Biomarkers in Dementia with Lewy Bodies

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Abstract

Background

Dementia with Lewy bodies (DLB) is the second commonest type of neurodegenerative dementia, but accurate antemortem diagnosis remains challenging, especially at the earliest (prodromal) disease stages and in the presence of mixed (Alzheimer) pathology. To investigate this we undertook two studies, an investigation of ^{123}I-FP-CIT imaging as a possible biomarker of prodromal DLB (at the mild cognitive impairment (MCI) stage), and a study of the effect of amyloid deposition measured by ^{18}F-Florbetapir PET on clinical phenotype in established DLB.

Methods

Prodromal DLB Study: 53 subjects with MCI and symptoms suggestive of Lewy body disease underwent comprehensive clinical and cognitive assessment and ^{123}I-FP-CIT SPECT imaging.

Amyloid Imaging Study: 22 DLB, 10 Alzheimer's disease (AD) and 15 control subjects underwent comprehensive clinical and cognitive assessment, MRI and ^{18}F-Florbetapir PET amyloid imaging.

Results

Prodromal DLB Study: An abnormal ^{123}I-FP-CIT scan was associated with increased rates of parkinsonism and RBD, but was not associated with a specific pattern of cognitive impairment. The pattern of ^{123}I-FP-CIT binding loss was symmetrical. Males were more likely to have an abnormal scan than females.

Amyloid Imaging Study: AD subjects displayed greater amyloid binding than DLB in frontal, temporal, cingulate and striatal regions. There were no significant differences between DLB and controls, but binding in DLB was intermediate between AD and controls in all regions. Frontal:Occipital binding ratio differentiated AD from DLB and controls. There were no consistent effects of amyloid on the phenotype of DLB subjects.

Conclusions

^{123}I-FP-CIT SPECT appears to be a marker of Lewy body disease in the prodromal stage. Longitudinal analysis is needed to determine its sensitivity and specificity.

Amyloid deposition is present in a proportion of DLB subjects, but is not associated with a clear difference in clinical phenotype. Longitudinal follow-up will determine whether it is associated with a difference in disease progression.

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Declaration

No portion of the work in this thesis has been submitted in support of an application for another degree or qualification at this or any other university or institute of learning. I confirm this thesis is my own work, and any assistance from others is acknowledged.

Professors Thomas and O'Brien were the Principal Investigators responsible for the original applications for grant funding and ethical approval for these studies. The author was responsible for the resubmission of documents to the ethics committee for the amyloid imaging study.

The author was responsible for the management of the projects under the supervision of Professors Thomas and O'Brien. This included the recruitment and clinical assessment of study subjects, formulating the hypotheses for this thesis and carrying out statistical analyses.

Neuropsychological assessment and carer questionnaires were carried out by Kirsty Olsen, the author and Nicola Barnett. Data entry and management was carried out by Kirsty Olsen. Clinical diagnosis was carried out by the author, Professor Thomas and Professor O'Brien.

The author carried out semiquantitative analysis of amyloid PET and FP-CIT SPECT scans under the supervision of Dr Michael Firbank and Dr Sean Colloby. The methods used for amyloid imaging were developed for this research by Dr Firbank and the author. The method of analysis of FP-CIT images was adapted from Dr Colloby's previous methods by Dr Colloby and the author. The measurement of hippocampal volume was performed by the author using a fully automated script developed by Dr Firbank.

Dr Firbank, Prof O'Brien, Dr Jim Lloyd, Dr George Petrides and the author performed visual analysis of the amyloid PET images. Dr Colloby, Prof Thomas, Dr Lloyd and the author performed the visual rating of the FP-CIT SPECT scans.

The author was responsible for the writing of the thesis.

Publications

The following publications reviewing prodromal dementia with Lewy bodies and amyloid imaging in Lewy body disorders were the result of background reading for this PhD and form the basis of the introductory chapters of this thesis. The publications do not contain any data from this thesis.

Donaghy, P., O'Brien, J.T. and Thomas, A. (2015) 'Prodromal dementia with Lewy bodies', *Psychological Medicine*, 45(2), pp. 259-268.

Donaghy, P., Thomas, A.J. and O'Brien, J.T. (2015) 'Amyloid PET Imaging in Lewy Body Disorders', *Am J Geriatr Psychiatry*, 23(1), pp. 23-37.

Donaghy, P.C. and McKeith, I.G. (2014) 'The clinical characteristics of dementia with Lewy bodies and a consideration of prodromal diagnosis', *Alzheimers Res Ther*, 6(4), p. 46.

Contents

List of Tables

List of Figures

Abbreviations

Aβ	Amyloid beta
ACE-R	Addenbrooke's Cognitive Examination Revised
AChI	Acetylcholinesterase inhibitor
AD	Alzheimer's disease
ADC	Apparent diffusion coefficient
AD-MCI	Alzheimer's disease mild cognitive impairment
ADNI	Alzheimer's Disease Neuroimaging Initiative
aMCI	Amnestic mild cognitive impairment
APOE	Apolipoprotein E
APP	Amyloid precursor protein
αSyn	Alpha-synuclein
AUC	Area under the curve
AVLT	Rey Auditory Verbal Learning Test
BADLS	Bristol Activities of Daily Living Scale
CAF	Clinician Assessment of Fluctuation
CDR	Clinical Dementia Rating Scale
CERAD	Consortium to Establish a Registry for Alzheimer's Disease
CIRS-G	Cumulative Illness Rating Scale for Geriatrics
Cr	Creatine
CRT	Choice reaction time
CSF	Cerebrospinal fluid
DCFS	Dementia Cognitive Fluctuations Scale
DeNDRoN	Dementia and Neurodegenerative Diseases Research Network
DICOM	Digital Imaging and Communications in Medicine
DLB	Dementia with Lewy bodies
DTBZ	Dihydrotetrabenazine
DVR	Distribution volume ratio
DWI	Diffusion weighted imaging
ECD	Ethyl cysteinate dimer
ESS	Epworth Sleepiness Scale
FAS	FAS verbal fluency test
FDG	Fluorodeoxyglucose

FP-CIT	n-fluoropropyl-2β-carbomethoxy-3β-(4-iodophenyl)nortropane
FTD	Frontotemporal dementia
FWE	Family-wise error
GCSI	Gastroparesis Cardinal Symptom Index
GDS	Geriatric Depression Scale
HC	Healthy control
IADL	Instrumental Activities of Daily Living Scale
LB	Lewy body
LBD	Lewy body dementia (DLB and PDD)
LB-MCI	Mild cognitive impairment due to Lewy bodies
LBSQ	Lewy Body Symptom Questionnaire
L-DOPA	Levodopa
LN	Lewy neurite
MBq	Megabecquerel
MCI	Mild cognitive impairment
MDS-UPDRS	Revised Unified Parkinson's Disease Rating Scale
MMSE	Mini Mental State Examination
MNI	Montreal Neuroimaging Initiative
MPRAGE	Magnetisation-prepared rapid gradient echo
MRI	Magnetic resonance imaging
MSA	Multiple System Atrophy
MSQ	Mayo Sleep Questionnaire
NAA	N-acetylaspartate
naMCI	Non-amnestic MCI
NEVHI	North-East Visual Hallucinations Interview
NFT	Neurofibrillary tangle
NIAAA	National Institute on Ageing/Alzheimer's Association
NifTI	Neuroimaging Informatics Technology Initiative
NPI	Neuropsychiatric Inventory
OR	Odds ratio
PD	Parkinson's disease
PDD	Parkinson's disease dementia
PD-MCI	Parkinson's disease with mild cognitive impairment
PET	Positron Emission Tomography

PiB	Pittsburgh Compound B
PSEN1	Presenilin 1
PSEN2	Presenilin 2
RBD	Rapid eye movement sleep behaviour disorder
rCBF	Regional cerebral blood-flow
REM	Rapid eye movement
ROI	Region of interest
SCARB2	Scavenger receptor B2
SD	Standard deviation
SMC	Subjective memory complaint
SNCA	α-synuclein gene
SPECT	Single photon emission computed tomography
SPM	Statistical Parametric Mapping
SRT	Simple reaction time
SUVR	Standardised uptake value ratio
UPDRS	Unified Parkinson's Disease Rating Scale
VaD	Vascular dementia

Chapter 1 Introduction

Dementia is a decline in cognitive function that interferes with a person's ability to undertake their usual activities, which is not explained by delirium or another psychiatric disorder (McKhann et al., 2011). It is one of the most important public-health issues confronting our society. Around 36 million people worldwide were living with dementia in 2010 and this will rise to over 110 million people by 2050 (Prince et al., 2013). Approximately 670,000 people in the UK have dementia, or 6.5% of the population over 65 years of age (Matthews et al., 2013). The prevalence rises with age, from around 1-2% in those aged 65-69 years to 10% in those aged 80-84 years (Matthews et al., 2013). This high prevalence is associated with significant distress for patients and carers, along with substantial financial costs to society (Wimo et al., 2013). In light of this it is unsurprising that finding a treatment for dementia is seen as a priority for national governments (G8, 2013).

There are many different dementia subtypes, but Alzheimer's disease, vascular dementia, dementia with Lewy bodies, Parkinson's disease dementia and frontotemporal dementia together account for the vast majority of cases (Stevens et al., 2002). Dementia with Lewy bodies (DLB) is the second most common type of neurodegenerative dementia after Alzheimer's disease (AD), accounting for over 4% of diagnosed cases (Vann Jones and O'Brien, 2014). It is characterised by the presence of visual hallucinations, parkinsonism, cognitive fluctuations, REM sleep behaviour disorder, sensitivity to antipsychotic medications and reduced striatal dopamine transporter levels on SPECT or PET imaging (McKeith *et al.*, 2005).

Recent unsuccessful trials of anti-amyloid therapies in AD have led some to suggest that commencing treatment after dementia has developed may be too late, as irreversible widespread neuronal damage has already occurred (Aisen et al., 2013). As a result, increasing attention has been given to the identification of dementia subtypes in their pre-dementia or prodromal phase. Identifying prodromal dementia enables researchers to examine the early pathophysiological processes involved in each dementia subtype and to develop and test treatments aimed at stopping or slowing these specific processes. Biomarkers that measure the presence of a specific brain pathology (e.g. amyloid PET imaging), or that are indicative of a particular dementia subtype (e.g.

striatal dopamine transporter reduction in DLB) may be important tools to identify the pathological subtype of dementia more accurately and much earlier in the disease process. This factor was the key driver behind the studies which comprise this thesis, which will investigate amyloid PET imaging in established DLB and striatal dopamine transporter imaging in prodromal DLB.

1.1 Dementia with Lewy Bodies

1.1.1 Epidemiology

Dementia with Lewy bodies (DLB) is the second most common type of neurodegenerative dementia after Alzheimer's disease (AD). A recent meta-analysis found that DLB accounts for 4.2% of dementia cases in community samples and 7.5% of cases in secondary care, with an incidence of 0.87 per 1000 person-years (Vann Jones and O'Brien, 2014). The increased prevalence seen in secondary care may be due to increased rates of neuropsychiatric symptoms and greater caregiver stress in DLB compared with AD (Walker et al., 2012). The actual prevalence and incidence of DLB may be significantly higher, due to under-diagnosis (Boot, 2013). One study that compared clinical diagnosis with pathological findings reported that only 52/162 cases of diffuse cortical LB disease (in the absence of significant neurofibrillary tangle (NFT) pathology) were classed as 'pure DLB' clinically (Nelson et al., 2010). In the overall sample 14.3% of dementia cases had 'diffuse neocortical type LB disease', with more than half of these also having significant NFT pathology.

1.1.2 Clinical features and diagnostic guidelines

DLB is a progressive cognitive disorder of sufficient severity to interfere with normal social or occupational function accompanied by other characteristic symptoms which will be discussed below (McKeith *et al.*, 2005).

1.1.2.1 Cognitive Impairment

The pattern of neuropsychological deficits seen in DLB is different from AD, with less marked memory impairment and more severe impairments of visuospatial, attentional and frontal-executive function (Metzler-Baddeley, 2007).

DLB is also associated with pronounced and clinically relevant fluctuations in arousal, cognition and function. Indeed, cognitive fluctuation is a core feature in the diagnostic criteria for DLB. Carers of those with DLB are more likely to report the person to have significant differences in function during the day, to be drowsy or to sleep during the day, to stare into space for long periods and to have periods where their flow of ideas seems disorganised (Ferman *et al.*, 2004; Lee *et al.*, 2014). These fluctuations in alertness and concentration can be demonstrated in computerised tests of reaction times (Ballard et al., 2001). Severe fluctuations can manifest as transient, unexplained episodes of loss of consciousness (McKeith *et al.*, 2005). Fluctuations may be most specific to DLB in the milder stages (MMSE >18) of cognitive impairment (Lee *et al.*, 2014).

1.1.2.2 Neuropsychiatric Symptoms

DLB is associated with a greater degree of neuropsychiatric symptoms than AD, and associated carer distress (Walker et al., 2012). Visual hallucinations are common in the early stages of DLB, unlike AD (Ferman *et al.*, 2013a). These tend to be vivid and most commonly feature people or animals (Aarsland et al., 2001a). Other visual symptoms seen in dementia, such as misperception and misidentification of family members tend to appear earlier in DLB than AD (Ferman *et al.*, 2013a). DLB is also associated with higher rates of auditory hallucinations, delusions and depression (Ballard *et al.*, 1999; Aarsland *et al.*, 2001a).

Rapid eye movement (REM) sleep behaviour disorder (RBD) is a parasomnia characterised by the enactment of dreams (e.g. punching, kicking, shouting out) that often results in injury (Schenck and Mahowald, 2002). It is strongly associated with the synucleinopathies (DLB, Parkinson's disease (PD), PD dementia (PDD) and multiple system atrophy (MSA)) (Iranzo et al., 2013), and can be a very early symptom of DLB (Boeve et al., 2003). RBD is one of the strongest predictors of a diagnosis of DLB at post mortem (Ferman et al., 2011).

1.1.2.3 Physical symptoms

DLB is associated with severe sensitivity to antipsychotic medications, with reactions including confusion, sedation, rigidity, immobility, features of neuroleptic malignant syndrome (fever, rigidity and raised serum creatinine kinase) and increased mortality

(McKeith *et al.*, 1992). Sensitivity can occur both with typical and atypical neuroleptics (Ballard et al., 1998).

Extrapyramidal symptoms are common in DLB (Del Ser *et al.*, 2000; Aarsland *et al.*, 2001b). Compared with Parkinson's disease, people with DLB are more likely to have predominant symptoms of postural instability and gait difficulty, rather than the tremor dominant phenotype (Burn et al., 2006). However, Ballard et al. investigated the signs most useful in identifying parkinsonism in DLB and found that rest tremor, action tremor, bradykinesia, decreased facial expression and rigidity were most characteristic of DLB (Ballard et al., 1997).

Autonomic features such as orthostatic hypotension, constipation and urinary incontinence are also common in DLB, and may be associated with increased mortality (Stubendorff et al., 2012).

1.1.3 Diagnostic Criteria

The diagnostic criteria for DLB were agreed by international consensus in 1996 (McKeith et al., 1996) and revised in 2005 (McKeith *et al.*, 2005). The current (2005) criteria are summarised in Table 1.1. A conclusive (definite) diagnosis of DLB can only be made at post-mortem, but a diagnosis of 'probable' or 'possible' DLB can be made based on clinical presentation. Three symptoms are defined as 'core features': recurrent visual hallucinations, spontaneous motor parkinsonism and fluctuations in cognition. Three 'suggestive features' are also identified: rapid eye movement sleep behaviour disorder (RBD), reduced dopamine transporter uptake in the basal ganglia on PET or SPECT imaging and severe neuroleptic sensitivity. The presence of two core features, or one core feature and one or more suggestive features in a person with dementia is sufficient for a diagnosis of probable DLB. Possible DLB is diagnosed if one core feature is present without other suggestive features, or if one or more suggestive features are present in the absence of core features.

DLB is clinically and pathologically similar to PDD. DLB and PDD are differentiated on the basis of the '1-year rule' (McKeith *et al.*, 2005), meaning that DLB is diagnosed if dementia develops prior to, or within one year of parkinsonism. PDD should be diagnosed if dementia develops more than one year after parkinsonism.

Table 1.1. Diagnostic criteria for DLB (from McKeith *et al.*, 2005)

1. Central feature

Dementia defined as progressive cognitive decline of sufficient magnitude to interfere with normal social or occupational function. Impairments of attention, executive and visuospatial function may be particularly prominent.

2. Core features

Fluctuating cognition with pronounced variations in attention and alertness

Recurrent visual hallucinations that are typically well formed and detailed

Spontaneous features of parkinsonism

3. Suggestive features

REM sleep behaviour disorder (RBD)

Severe neuroleptic sensitivity

Low dopamine transporter uptake in basal ganglia on SPECT or PET imaging

For a diagnosis of probable or possible DLB, dementia must be present.

Probable DLB: at least one core feature and one other feature (core or suggestive)

Possible DLB: one core feature and no suggestive features; or one or more suggestive features

4. Supportive features (commonly present but not proven to have diagnostic accuracy)

Repeated falls and syncope

Transient, unexplained loss of consciousness

Severe autonomic dysfunction, e.g., orthostatic hypotension, urinary incontinence

Hallucinations in other modalities

Systematized delusions

Depression

Relative preservation of medial temporal lobe structures on CT/MRI scan

Generalized low uptake on SPECT/PET perfusion scan with reduced occipital activity

Abnormal (low uptake) MIBG myocardial scintigraphy

Prominent slow wave activity on EEG with temporal lobe transient sharp waves

1.1.4 Prognosis

Rates of cognitive decline are comparable between clinically diagnosed DLB and AD, but mortality may be greater in DLB (Williams *et al.*, 2006; Garcia-Ptacek *et al.*, 2014). A Swedish Dementia Registry study found that DLB was associated with high levels of comorbidity including depression and stroke (Fereshtehnejad et al., 2014). In cohorts that have come to post-mortem, those with combined LB and AD pathology are seen to have had faster rates of decline than those with 'pure' AD or DLB (Olichney *et al.*, 1998; Kraybill *et al.*, 2005; Nedelska *et al.*, 2015a).

1.1.5 Genetics

Mutations in genes associated with AD (amyloid precursor protein [APP], presenilin 1 [PSEN1] and 2 [PSEN2], apolipoprotein [APOE]) and PD (α-synuclein [SNCA] and scavenger receptor B [SCARB2]) have been shown to be associated with Lewy body deposition and dementia (Meeus *et al.*, 2012; Bras *et al.*, 2014). In the general population, the APOE ε4 allele is the most important genetic risk factor for AD. Rates of the APOE ε4 allele in DLB are higher than in normal controls and similar to those in AD (Kobayashi *et al.*, 2011; Tsuang *et al.*, 2013). These findings support the hypothesis that AD, DLB and PD lie on a spectrum, with DLB showing pathologic and phenotypic traits of both conditions (Meeus et al., 2012). The SNCA and SCARB2 mutations found in DLB and PD are different, which may account for some of the phenotypic differences between these two diseases (Bras et al., 2014).

1.1.6 Neuropathology

Lewy bodies (LBs) and Lewy neurites (LNs) are the pathological hallmarks of DLB (Figure 1.1). These are abnormal filamentous protein inclusions containing α-synuclein (αSyn) found in the neuronal cytoplasm (LBs) and dystrophic neurites (LNs).

LBs and LNs are widely distributed throughout the peripheral and central nervous systems in DLB (Beach et al., 2009). The DLB consensus criteria define 3 patterns of deposition: brainstem, limbic, and diffuse neocortical (McKeith *et al.*, 2005). Cases are assigned a likelihood that the observed LB pathology explains the DLB clinical syndrome based on the extent of LB pathology (diffuse neocortical – most likely; brainstem – least likely) and the amount of AD pathology present (Table 1.2). AD pathology (Aβ plaques and NFTs) is often seen in DLB, this will be discussed in more

detail in chapter 3. The temporal progression of LB pathology will be discussed in chapter 2.

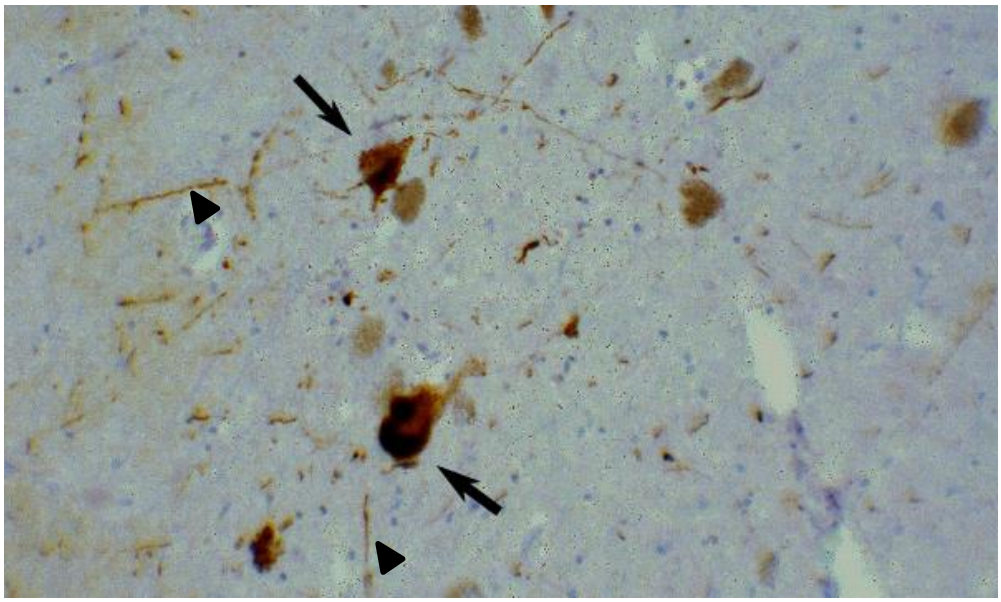

Figure 1.1. Lewy body and Lewy neurite pathology. Photomicrograph of Lewy bodies (arrows) and Lewy neurites (arrow heads) in the substantia nigra stained with α-synuclein antibody (made available by Suraj Rajan under creative Commons License http://commons.wikimedia.org/wiki/File:Substantia_nigra_with_Lewy_body_pathology.svg).

Table 1.2. Assessment of the likelihood that pathologic findings are associated with a DLB clinical syndrome (from McKeith *et al.*, 2005)

	NIA-Reagan rating of AD pathology		
Lewy body pathology	Low	Intermediate	High
Brainstem predominant	Low	Low	Low
Limbic	High	Intermediate	Low
Diffuse neocortical	High	High	Intermediate

1.1.7 Clinicopathologic Correlations

The severity of cognitive impairment seen in DLB does not correlate with the severity of LB pathology (Beach et al., 2009). This may be because smaller presynaptic accumulations of αSyn are responsible for the phenotype of DLB, rather than LBs and

LNs (Schulz-Schaeffer, 2010) or because the amount of LB pathology decreases in the later stages of DLB following cell death and reabsorption (Parkkinen et al., 2008). Visual hallucinations have been associated with LB pathology in limbic (Ferman *et al.*, 2013a) and temporal (Harding et al., 2002) areas.

1.1.8 Striatal Dopaminergic Imaging in DLB

1.1.8.1 Pathological basis of striatal dopaminergic imaging

DLB is associated with Lewy body pathology and cell death in the substantia nigra, with the resultant loss of nigrostriatal dopaminergic axons (Beach et al., 2009). The integrity of these nigrostriatal axons can be assessed by measuring the density of presynaptic dopamine transporters in the striatum using SPECT or PET imaging. A variety of ligands are available; the most commonly used is n-fluoropropyl-2β-carbomethoxy-3β-(4-iodophenyl)nortropane (FP-CIT). ^{123}I-FP-CIT SPECT binding has been shown to correlate with nigral dopaminergic neuronal density post-mortem (Colloby et al., 2012). Some drugs can affect FP-CIT binding, for example amphetamines, modafinil, bupropion and benzatropine (Booij and Kemp, 2008) but cholinesterase inhibitors do not affect the clinical utility of the scan (Taylor et al., 2007).

^{123}I-FP-CIT scans can be graded on a four point scale that was initially developed for the assessment of scan abnormalities in those with Parkinson's disease: 0 = normal; 1 = reduced putamen uptake in one hemisphere; 2 = symmetrical reduction in putamen uptake; 3 = bilateral reduction in putamen and caudate (Fig. 4.7). This scale reflects the pattern of disease progression in PD, with early ^{123}I-FP-CIT binding reductions in the putamen, often unilaterally. However, in DLB a different pattern of reduction is observed with less asymmetry between the two hemispheres and reduced binding throughout the striatum, rather than just the putamen (Walker *et al.*, 2004; Marquie *et al.*, 2014).

1.1.8.2 Clinical Utility of ^{123}I-FP-CIT SPECT

^{123}I-FP-CIT SPECT imaging can accurately identify striatal dopaminergic deficiency in Parkinson's disease and DLB (O'Brien et al., 2014b). A meta-analysis of four large studies found that ^{123}I-FP-CIT SPECT imaging has a sensitivity of 86.5% and a

specificity of 93.6% for the diagnosis of DLB (Papathanasiou et al., 2012). Only one of these studies compared imaging results with pathological diagnosis, finding sensitivity of 88% and specificity of 83% for visually rated scans, with specificity increasing to 100% when a semiquantitative measure was used (Walker et al., 2007). People with possible DLB and a positive ^{123}I-FP-CIT scan are more likely to convert to clinically diagnosed probable DLB than those with negative scans (O'Brien et al., 2009). ^{123}I-FP-CIT imaging increases clinicians' diagnostic certainty in dementia, particularly in the case of a positive scan (Walker *et al.*, 2015b)

^{123}I-FP-CIT scans can be positive in the absence of significant parkinsonism (McKeith et al., 2007) as up to 60% of nigrostriatal neurones can be lost prior to the development of motor symptoms (Cummings et al., 2011). A proportion of DLB cases have negative ^{123}I-FP-CIT scans, which may be due to a relative absence of pathology in the substantia nigra (Colloby et al., 2012), with the synuclein disease burden being predominantly in other parts of the brain or nervous system (Zaccai *et al.*, 2008). A significant proportion of frontotemporal dementia (FTD) cases have positive scans, most likely due to pathological changes in the substantia nigra and striatum (Morgan et al., 2012). ^{123}I-FP-CIT imaging cannot differentiate between DLB, PD, PDD or other parkinsonian syndromes such as MSA, progressive supranuclear palsy or corticobasal degeneration (Cummings et al., 2011).

1.1.8.3 Clinical Correlations

There is conflicting data on the relationship between ^{123}I-FP-CIT binding and clinical phenotype in DLB. Decreased FP-CIT binding in the right putamen was correlated with increased NPI apathy score in a mixed AD (n=14) and DLB (n=8) group, though this could be attributed to apathy and decreased ^{123}I-FP-CIT binding both being associated with DLB (David et al., 2008). Roselli et al. found a negative correlation with striatal uptake and NPI hallucinations score (n=18) (Roselli et al., 2009). They found significant correlation between caudate binding and NPI apathy, depression and delusion scores. Putamen binding was negatively correlated with UPDRS score. A more recent and larger study (n=51) using ^{123}I-PE2I found no relationship between dopamine transporter binding and MMSE score, Hoehn and Yahr score of PD severity, or the presence of hallucinations and fluctuations (Ziebell et al., 2013). However, a study involving serial scans 1 year apart found that decline in FP-CIT binding in the posterior

putamen was associated with decline in cognition; there was no correlation with change in UPDRS score (Colloby et al., 2005).

1.1.9 Other Biomarkers

1.1.9.1 Structural and Functional Imaging

DLB is associated with brain atrophy on structural imaging, particularly in subcortical structures. The medial temporal lobe is relatively spared compared with AD (Watson et al., 2009).

Imaging of cerebral perfusion and glucose metabolism can be achieved using SPECT and PET imaging. DLB is associated with hypoperfusion and hypometabolism, most marked in the posterior parietal and occipital regions, with relative sparing of the posterior cingulate and medial temporal areas, which are particularly affected in AD (Sinha et al., 2012). ^{18}F-FDG PET is more effective than perfusion SPECT at differentiating dementia from controls (Area under the curve (AUC) 0.93 v 0.72), and differentiating DLB from AD (AUC 0.80 v 0.58) (O'Brien et al., 2014a).
DLB with cognitive fluctuations is associated with lower functional connectivity in left frontoparietal, sensorimotor and temporal networks on functional MRI, without the disruption to the default mode network that is commonly seen in AD (Peraza et al., 2014).

1.1.9.2 Cardiac Imaging

^{123}I-MIBG cardiac scintigraphy uses a noradrenaline analogue to detect the loss of presynaptic sympathetic nerve terminals in the heart. The autonomic nervous system is an early site of LB pathology and MIBG has been shown to differentiate between DLB and AD. A systematic review of 8 studies with a total of 152 DLB patients found a pooled sensitivity of 98% and specificity of 94% for the differentiation of DLB and other dementias (Treglia and Cason, 2012). However, a more recent multicentre study with 61 cases of probable DLB found a lower sensitivity of 69% and a specificity of 89% (Yoshita *et al.*, 2015). The reason for this difference is not clear, though Yoshita and colleagues commented that the sensitivity was higher in the mild dementia group, potentially reflecting greater accuracy of clinical diagnosis of DLB at this stage. In more advanced disease AD cases may be more likely to mimic DLB with the presence of

symptoms such as visual hallucinations or parkinsonism, resulting in contamination of the DLB group.

Cardiac MIBG uptake can be abnormal in congestive cardiac failure, ischaemic heart disease and diabetic autonomic neuropathy (Chirumamilla and Travin, 2011). The results above were derived from cohorts that exclude those with heart failure and diabetes, both of which are common in the elderly, potentially limiting the generalisability of these results to a normal clinical population. A small study including patients with diabetes and heart failure found a sensitivity of 100% and specificity of 75% but there were only 4 non-LB dementia and 16 LB dementia cases in this sample (Slaets *et al.*, 2015). Although the sensitivity and specificity of MIBG appear comparable to FP-CIT SPECT, there is a need for large-scale studies in normal clinical populations along with post-mortem follow-up to determine its diagnostic accuracy.

1.1.9.3 Amyloid Imaging

Amyloid imaging in DLB is reviewed in Chapter 3.

1.1.9.4 Fluid biomarkers

There are no established fluid biomarkers for DLB. Conflicting reports of cerebrospinal fluid (CSF) αSyn level abnormalities in DLB have been published (Kasuga et al., 2012). This heterogeneity of results may reflect methodological differences in the measurement of αSyn, or cerebrospinal fluid contamination with red blood cells, which are relatively rich in αSyn (Kasuga et al., 2012). CSF $A\beta_{42}$ is decreased in both AD and DLB, consistent with the finding of amyloid deposition in some cases of DLB in PET imaging studies (Sinha et al., 2012). On the other hand, tau levels are higher in AD than DLB, consistent with a relative lack of tau pathology in DLB (Ferreira et al., 2014).
Several other potential biomarkers have been investigated including serum fatty acid binding protein levels and CSF calcium, magnesium and cocaine and amphetamine regulated transcript levels, but these require further investigation (Schade and Mollenhauer, 2014).

1.1.9.5 EEG

Increased EEG slow wave activity and increased frequency variability are seen in DLB compared with AD in posterior cortical areas (Bonanni et al., 2008). Increased frequency variability correlates with clinical measures of fluctuations (Walker *et al.*, 2000a).

1.1.10 Management

There are no treatments available that slow or reverse the pathologic progression of DLB. Symptomatic pharmacological treatments are available for some of the cognitive and non-cognitive symptoms of the disorder.

1.1.10.1 Cognitive symptoms

Reductions in cholinergic activity are greater in DLB than AD, and occur earlier in the disease course (Tiraboschi et al., 2002). These reductions may be the product of Lewy body pathology and neuronal loss in the nucleus basalis of Meynert (Lippa et al., 1999). Relationships have been found between reduced cholinergic activity and cognitive dysfunction (Tiraboschi et al., 2002) and visual hallucinations (Francis and Perry, 2007). Acetylcholinesterase inhibitors are the mainstay of drug treatment in DLB, both for cognitive and non-cognitive symptoms. Three double-blind, randomised controlled trials of acetylcholinesterase inhibitors in DLB have been reported. Rivastigmine was found to reduce neuropsychiatric symptoms (apathy, anxiety, delusions, hallucinations and aberrant motor behaviour), as well as improving scores in computerised tests of attention and memory (McKeith et al., 2000). Donepezil was found to improve cognitive function measured by the MMSE (Mori *et al.*, 2012; Ikeda *et al.*, 2015). This cognitive improvement was sustained over 52 weeks in an open-label continuation study (Mori et al., 2015). There is conflicting evidence on whether donepezil reduces neuropsychiatric symptoms (Mori *et al.*, 2012; Ikeda *et al.*, 2015).

The NMDA-receptor blocker memantine has also been investigated for its use in DLB. Two randomised, placebo-controlled trials in mixed DLB/PDD groups produced somewhat conflicting results. The first study found an improvement in global status in the combined DLB/PDD group, but no difference in the DLB subgroup, and no difference in neuropsychiatric symptoms measured by the Neuropsychiatric Inventory (NPI) (Aarsland et al., 2009). In the combined PDD/DLB group they did find an

improvement in computerised tests of memory and choice reaction time (Wesnes et al., 2014). The second, larger trial found an improvement in global status and a relative improvement in neuropsychiatric symptoms (delusions, hallucinations, night-time behaviour and appetite/eating disorders) in the DLB group treated with memantine (Emre et al., 2010). This study did not find any improvement in choice reaction time. Neither study found any significant improvement in activities of daily living scales.

1.1.10.2 Non-cognitive symptoms

The principal treatment for parkinsonism in DLB is levodopa, though the treatment may be less effective in DLB than PD (Molloy *et al.*, 2005; Lucetti *et al.*, 2010). Other therapies used in Parkinson's disease (e.g. dopamine agonists, anticholinergics, selegiline and amantadine) are unsafe due to side effects such as confusion, somnolence and hallucinations (Molloy et al., 2005). Levodopa may also be associated with similar side-effects, but is tolerated in the majority of patients (Molloy et al., 2005).

Antipsychotics are generally not recommended for psychotic symptoms in DLB as their efficacy is unproven (Aarsland et al., 2012), and they are associated with severe adverse effects (McKeith *et al.*, 1992; Ballard *et al.*, 1998). Where treatment with an antipsychotic is felt necessary quetiapine and clozapine may be least likely to cause adverse-effects, whereas antipsychotics with strong D_2-blocking actions are best avoided (e.g. typical antipsychotics, olanzapine, riperidone) (Boot *et al.*, 2013a).

The treatment of RBD in DLB is based on general guidelines for the management of RBD. The American Academy of Sleep Medicine recommends the use of clonazepam or melatonin (Aurora et al., 2010). Clonazepam is associated with greater side-effects, such as sedation, but also has a stronger evidence base.

1.1.11 Summary

DLB is a common form of dementia characterised by the presence of symptoms such as visual hallucinations, parkinsonism, cognitive fluctuations, RBD and sensitivity to neuroleptic medications. The pattern of cognitive impairment in DLB differs from AD, with less marked amnestic impairment and more marked visuospatial, attentional and frontal-executive impairments. αSyn-containing LBs and LNs are the pathological hallmarks of DLB. DLB is associated with less cortical atrophy than AD and cortical

hypoperfusion and hypometabolism tend to be more marked in occipital regions. Nigrostriatal dopaminergic and cardiac sympathetic denervation can be detected using SPECT imaging and are used in the diagnosis of DLB. The management of DLB is based on symptomatic treatment of cognitive impairment, parkinsonism, RBD and neuropsychiatric symptoms.

1.2 Alzheimer's Disease

1.2.1 Epidemiology

Alzheimer's disease (AD) is the most common type of dementia, accounting for around 70% of all cases (Reitz et al., 2011). Based on overall dementia prevalence figures from the Cognitive Function and Ageing Study, this would equate to around 470,000 people in the UK in 2011 (Matthews et al., 2013).

1.2.2 Clinical features and diagnostic guidelines

AD is characterised by a dementia of insidious onset and gradual progression, usually with prominent amnestic symptoms (McKhann et al., 2011). In some cases the most prominent deficits are in language, visuospatial or executive function, but by definition the deficit is not focal, and involves at least two domains of cognition. Neuropsychiatric symptoms are common in AD. The most common features include apathy, irritability and agitation (Craig et al., 2005). Hallucinations are less common and tend to occur later in the course of the illness (Ferman *et al.*, 2013a).

A definitive diagnosis of AD can only be made at post-mortem. Clinical criteria for 'probable AD' are displayed in Table 1.3. The diagnosis of probable AD is to some extent a diagnosis of exclusion, and cannot be made in the presence of evidence of other disorders such as vascular dementia, DLB or frontotemporal dementia. The updated National Institute on Ageing/Alzheimer's Association (NIAAA) criteria include the use of biomarkers for the diagnosis of 'probable AD dementia with evidence of the AD pathophysiological process' (McKhann et al., 2011). These are markers of amyloid pathology (low CSF $A\beta_{42}$ or positive amyloid PET imaging) and biomarkers of neuronal degeneration or injury (elevated CSF tau, hypometabolism in the temporo-parietal cortex on ^{18}F-FDG PET and disproportionate atrophy in the medial, basal and lateral

temporal lobe and medial parietal cortex). In recent years considerable attention has been given to the concept of prodromal AD, this will be discussed in Chapter 2.

1.2.3 Genetics

Early onset AD can cluster in families, showing an autosomal dominant pattern of inheritance. Three genes involved in amyloid production (APP, PSEN1 and PSEN2) have been identified that have high penetrance for early onset AD (Bettens et al., 2013). However, the majority of cases of AD are sporadic, with onset later in life. Many genetic and non-genetic risk factors (e.g. cerebrovascular disease, head injury and type 2 diabetes) have been identified (Reitz and Mayeux, 2014). The most clearly established genetic risk factor is the APOE gene. The APOE ε4 allele is associated with an increased risk of developing AD, whereas the APOE ε2 allele confers decreased risk. One copy of APOE ε4 increases the risk of developing AD by a factor of 3 and two copies increase the risk by a factor of 12 (Verghese et al., 2011). The mechanism of this effect is unknown and may be via amyloid-β (Aβ) metabolism or through another pathway such as tau phosphorylation, cell signalling or lipid metabolism (Verghese et al., 2011). Recent large genome-wide association studies have identified other genes that may be associated with AD, including genes involved in the immune system, APP pathways, tau pathways, and lipid metabolism (Reitz and Mayeux, 2014).

1.2.4 Neuropathology

The two most characteristic neuropathological findings, considered essential for the diagnosis of AD, are neurofibrillary tangles (NFTs) and Aβ plaques (Hyman et al., 2012). NFTs are intracellular accumulations of the microtubule-associated protein tau and are the pathology most strongly correlated with cognitive deficit in AD (Nelson et al., 2012). The Aβ peptide, like tau, is a normal cellular constituent. Aβ can form extracellular deposits containing various other proteins, lipids and cells. These can be classified as diffuse, focal or stellate (Duyckaerts et al., 2009). Aβ accumulation can also occur in vessel walls, termed cerebral amyloid angiopathy. Neuritic plaques are a type of focal plaque containing an Aβ core surrounded by dystrophic neurites. Post-mortem studies have found that this type of plaque is most closely associated with neuronal injury and cognitive deficit (Nelson et al., 2012).

The revised NIAAA Guidelines for the neuropathologic assessment of AD (Hyman et al., 2012) recommend a 3 dimensional ‘ABC’ approach – NFTs, amyloid plaque and

neuritic plaque pathology are each rated from 0-3 based on established criteria. These measures are then combined to give an overall measure of likelihood of AD (Table 1.4).

