1)	25.8 (7.0)	-.36	.723
SCOPA-AUT	4.3 (3.2)	18.6 (9.5)	1.00	**.011**

Independent samples t test, Mann Whitney U test and Fisher's exact test. Significant p values ($p<0.05$) in **bold.**

*Four pLOD excluded from BRAIN and two from FAB

A significant p value at Bonferroni adjustment would be $p<0.002$, calculated by 0.05 / number of clinical assessments

Abbreviations: MoCA= Montreal Cognitive Assessment, FAB=Frontal Assessment Battery, MDS- UPDRS= Movement Disorder Society Unified Parkinson's Disease Rating scale; UPDRS Motor examination= Unified Parkinson's Disease rating scale , LARS= Lille Apathy Rating Scale, BRAIN test= BRadykinesia Akinesia Incoordination Test; KS= kinesia score, AT; akinesia time,PD screening= Parkinson's Disease scale, HADS = Hospital Anxiety and Depression Scale; A=anxiety, DSM 5 SMD=DSM 5 criteria Patient Health Questionnaire 9 scale, RBDSQ= REM Sleep Behaviour Disorder single questionnaire, RBDQ= The REM sleep Behaviour Disorder screening questionnaire, UPSIT= University of Pennsylvania Smell Identification Test

Table 4-16 Mean clinical scores by DAT SPECT visual ratings in patients with LOD (pLOD)

Clinical assessments	Abnormal (n=7*)	Normal (n=22*)		*p*
	Mean (SD)			
Age in years	70.7 (8.0)	66.7 (7.2)	-1.24	.226
MoCA	22.1 (3.5)	26.2 (3.1)	2.95	**.006**
FAB	16.2 (1.9)	17.4 (0.7)	2.47	**.021**
MDS UPDRS Total	22.1 (15.0)	19.8 (12.9)	69.00	.709
MDS UPDRS ML-EDL	4.1 (3.8)	3.4 (4.3)	60.50	.409
MDS UPDRS nM- EDL	13.4 (10.6)	14.1 (8.3)	70.50	.746
MDS UPDRS ME	4.6 (2.2)	2.2 (2.7)	28.50	**.011**
Rigidity: Neck	1.2 (0.9)	0.1 (0.3)	18.50	**.002**
Rigidity: Lower extremity	0.8 (0.8)	0.1 (0.2)	38.00	**.048**
LARS	-19.0 (11.9)	-23.2 (9.7)	54.50	.258
BRAIN KS	38.1 (4.0)	56.0 (9.0)	3.78	**.002**
BRAIN AT	187.6 (119.5)	105.9 (27.3)	15.00	.245
PD Screening questionnaire	2.9 (2.7)	1.7 (1.7)	60.00	.409
HADS-D	9.3 (6.1)	6.7 (4.5)	61.00	.438
HADS-A	8.9 (5.1)	10.3 (5.5)	66.00	.600
DSM 5 SMD	9.1 (7.9)	9.5 (6.4)	71.50	.784
RBDSQ	4.4 (3.3)	4.9 (3.4)	73.00	.862
RBD1Q n (%)	3 (42.8)	7 (31.8)		.459
PDSS	102.7 (20.6)	93.0 (26.2)	-.89	.379
UPSIT	21.7 (4.9)	33.3 (3.2)	7.29	**<.001**
SCOPA-AUT	19.3 (11.2)	13.0 (7.3)	51.00	.199

Independent samples t test, Mann Whitney U test and Fisher's exact test. Significant p values ($p<0.05$) in **bold.**

A significant p value at Bonferroni adjustment would be $p<0.002$, calculated by 0.05 / number of clinical assessments

Abbreviations: MoCA= Montreal Cognitive Assessment, FAB=Frontal Assessment Battery, MDS- UPDRS= Movement Disorder Society Unified Parkinson's Disease Rating scale; UPDRS Motor examination= Unified Parkinson's Disease rating scale , LARS= Lille Apathy Rating Scale, BRAIN test= BRadykinesia Akinesia Incoordination Test; KS= kinesia score, AT; akinesia time,PD screening= Parkinson's Disease scale, HADS = Hospital Anxiety and Depression Scale; A=anxiety, DSM 5 SMD=DSM 5 criteria Patient Health Questionnaire 9 scale, RBDSQ= REM Sleep Behaviour Disorder single questionnaire, RBDQ= The REM sleep Behaviour Disorder screening questionnaire, UPSIT= University of Pennsylvania Smell Identification Test

Table 4-17 Mean clinical scores by DAT SPECT SQA ratings in patients with LOD (pLOD)

Clinical assessments	Abnormal (n=11*)	Normal (n=18*)		*p*
	Mean (SD)			
Age in years	71.5 (8.1)	65.4 (6.2)	-2.27	**.031**
MoCA	23.4 (3.9)	26.3 (2.9)	2.34	**.026**
FAB	16.8 (1.6)	17.4 (0.7)	1.43	.165
MDS UPDRS Total	21.5 (12.6)	19.7 (13.8)	84.50	.521
MDS UPDRS ML-EDL	3.5 (3.2)	3.6 (4.8)	80.50	.412
MDS UPDRS nM- EDL	14.4 (9.1)	13.7 (8.7)	97.50	.947
MDS UPDRS ME	3.5 (2.4)	2.3 (2.9)	60.50	.084
LARS	-22.5 (10.9)	-21.9 (10.0)	.15	.881
BRAIN KS	47.2 (14.5)	55.1 (7.4)	1.47	.162
BRAIN AT	145.8 (99.9)	110.7 (28.3)	35.00	1.000
PD Screening questionnaire	2.5 (2.2)	1.7 (1.9)	72.00	.238
HADS-D	8.3 (5.2)	6.7 (4.8)	84.50	.521
HADS-A	8.6 (4.8)	10.7 (5.6)	77.00	.340
DSM 5 SMD	8.4 (6.9)	10.0 (6.6)	84.00	.521
RBDSQ	5.5 (3.1)	4.4 (3.4)	79.00	.387
RBD1Q n (%)	5 (45.4)	5 (27.7)		.283
PDSS	91.4 (24.3)	97.8 (25.8)	.66	.512
UPSIT	25.8 (7.1)	33.3 (3.3)	3.90	**<.001**
SCOPA-AUT	18.6 (9.6)	12.0 (7.1)	55.00	**.049**

Independent samples t test, Mann Whitney U test and Fisher's exact test Significant p values ($p<0.05$) in **bold.**

A significant p value at Bonferroni adjustment would be $p<0.002$, calculated by 0.05 / number of clinical assessments

Abbreviations: MoCA= Montreal Cognitive Assessment, FAB=Frontal Assessment Battery, MDS- UPDRS= Movement Disorder Society Unified Parkinson's Disease Rating scale; UPDRS Motor examination= Unified Parkinson's Disease rating scale , LARS= Lille Apathy Rating Scale, BRAIN test= BRadykinesia Akinesia Incoordination Test; KS= kinesia score, AT; akinesia time,PD screening= Parkinson's Disease scale, HADS = Hospital Anxiety and Depression Scale; A=anxiety, DSM 5 SMD=DSM 5 criteria Patient Health Questionnaire 9 scale, RBDSQ= REM Sleep Behaviour Disorder single questionnaire, RBDQ= The REM sleep Behaviour Disorder screening questionnaire, UPSIT= University of Pennsylvania Smell Identification Test

4.4.3.1 One year follow up in participants with abnormal DAT SPECTs

All participants were invited back for a one year follow up of the clinical assessments to assess any progression in motor and non-motor features.

Of the 14 participants (11 pLOD and three HC) that were visually and semi quantitatively rated with **abnormal DAT SPECTs**, **three pLOD** completed the one year follow up and a breakdown of their results is shown in Table 4-18 and detailed below.

Five participants (one HC and four pLOD) did not reply when contacted, two participants had moved away or were away for an extended period of time at the follow up. The remaining four participants were not yet eligible for a one year follow up at the time.

Participant 1, who was visually rated with abnormal DAT SPECT and with abnormal uptake in the CN using the SQA, improved their cognitive score (MoCA), symptoms of apathy (LARS) and symptoms of anxiety (HADS-A). This participant also improved in sleep symptoms associated with PD (PDSS). Scores on the UPSIT, FAB, SCOPA-AUT and PD screening questionnaire remained unchanged. However, this participant **progressed** in non-motor and motor features measured by the MDS UPDRS, in symptoms of depression (DSM 5 SMD) and also in RBD related questionnaires.

Participant 3, who was visually rated with normal DAT SPECT and with abnormal uptake in the CN using the SQA also improved in their cognitive scores (MoCA) and symptoms of apathy (LARS). Participant 3 also improved in motor features associated with PD (in the self-rated PD screening questionnaire) and in symptoms of depression (DSM 5 SMD) and anxiety (HADS-A). Scores on the FAB

remained unchanged. However, this participant **progressed** non-motor and motor features measured by the MDS UPDRS and BRAIN test, in autonomic dysfunction (SCOPA-AUT) and in the UPSIT, progressing from a score of mild microsmia to moderate microsmia. Whilst scores in the RBDSQ decreased (indicating lack of progression), this participant scored “yes” after a one year follow up on the RBD1Q (unlike the year before). Sleep symptoms associated with PD also progressed (PDSS).

Participant 14 who was visually rated with abnormal DAT SPECT and with abnormal uptake in the PUT and CN using the SQA improved in their cognitive scores (MoCA), FAB, symptoms of apathy (LARS), depression (DSM 5 SMD) and anxiety (HADS-A). There was also improvement in non-motor features as self-measured by the MDS NM-EDL in two items. However, this participant **progressed** in motor features associated with PD (in the self-rated PD screening questionnaire and in the MDS UPDRS ME. Non-motor features also progressed as measured by the MDS nM EDL and also included; RBD (RBDSQ), sleep symptoms associated with PD (PDSS), autonomic dysfunction and the UPSIT. The RDB1Q remained unchanged.

Table 4-18 Results of the one year follow up in participants rated with an abnormal DAT SPECT

Participant and type	Age (years)	M=Male, F=Female	MoCA	FAB	MDS M-EDL	MDS nM EDL	MDS UPDRS	MDS UPDRS Total	LARS	BRAIN KS	BRAIN AT	PD Screening questionnaire	HADS-A	HADS-D	DSM 5 SMD	RBDSQ	RBD1Q	SCOPA-AUT	PDSS	UPSIT rating	DAT SPECT SQA rating	DAT SPECT visual rating
1	60	M								X	X									MM	C	AB
3	78	F																		MM	C	N
14	77	M																		A	P/C	AB

Key	
X	Missing data
P/C	PUT/CN
AB	Abnormal DAT SPECT
N	Normal DAT SPECT
	Patient with LOD
	Worsened score
	Improved score
	No change
UPSIT	
MiM	Mild microsomia
MM	Moderate microsomia
SM	Severe microsomia
A	Anosmia

4.4.4 Part Four: Comparison of methods:

4.4.5.1 Comparison of VR and SQA:

This section compares the DAT SPECT results of the visual ratings and the semi quantitative analysis (Table 4-19).

There was found to be good agreement between the ratings using Cohen's Kappa; $\kappa=0.664$ ($p<0.001$) when comparing normal (including equivocal) vs abnormal scans with agreement on ratings of 48 of 54 scans.

Scans rated as visually equivocal were classified as normal in four out of five cases and as abnormal in one case using the SQA. No scan that was rated as abnormal on visual rating was found to be classified as normal by the SQA. However, six scans rated as normal or equivocal visually were classified as abnormal by the age adjusted SQA (Table 4-19).

There were significant differences between participants with normal (including equivocal) and abnormal DAT SPECTS and visual rating in mean L and R PUT and CN SBRs ($p=<.001$). This was also the case for the mean SBRs of the DAT SPECTs using the SQA ratings ($p<.001$) (Table 4-20).

SBRs by type of VR

Table 4-21 provides a mean lowest PUT and CN SBRs based on the type of visual rating (normal, equivocal and all abnormal types) by HK (also shown visually in Figure 4-12 and Figure 4-13).

Mean lowest CN SBRs in those scans visually rated as abnormal type 1 type 2 (and with striatal loss on average tended to be below CN

SBRs for those scans rated as normal and also below DaTQUANT's control database mean SBRs. Mean lowest PUT SBRS in the visually rated abnormal scans; type 1 type 2 and striatal loss also tended to be below the mean lowest PUT SBRs in the scans rated as normal and the scans from DaTQUANT's control database.

The scans visually rated as equivocal (borderline abnormal), tended to have higher mean SBRs than all the abnormal types and similar to those rated as normal.

Figure 4-14 and Figure 4-15 visually demonstrate the SBRs in the L and R PUT and CN based on the visual ratings by HK.

Table 4-19 Comparison of visual ratings (VR) and semi quantitative ratings (SQA)

		HK Visual ratings			SQA total abnormal
		Normal	Equivocal	Abnormal	
SQA ratings based on PUT and CN SBRs	Normal	36	4	0	
	Equivocal	-	-	-	
	Abnormal	5	1	8	14
VR total abnormal				8	

SQA= semi quantitative analysis, PUT=putamen, CN=caudate nuclei

Table 4-20 Mean (SD) putamen (PUT) and caudate nuclei (CN) striatal binding ratios (SBRs) by type and method of rating

	Visual ratings			
Area	Normal	Abnormal		p
PUT (L)	2.46 (0.41)	1.80 (0.47)	4.44	**<.001**
PUT(R)	2.51 (0.43)	1.84 (0.48)	4.42	**<.001**
CN (L)	2.70 (0.39)	1.82 (0.48)	6.20	**<.001**
CN (R)	2.68 (0.42)	1.85 (0.22)	5.66	**<.001**
Lowest PUT	2.43 (0.41)	1.76 (0.47)	4.16	**<.001**
Lowest CN	2.62 (0.39)	1.76 (0.17)	6.07	**<.001**
	SQA ratings			
PUT (L)	2.55 (0.36)	1.82 (0.36)	6.52	**<.001**
PUT(R)	2.61 (0.37)	1.86 (0.37)	6.56	**<.001**
CN (L)	2.79 (0.32)	1.95 (0.22)	7.45	**<.001**
CN (R)	2.76 (0.38)	1.98 (0.25)	7.04	**<.001**
Lowest PUT	2.52 (0.35)	1.79 (0.37)	6.61	**<.001**
Lowest CN	2.70 (0.34)	1.89 (0.21)	8.27	**<.001**

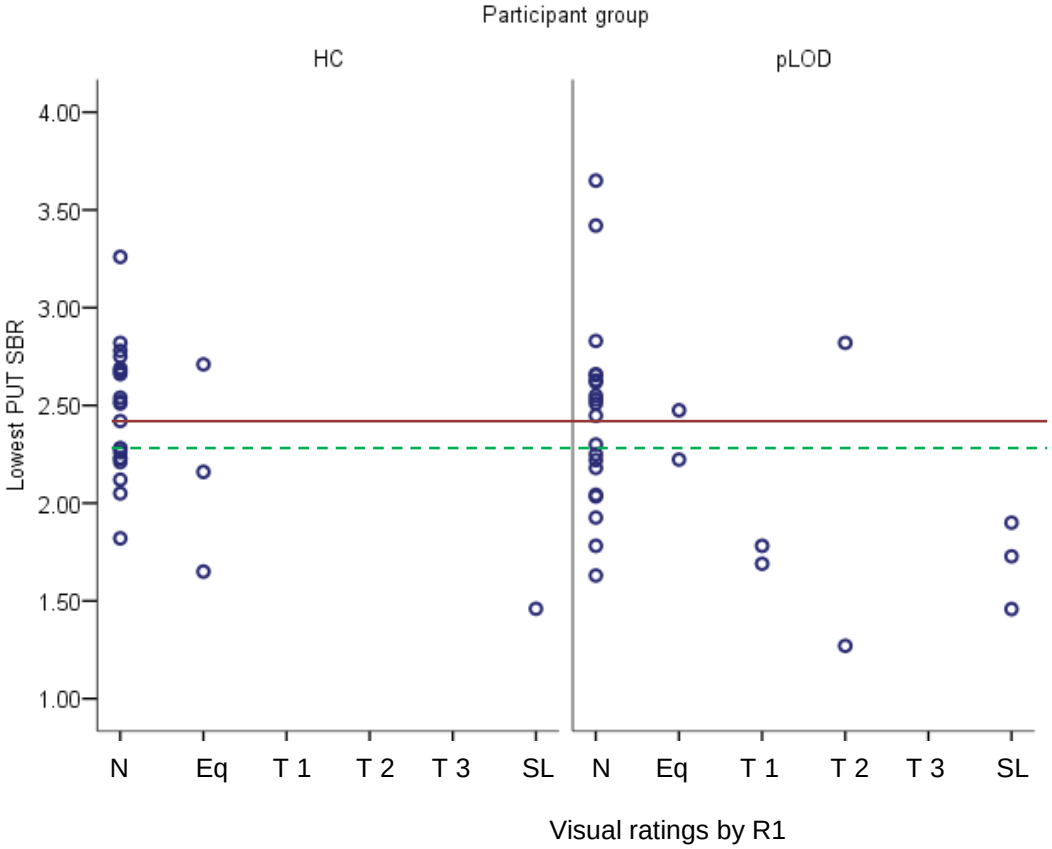
Independent samples t test. Significant p values (<0.05) in **bold**
PUT= putamen, CN= caudate nuclei, R= right side, L= left side, SQA=semi quantitative analysis

Table 4-21 Mean lowest putamen (PUT) and caudate nuclei (CN) striatal binding ratios (SBRs) by visual ratings

	Lowest CN SBR	Lowest PUT SBR
	Mean (SD) Median Range	
DaTQUANT normal controls		
Mean (SD)	2.61 (0.13)	2.32 (0.13)
Median	2.63	2.31
Range	0.55	0.50
LOD Study database n=54		
Normal (n=41)	2.61 (0.39)	2.45 (0.41)
	2.58	2.51
	1.70	2.02
Equivocal (n=5)	2.73 (0.31)	2.24 (0.39)
	2.59	2.22
	0.99	1.06
Abnormal Type 1 (n=3)	1.92 (0.049)	1.87 (0.07)
	1.92	1.74
	0.07	0.09
Abnormal Type 2 (n=2)	1.83 (0.12)	2.05 (0.11)
	1.83	2.05
	0.18	1.55
Abnormal Type 3 (n=0)	-	-
Striatal Loss (n=4)	1.65 (0.17)	1.64 (0.22)
	1.62	1.59
	0.37	0.44

SQA= semi quantitative analysis, PUT= putamen, CN= caudate nuclei, SD= standard deviation, n= number of participants, SBR= striatal binding ratios.
GE reporting guidelines: Normal=Two symmetric comma or crescent shaped focal regions of activity mirrored about the median plane, Abnormal type 1= Asymmetric activity, Abnormal type 2=Activity is absent in the putamen of both hemispheres and confined to the caudate nuclei, Abnormal type 3= Activity absent in the putamen of both hemispheres and greatly reduced in one or both caudate nuclei, Abnormal striatal loss= abnormal loss within striatal regions and an increase in background activity
Additional: Equivocal=Scans which are visually normal but with some uncertainty

Figure 4-12 Lowest putamen (PUT) striatal binding ratios (SBRs) in participants, based on their DAT SPECT visual rating (n=54)



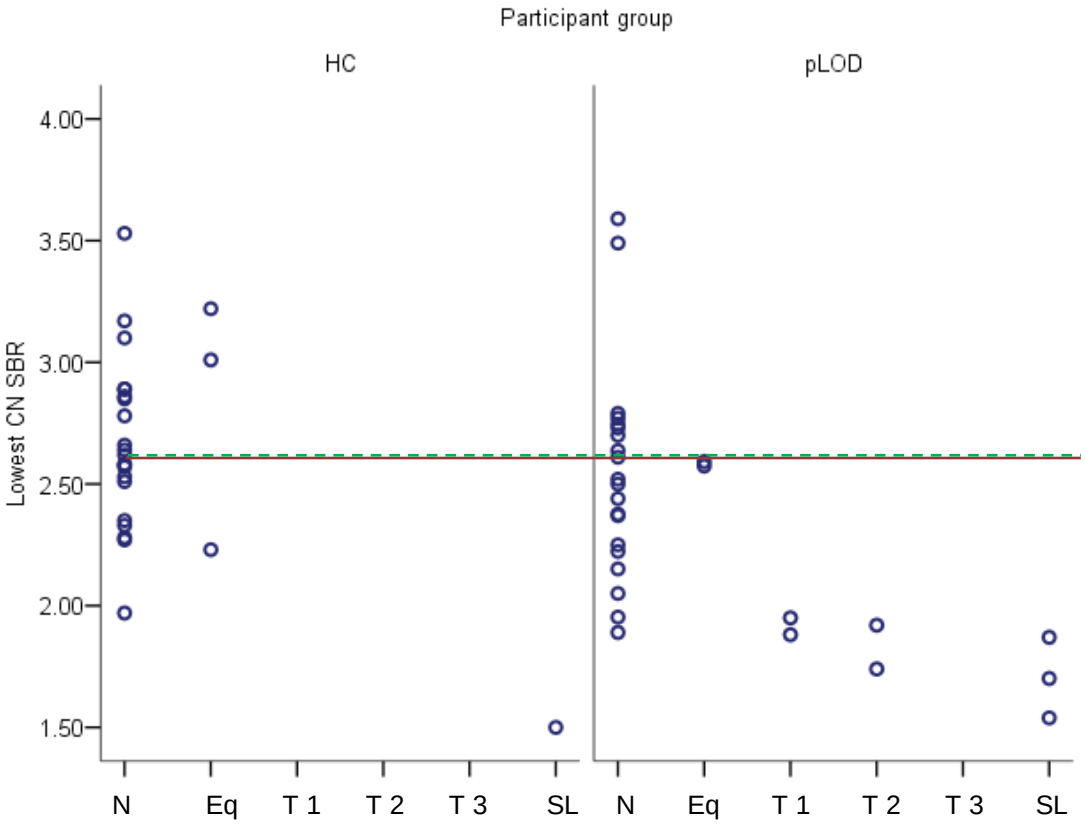
HC= healthy control, pLOD= patients with late onset depression

N- Normal, Eq- Equivocal, T1- abnormal type 2, T2- abnormal type 2, T3- abnormal type 3, SL- striatal loss

DaTQUANT control database mean lowest putamen SBR (2.32)

LOD study mean lowest putamen SBR (2.45) visually rated with normal scans

Figure 4-13 Lowest caudate nuclei (CN) striatal binding ratios (SBRs) in participants, based on their DAT SPECT visual rating (n=54)



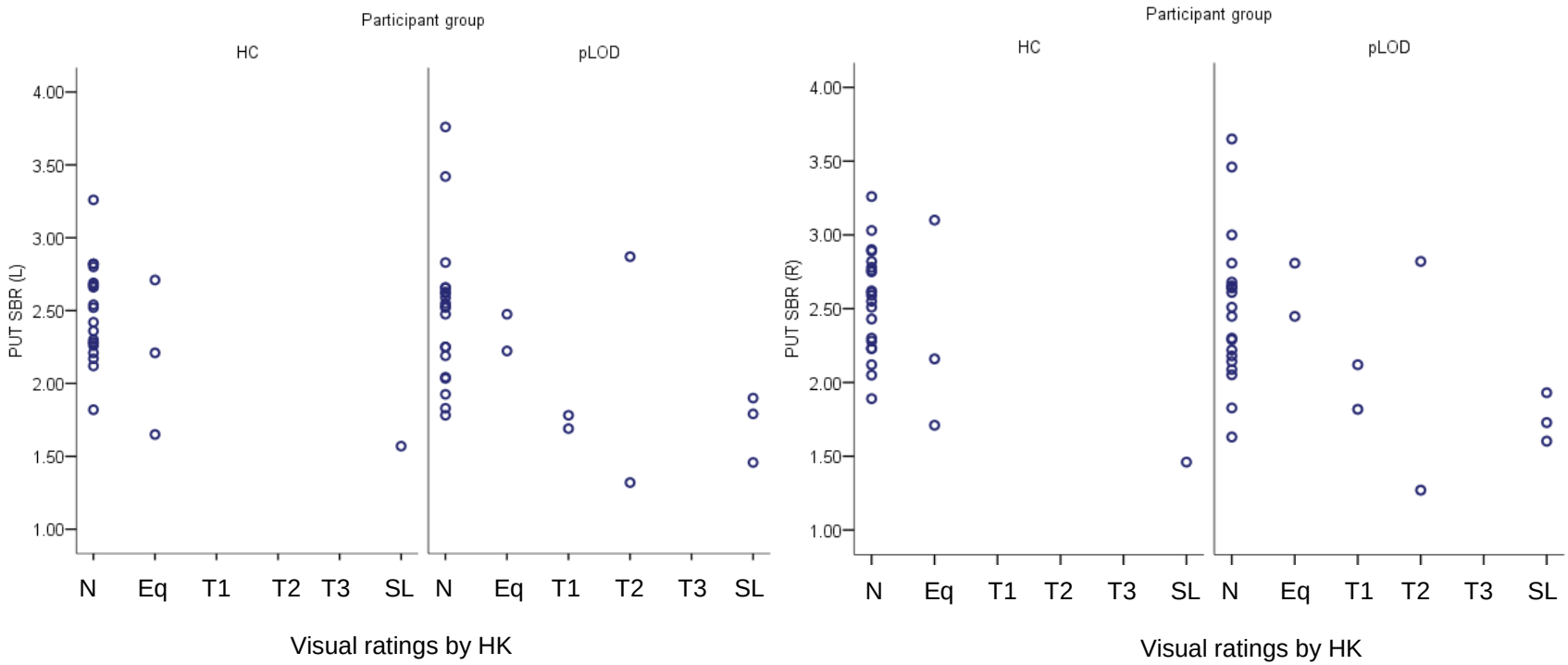
HC= healthy control, pLOD= patients with late onset depression

