

**Structural and diffusion MRI in dementia with Lewy  
bodies. A comparison with Alzheimer's disease and  
normal ageing**



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## Abstract

### Purpose:

Dementia with Lewy bodies (DLB) is a common form of dementia, yet its clinical features remain poorly understood. We investigated *in vivo* structural and micro-structural changes in DLB compared to Alzheimer's disease (AD) and normal ageing using magnetic resonance imaging (MRI) techniques and their relationship to clinical features.

### Methods:

Study subjects (35 DLB, 36 AD, 35 Controls) completed clinical and cognitive assessments and structural and diffusion tensor MRI scans. Voxel based morphometry (VBM) techniques were used to investigate patterns of regional grey matter atrophy. To investigate white matter tract change, diffusion tensor imaging (DTI) indices, fractional anisotropy (FA) and mean diffusivity (MD) were measured across the entire white matter skeleton using the tract based spatial statistics (TBSS) analysis.

### Results:

Groups were well matched for demographic and clinical factors, although, as expected, the DLB group also had more neuropsychiatric features, greater parkinsonism and more functional impairment than the AD group.

VBM analysis indicated a less diffuse pattern of grey matter atrophy in DLB than observed in AD, with areas of loss including the posterior and subcortical regions without significant frontal atrophy. DLB had greater preservation of the medial temporal lobe structures when compared to AD, which was associated with less impaired memory function.

The pattern of FA change in DLB compared to controls involved the parieto-occipital white matter tracts; in AD, the FA change was more diffuse. In DLB, an association was found between reduced FA and the phonemic fluency tasks in the white-matter tracts of the precentral gyrus, anterior cingulate and precuneus in DLB.

**Conclusions:**

Despite a similar level of dementia severity, the patterns of structural and diffusion tensor MRI changes in AD and DLB differ significantly. DLB was associated with less grey matter volume loss and less white matter tract change than AD along with differing regional patterns of change.

## Contents

Abstract .....	i
List of Figures .....	viii
List of Tables.....	x
Acknowledgements .....	xiii
Declaration .....	xiv
Abbreviations .....	xvi

## Chapter 1 Introduction ..... 1

1.1 Overview of Thesis Contents.....	2
1.2 Dementia with Lewy bodies.....	3
1.2.1 Epidemiology.....	3
1.2.2 Clinical features .....	4
1.2.2.1 Differential Diagnosis of DLB .....	6
1.2.2.2 Lewy body dementias – the relationship between DLB and PDD ...	6
1.2.2.3 Neuropsychological features.....	7
1.2.2.4 Neuropsychiatric features .....	8
1.2.2.5 Motor features .....	8
1.2.2.6 Sleep disorders .....	9
1.2.2.7 Autonomic dysfunction .....	9
1.2.3 Neuropathology.....	9
1.2.3.1 Lewy related pathology .....	9
1.2.3.2 Other pathological features of DLB.....	11
1.2.3.3 The Braak Hypothesis .....	11
1.2.3.4 Amygdala Lewy bodies.....	12
1.2.3.5 The role of Alzheimer’s pathology in DLB .....	12
1.2.3.6 Consensus criteria for the pathological diagnosis of DLB.....	12
1.2.3.7 Clinicopathological Correlations .....	13
1.2.4 Neurochemistry .....	14
Acetylcholine.....	14
Serotonin .....	15
Dopamine .....	15
1.2.5 Management of DLB .....	15

1.2.5.1	<i>Acetylcholinesterase inhibitors</i> .....	15
1.2.5.2	<i>Memantine</i> .....	16
1.2.5.3	<i>Treatment of Psychotic symptoms</i> .....	17
1.2.5.4	<i>Parkinsonism</i> .....	17
1.2.5.5	<i>Depression, REM sleep behaviour disorder and Autonomic failure</i> .....	18
1.2.6	<i>Summary</i> .....	18
1.3	<i>Alzheimer's disease</i> .....	19
1.3.1	<i>Clinical features</i> .....	19
1.3.2	<i>Risk factors and associations</i> .....	19
1.3.3	<i>Genetics</i> .....	20
1.3.4	<i>Pathological features</i> .....	20
1.3.5	<i>Imaging changes</i> .....	21
1.3.6	<i>Therapeutics</i> .....	22
1.3.7	<i>Summary</i> .....	23
<b>Chapter 2 Magnetic Resonance Imaging in Lewy body dementias</b> .....		<b>24</b>
2.1	<i>Introduction</i> .....	24
2.2	<i>Structural Magnetic Resonance Imaging</i> .....	24
2.2.1	<i>Cortical atrophy</i> .....	25
2.2.2	<i>Rate of cerebral atrophy</i> .....	27
2.2.3	<i>Medial temporal lobe</i> .....	28
2.2.4	<i>Subcortical structures</i> .....	33
2.2.5	<i>White matter hyperintensities</i> .....	34
2.3	<i>MR Diffusion Tensor Imaging (MR-DTI)</i> .....	36
2.4	<i>Proton Magnetic Resonance Spectroscopy (<sup>1</sup>H-MRS)</i> .....	39
2.5	<i>Functional MRI (fMRI)</i> .....	41
2.6	<i>Conclusions</i> .....	42