Table 1.3. Criteria for the diagnosis of probable AD (from the NIAAA criteria (McKhann et al., 2011))

1. Meets Criteria for dementia - Evidence of cognitive decline in at least two domains - Significant interference in ability to function at work or usual daily activities 2. In addition the dementia has the following characteristics - Insidious onset - Clear cut history of worsening cognition - The initial and most prominent cognitive deficits are in the areas of memory, language, visuospatial or executive function The diagnosis of probable AD should not be given when there is evidence of substantial cerebrovascular disease, core features of Lewy body disease, prominent features of frontotemporal dementia or primary progressive aphasia or another neurological or medical disease that may have a substantial effect on cognition.

The criteria are supported by findings from a neuropathologic cohort. 91% of those with high scores on semiquantitative measures for NFTs and neuritic plaques had moderate or severe dementia (Hyman et al., 2012). That said, some cases with significant AD pathology did not display signs of dementia before death. The importance of amyloid in the pathogenesis of AD remains controversial. The amyloid hypothesis posits that Aβ is the initiating factor of a cascade of events that leads to tau tangle formation, cell death and the clinical syndrome of AD (Karran et al., 2011). This is supported by evidence that Aβ is found in the brains of people clinically diagnosed with AD both at post-mortem and more recently during life using PET amyloid imaging (Jack et al., 2013). In addition, genes associated with early-onset Alzheimer's disease such as APP, PSEN1 and PSEN2 are known to be involved in the production of amyloid from APP, and the APOE ε4 gene may be involved in amyloid clearance (Karran et al., 2011). Set against this are the findings that amyloid load does not correlate well with disease progression, many people with plaques do not experience dementia and AD-like cognitive decline

and brain atrophy can occur in the absence of significant Aβ (Fjell and Walhovd, 2012). Perhaps most importantly, recent trials of anti-amyloid therapies have been unsuccessful, despite some agents demonstrating efficacy at Aβ removal from the brain (Sarazin et al., 2013). Large-scale neuroimaging studies currently underway may help to clarify the importance of amyloid in AD.

Table 1.4. Evaluation of AD pathological change according to NIAAA guidelines (Hyman et al., 2012).

Amyloid	Neuritic Plaque	Neurofibrillary Tangle Stage		
Plaque Phase	Score	0-II	III/IV	V/VI
0	0	Not	Not	Not
1/2	0/1	Low	Low	Low
	2/3	Low	Intermediate	Intermediate
3	0-3	Low	Intermediate	Intermediate
4/5	0/1	Low	Intermediate	Intermediate
	2/3	Low	Intermediate	High

The degree of amyloid plaque (Thal *et al.*, 2002), neuritic plaque (Mirra *et al.*, 1991) and neurofibrillary tangle (Braak and Braak, 1991) pathology is rated on three semiquantitative scales. The likelihood that this pathology is sufficient explanation for dementia is then classed as 'low', 'intermediate' or 'high'.

1.2.5 Imaging and other biomarkers

1.2.5.1 Structural Imaging

The early stages of AD are associated with hippocampal and entorhinal cortex atrophy on structural MRI, this is followed by more widespread medial temporal atrophy and involvement of the parietal and frontal lobes as well as subcortical structures such as the thalamus (Zakzanis et al., 2003). This atrophy is correlated with cognitive decline (Braskie and Thompson, 2014).

1.2.5.2 Functional Imaging

In AD impaired perfusion and glucose metabolism are observed in temporo-parietal areas, particularly the angular gyrus, the cuneus and the posterior cingulate cortex

(Herholz, 2011). Metabolism in the basal ganglia, motor cortex and visual cortex is well preserved.

1.2.5.3 Amyloid PET Imaging

Amyloid imaging using the ^{11}C ligand Pittsburgh Compound B (PiB) has been in use for a decade (Klunk et al., 2004). More recently, ^{18}F ligands such as Florbetapir, Flutametamol and Florbetaben have been developed, allowing amyloid imaging to become more widely used due to the longer half-life of ^{18}F (110 minutes) compared with ^{11}C (20 minutes) (Herholz and Ebmeier, 2011). These ligands bind to fibrillar amyloid and correlate well with Aβ plaque pathology assessed at autopsy, though some medial temporal lobe areas known to undergo early Aβ deposition, such as parahippocampal gyrus and occipitotemporal gyrus do not show high ligand binding (Jack et al., 2013).

The prevalence of amyloid deposition in cognitively healthy people increases with age (Jack et al., 2014). At the age of 70 around 23% of cognitively normal subjects, 49% of MCI subjects and 88% of people with AD have positive amyloid PET scans (Jansen *et al.*, 2015; Ossenkoppele *et al.*, 2015). MCI subjects and normal controls with significant amyloid pathology demonstrate more rapid brain atrophy and are more likely to convert to dementia than those with negative scans (Rowe *et al.*, 2013; Doraiswamy *et al.*, 2014; Huijbers *et al.*, 2015; Vemuri *et al.*, 2015). There have been inconsistent reports of correlation between amyloid ligand binding and cognitive impairment in AD, though the association may be stronger in MCI (Braskie and Thompson, 2013).

1.2.5.4 Fluid biomarkers

Compared with healthy controls, decreased $A\beta_{42}$, increased total tau and increased phospho-tau each have a sensitivity in the region of 80% and specificity between 80 and 90% for AD (Ferreira et al., 2014). Sensitivity and specificity can be increased by combining $A\beta_{42}$ and total tau markers. These markers are much less accurate in differentiating between AD and other dementias (Ferreira et al., 2014). The clinical utility of CSF biomarkers is limited by huge variability in results between different sites, possibly due to variations in procedures for the collection, processing and analysis of samples. Even within sites results can vary based on assay batches used (Vanderstichele et al., 2012). There are no validated blood biomarkers for AD at present (Rosen et al., 2013).

1.2.6 Management

The acetylcholinesterase inhibitors donepezil, galantamine and rivastigmine, along with the NMDA antagonist memantine are the mainstay of symptomatic treatment of cognitive impairment in AD (Tan et al., 2014). Non-drug treatment of the highly prevalent behavioural and psychological symptoms of AD should be used before medication except in severe behavioural disturbance. Drugs are considered a last option due to the prevalence of severe side effects, particularly with antipsychotic medications (Schneider *et al.*, 2005; Ihl *et al.*, 2011). Non-pharmacological interventions such as care-giver education and support may delay institutionalisation (Olazaran et al., 2010) and cognitive training and ADL training may improve specific areas of function (Olazaran et al., 2010). No disease modifying agent for AD has yet been established.

1.2.7 Summary

AD is characterised by the insidious onset and gradual progression of cognitive impairment, usually with prominent amnestic features. Aβ plaques and NFTs are found post-mortem in AD, but the pathophysiological significance of Aβ in the development of AD remains controversial. AD is associated with cortical atrophy on structural imaging, particularly in the medial temporal lobes; cerebral hypometabolism and hypoperfusion on SPECT or PET imaging and amyloid deposition on PET imaging. CSF Aβ, tau and phospho-tau can be used to differentiate AD from controls, but are less useful in differentiating between AD and other dementias. Cholinesterase inhibitors and memantine are used to improve cognitive function in AD, though no disease modifying agents are yet available.

Chapter 2 Prodromal Dementia with Lewy bodies

2.1 Introduction

The clinical condition of dementia, by definition a global cognitive decline with functional impairment, is now recognised as a diagnosis that can only be applied too late in the disease process to be useful for current and future therapeutic approaches which centre on disease modification. As a result, in recent years increasing research attention has been given to mild cognitive impairment (MCI) and the diagnosis of prodromal dementia. Petersen et al. (1999) described MCI as an entity with clinical characteristics intermediate between dementia and healthy controls, and high rates of conversion to dementia (most commonly Alzheimer's disease, because of the amnestic weighting of the MCI definition). Later, the diagnosis was refined to subcategorise MCI into amnestic and non-amnestic MCI (aMCI and naMCI respectively), depending on whether memory was significantly affected or not (Petersen *et al.*, 2001; Winblad *et al.*, 2004). aMCI was hypothesised to precede AD or vascular dementia, whereas naMCI was felt more likely to precede dementia with Lewy bodies (DLB), vascular dementia or frontotemporal dementia (Petersen, 2004). That said, these are descriptive terms, and do not imply any specific pathological cause for the cognitive impairment.

Following the disappointing results in recent trials of anti-amyloid therapies, some have hypothesised that such treatments may only be successful in the earliest stages of the disease (Aisen et al., 2013). This, coupled with the expectation that different types of dementia will require different treatments has led to the development of diagnostic criteria for prodromal dementia i.e. criteria that do specify the pathological cause of mild cognitive impairment. AD, the most common type of dementia, has received significant attention in this regard. Criteria for the diagnosis of MCI due to AD (Albert et al., 2011) and prodromal AD (Dubois et al., 2010) have been put forward. These diagnostic criteria are similar to previous descriptions of aMCI but also include validated disease biomarkers indicative of brain amyloid deposition and/or neuronal injury.

The existence of a prodromal phase of DLB is to be expected, given the insidiously progressive nature of the disorder, as with AD. There are validated consensus criteria for the clinical diagnosis of DLB (McKeith *et al.*, 2005). These display high positive

Table 2.1. Diagnostic features of DLB (from McKeith *et al.*, 2005)

Pattern of cognitive deficits – impairments of attention, executive and visuospatial function
Core features – spontaneous parkinsonism, complex visual hallucinations, fluctuating cognition
Suggestive features – REM sleep behaviour disorder (RBD), neuroleptic sensitivity, reduced dopamine transporter density in the striatum
Supportive features – repeated falls/syncope, transient unexplained loss of consciousness, autonomic dysfunction, depression, hallucinations, delusions
Imaging findings and other biomarkers – preservation of medial temporal lobe structures on structural imaging, reduced occipital perfusion, abnormal MIBG myocardial scintigraphy, EEG abnormalities

predictive value for the post-mortem neuropathological classification of intermediate- or high-likelihood DLB (Fujishiro et al., 2008). Prodromal DLB may display some of the features characteristic of established DLB (Table 2.1) (McKeith *et al.*, 2005; Troster, 2008). The identification of a DLB prodrome would enable investigation of the early pathophysiology of DLB and the development of treatments to interrupt these pathophysiological processes. Prodromal DLB may require different management from other dementia prodromes. For example, DLB may potentially be more responsive to

cholinesterase inhibition in its prodromal phase, given the early and widespread cholinergic losses seen in DLB compared with AD (Tiraboschi et al., 2002).

This chapter reviews the evidence available on the prodromal presentation of DLB, examining if mild cognitive impairment or other symptoms and biomarker abnormalities can be observed prior to the development of dementia. Before this, the neuropathology of DLB will be reviewed, as it may provide insights into the likely presentation of prodromal DLB.

2.2 The neuropathology of DLB

Knowledge of the temporal development of LB pathology may guide our hypotheses on the prodromal presentation of DLB. Much of the work on the pathology of Lewy body disease comes from research in Parkinson's disease. Just over a decade ago, Braak and colleagues proposed a staging system for Lewy pathology in Parkinson's disease (Braak et al., 2003). In a sample of brains from PD patients and asymptomatic individuals with LB disease they found that all could be classified into one of six stages of disease development. At the earliest stage (stage 1), LB pathology was confined to the dorsal motor nucleus of cranial nerves IX/X and the intermediate reticular zone of the medulla. Over the subsequent stages LB pathology ascended sequentially through the pons, midbrain and subcortical structures to finally affect the neocortex itself in stages 5 and 6. A key feature of Braak staging was that the sequential ascent of LB pathology from the brainstem to the neocortex was common to all cases – i.e. the cortex was not involved in every case, but where the cortex was affected, so were lower structures such as the limbic system and brainstem. Others have observed that LB pathology does not necessarily follow this pattern of distribution, particularly when cases are sampled from the general population, in which LB pathology can be found in higher centres such as the neocortex, despite sparing of lower regions (Parkkinen *et al.*, 2005; Zaccai *et al.*, 2008; Beach *et al.*, 2009; Frigerio *et al.*, 2011). Nevertheless, it is clear that some structures are particularly susceptible to LB pathology and may potentially act as 'sentinels' for the development of LB disease: the olfactory bulb (Beach et al., 2009); the dorsal motor nucleus of the vagal nerve and other brainstem structures (Braak et al., 2003) and the peripheral autonomic nervous system (Minguez-Castellanos *et al.*, 2007; Palma and Kaufmann, 2014), including the enteric nervous system (Braak et al., 2006).

As such, symptoms or biomarkers reflecting dysfunction in these areas may be some of the earliest indicators of DLB.

2.3 Epidemiological studies of rates of conversion from MCI to DLB

Four studies have prospectively followed-up patients with MCI for the development of DLB. However, these studies studied diverse populations with varying duration of follow-up. Unsurprisingly they found very different results.

Two studies based in tertiary referral centres found that 20-30% of MCI cases converting to dementia had probable DLB (Bombois *et al.*, 2008; Ferman *et al.*, 2013b). A memory-clinic cohort found 5.6% of MCI cases that later developed dementia had DLB (Palmqvist *et al.*, 2012). Conversely, a population-based cohort, the majority of whom were cognitively healthy at baseline, found no cases that converted to probable DLB, but 11% of dementia cases had possible DLB (Fischer *et al.*, 2007).

Despite the heterogeneity of these results, it is clear that at least some cases of DLB have an identifiable MCI stage. Interestingly, one study found that naMCI had a much higher annual conversion rate to DLB than aMCI (20% v. 1.5%) (Ferman *et al.*, 2013b). (Ferman *et al.*, 2013b).

In a post-mortem study of 134 patients who died with a diagnosis of MCI, 8 (6%) had cortical Lewy bodies (LBs); 5 of these 8 in the absence of vascular or AD pathology. A further 10% had nigral or limbic LBs (Schneider et al., 2009). Saito and Murayama (2007) found that, of 33 MCI cases showing degenerative pathology post-mortem, 6 (18%) had LB pathology, with half of these showing only DLB type changes. Another small study of an aMCI group found that 1/15 cases had transitional LB pathology post-mortem, along with some AD pathology (Petersen et al., 2006).

2.4 Clinical studies of the symptoms of prodromal DLB

Three prospective studies have compared the prevalence of core and suggestive features in MCI cases that later developed DLB (LB-MCI) to those that later developed AD (AD-MCI)

A longitudinal study of MCI (both amnestic and non-amnestic) found that those converting to DLB had higher rates of RBD, cognitive fluctuations and daytime

sleepiness than those that converted to AD (Ferman *et al.*, 2013b). 53% of DLB-MCI had one or more core features of LB disease at least one year before the estimated onset of cognitive symptoms, compared to 2% of AD-MCI. Two other longitudinal studies confirmed the increased prevalence of core features of LB disease and RBD in LB-MCI compared with AD-MCI (Cagnin *et al.*, 2015; Yoon *et al.*, 2015). The reported prevalence of each symptom varied between cohorts (Table 2.2), each of which used different methods to identify diagnostic features. It would seem that RBD is the most common diagnostic feature, but all symptoms appear to be relatively common.

Though two studies found significantly higher scores in the UPDRS in LB-MCI than AD-MCI, the severity of parkinsonism was very mild (mean UPDRS = 4.9 (Ferman *et al.*, 2013b) and 4.4 (Yoon *et al.*, 2015)).

Table 2.2. Prevalence of DLB diagnostic features in LB-MCI in longitudinal studies

	Ferman et al. 2013	Yoon et al. 2015	Cagnin et al. 2015
Visual Hallucinations (%)	-	39*	30*
Parkinsonism (%)	-	78*	63*
Fluctuations (%)	41*	22*	53*
RBD (%)	80*	67*	57*

* = significantly more common than AD-MCI comparison group

Three further studies (Auning *et al.*, 2011; Chiba *et al.*, 2012; Fujishiro *et al.*, 2013b) have asked patients with DLB and/or their carers to retrospectively report on the early symptoms of DLB (Table 2.3).

Auning et al. (2011) interviewed carers of patients newly diagnosed with mild DLB about the presenting symptoms of DLB (MMSE>20; n=61). Visual hallucinations (44%), gait problems (28%), tremor/stiffness (25%) and a tendency to fall (13%) were significantly more common in DLB compared with an AD control group, whereas memory problems were significantly less common. Fluctuating cognition was not offered as an option for presenting symptom. Carers did have the opportunity to report symptoms not on the preselected list, but any other reported symptoms were infrequent (<10%).

Chiba et al. (2012) asked patients and carers to fill in a survey of pre-defined symptoms without any additional instruction. They looked at the temporal onset of symptoms relative to memory loss. This was to allow comparison of DLB (n=34) and AD (n=32), both of which are associated with progressive memory impairment.

The most common symptoms present in the same year as the onset of memory loss were sleep rhythm change (62%), crying/shouting in sleep (62%), anosmia/hyposmia (41%), constipation (47%) and limb movements in sleep (35%; Table 2.3).

Of those symptoms that were more common in DLB than AD, the earliest to develop were constipation (mean = 9.4 years before memory impairment); crying/shouting during sleep (4.9 years); limb movements during sleep (3.9 years); anosmia/hyposmia (2.9 years) and nightmares (2.5 years).

The three symptoms taken to be most representative of DLB (due to high prevalence in DLB and relatively low prevalence in AD/controls) were crying/shouting during sleep, constipation and anosmia/hyposmia. One or more of these symptoms differentiated DLB from AD with a sensitivity of 0.71 and a specificity of 0.81. Increasing the threshold to two or more symptoms resulted in a decrease of sensitivity to 0.38 but an increase in specificity to 0.97. The questionnaire did not enquire about parkinsonism, hallucinations or fluctuations.

The same group later assessed the presence and time of onset of core features and eight symptoms of Lewy body disease in 90 patients with probable DLB (Fujishiro et al., 2013b). There was no comparison group. As with the previous study, the presence of each symptom at the onset of memory loss was recorded. This study found comparable rates of constipation, anosmia/hyposmia, RBD, depression and orthostatic dizziness to their previous study (Table 2.3). Lower rates of urinary incontinence were found (8%), and syncope was relatively rare (7%).

This study confirmed that constipation, anosmia and RBD often precede the onset of memory loss by several years. Visual hallucinations and extrapyramidal symptoms each were present in around one third of individuals at the onset of memory loss, though on average these symptoms developed 1.5 years after memory loss.

Table 2.3. Prevalence of key symptoms in retrospective clinical studies of prodromal DLB

	Auning et al. 2011 (% of DLB patients with symptom as a presenting symptom; n=61)	Chiba et al. 2012 (% of DLB patients with each symptom in year of onset of memory loss; n=34)	Fujishiro et al. 2013 (% of DLB patients with each symptom in year of onset of memory loss; n=90)
Cognitive Problems			
Memory	57	100	100
Problem- solving	33		
Language	16		
Neuropsychiatric Symptoms			
Visual Hallucinations	44*		31
Depression	34	24	19
Anxiety		26*	
Lack of motivation		26	
Locomotor Symptoms			
Tremor/stiffness	25*		
Gait problems	27*		
Falls	13*		
Extrapyramidal signs			31
Autonomic symptoms			
Constipation		47*	57
Orthostatic dizziness		24*	18
Urinary incontinence		27	8
Increased salivation		21*	
Sleep symptoms			
Sleep rhythm change		62*	
Crying/shouting in sleep		62*	
Limb movements		35*	
Nightmares		27*	
RBD			46
Other			
Anosmia/hyposmia		41*	38

* = significantly more common than AD (no comparison group in Fujishiro et al.)

2.5 Post-mortem studies of prodromal symptoms of DLB

Some post-mortem studies of established DLB have retrospectively assessed the chronological development of symptoms. Ferman et al. (2011) examined 98 patients with intermediate-high likelihood of DLB on post-mortem examination who had been part of a longitudinal study. On average, RBD preceded dementia by 6 years (with wide variation (SD=12 years), possibly reflecting some cases with very early onset RBD). Conversely visual hallucinations and parkinsonism followed the estimated dementia onset by an average of 2.6 and 1.8 years respectively. Another Mayo Clinic post-mortem study examined 52 patients diagnosed during life with probable or possible DLB (Fujishiro et al., 2008). The authors remarked that "RBD antedated the diagnosis of DLB in almost all cases in which RBD was noted", whereas the presence of notable visual hallucinations followed the development of dementia by an average of 2.8 years.

Both of the above studies recruited subjects when they had already been diagnosed with dementia and prospectively collected information through regular clinical and neuropsychiatric assessments. Two further studies specifically recruited non-demented subjects for prospective follow-up. These studies reported a different pattern of symptom development in prodromal DLB.

Jicha et al. (2010) enrolled cognitively normal patients for regular clinical follow-up and brain donation following death. Nine patients with neocortical DLB post-mortem and no significant AD or vascular pathology had an identified MCI phase during their illness. This group was compared with 12 patients with a post-mortem diagnosis of AD.

None of the AD-MCI group displayed parkinsonism, cognitive fluctuations or psychiatric symptoms (hallucinations/delusions/paranoia) during the MCI phase. 8/9 MCI-DLB demonstrated at least one of these features concurrent with the MCI diagnosis (parkinsonism n=5; fluctuations n=3; psychiatric symptom n=4).

DLB-MCI was associated with significant memory impairment, but the group performed better on immediate recall than AD-MCI. They were worse on phonemic fluency and tended toward being worse at trail-making, but were better at the Boston

Naming Test. It must be noted that, due to the strict inclusion criteria, these findings only represent the MCI phase of patients with later neocortical LB deposition (i.e. a subset of all those who have DLB, some of whom will not have cortical involvement) and without significant vascular or AD pathology.

In a similar study, Molano et al. (2010) identified 8 patients from their research databases that had been prospectively followed up after a diagnosis of MCI, and later were found have LB disease post-mortem (limbic- or neocortical-predominant). In the year of MCI diagnosis, or preceding this, 5 displayed parkinsonism and 3 had visual hallucinations, whereas none displayed fluctuations. RBD was present in 7 cases at the diagnosis of MCI, preceding it by up to 47 years.

7 cases developed dementia before death. Of these 5 had parkinsonism, 5 had hallucinations and 2 had fluctuations before the development of dementia.

The patients had a variety of MCI subtypes. Attention/executive function (n=6) and visuospatial function (n=6) were the cognitive domains most commonly affected.

2.6 Cognitive profile of prodromal DLB

Whereas amnestic impairments are by definition usually an early feature of AD, attention, executive and visuospatial impairments are more characteristic of DLB (Metzler-Baddeley, 2007). These differences are present in the prodromal stage, with non-amnestic MCI cases being 10 times more likely to convert to clinically diagnosed DLB than AD in one sample, and amnestic MCI 10 times more likely to convert to AD than DLB (Ferman *et al.*, 2013b). Single domain amnestic MCI was particularly unlikely to progress DLB, with 88% of those converting to DLB having impairment in attention and/or visuospatial function at baseline assessment. That said clinically significant memory impairment was present in over 30% of LB-MCI cases.

Two other longitudinal studies have investigated the pattern of cognitive deficits in MCI cases that later developed DLB (LB-MCI) compared with those that later developed AD (AD-MCI). LB-MCI displayed worse attention/executive function (trailmaking A, digit span, Stroop colour test), better memory (delayed recall, recognition) and worse visuospatial function (Rey complex figure test, number of angles in pentagon-drawing test) than AD-MCI (Cagnin *et al.*, 2015; Yoon *et al.*, 2015).

2.7 Biomarkers in Prodromal DLB

2.7.1 Striatal Dopamine Terminal Imaging

Striatal dopaminergic imaging is a sensitive and specific biomarker for the diagnosis of DLB (Papathanasiou et al., 2012) and is a suggestive feature in the 2005 diagnostic criteria (McKeith *et al.*, 2005). There has been little investigation into its use in the prodromal stage of DLB. One study performed PET dopamine terminal imaging on subjects with MCI with follow-up for the development of DLB (Albin et al., 2013). Of 27 MCI subjects, two had markedly reduced striatal ^{11}C-dihydrotetrobenazine binding. Both developed dementia at follow-up; one was classified as DLB, the other as frontotemporal dementia. However, 3/25 MCI subjects with normal ^{11}C-dihydrotetrabenazine scans also developed DLB.

2.7.2 Clinical Biomarkers

There is no available evidence on fluid biomarkers in prodromal DLB.

LB-MCI cases have significantly worse olfactory function than AD-MCI or stable MCI cases in the Cross-Cultural Smell Identification Test (area under the curve 0.84 for DLB-MCI v. AD-MCI and 0.91 for DLB-MCI v. stable MCI) (Yoon *et al.*, 2015).

2.7.3 Magnetic Resonance Imaging

In a pathological study discussed above (Molano et al., 2010), 3 cases had serial MRI. Compared with previously published data, hippocampal volumes at the time of MCI and rates of hippocampal atrophy were within the range of cognitively normal subjects.

A cross-sectional study of patients clinically diagnosed as prodromal DLB or prodromal AD found that prodromal AD displayed cortical thinning in the parietal lobes and left hippocampal gyri compared with prodromal DLB, whereas prodromal DLB displayed thinning in a small area of the right insula and pars opicularis (false discovery rate corrected) (Blanc *et al.*, 2015). There were no differences between prodromal DLB and healthy controls after correction for false discovery rate.

In a group of 170 patients with MCI, baseline subcortical hyperintensities on MRI were associated with an increased risk of developing mixed or vascular dementia, but not DLB or other dementia subtypes (Bombois et al., 2008).

A longitudinal MR spectroscopy study measured the ratios of N-acetylaspartate (NAA), Choline and myoinositol to creatine (Cr) in three cortical voxels (frontal, posterior cingulate and occipital) in MCI patients that later converted to DLB (n=10), AD (n-27) or remained stable (n=27) (Zhang *et al.*, 2015). AD-MCI subjects had lower NAA/Cr in the precuneus voxel than DLB-MCI suggesting greater neuronal integrity in prodromal DLB compared with prodromal AD. There were no differences between the groups in any other measurements.

Another study performed MR spectroscopy, diffusion weighted imaging (DWI) and perfusion imaging on 119 patients with MCI (Fayed et al., 2008). After follow-up subjects could be classified as AD (including mixed dementia, n=49); Lewy body dementia (LBD) (n=5; criteria not stated); MCI due to vascular disease (n=15); MCI due to depression (n=22); or MCI due to AD (n=28). There were no differences between LBD and the other groups in baseline spectroscopy or perfusion findings. On DWI in the right hippocampus, the LBD group higher baseline Apparent Diffusion Coefficient (ADC) values compared with the 3 MCI groups, indicating greater white matter disruption. The difference between LBD and AD approached significance (p=0.08). Values in the AD/mixed dementia group did not differ from the MCI groups. Baseline characteristics were not provided, so the findings could be due to differences between groups at baseline (e.g. age or severity of cognitive impairment).

2.7.4 PET Imaging

Clerici et al. (2009) performed ^{18}F-FDG PET on 16 patients with single domain aMCI and 14 patients with naMCI with executive dysfunction. These were compared with controls that were undergoing PET scans for cancer restaging. Of those who completed follow-up, 1/14 aMCI and 5/12 naMCI developed DLB. In a voxel-based analysis, the naMCI who developed DLB had heterogeneous patterns of hypometabolism compared to controls. The inferior and mesial frontal; anterior and posterior cingulate; superior temporal and inferior parietal areas were most frequently involved. Frontal hypometabolism may have been expected, given that executive dysfunction was one of

the inclusion criteria for the naMCI group. This may not be representative of all prodromal DLB.

Pardo et al. (2010) followed 19 army veterans with MCI for 3 years following baseline FDG-PET scans. Two developed DLB; both had an 'AD-like' pattern of hypometabolism (hypometabolism in medial parietal and lateral parietal regions) on visual inspection. Neither displayed occipital hypometabolism. Conversely, in another FDG-PET cohort of 45 subjects with MCI, two subjects displayed a 'DLB-like' pattern with occipital hypometabolism, and both later developed DLB (Cerami *et al.*, 2015).

Cases have been reported of occipital hypometabolism on ^{18}F-FDG PET imaging (Fujishiro et al., 2013a) or cardiac sympathetic denervation measured by ^{123}I-MIBG SPECT (Fujishiro et al., 2012) in non-demented individuals that later went on to develop DLB.

2.7.5 EEG

Resting state, eyes-closed EEG analysis was performed in 47 memory clinic cases of MCI, 20 of whom later developed DLB (Bonanni *et al.*, 2015). All of the DLB-MCI cases had baseline EEG abnormalities, whereas 93% of AD-MCI cases had a normal EEG. Dominant frequency variability was significantly greater in DLB-MCI group than the AD-MCI or stable MCI groups. The proportion of DLB cases was high because 21/47 cases were specifically selected because they had a core or suggestive feature of DLB in the MCI phase.

2.8 REM Sleep Behaviour Disorder

RBD is associated with high rates of conversion to dementia. Longitudinal studies have estimated that over half of patients with RBD go on to develop a neurodegenerative disorder which is nearly always a synucleinopathy (e.g. Parkinson's disease (PD), Parkinson's disease dementia, DLB, multi-system atrophy) if followed-up for more than a decade, rising to up to 93% if followed-up over longer periods (Postuma *et al.*, 2009; Iranzo *et al.*, 2013; Schenck *et al.*, 2013). In these studies, 14-39% of those who developed a neurodegenerative disorder were diagnosed with DLB.

Studies specifically looking at RBD in DLB have confirmed that RBD tends to precede cognitive symptoms by several years (Boeve *et al.*, 1998; Boeve *et al.*, 2003). In some cases the gap is over 25 years (Claassen et al., 2010). Core symptoms may develop earlier in DLB patients with RBD than those without RBD (Dugger et al., 2012).

2.8.1 Imaging in RBD to predict the development of DLB

Dang-Vu et al. (2012) performed ^{99m}Tc-ECD SPECT perfusion scanning on 20 patients with RBD who did not have dementia (though 13 had MCI), and compared these to 10 healthy controls. After an average follow-up of 3 years, 5 RBD subjects developed DLB (criteria not stated) and 5 PD. All those who developed DLB had an initial diagnosis of MCI. The PD/DLB group had increased baseline hippocampal regional cerebral blood-flow (rCBF) compared to the RBD group that did not develop neurodegenerative disease. The 5 DLB patients had increased hippocampal rCBF compared with controls. There were no significant differences between the PD and DLB groups. The PD/DLB group was on average 4.8 years older than the RBD subjects that did not develop disease.

Iranzo et al. (2010) performed striatal dopamine terminal binding of ^{123}I-FP-CIT and transcranial echosonography of the substantia nigra in 43 patients with RBD. Eight patients later developed neurodegenerative disease (5 PD, 2 DLB and 1 multi-system atrophy), all of whom had at least one abnormal imaging finding. 30% of those with an abnormal finding developed a neurodegenerative disease at 2.5 years, compared with 0% of those with 2 normal scans. Both DLB cases displayed substantia nigra hyperechogenicity and one had reduced striatal ^{123}I-FP-CIT uptake at baseline.

2.8.2 Other biomarkers in RBD to predict the development of DLB

Postuma and colleagues commenced a longitudinal study of RBD in 2004. After several years they have been able to identify baseline symptoms and signs that were associated with the development of neurodegenerative disease. In their latest report, 32 of 91 RBD subjects in their cohort had developed neurodegenerative disease (11 probable DLB, 4 possible DLB, 17 parkinsonism) (Postuma et al., 2013).

The RBD group that developed neurodegenerative disease reported greater baseline levels of urinary dysfunction, erectile dysfunction and constipation than controls (Postuma et al., 2013). They did not report more symptoms of orthostatic hypotension, but did have a greater postural drop in blood pressure. These abnormalities were present four or more years before the development of neurodegenerative disease. The results of the RBD without neurodegenerative disease group were intermediate between the controls and the disease group, possibly reflecting that some of this group were in the process of developing a neurodegenerative disease. Baseline postural hypotension and urinary dysfunction were significantly more common in the disease than the non-disease RBD group. ECG measures of autonomic dysfunction did not predict the development of neurodegenerative disease in RBD (Postuma et al., 2010).

Motor abnormalities assessed by the UPDRS, the alternate-tap test, the Purdue Pegboard and the timed up-and-go were all found to be abnormal in DLB three or more years before the diagnosis of dementia (Postuma et al., 2012). These tests appeared to be abnormal for longer periods before the development of DLB than PD. A multicentre study including this cohort found that patient-reported stooped posture (odds ratio (OR) 1.88) and tremor (OR 2.03) were more common in patients that went on to develop neurodegenerative disease (Postuma *et al.*, 2015b). Cardiovascular autonomic symptoms were also significantly more common (OR 1.28).

Patients that developed DLB or PD dementia had abnormal baseline colour vision and olfactory function, assessed using the Farnsworth-Munsell-100-Hue and University of Pennsylvania Smell Identification tests respectively (Postuma et al., 2011). These abnormalities were present at the first assessment, up to 5 years before the development of dementia. Those with both abnormal olfaction and colour vision had an estimated disease-free survival (i.e. no DLB, PD dementia or PD) of 18%, compared with 82% of those with normal function on both tests. A separate study found that olfactory impairment assessed with ‘Sniffin’ Sticks’ differentiated between those with RBD that later developed Lewy body disease and those that did not, with an area under the curve of 0.82 (Mahlknecht *et al.*, 2015).

In general, each of the abnormalities discussed above had high specificity but low sensitivity in identifying those with RBD that would go on to develop neurodegenerative disease. They were present some years before the diagnosis of

disease and tended to progress slowly. Postuma et al. (2015a) have suggested a combination of markers to predict risk of conversion from RBD to neurodegenerative disease. These could be used to identify patients at high risk of progression to DLB or PD. When olfaction, colour vision and motor tests were used, those with abnormalities in two of these areas had a 3-year conversion rate of 60%.

2.9 Discussion

2.9.1 Clinical presentation of prodromal DLB

The above evidence suggests that DLB can be preceded by an MCI phase before the development of dementia. Two studies that followed up participants with MCI for the development of DLB (Fischer *et al.*, 2007; Palmqvist *et al.*, 2012) reported figures similar to reported rates of DLB in clinically diagnosed dementia samples (Vann Jones and O'Brien, 2014); though rates varied greatly between studies, most likely due to recruitment from different clinical populations.

Although the data available are limited, the pattern of symptoms in prodromal DLB appears to differ from that of prodromal AD. Particular symptoms that are more frequent in prodromal DLB include RBD, autonomic dysfunction (including constipation and orthostatic dizziness), hyposmia, visual hallucinations and motor symptoms. Even without including core symptoms, prodromal DLB may be discriminated from prodromal AD with reasonable sensitivity and specificity (Chiba et al., 2012). The earliest symptoms of DLB are constipation, RBD and hyposmia. RBD has been demonstrated to precede DLB by decades in some cases. Table 2.4 shows approximate temporal relationships between symptoms in prodromal DLB from the evidence currently available. The order of symptom development is similar to that reported in PD (Gaenslen et al., 2011).

Prodromal DLB can present with either amnestic or nonamnestic cognitive impairment (Fischer *et al.*, 2007; Clerici *et al.*, 2009; Molano *et al.*, 2010), though naMCI is much more likely to progress to DLB than aMCI (Ferman *et al.*, 2013b). This is supported by findings in a recent study comparing neuropsychological measures at initial presentation (including MCI and mild dementia cases) (Yoshizawa et al., 2013). Those with ‘pure’ DLB pathology at post mortem had greater visuospatial impairment and less memory

impairment at initial assessment compared to 'pure' AD or mixed DLB+AD pathology groups.

There is conflicting evidence on when the core features of DLB develop. Two longitudinal post-mortem cohorts that recruited subjects with dementia found that core features developed after the onset of dementia (Fujishiro *et al.*, 2008; Ferman *et al.*, 2011). Conversely, two longitudinal post-mortem studies that recruited before the onset of dementia (Jicha *et al.*, 2010; Molano *et al.*, 2010), three longitudinal clinical studies (Ferman *et al.*, 2013b; Cagnin *et al.*, 2015; Yoon *et al.*, 2015) and two retrospective interview studies (Auning *et al.*, 2011; Fujishiro *et al.*, 2013b) found that core symptoms commonly develop before the onset of dementia.

These studies differed greatly in design and selection criteria, which may account for the differences in findings. Duration of dementia before death was notably different between some of the studies (≤4 years in Molano et al. (2010) v. 8-10 years on average in the two cohorts that recruited dementia patients (Fujishiro *et al.*, 2008; Ferman *et al.*, 2011)). This suggests that the studies may have recruited cohorts that were not clinically similar, or that diagnostic thresholds were different between the studies.

From this evidence, it appears that most cases of prodromal DLB will display clinical and neuropsychological characteristics similar to established DLB. The exact proportion of cases that conform to this phenotype remains to be established. In those cases that do not, other biomarkers may be needed to identify prodromal DLB.

2.9.2 Biomarkers of prodromal DLB

There has been little investigation into the use of imaging and other biomarkers to identify prodromal DLB. Indeed no studies were found that investigated CSF or blood biomarkers in prodromal DLB. Autonomic symptoms are common in prodromal DLB (Chiba et al., 2012), therefore objective biomarkers of autonomic function such as postural hypotension could potentially be useful in the diagnosis of prodromal DLB.

Table 2.4. Temporal order of symptom development in prodromal DLB

	Very Early (developing before cognitive symptoms)	Early (developing during MCI)	Late (developing around the time of conversion to dementia or later)
Symptoms	RBD	Memory impairment	Cognitive fluctuations
	Constipation	Parkinsonian symptoms	
	Hyposmia	Visual hallucinations	
	Depression	Anxiety	
	Urinary dysfunction		
	Erectile dysfunction		
Signs/neuropsychological findings/biomarkers	Orthostatic hypotension/dizziness	Attention/executive dysfunction	Occipital hypometabolism
	Impaired olfactory function	Visuospatial dysfunction	
	Minor motor abnormalities	Striatal dopaminergic denervation	
	Impaired colour vision	*Substantia nigra hyperechogenicity*	
		Increased hippocampal rCBF	

Items in italics reflect evidence from cohorts with RBD at baseline that may not be applicable to prodromal DLB as a whole

Dopamine terminal imaging, the most established biomarker for DLB, can be abnormal in mild DLB in the absence of clinical features of parkinsonism, suggesting that it may have a role in identifying prodromal DLB (Auning *et al.*, 2011; Siepel *et al.*, 2013). The only paper to investigate this (Albin et al., 2013) found that 1 of 2 MCI subjects with baseline striatal dopaminergic denervation later developed DLB. Three other subjects that developed DLB had normal dopamine terminal scans in the MCI phase. The same authors had previously reported a case of rapid striatal dopaminergic denervation around the time of onset of DLB (Albin and Koeppe, 2006).

The typical DLB pattern of occipital hypometabolism on FDG-PET is present in some cases of prodromal DLB, but others display a more 'AD-like' pattern (Clerici *et al.*, 2009; Pardo *et al.*, 2010; Cerami *et al.*, 2015). As in established DLB, cortical atrophy is less prominent in prodromal DLB compared with prodromal AD (Blanc *et al.*, 2015). With regards to other imaging modalities, raised hippocampal diffusivity on DWI compared with controls was found in one study, but this was not significantly greater than the prodromal AD group (Fayed et al., 2008).

In summary, it appears that striatal dopaminergic denervation and occipital hypometabolism are present in some, but not all patients with prodromal DLB. Further research is needed to evaluate the usefulness of these imaging modalities, and others known to be abnormal in established DLB. Cardiac ^{123}I-MIBG scintigraphy is sensitive and specific in differentiating mild DLB from mild AD (MMSE≥22) and may be a good biomarker in the prodromal stage given that the peripheral autonomic system is involved in the early stages of LB disease (Minguez-Castellanos *et al.*, 2007; Yoshita *et al.*, 2015).