N- Normal, Eq- Equivocal, T1- abnormal type 2, T2- abnormal type 2, T3- abnormal type 3, SL- striatal loss

- - - - - DaTQUANT control database mean lowest caudate nuclei SBR (2.61)

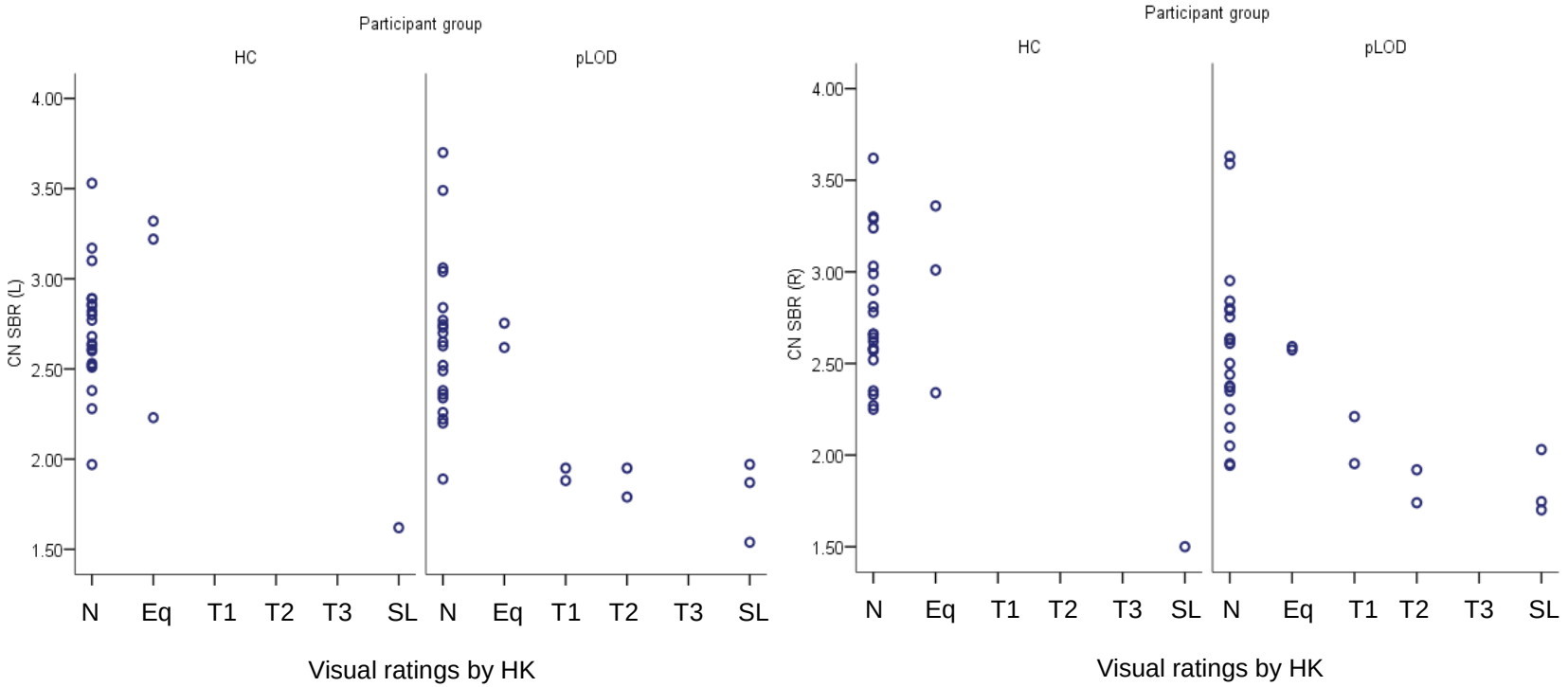
——— LOD study mean lowest caudate nuclei SBR (2.61) visually rated with normal scans

Figure 4-14 Left (L) and right (R) side putamen (PUT) SBRs by participant group, based on their DAT SPECT visual rating (n=54)



HC= healthy control, pLOD= patients with late onset depression, N- Normal, Eq- Equivocal, T1- abnormal type 2, T2- abnormal type 2, T3- abnormal type 3, SL- striatal loss

Figure 4-15 Left (L) and right (R) side caudate nuclei (CN) SBRs, by participant group, based on their DAT SPECT visual rating (n=54)



HC= healthy control, pLOD= patients with late onset depression, N- Normal, Eq- Equivocal, T1- abnormal type 2, T2- abnormal type 2, T3- abnormal type 3, SL- striatal loss

4.5 Discussion

In this current chapter, we found that in the overall group pLOD (n=29) had significantly reduced CN SBRs compared to HC (n=25).

Additionally, in the **overall pLOD vs HC group** comparisons;

- In comparison to HC, pLOD scored worse (when assessing group mean scores) on clinical assessments measuring non motor and motor symptoms (MDS UPDRS), RBD (RBDSQ), sleep and motor dysfunction associated with PD (PDSS, PD screening questionnaire) and symptoms of autonomic dysfunction (SCOPA-AUT).

When comparing DAT SPECT ratings:

- More pLOD than HC (seven pLOD vs one HC; p=0.042) were visually rated with an abnormal DAT SPECT. This was also found when the DAT SPECTs were rated using the SQA (11 pLOD vs three HC; p=.030)
- When comparing mean clinical scores between HC and pLOD rated with an abnormal SPECT using the SQA ratings; there were significant group differences in mean MDS UPDRS total, MDS UPDRS nM, HADS-D, HADS-A and DSM 5 SMD scores. There were also significant differences in the PDSS and SCOPA-AUT.

Within the pLOD

- Overall, pLOD with an **abnormal** DAT SPECT scored worse than pLOD with **normal** DAT SPECTs on group mean clinical scores associated with cognition (MoCA), subtle motor

abnormalities (BRAIN KS; MDS UPDRS ME) and hyposmia (UPSIT).

1. Group differences in SBRs

To discuss the first finding, we have found that pLOD have significantly reduced lowest and specifically R CN SBRs in comparison to HCs. Significant group differences in L CN SBR also missed significance p=.069. There were no significant group differences PUT SBRs. Whilst there has not been research available specific to the role of DA in LOD, research has investigated the role of DA in depression in general. A reduction in DA in the CN has been associated depression with Bowden et al (1997)'s study reporting reduced DA turnover in the CN (and nucleus accumbens) of depressed suicides. To our knowledge only Sarchiapone et al. (2006) have used the [^{123}I] FP-CIT tracer to investigate DAT binding ratios in patients with depression. Sarchiapone et al., 2006 reported reduced L and R SBRs in the CN and also the PUT in patients with a mean age 41.1 (SD 11.9) with major depression and anhedonia. Whilst anhedonia and apathy has been reported to be present in pLOD (Rapp et al., 2005) the patients in the current study on average did not score past abnormal cut offs for symptoms of apathy.

Studies using SPECT but other tracers include Wu et al., 2011 who reported significantly lower 99mTc-TRODAT-11 radio signal in the striatum of patients with depression (not LOD) and patients with PD (although the decrease was greater in those with PD). Additional research assessing DAT binding in PD has reported reduced 99mTc-TRODAT-11 radio signal in patients with PD and depression in comparison to patients with just PD (Weintraub et al., 2005).

Decreased DAT binding can potentially be explained by a reduced number of DATs per neuron due to a downregulation secondary to lower dopamine concentrations and impairment of dopaminergic transmission (Sarchiapone et al., 2006, Meyer et al., 2001). This explanation is consistent with reports of a decrease in DAT density when dopamine is chronically depleted (Kilbourn et al., 1992, Gordon et al., 1996, Sarchiapone et al., 2006). It can be suggested that the reduced CN SBRs in the current study in pLOD could be attributed to a similar process of impairment in dopaminergic transmission and whereby DA uptake sites downregulate in line with the reduced DA available. This process was also reported by Zigmond et al. (1990)'s study using mice.

An alternative hypothesis for the decrease in binding could also be attributed to dopaminergic depletion (Sarchiapone et al., 2006) with the depletion of DA attributed to (LB and lewy neurites (LBN) which are misfolded α- synuclein inclusions (Braak et al., 2003). Whilst post mortem research has been able to validate the degeneration of dopaminergic cells and presence of LB in patients with PD (Braak et al., 2003) this has not yet been investigated within patients with depression or LOD.

Vascular pathology may provide an explanation for the dopaminergic dysfunction, however as described in the methods, participants with significant cerebrovascular disease were excluded from the study due to the possibility of an abnormal SPECT caused by vascular lesions rather than a dopaminergic disruption. One pLOD who was rated with significant cerebrovascular disease (found on the Chapter 5 "MRI in LOD"), remained included in the analysis as their DAT SPECT was rated as visually and semi quantitatively normal.

1.2 Group clinical scores

The current chapter also found that pLOD scored significantly worse than HC on scales measuring non-motor and motor symptoms (MDS UPDRS) including RBD (RBDSQ), sleep and motor dysfunction associated with PD (PDSS, PD screening questionnaire) and symptoms of autonomic dysfunction (SCOPA-AUT). Differences measuring cognition (MoCA) and apathy (LARS) missed significance (p=.062; p=.058).

As discussed in Chapter 3 “Clinical correlates of LOD” there is an overlap of the symptoms present in both LOD (and depression in general) and neurodegenerative diseases including motor and autonomic dysfunction, as found in the current study. Whilst sleep disruptions can also be present in depression (not specific to LOD), RBD is not an associated symptom of LOD.
With regards to the physiological basis and pathology of these NMS (and motor symptoms) within PD, the Braak stage hypothesis has been able to provide some explanation (Braak et al., 2003) through a sequence of DA loss and consequent appearance of symptoms. Within PD, NMS are thought to take place prior to clinical motor symptomology through the involvement of LB and LBN in extranigral regions and affecting related functions. Gradually these LB and LBN reach the SN and over time cause depletion of DA with an approximate 50% (Fearnley and Lees, 1991) loss of DA neurons results clinical motor symptoms seen in PD. Fearnley and Lees (1991) further reported that clinical (motor symptoms) of PD occur four to five years post DA neuron loss (Berendse and Ponsen, 2009) also reported by Marek et al. (2011) using SPECT imaging.

Associations between motor dysfunction and SBRs

Significant associations between PD screening scores, motor experiences (measured by the MDS UPDRS M-EDL subscale) and PUT and CN SBRs were found in only pLOD. The associations may explained by symptoms of psychomotor dysfunction already associated with LOD (Rapp et al., 2005) which (amongst impaired cognitive function) is associated with psychomotor slowed speech and decreased movement (Buyukdura et al., 2011). The neurochemical basis of psychomotor retardation in depression has been discussed by Buyukdura et al. (2011); Shah et al. (1997) reported an association between a decrease in DA binding (using the ^{123}I IBZM tracer which is a D2/3 ligand) and psychomotor retardation in the striatum of patients with depression using SPECT. However DA binding did not correlate with severity of depression (as also found in the current study). Additionally, Martinot et al. (2001) (Buyukdura et al., 2011) reported decreased dopamine functioning in the L CN in depressed patients with psychomotor retardation (not with LOD) in comparison to HC when using [^{18}F]fluorodopa. These studies may provide an explanation for the associations between motor symptoms and in particular CN SBRs in pLOD with the association between motor symptoms and CN higher ($p=0.017$) than the association between motor symptoms and the PUT ($p=0.048$). Whilst in PD, motor symptoms begin with the PUT and progress to the CN as the disease progresses, the results from the SQA of the following study are suggestive of a different pattern of uptake and highlight the role of the CN in pLOD. As mentioned earlier, psychomotor retardation has been reported in pLOD (when compared with EOD) as has the role of the CN in depression so these results are in line with previous research in depression. *However* at this stage this pattern of dopaminergic dysfunction is not in line with the more common progression seen in PD. Further follow up through the use of

clinical and perhaps a follow up DAT SPECT is required to ascertain the pattern of dopaminergic degeneration and its association with that commonly seen in PD.

The clinical motor symptoms of PD are associated with a decrease in SBRs in both the PUT and later the CN as the disease progresses (GE Healthcare., 2012). Vriend et al., 2014's research into depression in PD reported that PUT SBRs and CN SBRs were associated with specific symptoms. Vriend et al., 2014 reported lower DAT binding in the R CN associated with depressive symptoms in those with PD and that motor symptoms were associated with low DA signaling to the PUT. In some part Vriend et al., 2014 may provide an explanation for the findings from the current study as pLOD had a decrease in R CN SBRs. Despite no significant group differences (pLOD and HC) between PUT SBRs, the association between PUT SBRs and motor dysfunction in only those with pLOD is indicative of an association between motor symptomology and PUT SBRs. Unlike Vriend et al., 2014 we did not find any associations between depressive symptoms (PHQ9) scores and CN or PUT SBRs suggestive of a lack of association between depression severity and CN SBRs in the current study. There were also no significant correlations found between duration of symptoms of depression/anxiety and SBRs. In the current study depression duration and severity varied throughout the group and is perhaps indicative of the lack of association between PHQ9 scores and CN and PUT SBRs.

2. DAT SPECT ratings

Further analysis investigated whether pLOD were rated with an abnormal DAT SPECT. Seven pLOD were visually rated with an abnormal SPECT in comparison to one HC; a significant difference ($p=.042$). In pLOD, balanced striatal loss was the most common pattern

of loss observed (n=three), followed by type 1 and type 2 (both n=two). DAT SPECT's are not usually conducted in the assessment or diagnosis of depression or LOD. The most common use of DAT SPECT is assessing patterns of uptake associated with PD and DLB related disorders as approved by guidelines (Bajaj et al., 2013). Balanced striatal loss is more often seen in patients presenting with DLB (Walker et al., 2004). Type 1 and type 2 ratings were more indicative of the typical pattern of loss in line with symptoms and pathology of PD (Marek et al, 1996; Tissingh et al, 1998).

The SQA which was used as an additional rating tool, also found significantly more pLOD than HC with an abnormal SPECT. In particular the SQA showed that significantly more pLOD than HC were rated with abnormal uptake in the R CN and with differences in the L missing significance (p=.065) when using the side specific ratings. There were no significant differences in the number of pLOD and HC rated with abnormal uptake in the PUT. The ratings fall into line with the group results of the reduced CN SBRs found in pLOD in comparison to HC.

Despite the SQA rating more participants with an abnormal SPECT, agreement between the visual and SQA ratings was good (κ=0.664 (p<0.001). There were also significant (p<.001) differences in PUT and CN SBRs between participants visually rated with a normal and abnormal DAT SPECT. A possible explanation for the SQA picking up more abnormal DAT SPECTs is twofold; 1) The SQA was able to identify very subtle uptake and especially with an exploratory cut off of one SD 2) The abnormal patterns of uptake were not applicable to the visual rating guidelines specific to patterns of uptake associated with PD/ DLB. For example, more pLOD were rated with a decrease in

uptake in the CN only using the SQA, which visually may have been hard to identify. Clinically, SQA has been recommended to objectively assess uptake (Darcourt et al., 2010) and can be useful alongside VR especially in challenging cases. In the current study, the SQA rated six SPECTS visually rated as normal and equivocal as abnormal. This discrepancy was probably due to the lower cut off of one SD used to identify abnormal SPECTs. Additionally, this may also provide an explanation for the SQA highlighting more pLOD with abnormal uptake in the CN (when compared with DaTQUANT's control database) whereas the visual ratings indicate more pLOD with a decrease in the PUT (abnormal type 1 and 2). The one SD cut off may have possibly identified subtle deficits in the CN which may not be visible to the eye and/or fall in line with the standardised rating criteria of abnormal type 1,2, 3 and striatal loss. However the visual ratings were the primary analysis for the study and less likely to pick up normal variants as "abnormal".

2.1 Clinical symptoms in abnormal DAT SPECTs

2.1.1 HC and pLOD with abnormal DAT SPECTs

Forty five per cent of pLOD and 33.3% of HC scored with “definite hyposmia” or RBD (out of the 14 abnormal SPECTs). Additionally, 27% of pLOD and no HC with abnormal SPECTS scored with **both** “definite hyposmia” and RBD.

Clinical scores between only HC and pLOD with an abnormal SPECT (SQA) were compared. There were no significant group differences when comparing the number of participants scoring past abnormal cut off thresholds for the assessments. Although not statistically significant, there were suggestions of increased RBD symptoms in pLOD in comparison to HC when comparing mean RBDSQ scores (p=.088) and the number of participants with a score of five or over (an abnormal score) (p=.096).

There were significant group differences in mean scores of the depression and anxiety (as measured by the HADS-D, HADS-A, DSM 5 SMD). There were also significant group differences in autonomic and sleep dysfunction (SCOPA-AUT and PDSS) and also in non-motor and motor measures (as indicated by MDS UPDRS Total scores).

As discussed earlier, autonomic and sleep dysfunction and slowness of movement are already associated with depression in general and have also been reported in the current pLOD group and may explain the differences with HC again in this subgroup of participants with abnormal DAT SPECTs. RBD and hyposmia are not symptoms commonly associated with a diagnosis depression or LOD.

There is established research exploring the role of DA using SPECT (as one of the explanations for the neurochemical basis) in RBD and hyposmia. Specific to olfactory dysfunction, Ponsen et al., 2004 reported reduced DAT binding in patients with idiopathic hyposmia and regarding RBD, Eisensehr et al. (2000) reported reduced DAT binding in idiopathic RBD patients.

Additionally, associations between olfactory dysfunction, RBD and PD (a disorder characterized by dopaminergic dysfunction) have been reported. Berendse et al 2001 reported abnormal reductions in hyposmic relatives of PD patients with 2/25 relatives subsequently developing PD in comparison to none of the normosmic relatives and Iranzo et al. (2010) reported a neurodegenerative disorder (PD, DLB and MSA) in 6/17 patients with RBD and reduced DAT binding when using SPECT. DLB (also associated with reduced DAT binding in the striatum) can also manifest with cognitive problems, subtle motor deficits and REM sleep behavior disorder (Roselli et al., 2009); the same features present in those with abnormal DAT SPECTs with Boeve et al. (2001) reporting that a- synucleinopathies have RBD and hyposmia.

Research into the relatives of the PD patients may also help explain the one HC visually rated with an abnormal DAT SPECT in the current study. Participant 13, who scored within normality on all of the clinical assessments had a family history of neurodegenerative disease; with a second degree relative with a diagnosis of PD. This family history may help to explain the abnormal DAT SPECT in this clinically “normal” participant at this stage. This participant was rated with abnormal uptake in the PUT and CN using the SQA. A follow up may help ascertain development of any symptoms. In addition, of the extra two

HC rated with an abnormal DAT SPECT using the SQA, one HC also had a relative with a neurodegenerative disease.

It can be suggested that the results of the current study are supportive of the research reported above with participants with abnormal DAT SPECTs (the majority of which are pLOD) with symptoms of olfactory dysfunction and RBD; both features which have been associated with decreased DAT binding but also with neurodegenerative diseases.

2.1.2 The pLOD with normal and abnormal DAT SPECTs

Group differences in mean clinical scores between pLOD with normal SPECTs and abnormal SPECTs (using the visual and SQA) were found when investigating the following clinical features; cognition (MoCA), frontal lobe dysfunction (FAB), subtle motor abnormalities (MDS UPDRS subscale ME, BRAIN KS). All of these features have been associated with LOD but in this case found to be higher in pLOD with an abnormal SPECT Specifically, there were significant group differences in the following items of the MDS UPDRS ME scale; neck rigidity (p=.002) and lower extremity rigidity (p=.048). In pLOD, associations between symptoms of motor dysfunction and CN and PUT SBRs were not significant however seem specific to pLOD with an abnormal DAT SPECT. Further suggestion of motor dysfunction in pLOD with an abnormal DAT SPECT were supported by the significant differences in BRAIN KS scores.

Associations between a decrease in DA binding and psychomotor retardation have been reported in other research previously, as explained earlier (Buyukdura et al., 2011, Shah et al., 1997, Martinot et

al., 2001). These studies may provide an explanation for the motor symptoms specific to pLOD rated with an abnormal DAT SPECT.

Additionally, pLOD with an abnormal DAT SPECT had significantly lower group mean scores on the UPSIT, indicative of hyposmia in comparison to pLOD with a normal SPECT. Furthermore, when comparing **HC** with normal and abnormal SPECTs, there were no significant differences in UPSIT scores (p=.163). As mentioned before, hyposmia is not commonly associated with a diagnosis of LOD and between pLOD and HC overall, no significant group differences were observed in the current study and specific only to pLOD with an abnormal DAT SPECT. Again, these findings are supporting of previous research reporting decreasing binding in the striatum in patients with hyposmia (but not depression) (Ponsen et al., 2004) with the results of this chapter demonstrating hyposmia to be specific to pLOD with a dopaminergic deficit.

Adjusting for the use of relevant medications

The current study adjusted for antidepressant use with research reporting a ten percent increase in SBRs through the use of SSRIs and SNRIs (Booij and Kemp, 2008). However, even when *not* adjusting SBRs and analysing the SBRs of the group as a whole, there remained significant differences in the lowest and right CN SBRs and with significantly more pLOD rated with abnormal R CN SBRs (n=11) when investigating the side specific ratings (p=.011).

Additionally, when excluding participants on medications which may affect clinical scores associated with RBD, autonomic dysfunction and motor features, there remained significant group differences between pLOD and HC with pLOD with higher scores on the RBDSQ, MDS

UPDRS ME and the SCOPA-AUT. However differences on the RBD1Q did miss significance after excluding participants on the relevant medications.

Limitations

With regards to the UPSIT, the standardised adult cut offs for mild, moderate, severe hyposmia and anosmia are gender and not age specific and have been used as a guideline to demonstrate the different degree of hyposmia between participants rated with an abnormal and normal DAT SPECT. For example, using the visual ratings, the majority of participants with abnormal SPECTs scored within severe microsmia whilst the majority rated with normal SPECTs scored with mild microsmia. In the abnormal DAT SPECT group age and gender specific percentiles have been given to show where these participants lie when compared to a normal database of controls. Furthermore, regardless of categorisation of the degree of hyposmia, there were significant differences between mean UPSIT scores between normal and abnormal DAT SPECTs in pLOD and when using a age and gender adjusted percentile for abnormality, 45% of pLOD in comparison 33% of HC scored with “definite hyposmia”.

A larger sample size is suggested with comparisons between abnormal and normal DAT SPECTs very small. This is also suggested by the differences in RBDSQ scores between abnormal pLOD and HC DAT SPECT groups missing significance (p=.088).

Follow up data in the current study sample is small, with only three pLOD with an abnormal SPECT followed up. However of the three pLOD with abnormal SPECTs that were followed up, all three had worsened in MDS UPDRS ME scores. Follow up of participants both

clinically and using SPECT imaging needs to be undertaken to better understand the long term clinical relevance in these participants.

Whilst efforts were made to ensure that the two imaging sites used the same SPECT scanner, some technical issues can still arise. Both hospitals had previous experience with [^{123}I] FP-CIT SPECT imaging and undergone previous phantom studies. However, the hospitals were not cross calibrated using a phantom specifically for the study. To reduce any variance between SPECT images from both sites, all the images were reconstructed using DaTQUANT, by the same researcher (HK) with age matched controls provided on DaTQUANT with reference values obtained using the same technique. .