<b>Chapter 3</b>	<b>Study aims, objectives and hypotheses.....</b>	<b>44</b>
3.1	Aims.....	44
3.2	Objectives and hypotheses.....	44
<b>Chapter 4</b>	<b>Clinical and Neuropsychological Characteristics .....</b>	<b>46</b>
4.1	Introduction.....	46
4.2	Methods.....	46
4.2.1	<i>Subjects</i> .....	46
4.2.2	<i>Assessment and Diagnosis</i> .....	47
4.2.3	<i>Clinical and Neuropsychological measures</i> .....	47
4.2.3.1	<i>Clinical Scales</i> .....	47
4.2.3.2	<i>Neuropsychological Assessment</i> .....	48
	Global Cognitive Measures.....	48
	Attention – CDR System.....	49
	Executive Function.....	50
	Praxis.....	50
	Memory.....	50
4.2.4	<i>Statistics</i> .....	51
4.3	Results.....	52
4.3.1	<i>Subject characteristics</i> .....	52
4.3.2	<i>Subject demographics</i> .....	52
4.3.3	<i>Clinical features</i> .....	53
4.3.3.1	<i>Dementia with Lewy bodies subject characteristics</i> .....	53
4.3.3.2	<i>Clinical scales</i> .....	56
4.3.3.3	<i>Other clinical variables</i> .....	58
4.3.4	<i>Neuropsychological Assessment</i> .....	59
4.3.4.1	<i>Attention</i> .....	59
4.3.4.2	<i>Praxis and Executive Function</i> .....	61
4.3.4.3	<i>Memory</i> .....	62
4.4	Discussion.....	64
4.4.1	<i>Clinical features</i> .....	64
4.4.2	<i>Neuropsychological changes</i> .....	66
4.5	Summary.....	68

<b>Chapter 5 Structural magnetic resonance imaging changes in dementia with Lewy bodies .....</b>	<b>69</b>
5.1 Introduction .....	69
5.2 Study specific methods .....	70
5.2.1 MRI data acquisition .....	70
5.2.2 VBM-DARTEL .....	70
5.2.3 Statistical Analysis .....	72
5.3 Results .....	72
5.3.1 Subject characteristics .....	72
5.3.2 Subject demographics .....	72
5.3.3 Total grey matter, white matter and intracranial volumes across groups .....	73
5.3.4 Differences in grey matter volume between DLB and Controls .....	74
5.3.5 Differences in grey matter volume between AD and Controls.....	75
5.3.6 Differences in grey matter volume between DLB and AD .....	76
5.3.7 Clinical correlations in DLB and AD .....	77
5.4 Discussion .....	79
<b>Chapter 6 Diffusion tensor imaging in dementia with Lewy bodies .....</b>	<b>83</b>
6.1 Introduction .....	83
6.2 Study specific methods .....	84
6.2.1 MRI data acquisition .....	84
6.2.2 DTI preprocessing and analysis .....	85
6.2.3 White matter hyperintensities.....	86
6.2.4 Statistics .....	87
6.3 Results .....	88
6.3.1 Subject characteristics .....	88
6.3.2 Subject demographics .....	88

6.3.3	<i>White matter hyperintensities</i> .....	88
6.3.4	<i>Fractional Anisotropy</i> .....	89
6.3.4.1	<i>Comparison of FA between DLB and Controls</i> .....	89
6.3.4.2	<i>Comparison of FA between AD and Controls</i> .....	91
6.3.4.3	<i>Comparison of FA between DLB and AD</i> .....	92
6.3.5	<i>Mean Diffusivity</i> .....	96
6.3.5.1	<i>Comparison of MD between DLB and Controls and AD and Controls</i> .....	96
6.3.5.2	<i>Comparison of MD between DLB and AD</i> .....	98
6.3.6	<i>Clinical Correlations</i> .....	98
6.3.6.1	<i>Episodic Memory</i> .....	98
6.3.6.2	<i>Letter Fluency</i> .....	100
	Relationship between FA and Letter fluency .....	100
	Relationship between MD and Letter Fluency .....	101
6.3.6.3	<i>Motor Parkinsonism in DLB</i> .....	102
	Relationship between FA and UPDRS-III.....	102
6.4	<i>Discussion</i> .....	104
<b>Chapter 7 Conclusions and future studies .....</b>		<b>109</b>
7.1	<i>Summary of main findings</i> .....	109
7.2	<i>Conclusions</i> .....	110
7.3	<i>Study strengths and limitations</i> .....	112
7.4	<i>Future directions</i> .....	113
<b>References</b> .....		<b>117</b>