2.9.3 RBD as a prodrome of DLB

RBD patients represent a particular cohort at risk for developing DLB. Poor olfaction and colour vision; autonomic and motor dysfunction; reduced striatal dopaminergic innervation on SPECT; substantia nigra hyperechogenicity and increased hippocampal perfusion may all help to predict those with RBD that will go on to develop DLB or PD (Iranzo *et al.*, 2010; Postuma *et al.*, 2010; Postuma *et al.*, 2011; Dang-Vu *et al.*, 2012; Postuma *et al.*, 2012; Postuma *et al.*, 2013; Mahlknecht *et al.*, 2015). None of these markers differentiate between those who will develop DLB from those who will develop PD. The evidence for these biomarkers is generally based on small DLB

samples and few of the findings have been replicated. Findings in RBD groups may not be generalizable to the wider prodromal DLB population.

2.9.4 Limitations

With the exception of three retrospective symptom questionnaire studies (Auning *et al.*, 2011; Chiba *et al.*, 2012; Fujishiro *et al.*, 2013b), most of the evidence above relates to small groups of DLB patients and these three studies did not systematically evaluate all symptom domains. Few findings have been replicated.

In general, the evidence is from clinical studies, without post-mortem verification of diagnosis. In some cases, this may have led to false positive or false negative results due to the misclassification of study subjects and such misclassification would in turn affect the apparent performance of biomarkers. Due to the heterogeneity of the data available, it is not possible at this stage to combine the data or objectively compare the reliability of conflicting findings. This prevents us from objectively testing whether or not DLB has a distinct prodrome using this data. Longitudinal studies will be required, first to develop criteria for the prodrome of DLB, and then to test their validity.

2.10 Conclusions

The evidence available, though limited, suggests that DLB has an identifiable prodromal phase. It may be possible to differentiate prodromal DLB from prodromal AD based on the presence of core and suggestive features of DLB, autonomic dysfunction and other biomarkers.

^{123}I-FP-CIT and ^{131}I-MIBG SPECT findings are abnormal in established DLB. It remains to be ascertained at what point in the evolution of the disease these findings become abnormal, and if these scans will be clinically useful in the identification of prodromal DLB.

Longitudinal studies are certainly needed to further characterise the clinical presentation of prodromal DLB and investigate the utility of biomarkers (including CSF biomarkers) in its identification. Interesting findings in RBD suggesting that olfactory, visual, autonomic and motor dysfunction; hippocampal hyperperfusion and substantia nigra hyperechogenicity may predict the development of DLB should be investigated in a ‘normal’ MCI group, not recruited in a specialist sleep disorders centre.

Characterisation of the DLB prodrome is vital to enable the identification of DLB patients in the prodromal stage. This will facilitate research into the pathophysiology of prodromal DLB and the development of treatments aimed at halting or reversing these pathophysiological processes.

Chapter 3 Amyloid imaging in Lewy body disorders

3.1 Introduction

The pathological hallmarks of LB disorders are Lewy bodies and Lewy neurites, but many cases also display AD pathology (amyloid-β (Aβ) plaques and neurofibrillary tangles (NFTs)) post-mortem (Kovari et al., 2009). The importance of AD pathology in LB disorders and its relationship to cognitive impairment is unclear. Post-mortem studies of confirmed DLB cases have reported that concurrent AD pathology measured by increased NFTs was associated with a lower likelihood of visual hallucinations and a lower chance of a DLB diagnosis during life (Merdes *et al.*, 2003; Weisman *et al.*, 2007; Walker *et al.*, 2015a). Similarly greater neuritic Aβ plaque pathology has been associated with fewer core features of DLB (Tiraboschi *et al.*, 2015). A combination of AD and LB pathology in dementia is associated with a lower occurrence of parkinsonism and hallucinations compared with LB pathology alone (Del Ser et al., 2001), and faster rates of cognitive decline (Kraybill et al., 2005) and brain atrophy (Nedelska *et al.*, 2015a). Greater cortical Aβ pathology has been associated with increased cognitive impairment (Nelson et al., 2009) and a shorter time from onset of parkinsonian symptoms to cognitive impairment (Ballard *et al.*, 2006; Selikhova *et al.*, 2009; Fujishiro *et al.*, 2010) in LB disorders.

DLB has been associated with a greater Aβ burden than Parkinson's disease dementia (PDD) and PD (Jellinger *et al.*, 2003; Aarsland *et al.*, 2004; Fujishiro *et al.*, 2010; Walker *et al.*, 2015a). It has been suggested that the co-occurrence of AD and LB pathology represents more than coincidence, and that there may be synergism, with Aβ increasing the propensity of α-synuclein to accumulate and aggregate (Pletnikova et al., 2005).

Post-mortem studies are by their nature cross sectional and tend to report end-stage disease. Thus patients with initial 'pure Lewy body pathology' may be found post-mortem to have significant AD pathology, although this may not have been related to their initial presentation. PET amyloid imaging, initially with ^{11}C-PiB (Pittsburgh compound B) and now with ^{18}F-labelled compounds, was developed to measure brain Aβ burden in vivo (Rowe and Villemagne, 2011). Post-mortem studies have demonstrated that amyloid imaging with these ligands correlates well with Aβ deposition in the form of neuritic and diffuse plaques, and amyloid angiopathy

(Ikonomovic *et al.*, 2008; Clark *et al.*, 2011; Sojkova *et al.*, 2011). Aβ deposition in vivo is consistently elevated in AD, and also in some healthy control subjects (Quigley *et al.*, 2011; Rosenberg *et al.*, 2013). This chapter will review all studies to date that have investigated amyloid imaging in LB disorders to examine the contribution of Aβ pathology to these disorders.

Terminology in this research field has been a subject of some debate (McKeith, 2009). In this chapter 'LB dementias' refers to both DLB and PDD. 'LB disorders' refers to all LB pathologies (in this chapter generally PD (+/- MCI), PDD and DLB).

3.2 Dementia with Lewy bodies

3.2.1 DLB vs controls

A summary of all PET amyloid imaging studies involving patients with Lewy body disease is provided in Table 3.1 at the end of this chapter. When compared with healthy controls, four studies have found DLB to be associated with significantly greater cortical ^{11}C-PiB binding on PET imaging (Edison *et al.*, 2008; Gomperts *et al.*, 2008; Gomperts *et al.*, 2012; Kantarci *et al.*, 2012b). A 32% greater uptake was found in one study (Edison et al., 2008), although 6 controls with raised ^{11}C-PiB binding had been excluded from analysis, as the aim of the study was to examine patterns of raised Aβ deposition in LB disorders.

Three studies failed to find a significant difference between DLB and controls in global or regional Aβ ligand binding (Foster *et al.*, 2010; Villemagne *et al.*, 2011; Siderowf *et al.*, 2014). However, in each study DLB displayed greater binding (mean cortical binding potential: 0.18 v. 0.08 (Foster et al., 2010); neocortex standardised uptake value ratio: 1.38 v. 1.26 (Villemagne et al., 2011); 1.32 v 1.02 (Siderowf *et al.*, 2014)). It should be noted that these studies contained relatively small DLB samples (n=6, n=7 and n=11 respectively) compared to those that found significant differences (n=8-21 (Edison *et al.*, 2008; Gomperts *et al.*, 2008; Gomperts *et al.*, 2012; Kantarci *et al.*, 2012b)).

Particular areas of increased Aβ ligand binding in DLB include frontal, parietal and cingulate areas, along with the striatum (Rowe *et al.*, 2007; Gomperts *et al.*, 2008; Claassen *et al.*, 2011; Gomperts *et al.*, 2012; Kantarci *et al.*, 2012b), with relative

sparing of the medial temporal lobe (Rowe *et al.*, 2007; Gomperts *et al.*, 2008; Kantarci *et al.*, 2012b).

3.2.2 DLB vs AD

Five studies have compared amyloid brain imaging in DLB and AD. The largest study (n=42) found global ^{11}C-PiB retention ratio to be significantly lower in DLB (Kantarci *et al.*, 2012b). Two other studies have also found lower cortical Aβ ligand binding in DLB compared with AD (Rowe *et al.*, 2007; Villemagne *et al.*, 2011) (distribution volume ratio: 1.7 v. 2.0 (Rowe et al., 2007); standardised uptake value ratio: 1.38 v. 1.93 (Villemagne et al., 2011)).

One study found AD to have significantly greater amyloid deposition than DLB only in the parietal and anterior cingulate regions (Siderowf *et al.*, 2014). Another study found no difference between DLB and AD, with very similar results in both groups in all cortical areas (Gomperts et al., 2008). In this study, the DLB group was on average 9 years older than the AD group, with significantly greater cognitive impairment, although results held after adjusting for age (but not cognition) in multivariate analysis.

3.2.3 DLB vs other LB disorders

Gomperts et al. (2012) compared DLB with the other Lewy body disorders: PDD, PD with mild cognitive impairment (PD-MCI) and PD. The study compared precuneus ^{11}C-PIB retention, as retention was particularly high in this region and correlated very highly with global retention (r=0.939). Mean precuneus ^{11}C-PiB distribution volume ratio was significantly higher in DLB than the other Lewy body disorders (DLB=1.49, PDD=1.28, PD-MCI=1.16, PD=1.16). This echoed their earlier findings of higher global cortical ^{11}C-PiB retention in DLB (Gomperts et al., 2008). Edison et al. (2008) also reported higher ^{11}C-PiB binding in DLB compared with PDD and PD, but did not report direct statistical testing between the groups. Another study comparing 3 DLB patients and 3 with multiple systems atrophy (MSA) found higher cortical ^{11}C-PiB binding in the DLB group (Claassen et al., 2011).

Three studies found no difference in Aβ ligand binding between DLB and PD (cortical standardised uptake value ratio: DLB=1.38, PD=1.14 (Villemagne et al., 2011); DLB=1.32, PD=1.12 (Siderowf *et al.*, 2014)); or PD, PD with MCI and PDD (mean

cortical binding potential: DLB=0.18, PDD=0.11, PD-MCI=0.08, PD=0.04 (Foster et al., 2010)). In these studies, although not significant, Aβ ligand binding in the groups bore the same relationship to each other seen in studies that did find a significant difference (DLB>PDD>PD).

When present, the pattern of Aβ ligand binding in Lewy body disorders is broadly similar to that seen in AD (Rowe *et al.*, 2007; Gomperts *et al.*, 2008; Maetzler *et al.*, 2008; Maetzler *et al.*, 2009; Kantarci *et al.*, 2012b; Petrou *et al.*, 2012; Shimada *et al.*, 2013), although one study found occipital cortex binding relative to overall binding to be lower in AD (Gomperts et al., 2008). A principle component analysis by Campbell et al. found that the pattern of deposition of amyloid in LB disease (PD-MCI, PDD and DLB) was more similar to that seen in controls than to the pattern seen in AD (Campbell *et al.*, 2013).

Amyloid imaging scans can be classified as amyloid positive or amyloid negative based on a quantitative threshold of amyloid ligand binding or visual rating of the scan, using thresholds derived from studies in AD. The differences between Lewy body diseases become more stark using this method. In a recent meta-analysis the prevalence of amyloid positive scans was 68% in DLB, compared with 34% in PDD and 5% in PD-MCI (Petrou *et al.*, 2015). A separate analysis which only included studies in which participant-level demographic data was available found the prevalence in DLB to be 51%, compared with 88% in AD and 24% in controls (Ossenkoppele *et al.*, 2015).

Meta-analyses in dementia (Ossenkoppele *et al.*, 2015), MCI and healthy older people (Jansen *et al.*, 2015) identify increasing age and the presence of the APOE ε4 genotype as the primary risk factors for amyloid positivity on PET imaging.

3.3 Parkinson's disease dementia

3.3.1 PDD vs controls/AD

All seven studies comparing Aβ ligand binding in PDD and controls have found no significant difference between the two groups (Edison *et al.*, 2008; Gomperts *et al.*, 2008; Maetzler *et al.*, 2008; Foster *et al.*, 2010; Jokinen *et al.*, 2010; Gomperts *et al.*, 2012; Edison *et al.*, 2013). In general PDD results were very similar to controls, with a

tendency toward a small number of outliers in the PDD group with high levels of cortical Aβ (Edison *et al.*, 2008; Maetzler *et al.*, 2009; Foster *et al.*, 2010; Gomperts *et al.*, 2012).

Maetzler et al. (Maetzler et al., 2008) compared PDD and AD after removing 2 PDD patients with markedly raised ^{11}C-PiB binding that they felt indicated a different disease entity to 'pure' PDD. The remaining PDD subjects demonstrated remarkably similar cortical Aβ binding to the control group and lower cortical and striatal binding than the AD group .The two high ^{11}C-PiB binding subjects had an 'AD-like' pattern of deposition. The only other study to compare PDD and AD found no outlying PDD subjects, with Aβ ligand binding significantly lower in PDD than AD (Gomperts et al., 2008).

3.3.2 PDD vs PD

Several studies have noted no significant difference in Aβ binding between PDD and PD (Edison *et al.*, 2008; Gomperts *et al.*, 2008; Foster *et al.*, 2010; Jokinen *et al.*, 2010; Gomperts *et al.*, 2012; Edison *et al.*, 2013) or PD with MCI (Gomperts *et al.*, 2012; Gomperts *et al.*, 2013) However, PDD consistently has a greater proportion of Aβ positive subjects when groups are divided in a binary fashion as positive or negative (Petrou *et al.*, 2015).

Relevant to this, Petrou et al. (Petrou et al., 2012) identified 40 patients with PD at risk for dementia (with MCI/older age /long duration of PD/prominent gait or balance impairments). On further testing 5/40 subjects were diagnosed as having mild dementia. When the subjects underwent amyloid imaging 4/5 subjects with dementia had elevated ^{11}C-PiB binding on visual inspection, compared with 2/30 patients with PD-MCI and 0/5 with PD and some other risk factor for dementia. This finding of increased rates of Aβ positive subjects in PDD groups compared with PD or controls, without any difference in mean cortical Aβ ligand binding, may be accounted for by a subgroup of outlying PDD subjects with high Aβ binding (Edison *et al.*, 2008; Maetzler *et al.*, 2009; Foster *et al.*, 2010; Gomperts *et al.*, 2012), or a different pattern of deposition in PDD that results in focal increases in binding without a significantly increased overall Aβ load (Gomperts et al., 2008).

3.4 Parkinson's Disease

Eight studies have compared amyloid imaging findings in PD and controls. One small study compared a group of PD patients with controls from a previous study and found the PD group had significantly lower ^{11}C-PiB binding in the frontal (^{11}C-PiB uptake/cerebellum: 1.10 v 1.30), parietal (1.15 v 1.35) and posterior cingulate (1.04 v 1.43) cortices (Johansson et al., 2008). The PD subjects were well known to the investigators, with no evidence of cognitive impairment on routine assessment. It may be that they represented a subgroup of particularly cognitively healthy individuals.

Other studies have found no difference between PD and controls (Edison *et al.*, 2008; Gomperts *et al.*, 2008; Foster *et al.*, 2010; Jokinen *et al.*, 2010; Villemagne *et al.*, 2011; Gomperts *et al.*, 2012; Siderowf *et al.*, 2014), although mean cortical Aβ ligand binding was marginally lower in PD in some (Foster *et al.*, 2010; Villemagne *et al.*, 2011; Gomperts *et al.*, 2012) (mean cortical binding potential: 0.04 v 0.08 (Foster et al., 2010), standardised uptake value ratio: 1.14 v 1.26 (Villemagne et al., 2011)). One study found ^{11}C-PiB binding to be higher in PD than controls (cortex:cerebellum ratio: 1.19 v 1.11, $p<0.05$) (Edison *et al.*, 2013).

Three studies comparing PD with AD have all found lower brain Aβ ligand binding in PD (Gomperts *et al.*, 2008; Johansson *et al.*, 2008; Villemagne *et al.*, 2011).

3.5 Relationship between Aβ and clinical picture

3.5.1 Cognitive impairment and dementia severity

Two studies have found a significant correlation between MMSE score and Aβ ligand binding when analysing data across multiple disease groups (DLB, PDD, PD: $r=-0.5$, $p=0.01$, $n=26$ (Gomperts et al., 2008); DLB, PD, AD, MCI, FTD, VaD, controls: $r=-0.49$, $p<0.0001$, $n=109$ (Villemagne et al., 2011)). However, over both studies, only the MCI group in Villemagne et al. (2011) displayed the same correlation within the group ($r=-0.76$, $p<0.0001$, $n=20$).

Other studies have considered PDD/DLB as a single group for analysis. In such a group the Aβ positive subjects were found to have a significantly lower MMSE than the Aβ negative group, but this group was also older (Maetzler et al., 2009). Conversely, Foster et al. (Foster et al., 2010) were not able to identify differences between Aβ positive and negative PDD/DLB groups in MMSE, NPI or cognitive fluctuations. However, pooling

the Aβ positive and Aβ negative PDD/DLB subjects (n=21) they found mean cortical and caudate Aβ binding correlated modestly with MMSE (cortical: r=-0.47, p=0.04; caudate: r=-0.44 p=0.05) and Clinical Dementia Rating Scale (CDR) global (cortical: r=0.55, p=0.01; caudate: r=0.51, p=0.02). No such correlation existed in PD or control groups. In PD with MCI, MMSE correlated strongly with caudate Aβ (r=-0.82, p=0.007, n=9) but not mean cortical Aβ (p=0.52).

In a population mostly consisting of PD-MCI, Aβ ligand binding was found to correlate with global cognitive scores (r=-0.55, p=0.0006, n=40) and Wechsler Adult Intelligence Scale score (r=-0.54, p=0.0004, n=40) (Petrou et al., 2012). However these results may have been strongly influenced by 4 PDD subjects with high levels of brain Aβ and cognitive impairment.

In a DLB-only group (n=18), Gomperts et al. (Gomperts *et al.*, 2012) found increased Aβ ligand binding to be associated with lower MMSE ($p<0.001$) scores and worse semantic memory (p=0.001). No such association was found in PDD, PD, PD-MCI or controls. A study with 11 DLB subjects found that amyloid positive subjects had significantly lower MMSE scores than amyloid negative subjects (14.4 v 25.3; p=0.046) (Siderowf *et al.*, 2014). Other studies found no relationship between Aβ and MMSE (Edison *et al.*, 2008; Shimada *et al.*, 2013) or dementia severity measured by CDR (Kantarci *et al.*, 2012b) in DLB.

One longitudinal study in 46 PD subjects with either MCI or no cognitive impairment found that baseline increased precuneus ^{11}C-PiB binding was related to decline in executive function (p=0.035), weakly related to decline in visuospatial function (p=0.06), and associated with a greater likelihood of transition to MCI or dementia (p=0.035) (Gomperts et al., 2013).

In summary, there is conflicting evidence surrounding the association of amyloid deposition with increased cognitive impairment in LB disorders. Where correlation has been reported, it has generally been modest, and often in samples containing disparate diagnostic groups. Further research is needed to clarify the association of amyloid deposition with cognitive impairment in each of the LB disorders.

3.5.2 Parkinsonism

In DLB and PDD, but not PD, higher relative Aβ ligand binding in the striatum (adjusted for overall binding) was associated in one study with better motor performance as measured by the UPDRS (DLB: r=-0.87, p=0.01, n=8; PDD: r=-0.90, p=0.005, n=7) (Gomperts et al., 2008). Other studies have found no correlation between global cortical (Maetzler *et al.*, 2009; Foster *et al.*, 2010; Kantarci *et al.*, 2012b; Shimada *et al.*, 2013) or precuneus (Gomperts *et al.*, 2012) Aβ binding and motor impairment. However, these studies did not report specifically assessing relative striatal binding. A longitudinal study found that baseline striatal or precuneus amyloid ligand binding in PD and PD-MCI did not predict future motor deterioration (Gomperts et al., 2013).

3.5.3 Hallucinations and visuospatial ability

In the two studies which have reported this, no relationship has been found between mean cortical (Kantarci *et al.*, 2012b) or occipital (Gomperts et al., 2008) Aβ binding and hallucinations.

In DLB, PDD and PD, but not AD or controls, relative parietal/posterior cingulate (but not occipital) binding was associated with impaired visuoperceptual ability as measured by the Benton visual form discrimination test (Gomperts et al., 2008).

3.5.4 Fluctuations

Fluctuations, measured using the Mayo Fluctuations Questionnaire, were not found to be associated with an increased Aβ ligand binding in DLB or PDD in two studies (Foster *et al.*, 2010; Kantarci *et al.*, 2012b).

3.5.5 RBD

No studies have reported investigating an association between RBD and Aβ ligand binding.

3.5.6 Disease onset, progression and treatment response

The first study of amyloid PET in DLB found that increased Aβ binding was correlated with a shorter time between the onset of cognitive impairment and diagnosis of DLB (r=-0.75, p=0.01, n=10), with no such relationship seen in AD (Rowe et al., 2007).

Maetzler et al. (Maetzler et al., 2009) found that in a combined DLB and PDD group, Aβ positive patients had an older age at onset of parkinsonism and dementia and had lower MMSE scores. The authors suggested that this may be an effect of increasing Aβ with increasing age, or that cortical Aβ deposition is associated with different disease mechanisms resulting in an older age of onset and more rapid clinical progression.

In a small group of treatment naïve patients, Graff-Radford et al. (2012) found that after treatment with acetylcholinesterase inhibitors Aβ positive patients (n=3) tended to remain stable or decline, whereas Aβ negative patients (n=4) tended to remain stable or improve.

3.5.7 Relationship with genetics, imaging findings and other biomarkers

An early study found the apolipoprotein (APOE) ε4 genotype was associated with increased ^{11}C-PiB binding across groups (including relatively large groups of AD and controls) but not within diagnostic groups (Rowe et al., 2007). Similarly, in a large study, Gomperts et al. (Gomperts *et al.*, 2012) found that APOE ε4 genotype and ^{11}C-PiB binding were correlated across the entire cohort (DLB, PDD, PD-MCI, PD, controls; r=0.49, p<0.0001). In a study involving DLB, PDD and PD subjects ^{11}C-PiB binding and CSF APOE levels were positively correlated (r^2=0.42, p<0.0001) (Vijayaraghavan *et al.*, 2014). The Aβ positive group (all of whom had dementia) were found to have increased rates of the APOE ε4 allele and lower CSF Aβ-42 concentrations compared with Aβ negative patients (Maetzler et al., 2009). These results are consistent with findings in AD subjects, those with MCI and apparently healthy older controls, that the APOE ε4 genotype is robustly associated with increased Aβ binding (Drzezga *et al.*, 2009; Fleisher *et al.*, 2013).

Shimada et al. (2013) examined the association between ^{11}C-PiB binding and cortical atrophy in a combined PDD/DLB group compared with AD and healthy controls. They found that of 6/15 PDD/DLB patients were ^{11}C-PiB positive. Compared to ^{11}C-PiB negative controls (^{11}C-PiB positive controls were excluded) the ^{11}C-PiB positive

PDD/DLB group demonstrated significant cortical atrophy, particularly in temporal and parietal areas, whereas the ^{11}C-PiB negative group did not. Using volume of interest analysis, the ^{11}C-PiB positive PDD/DLB group had lower parahippocampal grey matter volume than ^{11}C-PiB negative PDD/DLB subjects. There were no differences between the two groups in cognitive tests. Atrophy was not correlated with Aβ ligand binding in any group. The authors commented that Aβ deposition in PDD/DLB appeared to be associated with cortical atrophy in a pattern similar to that seen in AD, though the level of atrophy itself was not correlated with amyloid load and likely due to down-stream effects.

Four studies have compared amyloid imaging scans with ^{18}F-FDG PET in Lewy body disorders. One small study (n=3), found corresponding hypometabolism and amyloid deposition in several cortical areas (Claassen et al., 2011). Two studies in DLB and PDD reported brain hypometabolism (in areas such as the occipital and posterior parietotemporal lobes) in the absence of Aβ deposition and suggested that the two processes do not appear directly related, similar to the apparent dissociation between structural MR measures of atrophy and PET hypometabolism in DLB (Jokinen *et al.*, 2010; Kantarci *et al.*, 2012b; Sinha *et al.*, 2012). Consistent with this, a recent study compared PiB positive (n=5) and negative (n=5) DLB subjects and found very similar levels of brain metabolism in both groups (Ishii *et al.*, 2015). Loss of white matter integrity in the parieto-occipital region in DLB has also been found to be independent of Aβ pathology (Nedelska *et al.*, 2015b).

3.6 Reports of post-mortem examination after amyloid PET imaging

The first post-mortem report of a patient with DLB who had had an amyloid PET scan was by Bacskai et al. (2007). They found that amyloid imaging findings corresponded to post-mortem Aβ levels in brain homogenates measured using enzyme-linked immunosorbent assays. Much of the Aβ burden on imaging was due to cerebral Aβ angiopathy.

Burack et al. (2010) examined 3 patients with PDD, 2 of whom had extensive cortical ^{11}C-PiB uptake in PET scans before death. Both were found to have abnormal levels of

cortical Aβ (predominantly diffuse plaques) post-mortem. All cortical areas with PET mean cortical binding potential greater than 0.2 had severe plaque burden post-mortem. The case with no raised Aβ binding had minimal Aβ plaques but abundant cortical Lewy bodies, suggesting there is no significant binding of ^{11}C-PiB to LBs during amyloid PET scans.

Kantarci et al. (2012b) reported 3 cases of DLB with ante-mortem amyloid imaging. One had raised cortical ^{11}C-PiB binding (^{11}C-PiB retention ratio >1.6) and two had borderline binding (^{11}C-PiB retention ratio 1.4-1.6). The case with raised ^{11}C-PiB had sparse neuritic plaques but frequent diffuse plaques. The two borderline cases had sparse or moderate neuritic plaques (the level of diffuse plaques was not mentioned).

The case with raised ^{11}C-PiB underwent quantitative comparison of amyloid deposition measured by ^{11}C-PIB PET and post-mortem image analysis of immunostains of corresponding regions (Kantarci *et al.*, 2012c). There was a strong correlation between ^{11}C-PiB retention (18 months ante-mortem) and post-mortem Aβ density in the 17 ROIs analysed ($r=0.899$; $p < 0.0001$). Lewy body and tau density did not correlate with ^{11}C-PiB retention.

Ikonomovic et al. (2012) examined a case of probable DLB with a negative ^{11}C- PiB PET scan. Although post-mortem examination did identify Aβ plaques, they were infrequent and primarily diffuse rather than neuritic. The authors commented that the level of amyloid deposition necessary to elicit a positive ^{11}C-PiB PET scan is not yet clear. Post-mortem $A\beta_{42}$ concentration in brain homogenates correlated with ^{11}C-PiB retention in the ante-mortem PET scan ($r=0.72$, $p=0.009$), corroborating the finding of Kantarci et al. (2012b) above.

3.7 Discussion

3.7.1 Differences between diagnostic groups

In summary, all Lewy body disorders are generally associated with lower mean cortical Aβ ligand binding than AD. DLB is usually associated with higher mean cortical Aβ binding than PDD, PD or controls. There are no significant differences between PDD, PD and controls. When Aβ is present, the pattern of deposition in LB disorders may be

similar to that seen in AD, with deposition in frontal, parietal and cingulate areas, along with the striatum.

Two studies used ^{18}F-labelled tracers (Villemagne *et al.*, 2011; Siderowf *et al.*, 2014), all other studies used ^{11}C-PiB. The pattern of cortical binding with ^{18}F-Florbetaben was almost identical to that of ^{11}C-PiB (Villemagne et al., 2011).

Differences between diagnostic groups are more evident when subjects are classified as Aβ positive or negative based on visual rating or a quantitative threshold for tracer binding. AD has higher rates of amyloid positive scans than DLB, which in turn has higher rates than PDD (Ossenkoppele *et al.*, 2015; Petrou *et al.*, 2015). The difference between DLB and PDD is unlikely to be due to age, which was very similar between the two groups in this analysis (Petrou *et al.*, 2015). However, a confounding effect of APOE genotype cannot be ruled out. Interestingly, the observed rates in PD-MCI may be lower than those seen in other types of MCI, and even in healthy controls of the same age (Petrou *et al.*, 2015). From these findings we can conclude that whilst Aβ deposition in itself is neither necessary nor sufficient for the development of dementia in LB disorders, the presence of Aβ is more common in those with dementia, and relatively rare in those without dementia. These findings mirror results from post-mortem studies that have found greater Aβ deposition in DLB than PDD or PD (Harding and Halliday, 2001; Ballard *et al.*, 2006; Jellinger and Attems, 2008; Fujishiro *et al.*, 2010; Walker *et al.*, 2015a), and higher deposition in PDD than PD (Harding and Halliday, 2001; Compta *et al.*, 2011; Irwin *et al.*, 2012).

High levels of cortical Aβ are unusual in PD. Petrou et al. (Petrou et al., 2012) found that most of the small number of patients with Aβ positive PET scans in a PD cohort identified for being at risk of dementia actually, on closer examination, already had dementia. Similarly, in a post-mortem study of 129 cases of PD, 17 of 20 of patients that had Aβ plaque pathology rated CERAD grade B or C (Mirra et al., 1991) had dementia (85% v. 54% in the overall group) (Kempster et al., 2007). In another neuropathological study of 200 patients with an initial diagnosis of PD, higher CERAD scores were found almost exclusively in patients who developed dementia (CERAD scores PD: B=3%, C=0%; PDD: B=51%, C=33%) (Jellinger et al., 2002).

Diffuse neocortical or limbic Lewy body pathology is generally seen as the main substrate of dementia in LB disorders (Aarsland *et al.*, 2005; Emre *et al.*, 2007; Irwin *et al.*, 2012). The findings above suggest that the presence of Aβ confers a higher risk for the development of dementia in LB disorders. A possible explanation for this is that the combination of cortical Aβ and Lewy body pathology may have synergistic effects. Some post-mortem studies have found that increased Aβ is associated with increased α-synuclein levels in the brain in Lewy body disorders and AD (Pletnikova *et al.*, 2005; Lashley *et al.*, 2008; Compta *et al.*, 2011), although other studies have contradicted this (Ballard *et al.*, 2004; Aarsland *et al.*, 2005)

Interestingly, Aβ promotes the formation of α-synuclein oligomers and polymers in vitro (Masliah et al., 2001). Experiments in transgenic mice expressing human Aβ, tau and α-synuclein peptides have shown that the presence of Aβ increases the formation of α-synuclein neuronal inclusions (Masliah et al., 2001) and α-synuclein increases the deposition of both Aβ and tau (Clinton et al., 2010).

Thus, in LB disorders, the presence of significant Aβ may lead to a synergistic interaction with α-synuclein, resulting in widespread deposition of α-synuclein and Aβ, leading to cognitive impairment. This hypothesis may help explain the infrequency of significant Aβ deposition in PD without cognitive impairment and the increased rates of Aβ seen in DLB compared with PDD, as DLB by definition demonstrates dementia (an indicator of widespread neurodegeneration) earlier in the disorder. The apparent synergistic interaction between Aβ and α-synuclein is a possible target for therapeutic intervention, given our ability to identify the subset of Lewy body disease sufferers with Aβ deposition using PET imaging.

3.7.2 Relationship of Aβ to clinical picture

In imaging studies cognitive impairment has correlated positively with Aβ ligand binding across diagnostic groups (Gomperts *et al.*, 2008; Villemagne *et al.*, 2011). This may simply reflect that Aβ burden is high in AD and DLB and low in PD and controls. More interestingly, Aβ binding may be correlated with cognitive impairment in Lewy body dementia only-groups (Maetzler *et al.*, 2009; Foster *et al.*, 2010) and may predict cognitive decline in PD and PD-MCI (Gomperts et al., 2013). Some pathological studies support these findings. Patients with a combination of DLB and AD pathology post-mortem have been found to have had worse cognitive function (Nelson et al., 2009),

more severe dementia (Serby et al., 2003) and a faster rate of cognitive decline (Kraybill et al., 2005) than those with 'pure' DLB pathology. A correlation between cognitive impairment and Aβ deposition in PD and PDD groups has been found (Mattila *et al.*, 2000; Harding and Halliday, 2001; Compta *et al.*, 2011), although this link did not survive regression modelling in some studies (Mattila et al., 2000).

Conversely, other studies have found no correlation of MMSE with CERAD score in PD with or without cognitive impairment (Aarsland *et al.*, 2005; Braak *et al.*, 2005), and AD pathology in PDD has been found not to affect performance on MMSE or other neuropsychological tests (Sabbagh et al., 2009).

Imaging studies have found suggestive links between increased Aβ ligand binding and older onset of motor impairment and dementia (Maetzler et al., 2009), and a shorter interval between the onset of motor impairment and cognitive impairment (Rowe et al., 2007). There have been various, often contradictory pathological findings about the association of Aβ deposition with disease onset and progression in LB disorders.

Increased Aβ burden post-mortem has been associated with older age of onset and shorter survival in DLB, PD and PDD (Jellinger et al., 2002); and shorter duration of parkinsonism prior to the onset of dementia in PDD and DLB (Ballard *et al.*, 2004; Ballard *et al.*, 2006; Selikhova *et al.*, 2009; Fujishiro *et al.*, 2010; Compta *et al.*, 2011). Other studies found no correlation between Aβ burden age of onset, disease duration and age of death in DLB (Ballard *et al.*, 2004; Nelson *et al.*, 2009) or PD (Lashley et al., 2008); rate of decline in PD or PDD (Aarsland et al., 2005); or the interval between motor and dementia symptoms in PDD (Irwin et al., 2012). Some even found increased Aβ to be associated with longer disease duration in PD, although with higher dementia scores (Mattila et al., 2000). A large epidemiological study (Aarsland et al., 2007) found that the occurrence of dementia in PD was a function of age, and age of onset had no effect above this. Thus, in some studies (Fujishiro *et al.*, 2010; Compta *et al.*, 2011) the shorter duration of parkinsonism before dementia observed in Aβ positive patients could simply be a function of their older age.

Findings from amyloid imaging studies can drive hypotheses that should then be tested in other imaging studies as well as pathological studies. Similarly, pathological findings

(from which most of our current knowledge of the Aβ in LB disorders derives) will drive hypotheses in future imaging studies. Some other positive findings from these early imaging studies that should be tested in future studies include the links between striatal Aβ and Parkinsonism; parietal/posterior cingulate Aβ and visuoperceptual ability; and Aβ ligand binding, APOE genotype and CSF Aβ-42. Further studies are also needed to corroborate the finding that the other core symptoms of DLB, visual hallucinations and fluctuating cognition, are not related to Aβ deposition.

Based on current data it is not possible to make any firm conclusions on the influence of Aβ pathology on disease progression and clinical phenotype in Lewy body disorders. There are a few reasons for this. Aβ imaging studies have thus far, with two exceptions, been cross sectional rather than longitudinal. Studies vary in image acquisition, processing and analysis; clinical and imaging outcome measures used; cut off points for Aβ positive and negative cases; recruitment source (i.e. movement disorder or memory clinics); entry criteria for subjects; and processing of results (e.g. the removal of control/PDD subjects with raised amyloid from analysis (Edison *et al.*, 2008; Maetzler *et al.*, 2008; Shimada *et al.*, 2013)). Few studies have tested the same hypotheses using comparable outcome measures. Many studies also suffered from low sample sizes and sub-optimal measures such as the MMSE, which may not be sensitive to cognitive changes in LB disorders. These problems, and a tendency in some studies to report the results of statistical tests without summary data, prevented any quantitative meta-analysis of the results. Large-scale, prospective studies are needed to properly investigate the effect of Aβ burden on the onset, progression, severity and character of symptoms in LB disorders.

Neuropathological studies suffer from similar inconsistencies (e.g. in the use of grading rather than fully quantitative measures to assess brain pathology). Comparison between imaging and pathological studies is difficult as amyloid PET measures both diffuse and neuritic plaques, as well as amyloid angiopathy, whereas most pathological studies focus solely on neuritic plaques. Although ^{11}C-PiB has higher affinity for neuritic plaques, diffuse plaques account for most of the in vivo binding in LB disorders (Burack *et al.*, 2010; Kantarci *et al.*, 2012c).

3.7.3 Issues with PET amyloid imaging

Some authors have questioned the ability of PET amyloid imaging to adequately quantify amyloid burden, highlighting problems with current PET amyloid imaging technology including partial volume effects and non-specific binding of amyloid radioligands (Moghbel et al., 2012).

PET imaging has a relatively low resolution compared with MRI. This results in a relatively large voxel size. Given the thinness of the cerebral cortex, partial volume effects (where a voxel contains signal from both grey and white matter, or grey matter and CSF, for instance) are possible (Moghbel et al., 2012). This is further complicated by the presence of significant cortical atrophy in a proportion of subjects with cognitive impairment. Although this problem is by no means unique to PET amyloid imaging, it does raise doubts about the ability of PET imaging to accurately quantify amyloid burden in dementia (Villemagne et al., 2012). Despite this, the use of partial volume correction has been found not to significantly affect quantitative measures of amyloid ligand binding (Knesaurek *et al.*, 2013). Few of the studies reviewed here used such correction measures (Table 3.1).

Amyloid radioligands, particularly ^{18}F-labelled ligands, have high non-specific white-matter binding (Rowe and Villemagne, 2011). Studies comparing PiB binding (in vitro and in vivo) with amyloid burden measured by immunohistochemistry have demonstrated that PiB does not give a direct quantitative measure of cortical amyloid burden (Bacskai *et al.*, 2007; Svedberg *et al.*, 2009). Similarly, a study comparing in vivo PiB binding with amyloid burden post-mortem found cases where the precuneus was observed to have the highest PiB retention, despite other brain areas having markedly greater amyloid burden quantified by stereological assessment (Driscoll et al., 2012). It should be noted that, despite this, there was a strong correlation between ^{11}C-PiB binding and post-mortem amyloid burden in the precuneus, anterior cingulate and posterior cingulate, though not the hippocampus or orbitofrontal cortex (Driscoll et al., 2012). Thus, although ligand retention and amyloid burden are correlated in different brain areas within one subject (Kantarci *et al.*, 2012c) and between subjects (Clark *et al.*, 2011; Driscoll *et al.*, 2012), amyloid PET cannot be said to precisely quantify amyloid burden in each particular brain area. These issues should be borne in mind when interpreting and discussing amyloid PET findings.

Importantly, amyloid imaging ligands do not appear to bind significantly to cortical LBs in vitro (Fodero-Tavoletti *et al.*, 2012), and have been shown to accurately measure amyloid load in vivo in the presence of other pathologies, including LB (Burack *et al.*, 2010; Dugger *et al.*, 2014)

3.7.4 Tau pathology

Neuropathological studies have found that NFT pathology in DLB may be associated with a clinical picture more similar to AD than classical DLB (Ballard *et al.*, 2004; Fujishiro *et al.*, 2008). The degree to which high Aβ binding is also associated with a less classical DLB clinical phenotype is of interest; such patients may have a different prognosis, different levels of neuroleptic sensitivity and a different response to treatment.

There is a relative paucity of NFT pathology in LB disorders (Braak et al., 2005), but when present it is a reliable correlate of dementia (Jellinger *et al.*, 2002; Irwin *et al.*, 2012). Although imaging ligands for tau are not yet widely available, there are imaging correlates of tau pathology. Brain atrophy on MRI, particularly in the medial temporal lobe has been found to be associated with post-mortem tau pathology, measured quantitatively with tau antibody and image analysis or semi-quantitatively using Braak NFT staging (Braak and Braak, 1991; Whitwell *et al.*, 2008; Burton *et al.*, 2009). A recent study has found brain atrophy in amyloid positive, but not amyloid negative PDD/DLB subjects (Shimada et al., 2013), despite this, atrophy was not correlated with PiB binding. This may be because atrophy is the result of downstream effects, either of tau, α-synuclein, a combination of both, or another factor. A combination of amyloid imaging and MRI measures of atrophy (as a surrogate measure of tau pathology) may be important in any study wishing to investigate the influence of AD pathology in LB disorders.

3.8 Conclusions

Amyloid imaging studies have demonstrated that significant Aβ deposition is present in a proportion of DLB and PDD patients. Significant Aβ deposition appears to be relatively rare in PD. Dementia often occurs in the absence of Aβ, but there is some evidence that amyloid may be related to the onset or progression of cognitive symptoms

in these disorders, though current results are not conclusive. Large scale, prospective amyloid imaging studies may resolve some of these unanswered questions and clarify the importance of Aβ in LB disorders.