As mentioned earlier, an exploratory cut off of one SD was used to denote abnormal PUT and/or CN SBRs. This less stringent cut off was used for exploratory purposes, with the use of a higher (two SD) cut off commonly used in clinical cases. Taking this into account and to decrease the likelihood of including participants with normal SBRs into the abnormal SPECT group due to this low cut off, the primary analysis remains the visual analysis whereby eight participants were rated with abnormal uptake.

Nevertheless, this exploratory pilot study has provided further understanding of the clinical and neurochemical basis of LOD. We have observed reduced DAT binding in the CN in pLOD and with pLOD more likely to have abnormal patterns of DAT uptake usually associated with neurodegenerative disorders associated with a dopaminergic loss. However no significant associations between severity or duration of symptoms of depression and anxiety in pLOD with PUT and CN SBRs were found. Alongside frontal lobe dysfunction and subtle motor dysfunction, of particular note are symptoms of

hyposmia, not associated with LOD or depression and not found in the pLOD group as a whole but evident in pLOD with an abnormal SPECT with significantly lower mean UPSIT scores in comparison to pLOD with normal SPECTs. Long term follow up is needed to ascertain the development of these symptoms and whether they progress into clinical disorders; particularly with the suggestions of RBD with the clinical scores in the current study not a form of diagnosis and to assess symptomology associated with non-motor and motor features.

LOD can be regarded as a multisystem disorder with the involvement of a range of neurotransmitters including DA and extranigral structures. To assess additional pathology related to LOD, in the next chapter (Chapter 5 “MRI in LOD”) we investigate subcortical GM volumes and LL in those with LOD, through the use of MRI.

Chapter 5. **MRI in LOD**

5.1 MRI introduction

Research has reported associations between LOD and reduced volumes in regions including the HIPP, CN, PUT (Kempton et al., 2011) and the AMY (Egger et al., 2008). Other pathology includes an increase in ventricles (Dahabra et al., 1998, Hickie et al., 2005) and WML in comparison to those with EOD (Salloway et al., 1996, Hickie et al., 2005). The reported pathology has led studies to suggest that pLOD acquire AD or vascular dementia (Schweitzer et al., 2002); disorders which can also exhibit global, parietal and medial atrophy and WML with evidence of such pathology explored through the use of MRI (Pasquier et al., 1996, Scheltens et al., 1992, Wahlund L.O, 2001). Sequences which have frequently been used when observing the pathology of depression in later life or other later life diseases such as dementia include T1 weighted sequences which detect structural abnormalities such as atrophy, T2 fast field echo (FFE)/T2* useful for detecting micro bleeds and T2 weighted and flair attenuated aversion recovery (FLAIR) for observing WMH or WML (Disabato et al., 2014, Choi et al., 2017, Figiel et al., 1991, Wahlund et al., 2017).

5.2 Objectives

Objective 1: To compare visual assessments of global, parietal and medial temporal atrophy and the presence of WML in pLOD with HC. We hypothesise that pLOD in comparison to HC will present with greater atrophy and WML with reports of increased WML in pLOD in comparison to those with EOD (Salloway et al., 1996, Hickie et al., 2005). Atrophy in the brain is also widely reported in AD; another neurodegenerative disease. The reported pathology has led studies to suggest that pLOD have either underlying AD or vascular dementia (Schweitzer et al., 2002).

Objective 2: To compare the subcortical GM volumes in pLOD with HC. Regions include; 3rd and 4th ventricles and L and R; HIPP, AMY, CN and PUT. Additionally, to compare LL and global GM and global WM volumes. We hypothesise that pLOD with present with increased presence of LL in comparison to HC and greater ventricular volumes, as reported previously (Dahabra et al., 1998, Hickie et al., 2005). In addition we hypothesise that pLOD will present with decreased subcortical volumes in line with previous research reporting associations between LOD and reduced volumes in regions including the HIPP, CN, PUT (Kempton et al., 2011) and the AMY (Egger et al., 2008).

5.3 Methods

Patients

The same 54 participants included in Chapter 4 “DAT SPECT in LOD” were also invited to have an MRI. Out of these 54 participants, 48 participants also had the MRI, which included 20 HC and 28 pLOD.

Of these 48 participants, the majority of the MR scans were conducted at the Royal Free site (n=36), which used a Philips 1.5 T scanner whilst a Philips 3 T scanner was used at the Princess Alexandra Hospital, which was the secondary site (n=12). Participants attended the site which was most easily accessible to them.

Additional exclusion for the MRI were participants with the following; a pacemaker, artificial valve or stent, surgical clips in the head, cochlea implants, neuro stimulators or metal fragments in eyes. Participants with incidental findings which may affect the LL segmentation methods were excluded.

5.3.1 MRI methodology:

Image processing

The Imaging departments at both hospitals provided the raw data of the scans in a digital imaging and communications in medicine (DICOM) format. No image processing was required for the visual analysis of the scans. To prepare the scans for extraction of volumetric data, preprocessing and registration of the modalities was undertaken by Carole Sudre (UCL Institute of Neurology, Dementia Research Centre) in order for the LL to be obtained using the Bayesian Model Selection (BaMoS) framework (Sudre et al., 2015). Subcortical volumes were calculated by applying the Geodesic Information Flows

(GIF) framework (Cardoso et al., 2015). The volumes were provided in millimeter cube (mm^3).

The primary analysis for the MRI was the visual assessment. In addition, the scans were also analyzed semi quantitatively, by analysing total LL, volume of global WM and GM, and specific subcortical GM regions.

5.3.2.1 MRI Visual analysis: Rating scales

Visual assessments

The MRI scans were uploaded onto RadiAnt (https://www.radiantviewer.com), a DICOM viewing software which allowed the images of all the sequences to be viewed in axial, sagittal and coronal views.

The MRI scans were rated on a number of atrophy and wWML scales by two independent raters; HK (following training by SP; a consultant radiologist) and FK (a specialist registrar in clinical radiology) using the following website as guidance; http://www.radiologyassistant.nl/en/p43dbf6d16f98d/dementia-role-of-mri.html, 2012, which focuses on the use of MRI in the diagnosis of dementia and related neurodegenerative diseases.

Ratings for the following scales were given; Parietal atrophy (using the Koedam scale; (Koedam et al., 2011), global atrophy (using the GCA scale; (Pasquier et al., 1996) medial temporal atrophy (using the MTA scale; (Scheltens et al., 1992) and white matter lesions (using the Fazekas scale; (Wahlund L.O, 2001, Fazekas et al., 1987) as shown in Table 5-1 (adapted from http://www.radiologyassistant.nl/en/p43dbf6d16f98d/dementia-role-of-

mri.html, 2012). GCA, Koedam and Fazekas scores range from grade 0 (none or single) to grade 3 for severe. MTA scores range from 0 (none) to 4 (severe).

For additional analysis, the ratings of all the scales were also grouped in participants with a score of 0 or participants with a score of 1.

Visual assessment training

HK was trained by SP and agreement between the raters was measured on a sample of 6 participants. Agreement between HK and SP was good on the Fazekas κ =.788, $p<0.05$ and the GCA κ =.720, $p<0.05$. Agreement on the Koedam κ =.417, p=.270 was moderate, with disagreement on 2/6 scans.

Table 5-1 Rating scales used for visual assessment of the MRI scans

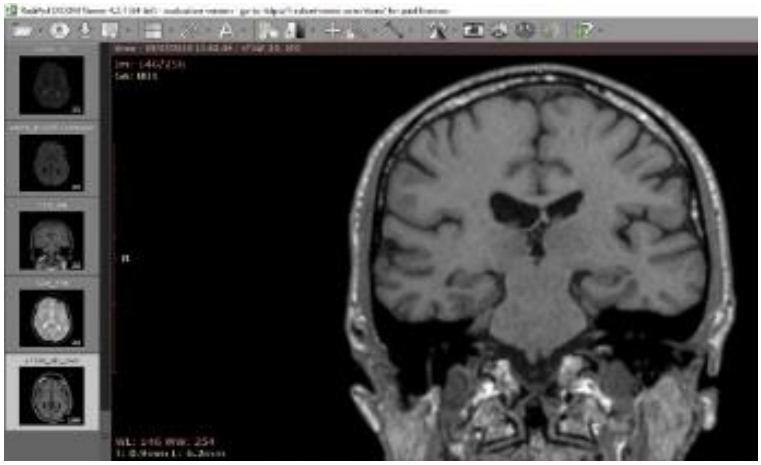
Grade	Global cortical atrophy (GCA scale)	Parietal atrophy (Koedam scale)	White matter lesions (Fazekas scale)	Medial temporal atrophy (MTA scale)
Grade 0	None	None	None or single WML lesion	No atrophy
Grade 1	Opening of sulci: mild atrophy	Mild parietal cortical atrophy	Multiple lesions (considered normal in elderly)	Only widening of choroid fissure
Grade 2	Volume loss of gyri (moderate atrophy)	Substantial parietal atrophy	Beginning confluency of lesions	Also widening of temporal horn of lateral ventricle
Grade 3	Severe “Knife blade” atrophy	“Knife blade” atrophy	Large confluent lesions	Moderate loss of hippocampal volume
Grade 4	N/A	N/A	N/A	Severe volume loss of hippocampus

WML= white matter lesion, N/A=not applicable

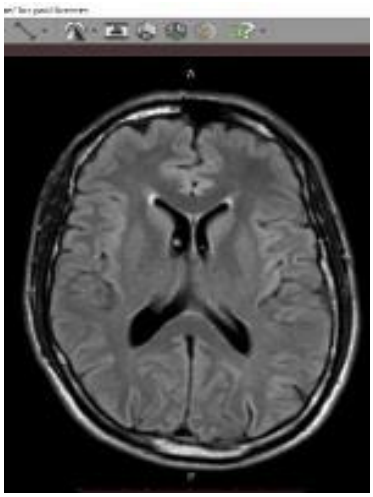
GCA scale; (Pasquier et al., 1996), Koedam scale; (Koedam et al., 2011), MTA scale; (Scheltens et al., 1992), Fazekas scale; (Wahlund L.O, 2001, Fazekas et al., 1987) (table adapted from http://www.radiologyassistant.nl/en/p43dbf6d16f98d/dementia-role-of-mri.html)

As recommended by http://www.radiologyassistant.nl/en/p43dbf6d16f98d/dementia-role-of-mri.html, 2012, the FLAIR sequence images were used to rate GCA scores, the FLAIR and T1 sequence images to rate Koedams, FLAIR or T2 sequence images to rate WML and the T1 sequences images to rate MTA (examples in Figure 5-1).

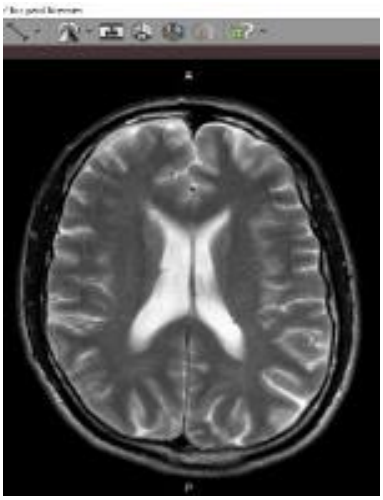
Figure 5-1 Example participant MR images (from the study), as shown on RadiAnt



Coronal 3D T1 slice



Axial FLAIR image



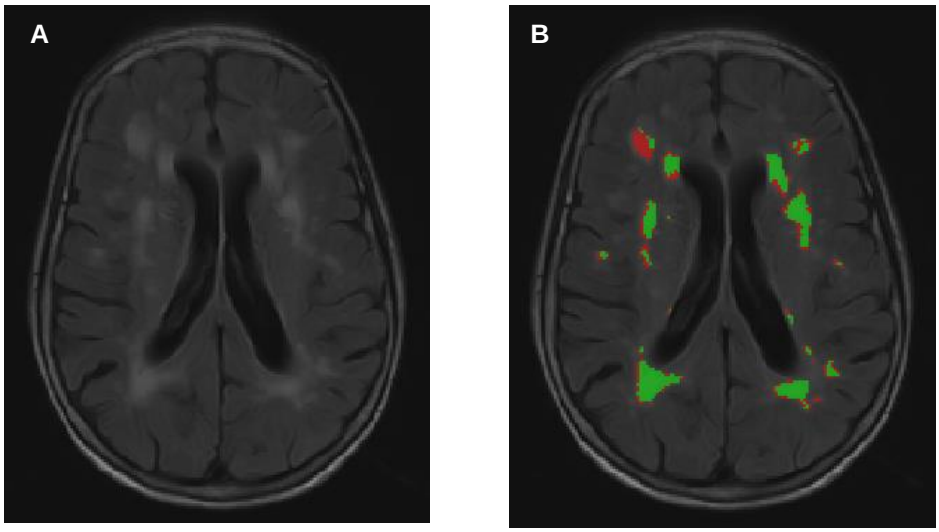
Axial T2 image

5.3.2.2. Regional grey matter volumes and white matter lesion load

LL

The T1 and FLAIR sequences were coregistered for optimal LL segmentation and the LL were then obtained using the using the BaMoS framework (Sudre et al., 2015). In short, BaMoS models jointly as an adaptive Gaussian Mixture Model in healthy appearing and unexpected observations. After convergence of the model, candidate lesion voxels are selected based on their distance to healthy appearing white matter. The resulting clusters are then classified as lesion or artifacts providing finally a probabilistic map that is integrated to obtain the final overall lesion volume. The BaMoS framework provides an automated reliable method for applying LL segmentation in comparison to manual segmentation which can suffer from inter and intra rater reliability (Sudre et al., 2015). Segmentation and scan quality were visually assessed by overlaying the obtained delineation onto the FLAIR image using ITKSnap [http://www.itksnap.org; Yushkevich et al. (2006) as shown in Figure 5-2. Due to an IF (as described in Chapter 2: Main study methodology), one HC was excluded from the LL analysis.

Figure 5-2 A: 3D T1 MRI scan registered with FLAIR and B: 3D T1 scan with segmentation overlay, with the green and red overlay demonstrating evidence of white matter lesion load
Both images are from the study and as displayed on ITK SNAP



Subcortical volumes

Subcortical volumes were calculated by applying the GIF framework, a method developed by (Cardoso et al., 2015) for brain parcellation. The GIF framework provides categorical labels for image parcellation by diffusing and mapping available examples (Cardoso et al., 2015). In this study, we examined the image parcellations for volumes of 3rd and 4th ventricles. In addition volumes of total R and L regions of the AMY and HIPP were calculated. Total HIPP volumes included the dentate gyrus, the ammonic subfields (CA1, CA2, CA3, and CA4), the prosubiculum, and the subiculum. The total volumes of the L and R CN and PUT were also calculated.

Global WM and GM volumes

Global WM and GM volumes were calculated by the sum of white and grey matter regions, respectively.

Total intracranial volumes (TIV) was calculated as sum of GM, WM and cerebrospinal fluid and was used as covariate when analysing group differences in subcortical GM volumes (i.e. HC vs p LOD).

Relevant clinical scores

Symptoms of depression using the DSM 5 SMD scale and **symptoms of anxiety** using the HADS scale were also measured to analyse associations with the subcortical GM volumes and LL. **Cardiovascular disease risk** (which may affect LL load) was calculated via the Q risk score calculator (https://qrisk.org/2017/, 2017), using the following participant details: age, sex, ethnicity, smoking status, diabetes status and evidence of; chronic kidney disease, atrial fibrillation, blood pressure treatment and Rheumatoid arthritis.

5.3.4 Statistical analysis

The main analysis was a comparison between pLOD and HC, using the ratings of the visual assessments and of the semi quantitative assessments. Histograms and Q-Q plots were used to visually assess the distribution of data. For comparison between categories, Chi-square tests or the Fisher's exact for small numbers. Group differences in participant demographics, relevant clinical assessment scores and SBRs from the DAT SPECT were analysed using independent sample t tests. Mann Whitney U tests were used for data not distributed normally.

The volumes and LL were log transformed for analysis and differences in LL, subcortical and global WM and GM volumes between groups were analysed using ANCOVA. The following variables were included as covariates; age, gender, TIV (to account for inter-individual variability) and the type of scanner used (as one site used a 1.5 T and another site a 3T scanner).

Scatterplots visually showed the dispersion of total LLs.

Weighted Cohen's Kappa was used to test interrater agreement between HK and FK's visual ratings of atrophy and WML in participants. Mann Whitney U tests were used to compare group mean scores of atrophy and WML. Histograms were used for a visual representation of the spread of ratings.

Associations between demographics, relevant clinical assessments and LL, and subcortical GM volumes in pLOD and HC were analysed by partial correlations (controlling for TIV and type of scanner used). To

assess for a linear relationship between relevant clinical assessments and subcortical GM volumes and LL in pLOD, linear regression analysis was run with TIV, the type of scanner used and age and gender included as covariates.

MRI and DAT SPECT

Associations between the volumes of the putamen and caudate nuclei and the SBR of the PUT and CN were analysed using partial correlations (controlling for age, TIV and type of scanner).

All analyses were conducted using IBM SPSS statistics 24.

5.4 MRI results

HC (n=20) had a mean age of 67.3 (8.4) with 35% of the group male and pLOD (n=28) were an average age of 67.1 (SD 7.5) years with 43% of the group male. Overall, pLOD scored with 16% risk of cardiovascular disease (CVD) in comparison to HC who had an average risk of 22% (Q risk score).

There were no significant differences ($p<0.05$) in age, gender and risk of CVD between pLOD and HC with MRIs (Table 5-2).

There were significant group differences in symptoms of depression, measured by the DSM 5 SMD scale ($p<.001$) and the HADS-D ($p<.001$) and symptoms of anxiety, measured by the HADS-A ($p<.001$). There were also significant group differences in the R ($p=.021$) and lowest ($p=.041$) CN SBRs.

Table 5-2 Mean group demographics, clinical assessment scores and DAT SPECT SBRs

	HC (n=20)	pLOD (n=28)		*p*
	Mean (SD)			
Age	67.3 (8.4)	67.1 (7.5)	427.5	.147
Male n (%)	7 (35)	12 (43)		.403
Q Risk score in %	22.3 (11.2)	16.2 (10.6)	180.0	.160
Clinical assessments				
DSM 5 SMD	1.32 (1.95)	9.38 (6.63)	66.00	**<.001**
HADS-D	7.03 (4.74)	1.25 (0.96)	44.00	**<.001**
HADS-A	2.68 (2.23)	2.68 (2.23)	73.00	**<.001**
DAT SPECT				
PUT (L) SBR	2.50 (0.39)	2.33 (0.55)	1.202	.236
PUT (R) SBR	2.56 (0.44)	2.37 (0.54)	1.279	.207
CN (L) SBR	2.73 (0.42)	2.48 (0.50)	1.787	.081
CN (R) SBR	2.77 (0.48)	2.43 (0.49)	2.381	**.021**
Lowest PUT SBR	2.47 (0.40)	2.30 (0.55)	1.179	.244
Lowest CN SBR	2.67 (0.45)	2.38 (0.49)	2.110	**.040**

Independent samples t test, Mann Whitney U, Fisher's exact. Significant p values ($p<0.05$) in **bold.**
DSM 5 SMD=DSM 5 criteria Severity Measure of Depression, HADS = Hospital Anxiety and Depression Scale; A=anxiety scale, D=depression, PUT= Putamen, CN= caudate nuclei, L= left side, R=right side, SBR= striatal binding ratio

5.4.1 Part One: MRI Visual analysis

Inter rater agreement between raters

Both HK and FK rated the MRI scans using the rating scales as described in Table 5-1 and their inter rater agreement was analysed (Table 5-3 to Table 5-6). There was moderate agreement using the weighted Cohen's kappa (k= .597, p<.001) between HK and FK, with overall agreement on the Fazekas ratings (measuring WML) in 35 out of 48 scans. There was also moderate agreement on the ratings of parietal atrophy, using the Koedem scale, (k= .578, p<.001) with agreement on 34 out of 48 scans and on ratings of global cortical atrophy (k= .576, p<.001) with agreement on 36 out of 48 scans. There was also moderate agreement on the ratings of medial temporal atrophy between HK and FK (k= .596, p<.001) with agreement on 36 out of 48 scans.

Table 5-3 Fazekas scores for white matter lesions by HK and FK

Fazekas		FK Visual ratings			
		0	1	2	3
HK visual ratings	0	6	1	0	0
	1	9	23	2	0
	2	0	1	5	0
	3	0	0	0	1

Table 5-4 Global cortical atrophy (GCA) scores by HK and FK

GCA		FK Visual ratings			
		0	1	2	3
HK visual ratings	0	5	5	0	-
	1	1	25	5	-
	2	0	1	6	-
	3	-	-	-	-

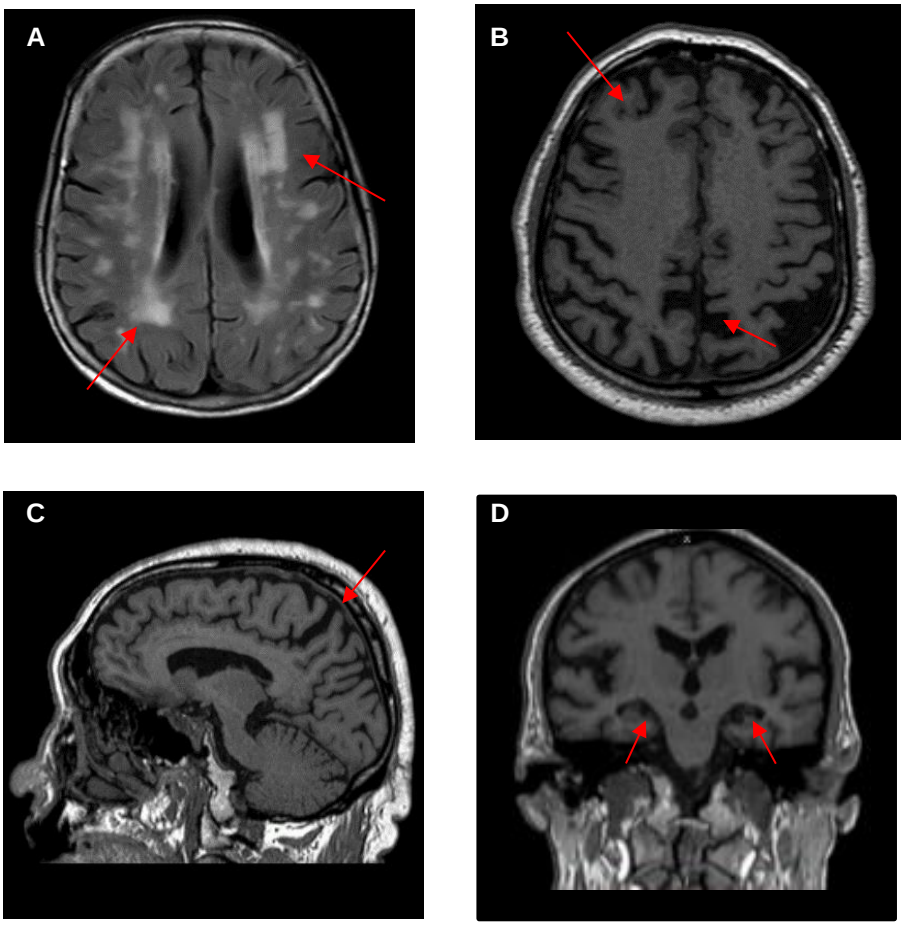
Table 5-5 Koedam scores for parietal atrophy by HK and FK

Koedam		FK Visual ratings			
		0	1	2	3
HK visual ratings	0	14	9	2	-
	1	1	14	2	-
	2	0	0	6	-
	3	-	-	-	-

Table 5-6 Medial temporal atrophy (MTA) by HK and FK

MTA		FK Visual ratings			
		0	1	2	3
HK visual ratings	0	28	8	0	0
	1	0	8	2	0
	2	0	0	0	2
	3	-	-	-	-

Figure 5-3 Examples of pathology found in participants



A: White matter lesions (rated using the Fazekas scale), **B:** Global cortical atrophy (rated using the GCA scale), **C:** Parietal atrophy (rated using the Koedam scale), **D:** Medial temporal atrophy (rated using the MTA scale). The red arrows demonstrate some (not all) examples of where abnormal pathology is present for each scale.

Table 5-7 and Table 5-8 display the ratings by HK and FK, by participant group.