## List of Figures

Figure 1.1. Classic Lewy body (green arrow) in the Substantia Nigra, X400 High Power Field, haematoxylin and eosin staining.* .....	10
Figure 1.2. alpha-synuclein immunohistochemistry X200 High Power Field. Cortical Lewy bodies (red arrows) and diffuse Lewy neurites (brown staining background) in the cingulate gyrus.* .....	10
Figure 2.1. Coronal view illustrating relative preservation of the Medial Temporal Lobe and hippocampus in dementia with Lewy bodies (right) compared with Alzheimer's disease (left).....	29
Figure 2.2. Representation of the diffusion tensor ellipsoid: 3×3 tensor matrix, eigenvalues $\lambda_1, \lambda_2, \lambda_3$ .....	36
Figure 2.3. Coronal view illustrating Diffusion Tensor Imaging in dementia with Lewy bodies. ....	37
Figure 4.1. Clinician's Assessment of Fluctuating Cognition. ....	55
Figure 4.2. CDR Computerised attentional task: response by group. ....	61
Figure 5.1. VBM-DARTEL pre-processing steps. ....	71
Figure 5.2. Three-dimensional surface renders showing the patterns of cortical GM loss in DLB (red) compared to controls; (A) $p < 0.001$ , uncorrected and (B) $p < 0.05$ , FWE-corrected.....	74
Figure 5.3. Three-dimensional surface renders showing the patterns of cortical GM loss in AD (red) compared to controls; (A) $p < 0.001$ and (B) $p < 0.05$ , FWE-corrected.....	75
Figure 5.4. Three-dimensional surface renders showing the areas of greater cortical GM loss in AD (red) compared to DLB ( $p < 0.05$ , FWE-corrected).....	76
Figure 5.5. Examples of coronal T1 images demonstrating medial temporal lobe atrophy in AD on the left and preservation of the medial temporal lobe structures on the right in DLB in (a) mild dementia and (b) moderate dementia. The left MTL structures are circled in red.....	76
Figure 5.6. Three-dimensional surface renders showing the areas of greater cortical GM loss in AD (red) compared to DLB ( $p < 0.05$ , FWE-corrected).....	77
Figure 6.1. Representation of the pre-processing steps involved in tract based spatial statistics method of analysis. ....	86
Figure 6.2. TBSS statistical maps for reduced FA in dementia with Lewy bodies. The areas of significant differences are represented in blue, overlaid onto the MNI template image and mean FA skeleton (green).....	90

- Figure 6.3. TBSS statistical maps for reduced FA in Alzheimer's disease. Areas of significant differences are presented in blue, overlaid onto the MNI template image and mean FA skeleton (green)..... 92
- Figure 6.4. Represents the results of the comparison between FA reduction in (a) DLB v AD and (b) AD v DLB. The areas of significant differences are represented in blue, overlaid onto the MNI template image and mean FA skeleton (green)..... 93
- Figure 6.5. Graphical representation of the mean Fractional Anisotropy (FA) within a white matter tract voxel in AD and DLB. Areas include: (a) left temporal, (b) fornix, (c) left thalamus and (d) right occipital. .... 95
- Figure 6.6. TBSS statistical maps for increased MD in (a) dementia with Lewy bodies and (b) Alzheimer's disease. Areas of significant differences ( $p < 0.005$ , corrected) are represented in blue overlaid onto the MNI template image and mean FA skeleton (green). .... 97
- Figure 6.7. Represents the relationship between Mean Diffusivity (MD) and Episodic Memory in (a) DLB and (b) AD. The areas that correlate significantly are represented in blue, overlaid onto the MNI template image and mean FA skeleton (green)..... 99
- Figure 6.8. Represents the relationship between Fractional Anisotropy and Letter fluency in DLB. The areas that correlate significantly are represented in blue, overlaid onto the MNI template image and mean FA skeleton (green). .... 100
- Figure 6.9. Fractional Anisotropy at MNI 23, 33, 25 (location is represented in the axial image by the red arrow) plotted against Letter Fluency in DLB..... 101
- Figure 6.10. Represents the relationship between MD and Letter fluency in DLB. The areas that correlate significantly are represented in blue, overlaid onto the MNI template image and mean FA skeleton (green). .... 102

## List of Tables

Table 1.1. Consensus Criteria for the Clinical diagnosis of dementia with Lewy bodies (McKeith et al., 1996).....	4
Table 1.2. Revised Consensus Criteria for the diagnosis of DLB (McKeith et al., 2005). .....	5
Table 1.3. Assignment of the likelihood that the pathologic findings are associated with the DLB clinical syndrome, (McKeith et al., 2005).....	13
Table 2.1. Structural MRI studies – Cortical Atrophy.....	27
Table 2.2. Structural MRI studies – Rate of Cerebral Atrophy. ....	28
Table 2.3. Structural MRI studies – Medial temporal lobe structures. ....	32
Table 2.4. Structural MRI studies – Subcortical structures. ....	34
Table 2.5. Structural MRI studies – White matter hyperintensities.....	35
Table 2.6. <sup>1</sup> H-MRS Studies.....	40
Table 4.1. CDR Attentional Task Battery.....	49
Table 4.2. Subject demographics. ....	53
Table 4.3. Clinical features of the DLB cases.....	54
Table 4.4. The Clinician Assessment of Fluctuation. ....	55
Table 4.5. Group UPDRS scores and motor parkinsonism.....	56
Table 4.6. Results of clinical scales by group.....	58
Table 4.7. Vascular risk factors in all groups.....	58
Table 4.8. Medications taken by dementia subjects.....	59
Table 4.9. CDR Computerised attentional task: response by group. ....	60
Table 4.10. Praxis and executive function task results by group. ....	62
Table 4.11. Results of the memory tasks by group. ....	63
Table 5.1. Demographics, clinical and cognitive measures.....	73
Table 5.2. Grey matter, white matter and total intracranial volumes across groups..	73
Table 5.3. Location and peak significance of GM volume reduction using VBM-DARTEL.....	78