Table 3.1. Summary of PET amyloid imaging studies including LB disease subjects (excluding neuropathological case studies)

Study	Population	Scans, ligand	Amyloid PET acquisition and image analysis	Major findings
Rowe et al. (2007)	10 DLB 17 AD 9 MCI 6 FTD 27 controls	MRI ^{11}C-PIB PET	90 min acquisition. ROI ^{11}C-PIB DVR v. cerebellum. Logan graphical analysis method.	PiB burden AD>DLB>controls. PiB burden correlated with shorter time from onset of symptoms to diagnosis in DLB. APOE ε4 genotype linked to Aβ burden across groups.
Edison et al. (2008)	13 DLB 12 PDD 10 PD 41 controls	MRI ^{11}C-PIB PET	60-90 min ^{11}C-PIB uptake ratio; ROI v. cerebellum. Voxel based comparison with threshold $p<0.001$.	DLB more likely to show raised Aβ burden compared with PDD or PD. Six control subjects were excluded from analysis because of high cortical Aβ ligand binding.
Gomperts et al. (2008)	8 DLB 7 PDD 11 PD 15 AD 37 controls	^{11}C-PIB PET	60 min DVR v cerebellum. Logan graphical analysis method.	PiB binding DLB>PDD/PD/controls. PiB binding in DLB comparable to AD. In LBD (DLB+PDD) relative striatal binding was associated with improved performance on UPDRS.
Johansson et al. (2008)	5 PD 16 AD 6 controls	^{11}C-PIB PET ^{11}C-L-DOPA PET	40-60 min ROI uptake ratio v. cerebellum.	PiB retention was greater in AD than PD in all cortical areas and striatum. Lower in PD than control in frontal, parietal and cingulate areas.
Maetzler et al. (2008)	10 PDD 6 AD 11 controls	^{11}C-PIB PET	42-72 min ROI SUVR v. cerebellum.	2/10 PDD had 'AD-like' pattern of amyloid deposition. Brainstem:posterior cingulate DVR: PDD>control, AD, 'AD-like' PD.
Maetzler et al. (2009)	9 DLB 12 PDD 14 PD	^{11}C-PIB PET	42-72 min ROI SUVR v. cerebellum.	PiB positive patients had lower CSF $A\beta_{42}$, higher APOE ε4 allele rate, all had dementia. Within dementia, PiB positive had lower MMSE scores.
Jokinen et al. (2010)	11 PDD 8 PD 24 controls	^{11}C-PIB PET ^{18}F-FDG PET MRI	60-90 min ROI uptake ratio v. cerebellum.	No significant difference between groups in any cortical area. PDD more likely to show 1+ cortical areas with increased PiB uptake.

Table 3.1 Continued

Study	Population	Scans, ligand	Amyloid PET acquisition and image analysis	Major findings
Foster et al. (2010)	6 DLB 15 PDD 9 PD-MCI 8 PD 9 controls	^{11}C-PIB PET MRI	60 min dynamic scan. ROI and mean cortical BP. Logan graphical analysis, cerebellum as reference.	No differences in mean cortical BP or regional BPs between groups. Correlation between caudate/cortical amyloid and MMSE in some groups.
Burke et al. (2011)	14 DLB 36 AD 25 FTD	^{11}C-PIB PET ^{11}C-DTBZ PET	80 min scan. ROI DVR v. cerebellum. Subjective visual assessment.	Only moderate concordance between clinical diagnosis and diagnosis based on scan results.
Claasen et al. (2011)	3 DLB 3 MSA 12 controls	^{11}C-PIB PET ^{18}F-FDG PET MRI	40-60 min acquisition. ROI v. cerebellum.	Higher binding found in DLB in all areas. Most marked in prefrontal, parietal, temporal and precuneus.
Villemagne et al. (2011)	7 DLB 5 PD 30 AD 20 MCI 11 FTD 4 VaD 32 controls	^{18}F-Florbetaben PET MRI	90-110 min acquisition. ROI SUVR v. cerebellar cortex	AD and MCI had higher neocortical and striatal binding than controls. No other significant differences between groups.
Gomperts et al. (2012)	18 DLB 12 PDD 14 PD-MCI 29 PD 85 controls	^{11}C-PIB PET	60 min dynamic acquisition. Logan graphical analysis method. ROI DVR v. cerebellum. SPM analysis corrected for multiple comparisons.	DLB exhibited higher binding than other groups, particularly in frontal and parietal areas. No differences found between non-DLB groups. PiB burden increased in APOE ε4 genotype across entire cohort. In DLB increased PiB binding was associated with decreased MMSE score and poorer semantic memory.

Table 3.1 Continued

Study	Population	Scans, ligand	Amyloid PET acquisition and image analysis	Major findings
Graff-Radford et al. (2012)	7 DLB	^{11}C-PIB PET MRI	40-60 min. ROI v. cerebellum. Partial volume correction.	Both subjects who declined were PiB positive; all 3 subjects who improved were PiB negative.
Kantarci et al. (2012)	21 DLB 21 AD 42 controls	^{11}C-PIB PET ^{18}F-FDG PET MRI	40-60 min acquisition. ROI v. cerebellum. Voxel based comparison using FWE correction. Partial volume correction.	DLB PiB binding was significantly lower than AD but significantly higher than controls. No relationship found between global PiB and motor impairment, dementia rating, visual hallucinations or duration of symptoms.
Petrou et al. (2012)	40 PD at risk for dementia	^{11}C-PIB PET ^{11}C-DTBZ PET MRI	80 min acquisition. Logan graphical analysis method. DVR v. cerebellum.	Cortical PiB was inversely correlated with overall cognitive score and WAIS score.
Ossenkoppele et al. (2012)	66 AD 30 MCI 15 SMC 18 FTD 5 DLB	^{11}C-PIB PET ^{18}F-FDG PET MRI	90 min dynamic scan. ROI BP v. cerebellum. For 12 patients 60-90 min SUVR ROI v cerebellum.	^{11}C-PIB PET contributed to diagnostic process in 86% of patients, mainly used to rule out AD.
Shimada et al. (2013)	8 DLB 7 PDD 13 AD 22 controls	^{11}C-PIB PET MRI	90 min dynamic scan. Logan graphical analysis method. ROI DVR v. cerebellum.	PiB positive LBD subjects demonstrated cortical atrophy compared with controls; greater atrophy in parahippocampal regions than PiB negative LBD subjects.
Gomperts et al. (2013)	35 PD 11 PD-MCI	^{11}C-PIB PET	60 min dynamic scan. Logan graphical analysis method. DVR v. cerebellum.	Higher PiB retention at baseline associated with progression to MCI or dementia and with deterioration in executive function. Motor deterioration not linked to baseline striatal or precuneus amyloid burden.

Table 3.1 Continued

Study	Population	Scans, ligand	Amyloid PET acquisition and image analysis	Major findings
Campbell et al. (2013)	10 DLB 43 PD/PDD 35 AD 67 controls	^{11}C-PIB PET MRI	60 min dynamic scan. ROI and cortical BP. Logan graphical analysis, cerebellum as reference.	Pattern of deposition of amyloid in LB disease was more similar to that seen in controls to the pattern seen in AD
Edison et al. (2013)	11 PDD 8 PD 24 controls	^{11}C-PIB PET ^{18}F-FDG PET MRI ^{11}C-PK11195 PET	90 min acquisition. ROI DVR v. cerebellum.	No difference between PD and PDD in cortical binding. PD, but not PDD, had greater binding than controls. No significant difference between controls and PD or PDD in a voxel-based analysis
Vijayaraghavan et al. (2014)	9 DLB 9 PDD 10 PD	^{11}C-PIB PET ^{18}F-FDG PET CSF markers	42-72 min ROI SUVR v. cerebellum.	^{11}C-PiB binding and CSF APOE levels were positively correlated
Ishii et al. (2015)	10 DLB 10AD	^{11}C-PIB PET ^{18}F-FDG PET	50-70 min ROI SUVR v cerebellum. Visual rating.	There are no differences in cortical metabolism between amyloid positive and amyloid negative DLB cases
Nedelska et al. (2015a)	30 DLB 30 AD 60 controls	^{11}C-PIB PET ^{18}F-FDG PET	40-60 min ROI SUVR v cerebellar grey matter	AD patients had higher Aβ binding than DLB. Loss of parieto-occipital white matter integrity in DLB is independent of Aβ deposition.
Siderowf et al. (2015)	11 DLB 5 PD 10AD 5 controls	^{18}F-Florbetapir PET	50-60 min Cortical SUVR v cerebellum Visual rating	AD>PD in all cortical regions AD>DLB in parietal and anterior cingulate areas only. DLB>controls in posterior cingulate only. Amyloid positive DLB had lower MMSE than amyloid negative. NB: broad range (MMSE 4-28)

BP = binding potential; DTBZ = dihydrotetrabenazine; DVR = distribution volume ratio; FDG = fluorodeoxyglucose; FWE = family-wise error; MRI = magnetic resonance imaging; PET = positron emission tomography; PIB = Pittsburgh compound B; ROI = region of interest; SMC = subjective memory complaint; SPM = statistical parametric mapping; SUVR = standardised uptake value ratio.

Chapter 4 Prodromal Dementia with Lewy Bodies: Aims, Hypotheses and Methods

4.1 Aims

The aim of this project is to characterise the phenotype of prodromal DLB. Patients with MCI who appear at risk of developing dementia with Lewy bodies will have a thorough baseline clinical and neuropsychological assessment and ^{123}I-FP-CIT dopaminergic imaging. They will then be reviewed annually for conversion to dementia. The baseline assessment of those that develop DLB can then be examined to identify predictors of conversion to DLB.

This thesis will examine the baseline data of this project to investigate the potential utility of ^{123}I-FP-CIT imaging as a biomarker for prodromal DLB. A comparison will be made between those with normal ^{123}I-FP-CIT scans and those with abnormal scans. Visual rating of ^{123}I-FP-CIT images will be compared with semi-quantitative analysis of striatal ^{123}I-FP-CIT binding to investigate whether visual rating is correlated with overall striatal binding.

4.2 Hypotheses

4.2.1 Primary Hypotheses

^{123}I-FP-CIT imaging is a biomarker of Lewy body disease, therefore those with MCI and an abnormal ^{123}I-FP-CIT scan will be more likely to have prodromal DLB than those with normal scans, and will have a phenotype similar to that seen in established DLB. This will be evidenced by:

1. The ^{123}I-FP-CIT abnormal group will have more core and suggestive diagnostic features of DLB

2. The ^{123}I-FP-CIT abnormal group will have higher scores in scales measuring these features

3. The ^{123}I-FP-CIT abnormal group will have worse visuospatial and executive function, but better memory on neuropsychological tests

4. The ^{123}I-FP-CIT abnormal group will have a higher frequency of other features associated with DLB e.g. anosmia, postural hypotension and constipation

4.2.2 Secondary Hypothesis

The pattern of nigrostriatal dopaminergic denervation in prodromal DLB will be similar to that seen in established DLB, and therefore:

5. Measures of asymmetry of ^{123}I-FP-CIT binding between the left and right striata will be low in the entire cohort.

4.3 Methods

4.3.1 Participants

Participants were recruited prospectively from NHS Trusts in the North of England. Patients were identified through direct referral from their treating teams; through the Dementias and Neurodegenerative Diseases Research Network (DeNDRoN) Research Case Register and through screening patient notes based on Mental Health Cluster, an instrument for categorising NHS Mental Health patients based on the type of illness they have and its severity (Department of Health, 2013). Patients categorised as 'Cluster 18' were screened. This refers to people with 'Cognitive Impairment (low need)' and is the Cluster most likely to be used for people with MCI.

All subjects needed to have sufficient English to complete cognitive testing. All subjects gave their written informed consent to take part in the study. The study received ethical approval from the National Research Ethics Service Committee North East - Newcastle & North Tyneside 2 (Research Ethics Committee Identification Number 12/NE/0290).

4.3.2 Inclusion/exclusion criteria

Subjects were ≥60 years old and fulfilled the NIAAA clinical criteria for MCI (Albert *et al.*, 2011) – concern regarding change in cognition, impairment in one or more cognitive domains, preservation of independence of function with minimal aids or assistance and not demented. In addition, all subjects had at least one symptom

suggesting they might have Lewy body disease. This included the core and suggestive features of DLB (spontaneous motor parkinsonism, cognitive fluctuations, recurrent visual hallucinations, REM sleep behaviour disorder, severe neuroleptic sensitivity), as well as other symptoms that may be more likely in Lewy body disease, such as auditory hallucinations and delusions. Given the concern that a large proportion of people with DLB are not identified during life the inclusion criteria were deliberately made broader to try and capture these patients. Thus patients were also included if they appeared to be developing a core or suggestive feature of DLB, though the full symptom was not yet present. Examples of this would be a rest tremor without other parkinsonian features, marked visual illusions or visuospatial dysfunction, daytime sleepiness that did not amount to fluctuations or new onset sleep disturbance without a clear RBD phenotype.

Subjects were excluded if they had an MMSE score <20; CDR score of >0.5; if they had developed parkinsonism more than one year prior to cognitive impairment or if they had evidence of clinical stroke or a serious neurological or mental condition that would affect their performance in study assessments. The 'one year rule' was used because patients developing parkinsonism more than one year prior to cognitive impairment would be classified as PDD rather than DLB if they progressed to dementia.

4.3.3 Baseline cognitive and clinical assessment

Volunteers underwent a thorough clinical and neuropsychological assessment to determine the symptoms they experienced and their severity.

4.3.4 Clinical assessment

Subjects were assessed by a doctor (PD) to confirm the diagnosis of MCI and suitability for the study. This included a neurological examination and the motor subscale of the Revised Unified Parkinson's Disease Rating Scale (MDS-UPDRS) to quantify the degree of parkinsonism present (Goetz *et al.*, 2008). The degree of physical comorbidity was rated using the Cumulative Illness Rating Scale for Geriatrics (CIRS-G) (Miller *et al.*, 1992).

North-East Visual Hallucinations Interview

The North-East Visual Hallucinations Interview (NEVHI) is a semi-structured interview that screens for hallucinations and investigates the emotions, cognitions and behaviours associated with hallucinations (Mosimann *et al.*, 2008).

Epworth Sleepiness Scale

The Epworth Sleepiness Scale (ESS) asks volunteers to rate their likelihood of falling asleep or dozing from 0 (never) to 3 (high chance) in eight different situations (Johns, 1991). Patients with DLB score higher in this test than those with AD or normal controls (Boddy *et al.*, 2007).

Geriatric Depression Scale

The 15 Item Geriatric Depression Scale (GDS) consists of 15 ‘yes’ or ‘no’ questions assessing the presence of symptoms of depression (D'Ath *et al.*, 1994). A cut-off of 5/6 gives a sensitivity of 86% and a specificity of 74% when compared with ICD-10 diagnosis (Almeida and Almeida, 1999).

Gastroparesis Cardinal Symptom Index

The Gastroparesis Cardinal Symptom Index (GCSI) is a rating scale of 9 symptoms of delayed stomach emptying (gastroparesis) grouped into three domains (nausea/vomiting, fullness/early satiety and bloating) (Revicki *et al.*, 2003).

Lewy body symptom questionnaire

All subjects also had the Lewy body symptom questionnaire (LBSQ). This is a list of 49 symptoms based on the Lewy Body Dementia Association’s Comprehensive Lewy Body Dementia Symptom Checklist (Lewy Body Dementia Association, 2014) along with other symptoms known to be associated with Lewy body disease. Some questions

were added after the start of the study. The symptoms of dry/painful eyes, double vision, difficulty reading because words and letters seem to move around the page and misjudging objects were added in light of new evidence (n=51) (Jefferis *et al.*, 2013). The symptoms poor balance, frequent falls and weak voice were added following a revision of the Lewy Body Dementia Association's checklist (n=43) (Lewy Body Dementia Association, 2014).

4.3.5 Neuropsychological tests

Addenbrooke's Cognitive Examination Revised

The Addenbrooke's Cognitive Examination Revised (ACE-R) is a 100-point examination, divided into five domains – orientation/attention, memory, verbal fluency, language and visuospatial function (Mioshi *et al.*, 2006). It has been shown to have good sensitivity and specificity for the identification of dementia. Mean scores of MCI subjects are intermediate between dementia and normal controls.

Verbal fluency

Letter and category fluency were tested. In letter fluency subjects must generate as many words (excluding proper nouns) beginning with the letter 'F' in one minute. This is then repeated for the letters 'A' and 'S'. Category fluency was tested as part of the ACER by asking the subjects to name as many animals as they could in one minute. Category fluency is impaired in both DLB and AD, whereas some studies have reported that letter fluency is more severely affected in DLB (Lambon Ralph *et al.*, 2001).

Trailmaking test

The trail-making test consists of two parts. In part A, subjects must connect numbered circles in ascending order using a pen. In part B, the sheet contains circles with numbers and letters. These must be connected in an alternating fashion: 1-A-2-B-3-C etc. Part A primarily assesses visuoperceptual abilities and attention, whereas part B also tests working memory and task-switching (Sanchez-Cubillo *et al.*, 2009). Those with autopsy

confirmed Lewy body pathology take longer to complete Part B than those with Alzheimer's disease, those with combined pathology have intermediate scores (Kraybill *et al.*, 2005).

Graded Naming

The Graded Naming Test requires subjects to recognise and name monochrome drawings of 30 objects (McKenna and Warrington, 2007). Most studies have found to be naming to be similarly impaired in DLB and AD (Troster, 2008), though a large study found greater impairment in DLB (Ferman *et al.*, 2006)

Rey AVLT

The Rey Auditory Verbal Learning Test (AVLT) (Rey, 1964) involves the subject trying to recall 15 words that are read out to them at a rate of one word per second (List A). They are given five trials, with the number of correct responses recorded after each trial (Trials 1-5). There is then a single trial with a new list of 15 words (List B). Following this the subject is asked to recall as many words as possible from the original list (Trial 6). After a 30 minute break the subject is again asked to recall as many words as possible from the list A (Delayed Recall). A list of 50 words is then read out, with the subject asked to identify those words that were on List A. The number of List A words correctly identified is recorded.

People with DLB score lower on the Rey AVLT than controls, but higher than those with AD (Ferman *et al.*, 2006).

4.3.6 Computerised cognitive tests

Computerised tests were performed using a laptop computer with 36cm screen placed approximately at eye-level. Patients held one or two response paddles depending on the test. All tests were operated through MATLAB and developed in Newcastle University by Michael Firbank, apart from the motion task which was developed by David Salmon and colleagues in UC San Diego. These tests aim to measure specific cognitive domains

that are known to be affected in DLB: visuospatial function and executive functions such as attention, processing speed and decision-making.

Simple and choice reaction time (Figure 4.1)

Simple Reaction Time (SRT): A white 'V' on a black background was presented to the participant 30 times in varying intervals. Each time the participant pressed the paddle as quickly as possible. The number of correct responses, average response time and standard deviation of response time were recorded.

Choice Reaction Time (CRT): A white arrow pointing to the right '>' or the left '<' was presented to the patient, who had to press a paddle corresponding to 'right' or 'left' each time. The number of correct responses, errors, average response time and standard deviation of response time were recorded.

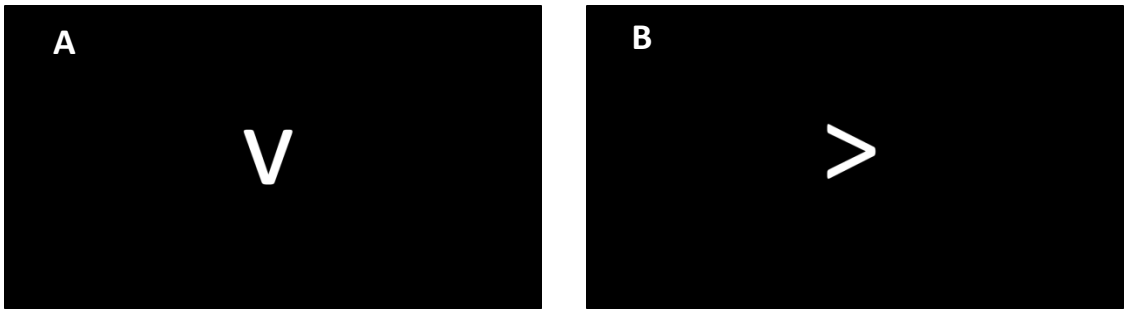

Figure 4.1. Visual stimuli presented in the (A) simple reaction time and (B) choice reaction time tasks.

The SRT and CRT both involve seeing a stimulus and physically responding to it. The CRT also contains another task – identifying the stimulus ('right' or 'left') and choosing an appropriate response based on this. The time taken to do this is called the Cognitive Processing Time and can be calculated as below:

$$Cognitive\ Processing\ Time = Choice\ Reaction\ Time - Simple\ Reaction\ Time$$

Compared to those with AD, people with DLB have been found to have longer reaction times indicating worse attention, greater variability in reaction times indicating greater fluctuation in attention, more errors in the CRT indicating impaired decision-making and longer cognitive processing times indicating slower processing speed (Ballard *et al.*, 2001).

Digit vigilance (Figure 4.2)

360 pseudorandom numbers were presented on screen at a rate of 2/second. Each time the number '9' appeared the patient pressed a paddle in their hand. A pale number '9' remained onscreen throughout. The number of correct responses, average response time and standard deviation of response time were recorded. DLB is associated with longer response times, greater variability in response times and fewer correct responses than AD (Ballard *et al.*, 2001).

Figure 4.2. The digit vigilance task. The bright number in the centre of the screen changed at a rate of 2/s. When it became a '9' subjects had to press a paddle as quickly as possible

Power of attention

Power of attention (Rowan *et al.*, 2007) is a summary measure of attention that combines the reaction times in the SRT, CRT and digit vigilance tasks. It is calculated as follows:

Power of attention = Simple Reaction Time + Choice Reaction Time + Digit Vigilance Response Time

Line angle discrimination (Figure 4.3)

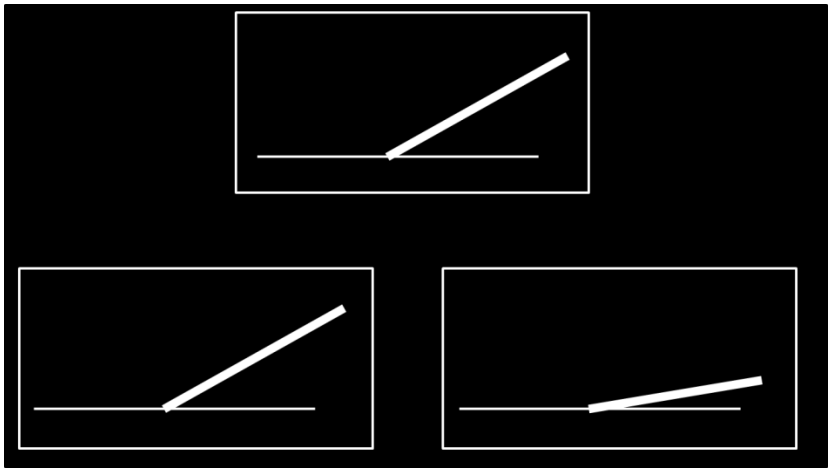

Figure 4.3. The angle discrimination task. Subjects had to choose which of the lower angles matched the angle at the top of the screen.

In this task of visuospatial function participants were presented with an angle at the top of the screen. At the bottom of the screen two angles were presented, one of which was the same as the top angle. Participants pressed 'left' or 'right' to select the option that they thought matched with the angle at the top of the screen. The difficulty modified based on the participant's responses. The program produced an estimate of the angle the participant could discriminate in degrees, where lower scores indicated better angle discrimination. People with DLB perform more poorly at this task than those with AD indicating worse visuospatial function (Wood *et al.*, 2013).

Motion task (Figure 4.4)

In the motion task moving white dots were presented on the screen for 1 second. A proportion of the dots were either moving horizontally to the right or to the left (signal). The rest of the dots were moving randomly (noise). The participant had to decide if the signal dots were moving to the right or the left. The program modified difficulty based on the participant's responses; the output was a threshold of the proportion of dots in the signal that the participant required to identify the direction of movement. Salmon and colleagues have found that people with DLB perform worse than those with AD in this task.

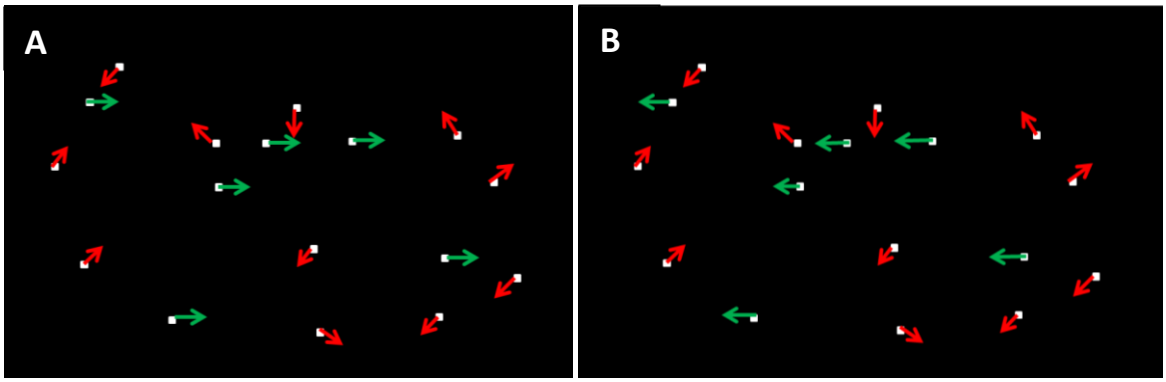

Figure 4.4. The motion task. Arrows are for illustration and were not present during the task. Subjects had to identify if the 'signal' dots (indicated with green arrows) were moving right (A) or left (B) amidst 'noise' dots (indicated with red arrows) moving in random directions.

4.3.7 Carer rating scales

NPI

The Neuropsychiatric Inventory assesses the frequency and severity of ten neuropsychiatric symptoms, as well as the level of carer distress caused by these symptoms (Cummings *et al.*, 1994). DLB cases have been found to score higher than AD cases in domains such as hallucinations, apathy and loss of appetite in the early stages of the disease (Ricci *et al.*, 2009).

Cognitive Fluctuations Scales

The recently developed Dementia Cognitive Fluctuations Scale (DCFS) enquires about four symptoms (variation in function, daytime sleepiness, daytime lethargy and overall level of consciousness) that were shown to differentiate DLB from AD (Lee *et al.*, 2014). Two further symptoms (staring into space and disorganised speech) were also rated (Ferman *et al.*, 2004).

The Clinician Assessment of Fluctuation (CAF) consists of two screening questions to identify impaired alertness and variation in levels of confusion. If present, these are then

rated on a scale of 0-4 for severity and duration. These two scores are then multiplied to give an overall score (Walker *et al.*, 2000a). Patients with DLB score higher than AD in this assessment (O'Brien *et al.*, 2014a).

Mayo Sleep Questionnaire

The Mayo Sleep Questionnaire (MSQ) is a carer-rated scale to identify REM sleep behaviour disorder (Boeve *et al.*, 2011). It involves questions relating to the presence of RBD and other causes of sleep disturbance, such as sleep apnoea. It can only be carried out with an informant that lives with the patient. The first question relates to the acting out dreams and has a sensitivity of 98% and specificity of 74% for the identification of RBD when compared with polysomnography (Boeve *et al.*, 2011).

Assessments of level of function

The Lawton Instrumental Activities of Daily Living Scale (IADL) (1969) measures a patient's current ability to perform eight tasks, regardless of whether the person regularly performs these tasks or not. The maximum score possible is 8, representing full function; the minimum possible score is 0, representing the highest level of functional impairment.

The Clinical Dementia Rating Scale (CDR) (Hughes *et al.*, 1982) involves a clinician rating the subject's level of impairment in six domains of function. Unlike the IADL scale, the rating is based on clinical judgement of the decline in function due to cognitive impairment, and not due to other factors such as physical impairment. An overall score can be calculated using an algorithm (Morris, 1993). A score of 1-3 is consistent with dementia; a score of 0.5 is consistent with MCI or dementia. A score of 0 is not consistent with dementia. The scores in each domain can be added to give a 'sum of boxes score'.

4.3.8 Presence of core and suggestive features of Lewy body disease

The presence or absence of core or suggestive features of Lewy body disease was determined by the reviewing clinician (PD) based on the clinical interview and carer questionnaires. These were rated using the definitions in the diagnostic criteria for dementia with Lewy bodies (McKeith *et al.*, 2005). These definitions are not fully operationalised and require some clinical judgement. The definitions used and scales that informed the decision are recorded in Table 4.1.

Table 4.1. Definitions of core and suggestive symptoms of Lewy body disease

Symptom	Definition	Scales used
Fluctuations	Fluctuating cognition with pronounced variations in attention and alertness	CAF DCFS
Visual hallucinations	Recurrent visual hallucinations that are typically well formed and detailed	NEVHI
Parkinsonism	Spontaneous features of parkinsonism, usually two or more of the following symptoms: rest tremor, rigidity, bradykinesia, parkinsonian gait, postural instability	UPDRS
RBD	Report of appearing to act out dreams – vocalising, flailing limbs and moving around the bed	MSQ
Neuroleptic sensitivity	Severe reaction to neuroleptic medication e.g. acute onset or exacerbation of parkinsonism and impaired consciousness	n/a

4.3.9 ^{123}I- FP-CIT SPECT imaging

Subjects were given 85mg Potassium Iodate thyroid protection 1 hour prior to the intravenous injection of 185MBq ^{123}I-2h-carbomethoxy-3h-(4-iodophenyl)-N-(3-fluoropropyl)-N-nortropane (FP-CIT; GE Healthcare). Subjects were imaged using a dual-detector gamma camera (Siemens Symbia S) with a low-energy high-resolution collimator. One-hundred and twenty 25 second projections were taken over 360° on a

128 x 128 matrix with slice thickness of (3.9mm). Image reconstruction was performed using ramp-filtered back-projection with a Butterworth filter (order 13, cut-off 0.3 cycles cm^{-1}) to produce the transverse sections. The reconstructed images, uncorrected for gamma ray attenuation, were subsequently transferred in the DICOM format to a personal computer for further analysis.

4.3.10 SPECT visual rating

Images were independently visually assessed by four raters (Alan Thomas, Sean Colloby, Jim Lloyd and Paul Donaghy) and classified into one of four categories (Figure 4.5) (Benamer *et al.*, 2000):

0 - Normal

1 - Unilateral reduction in putamen

2 - Bilateral reduction in putamen

3 - Bilateral reduction in caudate and putamen

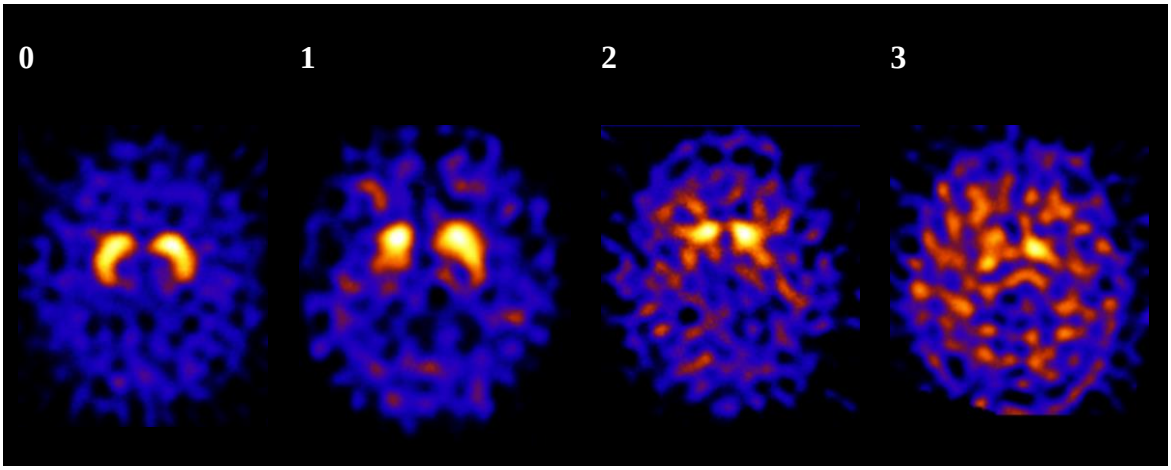

Figure 4.5. Visual Rating of FP-CIT scans (Benamer *et al.*, 2000)
0 - Normal. Normal tracer uptake bilaterally in putamen and caudate nuclei and largely symmetric
1 - Unilateral reduction in putamen. Asymmetric uptake with normal or almost normal putamen activity in one hemisphere and with a more marked reduction in the contralateral putamen
2 - Bilateral reduction in putamen. Significant bilateral reduction in putamen uptake with activity confined to the caudate nuclei.
3 - Bilateral reduction in caudate and putamen. Virtually absent uptake bilaterally affecting both putamen and caudate nuclei with relatively increased background activity.
Images are from study participants.

Following this a consensus panel was held to discuss scans in which there was not full agreement and decide on a consensus rating.

4.3.11 SPECT quantification

Files were processed in NifTI format using SPM 8 (www.fil.ion.ucl.ac.uk/spm/software/spm8/). Each image was positioned with the anterior commissure approximately at point 0,0,0. Each scan was spatially registered to a FP-CIT template image in Montreal Neuroimaging Initiative (MNI) space using the 'Normalise: Estimate and Write' function, preserving concentrations with trilinear interpolation and a cubic voxel size of 2mm. The registered images were then resliced using the 'Realign:Reslice' function.

An FP-CIT SPECT template previously developed in Newcastle was used establish striatal ROIs (Colloby *et al.*, 2004). The template was developed by co-registering the scans of 33 normal healthy controls to a MRI brain template image in MNI space. Thresholding of the template image was applied to produce bilateral striatal regions of interest (ROI) for automated analysis (Figure 4.6). A large occipital ROI was also manually drawn to assess non-specific binding. Both ROI maps were then applied to each subject's registered FP-CIT image using ITK-SNAP (www.itksnap.org), where uptake values for left striatum, right striatum and occipital activity were obtained. The striatal:occipital binding ratio was calculated as follows:

$$\frac{Mean\ Activity\ Left\ Striatum + Mean\ Activity\ Right\ Striatum}{2 \times Mean\ Occipital\ Activity}$$

The Asymmetry Index between the striata was calculated as follows:

$$\frac{Higher\ Striatal\ Activity - Lower\ Striatal\ Activity}{Mean\ Striatal\ Activity} \times 100\%$$

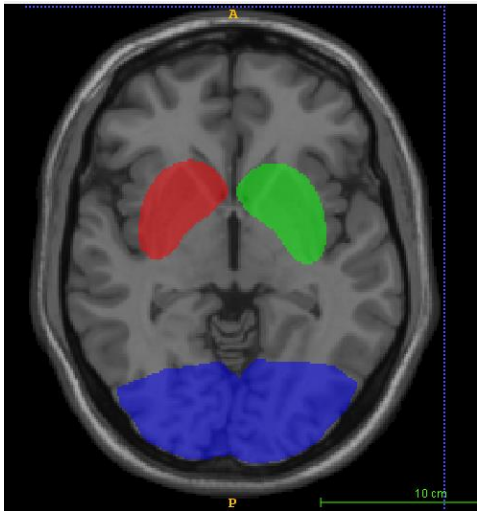

Figure 4.6. Striatal and occipital regions of interest overlaid on an MRI brain template image.

4.3.12 Statistics

Statistical analysis was completed using IBM SPSS Statistics 22 software. Normality was tested using the Shapiro-Wilk test. Comparisons between groups were carried out using t-tests or Mann-Whitney U tests depending on normality of the data. χ^2 or Fischer's Exact tests were used for categorical variables depending on cell size.

Correlation was carried out using Pearson's (for normally distributed data) or Kendall's tau (for non-normal data) correlation. Interrater and intrarater reliability in ^{123}I-FP-CIT SPECT visual rating was assessed using the Kappa statistic. A mean of all pair-wise kappa values was used to summarise agreement between the four raters.

A value of $p \leq 0.05$ was set as the threshold for statistical significance, uncorrected for multiple comparisons.

Statistical power was difficult to calculate for this analysis as the likelihood of a positive FP-CIT scan in this population was unknown. If 33% of participants had an abnormal scan a total sample size of 51 would be sufficient to detect differences between the groups with a large effect size ($\geq$0.8) with a statistical power of 0.8.

Chapter 5 Prodromal Dementia with Lewy Bodies: Results and Discussion

5.1 Recruitment

A large number of potential participants were screened for study suitability. Approximately 220 were approached, of whom 62 agreed to enter the study. Four participants were later found to be unsuitable, four withdrew and another died prior to completing the baseline assessment.

Table 5.1. Group demographics and symptom profile in the prodromal DLB study (SD in brackets)

N	53
Mean age (years)	76.3 (7.7)
Gender (% female)	40
Mean years of education	11.6 (2.7)
Mean MMSE score	26.5 (2.2)
Mean ACE-R score	78.5 (10.8)
ACE-R Attention/Orientation	17.1 (1.3)
ACE-R Memory	15.8 (5.1)
ACE-R Fluency	8.4 (3.1)
ACE-R Language	23.1 (3.0)
ACE-R Visuospatial	14.1 (2.1)
MDS-UPDRS	22.4 (15.3)
Hoehn and Yahr Stage	0.74 (1.2)
6-point UPDRS Score	2.9 (2.8)
Fluctuations (%)	37
Visual Hallucinations (%)	17
Parkinsonism (%)	32
RBD (%)	43
Neuroleptic sensitivity (%)	0
Abnormal FP-CIT SPECT (%)	36
Mean total core and suggestive features	1.7 (1.3)
Subjects with at least 1 core feature (%)	64
Subjects with at least 1 suggestive feature (%)	55
Subjects with at least 1 core and suggestive feature (%)	43

5.2 Group demographics and symptom profile

53 patients completed baseline assessment. The mean age was 76.3 years, 40% of the group was female and the mean MMSE was 26.5 (Table 5.1).

On average each patient had a mean of 1.7 (median 2) core or suggestive features of DLB. 13 patients (25%) had no symptoms that reached the threshold of a core or suggestive feature after a thorough clinical assessment (Table 5.2).

The most common diagnostic feature was REM sleep behaviour disorder (43%) followed by fluctuations (37%; Figure 5.1). No patient reported sensitivity to antipsychotics though only two patients (4%) were currently taking an antipsychotic medication.

Table 5.2. Number of core and suggestive features of LB disease in the prodromal DLB study subjects

No. of core or suggestive features	Frequency (n)
0	13
1	9
2	22
3	4
4	2
5	3

5.3 ^{123}I-FP-CIT SPECT visual rating

19 volunteers (36%) had an abnormal FP-CIT scan on visual rating (Figure 5.2). 8 subjects had grade 1 (unilateral loss in the putamen), 8 had grade 2 (bilateral putamen loss) and 3 had grade 3 (universal loss).

There was full agreement between the four raters in 35/53 scans (66%). The mean Kappa between the raters was 0.62 (substantial agreement).