1.1. Visual ratings by HK

There were no significant ($p<0.05$) differences between participant groups in mean ratings of atrophy (GCA, Koedam and MTA scale) or WML (Fazekas scale) in the visual ratings by HK;Table 5-9.

1.2 Visual ratings by FK

As with the ratings from HK, there were no significant ($p<0.05$) differences between participant groups in mean ratings of Koedam and the MTA scale or white matter lesions (Fazekas scale). However, there were significant differences in mean global atrophy ratings (GCA); $p=.014$, as shown in Table 5-10 with HC with a higher mean GCA score in comparison to pLOD.

Table 5-7 HK visual ratings in healthy controls (HC) and patients with LOD (pLOD) healthy controls (HC)

Scale	HC	pLOD
	n (%)	
Fazekas Score		
0	4 (20)	3 (11)
1	13 (65)	21 (75)
2	3 (15)	3 (11)
3	0 (0)	1 (3)
GCA Score		
0	3 (15)	7 (25)
1	12 (60)	19 (68)
2	5 (25)	2 (7)
3	0 (0)	0 (0)
Koedam Score		
0	12 (60)	13 (46)
1	5 (25)	12 (43)
2	3 (15)	3 (11)
3	0 (0)	0 (0)
MTA Score		
0	16 (80)	20 (71)
1	4 (20)	6 (21)
2	0 (0)	2 (7)
3	0 (0)	0 (0)
4	0 (0)	0 (0)

pLOD= patients with late onset depression, HC= healthy controls, GCA= global cortical atrophy, Fazekas= white matter lesions, Koedam= parietal atrophy, MTA= medial temporal atrophy

Table 5-8 FK visual ratings in healthy controls (HC) and patients with LOD (pLOD)

Scale	HC	pLOD
	n (%)	
Fazekas Score		
0	7 (35)	8 (29)
1	9 (45)	16 (57)
2	4 (20)	3 (11)
3	0 (0)	1 (3)
GCA Score		
0	1 (5)	5 (18)
1	11 (55)	20 (71)
2	8 (40)	3 (11)
3	0 (0)	0 (0)
Koedam Score		
0	5 (25)	10 (36)
1	9 (45)	14 (50)
2	6 (30)	4 (14)
3	0 (0)	0 (0)
MTA		
0	9 (45)	19 (68)
1	9 (45)	7 (25)
2	2 (10)	2 (7)
3	0 (0)	0 (0)
4	0 (0)	0 (0)

pLOD= patients with late onset depression, HC= healthy controls, GCA= global cortical atrophy, Fazekas= white matter lesions, Koedam= parietal atrophy, MTA= medial temporal atrophy

Table 5-9 Mean scores of visual ratings by HK

HK	HC (n=20)	pLOD (n=28)		*p*
	Mean (SD) Median			
Fazekas Score	0.95 (0.60) 1.00	1.07 (0.60) 1.00	258.0	.565
GCA Score	1.1 (0.64) 1.00	0.82 (0.55) 1.00	216.5	.117
Koedam Score	0.55 (0.76) 0.00	0.64 (0.69) 1.00	252.5	.524
MTA	0.20 (0.41) 0.00	0.36 (0.62) 0.00	252.0	.438

Mann Whitney U analysis

Table 5-10 Mean scores of visual ratings by FK

FK	HC (n=20)	pLOD (n=28)		*p*
	Mean (SD) Median			
Fazekas Score	0.85 (0.75) 1.00	0.89 (0.74) 1.00	274.0	.890
GCA Score	1.35 (0.59) 1.00	0.93 (0.54) 1.00	180.5	**.014**
Koedam Score	1.05 (0.76) 1.00	0.79 (0.69) 1.00	226.0	.221
MTA	0.65 (0.67) 1.00	0.46 (0.84) 0.00	220.0	.151

Mann Whitney U analysis. Significant ($p<0.05$) p values in **bold**

Dichotomising ratings of atrophy and white matter lesions

Group differences in ratings of 0 or 1 in all scales

The spread of visual ratings by HK and FK for each scale are shown in Figure 5-4-Figure 5-7, which also show that the majority of participants were rated with a score of 0 or a score of 1 in all the scales. Therefore, to assess further, differences between participants scoring only 0 or 1 were analysed. For example, when analsing the Fazkeas scores, only participants with none or single lesions (score of 0) or those with multiple lesions only (score of 1) were compared. A Fisher's exact test revealed no significant group differences in the Fazkeas ratings (p=.304). Similarly for GCA, Koedam and MTA scores, a Fisher's exact test revealed no significant group differences in GCA (p=.460) or the Koedam scale (p=.547) or the MTA (p=.189).

Medial temporal atrophy

FK additionally provided separate ratings for left and right medial temporal atrophy. No significant group differences were observed between mean left and right MTA scores (Table 5-11).

Table 5-11 Left and right MTA ratings by FK

FK				
Scale	HC (n=20)	pLOD (n=28)		*p*
	Mean (SD) Median			
MTA Left	0.30 (0.57) 0.00	0.32 (0.81) 0.00	-.101	.920
MTA Right	0.60 (0.68) 0.50	0.39 (0.73) 0.00	.990	.327

Mann Whitney U test between groups

Figure 5-4 HK and FK Fazekas ratings of white matter lesions, by participant group (patients with late onset depression; pLOD n=28 and healthy controls; HC n=20)

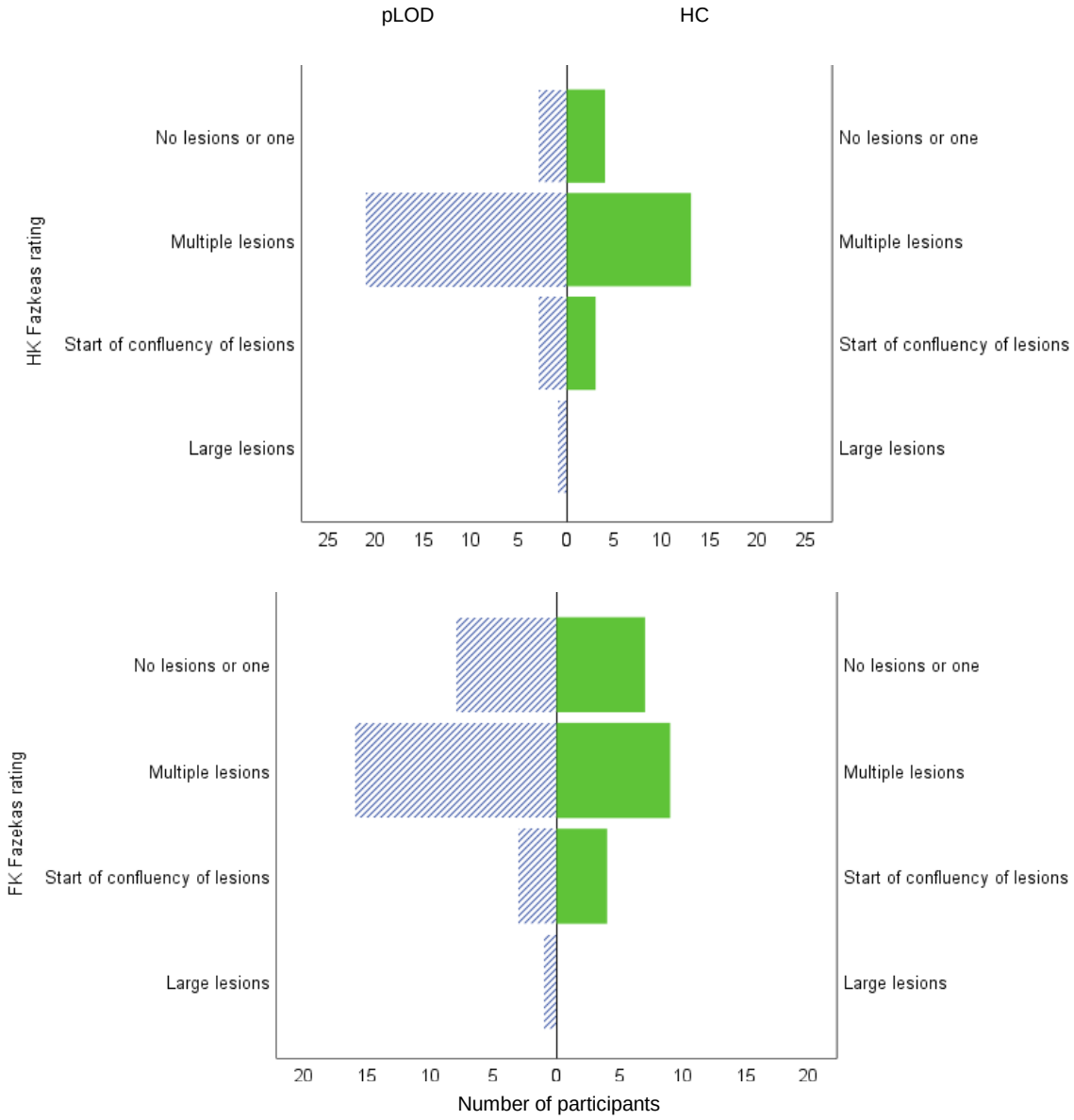


Figure 5-5 HK and FK Koedam ratings of parietal atrophy, by participant group (patients with late onset depression; pLOD n=28 and healthy controls; HC n=20)

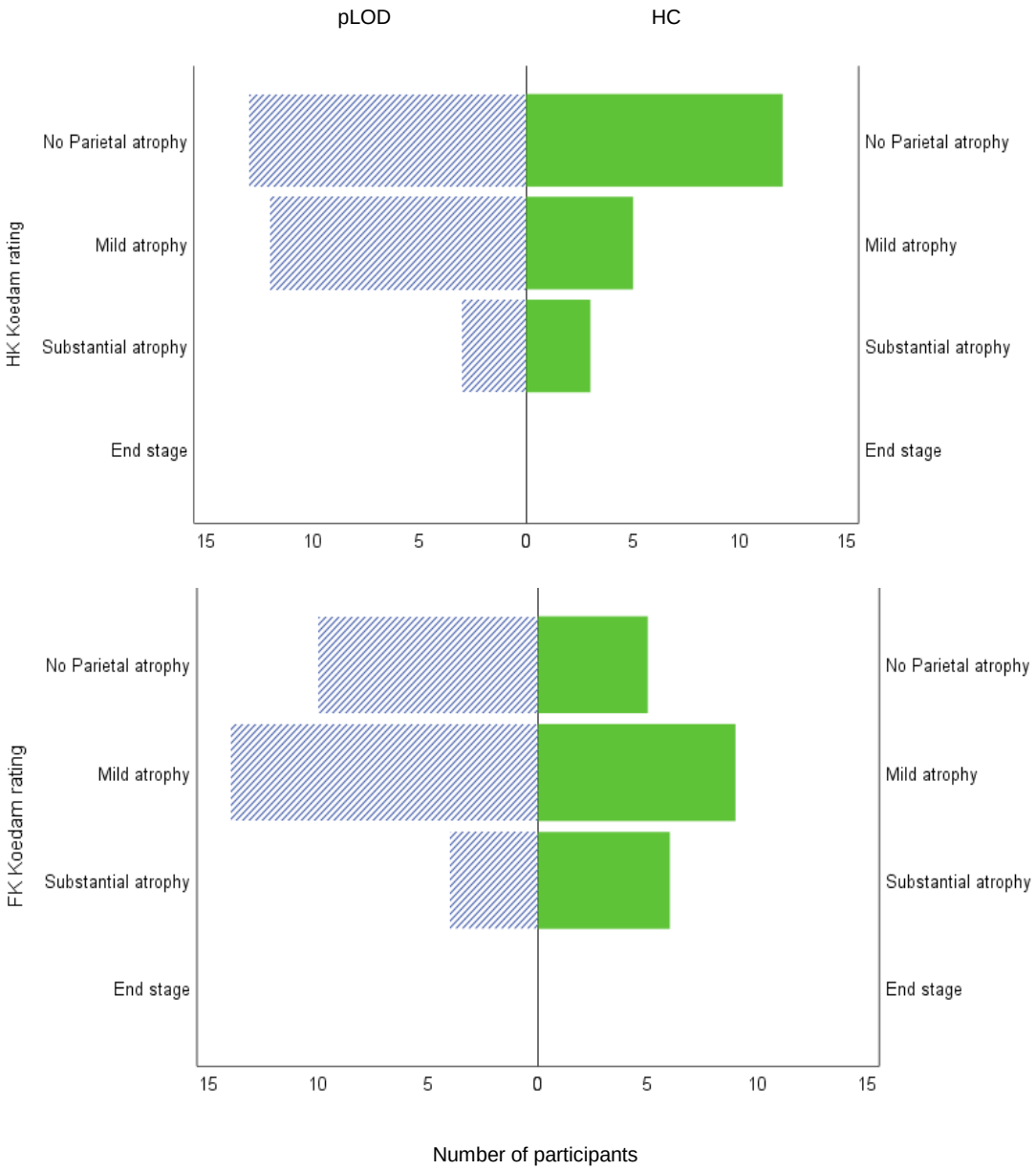


Figure 5-6 HK and FK global cortical atrophy ratings, by participant group (patients with late onset depression; pLOD n=28 and healthy controls; HC n=20)

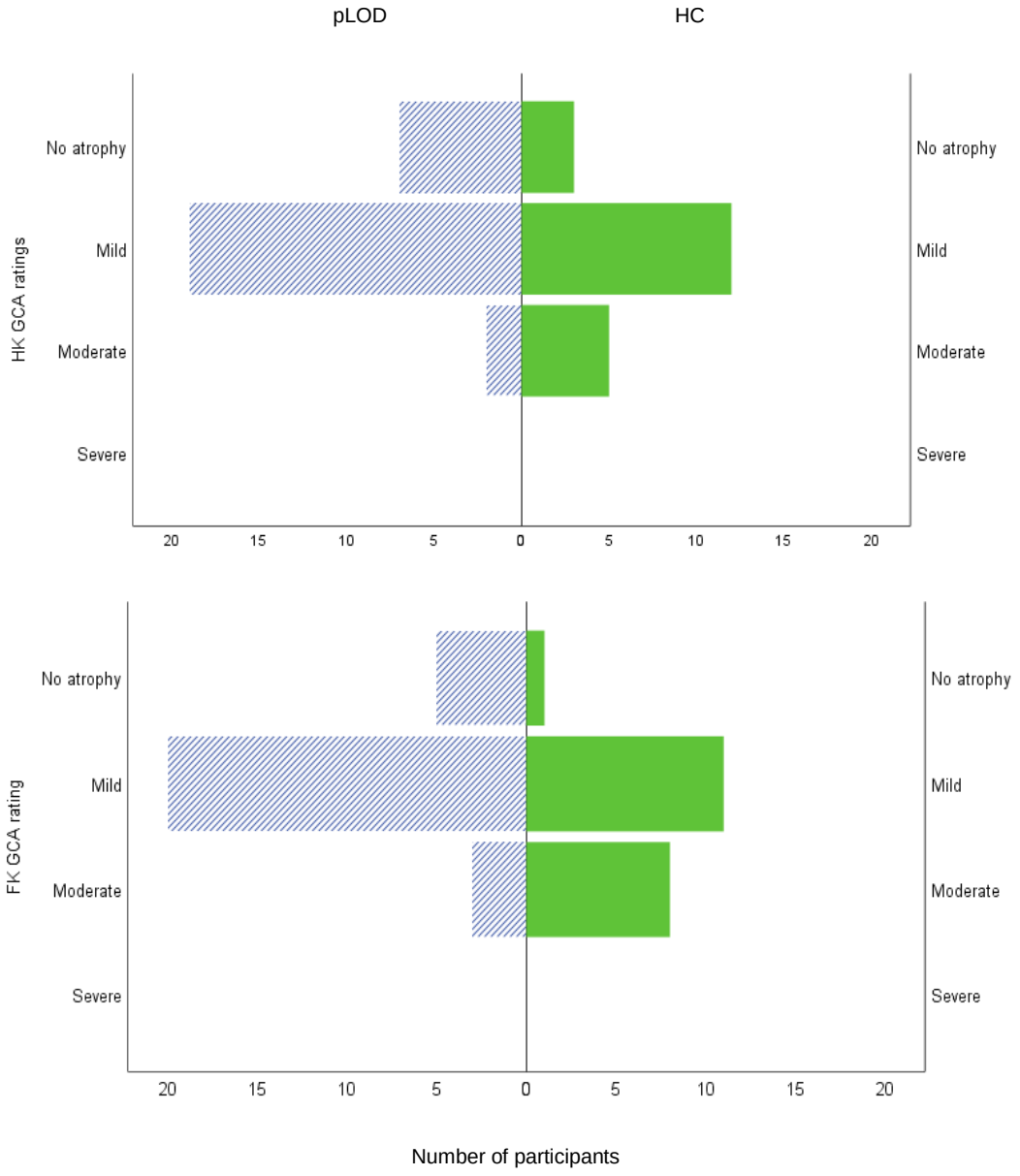
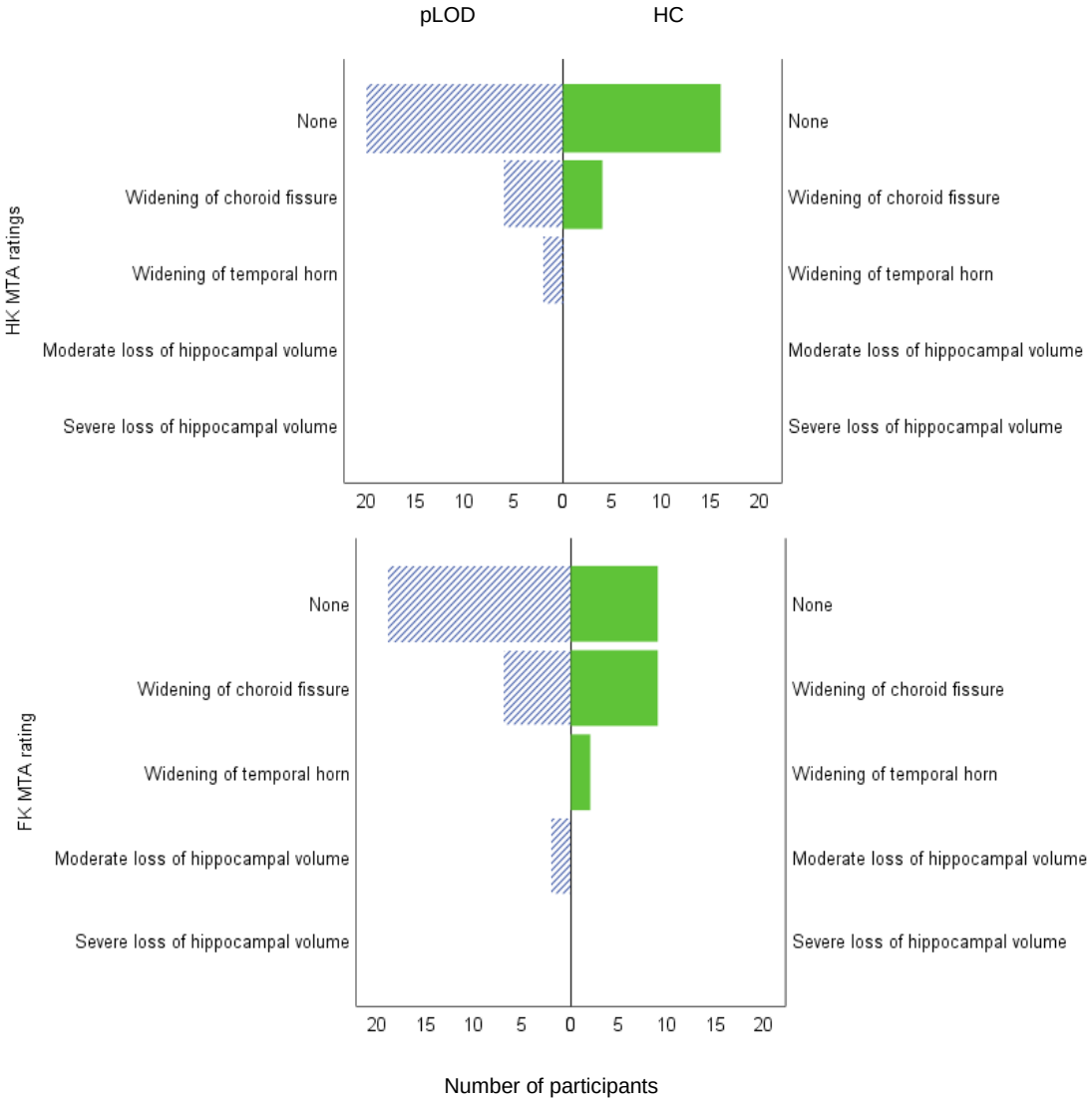


Figure 5-7 HK and FK medial temporal atrophy (MTA) rating, by participant group (patients with late onset depression; pLOD n=28 and healthy controls; HC n=20)



5.4.2 Part Two: Subcortical GM volumes and LL analysis

Group differences (HC vs pLOD) in the subcortical GM were analysed (Table 5-12) using ANCOVA. Group differences in the right HIPP and the right amygdala missed significance (p=.079, p=.070). No significant group differences were found including in LL with Figure 5-8 displaying the spread of LL in pLOD and HC.

Table 5-12 Comparison of MRI findings between pLOD and HC

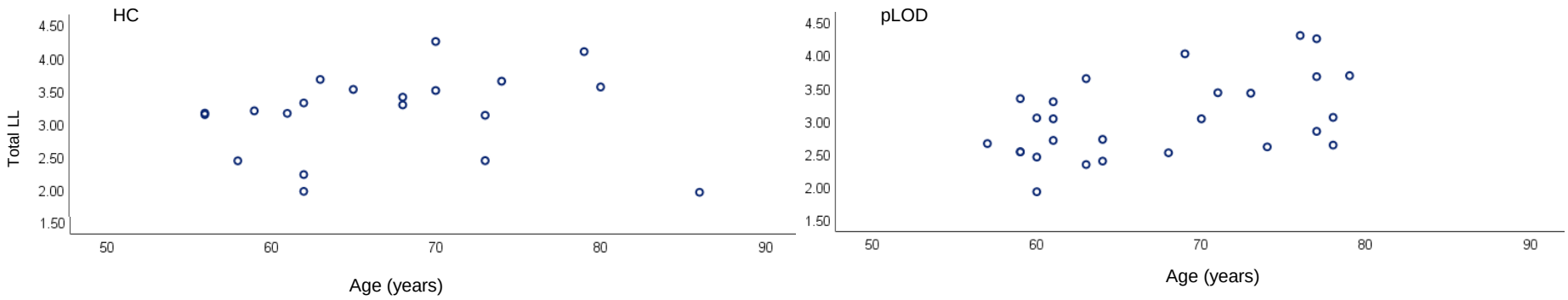
Region	HC	pLOD		*p*
	Mean mm^3 (SD) Median [1st IQ; 3rd IQ]			
Third Ventricle	3.15 (0.12) 3.18 [3.04; 3.25]	3.11 (0.15) 3.11 [3.01;3.22]	1.744	.195
Fourth Ventricle	3.27 (0.10) 3.26 [3.19;3.36]	3.27 (0.11) 3.27 [2.20;3.33]	.518	.476
Right Caudate	3.49 (0.05) 3.48 [3.43;3.51]	3.49 (0.05) 3.48 [3.46;3.53]	.536	.469
Left Caudate	3.47 (0.05) 3.46 [3.43;3.51]	3.46 (0.05) 3.45 [3.41;3.49]	.020	.889
Right Putamen	3.59 (0.02) 3.59 [3.57;3.62]	3.59 (0.04) 3.59 [3.55;3.63]	1.763	.192
Left Putamen	3.60 (0.03) 3.60 [3.58;3.63]	3.60 (0.05) 3.60 [3.56;3.63]	1.288	.264
Right Hippocampus	3.58 (0.03) 3.57 [3.54;3.61]	3.55 (0.04) 3.56 [3.52;3.58]	3.273	.079
Left Hippocampus	3.55 (0.03) 3.55 [3.53;3.59]	3.54 (0.05) 3.54 [3.50;3.59]	.576	.453
Right Amygdala	3.21 (0.03) 3.21 [3.2;3.24]	3.18 (0.05) 3.20 [316;3.21]	3.477	.070
Left Amygdala	3.21 (0.03) 3.21 [3.18;3.24)	3.19 (0.05) 3.19 [3.17;3.24]	1.454	.235
Total white matter	5.61 (0.05) 5.61 [5.57;5.65]	5.61 (0.05) 5.61 [5.58;5.65]	1.107	.300
Total grey matter	5.65 (0.03) 5.65 [5.63;5.67]	5.64 (0.03) 5.64 [5.62;5.67]	.047	.830
Total LL	3.16 (0.63) 3.25 [2.62;3.56]	3.04 (0.60) 3.04 [2.54;3.43]	.002	.963

Analysis of variance (ANCOVA) with TIV, type of scanner, age and gender as a covariate,

A significant p value with Bonferroni adjustment would be $p<0.003$, calculated by 0.05 / all regions in Table 5-13

mm^3=millimeters cube, LL= white matter lesion load, SD= standard deviation.