Table 6.1. Demographics, clinical and cognitive measures. ....	88
Table 6.2. White matter hyperintensity burden across groups. ....	89
Table 6.3. Location and significance of Fractional Anisotropy (FA) reduction across groups. The table shows voxel level significance, MNI coordinates and anatomical region. ....	94
Table 6.4. Locations and significance of the correlation between fractional anisotropy (FA) and the Unified Parkinsons Disease Rating Scale-III in dementia with Lewy bodies. The table shows voxel level significance (p<0.05, uncorrected) MNI coordinates and anatomical region. ....	103

## **About the author**

I completed my medical degree at Monash University, Melbourne, Australia in 2000. Following my intern year, I embarked on specialist physician training with the Royal Australasian College of Physicians. I completed my advanced training in Geriatric Medicine and was subsequently admitted to Fellowship with the Royal Australasian College of Physicians in 2009. My clinical interest in ageing, cognitive impairment and dementia has developed throughout the course of my training and has ultimately led me to research in dementia, at the Institute for Ageing and Health, Newcastle University, UK.

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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 of this or any other university or other institute of learning.

Professor Andrew M. Blamire and Professor John T. O'Brien were the principal investigators responsible for the original grant application to secure funding and ethical approval for the project. The Sir Jules Thorn Charitable Trust have provided funding for the project (05/JTA).

The author was involved in coordinating and managing all aspects of the clinical application phase of the project. This included finalising the study protocol, formulating the detailed study hypotheses, recruitment and assessment of study subjects (including the UPDRS III), management and coordination of demographical, clinical and neuropsychological data.

Clinical diagnosis of patients was carried out by the author (RW), Professor John O'Brien (JO'B) and Dr Robert Barber (RB). Josh Wood (Assistant Psychologist) and members of North-East DeNDRoN, assisted with the recruitment and assessment of study participants.

Neuropsychological assessment and carer based questionnaires were undertaken by the author, Josh Wood, or Clinical Trials Officers from North-East DeNDRoN, Karen Morgan and Barbara Wilson. Jenny Brown and Christine Jackson (NE-DeNDRoN) provided administrative support for the study and Josh Wood assisted with demographical, clinical and neuropsychological data entry.

Professor Andrew Blamire and Dr Jiabao He (MR Physics) developed the MRI protocol. Acquisition of the MRI data was performed by radiographers, Louise Morris, Carol and Tim Hodgson at the Newcastle Magnetic Resonance Centre with initial MRI data processing undertaken by Dr Benjamin Aribisala, Newcastle Magnetic Resonance Centre.

Throughout the period of research, and in collaboration with Professor O'Brien and Professor Blamire, the author was responsible for making further modifications to the study objectives. The author carried out study specific post-processing of the

MRI data and all image analyses with support from Dr Sean Colloby and Dr Michael Firbank (medical physicists). Dr Sean Colloby and Dr Robert Barber completed the visual rating of white matter hyperintensities and the author analysed the data. The author was responsible for the analysis and interpretation of the clinical and neuropsychological data with some advice provided by Dr Sean Colloby and Associate Professor Ray Watson (Statistician). The author was responsible for the writing of the thesis.



## Abbreviations

**<sup>1</sup>H-MRS:** Proton magnetic resonance spectroscopy

**5HT:** 5-hydroxytryptamine (serotonin)

**5-item UPDRS:** UPDRS motor subscale components include tremor at rest, bradykinesia, action tremor, facial expression and rigidity