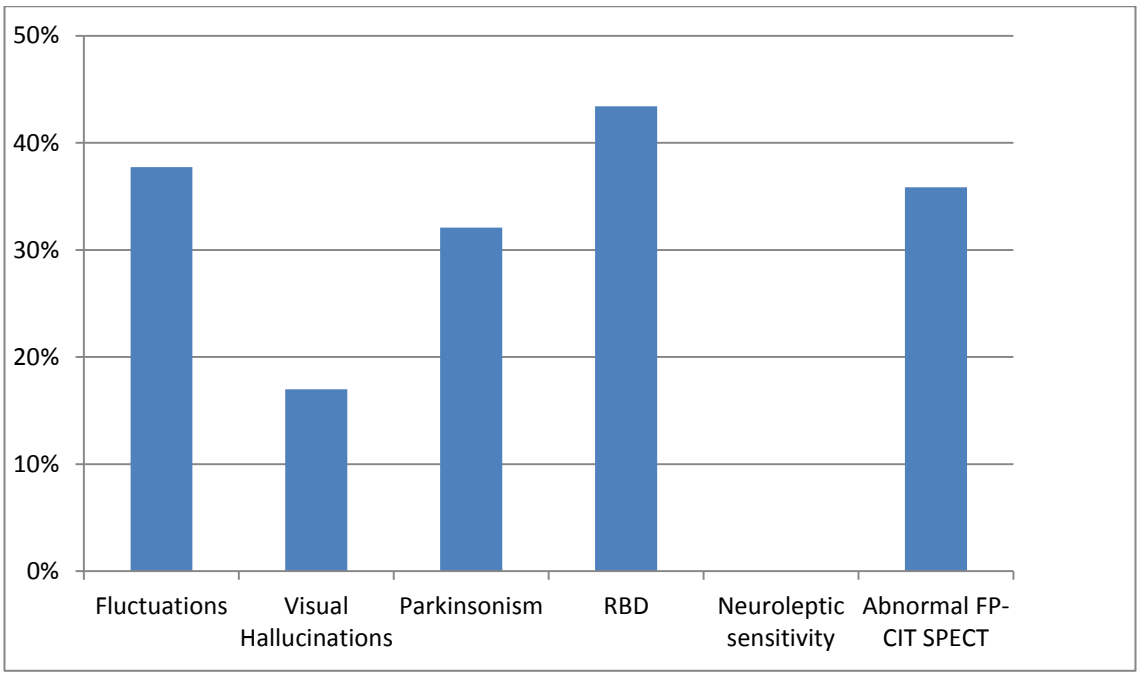

Figure 5.1. Frequency of each core and suggestive feature of Lewy body disease in the prodromal DLB study.

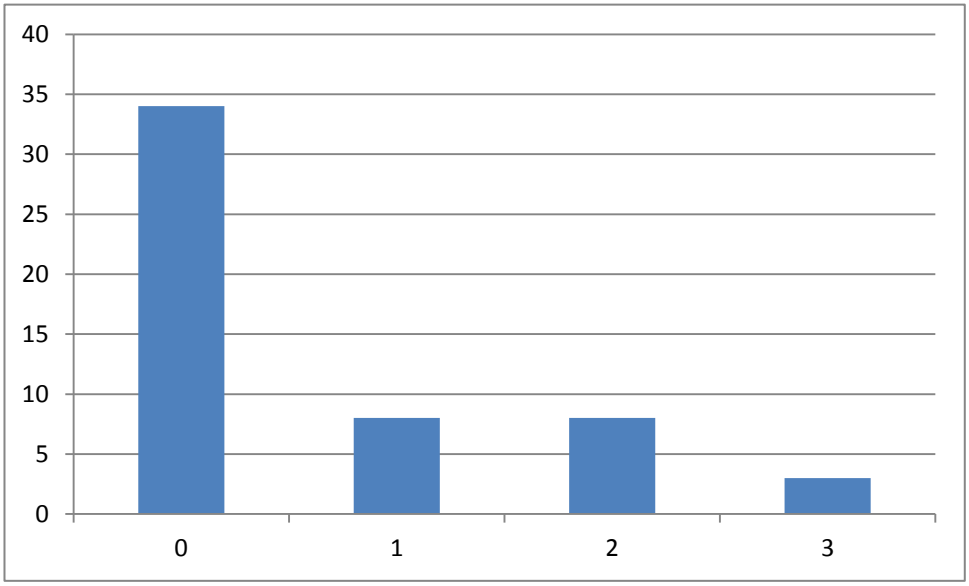

Figure 5.2. Frequency of FP-CIT SPECT visual rating grade in the prodromal DLB study. 34 subjects had a normal scan (64%), 19 (36%) had an abnormal scan (n=8 Grade 1, n=8 Grade 2, n=3 Grade 3).

5.4 Comparison of FP-CIT normal and abnormal subjects

5.4.1 Demographics

Those with abnormal scans were of similar age to those with normal scans but were more likely to be male (89% v. 46%; p=0.001) and to be taking medications for parkinsonism (32% v 6%; p=0.02). Rates of severe co-morbidity measured by the CIRS-G were low in both groups, with few domains rated as category 3 (severe/constant significant disability) and none at category 4 (severe impairment in function). The FP-CIT abnormal group tended towards slightly lower overall levels of co-morbidity than the FP-CIT normal group (CIRS-G 8.0 v 10.2; p=0.08). There were no differences between those with normal and abnormal scans in terms of prescription of cholinesterase inhibitors or loss of function measured by the CDR or IADL scales (Table 5.3).

Table 5.3. Demographics of FP-CIT normal and abnormal groups

	FP-CIT Normal	FP-CIT Abnormal	
	Mean (SD)	Mean (SD)	p
n	34	19	-
Age	76.7 (8.5)	75.6 (6.1)	0.63
Gender (% female)	56	11	**0.001**
Number of years education	11.4 (2.7)	12.0 (2.7)	0.15
Cholinesterase Inhibitor prescribed (%)	38	42	0.78
Levodopa prescribed (%)	6	32	**0.02**
CDR total Score	0.4 (0.2)	0.5 (0.1)	0.36
IADL Total (n=47)	6.5 (1.6)	6.1 (2.0)	0.59
CIRS-G Total score	10.2 (4.3)	8.0 (4.6)	0.08
Average CIRS domains at Level 3	0.5 (0.7)	0.3 (0.6)	0.11
Average CIRS domains at Level 4	0 -	0 -	-

5.4.2 Presence of core and suggestive features of LB disease

The presence or absence of core and suggestive symptoms of LB disease was determined for each volunteer as described in section 4.3.8. All volunteers with an abnormal FP-CIT scan also had at least one other LB feature (Figure 5.3). The majority

of those with normal FP-CIT scans had zero or one LB feature. The average number of features (not including abnormal FP-CIT scan) in the abnormal scan group was 1.9 compared with 1.0 in those with normal scans ($p<0.01$). The specific symptoms associated with an abnormal scan were parkinsonism (58% v 18%; p=0.003) and REM sleep behaviour disorder (68% v 29%; p=0.006; Figure 5.4). There were no differences between the groups in the rate of cognitive fluctuations (Abnormal v normal = 42% v 35%; p=0.62) or visual hallucinations (Abnormal v normal = 21% v 15%; p=0.71).

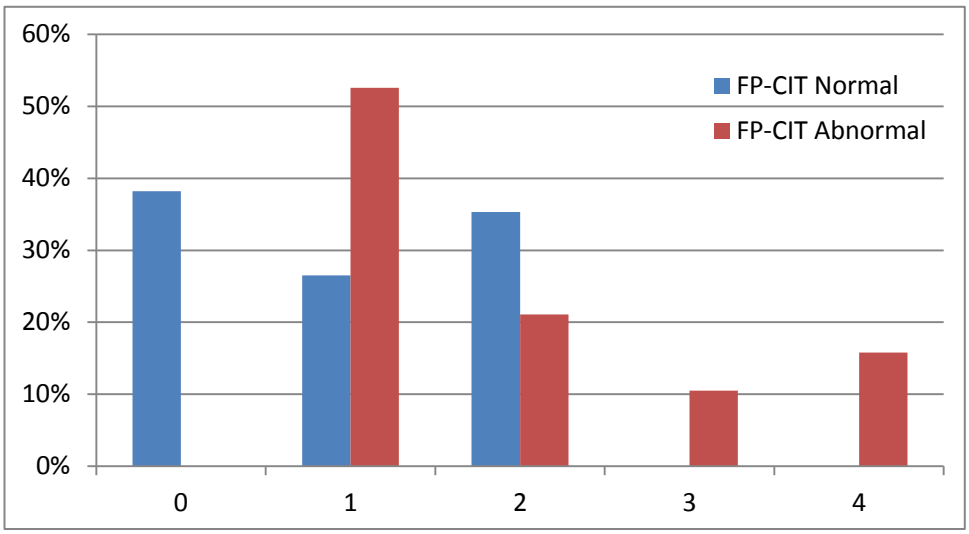

Figure 5.3. The number of core and suggestive clinical features of LB disease experienced by subjects in the normal and abnormal FP-CIT groups.

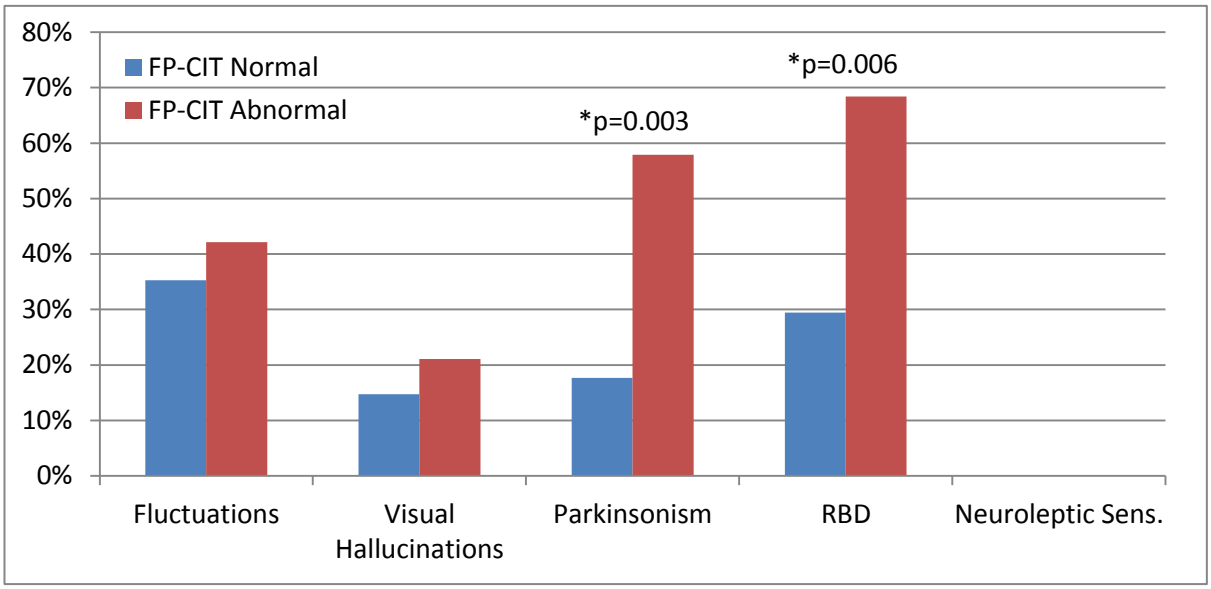

Figure 5.4. The proportion of subjects experiencing core and suggestive features of LB disease in the normal and abnormal FP-CIT groups. Those with abnormal scans were significantly more likely to experience parkinsonism (58% v 18%; p=0.003) and REM sleep behaviour disorder (68% v 29%; p=0.006).

5.4.3 Cognitive tests

One patient could not complete the Rey AVLT. Two patients could not complete Trails A, 22 could not complete Trails B.

There were no differences between those with normal and abnormal scans in the ACE-R or any of its cognitive subdomains (Table 5.4). There were no differences in memory measured by the Rey AVLT, verbal fluency measured by the 'FAS' test or word retrieval measured by the graded naming test. There were no differences in executive function measured by the Trails B, but this test had a significant floor effect, with 58% of those with abnormal and 38% of those with normal FP-CIT SPECT being unable to complete the test within 5 minutes (Tombaugh et al. 2004).

Table 5.4. Cognitive test scores in FP-CIT normal and abnormal groups.

	FP-CIT Normal		FP-CIT Abnormal		
	Mean	(SD)	Mean	(SD)	p
MMSE	26.4	(2.4)	26.7	(1.7)	0.65
ACE-R total	78.4	(12.0)	78.5	(8.6)	0.97
ACE-R attention and orientation	16.9	(1.4)	17.4	(1.1)	0.22
ACE-R memory	15.6	(5.5)	16.2	(4.4)	0.70
ACE-R fluency	8.9	(3.0)	7.6	(3.0)	0.15
ACE-R language	22.8	(3.5)	23.6	(1.7)	0.96
ACE-R visuospatial	14.2	(2.1)	13.8	(2.2)	0.60
Rey AVLT Trial 1	3.9	(1.8)	4.0	(1.7)	0.85
Rey AVLT Trial 6	3.9	(3.5)	4.6	(3.4)	0.47
Rey AVLT Delay	3.2	(3.8)	3.8	(3.4)	0.38
Rey AVLT Recognition	11.6	(2.7)	11.3	(2.6)	0.65
Verbal Fluency (FAS) score	30.4	(15.8)	29.4	(14.3)	0.94
Trails A (s)	64.4	(34.9)	76.7	(41.0)	0.34
Trails B (s)	134.6	(72.3)	158.6	(72.5)	0.25
Failed to complete Trails B (%)	38		58		0.17
Graded naming test	16.9	(7.5)	16.6	(5.7)	0.87

5.4.4 Computerised tests of cognitive function

One patient could not complete the digit vigilance task due to double vision. Computer errors resulted in the loss of data for one patient in the reaction time tests and seven patients in the angle discrimination task.

There were no differences between the groups in simple reaction time, choice reaction time, or the variation in these measures assessed by standard deviation (Table 5.5). Cognitive processing time did not differ between the groups. Those with abnormal scans were more likely to make errors on the choice reaction time task. They also tended towards being more likely to miss a stimulus in the digit vigilance test, though their response times were not different to the normal group. There was no difference in power of attention between the two groups.

There were no differences between the groups on the two measures of visuospatial function – the angle and motion tasks.

Table 5.5. Computerised Cognitive Test Scores in FP-CIT normal and abnormal groups.

	FP-CIT Normal		FP-CIT Abnormal		
	Mean	(SD)	Mean	(SD)	p
Simple Reaction time (ms)	452.6	(214.0)	388.4	(135.2)	0.43
Simple reaction time SD (ms)	158.8	(149.6)	96.2	(71.7)	0.26
Choice reaction time (ms)	774.7	(338.1)	709.1	(197.5)	0.89
Choice reaction time SD	202.2	(149.6)	194.3	(87.0)	0.54
Choice reaction time number of errors	1.7	(1.7)	3.8	(6.1)	**0.03**
Cognitive processing time	322.1	(239.0)	320.7	(116.2)	0.27
Digit vigilance number got (max 36)	32.4	(5.0)	29.9	(5.3)	**0.053**
Digit vigilance mean time (ms)	566.6	(73.8)	581.5	(65.2)	0.48
Digit vigilance SD (ms)	109.5	(44.5)	113.2	(37.7)	0.76
Digit vigilance false positives	4.7	(6.5)	2.9	(2.8)	0.38
Power of attention (ms)	1793	(556)	1670	(373)	0.78
Angle discrimination threshold (^{o})	23.2	(18.1)	22.2	(14.3)	0.60
Motion Task Threshold	0.72	(0.25)	0.64	(0.30)	0.47

5.4.5 Symptom scales

Six patients did not have a carer or informant to complete the carer questionnaires. 21 did not have a cohabitee to complete the MSQ, though a collateral history could be obtained from another family member in some cases to allow a clinical diagnosis of RBD (Figure 5.1). Two patients did not have a postural BP measurement.

There were no differences between the groups in any NPI domain. There was no evidence of any difference in the severity of hallucinations measured by the NEVHI or depressive symptoms measured by the GDS (Table 5.6).

Table 5.6. Symptom Scales in FP-CIT normal and abnormal groups

	FP-CIT Normal		FP-CIT Abnormal		
	Mean	(SD)	Mean	(SD)	p
DCFS total	7.3	(3.1)	7.8	(3.3)	0.40
Clinician Assessment of Fluctuation	1.1	(2.1)	2.5	(3.4)	0.07
Epworth Sleepiness scale	7.3	(5.5)	9.4	(4.3)	0.06
MSQ RBD Question (% yes)	50	(n=14)	61	(n=18)	0.53
NPI total	12.5	(11.1)	11.2	(10.4)	0.72
NPI Distress total	6.5	(7.0)	5.7	(7.5)	0.48
GDS	3.5	(3.3)	3.2	(2.4)	0.76
NEVHI	3.0	(4.4)	2.9	(3.8)	0.90
GCSI total	0.4	(0.6)	0.1	(0.3)	0.12
MDS-UPDRS	18.6	(13.1)	29.2	(17.0)	**0.02**
Hoehn and Yahr Stage	0.5	(1.1)	1.2	(1.3)	**0.04**
6-point UPDRS score	2.3	(2.3)	4.1	(3.2)	**0.01**
Standing - Lying Systolic BP	3.8	(20.1)	-10.1	(22.7)	**0.01**
Standing - Lying Diastolic BP	5.0	(8.9)	-4.0	(11.0)	**0.003**

The abnormal group tended towards higher scores on one fluctuations scale, the CAF (2.5 v 1.1; p=0.07) but not the DCFS (7.8 v 7.3; p=0.40). They also tended toward increased daytime sleepiness measured by the Epworth Sleepiness scale (9.4 v 7.3; p=0.06).

There were no differences between the groups on the MSQ Question 1 which has proven reliability for the identification of RBD. However 21 subjects (40%) did not have an appropriate informant to complete the questionnaire, and these were almost entirely in the FP-CIT normal group (n=20/21).

Unsurprisingly the abnormal FP-CIT SPECT group scored higher on the MDS-UPDRS (29.2 v 18.6; p=0.02) and Hoehn and Yahr (1.2 v 0.5; p=0.04) scales. They also had a small but statistically significant drop in postural BP, compared to the rise seen in the normal group (systolic (mmHg): -10.1 v 3.8, p=0.01; diastolic (mmHg): -4.0 v 5.0, p=0.003).

5.4.6 Symptom questionnaire

Those with abnormal scans were more likely to report parkinsonian symptoms such as shuffling (68% v 29%; p=0.006), drooling (58% v 29%; p=0.04), loss of sense of smell (53% v 24%; p=0.03) and change in posture (79% v 35%; p=0.04). They also reported the symptoms of RBD (involuntary movements in sleep 74% v 26%, p=0.001; Acting out dreams 58%v 29%, p=0.04; crying out in sleep 63% v 24%, p=0.004)) and insomnia (42% v 15%; p=0.04). They tended towards being more likely to report constipation (53% v 29%; p=0.09), slowness of movement (68% v 44%; p=0.09) and a change in handwriting (74% v 50%; p=0.09; Figures 5.5 and 5.6).

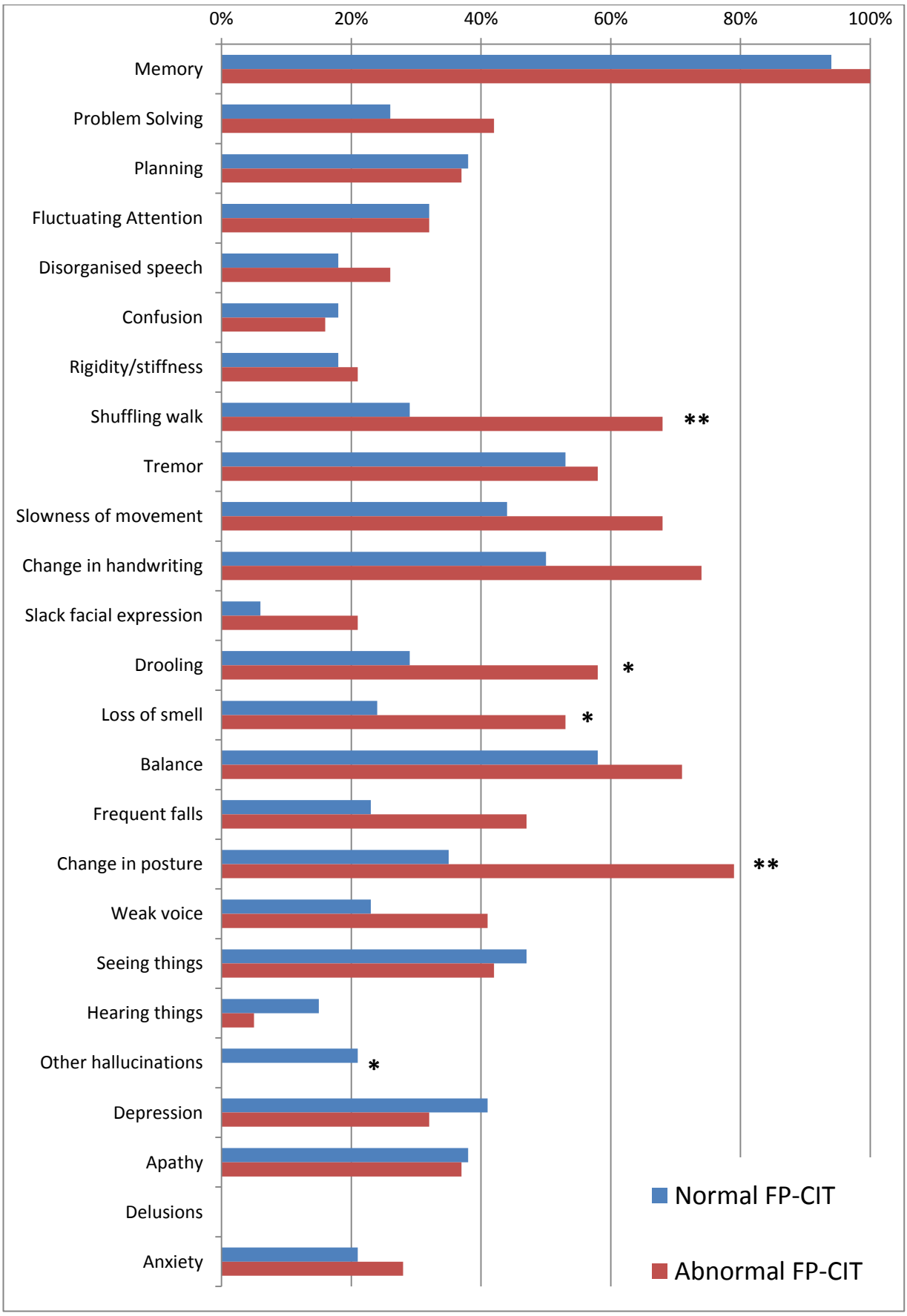

Figure 5.5. Cognitive, parkinsonian and psychiatric symptoms in FP-CIT normal and abnormal groups. (*=p<0.05; **=p<0.01))

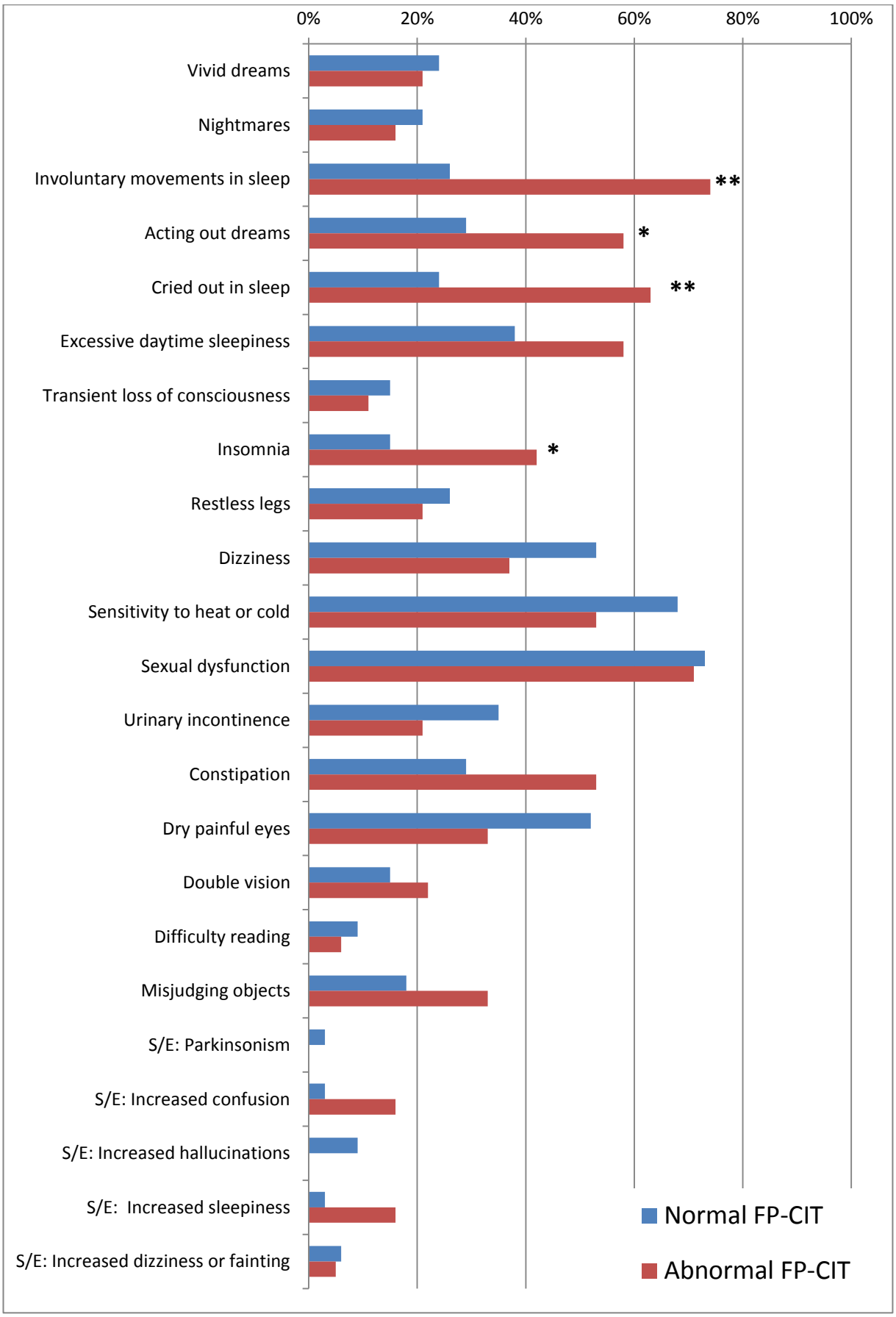

Figure 5.6. Sleep, autonomic and visual symptoms in FP-CIT normal and abnormal groups. (S/E = side effects to medications; *=p<0.05; **=p<0.01))

5.5 Charactieristics of FP-CIT binding assessed by quantification.

Fully-automated quantification was successful in 51/53 scans. In one scan the patient was incorrectly positioned, resulting in the edge of the bounding box being close to the striata. This was satisfactory for visual rating but SPM was unable to successfully realign the scan. In another scan realignment failed because there was exceptionally low uptake in both striata and therefore insufficient signal to allow realignment.

FP-CIT positive patients had a lower mean striatal:occipital FP-CIT binding ratio compared to those with normal scans (1.61 v 2.10; p<0.001; Figure 5.7). Visual rating grade was inversely correlated with mean striatal binding (Kendall's tau B correlation co-efficient = -0.59; p<0.001). There was some overlap in striatal binding between those with normal and abnormal scans (Figure 5.8). There was no correlation of mean striatal binding with age (Pearson's r=-0.06; p = 0.69).

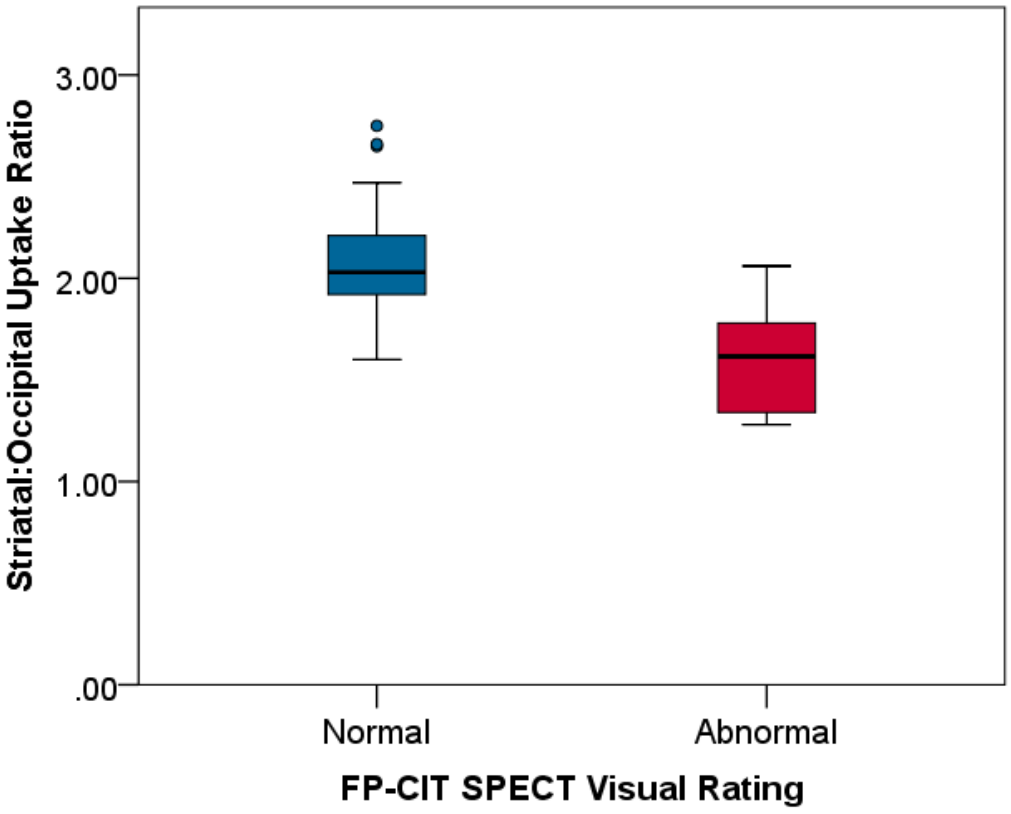

Figure 5.7. Quantification of striatal binding relative to non-specific occipital binding in FP-CIT normal and abnormal groups. FP-CIT abnormal patients had a lower mean striatal:occipital FP-CIT binding ratio compared to those with normal scans (1.61 v 2.10; p<0.001)

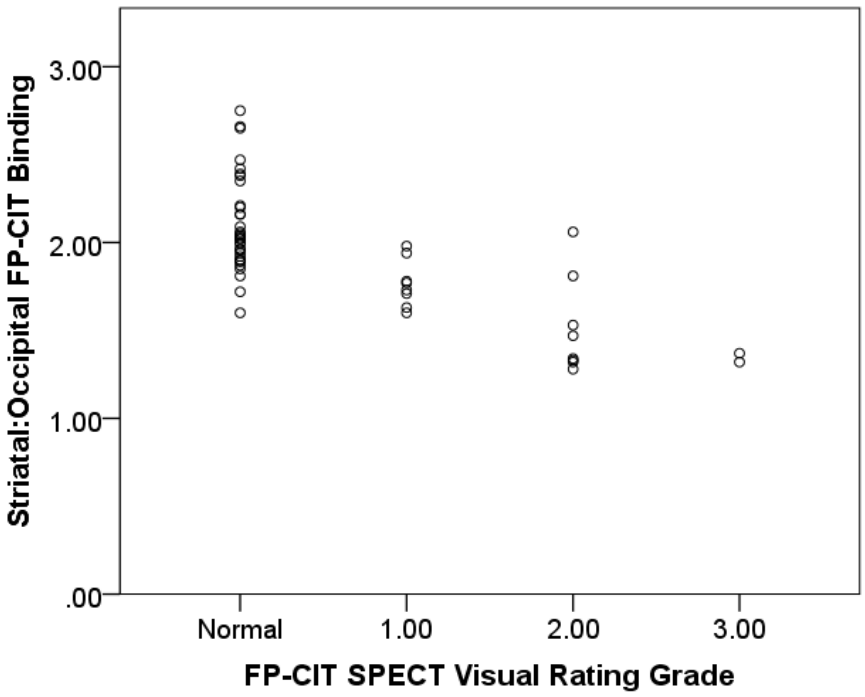

Figure 5.8. Comparison of striatal FP-CIT binding and visual rating grade in the prodromal DLB study. Visual rating grade was correlated with striatal binding (Kendall's tau B correlation co-efficient = -0.59; p<0.001).

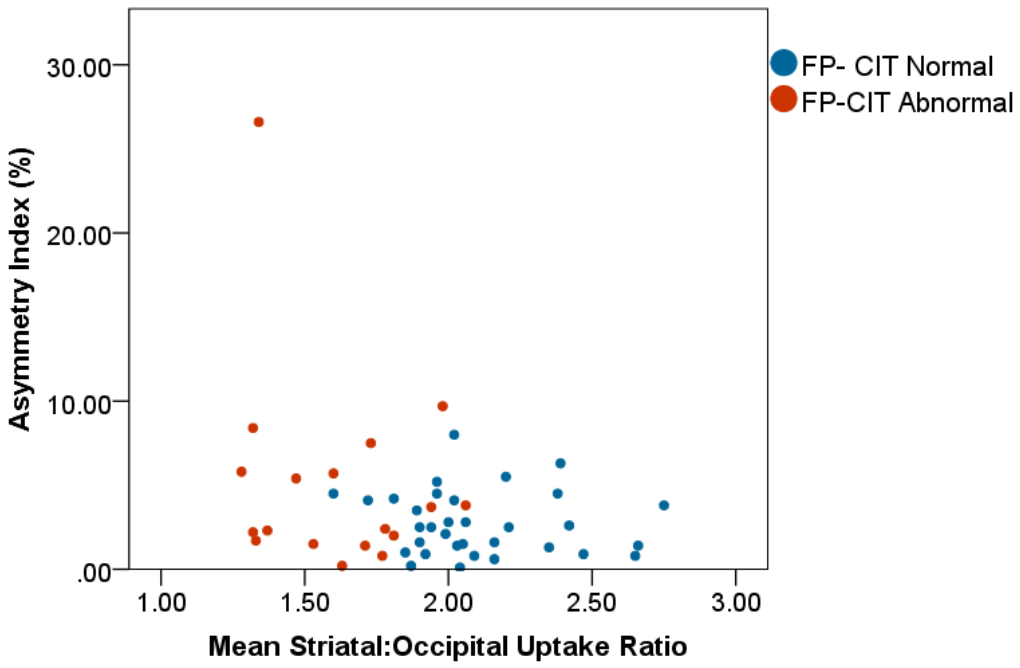

Figure 5.9. Comparison of mean striatal uptake with asymmetry in the prodromal DLB study. Few subjects had significant asymmetry and there was no significant difference in asymmetry between those with normal and abnormal scans (2.7% v 5.1%; p=0.16)

There was little asymmetry between the left and right striata in the group, with only one patient having an asymmetry index above 10% (Figure 5.9). The mean asymmetry index score was 2.7% in the normal group and 5.1% in the abnormal group (p=0.16).

5.6 Comparison of genders

Given the unexpectedly large difference in the proportion of males and females classified as FP-CIT abnormal, a comparison of males and females was carried out.

5.6.1 Demographics

Table 5.7. Demographics in males and females in the prodromal DLB study

	Male	Female	
	Mean (SD)	Mean (SD)	P
n	32	21	-
Age	74.1 (7.4)	79.7 (7.0)	**0.008**
Number of years education	12.2 (2.9)	10.6 (1.9)	**0.018**
ACE-R total	79.3 (11.2)	77.2 (10.2)	0.50
Cholinesterase inhibitor prescribed (%)	41	38	0.85
Levodopa prescribed (%)	19	10	0.46
CDR total	0.4 (0.2)	0.5 (0.2)	0.82
IADL	6.2 (1.8)	6.6 (1.6)	0.42
CIRS-G total	9.0 (5.1)	10.1 (3.3)	0.34
MDS-UPDRS	21.4 (17.4)	23.9 (11.8)	0.19
Hoehn and Yahr stage	0.8 (1.2)	0.7 (1.3)	0.77
6-point UPDRS score	3.0 (3.1)	2.8 (2.4)	0.81
Mean Striatal:Occipital Ratio	1.80 (0.3)	2.13 (0.3)	**0.001**
Total Core and Suggestive symptoms (/5)	1.5 (1.1)	1.0 (0.9)	0.09

Females were on average almost five years older than males. There were no differences between the genders in MDS-UPDRS score or Hoehn and Yahr Stage, though males were almost twice as likely to be taking medications for parkinsonism (p=0.46; Table

5.7). There were no differences between the groups in MCI severity measured by CDR and IADL scores and comorbidity measured by the CIRS-G.

5.6.2 Presence of core and suggestive features of LB disease and striatal FP-CIT binding

Males were more likely to have a positive FP-CIT scan (53% v 10% p=0.001), and semi-quantified striatal binding was significantly higher in females (2.13 v 1.80; p= 0.001; Figure 5.11). Males were more likely to have RBD (59% v 19%; p=0.004) and approached having more core and suggestive features than females (exluding FP-CIT imaging; 1.5 v 1.0; p=0.09; Figure 5.10). There were no significant differences between the genders in the presence of fluctuations, visual hallucinations or parkinsonism.

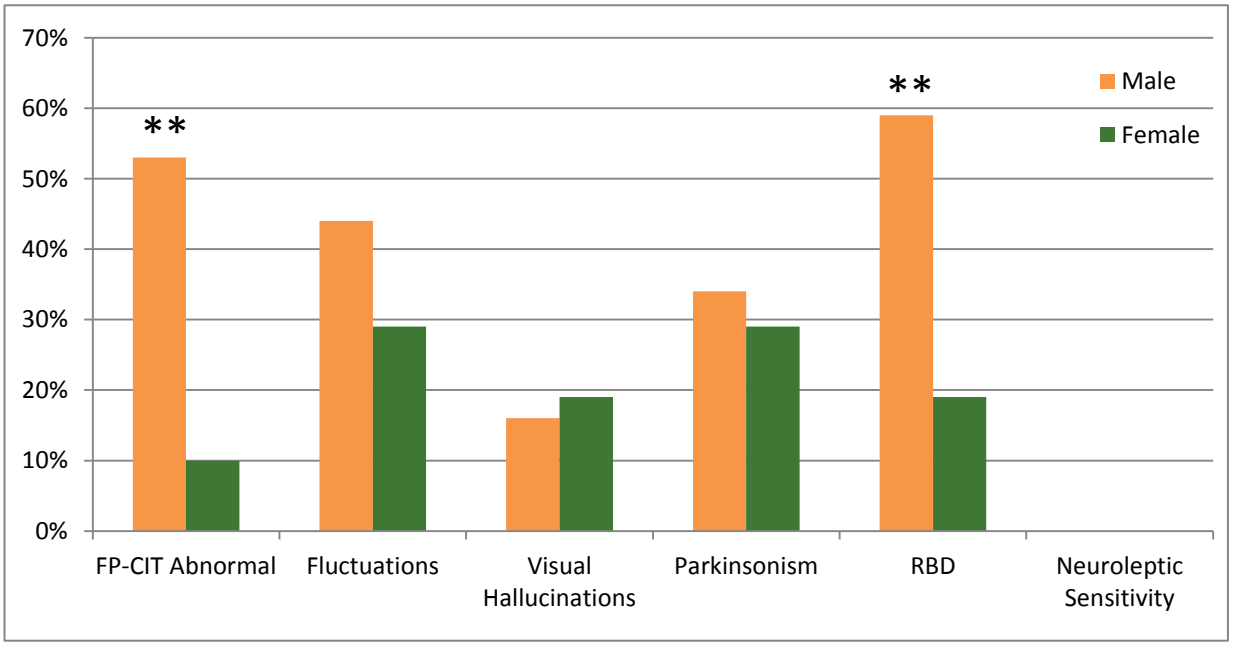

Figure 5.10. Frequency of core and suggestive features of Lewy body disease in males and females in the prodromal DLB study. Males were more likely to have a positive FP-CIT scan (53% v 10% p=0.001) and RBD (59% v 19%; p=0.004). There were no significant differences between the genders in the presence of fluctuations, visual hallucinations or parkinsonism

Given the gender disparity in FP-CIT imaging it could be concluded that either fewer females than males in our study have LB disease, or that females with early LB disease

show less nigrostriatal denervation. If females are more likely to have a substantia nigra-sparing form of LB disease, then FP-CIT negative females in our cohort should be more likely to have prodromal DLB than FP-CIT negative males. A post-hoc analysis was carried out to investigate if FP-CIT negative females had a clinical profile more similar to DLB compared with FP-CIT negative males.