Figure 5-8 Scatterplots showing the white matter lesion load (LL) in healthy controls (HC) (n=19) and patients with late onset depression (pLOD) (n=28)



Associations between MRI findings and demographic and clinical values

Correlations between the MRI findings and demographic and clinical values are shown in Table 5-13 and Table 5-14.

Significant correlations ($p<0.05$) are highlighted in bold. All correlations included type of scanner used and TIV as covariates.

However, when also controlling for age and gender (alongside type of scanner used and TIV) the significant correlation (shown in Table 5-14) between global WM and Q risk score in HC missed significance at $p<0.05$ (r .50, p=.080).

Table 5-13 Associations between demographic and clinical variables and subcortical grey matter volumes in HC and pLOD

	Regions r, p							
	L CN	R CN	L PUT	R PUT	L HIPP	R HIPP	L AMY	R AMY
HC								
Age	-.27, .317	-.23, .410	-.12, .647	-.29, .294	-.06, .807	-.22,.415	-.51, .051	-.27, .330
Gender	.31, .252	.37, .167	.18, .508	.25, .351	.12, .659	.03, .903	.07, .792	-.20, .472
Q risk score	.35, .195	.32, .234	.28, .304	.37, .172	.11, .696	.13, .645	.16, .562	-.29, .288
pLOD								
Age	.03, .891	.14, .524	-.36, .100	-.33, .124	-.48, **.021**	-.54, **.009**	-.55, **.008**	-.62, **.002**
Gender	.24, .268	.26, .242	.19, .390	.12, .593	-.05, .807	.09, .688	-.04, .841	.03, .886
Q risk score	.30, .172	.22, .324	.21, .341	.16, .467	-.10, .651	-.15, .507	-.25, .256	-.03, .895
DSM 5 SMD score	-.16, .430	-.12, .538	.27, .181	.18, .356	.17, .406	.16, .433	.10, .600	.22, .263
HADS Anxiety score	-.02, .891	-.02, .914	.28, .160	.20, .307	.14, .492	.08, .697	-.03, .862	.03, .860

Partial correlation controlling for TIV and type of scanner used. Significant correlations ($p<0.05$) are in **bold**
L= left, R=right, CN= caudate nuclei, PUT=putamen, HIPP= hippocampus, AMY= amygdala, SBR= striatal binding ratio

Table 5-14 Associations between demographic and clinical variables and global grey matter and white matter volumes and white matter lesions in HC and pLOD

	Regions r, p				
	3rd ventricle	4th ventricle	global GM	global WM	LL
HC					
Age	.31, .248	-.28, .305	-.53, **.041**	-.62, **.013**	.05, .846
Gender	-.08, .773	-.05, .838	.14, .603	.56, **.029**	.09, .761
Q risk score	-.16, .568	-.07, 778	-.27, .328	.58, **.022**	.35, .219
pLOD					
Age	.81, **<.001**	.09, .684	-.68, **<.001**	-.41, .052	.33, .139
Gender	.01, .963	.21, .340	.10, .657	.11, .608	.01, .938
Q risk score	.02, .911	.19, .386	-.17, .446	.05, .821	.27, .226
DSM 5 SMD score	-.13, .608	-.01, .945	.21, .391	.28, .247	-.14, .544
HADS Anxiety score	-.08, .750	.16, .519	.06, .801	.37, .131	-.07, .756

Partial correlation controlling for TIV and type of scanner used. Significant correlations ($p<0.05$) are in **bold**
GM= grey matter, WM=white matter, LL=white matter lesions

Relationship between symptoms of depression and anxiety and subcortical grey matter volumes

The relationship between symptoms of depression (measured by the DSM 5 severity measure of depression; DSM 5 SMD) and anxiety (measured by the Hospital and anxiety questionnaire; anxiety subscale; HADS-A) and subcortical grey matter volumes in pLOD were explored. Age and gender, TIV and type of scanner were included in all the adjusted models as covariates.

Linear regressions showed no significant ($p<0.05$) relationships in either crude or adjusted models (Table 5-15 and-Table 5-18).

There were also no significant relationships ($p<0.05$) between L and R CN and L PUT volumes and symptoms of depression (Table 5-16 and Table 5-18).

There were no significant ($p<0.05$) relationships (using linear regressions) found between symptoms of anxiety and subcortical grey matter volumes within pLOD in either crude or adjusted models (Table 5-17).

Table 5-15 Symptoms of depression (using the DSM 5 SMD) and relationship with subcortical grey matter volumes of interest within patients with LOD (pLOD) only

Predictor	Model	Dependent β standardised coefficient (p)						
		3rd ventricle	4th ventricle	R HIPP	L HIPP	R AMY	L AMY	Total LL
DSM 5 SMD score	Crude	-.12 (.527)	.04 (.841)	.06 (.741)	.08 (.663)	.13 (.493)	.03 (.842)	-.29 (.134)
	Adjusted	.20 (.226)	.16 (.491)	-.03 (.849)	.03 (.878)	-.02 (.892)	-.15 (.490)	-.01 (.957)

DSM 5 SMD= DSM 5 severity measure of depression, HIPP= Hippocampus, AMY=amygdala, total LL= white matter lesion load, R=right, L=Left

Crude: Model contains only DSM 5 SMD scores as the predictor, Adjusted: Model includes DSM 5 SMD scores and the following other predictor (covariate) variables; age, gender, type of scanner used (1.5 tesla or 3 tesla) and total intracranial volume (TIV): to account for variances in intracranial volume across participants.

Table 5-16 Symptoms of depression (using the DSM 5 SMD) and relationship with left and right caudate nuclei (CN) and left and right putamen (PUT) volumes within patients with LOD (pLOD) only

Predictor	Model	Dependent β standardised coefficient (p)			
		R CN	L CN	R PUT	L PUT
DSM 5 SMD score	Crude	-.22 (.250)	-.23 (.229)	.09 (.924)	.07 (.689)
	Adjusted	-.04 (.866)	-.15 (.499)	-.02 (.871)	.03 (.845)

DSM 5 SMD= DSM 5 severity measure of depression, CN=caudate nuclei, PUT=putamen, total LL= white matter lesion load, R=right, L=Left
Crude: Model contains only DSM 5 SMD scores as the predictor, Adjusted: Model includes DSM 5 SMD scores and the following other predictor (covariate) variables; age, gender, type of scanner used (1.5 tesla or 3 tesla) and total intracranial volume (TIV): to account for variances in intracranial volume across participants.

Table 5-17 Symptoms of anxiety (using the HADS anxiety) and relationship with subcortical grey matter volumes and white matter lesion load within patients with LOD (pLOD) only

Predictor	Model	Dependent β standardised coefficient (p)						
		3rd ventricle	4th ventricle	R HIPP	L HIPP	R AMY	L AMY	Total LL
HADS-A score	Crude	-.08 (.652)	.19 (.326)	.08 (.670)	.68 (.499)	.11 (.563)	.05 (.778)	-.19 (.336)
	Adjusted	.04 (.777)	.22 (.331)	.01 (.912)	.12 (.518)	-.07 (.628)	-.11 (.477)	.04 (.858)

HADS-A= Hospital and anxiety scale; anxiety score only, HIPP= Hippocampus, AMY=amygdala, total LL=white matter lesion load, R=right, L=Left

Crude: Model contains only HADSA scores as the predictor, Adjusted: Model includes HADSA scores and the following other predictor (covariate) variables; age, gender, type of scanner used (1.5 tesla or 3 tesla) and total intracranial volume (TIV): to account for variances in intracranial volume across participants.

Table 5-18 Symptoms of depression (using the HADS anxiety) and relationship with left and right caudate nuclei (CN) and left and right putamen (PUT) volumes within patients with LOD (pLOD) only

Predictor	Model	Dependent β standardised coefficient (p)			
		R CN	L CN	R PUT	L PUT
HADS-A score	Crude	-.59 (.557)	-.34 (.734)	.13 (.496)	.19 (.330)
	Adjusted	.01 (.944)	-.09 (.967)	.03 (.850)	.08 (.597)

HADS-A= Hospital and anxiety scale; anxiety score only, CN=caudate nuclei, PUT=putamen
Crude: Model contains only HADSA scores as the predictor, Adjusted: Model includes HADSA scores and the following other predictor (covariate) variables; age, gender, type of scanner used (1.5 tesla or 3 tesla) and total intracranial volume (TIV): to account for variances in intracranial volume across participants.

5.4.3 Part Three: MRI and DAT SPECT

Volumes and SBRs of the PUT and CN

There were no significant correlations between the SBRs and volumes of the PUT and CN; as shown in Table 5-19.

Table 5-19 Volumes and putamen (PUT) and caudate nuclei (CN) striatal binding ratios (SBRs) in abnormal DAT SPECTs

	Regions r, p			
	L CN	R CN	L PUT	R PUT
HC				
Lowest mean PUT SBR	-.29, .295	-.21, .437	-.28, .308	-.09, .734
Lowest mean CN SBR	-.44, .101	-.23, .406	-.51, .049	-.44, .099
PUT L SBR	-.13, .631	-.03, .911	-.15, .578	.01, .949
PUT R SBR	-.25, .353	-.45, .092	-.25, .353	-.41, .128
CN L SBR	-.12, .665	-.32, .243	-.34, .244	-.39, .150
CN R SBR	-.47, .073	-.28, .299	-.50, .053	-.37, .166
pLOD				
Lowest mean PUT SBR	-.00, .996	.04, .851	.02, .919	.10, .643
Lowest mean CN SBR	.25, .251	.22, .310	.24, .275	.32, .137
PUT L SBR	.04, .860	.07, .750	.05, .806	.13, .541
PUT R SBR	-.00, .971	.07, .750	.06, .772	.13, .537
CN L SBR	.25, .244	.18, .389	.12, .574	.19, .376
CN R SBR	.24, .274	.22, .307	.27, .210	.35, .111

Partial correlations (controlling for total intracranial volume; TIV, type of scanner used and age)
DAT SPECT= Dopamine transporter single photon emission computerised tomography, pLOD=patients with late onset depression, *n* = number of participants, r= correlation, p= p value

5.5 Discussion

In the current study, there were no significant group differences in visual ratings of atrophy or WML. There were no significant group differences in LL and global GM and WM volumes.

In pLOD:

- Age was significantly associated with volumes of the 3rd ventricle, the right HIPP the right AMY and **global GM.**

In HC:

- Age was significantly associated with global WM and GM
- Gender was associated with global WM.

There was no relationship between symptoms of depression/anxiety, and volumes or LL in pLOD.

Visual ratings

The visual analysis of the MRI scans was conducted by HK (researcher trained by a radiologist) and FK (a specialist registrar in clinical radiology). The moderate agreement on all of the rating scales can be explained by the rating experience between the raters. HK was trained by a radiographer and with good agreement, albeit this was on a smaller sample. Despite the moderate agreement, group differences were present only in the group mean GCA ratings (as discussed later). The visual analysis of the MR scans by HK found no significant group differences in ratings of global, parietal and medial temporal atrophy. Whilst more pLOD than HC were rated with WML (rating of 1 or above), this was not significantly higher than HC (and also within normal age related pathology). The majority of participants were rated

with a Fazekas score of 1 (indicating multiple lesions); pLOD (75%) than HC (43%). These findings were also reported by FK who rated 57% of pLOD and 45% of HC with grade 1. However, there were no significant group differences in mean average scores or the number of participants with a score of 1 (vs 0).

Using the visual ratings by HK, the majority of both pLOD and HC scored with a rating of 1 (mild atrophy) on GCA and parietal atrophy which is also in keeping with natural age related changes. The majority of participants scored 0 on the MTA (no medial temporal atrophy) and with no significant group differences between left or right MTA scores. FK, however found significant group differences in global atrophy with HC on average scoring higher on the global atrophy scales. The mean score of 1.35 (SD .05) was in line with natural age related changes.

LLD and LOD has been associated with the presence of WML (Godin et al., 2008, O'Brien and Ames, 1996, Figiel et al., 1991). Although not all research has reported an association between cerebrovascular pathology and LOD (Aizenstein et al., 2011, Tsopelas et al., 2011). Tsopelas et al. (2011) suggested that their large sample study was more representative of a normal population rather than previous studies looking in pathology into LOD which focus on specific samples (such as those with major depression).

The ratings scales used to visually rate atrophy and WML are largely used to assess pathology associated with AD and were used for exploratory purposes in the current study. Perhaps the lack of significant group differences (in WML and atrophy) can be attributed the pathology not being currently relevant to the current sample of patients especially who at this stage, were not exhibiting any symptoms of associated with or diagnosed with dementia. Although with studies suggesting up to 40% of pLOD develop dementia (including AD,

vascular disease and DLB (Schweitzer et al., 2002, Fiske et al., 2009), perhaps any changes at this stage of LOD are subtle. This is suggested by the non-significant differences in WMH between groups despite pLOD tending to score with WMH in comparison to HC.

Subcortical GM volumes

Volumetric analysis was used to investigate specific GM volumes previously associated with the pathology of LOD. There were no significant group differences in the volumes of the subcortical GM regions or global GM and WM.

Group differences in R HIPP volumes missed significance (p=.079). The role of the HIPP has been associated with depression in general (Egan et al., 2003, Kalia, 2005). Specific to LOD, Hickie et al. (2005) reported reduced total HIPP volumes in pLOD in comparison to those with EOD and HC using a 1.5 T scanner after controlling for age and whole brain volume (however decreased volumes were also found in EOD patients in comparison to HC). Additionally, Bonferroni corrected analysis revealed no differences between EOD and LOD groups in L HIPP volumes although differences remained between HC and both LOD and EOD as a group. Significant differences between total and R volumes remained between LOD and HC however not EOD and LOD or EOD and HC groups. The authors suggested that the onset of depression reflects an early phase of a dementia such as AD or vascular dementia and is perhaps not specific to age of onset (i.e. LOD or EOD). In our current the study group differences missing significance in the R HIPP may be due to smaller numbers (n=28) in comparison to 41 pLOD investigated by Hickie et al. (2005). Additionally, we did not have an EOD comparison group in the current study to verify whether differences were specific to LOD or just between depression and HC in general. Decreased subfield HIPP

volumes have also been reported in pLOD. Choi et al. (2017) reported a reduction in both subfield (CA1, CA2, CA3, CA4, DG) and whole volumes when using a 3 T scanner and using ANCOVA accounting for age, gender and TIV and Sivakumar et al. (2015) who also used a 3 T scanner reported reductions of the posterior HIPP volume indicating sub region specific HIPP abnormalities in pLOD compared with HC and after controlling for age gender and TIV. In addition, Lebedeva et al. (2015) reported thinning in the parahippocampal regions in pLOD when compared with controls using a 1.5 T from three different vendors (phantom scanning was performed to assess interscanner reliability). HIPP volumes were normalised using the subject's intracranial volume, although the results were not significant after controlling for age and gender.

It is apparent that there is mixed research reporting an association between pLOD and reduced HIPP volumes. For the current study it can be suggested perhaps a small size or lack of severity of symptoms can account for the lack of significance (which will be discussed later). Visual MTA ratings (which include assessing the height of hippocampal formation) by HK and FK did also not reveal any significant differences at this stage with no differences between left or right MTA ratings (as discussed earlier).

Additionally, reduced volumes of the HIPP have also been associated with cognitive impairment and dementia. Cognitive impairment has been reported in both those with LOD (Naismith et al., 2003) but also in the prodromal (and developed) phase of neurodegenerative disease such as AD and Parkinsonian disorders (Darweesh et al., 2017, Chaudhuri et al., 2006) with reduced HIPP volume reported in patients with PD (Laakso et al., 1996, Chaudhuri et al., 2006).

Group differences between R AMY volumes, also did not reach significance (p=.070). Egger et al. (2008) reported a significant decrease of volume in the R AMY in those with pLOD (first depression onset after 60) in comparison to HC. Whilst small sample size may again be attributed to lack of significance in the current study, to perhaps explain our findings, Burke et al. (2011) reported significant differences between AMY volumes between those with EOD and pLOD, however after controlling for age, gender and cerebral matter in pLOD and the EOD group, concluded that AMY volumes were smaller in those with depression regardless of onset.

Based on the findings of Burke et al. (2011) it can be suggested that perhaps reduced AMY volumes may be applicable to LLD in general rather than within EOD and LOD. Alternatively, some imaging studies have defined LOD as onset after the age of 60 years of age (Egger et al., 2008) and consequently investigating an older population sample in comparison to the sample in the current study (who have onset after the age of 55).

LL

Additionally, there were no significant group differences in LL, as also reported in the visual analysis. The results of the current study fall in line with the findings from Tsopelas et al. (2011) who reported no link between LLD and cerebrovascular pathology.

Alternatively, instead of rating general WMH, perhaps the presence of WMH in specific regions could have been explored. For example, Figiel et al. (1991) reported a higher occurrence of CN and large WMH in patients with depression onset after the age of 60 in comparison to those of early onset.

Age and subcortical GM volumes in pLOD

In the current study, we found significant associations between age and **global GM** in both pLOD and HC. Only age in HC also significantly correlated with global WM, with age and global WM in pLOD missing significance (p=.052). Gender also correlated with global WM in HC only. When assessing **specific** subcortical volumes, age significantly correlated with left and right HIPP and AMY volumes in pLOD. L AMY volumes correlating with age missed significance in HC (p=.051).

Soares et al. (2014) reported that depressed mood and stressful events increase the effect of age on the brain structure. Lebedeva et al. (2015) also reported that LOD women had significantly more extensive cortical thinning in the lateral prefrontal cortex compared with EOD women using age, education, and MMSE score as covariates, suggesting an association with depression and increased aging effect on the brain cortex although specific to women with LOD. Specific to the volumes of the HIPP and AMY, Alexopoulos (2005) also suggested that age related changes may contribute to comprising the integrity of the AMY and HIPP (amongst other regions) and consequent depression, in the elderly.

However in the current study **global** GM and WM was also associated with age in HC suggestive of an age related process rather than a pathological process. Based on the results of the current study it can be suggested that perhaps depressed mood increases the effect of ageing particularly in those areas already associated with depression; the AMY and HIPP (and also ventricular volume; discussed below).

Age is also a risk factor for neurodegenerative diseases including PD and dementia. As mentioned earlier, research has reported associations between LOD and dementia; perhaps suggestive of a neurodegenerative component of LOD. Karas et al. (2004) reported lower global GM volume in patients with AD in comparison to HC. Chagas et al. (2017) reported decreased volume and/or reduction of cortical thickness, the AMY, and cerebellar WM (amongst other regions) in patients with a lifetime of major depressive disorder and PD. The authors suggested current and lifetime MDD has a negative impact on the neurodegenerative process of PD, linking these two later life disorders.

Ventricular volume

Although there were no significant group differences in mean ventricular volumes, in only pLOD, age correlated with the volumes of the 3rd ventricle. Dahabra et al. (1998) reported pLOD had larger third and lateral ventricles alongside greater frequency and severity of subcortical WML. Additionally, Alexopoulos et al. (1992) suggested that the pathology found in pLOD may have a stronger association with neurologically dementing disorder in comparison to those with EOD; who were more like to have significantly smaller ventricular and less sulcal widening in comparison to patients with dementia.

Relationship with symptoms of depression and anxiety

Any relationship between symptoms of depression and anxiety with subcortical grey matter volumes and LL were also investigated. No significant associations between symptoms of anxiety or depression with volumes or LL were found. Sivakumar et al. (2015) reported that depression severity has a significant negative correlation with volumes

of right and left posterior HIPP. However in the current study, on average pLOD had a DSM 5 SMD score of mild depression and there were no significant correlations with severity of depression (DSM 5 SMD) and anxiety (HADS-A) with volumes of the HIPP (with lack of severity of symptoms perhaps providing an explanation for the lack of significance in HIPP volumes between groups).

Barber et al. (1999) reported that in all patients with dementia, frontal WMH were associated with higher depression scores. In the current study, however associations between LL and symptoms of depression and anxiety were not found. This may be due to the reduced WML found, with the majority of visual ratings of WMH with a score of 1 (as discussed earlier).

MRI and DAT SPECT

As with the other subcortical GM regions, there were no significant group differences between PUT and CN volumes. Greenwald et al. (1997) reported that pLOD had significantly more L medial temporal and L CN atrophy than EOD patients and Kempton et al. (2011) reported that depression was associated with a reduced volume in the CN and PUT (alongside the hippocampus, cingulate and orbitofrontal cortex).

In addition, a comparison between PUT and CN volumes and SBRs were also of particular interest with the previous chapter of our study reporting a decrease in the SBRs of the CN in those with LOD in comparison to HC and with previous reports of decreased SBRs in the striatum in those with depression (Sarchiapone et al., 2006, Meyer et al., 2001).

However, no significant associations between volumes and SBRs of the PUT and CN were found. The lack of any associations between SBRs and PUT and CN volumes in pLOD in the current sample is suggestive of a neurochemical process in pLOD no with no significant group differences in volumes however significant group differences in lowest and R SBRs in the current study sample.

The results of the current study can be suggested to reflect previous studies investigating depression and LOD with a mixture of significant (associations between age and specific subcortical volumes) and non-significant (no significant group differences in WML) results. It is difficult to make comparisons between studies due to the varied onset ages used to categorise LOD, use of LLD (which includes EOD) and with the etiology of LOD itself also heterogeneous. Severity of depression also need to be considered with research reporting associations between depression severity and the effect on the brain (Sivakumar et al., 2015).

Limitations

A limitation for the current study is the use of different MR scanners. The majority of the MR scans were conducted at the Royal Free site, using a 1.5 T scanner whilst a 3 T scanner was used at the secondary research site. Differences in the volumetric analysis could potentially be affected, with the 3 T more sensitive to picking up details in comparison to the MR scans obtained from the 1.5 T.

To conclude, at this stage, overall no significant differences between atrophy, WML and volumes of global and subcortical GM (and WM) were observed between groups. Notable findings include the role of age in ventricular and HIPP and AMY volumes in pLOD which require further investigation with age also playing a role in neurodegenerative

disorders and research reporting LOD and consequent dementia (Schweitzer et al., 2002). A larger sample size and long term follow up is needed to ascertain the significance and development of these findings.

Chapter 6. **Final Discussion**

The current study aimed to explore whether pLOD presented with a dopaminergic dysfunction and with clinical features associated with PD.

When investigating non motor and motor features associated with PD ("Chapter 3: Clinical correlates of LOD"), mean group clinical scores for pLOD were significantly worse than HC. In particular, there were significant group differences in RBD scores (p=0.05), which persisted in pLOD even when controlling for those patients on relevant anti-depressants. RBD has been commonly reported in prodromal PD (Chaudhuri and Schapira, 2009) however is not associated with LOD. Research into the symptoms of prodromal PD has reported that participants with RBD and a dopaminergic dysfunction are at higher risk of PD later in life (Iranzo et al. (2010). In the current study we have demonstrated symptoms associated with prodromal PD present in pLOD, however further tests are required (i.e. polysomnography) to accurately diagnose RBD.

Next we investigated whether pLOD had reduced striatal dopaminergic uptake in comparison to HC ("Chapter 4: DAT SPECT in LOD"). Using the SQA, we identified that overall pLOD had reduced CN SBRs in comparison to HC, in line with findings from previous literature reporting decreased DA functioning in patients with depression (Bowden et al., 1997). Additionally we also reported associations between SBRs and clinical motor scores with previous research also similarly reporting associations between psychomotor retardation and DA functioning in depression (Martinot et al., 2001, Buyukdura et al., 2011).

A novel finding was using the visual (primary) analysis, where we reported that seven pLOD in comparison to one HC were rated with an abnormal DAT SPECT with patterns of uptake commonly associated with PD (abnormal type 1 and type 2 ratings) and DLB (striatal loss rating) (Benamer et al., 2000, Walker et al., 2004).