**AChE:** Acetylcholinesterase.

**AC-PC:** Anterior commissure – posterior commissure

**AD:** Alzheimer’s disease

**ADAS-cog:** Alzheimer’s disease assessment scale – cognitive subscale

**ADC:** Apparent diffusion coefficient

**ADL:** Activities of daily living

**ANOVA:** Analysis of Variance

**APOE:** Apolipoprotein

**APP:** Amyloid precursor protein

**A $\beta$ :** Beta-amyloid

**BOLD:** Blood oxygen level dependent

**Bristol ADL:** Bristol Activities of Daily Living;

**BVMT:** Brief Visual Memory Task

**CA1–4:** *Cornu Ammonis*1–4

**CAF:** Clinician’s assessment of fluctuation

**CAMCOG:** Cambridge Cognitive Examination

**CAMCOG-EF:** Cambridge Cognitive Examination executive function subscale

**CBD:** Corticobasal degeneration

**CDR:** Cognitive Drug Research

**CGIC:** Clinical global impression of change

**Cho:** Choline

**CogRT:** Cognitive reaction time

**Cr:** Creatine

**CRT:** Choice reaction time

**CSF:** Cerebrospinal fluid

**CT:** Computerised tomography

**DARTEL:** Diffeomorphic anatomical registration through exponentiated Lie algebra

**df:** degrees of freedom

**DLB:** Dementia with Lewy bodies

**DSM:** Diagnostic statistical manual

**DTI:** Diffusion tensor imaging

**DWMH:** Deep white matter hyperintensities

**EEG:** Electroencephalogram

**EPI:** Echo planar imaging

**FA:** Fractional anisotropy

**FDG:** fluorodeoxyglucose (<sup>18</sup>F)

**FLAIR:** Fluid attenuated inversion recovery

**FLIRT:** FMRIB Linear Image Registration Tool

**fMRI:** Functional magnetic resonance imaging

**FMRIB:** Functional MRI of the brain

**FNIRT:** FMRIB Non-linear Registration Tool

**FP-CIT:** 2β-carbomethoxy-3β-4-iodophenyl-N-3-fluoropropyl-nortropane

**FSL:** Functional MRI of the brain (FMRIB) software library

**FWE:** Family-wise error

**FWHM:** Full width at half maximum

**GDS:** Geriatric Depression Scale

**Glx:** Glutamine/glutamate

**GM:** Grey matter

- Hb:** Haemoglobin
- HMPAO:** Hexamethyl propylenamine oxime
- HVLT:** Hopkins verbal learning test
- ILF:** Inferior longitudinal fasciculus
- JHU:** John Hopkins University
- LB:** Lewy body
- LBD:** Lewy body dementias
- LN:** Lewy neurites
- MATLAB:** Matrix Laboratory computer package, published by Math-Works
- MCI:** Mild cognitive impairment
- MD:** Mean diffusivity
- mI:** Myo-inositol
- MIBG:** <sup>123</sup>I-metaiodobenzyl guanidine
- mM:** Millimolar
- MMSE:** Mini-mental state examination
- MNI:** Montreal Neurological Institute
- MR:** Magnetic Resonance
- MRI:** Magnetic Resonance Imaging
- MTA:** Medial temporal atrophy
- MTL:** Medial temporal lobe
- MTR:** Magnetisation transfer ratio
- n:** Number of subjects.
- NA:** Not applicable.
- NAA:** N-acetyl aspartate
- NART:** National adult reading test
- NC:** Normal control
- NFT:** Neurofibrillary tangles

**NIA:** National Institute of Ageing

**NINCDS-ADRDA:** National Institute of Neurological and Communicative Diseases and Stroke and the Alzheimer's Disease and Related Disorders Association

**NMDA:** N-methyl D-aspartate

**NPI:** Neuropsychiatric Inventory

**ns:** not significant

**p value:** Probability value

**PD:** Parkinson's disease

**PDD:** Parkinson's disease dementia

**PET:** Positron emission tomography

**PGSE:** Pulsed Gradient Spin Echo

**PIB:** Pittsburgh Compound-B

**PIGD:** Postural instability gait difficulty

**PSEN:** Presenilin

**PSP:** Progressive supranuclear palsy

**PVH:** Periventricular hyperintensities

**RBD:** Rapid eye movement sleep behavior disorder

**REM:** Rapid eye movement

**ROI:** Region of interest

**RVH:** Recurrent visual hallucinations

**SD:** Standard deviation

**SPECT:** Single photon emission computerised tomography

**SPM:** Statistical Parametric Mapping

**SRT:** Simple reaction time

**T:** Tesla

**T1:** Spin-lattice relaxation time

**T2:** Spin-spin relaxation time

- TBSS:** Tract based spatial statistics
- TD :** Tremor dominant
- TFCE:** Threshold-free cluster enhancement
- TIV:** Total intracranial volume
- VaD:** Vascular dementia
- VBM:** Voxel based morphometry
- VH:** Visual hallucinations
- Vig:** Digit vigilance
- VOI:** Voxel of interest
- WM:** White matter
- WMH:** White matter hyperintensities

## **Chapter 1 Introduction**

Dementia currently affects approximately 7% of those over the age of 65 and 30% of people over the age of 80 and it is estimated that the prevalence of dementia will double over the next 30 years (Knapp and Prince, 2007). In the UK alone, it is estimated that over a million people will be affected by 2025. Dementia is associated with significant morbidity and mortality and has therefore become a national health and social care priority.

Dementia has many forms, of which Alzheimer's disease (AD) and dementia with Lewy bodies (DLB) are the most common. Distinguishing between AD and DLB can be difficult during life as clinical symptoms often overlap. However, establishing an accurate clinical diagnosis is important in order to improve patient care. Additional markers are therefore required, which, when combined with clinical information can improve diagnostic accuracy.