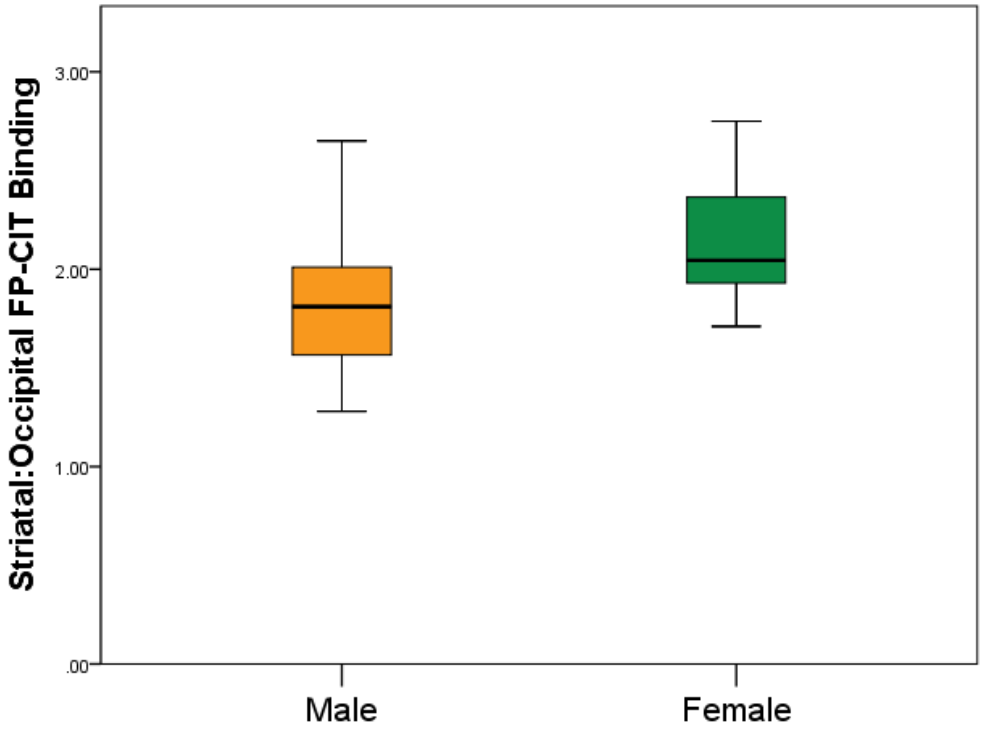

Figure 5.11. Comparison of Striatal FP-CIT binding in males and females in the prodromal DLB study. Striatal binding was significantly higher in females (2.13 v 1.80; p= 0.001).

5.7 Comparison of males and females with normal FP-CIT scans

5.7.1 Demographics

As with the whole sample, females were older than males and had fewer years in education (Table 5.8). There were no differences between the genders in the prescription of cholinesterase inhibitors or medications for parkinsonism and no difference in severity of MCI or the presence of comorbidities.

Table 5.8. Demographics of FP-CIT negative males and females

	Male	Female	
	Mean (SD)	Mean (SD)	p
n	15	19	-
Age	73.1 (8.6)	79.6 (7.4)	**0.02**
Number of years education	12.4 (3.1)	10.5 (2.0)	**0.04**
Cholinesterase inhibitor prescribed (%)	33	42	0.60
Levodopa prescribed (%)	0	11	0.49
CIRS-G total score	10.1 (5.4)	10.3 (3.4)	0.91
CDR total Score	0.4 (0.2)	0.5 (0.2)	0.34
IADL	6.4 (1.6)	6.6 (1.6)	0.57

5.7.2 Core and suggestive features of DLB

There was no difference between the two groups in mean number of core and suggestive symptoms (male 1.0 v female 0.95; p=0.86). There were no significant differences in the rates of any symptom (p≥0.20), though males had relatively high rates of fluctuations and RBD, but low rates of visual hallucinations and parkinsonism (Figure 5.12).

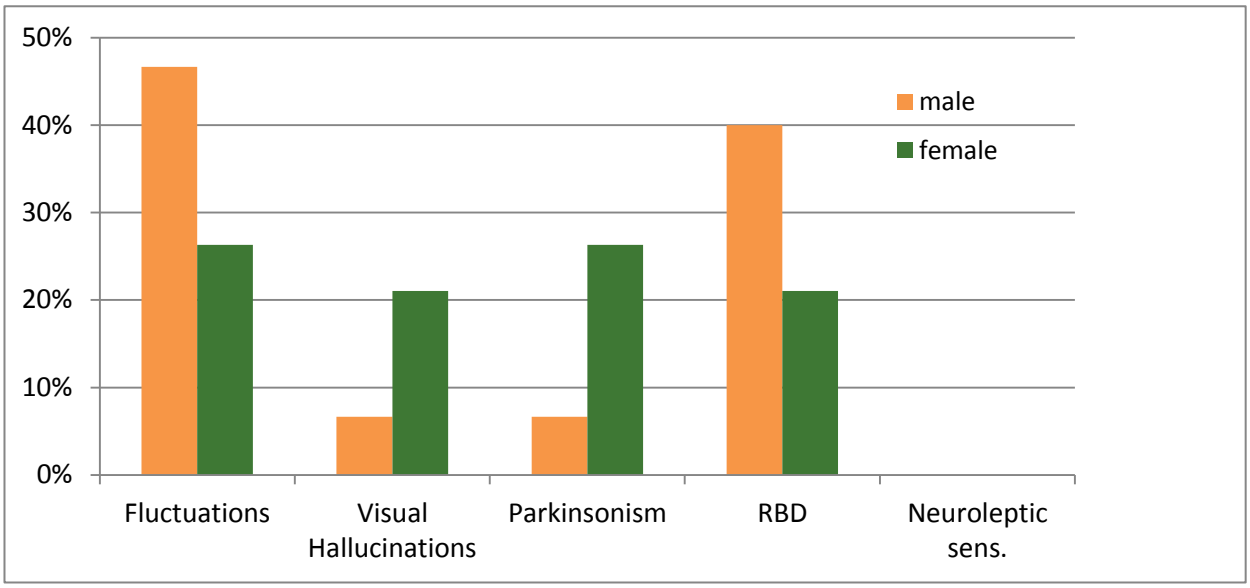

Figure 5.12. Rates of core and suggestive symptoms in FP-CIT negative males and females. There were no significant differences in the rates of any symptom (p≥0.20).

5.7.3 Cognitive tests

There was no difference between the groups in global cognitive function assessed by the ACER, but FP-CIT negative females had lower scores in the visuospatial domain (13.6 v 14.9; p=0.04).There was no difference between the groups in memory, verbal fluency, executive function measured by Trails B or naming (Table 5.9).

To account for demographic differences between the genders, linear regression was carried out with age, years in education and gender as independent variables. Gender was not associated with differences in any cognitive test (p>0.20 in all cases) including the ACE-R visuospatial domain (β=-0.22, p=0.26, n=34).

Table 5.9. Cognitive Tests in FP-CIT negative males and females

	Male		Female		
	Mean	(SD)	Mean	(SD)	p
MMSE	26.3	(2.8)	26.4	(2.1)	0.86
ACE-R total	79.0	(14.1)	78.0	(10.4)	0.80
ACE-R attention and orientation	16.7	(1.7)	17.1	(1.2)	0.78
ACE-R memory	15.7	(6.0)	15.5	(5.3)	0.89
ACE-R fluency	9.1	(3.5)	8.7	(2.8)	0.72
ACE-R language	22.5	(4.2)	23.0	(2.9)	0.76
ACE-R visuospatial	14.9	(1.9)	13.6	(2.1)	**0.04**
Rey AVLT Trial 1	3.9	(2.3)	3.8	(1.4)	0.97
Rey AVLT Trial 6	4.0	(3.7)	3.9	(3.3)	0.99
Rey AVLT Delay	3.1	(4.6)	3.3	(3.1)	0.42
Rey AVLT Recognition	11.2	(3.2)	11.8	(2.3)	0.71
Verbal Fluency (FAS) score	34.6	(15.4)	27.1	(15.8)	0.17
Trails A (s)	59.3	(34.9)	68.4	(35.3)	0.32
Trails B (s)	137.9	(90.5)	131.2	(52.5)	0.75
Failed to complete Trails B (%)	33		42		0.60
Graded naming test	17.4	(9.9)	16.5	(5.1)	0.34

5.7.4 Computerised tests of cognitive function

FP-CIT negative females had slower reaction times than males in the simple reaction time (489ms v 404ms; p=0.006) and the digit vigilance (593ms v 534ms; p=0.002) tasks, and approached having a worse overall power of attention (1849ms v 1720 ms (p=0.06). There was no difference in choice reation time, variability of reaction times, or numbers of mistakes in the reaction time and vigilance tasks. Females had worse visuospatial function both in the angle discrimination (31.6 v 11.4; p=0.001) and motion (threshold 0.80 v 0.63; p=0.04) tasks (Table 5.10).

Linear regression was again carried out with age, years in education and gender as independent variables. The association of gender with a difference in digit vigilance time (β=0.48, p=0.02, n=34) and angle discrimination threshold (β=0.51, p=0.02, n=29) remained. There was no significant association in simple reaction time (β=0.32, p=0.10, n=33), motion task threshold (β=0.30, p=0.19, n=34) or any other computerised test variable.

Table 5.10. Computerised Tests in FP-CIT negative males and females

	Male		Female		
	Mean	(SD)	Mean	(SD)	p
Simple Reaction time (ms)	403.8	(216.0)	488.6	(210.9)	**0.006**
Simple reaction time SD correct (ms)	116.4	(100.0)	190.1	(173.6)	0.13
Choice reaction time (ms)	784.8	(392.3)	767.4	(303.1)	0.32
Choice reaction time SD	225.0	(189.1)	185.3	(115.1)	0.87
Choice reaction time number of errors	1.5	(1.6)	1.8	(1.8)	0.63
Cognitive processing time	381.0	(319.8)	278.7	(151.6)	0.42
Digit vigilance number got (max 36)	32.9	(4.9)	32.0	(5.1)	0.58
Digit vigilance mean time (ms)	533.7	(55.8)	592.6	(77.1)	**0.02**
Digit vigilance SD (ms)	96.6	(34.0)	119.6	(49.8)	0.14
Digit vigilance false positives	4.5	(3.3)	4.9	(8.3)	0.23
Power of attention (ms)	1720	(578)	1849	(548)	0.06
Angle discrimination threshold (^{o})	11.4	(8.3)	31.6	(18.6)	**0.001**
Motion Task Threshold	0.63	(0.28)	0.80	(0.21)	**0.04**

5.7.5 Symptom scales

There were no differences between the groups in the NPI or in specific measures of fluctuations, sleepiness, hallucinations or gastroparesis (Table 5.11). Females recorded more depressive symptoms on the GDS (4.5 v 2.3; p=0.047) and significantly higher MDS-UPDRS scores (23.3 v 12.7; p=0.004). There was no difference between the groups in lying and standing BP. Only 10 males and 4 females had a suitable informant to complete the MSQ, so this was not included in the statistical analysis. There were no significant differences in any NPI subscale, though 21% of informants for females reported delusions compared with 0% for males (p=0.07).

Linear regression with age, years in education and gender as independent variables found no association between age and any variable in Table 5.11 including GDS score (β=.31, p=0.10, n=34), MDS-UPDRS score (β=0.28, p=0.14, n=34) or 6-point UPDRS score (β-0.17, p=0.40, n=34).

Table 5.11. Symptom Scales in FP-CIT negative males and females

	Male		Female		
	Mean	(SD)	Mean	(SD)	p
DCFS total	7.4	(3.3)	7.2	(3.0)	0.95
Clinician Assessment of Fluctuation	0.9	(1.9)	1.4	(2.3)	0.70
NPI total	13.3	(12.7)	11.8	(9.7)	0.73
NPI Distress total	6.1	(6.9)	6.9	(7.3)	0.57
GDS	2.3	(1.9)	4.5	(3.9)	**0.047**
NEVHI	2.5	(3.9)	3.4	(4.8)	0.66
Epworth Sleepiness scale	6.1	(4.4)	8.3	(6.2)	0.23
GCSI total	0.3	(0.7)	0.4	(0.5)	0.18
MDS-UPDRS	12.7	(12.0)	23.3	(12.2)	**0.004**
Hoehn and Yahr stage	0.1	(0.5)	0.8	(1.4)	0.30
6-point UPDRS score	1.6	(2.0)	2.8	(2.5)	**0.02**
Standing - Lying Systolic BP	5.1	(13.6)	2.7	(24.9)	0.88
Standing - Lying Diastolic BP	6.4	(7.3)	3.8	(10.2)	0.41

5.7.6 Symptom questionnaire

FP-CIT negative females were more likely to report frequent falls (40% v 0%; p=0.02), a change in posture (53% v 13%; p=0.02) and dizziness (74% v 27%; p=0.01). They tended toward being more likely to have auditory hallucinations (26% v 0%; p=0.053) and to have difficulty moving around due to misjudging objects (38% v 6%; p=0.07; Figures 5.13 and 5.14).

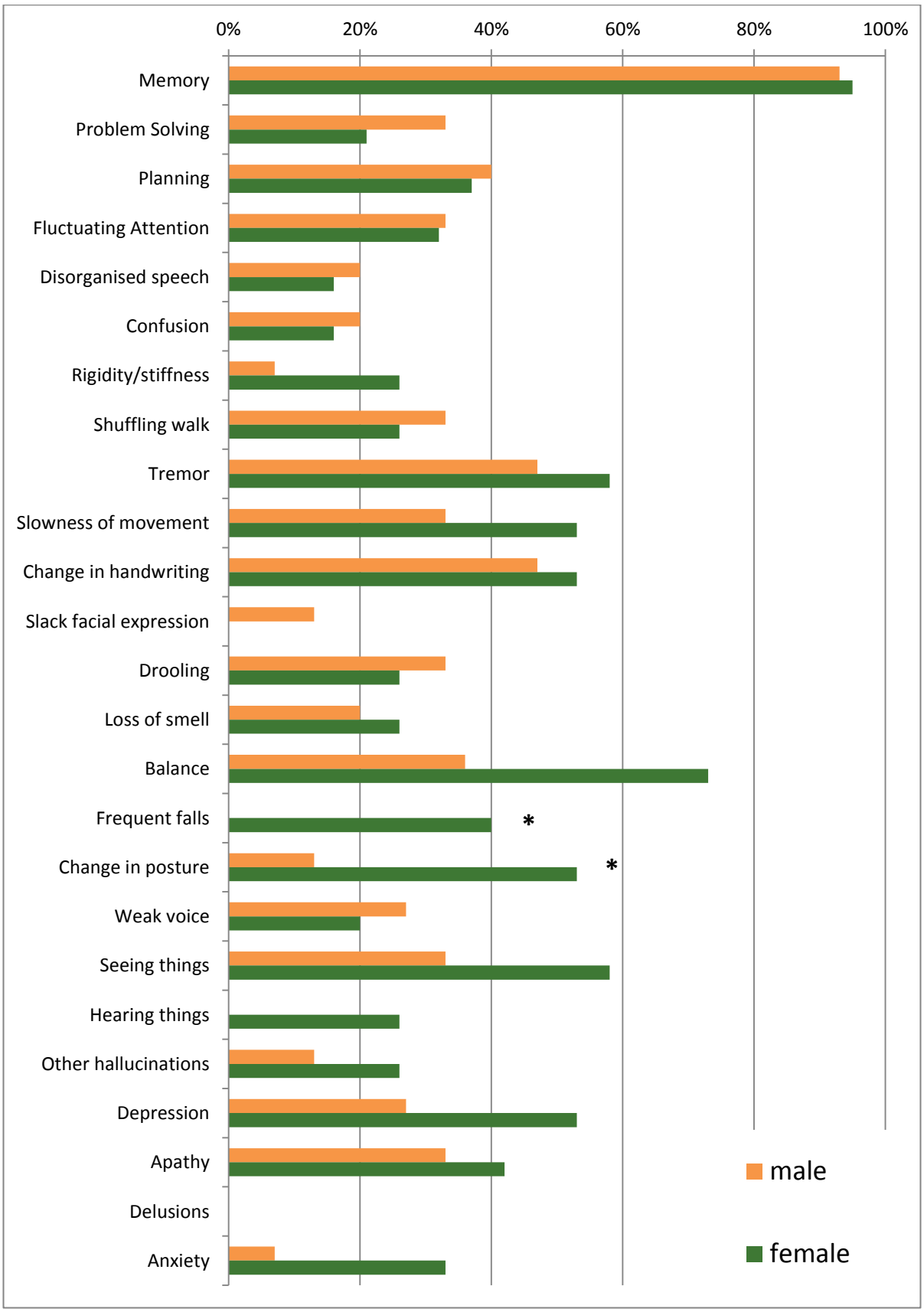

Figure 5.13. Cognitive, parkinsonian and psychiatric symptoms in FP-CIT negative males and females. (*=p<0.05)

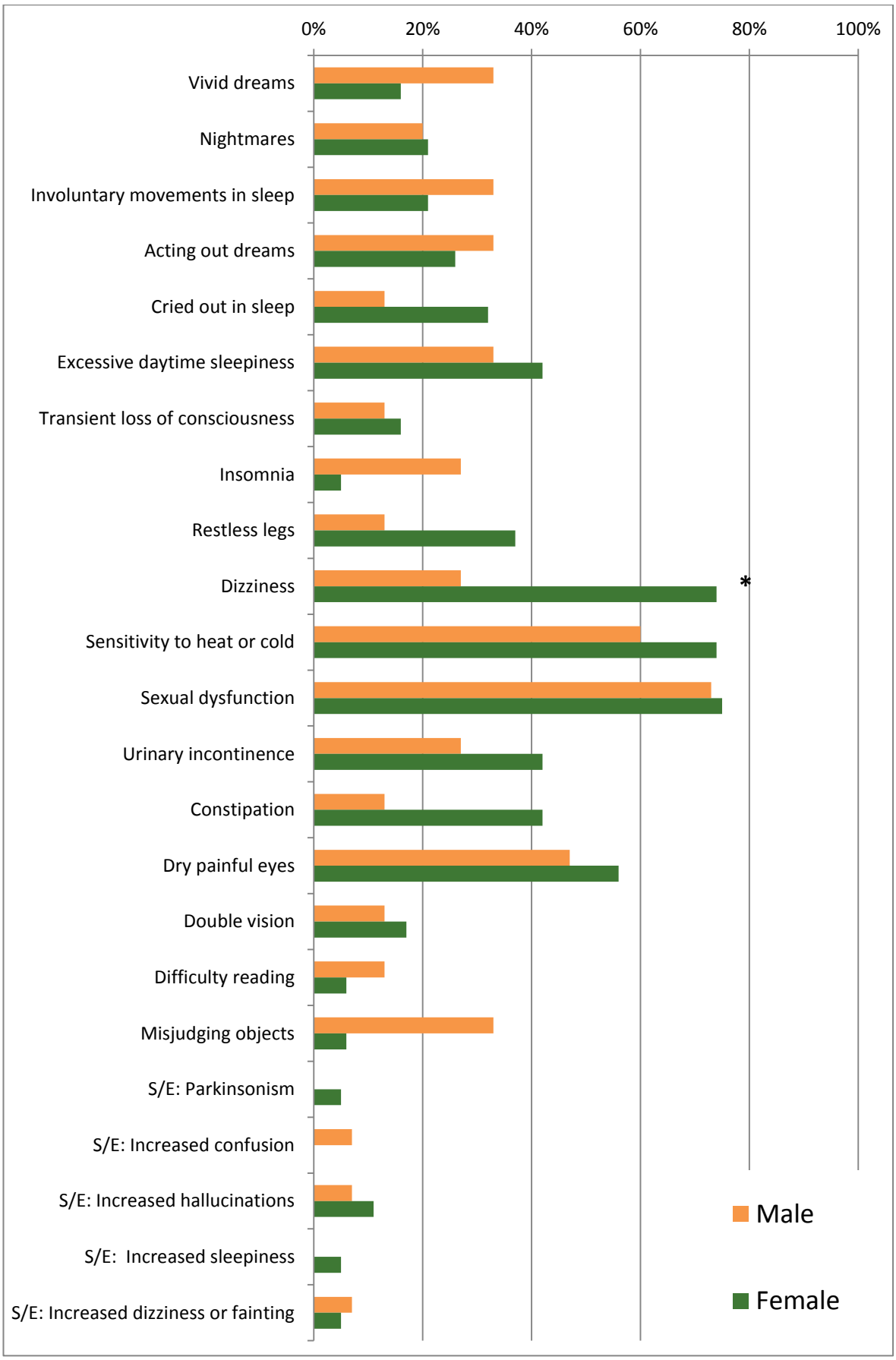

Figure 5.14. Sleep, autonomic and visual symptoms in FP-CIT negative males and females. (S/E = side effects to medications; *=p<0.05)

5.8 Discussion

The primary hypothesis in this analysis was that ^{123}I-FP-CIT imaging is a biomarker for prodromal DLB and will therefore be associated with a clinical profile similar to that seen in established DLB, albeit in a less severe form.

5.8.1 Hypothesis 1: The ^{123}I-FP-CIT abnormal group will have more core and suggestive diagnostic features of DLB

The presence or absence of the diagnostic features of DLB was determined by the reviewing clinician (PD). Abnormal ^{123}I-FP-CIT imaging was indeed associated with an increased number of core or suggestive features of DLB, particularly parkinsonism and RBD. All subjects with an abnormal scan were found to have at least one other core or suggestive feature of DLB. This supports the hypothesis that ^{123}I-FP-CIT imaging is a biomarker of Lewy body pathology in the MCI phase.

5.8.2 Hypothesis 2: The ^{123}I-FP-CIT abnormal group will have higher scores in scales measuring these features

Revised UPDRS (MDS-UPDRS) scores were higher in the abnormal ^{123}I-FP-CIT group. However an affirmative answer to the RBD screening question of the Mayo Sleep Questionnaire was common in both groups.

Despite the absence of clinically detected differences in the rate of cognitive fluctuations, scores in the Clinician Assessment of Fluctuation and Epworth Sleepiness scales approached being significantly greater in those with abnormal scans. However, similar proportions of both groups had clinically significant scores. Only 3 in the normal group and 4 in the abnormal group scored above the CAF threshold of ≥5 (Walker *et al.*, 2000a). A higher proportion (8 normal, 10 abnormal) scored above an Epworth Sleepiness Scale threshold of ≥10 previously found to differentiate DLB from Controls (Boddy *et al.*, 2007). Long-term follow-up will ascertain if lower thresholds in these scales can identify fluctuations in prodromal LB disease. It should be noted that other conditions such as vascular cognitive impairment may be associated with fluctuation (Walker *et al.*, 2000a), limiting the specificity of these scales. The dementia cognitive fluctuations scale did not detect any difference between the groups. There were no differences between the groups in the Visual Hallucination Inventory.

In summary, the two specific features associated with abnormal ^{123}I-FP-CIT imaging were parkinsonism and RBD. ^{123}I-FP-CIT imaging is a measure of nigrostriatal dopaminergic nerve degeneration, thought to be the direct cause of parkinsonism in DLB and therefore the association between the two is unsurprising. RBD is also thought to be caused by brainstem LB pathology (Boeve *et al.*, 2007). Thus it seems that ^{123}I-FP-CIT imaging may be a biomarker of brainstem LB pathology more widely. The reticular activating system, including the midbrain reticular formation, is thought to be an important factor in fluctuations in DLB (Ferman *et al.*, 2014). This may explain a tendency toward increased severity of fluctuating cognition and daytime sleepiness in the abnormal ^{123}I-FP-CIT group. Despite this, fluctuations were not more common in the ^{123}I-FP-CIT abnormal group. There were also no differences in visual hallucinations between the two groups. There are at least two potential explanations for these findings:

1. Some cases of prodromal DLB do not display significant substantia nigra pathology.

The Braak hypothesis suggests that Lewy body pathology progresses in a predictable sequence from the brainstem to subcortical structures and finally to the neocortex (Braak *et al.*, 2003). We know from post-mortem studies that this does not hold in all cases, and limbic and cortical areas can be affected without involvement of the substantia nigra (Zaccai *et al.*, 2008). Furthermore, the severity of synucleinopathy in the brain is only weakly correlated with substantia nigra neuronal loss in DLB (Beach *et al.*, 2009).

Visual hallucinations are associated with LB disease in the amygdala, parahippocampal gyrus and temporal cortex (Harding *et al.*, 2002). Fluctuating cognition has also been associated with cortical LB pathology (Schneider *et al.*, 2012). Thus, in cases with neocortical involvement and relatively little brainstem LB pathology hallucinations and cognitive fluctuations would be expected to be common, and parkinsonism, RBD and abnormal ^{123}I-FP-CIT imaging relatively rare. This would explain the absence of any difference in rates of hallucinations and fluctuations between the ^{123}I-FP-CIT normal and abnormal groups. Therefore ^{123}I-FP-CIT imaging may detect prodromal LB disease in cases with brainstem involvement and symptoms such as parkinsonism and RBD.

^{123}I-FP-CIT imaging may not be useful in detecting other patterns of LB pathology with early cortical involvement and symptoms such as hallucinations and fluctuations.

2. Some visual hallucinations and fluctuations in our group were caused by non-Lewy body pathology.

Symptoms such as visual hallucinations, fluctuations and parkinsonism are characteristic of DLB, but can occur in other disorders such as AD and vascular dementia (Walker *et al.*, 2000b; Ferman *et al.*, 2013a; Kaur *et al.*, 2013).Thus it seems probable that some of the cohort with normal FP-CIT scans may have visual, cognitive or motor symptoms mimicking LB features but not caused by LB pathology. One potential factor would be vascular disease. This is supported by the finding of higher CIRS-G scores in the domains of 'Heart' and 'Vascular' in the ^{123}I-FP-CIT normal group (CRIS-G Heart+Vascular: Normal 2.3 ±1.6, Abnormal 1.3 ±1.5; p=0.04), though MRI assessment of cerebrovascular disease was not included in this study.

Each of the two explanations above is probably true for a proportion of those with normal ^{123}I-FP-CIT scans i.e. some of those with normal scans have a substantia nigra sparing pattern of LB deposition, others do not have LB disease at all but have symptoms such as parkinsonism, hallucinations, fluctuations and RBD caused by other pathology such as vascular disease. The proportion of ^{123}I-FP-CIT normal participants in this cohort that actually have prodromal DLB will be established in longitudinal follow-up.

5.8.3 Hypothesis 3: The ^{123}I-FP-CIT abnormal group will have worse visuospatial and executive function, but better memory on neuropsychological tests

We hypothesised that abnormal ^{123}I-FP-CIT imaging would be associated with the typical DLB cognitive profile of decreased attention, executive and visuospatial function, but relatively preserved memory. No differences were detected between the two groups in any ACER subdomain, memory measured by the Rey AVLT delayed recall or recognition, attention/executive function measured by the Trails Test, verbal fluency or graded naming. Executive dysfunction was common in the overall cohort,

with 38% of those with normal and 58% of those with abnormal scans being unable to complete Trails B. Computerised tests of cognition did find some evidence of executive dysfunction with increased errors in the choice reaction time task in the abnormal ^{123}I-FP-CIT group, along with a tendency towards decreased attention in the digit vigilance task. Features previously found in DLB such as visuospatial dysfunction (Wood *et al.*, 2013), slowed cognitive processing, longer reaction times and increased variation in reaction times (Ballard *et al.*, 2001) were not seen in the ^{123}I-FP-CIT abnormal group.

We cannot say at this point whether the full neuropsychological profile of DLB is not expressed in the prodromal stage, or whether it is not discernible here due to a proportion of those in the ^{123}I-FP-CIT normal group having prodromal DLB, that is having Lewy body disease but without sufficient substantia nigra involvement to become abnormal on ^{123}I-FP-CIT imaging. The neuropsychological scales used often show clear differences between established DLB and AD, but may not be sensitive to changes in the early stages of LB disease, where cognitive deficits are by definition milder than those seen in established disease.

5.8.4 Hypothesis 4: The ^{123}I-FP-CIT abnormal group will have a higher frequency of other features associated with DLB e.g. anosmia, postural hypotension and constipation

5.8.4.1 Olfactory system

The olfactory system thought to be one of the earliest sites of LB deposition (Beach *et al.*, 2009) and decreased sense of smell has been found to be an early symptom of DLB (Chiba *et al.*, 2012; Fujishiro *et al.*, 2013b). Consistent with this, loss of sense of smell was found to be more common in the FP-CIT abnormal group.

5.8.4.2 Peripheral autonomic Nervous System

The peripheral autonomic nervous system is susceptible to early involvement in LB disease (Minguez-Castellanos *et al.*, 2007) and orthostatic dizziness and hypersalivation have been reported as early symptoms in DLB (Chiba *et al.*, 2012). The ^{123}I-FP-CIT abnormal group were more likely to report drooling but not dizziness, though they did have a significantly greater fall in blood pressure on standing. The magnitude of this fall

(10/4 mmHg) may not be clinically significant. Other autonomic symptoms such as sexual dysfunction, urinary incontinence and sensitivity to heat or cold were relatively common in both groups.

5.8.4.3 Enteric System

LBs can be found in the enteric nervous system in the early stages of LB disease and in asymptomatic individuals. Constipation has previously been reported to be significantly more common in DLB than AD in a survey carried out in Japan (47% v. 16%) (Chiba *et al.*, 2012). There was no significant difference in the rates of constipation between the ^{123}I-FP-CIT abnormal and normal groups in this cohort (53% v 29%). This may reflect a lack of specificity of constipation as a marker of LB pathology in populations with high background rates of constipation, compared with the low rate of 17% of healthy older people in the Japanese cohort.

Delayed gastric emptying is common in established PD (Heetun and Quigley, 2012) but symptoms of delayed gastric emptying measured by the GCSI were very infrequent in both groups in our cohort, with only two subjects scoring above the threshold of 1.90.

5.8.4.4 Visual Symptoms

Visual symptoms such as dry/painful eyes, double vision, difficulty reading and misjudging objects have been reported in DLB (Jefferis *et al.*, 2013) but these did not differentiate the ^{123}I-FP-CIT normal and abnormal groups.

5.8.4.5 Neuropsychiatric Symptoms

Depression is a supportive feature for the diagnosis of DLB (McKeith *et al.*, 2005) and has been reported to be more common in DLB than AD (Ballard *et al.*, 1999; Boot *et al.*, 2013b). Despite this, the ^{123}I-FP-CIT abnormal group did not score higher on the GDS. There were no differences between the groups in NPI overall score or carer distress.

The ^{123}I-FP-CIT normal group were more likely to report 'other hallucinations' (olfactory, gustatory or tactile; 21% v. 0%). The reason for this increased incidence is not yet clear but may be related to the pathological heterogeneity of the ^{123}I-FP-CIT normal group.

In summary, the above evidence shows that hyposmia and drooling are more common in those with abnormal ^{123}I-FP-CIT imaging, supporting the hypothesis that it is a biomarker of prodromal DLB. There were no differences between the groups in other autonomic, enteric, visual and neuropsychiatric symptoms. In general, most symptoms associated with LB disease were relatively common in the ^{123}I-FP-CIT normal group. A lack of specificity may limit the usefulness of these symptoms to identify people with prodromal DLB but their presence may increase confidence in the diagnosis of DLB in clinical settings, particularly in the early stages of dementia (Donaghy and McKeith, 2014).

Quantitative measures of dysfunction may be more useful in differentiating normal ageing from pathological changes. Abnormal olfaction measured by the University of Pennsylvania Smell Identification Test and abnormal colour vision measured by the Farnsworth-Munsell test are both associated with an increased hazard ratio for the development of DLB in people with idiopathic RBD (Postuma *et al.*, 2015a). The same authors found that using abnormalities in olfaction, colour vision and motor function to select patients could increase the three year conversion to neurodegenerative disease from 30% to 60%. Research is beginning to emerge on the utility of similar tests in MCI cohorts, with the Cross-Cultural Smell Identification Test differentiating DLB-MCI from AD-MCI with an area under the receiver operating characteristic of 0.84 (Yoon *et al.*, 2015). A similar battery to that developed by Postuma et al. for RBD could be developed for use in MCI cohorts to identify those at highest risk of conversion to DLB.

5.8.5 Hypothesis 5: Measures of asymmetry of ^{123}I-FP-CIT binding between the left and right striata will be low in the entire cohort.

There was little asymmetry in striatal binding in the cohort. Only one subject had an asymmetry index greater than 10%. This is similar to reports in established DLB, and different to the asymmetrical binding pattern often seen in PD (Walker *et al.*, 2004).

This poses some difficulties in the visual classification of scans. The mildest grade of abnormality in PD is defined as asymmetry in the putamina (Benamer *et al.*, 2000). If a more symmetrical pattern of loss occurs in DLB than PD, early disease may be more difficult to visualise. Quantitative measures may be helpful to detect subtle, symmetrical decline in ^{123}I-FP-CIT binding. This is somewhat complicated by natural variation in ^{123}I-FP-CIT binding and differences associated with age and gender. Good data on normal ranges are beginning to become available from large-scale studies of normal cohorts (Varrone *et al.*, 2013), but to apply these to study cohorts requires scanning and processing procedures that replicate those used to acquire the normal data.

Those with abnormal ^{123}I-FP-CIT scans unsurprisingly had lower overall striatal binding than those with normal scans and visual rating grade was inversely correlated with striatal binding. That said, there was significant overlap between those with normal and abnormal scans and no evidence of a clear 'intermediate' group between those with normal and abnormal scans. Longitudinal follow-up may allow us to identify a threshold of binding that identifies individuals at risk of developing DLB based on our quantification methods.

5.8.6 Gender differences

More than half of males (53%), but few females (10%) had an abnormal ^{123}I-FP-CIT scan. This degree of difference was unexpected. Despite being older, females had ^{123}I-FP-CIT binding ratios 18% higher than males on average, larger than the approximately 10% difference noted a normal cohort (Varrone *et al.*, 2013). Varrone et al. suggested this may be due to differences in striatal size, with females having the same number of dopamine transporters as males, but smaller striata, resulting in higher ^{123}I-FP-CIT uptake per unit volume. However an expected 10% difference in striatal binding would not seem sufficient to explain a five-fold greater likelihood of an abnormal scan on visual rating. In addition, RBD was found to be significantly more common in males than females (59% v 19%). These results suggest that either females in our study are less likely to have prodromal DLB than males, or that females with prodromal DLB are less likely to have brainstem involvement, and therefore less likely to have RBD or a positive FP-CIT scan.

A meta-analysis of epidemiological studies found DLB to be equally common in males and females (Vann Jones and O'Brien, 2014). If LB disease is equally common in males and females in our cohort, we would expect that a greater proportion of the ^{123}I-FP-CIT normal females have prodromal DLB than the ^{123}I-FP-CIT normal males. A post-hoc comparison was carried out to test this hypothesis.

We expected that ^{123}I-FP-CIT normal females would have a more 'Lewy body' phenotype than males if this hypothesis was correct. However, there were no differences between ^{123}I-FP-CIT normal males and females in the number of core or suggestive features present. Females did score higher on the MDS-UPDRS, but there were no differences in quantitative scales for other core and suggestive features. Females recorded slower reaction times in tests of attention. It is not clear if this was due to attentional deficit or motor impairment. Females consistently scored lower on visuospatial tasks (ACE-R visuospatial subdomain, angle task and motion task). There was no evidence of executive dysfunction measured by Trails B or the choice reaction time test.

Females with normal FP-CIT had higher scores on the GDS and were more likely to report dizziness, but there was no difference in postural BP change, other autonomic symptoms such as constipation or other associated symptoms such as hyposmia and drooling.

^{123}I-FP-CIT normal females were older than ^{123}I-FP-CIT normal males by an average of 6.5 years. This may be a factor in the differences in visuospatial function, reaction times and MDS-UPDRS scores between the genders. However, females did have slower response times in the digit vigilance tasks and worse visuospatial function on the angle discrimination task even after linear regression to account for the effect of age and years in education. In any case there was no clear pattern of increased LB features in the female group. This suggests that in our overall cohort males were more likely to have LB disease than females. Alternatively, females may have a less clear LB phenotype in the prodromal stage of DLB, with fewer core and suggestive features (RBD in particular) and greater visuospatial dysfunction. This remains speculative, and longitudinal follow-up is needed establish whether or not this is the case.

5.9 Strengths and limitations of study

This is the first cohort that we are aware of that specifically examines the phenotype of people with MCI who are at risk of developing DLB. The cohort is well characterised both clinically and neuropsychologically. As there is little evidence for the presentation of prodromal DLB this study is by nature exploratory. We have been inclusive in our selection criteria in an effort not to exclude different presentations of prodromal DLB. Recruiting patients with symptoms that do not reach the threshold of core or suggestive features of DLB may increase the heterogeneity of the cohort, but will also allow us to chart the development of these features in some subjects. Longitudinal follow-up will clarify which subjects are in the prodromal stages of DLB.

No statistical correction was made for multiple comparisons. In this exploratory study priority was given to reducing the likelihood of type II errors, where potentially clinically significant findings remain undetected. Our positive findings require confirmation in independent cohorts.

The ^{123}I-FP-CIT normal and abnormal groups were very similar in age and overall cognitive function, enabling a direct comparison between the groups. This has allowed us to conclude that ^{123}I-FP-CIT is a biomarker of prodromal DLB, though the sensitivity and specificity of this marker will not be clear until longitudinal follow-up is complete.

40% of the cohort was treated with cholinesterase inhibitors. This reflects a willingness from some clinicians to treat visual hallucinations and fluctuations with cholinesterase inhibitors in the absence of dementia, and also a trend towards earlier treatment of cognitive impairment over the past several years. During screening for this study we found that in some cases a diagnosis of dementia was made by clinical teams despite recording an absence of any functional impairment. For the purposes of this study we applied the NIAAA criteria for MCI to determine suitability to participate (Albert *et al.*, 2011). Those on treatment with cholinesterase inhibitors had similar levels of functional impairment measured by the IADL and CDR scales (CDR in those on treatment = 0.48 v. 0.42 in those not on treatment ($p= 0.32$); IADL = 5.8 v. 6.7 ($p= 0.14$)).

We included subjects with MMSE ≥20 provided they satisfied NIAAA criteria for MCI i.e. preservation of independence with minimal aids or assistance. Whilst many people with an MMSE score between 20 and 25 will have dementia, some are not yet functionally impaired, and their relatively low score may reflect low prior educational

attainment. Those with MMSE <25 (n=9) had similar levels of functional impairment to those with scores ≥25 (n=44). CDR in those with MMSE<25 = 0.50 v. 0.43 in those with MMSE ≥25 (p=0.54); IADL = 6.3 v 6.3 (p=0.80).

A complete dataset was not possible in all participants, principally due to the absence of a suitable informant, and in some cases due to technical errors in the computerised tests. Only two ^{123}I-FP-CIT scans failed automatic quantification. One of these was clearly abnormal, with extremely low binding. Such cases would not pose any problem in clinical practise. The other subject was incorrectly positioned in the scanner.

Clinical assessment was carried out prior to ^{123}I-FP-CIT reporting, preventing the biasing of clinical assessment. However, in some cases the clinician was aware of the ^{123}I-FP-CIT result e.g. if a scan had been carried out as part of their routine care prior to assessment.