Additionally, we further identified that pLOD who a dopaminergic deficit had lower cognitive scores, subtle motor abnormalities and anosmia in comparison to pLOD without a dopaminergic deficit. Alongside RBD, anosmia is one of the most common symptoms associated with prodromal PD. Research has additionally reported that relatives of PD patients with anosmia and a dopaminergic deficit are more likely to develop PD (Berendse et al 2001) and that those with hyposmia also present with a dopaminergic deficit (Jennings et al., 2017, Ponsen et al., 2004).

Previous literature has suggested a 50% loss of the nigral dopamine neurons (Fearnley and Lees, 1991) at the time of clinical symptoms. We have identified in a group of pLOD who have a dopaminergic deficit associated with PD with additional increased presence of non motor symptoms compared to those without this dopaminergic deficit and that these changes may represent the “prodromal phase” of PD.

Moreover, we investigated further pathology using MR scans (Chapter 5: MRI in LOD) however no significant group differences were found in presence of WML, atrophy or subcortical volumes. These results may be due to either no differences between the pLOD and HC groups in these structures or alternatively, that the visual ratings, which were those commonly used when identifying pathology associated with AD, are not sensitive enough for changes in this group of pLOD. Furthermore, if only a subgroup of pLOD have these changes, they may not have been detected in this small study sample.

Limitations

Some methodological limitations need to be noted. Both research sites used the same DAT SPECT scanners and all the SPECTs were reconstructed by the same researcher (HK) using DaTQUANT. However the SPECTs were conducted at two different sites posing potential differences with phantom scanning not specifically undertaken by each site for the purpose of the study. Additionally, when conducting the MR scans, one site used a 1.5 T scanner and the second site using a 3 T scanner (which would have been more accurate in assessing any subtle abnormalities).

The recruitment strategy may also have identified a biased sample of HC; when using the SQA, three HC were found to have a dopaminergic deficit. HC were recruited via public advertising with perhaps those HC more likely to join who may have been particularly concerned with a neurodegenerative disease and two out of the three HC had relatives with a neurodegenerative disease.

Additionally, using the SQA we reported a decrease in CN SBRs in pLOD with stronger associations between CN SBRs (than PUT SBRs) and subtle motor abnormalities. Although previous research has reported associations between CN SBRs and psychomotor retardation in depression this is not in line with the common pattern of dopaminergic deficit observed in PD (with a loss in the PUT then progressing to a loss in the CN). However the results may indicate the importance of the role of DAT functioning in the CN in pLOD.

Furthermore, there were discrepancies between the SQA and the visual ratings with the SQA reporting a decrease in mean CN SBRs in pLOD but with the visual ratings demonstrating an additional decrease also in the PUT (however only in those rated with abnormal SPECT).

These differences are likely to be explained by differences in the techniques.

A two SD cut off is more commonly used in clinical cases when using SQA however in the current study we used a lower one SD cut off from age matched controls for exploratory purposes and to assess subtle deficits in this prodromal stage. Consequently, the SQA highlighted six additional participants with an abnormal SPECT using a one SD cut off. To avoid over rating normal variants as abnormal when using the SQA one SD cut off, the visual analysis remains the primary analysis for the study with visual interpretations recommended for clinical DAT SPECTs in clinical practice (Djang et al., 2012).

Although the results of the current study and previous literature investigating symptoms of LOD are suggestive of LOD as a separate disorder with specific symptomology also often associated with other neurodegenerative diseases, a further age-matched EOD sample should also be used as a comparison sample. This is to ascertain the differences in depression with first onset after the age of 55 years and that of depression in late life with earlier onset. Additionally, further follow up is also required to confirm risk of PD.

Future research

Using DAT SPECT we have shown that 24% of pLOD have a dopaminergic deficit, putting them at higher risk of developing PD/DLB in the future. However depression in later life is also a risk factor for dementia (Schweitzer et al., 2002) including AD and therefore some of these pLOD who do not have a dopaminergic deficit could be preclinical AD cases. Using further imaging techniques and in particular amyloid imaging in this DAT SPECT positive cohort would provide further opportunity to predict which patients will develop a

neurodegenerative disease, and what type (e.g. PD, AD, DLB). Previous and current research has found a link between preclinical AD and increased levels of amyloid deposition. Evidence of amyloid accumulation (evidenced by cerebrospinal fluid or PET imaging) has been defined as Stage 1 of preclinical AD according to the US National Institute on Aging and the US Alzheimer's Association framework (Sperling et al 2009). Underlying pathology can be known to start 10 to 20 years before clinical signs of dementia appear (Holtzman et al., 2011) with LOD duration in the current pLOD ranging from 0.5-10 years.

Using a combination of clinical and imaging methods the current study has provided further information on already established (apathy, autonomic dysfunction) but also new (RBD and anosmia) clinical features associated with LOD and evidence of a dopaminergic deficit in pLOD. These findings overlap with the features seen in those with prodromal PD and suggest that pLOD are at increased risk of PD. This is supported by the fact that those pLOD with a dopaminergic deficit had greater presence of clinical features of prodromal PD that those pLOD without a dopaminergic deficit. Whilst a larger sample size and follow up is required to will ascertain the long term significance of these findings, the results have provided new information about LOD as a disorder and its association between LOD and symptoms of prodromal PD and findings of dopaminergic deficit seen in prodromal PD.

Chapter 7. **A one-year audit on indications for DAT SPECT and the use of visual and semi quantitative methods**

7.1 Introduction

PS such as PD, multiple system atrophy (MSA) and progressive supranuclear palsy (PSP) are associated with a dopaminergic deficit in the nigrostriatal pathway.

Whilst clinical assessment is used for a diagnosis of PS, the DAT SPECT scan can be used to clarify uncertain diagnosis and guide consequent management. As an invasive and expensive procedure, DAT SPECT or DaTSCAN/DaTscan (GE healthcare, Amersham, UK) should only be requested for licensed indications such as those outlined by the FDA (2011), European Medicines Agency (EMA) (2011) (Bajaj et al., 2013) and the Society of Nuclear Medicine (SNM) (Djang et al., 2012).

Guidelines have advised that visual interpretation is usually sufficient for clinical interpretation (Djang et al., 2012) of a DAT SPECT. However, SQA can be useful alongside VR especially in challenging cases and has been recommended to objectively assess uptake (Darcourt et al., 2010).

7.2 Objectives

Objective 1: Compare indications for DAT SPECT requests with published guidelines, in one large NHS trust over one year.

Objective 2: Compare the DAT SPECT visual ratings with SQA.

7.3 Methods

Patients

Inclusion criteria

Patients (male or female, any age), referred for a DAT SPECT from 1st September 2016 to 30th September 2017 from The Royal Free hospital NHS trust; which includes The Royal Free, Barnet and Chase Farm hospitals. For the second part of the audit, only DAT SPECTs which were available in their raw format were included so they could be reconstructed for the SQA, for the purpose of the audit.

Exclusion criteria

Patients that were scanned at The Royal Free hospital NHS trust but **not referred** from the trust (and from other hospitals).

Data acquisition

A list of the DAT SPECTs conducted at the Royal Free Trust hospitals and the SPECT reports were collated from the nuclear medicine departments from The Royal Free Hospital and Barnet hospital (which also included data from Chase Farm hospital).

Indications were available on the DAT SPECT report, however they were brief in many cases. Therefore to correctly identify the referral and indication, preclinical information was additionally obtained from

patient records using the hospital databases (electronic document and records management; EDRM). Patient demographic information such as age and gender and follow up and management information was also obtained from patient records.

Prior to the audit

SPECT image acquisition and processing

All the DAT SPECTs had been conducted on the Siemens Symbia Dual headed scanners, available at the Royal Free hospital and Barnet hospital.

The scans were reconstructed by the hospitals using The Brain Registration & Analysis Software Suite (BRASS) manufactured by HERMES Medical. In cases of visual uncertainty, NMP used SQA using BRASS (SQA-B) to obtain SBRs to assess the scans semi quantitatively.

For the audit

For the second part of the audit, DAT SPECTs (which were available in their raw format; DICOM) were reconstructed (by HK) using GE's quantification software, DaTQUANT (GE Healthcare 2012). Image reconstruction was applied with OSEM 2 x 10 with a Butterworth filter 0.6 cut off.

7.3.1 Analysis of the audit

Comparison with licensed indications

Licensed indications and those indications out of license were those outlined by the **European Medicines Agency (**EMA) (2011), **Food and Drugs Administration (**FDA) (2011) and **Society of nuclear medicine** (SNM); (Djang et al., 2012, Bajaj et al., 2013):

European Medicines Agency (EMA) main licensed indications

1. DaTSCAN is indicated for detecting loss of functional dopaminergic neuron terminals in the striatum in Cases with clinically uncertain Parkinsonian Syndromes, in order to help differentiate Essential Tremor from Parkinsonian Syndromes related to idiopathic Parkinson's disease, Multiple System Atrophy and Progressive Supranuclear Palsy.
2. DaTSCAN is indicated to help differentiate probable dementia with Lewy bodies from Alzheimer's disease.

Food and Drugs Administration (FDA) main licensed indications

DaTSCAN may be used to help differentiate:

1. ET from tremor due to parkinsonian syndromes (idiopathic Parkinson's disease, multiple system atrophy and progressive supranuclear palsy).

Society of Nuclear Medicine (SNM) other licensed indications

1. Differentiation of presynaptic Parkinsonian syndromes from parkinsonism without presynaptic dopaminergic loss, such as drug-induced parkinsonism or psychogenic parkinsonism

Indications out of license are:

1. DaTSCAN is unable to discriminate between Parkinson's disease, Multiple System Atrophy and Progressive Supranuclear Palsy (EMA).
2. DaTSCAN is unable to discriminate between dementia with Lewy bodies and Parkinson's disease dementia (FDA).

DAT SPECT ratings

Prior to the audit:

The DAT SPECTs had already been rated for clinical purposes by nuclear medicine physicians (NMP) who provided descriptive reports of the pattern of uptake visible (as available on the DAT SPECT reports).

SQA by NMP

For scans that were also semi quantitatively analysed using The Brain Registration & Analysis Software Suite (BRASS) (SQA-B) (in cases of visual uncertainty), uptake under the following values was considered abnormal; CN; <1.37 and PUT; <1.35 (Pencharz et al., 2014) and were used by the nuclear medicine department at the Royal Free Hospital. Using BRASS, and with scans of 109 patients, Pencharz et al., 2014 calculated these specific cut off values for the PUT and CN by first measuring the minimum value for the CN and PUT; which were 1.43 and 1.41 respectively. However to include a margin of error, the following values of 1.37 and 1.35 to classify abnormal scans were used and with the authors reporting that the use of these values reduced the number of scans that were recorded as equivocal.

For the purpose of the audit

From the descriptions of the DAT SPECT uptake (provided in the reports) by the NMP, the SPECTs were then categorised in ratings of; normal, equivocal and abnormal by HK for the purpose of the audit.

VR by HK

To provide additional information regarding the specific patterns of uptake in the DAT SPECT, HK, who was blinded to all patients information except for age and gender (in line with the ratings by the NMP), also visually rated available DAT SPECTs (which were reconstructed for the purpose of the audit; below), using the rating criteria described in Chapter 4 “DAT SPECT in LOD”.

The colour scale in which the scans were rated in was GE colour, Rainbow, Cool (as shown in Figure 7-6).

Semi quantitative analysis of the DAT SPECTs

Available DAT SPECTs were semi quantitatively analysed using GE’s SQA software, DaTQUANT; specifically obtaining the SBRs for the PUT and CN and comparing these patients SBRs against the same control database available on DaTQUANT and as described in Chapter 4 “DAT SPECT in LOD”.

SBR cut off for abnormal uptake

Two standard deviation (SD) cut off

DAT SPECT scans were rated with AU using the SQA if they had L or R SBRs in the PUT or CN two SDs below SBRs of the same region and side of age matched control SBRs (available from DaTQUANT).

A cut off of two SDs from the mean for abnormal uptake has been considered appropriate in previous studies comparing visual and semi quantitative methods in DAT SPECT (Makinen et al., 2016).

Additionally, in the current audit, two SDs was used after investigating the number of cases rated with AU using one SD and 1.5 SDs cut offs and comparing with the visual ratings. Table 7-8 shows the number of DAT SPECTs rated as abnormal using the different cut offs and in comparison with the NMP visual ratings. Using a cut off of two SDs provided the best agreement with the visual ratings in identifying abnormal and normal SPECTs and hence this cut off was the most appropriate to use.

Calculating abnormal DAT SPECTs

Patients with PUT or CN SBRs two SD below the mean PUT and CN SBRs, of the same side (L or R) of controls of the same age in the control database were grouped into those with AU for their age. Patients with PUT or CN SBRs above or equal to the mean PUT and CN SBRs, of the same side (L or R) of controls of the same age in the control database were grouped into those with NU for their age. (Formula 7.1).

Formula 7.1: Calculating participants with abnormal and normal uptake

$$Abnormal\ uptake = \\ Participant\ left\ or\ right\ SBR < left\ or\ right\ age\ matched\ control\ \ SBR - 2\ SD$$

$$Normal\ uptake = \\ Participant\ left\ or\ right\ SBR \geq left\ or\ right\ age\ matched\ control\ \ SBR - 2\ SD$$

SBRs= putamen and/or caudate nuclei striatal binding ratios

Percentage of deviation from age matched mean SBRs

DaTQUANT also calculates the percentage that patient PUT and CN SBRs deviate from mean SBRs in age matched controls. The percentage of deviation was observed in scans visually rated as equivocal and to gain a better understanding of the scans where there were discrepancies between VR and the SQA. The percentage of deviation is calculated using Formula 7.2 below (GE healthcare, 2012). Deviations were calculated separately for each region (PUT or CN).

Formula 7.2 Calculating the percentage of deviation from mean SBRs from DaTQUANT's control database

$$Deviation = (Measured\ SBRs - Mean\ SBRs)/Mean\ SBRs * 100\%$$

Measured= Measured Case PUT or CN SBRs, Mean= Control database mean PUT or CN SBRs

Finally, lowest PUT and CN SBRs were also calculated, as described in Chapter 4 "DAT SPECT in LOD" to assess the SBRs in the scans visually rated as normal and abnormal.

7.3.2 Statistical analysis

The main analysis was a comparison between the indications used by the trust with licensed indications. Referrals and the number of correctly licensed indications were tabulated. Histograms were used to display medication use and effect on management following the DAT SPECT.

VR and SQA

Agreement between the DAT SPECTs rated abnormally using the VR and the SQA was analysed using Cohen's Kappa analysis.

All analyses and graphs were conducted using IBM SPSS statistics 24.

7.4 Audit results

Patients

Seventy four patients met the inclusion criteria of referral for a DAT SPECT from 1st September 2016 to 30th September 2017 from The Royal Free hospital NHS trust which includes The Royal Free, Barnet and Chase Farm hospitals. Follow up information was obtained from the time of the DAT SPECT until the time of when information was obtained for the audit; April 2018.

The 74 patients included in the audit were an average age of 70 (SD 11.7) years old, with ages ranging from 34-89 years of age. Forty six males and 28 females were included.

7.4.1 Part One: Indications for the DAT SPECT

1.1 Referrals and indications requested

The majority of referrals were from Neurology (n=56), followed by Geriatric medicine (n=13). Other referring specialities Nephrology, Medical oncology and Gynaecology (n=3) and 2 referrals were from unknown sources.

All referrals met licensed indications (Table 7-1). The most frequently requested indication was “Clinically uncertain PS” (n=49), followed by “Presynaptic Parkinson Syndromes vs Parkinsonism without presynaptic dopaminergic loss” (n=13) and “Essential tremor (ET) vs tremor due to parkinsonian syndromes” (n=12).

Table 7-1 Referrals and indications requested

Referrals	n
Neurology	56
Geriatric medicine	13
Other	3
Unknown	2
Licensed indications	**n**
Clinically uncertain Parkinsonian syndromes	**49**
iPD	29
MSA or PSP	14
DLB	6
Presynaptic Parkinson Syndromes vs parkinsonism without presynaptic dopaminergic loss	**13**
Drug induced	7
Vascular	5
Functional	1
Essential tremor (ET) vs tremor due to parkinsonian syndromes (including dystonic tremor)	**12**

iPD=idiopathic Parkinson's disease, MSA=Multiple system atrophy, PSP= Parkinson's supranuclear palsy, DLB=dementia with Lewy bodies

1.2 DAT SPECT results

The descriptive DAT SPECT reports by the NMP were categorised into normal, equivocal and abnormal by HK. NMP had rated 33 of the DAT SPECTs as visually normal including nine SPECTs rated as equivocal (within normal limits but mild asymmetry or reduction). Forty one DAT SPECTs had been rated as abnormal including five cases with vessel disease, infarcts or strategic lesions suggested as the cause of disrupted uptake in the SPECT (Table 7-2). The reports also revealed that the NMP had performed semi quantitative analysis using SQA-B on two cases. The SQA-B confirmed the visual ratings by the NMP (Table 7-5).

Impact of DAT SPECT on initial diagnosis

Following the DAT SPECT and looking back at the initial diagnoses, diagnoses of 71 out of the 74 cases were either confirmed or clarified (in the cases where there was uncertainty surrounding a PS or differentiating between ET and PS or drug induced vs PS symptoms). Two diagnoses remained unsure even after the DAT SPECT and one initial diagnosis was refuted following the scan.

In those with follow up and whom the diagnosis was mentioned, the diagnosis remained unchanged in 47 patients, with no follow up information regarding diagnosis available in 11 patients and unclear information in 16 patients (Table 7-4).

In **normal** DAT SPECTs

In patients visually rated with normal SPECTs (n=33), 21 diagnosis were confirmed (not a PS), nine were clarified as essential tremor and not PD and three were clarified as drug induced and not PD. At a follow up, the majority of diagnoses remained the same (n=19). In six patients there was no follow up available and in eight patients any follow up diagnosis was unclear (Table 7-4).

Recommendations

Other information obtained from the DAT SPECT reports included recommendations by the NMP. Three cases had been recommended for a follow up DAT SPECT and six recommended for MRI. Two DAT SPECTs were able to be rated but were criticised with suboptimal imaging.

Recommended for follow up

As mentioned above, in three cases follow up was recommended and two of these SPECTs had been visually rated by the NMP as normal or equivocal and one DAT SPECT as abnormal; although possibly due to an ischaemic infarct. Follow up information regarding a second DAT SPECT was not available at the time of the audit however an MRI was conducted in two of the cases with small vessel disease found in the SPECT with the suspected infarct.

Of these three SPECTs, the NMP had also conducted SQA-B which had also confirmed a normal scan (case 222; Table 7-5) alongside the visual analysis.

For the purpose of the audit, semi quantitative analysis using DaTQUANT on this scan also confirmed a normal report. The other two SPECTs were unavailable for analysis using DaTQUANT.

Impact of DAT SPECT on management

Seventy one patients had additional follow up information available regarding management (Table 7-6). Of these patients, 45 had a change in management which included 35 patients with a change in medication and five patients with a clinical referral and medication change. Additionally, five patients were referred to a specialist clinic. Ten patients did not require any change in management as the DAT SPECT confirmed a non-PS with no follow up required.

Medication use

Forty patients had a change in medication which included starting, stopping, increasing or decreasing medication, as shown in Figure 7-1.

Use of Levodopa (L-DOPA)

Within those patients (n=40) that had a change in medication following the DAT SPECT, 15 started levodopa (L-DOPA), three stopped (**not** due to a poor response), three started L-DOPA with another medication three patients increased their L-DOPA .

Table 7-2 DAT SPECT visual ratings breakdown (n=74)

Rating	Cases *n*
Normal	33
Equivocal	9
Abnormal	41
Suggested vascular cause	5

Table 7-3 Impact of DAT SPECT on initial diagnosis

Impact of DAT SPECT on initial	**All** DAT SPECTs	Normal DAT
	Number of Cases	
Confirmed or clarified	71	33
Refuted	1	
Still unsure	2	
MRI recommended in DAT report	6	1
Follow up DAT SPECT recommended	3	1
Suboptimal imaging	2	

Confirmed = Initial diagnosis of a suspected PS confirmed, Clarified=i.e. ET or PD, drug induced or PD, vascular or PD, unsure if PS
Refuted= Initial diagnosis changed
Still unsure=Initial diagnosis not confirmed, changed nor clarified from scan

Table 7-4 Follow up diagnosis following DAT SPECTs

	All DAT SPECTs	Normal SPECTs
Unchanged	47	19
No follow up diagnosis available	11	6
Unclear from notes	16	8

Table 7-5 Semi quantitative analysis using BRASS (SQA-B) by NMP to confirm visual ratings prior to the audit

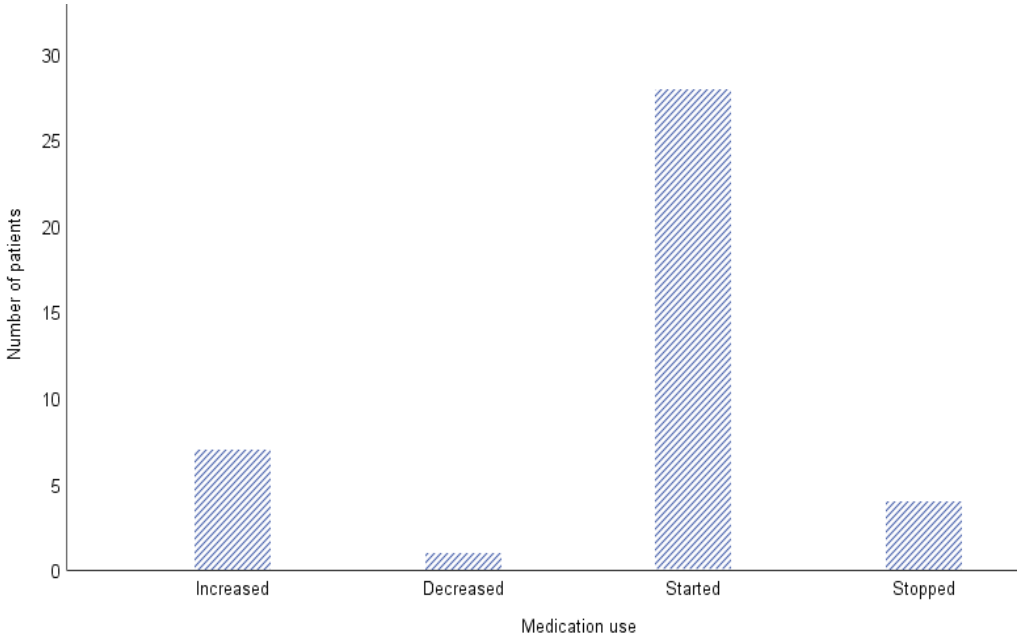
	Percentage of deviation from age matched control mean SBRs					
Case	L PUT	R PUT	L CN	R CN	NMP rating	SQA-B rating
222	+20	+8	+19	+16	Equivocal	Normal
240	-58	-65	-43	47	Abnormal	Abnormal

Table 7-6 Change in management following the DAT SPECT (n=45*)

Management	Patients *n*
Change in medication	35
Clinic referral	5
Change in medication and clinic referral	5

*number of patients with a change in management

Figure 7-1 Medication change in patients



7.4.2 Part Two: HK VR

The 41 scans available for SQA were reconstructed using DaTQUANT, and rated by HK, with the ratings compared with the ratings of the NMP.

Table 7-7 shows a breakdown of the visual ratings by HK. Fifteen scans were rated as normal and eight DAT SPECTs as equivocal.

Eighteen scans were rated with abnormal uptake which included five with type 1 uptake (activity in the region of the PUT of one hemisphere absent or greatly reduced with respect to the other), seven with type 2 (activity absent in the PUT of both hemispheres and confined to the CN), five with type 3 (activity absent in the PUT of both hemispheres and greatly reduced in one or both CN) and one scan rated with striatal loss (balanced striatal loss to the CN and PUT).

Figure 7-2 and Figure 7-3 display the lowest PUT and CN SBRs according to the visual ratings by HK to provide a comparison of the SBRs by ratings.