Neuroimaging can investigate both brain structure and function. Magnetic resonance imaging (MRI) offers a non-invasive method to study cerebral changes in more detail. It can provide highly detailed information about the structure of the brain without using ionizing radiation, and has been utilised in studies of AD and DLB. MR technology and methods of analysis continue to improve with MRI becoming a more powerful tool for detecting neuronal structural and functional change. Diffusion tensor imaging is a more recently developed MRI technique which reflects the white matter tracts, the microstructure of the brain. It has potential to be more sensitive to early and possibly preclinical change. By investigating the structural and micro-structural alterations of these conditions and studying the neurobiological correlates we can add to the weight of clinical information and potentially improve the diagnostic accuracy.

This thesis describes a study in which structural and diffusion MR was used to investigate brain changes in DLB and AD subjects together with a group of similarly aged controls.

## 1.1 Overview of Thesis Contents.

**Chapter 1.** Introduction – describes DLB and AD, outlining their aetiology, clinical features, pathological changes and management.

**Chapter 2.** MRI in Lewy body dementias – provides a review of the MRI literature in Lewy body dementias. Structural imaging changes are presented, then diffusion tensor imaging, proton magnetic spectroscopy and functional MRI.

**Chapter 3.** Study aims – outlines the aims, objectives and hypotheses of the research undertaken in this thesis.

The following 3 chapters present and discuss the results of the study.

**Chapter 4.** Clinical and neuropsychological features of DLB – presents the methods of study recruitment and provides a detailed account of the clinical and neuropsychological features of the study cohort.

**Chapter 5.** Structural imaging changes in DLB – focuses on the patterns of MRI grey matter atrophy in DLB compared to AD and age-matched controls using voxel based morphometry analysis methods.

**Chapter 6.** Diffusion tensor imaging in DLB – focuses on MRI white matter tract changes in DLB compared to AD and age-matched controls using tract based spatial statistics analysis method. Correlations with key clinical and neuropsychological data are presented and discussed.

**Chapter 7.** Conclusions and future studies – presents the conclusions drawn from the structural and diffusion imaging studies in assessing DLB, including the study strengths and limitations with a discussion of potential future directions.

## 1.2 Dementia with Lewy bodies

DLB is the second commonest cause of degenerative dementia in older people, only AD being more common. Despite this, its clinical features are poorly understood and under recognition is common. An accurate clinical diagnosis is important in order to improve individual patient care and is essential for furthering research into the disorder.

This section summarises the clinical and pathological features of DLB as well as the current management strategies. The diagnostic difficulties between DLB and AD and the relationship between DLB and Parkinson's disease dementia (PDD) will also be discussed.

### 1.2.1 Epidemiology

Prevalence estimates of DLB rely on referrals to specialty health service providers or autopsy case series and vary from between 15% to 35% of cases of dementia, suggesting that it is the second most common form of dementia (McKeith et al., 2004). In a UK community based prevalence study of dementia subtypes in those over 65, Stevens *et al.* (2002) reported that almost 10% of the sample fulfilled consensus criteria for probable DLB and 30.5% for either possible or probable DLB.

DLB is more common in men and the average age of onset is 75 years, although there is wide variation (Geser et al., 2005). Dementia is the most frequent clinical presentation, although psychiatric symptoms; including visual hallucinations, delusions and depression and autonomic dysfunction and falls can also be early features (McKeith et al., 2004).

Overall the disease progresses at a rate comparable to that seen in AD. A study by Ballard *et al.* (2001b) of patients referred to Old Age Psychiatry dementia services, showed that the rate of cognitive decline was approximately 4 mini-mental state examination (MMSE) points and 12-14 Cambridge Cognitive Assessment (CAMCOG) points per year in AD, DLB and vascular dementia with no significant differences between the groups. However, more recent studies suggest greater functional decline, resource use and reduced quality of life in DLB compared to AD (Bostrom et al., 2007b; Bostrom et al., 2007a). Parkinsonism tends to worsen at a similar rate to that seen in PD, approximately 10% per year and the average survival time is also similar to



that of AD with some DLB patients showing a more rapid progression and death within 1-2 years of onset (Geser et al., 2005).

### 1.2.2 Clinical features

Internationally agreed clinical diagnostic criteria for DLB were first established by consensus in 1996 following the publication of clinical features characteristic of the disorder which was based on several different case series of DLB subjects (McKeith et al., 1996). The criteria were then validated by several groups and generally found to have high specificity, up to 100% in larger referral centres, although they lacked sensitivity meaning that the diagnosis will be missed in many cases (up to 50%) during life (Litvan et al., 2003). This was the main reason that they were revised in 2005, with the aim to help improve case detection (see summary Tables 1.1 and 1.2) (McKeith et al., 2005; McKeith et al., 1996).

The initial consensus criteria included the central feature of progressive cognitive decline of sufficient magnitude to interfere with social or occupational function, along with a triad of core clinical features of the condition: (1) recurrent visual hallucinations, (2) cognitive fluctuation and (3) spontaneous motor parkinsonism, with at least 2 of the core features required to make a clinical diagnosis of probable DLB (Table 1.1).