5.10 Future directions

Longitudinal follow-up of this cohort will determine the sensitivity and specificity of ^{123}I-FP-CIT imaging in prodromal DLB, along with the diagnostic utility of the other neuropsychological and clinical scales assessed. We may be able to identify clinical phenotypes that reflect emergent core or suggestive features of LB disease e.g. visual phenomena such as misidentification or passage hallucinations preceding fully formed visual hallucinations.

In established DLB ^{123}I-FP-CIT imaging has a sensitivity of 89% and a specificity of 81% (O'Brien *et al.*, 2014b). Sensitivity could be expected to be lower in prodromal DLB as nigrostriatal pathology and neuronal loss will be less well developed. The prevalence of LB features, in particular RBD, in the ^{123}I-FP-CIT normal group suggests that some of this group also have LB disease. Semi-quantitative analysis may identify subtle abnormalities not detected by visual rating.

The pattern of signal loss in the striatum may differ between DLB and PD, with less asymmetry and a lower caudate:putamen binding ratio in DLB (Walker *et al.*, 2004; Marquie *et al.*, 2014). The relative lack of brain structural information contained in a

^{123}I-FP-CIT image makes automated analysis of striatal subdivisions difficult. We plan to do this using semi-automated software with user-positioning of caudate and putamen templates. This will allow us to ascertain whether a particular pattern of loss is seen in early LB disease. However, the evidence from established DLB suggests that losses are global, rather than being restricted to the putamen (Walker *et al.*, 2004). This will affect the visual rating of scans. We found good agreement on visual rating (mean Kappa = 0.62) but many scans were considered difficult to rate due to a mild general decrease in striatal binding in the absence of any more localised signal loss. This is more difficult to visualise than the unilateral loss in the putamen seen in the early stages of PD. The development of thresholds for normal binding using semi-quantitative analysis may help to identify when overall striatal binding has decreased.

More work is needed to investigate other potential biomarkers of prodromal DLB. Biomarkers that investigate structures affected early in the disease process may be the most useful. The peripheral autonomic nervous system can be investigated using MIBG imaging of cardiac sympathetic denervation (Fujishiro *et al.*, 2012). Biopsies of peripheral nerves in the skin or gut may allow us to directly identify αSyn pathology (Beach *et al.*, 2010; Wang *et al.*, 2013). We plan to examine cardiac MIBG and skin autonomic nerve αSyn as potential markers of LB disease in the near future. Findings in RBD suggest that olfactory and visual dysfunction, as well as subtle motor impairments may be markers of prodromal LB disease. No one biomarker is likely to prove sensitive and specific enough in isolation. A two-stage process may be necessary, where initial sensitive, non-invasive and inexpensive markers (e.g. visual/olfactory/motor function tests, symptom questionnaires) are used to identify patients at risk and then a second more specific but potentially more expensive or invasive test (e.g. ^{123}I-FP-CIT imaging, biopsy) is used to confirm the presence of LB disease.

Chapter 6 Amyloid Imaging Aims, Hypotheses and Methods

6.1 Aims

The aim of this project is to determine the prevalence and pattern of amyloid deposition in DLB, and whether amyloid deposition influences clinical phenotype. Subjects had a thorough baseline clinical and neuropsychological assessment followed by amyloid PET imaging using Florbetapir. Where possible subjects also had an MRI scan.

6.2 Hypotheses

6.2.1 Primary hypotheses

1. The proportion of DLB patients with positive amyloid scans on visual rating will be greater than controls and less than AD.
2. Semiquantitative measures of amyloid deposition will be greater in DLB than controls and less than AD.

6.2.2 Secondary hypotheses

We will test if amyloid deposition is associated with a clinical phenotype with similarities to AD, and faster disease progression.

3. Amyloid positive DLB cases will have greater memory impairment and less visuospatial, attention and executive impairments than amyloid negative DLB cases.
4. Amyloid positive DLB cases will score lower in scales measuring hallucinations, fluctuations and parkinsonism
5. Amyloid positive DLB cases will have smaller hippocampi than amyloid negative cases
6. Amyloid positive DLB cases will have a shorter duration of illness than amyloid negative cases

We will also examine correlations between clinical measures and amyloid binding in DLB.

6.3 Methods

6.3.1 Participants

Participants were recruited prospectively from NHS Trusts in the North of England. Patients were identified through direct referral from their treating teams; through the Dementias and Neurodegenerative Diseases Research Network (DeNDRoN) Research Case Register and through screening patients' notes in memory clinics. Control subjects were recruited through the DeNDRoN Case Register or were partners of participants.

All subjects needed to have sufficient English to complete cognitive testing. All subjects with capacity gave their written informed consent to take part in the study. In the case of subjects with dementia who lacked capacity their participation in the study was discussed with a consultee. The study received ethical approval from the National Research Ethics Service Committee North East - Newcastle & North Tyneside 2 (Research Ethics Committee Identification Number 13/NE/0064).

6.3.2 Inclusion/exclusion criteria

Dementia subjects were ≥60 years old and had a diagnosis of probable DLB or probable AD confirmed by two clinicians based on current diagnostic criteria (McKeith *et al.*, 2005; McKhann *et al.*, 2011), with an MMSE score ≥12. Subjects taking anti-cholinesterase drugs or memantine were stable for three months before baseline assessment. Control patients had an MMSE ≥26 and no signs of dementia.

Subjects were excluded if they had a major concurrent psychiatric illness; severe physical illness that would limit their ability to fully participate in the study; contraindications to PET imaging; history of other significant neurological illness including stroke; medications that may significantly interfere with cognitive testing (e.g. high dose benzodiazepines), previous experimental treatment with an amyloid-targeting agent or current treatment with any other investigational agent. AD and control patients were excluded if they had had a diagnosis of Parkinson's disease.

6.3.3 Baseline cognitive and clinical assessment

The clinical and neuropsychological assessments carried out in this study are listed in table 6.1. The assessment was identical to that carried out in the Prodromal Study (see

sections 4.3-4.7) with the following exceptions: The Bristol Activities of Daily Living Scale (BADLS) (Bucks *et al.*, 1996) was used in this study. This rates a person's ability to perform 20 daily tasks on a scale from 0 (able to perform task or not applicable) to 3 (unable to perform task). A maximum score of 60 represents the highest level of functional impairment. The Northeast Visual Hallucinations Inventory, Clinical Dementia Rating Scale, Epworth Sleepiness Scale and Mayo Sleep Questionnaire were not used in this study.

Table 6.1. Clinical and neuropsychological assessment in the amyloid imaging study

Clinical Assessment	Cognitive Assessment
Revised Unified Parkinson's disease Rating Scale Motor Sub-scale	Addenbrooke's Cognitive Examination - Revised
Neuropsychiatric Inventory	FAS Verbal Fluency
Geriatric Depression Scale	Trail-making Test A and B
Clinician Assessment of Fluctuations	Graded Naming Test
Dementia Cognitive Fluctuations Scale	Rey Auditory Verbal Learning Test
Functional Assessment	**Computerised Tests**
Instrumental Activities of Daily Living Scale	Simple and choice reaction time
Bristol Activities of Daily Living Scale	Digit vigilance
	Line angle discrimination
	Motion Task

6.3.4 Imaging

PET

Subjects were given a 370MBq intravenous injection of ^{18}F-Florbetapir (Amyvid) 30-50 minutes prior to scanning. Subjects were imaged using a PET-CT scanner in list mode (Siemens Biograph-40) for 15 minutes (3 x 5 minute frames), 30-50 minutes after injection. Images were reconstructed using iterative reconstruction (4 iterations, 16 subsets), with a 168x168 matrix size, 2.04x2.04mm pixel size, 3mm slice thickness, and 3mm post-reconstruction Gaussian filter. Attenuation correction was performed utilising CT scan data.

CT

CT scans were obtained immediately before the PET images. CT dose was minimised using the Siemens CAR Dose 4D protocol with 50mAsec target dose, 0.5sec gantry rotation time, 0.8mm beam pitch, 0.6mm slice thickness and scan duration of 19 seconds. Images were reconstructed with 3mm slice thickness to match the PET images. For subjects without MR imaging, CT scans were also reconstructed with a 1mm slice thickness for use as a background scan for visual rating.

MRI

MRI scans were performed in all subjects unless contraindicated. Scans were acquired on a 3T whole body MR scanner (Achieva scanner; Philips Medical Systems), with body coil transmission and eight channel head coil receiver. Images acquired included a 3D sagittal magnetisation-prepared rapid gradient echo (MPRAGE) sequence (repetition time 8.3ms, echo time 4.6ms, flip angle 8^{o}, inversion delay 1250 ms, imaging time 4.5mins). The sagittal acquisition matrix was 216x240, giving a voxel size of 1x1x1mm.

6.3.5 Image processing

MRI and PET images were analysed in NifTI format, using SPM 8 (www.fil.ion.ucl.ac.uk/spm/software/spm8/). Each image was manually repositioned

with the anterior commissure approximately at point 0,0,0. A mean PET image was obtained by co-registering the three 5 minute scans. This mean scan was then co-registered with the native space MRI. MRI images were segmented into white matter, grey matter and CSF. Grey and white matter images were smoothed using a 4mm full width half-maximum Gaussian kernel. Region of interest (ROI) maps were developed to mirror those used in the Alzheimer's Disease Neuroimaging Initiative (ADNI) (Landau and Juagust, 2014) using the MarsBar (www.marsbar.sourceforge.net) region map (Table 6.2) (Tzourio-Mazoyer *et al.*, 2002). ADNI regions were used because this study represents the largest cohort of Florbetapir PET images to date and so was felt to be the most robust validated dataset as a comparator. From this cohort thresholds for abnormal levels of amyloid deposition have been developed that can be easy transferred to other datasets such as this cohort.

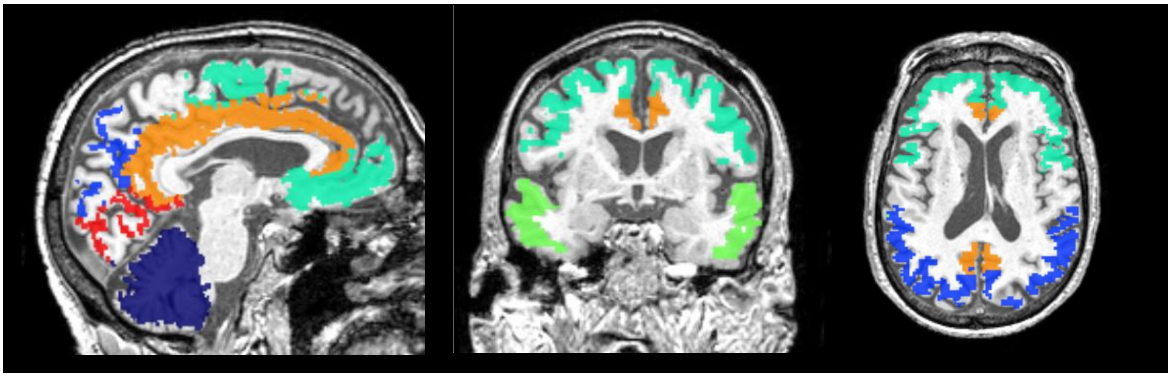

Figure 6.1. Regions of interest used in amyloid imaging. Frontal (teal), cingulate (orange), temporal (green), parietal (blue), occipital (red) and cerebellar (navy) areas are displayed.

In addition to the frontal, temporal, parietal, cingulate and cerebellar areas used in ADNI, striatal and occipital regions were also included (Figure 6.1). These maps were transformed into native space for each subject. Cortical and white matter areas within these regions were identified using a threshold of 0.5 on the smoothed grey and white matter segmentation images. Mean PET uptake values within the ROIs were then determined. Standardised uptake volume ratios (SUVR) were calculated for each region by dividing mean PET uptake in the region by mean PET uptake in the cerebellum. The cerebellum has been the usual reference region for cross-sectional data in ADNI (Jagust *et al.*, 2015). No partial volume correction was made as this has been found not to significantly change images (Knesaurek *et al.*, 2013).

Table 6.2. Cortical regions of interest and corresponding MarsBar regions

Region of interest	MarsBar Regions
Frontal lobe	Superior frontal gyrus
	Middle frontal gyrus
	Inferior frontal gyrus
	Supplementary motor area
	Paracentral lobule
	Gyrus rectus
	Olfactory cortex
Temporal lobe	Superior temporal gyrus
	Herschel gyrus
	Middle temporal gyrus
	Inferior temporal gyrus
Parietal lobe	Superior parietal gyrus
	Inferior parietal, but supramarginal and angular gyri
	Angular gyrus
	Supramarginal gyrus
	Precuneus (middle and superior areas)
Cingulate lobe	Anterior cingulate and paracingulate gyri
	Median cingulate and paracingulate gyri
	Posterior cingulate gyrus
	Precuneus (inferior area)
Occipital lobe	Superior occipital gyrus
	Middle occipital gyrus
	Inferior occipital gyrus
	Cuneus
	Calcarine fissure and surrounding cortex
	Lingual gyrus
	Fusiform gyrus

Hippocampal volumes were obtained from the MPRAGE scan using a fully-automated script in SPM which has been validated against manual tracing and used in many other studies (Firbank *et al.*, 2008). This script spatially normalises the MRI image to

standard space, following which grey matter pixels are identified within a predefined hippocampal region to segment the hippocampi.

6.3.6 Visual rating

Images were reviewed by 5 raters (John O'Brien, Michael Firbank, George Petrides, Jim Lloyd and Paul Donaghy), all of whom had completed training in amyloid image reading. Raters were blinded to all clinical data. Six scans were duplicated in the dataset to test intrarater reliability. Scans were classified as positive or negative based on the method developed by the manufacturer (Eli Lilly, 2012), summarised as follows. Images were viewed in transaxial orientation (with access to other views where necessary) in a black-white scale. The occipital, temporal, parietal and frontal areas were reviewed and classified as normal if the signal from the cortex was less than the signal from the white matter (clear grey white contrast) and abnormal where there was a reduction or loss of grey-white contrast, or where the signal from the grey matter was greater than the white matter. Subjects were classified as amyloid positive if there were two or more brain regions (each larger than a single gyrus) with reduced or absent grey-white contrast or one area in which grey matter activity clearly exceeded white matter (Figure 6.2). All scans were rated independently, following which a consensus meeting was held to discuss those scans in which there was disagreement (defined as a 3/2 split) on whether the scan was positive or negative and a final decision reached.

6.3.7 Statistics

Statistical analysis was completed using IBM SPSS Statistics 22 software. Normality was tested using the Shapiro-Wilk test. Comparisons between diagnostic groups were carried out using one-way ANOVA or Kruskall-Wallace tests depending on normality of the data. Where significant, post hoc pairwise comparisons were carried out using the Tukey's Test or Mann-Whitney U tests with a Bonferroni correction (α=0.05). Comparisons between amyloid positive and negative DLB cases were carried out using t-tests or Mann-Whitney U tests. χ^2 or Fischer's Exact tests were used for categorical variables depending on cell size.

Correlation was carried out using Pearson's r (for normally distributed data) or Kendall's tau (for non-normal data) correlation. Linear regression was used to adjust for

the effect of age and years in education on specific variables when correlation was observed. Interrater and intrarater reliability in amyloid PET scan visual rating were assessed using the Kappa statistic. A mean of all pair-wise kappa values was used to summarise agreement between the five raters. Unless otherwise stated, a p value of ≤0.05 was set as the threshold for statistical significance, uncorrected for the number of statistical tests performed.

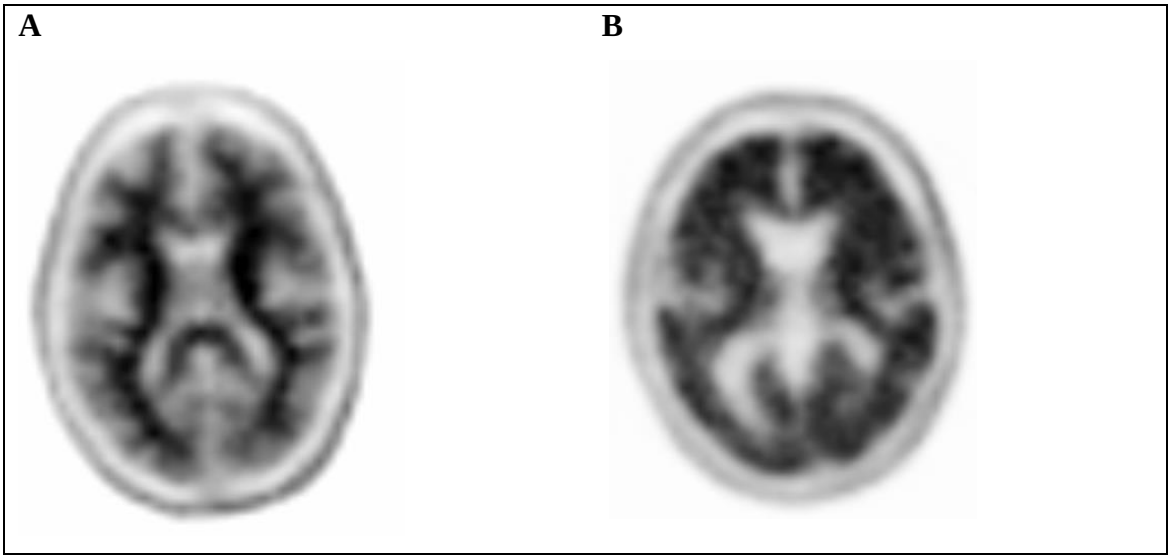

Figure 6.2. Examples of negative (A) and positive (B) amyloid PET imaging scans. **A** displays the normal contrast between high white matter binding (dark) and low grey matter binding (light) seen in controls and some DLB patients. **B** shows loss of grey-white contrast in a subject with AD.

The primary aim of this study is to compare amyloid binding in DLB subjects with AD and controls. Sample sizes of DLB (n=20), control (n=10) and AD (n=10) would be sufficient to detect differences with an effect size of >1.1 with statistical power of 0.8. The difference between these diseases in Florbetapir binding is not yet known. However, using the effect sizes reported with another ^{18}F-labelled ligand, Florbetaben, this study would be powered to find differences between AD and DLB (effect size = 1.8) but perhaps not differences between DLB and controls (effect size = 0.8) (Villemagne *et al.*, 2011). This sample size would have a power of 0.78 to detect differences in the proportion of amyloid positive scans between DLB and AD given the expected rates of 50% in DLB, 90% in AD and 25% in controls (Ossenkoppele *et al.*,

2015). This sample size would not be sufficient to reliably detect the expected difference between DLB and controls (power=0.29).

Comparisons will be made between amyloid positive and negative DLB cases. A sample size of 20 (n=10 in each group) would be sufficient to detect large differences between the groups (effect size >1.3) with statistical power of 0.8.

Chapter 7 Amyloid Imaging Results and Discussion

7.1 Recruitment

A large number of clinical notes were screened to identify patients with DLB and AD. 141 dementia patients were approached to participate in the study, of whom 40 agreed to enter. Four patients scored below the threshold for MMSE, another patient was found to have an atypical presentation of AD and was excluded. One patient had a major stroke and another withdrew before completing baseline. One patient did not tolerate PET imaging. In total, 32 subjects with dementia (22 DLB and 10 AD) completed baseline amyloid PET imaging, 29 of them also had an MRI scan. Two of the DLB participants were unable to have an MRI due to the presence of pacemakers. One AD participant failed to tolerate MR imaging. Subjects without MR imaging were included in the visual rating but not in the quantification of amyloid PET images. 15 control subjects also completed baseline PET and MRI imaging successfully.

7.2 Comparison of DLB, AD and controls

7.2.1 Demographics

Table 7.1. Demographics of DLB, AD and controls

	Control	AD	DLB	
	Mean (SD)	Mean (SD)	Mean (SD)	p
n	15	10	22	-
Age	75.2 (6.9)	75.9 (5.2)	76.5 (6.4)	0.84
Gender (% female)	27	20	18	0.70
Years in education	12.4 (3.0)	11.7 (2.8)	11.0 (3.2)	0.47
MMSE	29.0 (1.1)	20.2 (5.0)	20.3 (4.7)	$<0.001^{a,c}$
CIRS-G	6.3 (3.5)	7.0 (3.1)	11.2 (4.0)	$0.001^{a,b}$
CIRS-G Neurological	0.2 (0.8)	0 (0.0)	1.1 (1.2)	$0.003^{a,b}$
CIRS-G Heart + Vascular	2.2 (1.3)	0.9 (1.2)	2.0 (1.8)	0.09
AChI/Memantine (%)	0	100	100	-
Levodopa (%)	0	0	55	-

a = significant difference DLB v control; *b* = significant difference DLB v AD; *c* = significant difference AD v control. Post-hoc Tukey's Test, α=0.05.

AChI=acetylcholinesterase inhibitor.

Demographic variables are shown in Table 7.1. There were no significant differences between control, AD and DLB subjects in age, gender or number of years in education. There was no difference between AD and DLB cases in overall cognitive function measured by the MMSE. DLB cases scored higher on the CIRS-G scale of co-morbidity. There was no significant difference in CIRS-G heart + vascular subscores. As expected, the DLB group scored higher than AD or controls in the neurological subscore (which included parkinsonism). All dementia patients were taking a cholinesterase inhibitor or memantine. 55% of DLB patients were on levodopa treatment for parkinsonism.

7.2.2 Cognitive tests

2 AD and 12 DLB failed to complete Trails A; 7 AD and 18 DLB failed to complete trails B. Therefore trails data was not analysed. One AD and one DLB subject did not wish to complete the Rey AVLT.

DLB subjects scored significantly worse than controls on every test except Rey AVLT recognition (DLB 11.4 v Control 12.9; p=0.31). AD subjects scored worse than controls on every test except the FAS, and in this test the difference was only marginally above the threshold for significance (p=0.051).

Overall cognitive function was very similar between AD and DLB (mean ACE-R = 60.3 v 59.7; p=0.99). DLB scored higher than AD in the ACE-R memory domain, and approached a significantly higher score in the Rey AVLT recognition test (11.4 v 7.4; p=0.06). There were no other differences between the two groups in any other cognitive test (Table 7.2).

7.2.3 Computerised tests of cognitive function

Nine patients did not have angle task data due to a computer error (4 DLB, 2 AD, 3 controls). A further 5 DLB subjects were unable to complete this task. One AD and four DLB subjects could not complete the digit vigilance task. Eight DLB subjects could not complete the motion task. One DLB subject did not complete the reaction time task.

Table 7.2. Cognitive tests in DLB, AD and controls

	Control		AD		DLB		
	Mean	(SD)	Mean	(SD)	Mean	(SD)	p
ACE-R Total	94.7	(2.8)	60.3	(17.8)	59.7	(16.7)	$<0.001^{a,c}$
ACE-R Attention/Orientation	18.0	(0.0)	12.7	(4.0)	12.6	(3.40	$<0.001^{a,c}$
ACE-R Memory	23.6	(1.9)	7.7	(3.7)	12.1	(5.7)	$<0.001^{a,b,c}$
ACE-R Fluency	12.0	(1.5)	6.4	(3.9)	5.6	(3.2)	$<0.001^{a,c}$
ACE-R Language	25.4	(0.9)	21.6	(5.0)	21.1	(3.3)	$<0.001^{a,c}$
ACE-R Visuospatial	15.7	(0.6)	11.9	(3.7)	8.8	(3.7)	$<0.001^{a,c}$
Rey AVLT Trial 1	5.7	(1.7)	1.8	(1.6)	2.2	(2.3)	$<0.001^{a,c}$
Rey AVLT Trial 6	9.1	(2.7)	0.4	(1.3)	2.2	(2.3)	$<0.001^{a,c}$
Rey AVLT Delay	8.3	(2.7)	0.4	(0.7)	1.6	(2.1)	$<0.001^{a,c}$
Rey AVLT Recognition	12.9	(1.8)	7.4	(4.2)	11.4	(2.4)	$<0.01^{c}$
Verbal Fluency (FAS) score	50.1	(12.8)	29.8	(16.4)	21.6	(15.1)	$<0.001^{a}$
Graded Naming Test	24.1	(2.3)	14.0	(7.8)	13.9	(7.9)	$<0.001^{a,c}$

a = significant difference DLB v control; *b* = significant difference DLB v AD; *c* = significant difference AD v control. Post-hoc Tukey's Test, α=0.05.

DLB subjects were slower and showed greater variability in reaction times in the simple reaction time (SRT), choice reaction time (CRT) and digit vigilance tasks and overall power of attention compared with controls (Table 7.3). They also demonstrated worse visuospatial function on the angle discrimination and motion tasks. There were no significant differences between DLB and AD subjects in any test, though in every measurement apart from CRT errors and cognitive processing time their scores were worse than AD. This difference approached significance in the digit vigilance task (digit vigilance number correct DLB=21.2, AD=30.9, p=0.08; digit vigilance time DLB=736ms, AD=587ms, p=0.09).

Two DLB subjects had a high false positive rates in the digit vigilance task (>72), but excluding these subjects did not change the overall result. Four DLB subjects had a negative cognitive processing time, suggesting a learning effect following the SRT task resulting in faster responses to the CRT task than the SRT task.

Table 7.3. Computer tests in DLB, AD and controls

	Control		AD		DLB		
	Mean	(SD)	Mean	(SD)	Mean	(SD)	p
SRT (ms)	341	(53)	424	(148)	767	(567)	<0.01[a]
SRT SD (ms)	67	(29)	208	(218)	569	(693)	<0.001[a,c]
SRT number correct	29.6	(0.8)	29.0	(0.9)	27.2	(3.6)	0.02[a]
CRT (ms)	564	(68)	962	(466)	1105	(537)	<0.001[a,c]
CRT SD (ms)	107	(36)	322	(212)	527	(479)	<0.001[a,c]
CRT errors	1.6	(1.8)	6.2	(7.8)	5.4	(4.7)	0.03[a,c]
Cognitive Processing Time (ms)	223	(63)	539	(404)	338	(442)	0.03[c]
Digit vigilance number correct	35.5	(1.0)	30.9	(5.3)	21.2	(7.4)	<0.001[a,c]
Digit vigilance time (ms)	516	(54)	587	(111)	736	(183)	<0.001[a]
Digit vigilance SD (ms)	69	(33)	172	(127)	258	(130)	<0.001[a,c]
Digit vigilance false positives	1.1	(1.1)	6.0	(4.8)	19.9	(30.1)	<0.001[a,c]
Power of attention (ms)	1421	(143)	1848	(519)	2604	(822)	<0.001[a]
Angle Task (^{o})	8.4	(3.3)	25.5	(22.4)	43.3	(31.6)	0.001[a]
Motion Task	0.56	(0.30)	0.80	(0.27)	0.96	(0.07)	<0.01[a]

SRT =simple reaction time, CRT = choice reaction time. *a* = significant difference DLB v control; *b* = significant difference DLB v AD; *c* = significant difference AD v control. Post-hoc Tukey's Test, α=0.05.

7.2.4 Symptom scales

One DLB participant did not have carer distress scores for the NPI as he lived alone. Other questionnaires were filled out by a member of day hospital staff who knew him well.

Unsurprisingly DLB subjects scored higher than AD subjects in the CAF and DCFS fluctuations scales, and also in the Geriatric Depression Scale (Table 7.4). DLB cases scored higher in the NPI hallucination domain, and approached higher scores in the depression domain. No other NPI domain approached significant difference (p>0.2). DLB cases had greater functional impairment measured by the IADL and BADL scales.

Table 7.4. Symptom scales in DLB, AD and controls

	AD		DLB		
	Mean	(SD)	Mean	(SD)	p
CAF	1.4	(3.0)	6.5	(4.6)	<0.01
DCFS total	7.0	(3.2)	11.7	(3.3)	<0.01
Geriatric depression scale	1.4	(1.2)	4.4	(2.2)	<0.001
NPI total hallucinations	0.0	(0.0)	2.8	(2.9)	0.001
NPI total depression	0.2	(0.4)	1.6	(1.8)	0.06
NPI total	10.2	(8.8)	19.3	(16.2)	0.11
NPI distress total	4.7	(5.0)	8.1	(9.0)	0.35
BADL	11.8	(7.6)	21.7	(12.1)	0.03
IADL	4.4	(1.4)	2.9	(1.9)	0.04

7.2.5 Parkinsonism, autonomic and enteric function

Table 7.5. Parkinsonism, orthostatic BP changes and gastroparesis in DLB, AD and controls

	Control		AD		DLB		
	Mean	(SD)	Mean	(SD)	Mean	(SD)	p
MDS-UPDRS	5.3	(3.1)	12.9	(4.8)	47.5	(18.5)	<0.001[a,b]
Hoehn and Yahr Stage	0		0		2.2	(1.3)	<0.001[a,b]
6-point UPDRS	0.8	(0.4)	2.0	(1.5)	7.9	(3.6)	<0.001[a,b]
Systolic BP change (mmHg)	7.0	(17.6)	8.9	(19.6)	-11.7	(23.3)	0.01[a,b]
Diastolic BP change (mmHg)	9.0	(8.2)	8.1	(11.2)	-0.6	(10.5)	0.02[a]
GCSI	0.19	(0.36)	0.25	(0.44)	0.41	(0.46)	0.10

(*a* = significant difference DLB v control; *b* = significant difference DLB v AD; *c* = significant difference AD v control. Post-hoc Tukey's Test, α=0.05)

Three DLB patients did not have postural BP checked as they were unable to stand. As expected DLB cases had higher score in the UPDRS, 6-point UPDRS and Hoehn & Yahr Scale (Table 7.5). They had a significantly greater fall in systolic BP than AD and controls, and a significantly greater fall in diastolic BP than controls. There were no

differences between the groups in GCSI scores. Only one patient in the entire cohort scored above the threshold for abnormality on the GCSI.

7.3 Imaging results

7.3.1 Visual rating

There was absolute consensus between all five raters in 35/53 scans and majority read (4:1) in 9, with only 9 scans needing discussion at the consensus meeting. The mean kappa in the group was 0.65, indicating a good level interrater reliability. Intrarater reliability was also good, with a kappa of 0.66.

All AD subjects scans were visually rated as amyloid positive, a significantly greater proportion than DLB (46%; $p<0.01$; n=32, Fisher's Exact Test)) and control subjects (20%; $p<0.001$; n=25, Fisher's Exact Test). The difference between DLB and controls was not significant ($p=0.11$; $\chi^2=2.54$; df=1, n=37).

7.3.2 *Semiquantification of amyloid PET scans*

Table 7.6. Regional amyloid binding relative to cerebellum in DLB, AD and controls

	Control		AD		DLB		
	Mean	(SD)	Mean	(SD)	Mean	(SD)	p
Parietal	1.12	0.17	1.42	0.13	1.23	0.21	$<0.01^{c}$
Frontal	1.13	0.18	1.50	0.19	1.24	0.22	$<0.01^{b,c}$
Temporal	1.13	0.15	1.44	0.19	1.23	0.20	$<0.01^{b,c}$
Cingulate	1.20	0.22	1.55	0.19	1.31	0.24	$<0.01^{b,c}$
Occipital	1.10	0.12	1.30	0.15	1.18	0.15	$<0.01^{c}$
Striatal	1.13	0.13	1.41	0.18	1.19	0.16	$<0.01^{b,c}$
Mean Cortical	1.14	0.18	1.48	0.17	1.25	0.21	$<0.01^{c}$
Visual Rating positive (%)	20		100		46		$<0.001^{b,c}$
APOE ε4 allele present (%)	33		90		68		$0.01^{a,c}$
Hippocampal Volume (mL)	2.75	0.35	1.59	0.50	2.25	0.55	$<0.001^{a,c}$

a = significant difference DLB v control; *b* = significant difference DLB v AD; *c* = significant difference AD v control. Post-hoc Tukey's Test, α=0.05

There were no significant differences in normalised amyloid binding in any region between DLB and controls (Table 7.6; Figure 7.1). AD cases had greater binding than controls in all regions, and greater binding than DLB in frontal, temporal and cingulate cortices and the striatum. Mean cortical binding (p=0.053) and parietal binding (p=0.06) showed a trend approaching statistical significance, being greater in AD than DLB.

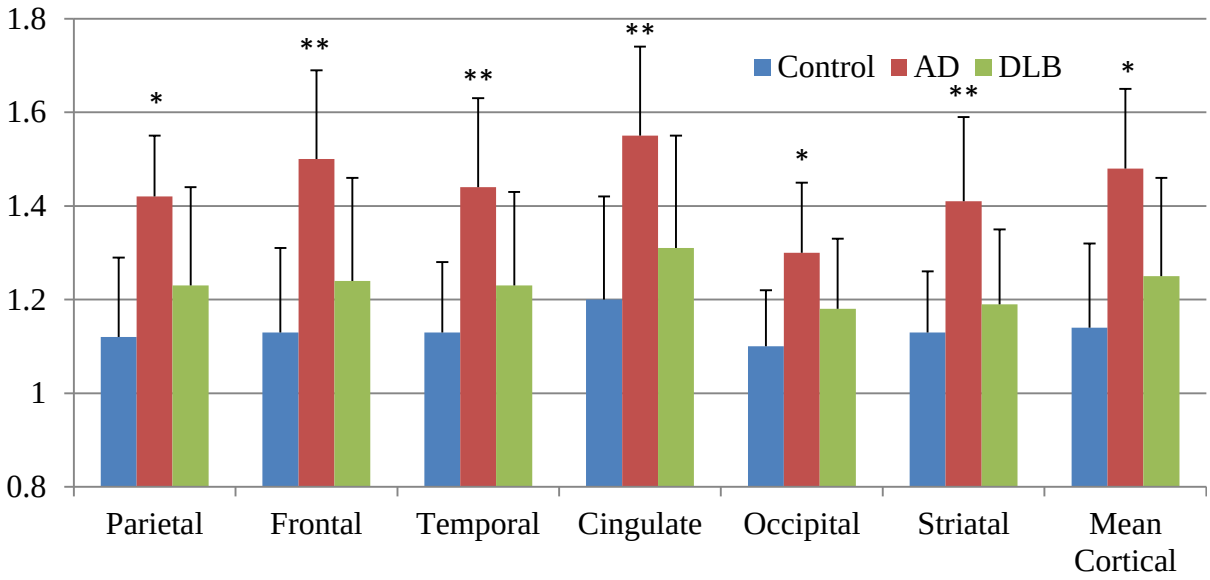

Figure 7.1. Amyloid ligand binding in six brain regions and overall cortical binding (normalised to whole cerebellum reference region) in DLB, AD and controls. Error bars represent standard deviation. (*=AD>control; **=AD>DLB & control; p<0.05)

The APOE ε4 allele was more common in DLB (68%) and AD (91%) than control subjects (33%; DLB v control p=0.04, χ^2=4.36, df=1, n=37; AD v control p=0.01, n=25, Fisher's Exact Test). The difference between AD and DLB was not statistically significant (p=0.38, n=32, Fisher' Exact Test). Across the entire cohort, presence of the APOE ε4 allele was associated with positive visual rating of the amyloid PET scan (83% v 42%; p=0.004; χ^2=8.33; df=1, n=47).

DLB and AD had smaller hippocampi than controls, DLB approached having significantly larger hippocampi than AD (2.25 v 2.75mL; p=0.06; Table 7.6).

7.3.3 Pattern of amyloid deposition

Table 7.7. Regional amyloid binding relative to mean cortical binding in DLB, AD and controls

	Control		AD		DLB		
	Mean	(SD)	Mean	(SD)	Mean	(SD)	p
Relative parietal	0.98	0.03	0.97	0.04	0.98	0.05	0.51
Relative frontal	0.99	0.02	1.01	0.02	0.99	0.03	0.07
Relative temporal	0.99	0.03	0.97	0.02	0.99	0.04	0.40
Relative cingulate	1.04	0.04	1.05	0.03	1.04	0.03	0.81
Relative occipital	0.97	0.07	0.88	0.07	0.95	0.07	0.01[c]
Relative striatal	1.00	0.07	0.95	0.07	0.96	0.09	0.34
Frontal:Occipital Ratio	1.02	0.08	1.15	0.12	1.05	0.09	0.01[b,c]

a = significant difference DLB v control; *b* = significant difference DLB v AD; *c* = significant difference AD v control. Post-hoc Tukey's Test, α=0.05

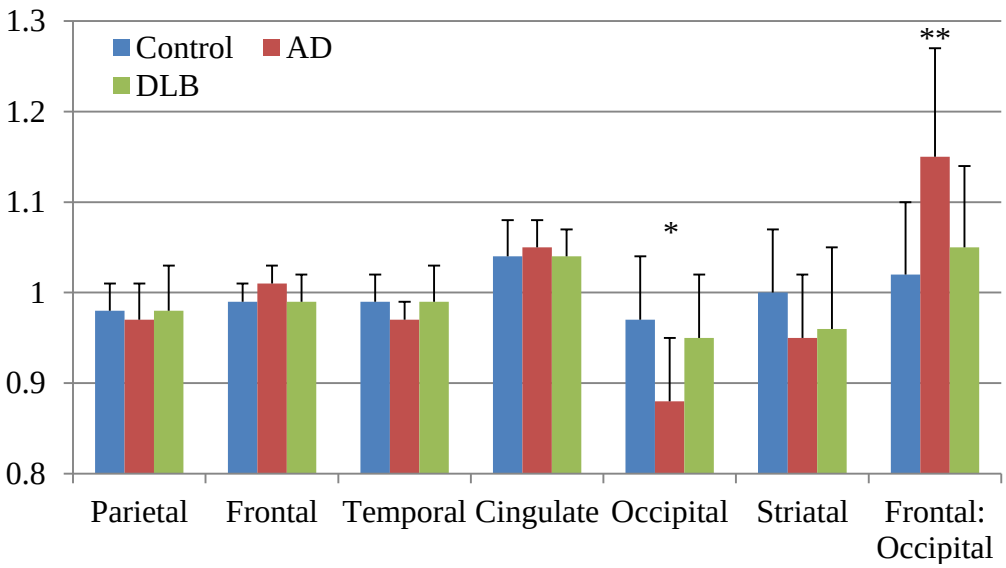

Figure 7.2. Relative amyloid ligand binding in six brain regions relative to overall cortical binding, and frontal:occipital binding ratio in DLB, AD and controls. Error bars represent standard deviation (* = significant AD v control; ** = significant AD v DLB & control; $p<0.05$).

To compare the pattern of amyloid deposition across disease categories binding in each region was compared to overall mean cortical binding in that individual (Table 7.7, Figure 7.2). Relative occipital binding differed across groups, being significantly lower in AD compared with controls (p=0.01) and approaching significance compared with DLB (p=0.06). In a post-hoc analysis frontal:occipital ratio was found to be greater in AD than DLB or controls (AD v DLB p=0.03; AD v controls p=0.01). When only amyloid positive DLB cases were included the difference was not statistically significant (DLB 1.09 v AD 1.15; p=0.21).

7.3.4 Comparison of visual rating and quantification

A comparison of visual rating and quantification can be seen in figure 7.3. Scans with a cortical SUVR>1.11 are classed as amyloid positive in ADNI (Landau and Juagust, 2014). When this was compared with visual rating there was agreement in 36/44 cases (kappa 0.64; p<0.001). Seven subjects visually rated as negative were above the ADNI-defined SUVR threshold of 1.11 (4 DLB, 3 controls). One DLB subject was visually rated as positive but had a cortical SUVR below threshold.