Table 7-7 Breakdown of visual ratings by HK

Rating	n (n=41)
Normal	15
Equivocal	8
Abnormal type 1	5
Abnormal type 2	7
Abnormal type 3	5
Striatal loss	1

Figure 7-2 Lowest putamen striatal binding ratios (PUT SBRs), by HK's visual ratings (n=41)

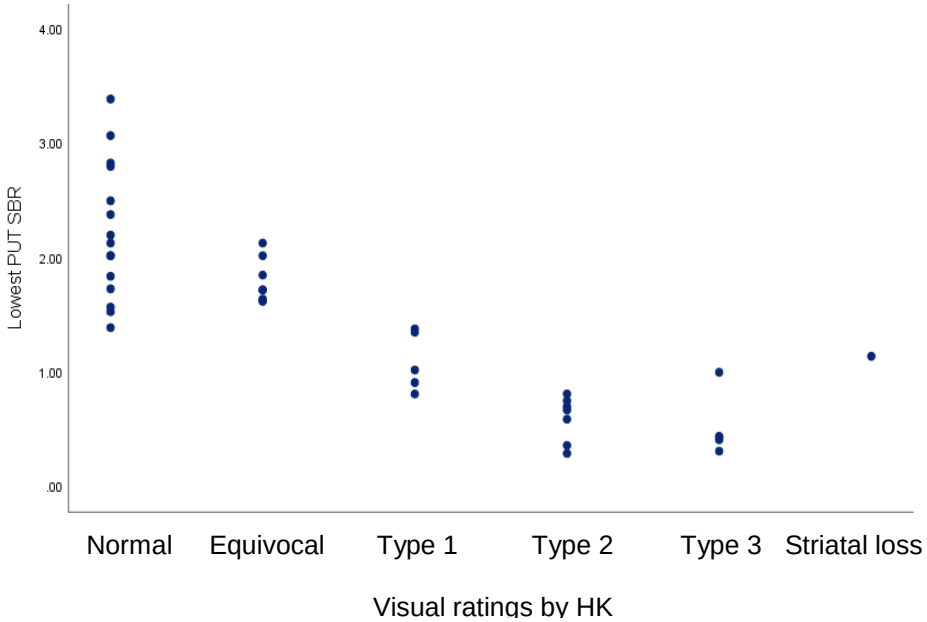
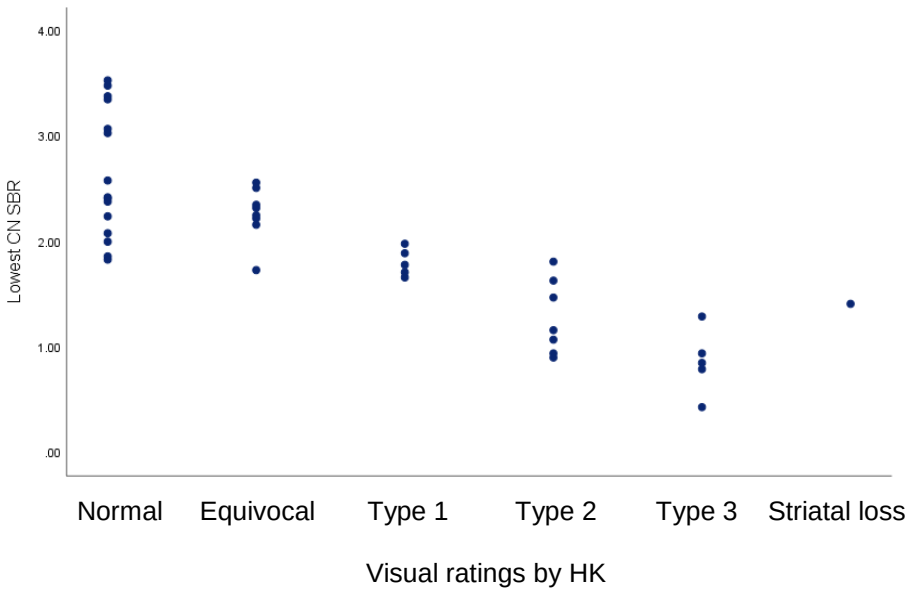


Figure 7-3 Lowest caudate nuclei striatal binding ratios (CN SBRs), by HK's visual ratings (n=41)



Inter rater agreement between HK and NMP visual ratings

There was very good inter rater agreement between the visual ratings by HK and the NMP; k=.851 p<.001 with agreement on 38 out of 41 SPECTs (with SPECTs rated as equivocal, as normal).

Discrepancies between HK and NMP ratings

Two SPECTs (case 214 and 256) rated as abnormal by HK were rated as normal by the NMP. One SPECT (case 182) that was rated as equivocal by HK was rated as abnormal by the NMP and detailed in Table 7-9.

Figure 7-4 also shows the NMP ratings of the scans visually rated as equivocal by HK. Four out of the eight scans were also rated as equivocal by the NMP; the same four rated as equivocal by HK. In particular, case 182 was rated as abnormal by the NMP. The percentage of deviations from age matched mean SBRs in these two patients using the SQA are shown in Table 7-9.

SQA

The visual ratings by HK were also compared with the SQA using the two SD cut off. There was very good agreement between the VR by HK and the SQA rating; k=.849, p=<.001, with agreement on 38 out of 41 scans.

Discrepancies between HK and the SQA included 3 SPECTs (case 215, 214, 256) rated as abnormal by HK and as normal by the SQA. These SPECTs are shown in Figure 7-7 and detailed in Table 7-9.

All of the scans rated as equivocal by HK were rated as normal using the SQA (within two SDs of the mean of age matched control SBRs).

Percentages of deviations from the control database SBRs in all SPECTs with discrepancies in ratings between HK, NMP and the SQA are shown in Appendix H.

7.4.3 Part Three: Comparison of NMP visual ratings and the semi quantitative analysis

Comparison between the visual ratings by the NMP, HK (normal (including equivocal) and abnormal) and the SQA in the available 41 SPECTs were also analysed.

Semi quantitative analysis cut offs

Agreement between the NMP and the SQA using the two SD cut off was excellent ($k=0.898$, $p<.001$) and with disagreement on the ratings of two scans. Using the one and 1.5 SD cut off, the same scans were rated with AU and both cut offs had lower agreement than a two SD cut off when compared with the NMP visual ratings; $k=0.799$, $p<.001$.

Two cases (182 and 215) were rated as abnormal visually by the NMP but as normal when using the SQA two SD cut off. A breakdown of the percentage of deviation from age matched control SBRs are shown in Table 7-9. These two cases were also rated as normal when using the one and 1.5 SD cut off. Figure 7-6 and Figure 7-7 display the DAT SPECT scans of these two cases (as reconstructed by HK, for the purpose of the audit, using DaTQUANT).

Visually equivocal scans by the NMP

Cases visually rated with a normal DAT SPECT (based on the reports by the NMP) included nine SPECTs that were categorised as equivocal. Of those SPECTs categorised with an equivocal by the NMP, HK rated five SPECTs as normal and four also as equivocal (Figure 7-5).

A list of the percentages that SBRs in these patients deviated from mean SBRs from age matched controls from DaTQUANT's control database are shown in Appendix H.

Table 7-8 Comparison of SQA ratings using different SDs with NMP visual ratings

SD	Rating	NMP visual ratings		
		Normal	Abnormal	Total abnormal SQA
1 SD	Normal	22	2	
	Abnormal	2	15	**17**
1.5 SD	Normal	22	2	
	Abnormal	2	15	**17**
2 SD	Normal	24	2	
	Abnormal	0	15	**15**

Table 7-9 Summary table of key cases discussed (where there were discrepancies between normal (including equivocal) and abnormal ratings between the visual raters and/or the SQA).

All DAT SPECTs in the table were rated as **normal** using the SQA (two SD cut off)

	Raters			Percentage of deviation from age matched control mean SBRs						
Case	NMP	HK	1/1.5 SD	L PUT	R PUT	L CN	CN R	Indication	DAT report	Follow up
182	A	E	N	+11	+5	+14	+13	Drug vs PS	Mild but definite reduction in right putamen	Diagnosed with PD-antipsychotics exacerbated systems. Started L-DOPA.
215	A	A	N	-18	+10	-6	+27	Uncertain PS	Reduction in left putamen	No follow up.
214	N	A	N	-10	-20	-0	-11	Uncertain PS	Normal study-normal uptake and normal background activity	No follow up.
256	N	A	A	-27	-34	-30	-21	Drug vs PS	Normal study-normal uptake and normal background activity	No clear diagnosis-AD? Not PD. Stopped L-DOPA-as patient fine without.
236	N	E	A	-27	-21	-15	-7	Uncertain PS	Normal study-normal uptake and normal background activity	No follow up.

Abbreviations: A= abnormal, E= equivocal, N=normal, NMP= Nuclear medicine physicians, 1/1.5 SD= 1 and 1.5 standard deviation cut off, SQA= Semi quantitative analysis, L/R=Left/Right side, PUT=putamen, CN=caudate nuclei, SBRs= Striatal binding ratios, PS= Parkinsonian syndrome. PD=Parkinson's disease, AD=Alzheimer's disease, L-DOPA=Levodopa medication

Figure 7-4 Ratings by NMP on DAT SPECTs rated as equivocal by HK
All DAT SPECTs were rated as normal using the SQA (2 SD cut off)

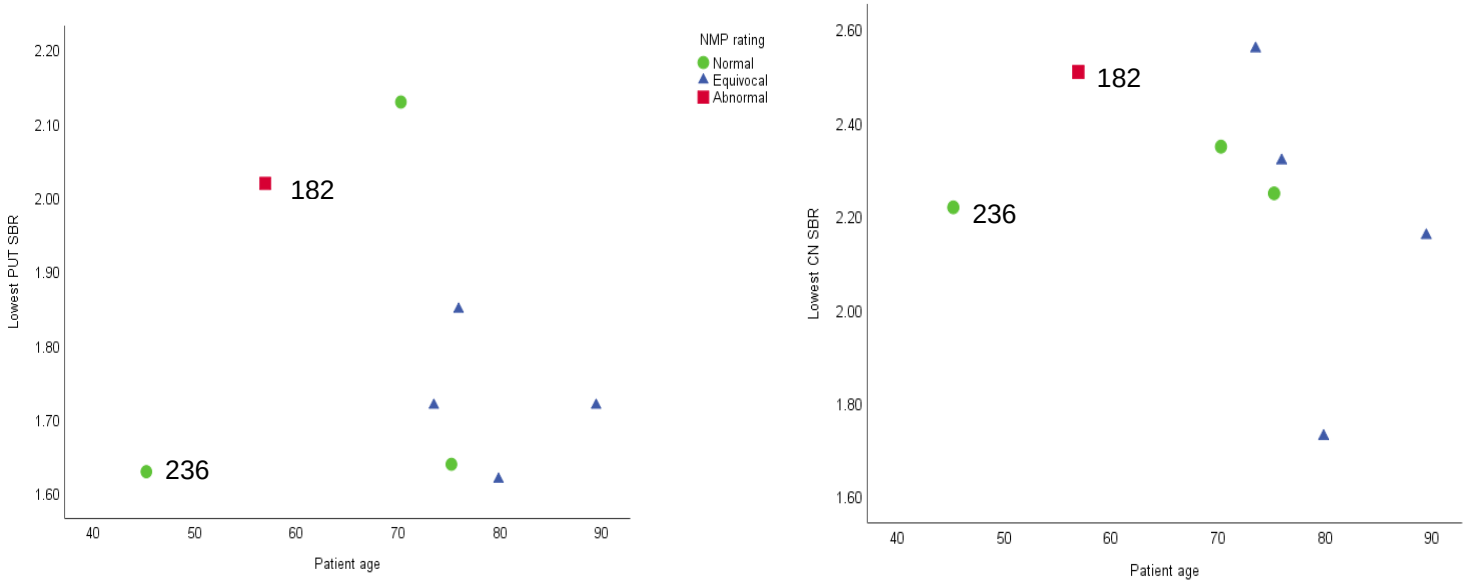


Figure 7-5 Ratings by HK on DAT SPECTs rated as equivocal by NMP

All DAT SPECTs were rated as normal using the SQA (2 SD cut off)

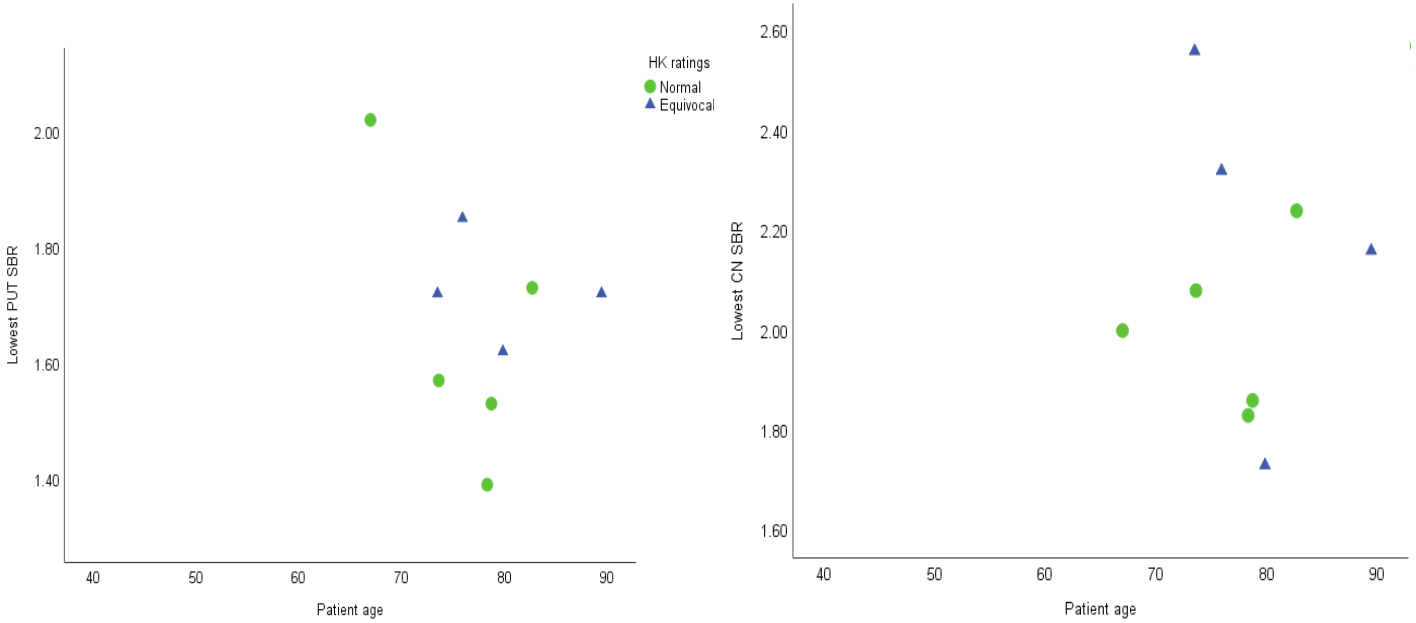


Figure 7-6 Case 182, a 57 year old female; the only patient with increased SBRs in the PUT and CN using the SQA but rated as abnormal by the NMP (and equivocal by HK)

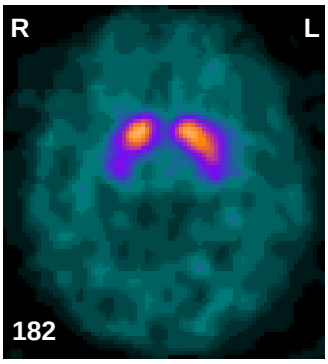
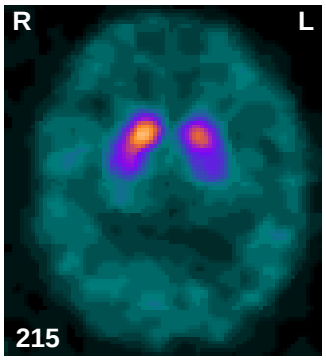
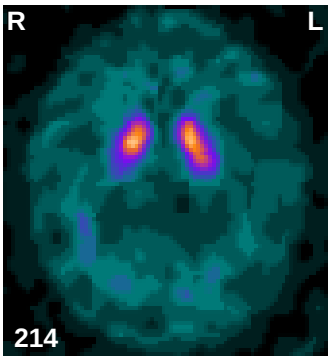
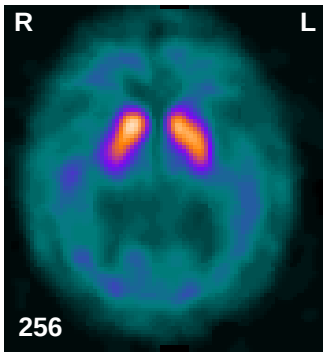
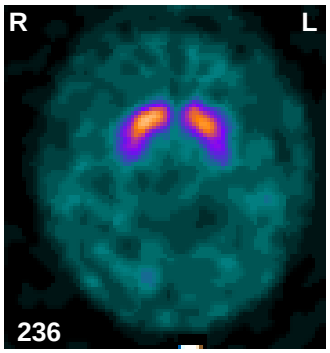


Figure 7-7 DAT SPECTs described in Table 7-9.
All rated as **normal** using the SQA (2 SD cut off).
Case 215, a 73 year old female, Case 214, a 79 year old male, Case 256, a 73 year old female and Case 236, a 45 year old male.









7.5 Discussion

All the referrals from the current audit met licensed indications, demonstrating correct use of the DAT SPECT in all cases. 44.6% cent of patients were reported with a normal SPECT and 55.4% with an abnormal SPECT and in almost all cases (95.9%), the SPECT helped to confirm or clarify the initial diagnosis. Follow up was conducted in 91.9% of patients; 63% had a change in management with a change in medication in the majority of patients. Post DAT SPECT, where available, follow up diagnosis remained unchanged in 63.5% of patients with no follow up diagnosis available in 14.9% of patients and unclear information in 21.6% of patients.

There was very good inter rater agreement between visual ratings by NMP and HK (k=.851). Agreement between visual by the NMP and the SQA using a two SD cut off was very good (k=0.898).

Indications

To our knowledge, the most recent (and available) audit of DAT SPECTs was the National DaTSCAN audit which was conducted in 2015 by the British Nuclear Medicine Society (BNMS). The BNMS audit (BNMS, 2015) consisted of data from 504 patients from 84 centres (The Royal Free Hospital Trust did not take part in this audit).

Results of the current audit were largely in line with the results reported by the BNMS. In the current audit 74.3 % of referrals were to clarify clinically uncertain PS (which included; PD, MSA, PSP) and clarifying ET. Similarly, the BNMS reported confirmation or exclusion of PD or PS to be most common indication (77.8 %).The next most common indication in the current audit was clarifying presynaptic PS from Parkinsonism without presynaptic dopaminergic loss (17.5%); which

included suspected drug induced PD (9.4%). Suspected drug induced PD was attributed to 5.5% of referrals in the BNMS audit. Other indications within the clarifying presynaptic PS from Parkinsonism without presynaptic dopaminergic loss category in the current audit included functional (1.4%) and vascular causes (6.8%).

The BNMS audit attributed 3.6% of requests to clarifying vascular causes. In the BNMS audit indications for suspected DLB tended to be higher covering 12.5% of requests with 8% per cent of the referrals clarifying DLB in the current audit.

DAT SPECT visual ratings

Of the 71 DAT SPECTs included in the audit, 44.6% were rated as normal and 55.4% were rated as abnormal by the nuclear NMP. The abnormal scans included 2% which attributed a dopaminergic deficit due to vascular pathology. Demonstrating the usefulness of reporting potential vascular causes, one of the critiques raised by the reviewers of the 2015 BNMS audit was the failure to consider vascular lesions as the cause of asymmetry in SPECTs rated as abnormal.

Inter rater agreement

The ratings by the NMP had very good agreement with the secondary rater, HK, who also visually rated the scans, with agreement on 38 out of 41 scans. A breakdown of the scans by HK revealed that the majority of the abnormal scans were rated with characteristic patterns of PD; abnormal type 1 (activity in the region of the PUT of one hemisphere absent or greatly reduced with respect to the other), type 2 (activity absent in the PUT of both hemispheres and confined to the CN) and type 3 (activity absent in the putamen of both hemispheres and greatly reduced in one or both CN). Despite 8% referrals

requesting a DAT SPECT to clarify DLB, only one scan was rated with balanced loss in both hemispheres (striatal loss rating), potentially (not always) indicative of DLB; with research reporting a balanced loss more evident in DLB than PD (Walker et al., 2004). This rating was only reported by HK and not the NMP.

Other results to note include suboptimal imaging in 2.7% of cases with no specific details. Suboptimal imaging was also reported in a minority of cases in the BNMS audit (4%) relating to low counts, over-smoothing of images, un-corrected tilt and patient motion artefact.

Impact on diagnosis and management

Following the DAT SPECT, diagnoses of 71 out of the 74 cases were either confirmed or clarified (in the cases where there was uncertainty surrounding a PS or differentiating between ET and PS or drug induced vs PS symptoms). Two diagnoses remained unsure even after the DAT SPECT and one initial diagnosis was refuted following the scan.

Follow up diagnosis

Follow up diagnosis remained unchanged in the majority of cases (n=47) with no follow up information regarding *diagnosis* available in 11 cases and unclear information in 16 of cases (Table 7-4).

In patients visually rated with **normal** SPECTs (n=33), All diagnoses were clarified (i.e. clarifying uncertain a PS, essential tremor and not PD or drug induced and not PD). At a follow up, the majority of diagnoses remained the same (i.e. confirmed ET or drug induced PD); n=19. In 6 cases there was no follow up available and in eight cases any follow up diagnosis was unclear.

With the majority of diagnoses (57.5%) unchanged in the follow ups post (a normal) DAT SPECT, it can be suggested that the SPECTs were correctly requested and helped confirm or clarify the presence of a presynaptic dopaminergic deficit. In the cases of lack of follow up information or unclear diagnosis, this may have been due to no change in initial diagnosis but this cannot be confirmed. Therefore although 44.6% of the SPECTs requested were normal, they were used appropriately.

A study by Booij et al. (2001) which assessed follow up diagnosis post DAT SPECT in in patients with inconclusive parkinsonism but with normal DAT SPECTS also reported essential tremor and drug induced post synaptic parkinsonism (amongst other non-presynaptic diagnosis) as common diagnoses in patients reported with normal SPECTs.

Recommended for follow up

In three cases, a follow up DAT SPECT was recommended by the NMP. Two of these SPECTs had been visually rated by the NMP as normal or equivocal and one as abnormal; but possibly due to an ischaemic infarct. Follow up information regarding a second DAT SPECT was not available at the time of the audit and perhaps a longer follow up period is required to ascertain final diagnoses. For example, Catafau and Tolosa (2004), who also investigated diagnosis and management following DAT SPECTs suggested (for their own study), a 24 month follow up in patients without definitive diagnosis to allow a formal validation of changes in management and diagnosis post SPECT.

Impact on management

Follow up information regarding **management** was available in the majority of patients (97%) and following the DAT SPECT.

Of the patients with follow up information, 63% had a change in management. Of the 63%, it is evident that the DAT SPECT had a large effect on management of medication with 77.7% of patients experiencing a change in medication and 11.1% of patients with both a change in medication and specialist clinical referral. Additionally 11.1% of patients were referred just to a specialist clinic. In terms of medication, 37.5% started L-DOPA, 7.5% increased L-DOPA dosage and 7.5% started L-DOPA alongside another medication. Graebner et al. (2017)'s study into the clinical impact of the DAT SPECT also reported a large effect on management with a change in management in 24 out of 27 cases following a DAT SPECT which specifically included changes in medication in 18 patients and psychological impacts for 11 patients.

Parts 2 and 3: Comparison between ratings

Visual ratings by the NMP and by HK

In the current audit, there was very good agreement between the VR of the DAT SPECTs (normal (including equivocal) and abnormal) between HK and the NMP. There were discrepancies between the raters on **three** SPECTs; two (case 214 and 256) of which were rated as abnormal by HK but rated as normal by NMP and one SPECT (case 182) rated as abnormal by NMP but normal by HK. Patterns of asymmetrical uptake in the PUT were evident in these scans; as also confirmed using the SQA. For example, case 214, who was rated

abnormal by HK (but normal by NMP) had a -10 decrease in the L PUT and a -20 decrease in the R PUT which visually could be interpreted as asymmetrical. There was no follow up available to ascertain final diagnosis.

Case 256 was rated with balanced striatal loss and an increase in background activity by HK with the low deviations in both the L and R PUT and CN (Table 7-9) reflecting the rating. This SPECT was rated as normal by NMP. The use of SQA with regards to this patient will be discussed below ("*Comparison between VR and the SQA*") with Djang D (2004) suggesting that SQA can be seen as an aid to visual interpretation; increasing diagnostic accuracy. A similar pattern of uptake which lead to a discrepancy between raters was also reported in the 2015 BNMS audit whereby the initial report had concluded normal activity but with clinical reviewers reporting decreased binding in the putamen bilaterally and increased **non-specific binding**.