---

#### *Central Feature (essential)*

---

- Dementia defined as progressive cognitive decline of sufficient magnitude to interfere with normal social or occupational function.
- 

#### *Core Features*

**Probable DLB – 2 core features**

**Possible DLB – 1 core feature**

---

- Fluctuating cognition with pronounced variations in attention and alertness
  - Recurrent visual hallucinations
  - Spontaneous features of parkinsonism
- 

#### *Supportive features*

---

- Repeated falls
  - Syncope
  - Transient loss of consciousness
  - Neuroleptic sensitivity
  - Systematised delusions
  - Hallucinations in other modalities
- 

Table 1.1. Consensus Criteria for the Clinical diagnosis of dementia with Lewy bodies (McKeith et al., 1996).

In the revised version, the 3 core features remain unchanged, however, greater weighting has been given to neuroleptic sensitivity reactions, reduction of the dopamine transporter on imaging and the presence of rapid eye movement (REM) sleep behaviour disorder by including them as ‘suggestive’ features (Table 1.2), one of which (in the presence of at least one core feature) is now sufficient for a diagnosis of probable DLB.

<b><i>Central Feature (essential)</i></b>
➤ Dementia defined as progressive cognitive decline of sufficient magnitude to interfere with normal social or occupational function.
<b><i>Core Features</i></b>
<b>Probable DLB – 2 core features</b>
<b>Possible DLB – 1 core feature</b>
➤ Fluctuating cognition with pronounced variations in attention and alertness
➤ Recurrent visual hallucinations
➤ Spontaneous features of parkinsonism
<b><i>Suggestive features</i></b>
<b>Probable DLB – 1 or more suggestive feature with 1 or more core feature</b>
<b>Possible DLB – 1 or more suggestive feature</b>
➤ REM sleep behaviour disorder
➤ Severe neuroleptic sensitivity
➤ Low dopamine transporter uptake in basal ganglia (SPECT or PET)
<b><i>Supportive features</i></b>
➤ Repeated falls and syncope
➤ Transient unexplained loss of consciousness
➤ Severe autonomic dysfunction
➤ Hallucinations in other modalities
➤ Systematised delusions
➤ Depression
➤ Relative preservation of medial temporal lobe structures on CT/MRI scan
➤ Generalised 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

Table 1.2. Revised Consensus Criteria for the diagnosis of DLB (McKeith et al., 2005).

The addition of disease biomarkers to the revised criteria was an important step forward. Abnormal dopamine transporter imaging is extremely helpful to distinguish between AD and DLB. However, it remains somewhat limited by its sensitivity, which peaks at approximately 80% (McKeith et al., 2007), as well as possible inaccuracy of the gold standard (clinical consensus criteria) against which the biomarker has been judged, the exception being the studies of Walker and Walker (2009) who found 100% sensitivity

when judged against autopsy. This may, in part, be because not all DLB cases have loss of the dopamine transporter. Preservation of hippocampal and medial temporal lobe volume on MRI (Burton et al., 2009; Whitwell et al., 2008; Barber et al., 1999a) and occipital hypoperfusion and hypometabolism without occipital atrophy on MRI imaging have been consistent findings in imaging studies of DLB (Middelkoop et al., 2001; Ishii et al., 1999; Imamura et al., 1997). These were incorporated into the revised criteria for DLB as supportive features as the findings currently lack diagnostic specificity. Scintigraphy with  $^{123}\text{I}$ -metaiodobenzyl guanidine (MIBG) is a method that can measure postganglionic sympathetic cardiac innervation. This has been shown to be reduced in DLB compared with AD and suggested to be more reliable than orthostatic testing, although further validation studies are indicated (Idiaquez and Roman, 2011; King et al., 2011; Treglia and Cason, 2010).

Further development of disease biomarkers is therefore needed, which, when combined with the clinical features of the DLB syndrome may help improve case detection (Sinha et al., 2011).

### ***1.2.2.1 Differential Diagnosis of DLB***

The main differential diagnoses are (1) other dementias (most especially AD), (2) delirium, (3) the spectrum of other disorders with parkinsonism, such as progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD) or (4) those with psychiatric features such as delusional disorders or depression with psychosis.

### ***1.2.2.2 Lewy body dementias – the relationship between DLB and PDD***

Dementia is a frequent complication of PD. The risk of progression to dementia increases with age and affected over 70% of PD patients in an 8 year prospective longitudinal study, six times the rate of healthy controls (Aarsland et al., 2003). The clinical and neuropathological phenotype of DLB and PDD are similar with no specific features reliably separating the conditions (Lippa et al., 2007). Since the discovery of alpha synuclein the relationship between PD, DLB and PDD has been able to be further established and they probably represent differing points along a spectrum of Lewy body disorders (Lippa et al., 2007).