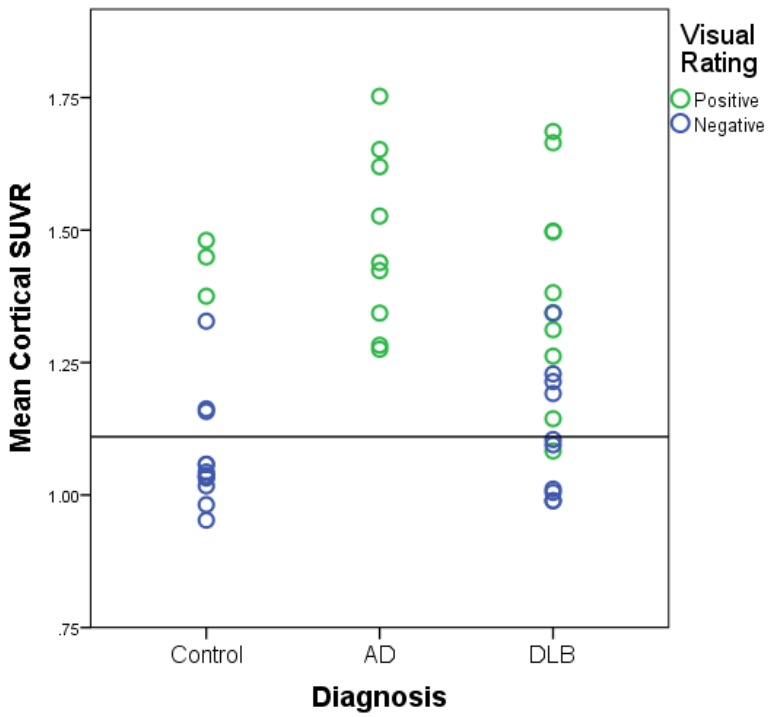

Figure 7.3. A comparison of visual rating and quantification of amyloid imaging. The horizontal line reflects the ADNI-defined cut off of 1.11.

7.4 Comparison of amyloid positive and negative DLB cases based on visual rating

7.4.1 Demographics

There were no differences between amyloid negative and amyloid positive DLB subjects in age, gender, overall cognitive function, prescription of levodopa or presence of the APOE ε4 gene (Table 7.8). Amyloid positive cases had significantly more years in education (12.7 v 9.5; p=0.01). There were no significant differences in duration of illness measured by time since diagnosis or duration of memory impairment. Two DLB subjects were unable to give a date of onset of memory problems, and did not have a carer that was able to do so.

Table 7.8. Demographics and duration of illness in amyloid positive and negative DLB subjects

	Amyloid negative	Amyloid positive	
	Mean (SD)	Mean (SD)	p
n	12	10	-
Age	75.8 (6.3)	77.2 (6.7)	0.63
Gender (% female)	17	20	1.00
Years in education	9.5 (2.5)	12.7 (3.2)	**0.01**
MMSE	19.6 (3.6)	21.2 (5.9)	0.46
CIRS-G	11.6 (4.4)	10.8 (3.7)	0.87
AChI/Memantine (%)	100	100	na
Levodopa (%)	50	60	0.69
Time since diagnosis (months)	31.3 (26.1)	20.8 (13.7)	0.27
Duration memory impairment (months)	71.6 (43.8)	62.4 (55.9)	0.41
APOE ε4 Allele Present (%)	58	80	0.38

AChI=acetylcholinesterase inhibitor

7.4.2 Cognitive tests

There were no differences between the two groups in any ACE-R cognitive domain, memory measured by the Rey AVLT or graded naming (Table 7.9). The amyloid positive group scored significantly higher on the FAS verbal fluency test (28.0 v 16.3;

p=0.02). The FAS test is known to vary according to educational level (Tombaugh *et al.*, 1999). When multiple linear regression was used with years in education and the presence or absence of amyloid as independent variables there was no effect of amyloid imaging result on FAS score (β=0.16; p=0.48), though years in education approached having a significant effect (β=0.46; p=0.051).

Table 7.9. Cognitive tests in amyloid positive and negative DLB subjects

	Amyloid negative		Amyloid positive		
	Mean	(SD)	Mean	(SD)	p
ACE-R Total	57.7	(14.8)	62.1	(19.3)	0.55
ACE-R Attention/Orientation	11.8	(2.8)	13.7	(4.0)	0.19
ACE-R Memory	12.4	(5.5)	11.8	(6.2)	0.81
ACE-R Fluency	4.8	(2.8)	6.5	(3.6)	0.22
ACE-R Language	20.7	(3.7)	21.5	(2.8)	0.92
ACE-R Visuospatial	8.9	(2.6)	8.6	(4.9)	0.86
Rey AVLT Trial 1	1.9	(1.9)	2.6	(2.8)	0.51
Rey AVLT Trial 6	2.3	(1.9)	2.0	(2.8)	0.50
Rey AVLT Delay	1.3	(1.4)	1.9	(2.8)	1.00
Rey AVLT Recognition	11.2	(2.7)	11.8	(1.9)	0.57
Verbal Fluency (FAS) score	16.3	(13.7)	28.0	(14.8)	**0.02**
Graded Naming Test	13.8	(8.2)	14.1	(8.1)	0.92

*Data uncorrected for years in education

7.4.3 Computerised tests of cognitive function

Amyloid negative subjects had much slower simple reaction times than amyloid positive (998ms v 473ms; p=0.049; Table 7.10). Variation in reaction time approached significance (837ms v 211ms; p=0.06). There were no differences in the digit vigilance task, a very similar test to the SRT, and no differences in the choice reaction task, overall power of attention or the angle discrimination task. Both groups had poor scores in the motion task, particularly the amyloid negative group (amyloid negative 0.99 v amyloid positive 0.90; p=0.10).

Given the association of education level with cognitive test scores linear regression was carried out with amyloid imaging result and years in education as independent variable for each cognitive and computerised tests in tables 7.9 and 7.10. Only digit vigilance false positive rate (β=0.57; p=0.04) showed a significant effect of amyloid deposition. The ACE-R memory subdomain (β=-0.41; p=0.06) and cognitive processing time (β=0.46; p=0.08) showed a trend towards significance.

Table 7.10. Computer tests in amyloid positive and negative DLB subjects

	Amyloid negative		Amyloid positive		
	Mean	(SD)	Mean	(SD)	p
SRT (ms)	988	652	473	220	**0.05**
SRT SD (ms)	837	792	211	294	0.06
SRT number correct	26.5	4.3	28.2	2.5	0.51
CRT (ms)	1246	628	918	334	0.19
CRT SD (ms)	592	552	440	377	0.65
CRT errors	5.0	5.3	6.0	4.0	0.46
Cognitive Processing Time (ms)	258	515	445	319	0.35
Digit vigilance number correct	18.1	7.8	23.6	6.4	0.12
Digit vigilance time (ms)	751	172	724	200	0.70
Digit vigilance SD (ms)	241	144	271	124	0.63
Digit vigilance false positives	7.0	7.4	30.2	37.5	0.06
Power of attention (ms)	2727	(973)	2116	(571)	0.13
Angle Task (^{o})	49.1	34.4	36.6	29.7	0.50
Motion Task	0.99	0.02	0.92	0.09	0.10

*Data uncorrected for years in education. SRT =simple reaction time, CRT = choice reaction time.

7.4.4 Symptom scales

There were no differences between the groups in scores on the fluctuation or depression scales (Table 7.11). There was also no difference in NPI overall score, any NPI subdomain, NPI distress score or functional impairment measured by the BADL or IADL scales.

Table 7.11. Symptom scales in amyloid positive and negative DLB subjects

	Amyloid negative		Amyloid positive		
	Mean	(SD)	Mean	(SD)	p
CAF	5.8	(4.5)	7.4	(4.9)	0.38
DCFS total	12.4	(3.3)	10.9	(3.3)	0.30
Geriatric depression scale	4.6	(2.7)	4.2	(1.5)	0.68
NPI total hallucinations	2.8	(2.7)	2.7	(3.2)	0.72
NPI total depression	1.4	(1.6)	1.7	(2.2)	1.00
NPI total	17.0	(9.3)	22.1	(22.1)	0.77
NPI distress total	5.8	(6.4)	11.1	(11.3)	0.35
BADL	22.8	(12.3)	20.3	(12.4)	0.77
IADL	2.9	(1.9)	2.9	(2.0)	0.98

7.4.5 Parkinsonism, autonomic and enteric function

There were no differences between the groups in levels of parkinsonism or orthostatic blood pressure changes (Table 7.12). The amyloid negative group approached having more symptoms of gastroparesis but as previously mentioned only one patient had a clinically significant score.

Table 7.12. UPDRS, orthostatic blood pressure changes and GCSI in amyloid positive and negative DLB subjects

	Amyloid negative		Amyloid positive		
	Mean	(SD)	Mean	(SD)	p
MDS-UPDRS	45.3	(15.2)	50.1	(22.3)	0.56
Hoehn and Yahr Stage	2.1	(1.4)	2.3	(1.2)	0.92
6-point UPDRS	7.0	(2.8)	8.9	(4.3)	0.23
Systolic BP change (mmHg)	-8.9	(23.5)	-14.9	(24.0)	0.59
Diastolic BP change (mmHg)	1.5	(9.0)	-2.9	(12.0)	0.37
GCSI	0.57	(0.56)	0.22	(0.21)	0.06

7.4.6 Hippocampal volume

There was no difference in hippocampal volume between the amyloid positive and negative groups (positive 2.25 v negative 2.26 mL; p=0.96). Mean cortical amyloid was inversely correlated with hippocampal volume in the DLB group (Pearson's r=-0.573; p=0.008) but not AD (r=0.06; p=0.87) or controls (r=0.38; p=0.16). This association did not survive linear regression with age as an independent variable (β=-0.22; p=0.25).

7.5 Correlation of amyloid binding with clinical picture in DLB

We tested correlations between amyloid binding and clinical phenotype that had previously been reported. These analyses are based on correlations within DLB subjects only.

7.5.1 Overall cognitive function and cortical or striatal binding

There was no correlation between mean cortical or striatal binding and overall cognitive function measured by the MMSE or ACE-R, or functional impairment measured by the BADL and IADL scales (p>0.3 for all correlations).

7.5.2 Memory

Higher mean cortical amyloid binding was associated with lower ACE-R retrograde memory scores (questions on current and past UK Prime Ministers and USA Presidents) (Kendall's tau = -0.38; p=0.03). This association was strengthened after linear regression with age and years in education (β=-0.58; p=0.01). Correlation between cortical binding and overall ACE-R memory subscore approached significance (Pearson's r=-0.418; p=0.07), indicating a trend towards lower memory scores in amyloid positive subjects. When entered into regression with age and years in education this association was significant (β=-0.50; p=0.02). There was no significant correlation between amyloid and memory assessed by the Rey AVLT delayed recall or recognition.

7.5.3 Parkinsonism and striatal binding

There was a positive correlation between striatal amyloid binding and 6-point UPDRS score (Pearson's r=0.48; p=0.03), this correlation held after linear regression with age (Coefficient=0.61; p=0.008). There was no relationship between striatal binding and total UPDRS score (Pearson's r=0.25; p=0.28) or Hoehn & Yahr Stage (Kendall's tau=-0.13; p=0.47). Contrary to a previous report (Gomperts *et al.*, 2008), relative striatal binding did not show any correlation with parkinsonism, (UPDRS: Pearson's r=0.20; p=0.41).

7.5.4 Visuospatial function and parietal, cingulate, occipital binding

There was a negative correlation between scores on the visuospatial subdomain of the ACE-R and relative cingulate binding (Pearson's r=-0.49; p=0.03) but a positive correlation with relative parietal binding (Pearson's r=0.47; p=0.04). Only the positive correlation with parietal binding held after regression with age and years in education (p=0.01). There was no significant relationship between the computerised tasks of visual function and cortical binding in the parietal, cingulate or occipital areas.

7.6 Discussion

7.6.1 Clinical and neuropsychological characteristics of participants

DLB and AD groups were well matched for age, gender, level of education and overall cognitive function. DLB subjects performed better than those with AD in the ACE-R memory sub-score, but there were no other significant differences between DLB and AD in any other cognitive test. This was likely due to the relatively low numbers in the cohort, particularly of AD patients. Although not significant, the DLB group had worse visuospatial function and attention in almost every test of these domains. The Trail-making Test was not effective in measuring executive function in this cohort due to a marked floor-effect. Only 22% of the dementia cases were able to successfully complete Trails B.

The DLB group scored higher in scales measuring the severity of core features of DLB: hallucinations, fluctuations and parkinsonism. This is unsurprising, as these patients are diagnosed as DLB based on the presence of these symptoms. Depression is a supportive

feature of DLB in the consensus diagnostic criteria (McKeith *et al.*, 2005), and the DLB group were found to have higher GDS scores than the AD group.

7.6.2 APOE genotype

A recent large genetic study including 667 pathologically confirmed DLB cases confirmed APOE genotype as a strong risk factor in DLB (Bras *et al.*, 2014). As has previously been reported, the APOE ε4 genotype was more common in AD and DLB than controls (Kobayashi *et al.*, 2011), though the rates here were higher in all groups than previously reported. Kobayashi et al. and a recent meta-analysis of amyloid imaging (Ossenkoppele *et al.*, 2015) found the APOE ε4 allele in 40-50% of DLB cases compared with 68% in this cohort. This may be partly explained by the higher APOE ε4 allele frequencies observed in more northern parts of Europe compared to southern Europe (Lucotte *et al.*, 1997) but selection bias may be a more important factor. Anecdotally we notice that participants are often motivated to take part in research because other family members have suffered from dementia. Therefore we may be more likely to recruit people with a genetic predisposition towards dementia, such as the APOE ε4 allele.

7.6.3 Hypothesis 1: The proportion of DLB patients with positive amyloid scans on visual rating will be greater than controls and less than AD.

When amyloid PET scans were visually rated 100% of AD cases were amyloid positive, compared with 46% of DLB and 20% of controls. These figures are consistent with a recent meta-analysis that found 88% of AD, 51% of DLB and 24% of control cases to be amyloid positive (Ossenkoppele *et al.*, 2015). The relatively even division of the DLB group into two halves enabled a comparison to be made between amyloid positive and amyloid negative cases.

Carriers of the APOE ε4 allele were more likely to be amyloid positive across the whole cohort, but there was no effect of APOE ε4 in controls or DLB examined independently. The association between APOE ε4 and DLB may be related to the association of APOE ε4 with amyloid deposition, or the promotion of αSyn aggregation by APOE (Verghese *et al.*, 2011).

7.6.4 Hypothesis 2: Semiquantitative measures of amyloid deposition will be greater in DLB than controls and less than AD.

AD cases displayed the highest amyloid deposition with a mean cortical amyloid burden significantly greater than controls and approaching significantly greater than DLB. AD had greater amyloid deposition than controls in all brain areas assessed, and greater than DLB in frontal, temporal, cingulate and striatal regions. There was no difference between DLB and controls in any region, but DLB scored intermediate between AD and controls in every measure.

The majority of previous studies have found significantly greater mean cortical binding in AD than DLB (Rowe *et al.*, 2007; Villemagne *et al.*, 2011; Kantarci *et al.*, 2012b). One study found differences only in the parietal and posterior cingulate regions (Siderowf *et al.*, 2014) and another found no differences (Gomperts *et al.*, 2008). However these studies had relatively low numbers of DLB cases (n=11, and 8 respectively) and in one study the DLB group was nine years older than the AD group (Gomperts *et al.*, 2008). Similarly, amyloid binding has been found to be greater in DLB than controls in some studies (Edison *et al.*, 2008; Gomperts *et al.*, 2008; Gomperts *et al.*, 2012; Kantarci *et al.*, 2012b), with no significant differences reported in smaller studies (n=6-11 DLB subjects) (Foster *et al.*, 2010; Villemagne *et al.*, 2011; Siderowf *et al.*, 2014).

This study used ^{18}F-Florbetapir, whereas previous studies have used ^{11}C-PiB with one exception (Villemagne *et al.*, 2011). The signal to noise ratio is greater in PiB than in Florbetapir (Landau *et al.*, 2014), this may explain the lack of statistical significance between DLB and controls in our findings despite the relatively large sample size compared with previous studies. Nevertheless we found amyloid deposition in DLB to be intermediate between AD and controls, which is consistent with previous research.

Though amyloid binding was numerically higher in AD than controls and DLB in every cortical area, when regional binding was normalised to overall cortical binding, AD cases were found to have relatively low occipital binding. This has previously been reported in one amyloid imaging study (Gomperts *et al.*, 2008) and is consistent with pathological findings or relatively low occipital Aβ load in AD compared with other cortical areas (Walker *et al.*, 2015a). We calculated the frontal:occipital ratio for each subject and found that this was significantly higher in AD than DLB or controls. This did not hold when only amyloid positive DLB subjects were compared with AD,

suggesting that where amyloid deposition occurs in DLB the pattern is similar to that in AD. This is consistent with previous studies that have found similar patterns of deposition in amyloid positive DLB and AD (Kantarci *et al.*, 2012b; Shimada *et al.*, 2013).

The distribution of amyloid binding in DLB was similar to that reported in a neuropathological cohort diagnosed with DLB during life, but fulfilling neuropathological criteria for AD and DLB post mortem (Walker *et al.*, 2015a), with the highest amyloid levels in the cingulate and lowest in the occipital cortex. However, the difference between these two areas was much higher in the neuropathological study (cingulate:occipital ratio 2.28 v. 1.11 in this study), most likely due to a combination of greater amyloid deposition in end-stage disease and an increase in signal to noise ratio in post mortem quantification compared with imaging studies.

7.6.5 Comparison of visual rating and cortical SUVR threshold

One scan visually rated as positive was classified as negative using the ADNI cortical SUVR threshold of 1.11. Four DLB and three control cases were categorised as amyloid positive despite being visually classified as negative. This is consistent with a previous report of 250 scans from a phase 1b trial in AD, which found visual rating to be more conservative, producing more amyloid negative results (Klein *et al.*, 2014). The use of visual rating in this study is justified, as it has been shown to have diagnostic validity in AD, and predictive validity for the conversion of MCI to AD (Schreiber *et al.*, 2015). However, quantification may detect lower levels of amyloid deposition that are clinically significant (Schreiber *et al.*, 2015). As such, a comparison of those with amyloid above and below the ADNI threshold of 1.11 may be a more accurate measure of the effect of amyloid in DLB. However, as a significant majority of DLB cases fall above this threshold a much larger sample would be necessary to test this.

7.6.6 Hippocampal volume

AD and DLB both had smaller hippocampi (and so greater hippocampal atrophy) than controls. AD approached having greater atrophy than DLB. These findings are

consistent with previous reports of medial temporal lobe atrophy in DLB that is greater than controls but less than AD (Watson *et al.*, 2009).

7.6.7 Hypothesis 3: Amyloid positive DLB cases will have greater memory impairment and less visuospatial, attention and executive impairments than amyloid negative DLB cases.

The amyloid positive group had more years in education compared to the amyloid negative group. Despite this there were no differences between the two groups in the vast majority of cognitive tests. This may be due to increased cognitive reserve in the amyloid positive group masking the cognitive effects of amyloid deposition (Amieva *et al.*, 2014). Initial differences between the groups in verbal fluency and simple reaction time did not survive linear regression with years in education and visual rating as independent variables. This regression analysis found those with amyloid deposition to have significantly more digit vigilance false positive responses, and a trend toward slower cognitive processing in the choice reaction task and worse memory function on the ACE-R (but not the Rey AVLT). Overall there was no difference between the groups in the vast majority of cognitive tests. Similarly, amyloid imaging has previously been found not to correlate with cognitive impairment in AD (Jagust *et al.*, 2009).

This is the largest comparison of amyloid positive and negative DLB subjects to date, and includes significantly more neuropsychological comparisons than most previous reports. Despite the large number of outcome measures tested there were very few differences between the groups, even without correcting for multiple comparisons. We hypothesised that those with amyloid deposition would have a phenotype less typical of DLB, with some features of AD, i.e. worse memory function, less marked attention/executive and visuospatial dysfunction. There was no consistent evidence for this. Therefore, people with clinically diagnosed DLB and amyloid deposition appear to have the same cognitive phenotype as those without amyloid.

7.6.8 Hypothesis 4: Amyloid positive DLB cases will score lower in scales measuring hallucinations, fluctuations and parkinsonism

There were no differences between amyloid positive and negative DLB subjects in scales measuring fluctuations, hallucinations, parkinsonism, depression, other

neuropsychiatric symptoms and postural BP changes. Amyloid positive subjects approached having lower scores in the GCSI, but only one subject had a clinically significant score.

We hypothesised that those with amyloid deposition would have less severe parkinsonism, fluctuations and hallucinations, but found no evidence for this. Consistent with our findings in cognitive tests we found no evidence that amyloid deposition was associated with a different clinical phenotype in DLB. That said, the DLB group in this study necessarily had core and suggestive features present, as they had received a clinical diagnosis of probable DLB. Our study criteria would exclude any potential patients with mixed amyloid and αSyn pathology but no diagnostic features of DLB. Neuropathological studies do not suffer from this bias. A recent study of cases with a post-mortem diagnosis of DLB found that all three core features of DLB were less frequent in cases with moderate to frequent neuritic Aβ plaques compared to those with no or sparse neuritic plaques (Tiraboschi *et al.*, 2015), suggesting that Aβ may have an effect on the clinical profile of DLB. Despite this over 75% of cases with advanced neuritic plaques disease were clinically diagnosed as DLB.

7.6.9 Hypothesis 5: Amyloid positive DLB cases will have smaller hippocampi than amyloid negative cases

There was no difference in hippocampal volume between the amyloid positive and negative groups. However, hippocampal volume was inversely correlated with amyloid deposition. It is known that amyloid deposition increases with age (Jansen *et al.*, 2015) and hippocampal volume decreases with age (Frisoni *et al.*, 2008), therefore any inverse correlation between amyloid and hippocampal atrophy may be due to age. The association between the two variables in this cohort did not survive linear regression with age as an independent variable.

Hippocampal atrophy was evident in the DLB group compared to controls but we found no direct link between amyloid and atrophy. Atrophy may be due to other types of pathology, particularly tau neurofibrillary tangles (Kantarci *et al.*, 2012a; Nedelska *et al.*, 2015a).

7.6.10 Hypothesis 6: Amyloid positive DLB cases will have a shorter duration of illness than amyloid negative cases

Amyloid and αSyn pathology have been shown to interact synergistically in vitro and in mouse models (Masliah *et al.*, 2001). It has been hypothesised that this interaction may result in more rapid cognitive decline in those with both pathologies. The lack of cognitive differences found in this study suggests that amyloid does not have a significant impact in the severity or pattern of cognitive impairment in DLB. However, there are three reasons for us to be cautious of making this conclusion. Cross-sectional analysis may be insensitive to detect such differences, as those with more severe impairment due to co-existing amyloid and αSyn may be more likely to refuse participation in the study. Also, those with amyloid pathology had more years in education, which may have masked the effect of amyloid deposition. Finally, those with amyloid deposition had a shorter duration of illness (21 v 31 months). Though not statistically significant, this raises the possibility that those with amyloid deposition have declined more quickly. Cognitive tests in this cohort will be repeated after one year. If amyloid is associated with more rapid cognitive decline this may be detected at follow-up assessment.

It is difficult to assess the rate of cognitive decline from cross-sectional imaging studies or retrospective pathological studies. Previous studies have used a variety of proxy measures for rate of cognitive decline such as the interval between motor and cognitive impairment, age of onset, disease duration and age of death (Jellinger *et al.*, 2002; Ballard *et al.*, 2004; Rowe *et al.*, 2007; Maetzler *et al.*, 2009; Nelson *et al.*, 2009; Foster *et al.*, 2010). Unsurprisingly findings differ between these heterogeneous studies, but longitudinal studies such as this one may be able to give a definitive answer to this question.

7.6.11 Correlation of amyloid with clinical variables

We tested correlations between amyloid binding and clinical variables within the DLB group that had previously been reported in the literature. AD and control subjects were excluded to avoid confounding relating to differences between diagnostic groups rather than the direct or indirect effects of amyloid.

With the exception of global cognitive function (e.g. measured by the MMSE) these correlations had been reported in single studies without any corroboration in

independent cohorts and were unlikely to have been a priori hypotheses. The chance of Type 1 errors in these post-hoc tests is relatively high, particularly where multiple clinical and neuropsychological measures may be compared with multiple cortical regions. We sought to independently verify these findings in our cohort, with the expectation that we would not replicate the most of the findings.

7.6.11.1 Cognitive and functional impairment

We found no evidence to support previous findings of correlation between cortical or striatal binding and summary measures of cognitive or functional impairment. There was conflicting evidence from previous studies regarding this, and reported correlations were often modest. Our findings support previous research that there is no link between amyloid burden and overall cognition (Edison *et al.*, 2008; Shimada *et al.*, 2013) or functional impairment (Kantarci *et al.*, 2012b) in DLB. Where correlation has been reported it has generally been in diagnostically heterogeneous cohorts including healthy controls and PD, both of which have high cognitive function and low rates of amyloid (Gomperts *et al.*, 2008; Maetzler *et al.*, 2009; Villemagne *et al.*, 2011). Any correlation in these cohorts may be due to these group differences rather than the effect of amyloid itself. One study found an association between higher amyloid binding in the precuneus and lower MMSE in DLB, but the strength of the association was not reported (Gomperts *et al.*, 2012). This study measured precuneus amyloid deposition because amyloid deposition is particularly high in this area and is highly correlated with global retention. Precuneus binding was not measured in our study.

7.6.11.2 Semantic memory

Few amyloid imaging studies have undertaken sufficient neuropsychological testing to investigate cognitive sub-domains. One study reported an association between cortical amyloid deposition and worse scores in tests of semantic memory, but no association with episodic memory (Gomperts *et al.*, 2012).

In our study semantic memory was tested in the 'Retrograde Memory' section of the ACE-R. This was inversely correlated with cortical amyloid deposition. It appears that amyloid deposition may indeed be associated with worse semantic memory, though we should bear in mind that the ACE-R retrograde memory assessment consists only of

four points (of 26 total points for memory), and is not a rigorous test of semantic memory.

We also found inverse correlation between amyloid and ACE-R overall memory scores after regression with age and years in education as variables. However, in accordance with Gomperts et al. we found no association with episodic memory assessed using the Rey AVLT.

7.6.11.3 Parkinsonism

One previous study has reported relative striatal binding to be inversely correlated with UPDRS scores (Gomperts *et al.*, 2008). The rationale for examining relative binding rather than overall striatal amyloid deposition was not stated.

We found no association between relative striatal binding and UPDRS scores, or striatal binding (not corrected for overall binding) and overall UPDRS or Hoehn and Yahr stage. We did find a direct correlation between striatal binding and 6-point UPDRS scores, contrary to the previous report of inverse correlation between striatal binding and parkinsonism. The relationship between striatal amyloid deposition and motor impairment has not been explored in other imaging or post mortem studies. The findings of Gomperts et al. were in a small DLB group (n=8), and were not replicated in our cohort. Our finding of direct correlation is perhaps more plausible but it requires replication in other cohorts.

7.6.11.4 Visuospatial function

Gomperts et al. (2008) also found an association between increased amyloid in the parietal/posterior cingulate region relative to overall binding hand impaired visuoperceptual ability in the Benton Visual Form Discrimination Test. There was no correlation between cingulate or parietal amyloid binding and computerised tests of visuospatial function in our cohort, though this lacked statistical power, with data available for only 11 and 13 DLB patients in the angle discrimination and motion tasks respectively. Relative cingulate binding was associated with worse visuospatial function measured in the ACE-R, but this did not survive regression with age. Contrary to the finding of Gomperts et al. we found a surprising positive correlation between ACE-R visuospatial performance and relative parietal amyloid binding which did survive a

regression analysis. This seems biologically implausible, and may well be a Type 1 error. Due to the small numbers of patients involved and the conflicting findings between our study and that of Gomperts et al. no conclusions can be drawn on the effect of cingulate and parietal amyloid deposition on visuospatial function in DLB. This should be investigated in other cohorts.

7.7 Summary

DLB was associated with a lower cortical burden of amyloid in most regions than AD. Though there were no statistically significant differences in amyloid deposition between DLB and controls, deposition in DLB was intermediate between AD and controls in all regions. This is consistent with previous findings in DLB. Where significant amyloid deposition occurs in DLB, the pattern of deposition appears grossly similar to that of AD.

There were no consistent differences in the cognitive or clinical phenotype of amyloid positive and negative DLB cases, and no difference in hippocampal atrophy. Correlation between hippocampal atrophy and amyloid was likely due to the confounding effects of age, and other pathology such as tau may be responsible for the atrophy seen in DLB. These findings replicate neuropathological studies that found no influence of overall amyloid load on the ability to diagnose DLB in life (Tiraboschi *et al.*, 2015; Walker *et al.*, 2015a). When only neuritic plaques are considered, amyloid pathology may be associated with decreasing rates of core features of DLB (Tiraboschi *et al.*, 2015), but amyloid imaging agents bind to diffuse amyloid plaques and vascular amyloid and so cannot be used to test these findings in life.

Previous findings of a correlation between amyloid deposition and clinical phenotype (Gomperts *et al.*, 2008; Maetzler *et al.*, 2009; Villemagne *et al.*, 2011; Gomperts *et al.*, 2012) were generally not replicated in our cohort, apart from a correlation between semantic memory impairment and cortical amyloid deposition. This is consistent with other imaging studies that found no link between amyloid burden and cognitive (Edison *et al.*, 2008; Shimada *et al.*, 2013) or functional (Kantarci *et al.*, 2012b) impairment.

The presence of amyloid was not associated with lower cognitive scores to suggest a synergistic effect between amyloid and αSyn. That said, there was a non-significant 10 month difference in disease duration between the groups which may potentially be

clinically relevant. Also, the amyloid positive group had more years in education, which is known to protect against decline in cognitive function in dementia (Amieva *et al.*, 2014). Longitudinal follow-up will be necessary to determine whether those with amyloid deposition deteriorate at a faster rate than those without amyloid.

7.8 Strengths and limitations of study

This is one of the largest studies of amyloid imaging in DLB to date. All subjects have had a detailed cognitive and clinical assessment. All but three subjects had an MRI, allowing for accurate region of interest analysis of amyloid ligand binding. As far as possible we followed the analysis methods used in ADNI (Landau and Juagust, 2014), allowing us to compare our cohort with well-defined thresholds for abnormality. The DLB group was sufficiently large and well balanced between amyloid positive and negative to allow a direct comparison between the two.

We did not have a complete dataset for all patients due to patient inability to complete certain tests and computer error. This, and the low number of participants in the AD group may have reduced the power of the study to find differences between the AD and DLB groups, and between the amyloid positive and negative DLB cases. This particularly affected the computerised measures of visuospatial function – the angle and motion tasks. Comparison with some previous studies in DLB may be impaired by the use of different cortical region maps to define regions of interest.

Amyloid imaging agents, particularly ^{18}F-labelled agents such as Florbetapir, have high nonspecific white matter binding (Rowe and Villemagne, 2011). Partial volume effects can occur if white matter (high signal) or CSF (low signal) are present in a voxel identified as predominantly grey matter. Despite this, the use of partial volume correction does not appear to improve the accuracy of results (Knesaurek *et al.*, 2013) and is not performed in the ADNI cohort.

Emerging evidence suggests that white-matter reference regions may be better than cerebellar reference regions when quantifying changes in amyloid deposition over time (Chen *et al.*, 2015; Landau *et al.*, 2015). This may be due to greater accuracy in cerebral white matter measurement than pontine or cerebellar areas due to their relatively peripheral positioning in the imaging field, or the relative stability of white matter compared to the cerebellum over time. We used the whole cerebellum region as this is

the only region that has been validated against post-mortem amyloid measurement (Clark *et al.*, 2011) and is still recommended for cross-sectional comparisons (Chen *et al.*, 2015). It is also the reference region used in most previous studies of amyloid imaging in DLB (Table 3.1).

Results from Florbetapir are strongly correlated with those from PiB, though there is greater specific cortical binding in PiB (Landau *et al.*, 2013). Amyloid ligands such as PiB and Florbetapir do not give a direct measure of amyloid burden and may overestimate binding in specific regions e.g. the precuneus (Driscoll *et al.*, 2012). However, post mortem examination has confirmed that Florbetapir signal is correlated with amyloid density measured by immunochemistry (Clark *et al.*, 2011). Crucially, this correlation does not seem to be affected by the presence of other brain pathology including LB disease (Dugger *et al.*, 2014).

7.9 Future directions

Although a substantial cohort compared to other published data, we are continuing to recruit to this study. A larger cohort may show statistically significant differences in some of the trends seen in this cohort, and negate some spurious findings. There were few DLB subjects that were below the threshold for abnormality of quantitative assessment of amyloid binding. A larger cohort may allow us to compare amyloid positive and negative based on a quantitative threshold rather than visual rating.

There is no evidence from this study that amyloid deposition is associated with a different pattern of clinical or neuropsychological impairment in DLB. We cannot conclude that amyloid is not associated with more rapid decline in DLB. All clinical and cognitive assessments will be repeated after one year. At this point any effect of amyloid on disease progression in DLB may be evident. In the absence of any effect of amyloid, the effect of APOE could be investigated, given the possibility of interaction between APOE and αSyn (Verghese *et al.*, 2011). Neuropathological studies have found that tau is more closely related to clinical phenotype in DLB than amyloid (Merdes *et al.*, 2003; Weisman *et al.*, 2007; Walker *et al.*, 2015a). Therefore novel tau imaging ligands may be more likely to identify DLB subgroups with differing symptoms and cognitive profiles.

The pattern of amyloid deposition in amyloid positive DLB and AD was similar in a region of interest analysis. A voxel-based analysis may be better able to identify more subtle differences between the two diseases within regions.

Until in vivo synuclein imaging ligands become available, pathological studies are required to assess whether the presence of amyloid is associated with a lower likelihood of the presence of LB clinical features in the presence of αSyn pathology. This study found no difference in the presence of LB clinical features but this may be due to selection bias, as all our DLB subjects by definition have at least two diagnostic features of DLB.

Dissecting the contributions of different brain pathologies to the clinical presentation and progression of dementia was previously only possible through post mortem studies. Post mortem studies by nature tend to assess late stage disease, and therefore may not identify the pathological processes that are important in the initial development of disease. Amyloid imaging allows us to examine the importance of amyloid deposition in vivo. Similar ligands have been developed for tau (Villemagne *et al.*, 2015) and ligands are in development for the imaging of αSyn (Zhang *et al.*, 2014). Over the coming years research studies using these and other ligands should shed more light on the pathophysiology of dementia with Lewy bodies, including interactions between different pathologies.

Chapter 8 Conclusions

These studies investigated the association of two imaging biomarkers with clinical phenotype at different stages of cognitive impairment in Lewy body disease. We investigated the use of ^{123}I-FP-CIT SPECT as a potential diagnostic biomarker of prodromal DLB. We used ^{18}F-Florbetapir amyloid PET to examine the clinical implications of concurrent amyloid deposition in DLB.

We found that 36% of MCI cases with Lewy body symptoms had an abnormal ^{123}I-FP-CIT scan, and those with abnormal scans had more core features of LB disease than those with normal scans. The relatively high frequency of abnormal scans and their association with symptoms suggestive of LB disease suggests that ^{123}I-FP-CIT imaging shows promise as a potential biomarker for prodromal DLB. That said, several cases with normal scans also had significant LB features. Some of these may represent cases of prodromal DLB with relative sparing of the substantia nigra. Other diagnostic biomarkers will be necessary to identify such cases.

Contrary to observations in PD, there was little asymmetry in ^{123}I-FP-CIT imaging between the left and right striata (Walker *et al.*, 2004). Early losses that affect the entire striata in both hemispheres are more difficult to visualise than focal losses, potentially limiting the utility of visual assessment of images, the standard method of interpreting clinical scans currently. Semiquantitative measurements may aid differentiation between normal and abnormal scans but reference ranges for these will need to take into account gender and age differences in FP-CIT binding (Varrone *et al.*, 2013). We found even greater differences than expected between the genders in this cohort. It remains to be seen whether this reflects a difference in the pathophysiology of prodromal DLB between males and females, or simply lower rates of LB disease in females in this cohort.

Longitudinal follow-up of these subjects to dementia, and eventually autopsy, will identify the sensitivity and specificity of visually rated ^{123}I-FP-CIT scans to detect prodromal DLB in MCI with Lewy body symptoms. This may also aid the development of gender and age-specific thresholds for abnormality in semiquantified images. A very large sample size would be needed to determine the sensitivity and specificity of this

marker in a general MCI population (i.e. not selected for the presence of Lewy body symptoms) as only 5-10% of cases would be expected to convert to DLB in longitudinal follow-up (Vann Jones and O'Brien, 2014). This type of study would be extremely expensive and possibly unnecessary, as FP-CIT is unlikely to be used as a diagnostic tool in those without symptoms of Lewy body disease. A more feasible and useful study may be to investigate a group of LB-MCI cases alongside AD-MCI cases as a comparator group.

Recent diagnostic criteria have highlighted the use of imaging biomarkers to increase diagnostic certainty in dementia (McKhann *et al.*, 2011) and MCI related to Alzheimer's disease (Albert *et al.*, 2011). The accurate identification of prodromal DLB is important for research studies wishing to investigate the pathophysiology of prodromal DLB and trial drugs targeting LB pathology. Postuma et al. (2015a) have demonstrated that the use of three simple clinical biomarkers (olfaction, colour vision and motor function) can help to identify subjects with RBD at high risk of developing a neurodegenerative disease. A similar process may be used to identify subjects with MCI at high risk of developing DLB. It may be that FP-CIT has a role in this, but less expensive biomarkers such as olfactory dysfunction (Yoon *et al.*, 2015) may be preferable where large numbers of people are being screened. A two-stage process may be an effective way to identify prodromal DLB, with inexpensive, sensitive markers in the first stage (e.g. olfactory dysfunction, mild motor impairments) followed by a potentially more expensive and invasive, but more specific markers in the second stage (Donaghy and McKeith, 2014). ^{123}I-FP-CIT SPECT is a candidate for this, along with cardiac MIBG and nerve biopsy for the presence of αSyn (Treglia and Cason, 2012; Wang *et al.*, 2013).

In addition to use as diagnostic markers, imaging ligands can be used to investigate the pathophysiology of dementia and the effect of mixed pathology on clinical and cognitive features. Amyloid imaging is a useful tool to examine the effect of amyloid pathology in DLB, following post mortem and in vitro evidence of possible interactions between Aβ and αSyn (Masliah *et al.*, 2001; Fujishiro *et al.*, 2010). Consistent with other studies, we found that amyloid deposition in DLB is intermediate between AD and controls. The pattern of amyloid deposition in AD was different from DLB or

controls, with relatively high binding in frontal and low binding in occipital regions. However, we found that amyloid positive DLB cases may have a more 'AD-like' pattern of deposition.

The presence of amyloid pathology had little effect on clinical phenotype in DLB, though longitudinal follow-up may demonstrate that amyloid deposition has an effect on the speed of clinical progression. Our findings, albeit on a relatively small cohort, suggest that recognition of DLB is not more difficult in the face of concurrent amyloid deposition, and conversely that there are not obvious clinical or cognitive features that will allow in vivo identification of amyloid pathology in DLB. However, this cross-sectional study could not examine possible effects on disease course, which would have important therapeutic impact. If amyloid is associated with a faster rate of decline then amyloid and the interaction between amyloid and αSyn may be targets for treatment in DLB. If such treatments were developed, amyloid imaging would then be useful as a pathological biomarker to identify people who would benefit from such treatments. If there is no effect of amyloid on rate of progression, then such drugs would be unlikely to be beneficial and resources could be directed towards developing treatments that target other pathophysiological processes.

Over the coming years the already available ligands for tau (Villemagne *et al.*, 2015) and the prospect of ligands in the future for other pathologies such as αSyn (Zhang *et al.*, 2014) will shed new light on the pathophysiology of dementia, including the effect of comorbid pathologies, which are present in most cases of dementia (Schneider *et al.*, 2007). These and other biomarkers will also be used in therapeutic studies to identify cohorts with specific brain pathology prior to the development of dementia and to monitor the effects of treatments targeting these pathologies (Hampel *et al.*, 2014). If these studies identify treatments for dementia, imaging and other biomarkers will then be important in identifying the pathological processes present in an individual and deciding the specific treatment suitable for that person.

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