HK rated eight scans as equivocal. Four of these SPECTs were also interpreted as equivocal by the NMP. Case 182 in particular (discussed further in the case report below) was rated as abnormal and three remaining scans as normal by NMP. The NMP rated nine scans as equivocal; all of which were rated as normal or equivocal also by HK.

Overall the very good agreement between the raters has been supported by previous studies also visually interpreting DAT SPECTs (Papathanasiou et al., 2012, Seibyl et al., 2014, Bajaj et al., 2013). Small discrepancies between raters are not uncommon with Papathanasiou et al. (2012) also reporting that with regards to their study (investigating interobserver reliability in DAT SPECT), any discrepancies between three blinded raters in four out of 89 cases largely stemmed from misinterpreting small equivocal defects in the

putamina as abnormal. Additionally, as an alternative explanation for discrepancies between raters, Bajaj et al. (2013) discussed that interpretation of the DAT SPECT can sometimes be challenged due to a number of factors including subtle anatomic asymmetry being mistaken for pathological uptake (in less experienced raters).

Comparison between the VR and the SQA

In the current audit, inter rater agreement between the visual ratings by the NMP and the SQA was very good, with agreement on 39 out of the 41 scans. SQA-B was also used by the NMP prior to the audit in two cases (details in Table 7-5) and discussed below).

Case 222 was rated with an equivocal DAT SPECT and the SQA-B confirmed normal uptake. When conducting SQA using DaTQUANT on this SPECT, the percentage of deviation from the control database showed very high uptake however some asymmetry between L and R hemispheres which may explain the equivocal rating by the NMP. The second case (case 240) displayed very low deviations from the control database and was also visually rated as abnormal. A potential reason for also running SQA-B on the SPECT despite the abnormal visual rating may have been due to the balanced loss in the left and right CN which visually may have been difficult to interpret. In both cases, the SQA-B was able to confirm the VR, with Darcourt et al. (2010) suggesting the use of SQA complementary to the visual ratings, to objectively assess uptake.

When analysing the 41 SPECTs for the purpose of the current audit, discrepancies between the NMP VR and the SQA (obtained by DaTQUANT) and using a two SD cut off were found in two SPECTs

(case 182 and 215). Both SPECTs had been visually rated as abnormal by the NMP but as normal using the SQA two SD cut off (both scans shown in Figure 7-7 and with details in Table 7-9).

Case 215, a 73 year old female, had reduced uptake in L PUT and L CN when compared to the control database; demonstrated by the reduced percentages of deviations. The clear asymmetry between the L (-18%) and R PUT (+10) can explain the visual interpretation of the SPECT (by both NMP and HK). However using the SQA (and all the cut offs one, 1.5 and two SD), this SPECT was rated within normality. There was no follow up available to assess progression; alternatively this may be indicative of a lack of progression of symptoms.

Case 256 (rated abnormal by HK only) and normal using the two SD cut off was a 73 year old female an overall decrease in SBRs in the L and R PUT and CN especially in comparison to a normal database, yet not enough decrease to be rated SQ normal (When using a two SD cut off). Makinen et al. (2016)'s study investigating visual vs automated analysis of DAT SPECTs in PD elaborated on 12 cases whereby there were discrepancies between visual and automated methods. The authors of the study reported that in these cases (where visual analysis was abnormal and the automated; normal) patients were significantly older compared to other patients with significantly lower (17.6 %) lower mean striatal tracer binding compared to normal scans but significantly higher binding (62.7%). Additionally, after a follow-up of at least 4.5 years, none of these patients developed a PS.

With regards to the current audit, any small discrepancies between raters may also be attributed to the use of different methods. For example the NMP will have rated the DAT SPECTs reconstructed using BRASS whilst the SPECTs rated by HK were reconstructed

using DaTQUANT. The overall agreement between the raters however is indicative of good agreement between the two methods.

Use of SQA in equivocal scans

Nine DAT SPECTs were rated with equivocal uptake (within normal limits but with mild reduction or asymmetry) by the NMP. The percentage of deviation from the normal database were observed in these scans to assess the use of SQA in equivocal scans. What was evident in the majority of the scans were the percentages of very high levels of uptake (indicative of a very normal scan) but the asymmetry which may explain why the scans were rated as equivocal. Similarly, an additional four DAT SPECTs rated as equivocal by HK were rated with normal uptake using the two SD cut off; highlighting the good agreement between visual ratings and the SQA in the majority of cases.

SQA; SD cut offs

Although agreement between the visual ratings by the NMP and the SQA using the one and 1.5 SD was high, a cut off of two SD was found to have the highest agreement with the visual ratings and chosen as the cut off for abnormal SPECTs. The impact of using different SDs will be discussed further below.

There was disagreement on 2 SPECTs between the one/1.5 SD and two SD cut off, with SPECTs of case 256 and 236 rated as abnormal if using the one/1.5 SD cut off. Both of these SPECTs were rated as normal by NMP and 256 as abnormal and 236 as equivocal by HK.

Case 256, a 73 year old female (as mentioned earlier) had an overall decrease in dopaminergic activity. When applying the two SD cut off this SPECT was rated as normal. The indication for this SPECT was

drug vs PS and the normal DAT SPECT report by NMP lead to a conclusion of confirmation of no evidence of PD. However the follow up report also states that this patient did not have a clear diagnosis following the SPECT with AD suggested.

Case 236, a 45 year old male, did not meet the threshold for abnormality using a two SD cut off but all had an over reduction in dopaminergic activity when compared with the SBRs of age matched controls and abnormal when using a one/1.5 SD cut off. This case was referred for a DAT SPECT due to uncertain PS and with no follow up available. Despite the lower SBRs in comparison to the other SPECTs this case was rated as normal and equivocal respectively, by the NMP and HK (as shown in Figure 7-4).

With regards to these two cases perhaps longer term follow up would have been useful. For example, cases of patients with parkinsionian symptoms, with normal SPECTs scan but then later being diagnosed with a PS have been reported. For example, in the study by Booij et al. (2001), a subject suspected of young-onset parkinsonism with a normal [^{123}I]FP-CIT SPECT was clinically diagnosed with multiple system atrophy in a later follow-up (due to reduced [^{123}] IBZM **postsynaptic** striatal uptake two weeks after the [^{123}I] FP-CIT SPECT). The authors of the study suggested this could be a rare case of a form of multiple system atrophy where only the postsynaptic (mainly putaminal) dopaminergic system was affected (and not yet the presynaptic system). Alternatively, a cut off of one SD is very low and in this case the same patients were rated with normal or abnormal DAT SPECTs when using one or 1.5 SDs.

Case report: Patient 182

Case 182 was referred for a DAT SPECT to clarify Parkinsonism due to use of antipsychotics or a dopaminergic deficit.

This DAT SPECT was rated as abnormal by NMP but normal (equivocal) by HK. Using the SQA, patient SBRs were deviating from mean control SBRs by +11 in the L PUT and +5 in the R PUT and with the NMP report stating (paraphrased) a “definite reduction in the R PUT”

The follow up of this patient, reported a diagnosis of PD and with the patient starting medication.

Whilst the SQA was able to demonstrate the asymmetry present between the L and R PUT SBRs, the SBRs of this patient were not found to be below the mean SBRs of age matched controls. HK’s rating of equivocal (and not definitely normal) with regards to this scan also highlights the visible reduction in uptake although, which was thought to be within normal limits rather than abnormal (as rated by the NMP).

The relatively intact SBRs, indicated by the SQA yet a diagnosis of PD can possibly be explained by the use of antipsychotics exacerbating very early PD symptoms.

Acton et al (2006) reported that the human visual system can identify extremely subtle patterns in the data, which simple regions of interest analysis cannot detect perhaps suggestive of the use of visual over SQA analysis. This would be another case which would be of interest for further follow.

To conclude, indications were correctly used and often made an impact on management and diagnosis. When following up the SPECTs rated

as **normal** by the NMP, the majority of diagnoses remained the same (n=19) indicating usefulness of the DAT SPECT.

Agreement between visual ratings and semi quantitative analysis was very good and the use of SQA in the current audit has been useful in confirming SPECTs rated as equivocal. The importance of further and long term follow up of patients to ascertain final diagnoses following a DAT SPECT has been highlighted; especially in cases where there were discrepancies between the visual read and the SQA and inconclusive diagnoses. The usefulness of the DAT SPECT however remained in 97% of cases where the SPECT clarified diagnosis based on the indication requested.

Appendices

Appendix A

Biobank criteria for incidental brain MRI findings

Table A4i: Incidental findings on brain MRI

Potentially serious for feedback	Not for feedback
Acute brain infarction	Asymmetrical ventricles
Acute hydrocephalus	Chiari malformation[4]
Acute intracranial haemorrhage[1]	Chronic hydrocephalus
Arachnoid cyst[3]	Developmental anomalies (including venous anomalies)
Colloid cyst of third ventricle	Lipoma of corpus callosum
Intracranial mass lesion[2]	Non-acute brain infarction
Mastoiditis	Non-specific white matter hyperintensities
Suspected intracranial aneurysm or vascular malformation	Regional or global atrophy
	Suspected demyelination

[1] Not old bleeds, or micro-bleeds only detected on gradient recalled echo sequences
[2] Except meningiomata in locations considered highly unlikely to cause problems
[3] Only if large and considered likely to increase the risk of developing a subdural haematoma
[4] Descent of part of the cerebellum +/- brainstem below the foramen magnum

Incidental finding list from the biobank criteria 30th September 2016

Appendix B

Medications taken by participants that may affect clinical scores

Clinical domain affected (clinical scale affected)	Medication type	pLOD on these medications excluded	HC on these medications excluded
RBD (RBDSQ, RBD1Q)	*Antidepressants*	Citalopram Sertraline Escitalopram Venlafaxine Fluoxetine Nortriptyline Amytriptyline Paroxetine	-
	Neuroleptics	Olanzapine Risperidone	-
Parkinsonism (MDS UPDRS ME)	*Neuroleptics and anti - hypertensives*	Olanzapine Risperidone Amlodipine	 Amlodipine
Hypotension/autonomic dysfunction (SCOPA-AUT)	*Antidepressants, Neuroleptics, anticonvulsants, anti-hypentensives*	Nortiptyline Risperidone Olanzapine Pregabalin Lisinopril Atenolol Losartan Amlodipine Ramipril Bisoprlol Bendroflumethiazide	 Amlodipine Ramipril Bisoprlol Bendroflumethiazide

Abbreviations: RBD= REM sleep behaviour disorder, MDS- UPDRS ME = Movement Disorder Society Unified Parkinson's Disease Rating scale Motor examination,RBDSQ= REM Sleep Behaviour Disorder single questionnaire, RBDQ= The REM sleep Behaviour Disorder screening questionnaire, SCOPA-AUT= Scales for Outcomes in Parkinson's disease- Autonomic.

Appendix C

Visual and semi quantitative DAT SPECT ratings when SBRs have not been adjusted for antidepressant use

Dichotomized ratings of putamen and caudate nuclei striatal binding ratios

	n (%) of participants		
Rating	pLOD	HC	p
Normal	21 (72)	22 (88)	
Abnormal	8 (28)	3 (12)	0.14

Side specific age adjusted semi quantitative analysis ratings of putamen striatal binding ratios

	n (%) of participants		
Putamen left			
Rating	pLOD	HC	p
Normal	25 (86)	22 (88)	
Abnormal	4 (14)	3 (12)	.316
Putamen right			
Normal	26 (90)	22 (88)	
Abnormal	3 (10)	3 (12)	.316

Side specific age adjusted semi quantitative analysis ratings of caudate nuclei striatal binding ratios

	n (%) of participants		
Caudate left			
Rating	pLOD	HC	p
Normal	23 (79)	23 (92)	
Abnormal	6 (21)	2 (8)	.065
Caudate right			
Normal	22 (76)	24 (96)	
Abnormal	7 (24)	1 (4)	.011

Appendix D

Correlations between lowest SBRs and clinical scores when excluding participants on the relevant medications

Clinical assessments	HC (n=25*)	pLOD (n=29*)	HC (n=25*)	pLOD (n=29*)
	PUT SBR (r,p)	PUT SBR (r,p)	CN SBR (r,p)	CN SBR (r,p)
MDS UPDRS ME	-.28 (.251)	.36 (.084)	-.43 (.074)	.27 (.195)
RBDSQ	.34 (.112)	-.26 (.399)	.32 (.138)	.06 (.851)
RBD1Q	.19 (.375)	.25 (.434)	.41 (.053)	.14 (.663)
SCOPA-AUT	.37 (.124)	.14 (.543)	.40 (.092)	-.06 (.801)
SCOPA-AUT²	.37 (.124)	.14 (.507)	.40 (.092)	-.08 (.818)

Partial correlations controlling for age and gender

*The following were excluded; 5 HC and 3 pLOD in the MDS UPDRS ME, 15 pLOD in the RBDSQ and RBD1Q and 5 HC and 5 pLOD in the SCOPA AUT and 7 in the SCOPA-AUT² (which includes patients taking Olanzapine, Risperidone and Pregabalin).

Appendix E

Breakdown of UPDRS ME ratings by DAT SPECT visual ratings in patients with LOD (pLOD)

MDS UPDRS ME	Abnormal (n=7*)	Normal (n=22*)		*p*
	Mean (SD)			
Rigidity Neck	1.28 (0.95)	0.13 (0.35)	18.50	**.002**
Rigidity UE	0.14 (0.37)	0.04 (0.21)	69.50	.709
Rigidity LE	0.85 (0.89)	0.09 (0.29)	38.00	**.048**
Speech	0.00 (0.00)	0.00 (0.00)	77.00	1.00
Facial expression	0.28 (0.48)	0.00 (0.00)	55.00	.280
Finger tapping	0.71 (0.95)	0.22 (0.42)	56.50	.304
Hand movement	0.00 (0.00)	0.04 (0.21)	73.50	.862
Pronation Supination	0.14 (0.37)	0.09 (0.29)	73.00	.862
Toe tapping	0.14 (0.37)	0.77 (1.50)	58.50	.354
Leg agility	0.00 (0.00)	0.13 (0.35)	55.50	.600
Arising from chair	0.00 (0.00)	0.09 (0.29)	70.00	.746
Gait	0.14 (0.37)	0.09 (0.29)	73.00	.862
Freezing of gait	0.00 (0.00)	0.00 (0.00)	77.00	1.00
Postural stability	0.00 (0.00)	0.04 (0.21)	73.50	.862
Global spontaneity of movement	0.42 (0.53)	0.13 (0.35)	54.50	.258
Posture	0.28 (0.48)	0.09 (0.29)	62.00	.469
Postural tremor	0.00 (0.00)	0.09 (0.42)	73.50	.862
Kinetic tremor	0.00 (0.00)	0.00 (0.00)	77.00	1.00
Rest tremor UE	0.14 (0.37)	0.04 (0.21)	69.50	.709
Rest tremor LE	0.00 (0.00)	0.00 (0.00)	77.00	1.00
Rest tremor Jaw	0.00 (0.00)	0.04 (0.21)	73.50	.862
Constancy of rest	0.00 (0.00)	0.00 (0.00)	77.00	1.00

Abbreviations: MDS- UPDRS ME = Movement Disorder Society Unified Parkinson's Disease Rating scale Motor examination, UE= Upper extremity, LE= Lower extremity

Appendix F

Mean clinical assessment scores between HC by DAT SPECT rating (SQA)

Clinical assessments	Abnormal (n=3)	Normal (n=22*)		*p*
	Mean (SD)			
Age in years	68.3 (9.9)	69.9 (9.1)	.278	.784
MoCA	28.3 (2.1)	26.6 (2.6)	-1.081	.291
FAB	16.7 (.1.5)	16.8 (1.4)	.174	.863
MDS UPDRS Total	4.3 (4.9)	6.6 (6.3)	24.000	.450
MDS UPDRS ML-EDL	0.7 (1.2)	1.5 (2.3)	27.000	.663
MDS UPDRS nM- EDL	2.0 (1.0)	4.4 (3.8)	21.000	.353
MDS UPDRS ME	1.7 (2.9)	0.7 (1.2)	31.500	.906
LARS	-29.3 (4.5)	-25.9 (4.8)	1.22	.318
BRAIN KS	59.5 (6.6)	57.6 (8.8)	-.357	.726
BRAIN AT	78.8 (29.7)	106.4 (56.8)	12.000	.250
PD Screening questionnaire	0.3 (0.6)	1.0 (1.3)	25.000	.550
HADS-D	1.0 (1.0)	1.3 (0.9)	28.500	.723
HADS-A	2.0 (2.0)	2.8 (2.3)	27.000	.663
DSM 5 SMD	0.7 (1.2)	1.4 (2.0)	27.000	.663
RBDSQ	1.7 (1.5)	2.3 (2.3)	29.000	.783
RBD1Q n (%)	0	2		.761
PDSS	130.8 (15.6)	127.2 (13.1)	-.435	.688
UPSIT	24.0 (10.1)	30.0 (6.4)	1.44	.163
SCOPA-AUT	4.3 (3.2)	8.2 (5.4)	19.000	.273

Independent samples t test, Mann Whitney U and Fisher's exact test
*Seven excluded from the BRAIN test
Abbreviations: MoCA= Montreal Cognitive Assessment, FAB=Frontal Assessment Battery, MDS- UPDRS= Movement Disorder Society Unified Parkinson's Disease Rating scale; ML EDL= Motor Aspects of Experiences of Daily Living, nM-EDL= Non Motor Aspects of Experiences of Daily Living, UPDRS Motor examination= Unified Parkinson's Disease rating scale , LARS= Lille Apathy Rating Scale, BRAIN test= BRadykinesia Akinesia Incoordination Test; KS= kinesia score, AT; akinesia time,PD screening= Parkinson's Disease scale, HADS = Hospital Anxiety and Depression Scale; A=anxiety, DSM 5 SMD=DSM 5 criteria Severity Measure of Depression, RBDSQ= REM Sleep Behaviour Disorder single questionnaire, RBDQ= The REM sleep Behaviour Disorder screening questionnaire, PDSS= Parkinson's disease Sleep Scale, UPSIT= University of Pennsylvania Smell Identification Test, SCOPA-AUT= Scales for Outcomes in Parkinson's disease- Autonomic.

Appendix G

Raw clinical scores for each participant rated with an abnormal DAT SPECT (visual and SQA ratings)

	Pt 1	Pt 2	Pt 3	Pt 4	Pt 5	Pt 6	Pt 7
Participant group	pLOD	pLOD	pLOD	pLOD	pLOD	pLOD	pLOD
Age /Gender	60/M	59/F	78/F	75/M	63/M	79/M	76/M
MoCA	23	29	21	23	22	25	19
FAB	16	18	17*	17*	N/A	13	N/A
MDS UPDRS Total	17	28	18	26	50	26	26
ML-EDL	2	5	2	2	11	1	7
nM- EDL	10	22	13	21	34	17	15
MDS UPDRS	5	1	3	3	5	8	4
LARS	-30	-21	-34	-33	-15	-21	5
Average BRAIN KS	N/A	64.5	42.5	-888	33	41	N/A
Average BRAIN AT	N/A	81	112	-888	350	172	N/A
PD Screening Q	5	3	2	1*	6	1*	6
HADS Anxiety	10	15	7	8	16	14	7
HADS Depression	11	10	7	2	15	14	16
DSM 5 SMD	16	15	5*	5*	22	12	3
LOD Duration (years)	2		1		4	2	2
Antidepressant use		SNRI	SSRI	SSRI	SSRI	SNRI	
RBDSQ	3*	8	6	10	9	2	0
RBD1Q	N	N	N	N	Y	N	N
PDSS	98	68	96	65	70	103	112
UPSIT	26	35	33	36	19	25	21
UPSIT age and gender percentile/ olfactory diagnosis	13th /MM	28thh /N	57th /MiM	87th /N	11th /SM	27th /SM	23rd /SM
SCOPA-AUT	28	28	15	11	39	11	20
Visual Ratings	A	N	N	N	A	A	A
Left and right side striatal binding ratios (SBRs) using DaTQUANT							
Left PUT SBR	2.87	2.04	2.03	1.78	1.78	1.79	1.90
Right PUT SBR	2.82	2.14	2.09	1.83	1.82	1.73	1.93
Left CN SBR	1.95	2.22	1.89	2.26	1.88	1.97	1.87
Right CN SBR	1.92	2.35	1.94	1.95	1.95	1.70	2.03

	Pt 8	Pt 9	Pt 10	Pt 11	Pt 12	Pt 13	Pt 14
Participant group	HC	pLOD	HC	pLOD	pLOD	HC	pLOD
Age /Gender	73/ M	64/M	74/M	78/M	76/M	56/M	77/M
MoCA	26*	24	29	29	16	30	26*
FAB	17*	18	15	18	17*	18	17*
MDS UPDRS Total	10	14	2	9	1	1	21
ML-EDL	2	3	0	1	0	0	5
nM- EDL	3	8	2	8	0	1	10
MDS UPDRS	5	3	0	0	1	0	6
LARS	-29	-20	-34	-27	-22	-25	-30
Average BRAIN KS	52	N/A	62	71	37	64.5	41.5
Average BRAIN AT	70	N/A	111.5	77	166.5	55	62
PD Screening Q	1	1	0	2	0	0	1
HADS Anxiety	0	5	0	3	1	4	9
HADS Depression	1	3	0	7	3	2	3
DSM 5 SMD	0	7	0	3	0	2	4
LOD Duration (years)	-	10	-	1	6	-	2
Antidepressant use	-		-		SNRI	-	
RBDSQ	0	7	2	5	3*	3*	7
RBD1Q	N	Y	N	Y	N	N	Y
PDSS	137.5	89	142	57	136	113	111
UPSIT	15	29	22	28	18	35	15
UPSIT age and gender percentile/ olfactory diagnosis	8th /A	15th /MM	23rd /SM	32nd /MM	15th /A	56th /N	10th /A
SCOPA-AUT	2	13	3	16	6	8	18
Visual Ratings	N	A	E	E	A	A	A
Left and right side striatal binding ratios (SBRs) using DaTQUANT							
Left PUT SBR	1.82	1.69	1.65	1.83	1.60	1.57	1.32
Right PUT SBR	1.89	2.12	1.71	1.63	1.46	1.46	1.27
Left CN SBR	1.97	1.95	2.23	2.20	1.54	1.62	1.79
Right CN SBR	2.25	2.21	2.34	2.05	1.75	1.50	1.74

Appendix H

All discrepancies between nuclear medicine physicians (NMP), HK and the semi quantitative analysis (SQA). All scans were rated as normal using the 2 SD cut off

	Raters			Percentage of deviation from age matched control mean SBRs						
Case	NMP	HK	1/1.5 SD	L PUT	R PUT	L CN	CN R	Indication	DAT report	Follow up
182	A	E	N	+11	+5	+14	+13	Drug vs PS	Mild but definite reduction in right putamen	Diagnosed with PD-antipsychotics exacerbated systems. Started L-DOPA.
183	E	N	N	+10	+17	+20	+17	Uncertain PS	Mildly increased background uptake. No evidence of PS	Unclear
184	E	N	N	-6	+7	-4	-4	Uncertain PS (DLB)	Slight asymmetry in striata but not indicative of DLB/PS	Unclear
185	E	N	N	+13	-9	+5	+2	DLB vs vascular	Minimal asymmetry between L and R PUT	Unclear

211	N	E	N	+0	+14	+20	+12	Uncertain PS (DLB)	Normal	Mild cognitive impairment
215	A	A	N	-18	+10	-6	+27	Uncertain PS	Reduction in left putamen	No follow up.
214	N	A	N	-10	-20	-0	-11	Uncertain PS	Normal study- normal uptake and normal background activity	No follow up.
224	E	N	N	+16	+14	-1	-3	ET from PD	Very subtle reduction in R posterior putamen	Essential tremor
226	E	N	N	-14	-16	-4	+3	Uncertain PS	Slight heterogeneity bilaterally may be vessel disease	Likely ET but under observation for progression to PD. MRI showed severe atrophy.
256	N	A	A	-27	-34	-30	-21	Drug vs PS	Normal study- normal uptake and normal background activity	No clear diagnosis **not** PD. Thought to be AD. Stopped L-DOPA-as the patient seemed fine without it.
236	N	E	A	-27	-21	-15	-7	Uncertain PS	Normal study- normal uptake and normal background activity	No follow up.
244	N	E	N	+24	+39	+33	+15	Uncertain PS (DLB)	Mild asymmetry in L PUT- small vessel disease suggested	No evidence of MRI. No follow up
265	E	E	N	+3	-3	-7	-13	Uncertain PS	Mild reduction in R PUT tail	No clear diagnosis, symptoms improved

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