Given the similarity between DLB and PDD, the so called ‘1-year rule’ between the development of motor symptoms and dementia was established by consensus in order to

separate the two conditions (McKeith et al., 2005). The diagnosis of PDD is appropriate where dementia develops in established PD and DLB where cognitive impairment precedes or coincides within a year of the development of motor parkinsonism (McKeith et al., 2005). This rather arbitrary approach was deemed necessary particularly for clinical research purposes so as not to confound data pooling and allow for comparison across studies (McKeith et al., 2005). It is also a useful clinical descriptor for two disorders that can have very different initial clinical presentations. In pathological studies, a collective approach including both PDD and DLB in a Lewy body dementia or  $\alpha$ -synucleinopathy group may be more appropriate (McKeith et al., 2005). In terms of clinical practice, it has been recommended that strict application of the 1-year rule may not be helpful and that the most predominant and problematic symptom (dementia or movement disorder) might best define the patient (McKeith et al., 2005).

### ***1.2.2.3 Neuropsychological features***

Typically DLB manifests as recurrent confusion on a background of progressive cognitive decline (McKeith et al., 2004). In the early stages, the cognitive profile is one of predominantly subcortical neuropsychological impairment with significant attentional and visuo-perceptual difficulties (Metzler-Baddeley, 2007). Generally, patients with DLB perform better than those with AD on tests of memory but worse on visuo-spatial performance tasks.

Global measures (MMSE) are usually comparable to AD patients at a similar stage of dementia (McKeith et al., 2003). Patients with DLB however, have disproportionately more severe deficits in tasks of attention and executive function when compared to AD (Collerton et al., 2003).

Cognitive fluctuations represent one of the core features for the clinical diagnosis of DLB and are largely a result of problems with attention (McKeith et al., 2005). Cognitive ability in DLB can vary over minutes, hours, or days and such cognitive fluctuations occur in up to 90% patients (McKeith et al., 1996). Computer-based tasks of attention have detected variations in DLB task performance on a second to second basis, greater than observed in AD (Ballard et al., 2001a). However, accurate detection of cognitive fluctuation in DLB remains a challenge. This was highlighted in a study of the clinical symptoms of DLB which found that it was the most difficult feature to

accurately assess, with low inter-rater reliability reported (Mega et al., 1996). To help address this, scales were developed to try to objectively assess fluctuation and are currently in use (Ferman et al., 2004; Walker et al., 2000b).

Impaired visual perception is a consistent finding in DLB (Metzler-Baddeley, 2007). To quantify this, Mosimann *et al.* (2004) aimed to quantify visual discrimination, space-motion and object form perception in patients with DLB, PDD and AD matched for overall dementia severity. They found that all aspects of visuo-perceptual function were impaired in DLB and PDD subjects who performed worse than patients with AD. Patients with DLB and hallucinations were also more impaired on the visual perception tasks than those without hallucinations (Mosimann et al., 2004).

Language can also be affected, manifest early as difficulty verbalizing simple words which may relate to fronto-subcortical dysfunction. In the later stages of the disease, deficits are more global and can be indistinguishable from AD (Geser et al., 2005).

#### ***1.2.2.4 Neuropsychiatric features***

Visual hallucinations are common in patients with DLB (McKeith et al., 2005). The visual hallucinations are quite characteristic; typically recurrent, highly detailed and often involve children or animals. The reaction of a person with DLB experiencing hallucinations can vary widely from indifference to highly distressing (McKeith et al., 2004). The misidentification of objects or visual illusions is also common and often overlaps with visual hallucinations, suggesting a common mechanism.

Other neuropsychiatric features that occur in patients with DLB include auditory hallucinations, delusions, depression, anxiety and apathy (McKeith et al., 2005).

#### ***1.2.2.5 Motor features***

Motor parkinsonism is a syndrome of bradykinesia, rigidity, rest tremor and gait and postural reflex abnormalities. It is a prominent feature of DLB, occurring in up to 75% of patients (McKeith et al., 2005). Severity of parkinsonism in DLB is similar to that of idiopathic PD, however, the subtypes differ (Burn et al., 2006; Burn et al., 2003). In DLB, parkinsonism is typically axial with prominent gait abnormality and postural instability, commonly referred to as the postural instability gait difficulty (PIGD) subtype (Burn et al., 2003). Tremor is also usually bilateral and less pronounced than in PD rather than the asymmetrical tremor dominant (TD) subtype seen more commonly in

PD (McKeith et al., 2004). Burn *et al.* (2003) found that the PIGD subtype account for 69% of DLB cases, significantly more than in PD (38%).

#### **1.2.2.6 Sleep disorders**

REM sleep behaviour disorder (RBD) is a parasomnia which can precede the onset of dementia or parkinsonism by many years (Boeve, 2010; Turner, 2002). It involves the loss of normal skeletal muscle atonia during REM sleep which can be confirmed clinically by polysomnography (Boeve, 2010). A suggestive history is usually obtained from the patient's partner. Patients appear to be acting out their dreams, flailing, vocalizing and moving about the bed, sometimes violently. RBD is usually associated with an underlying synucleinopathy – PD, DLB or Multisystem Atrophy and rarely other neurodegenerative diseases (Boeve et al., 2003).

Other relatively common sleep disorders associated with DLB include excessive daytime sleepiness, insomnia, circadian dysrhythmia, obstructive sleep apnoea, restless legs, nightmares and confusion on waking (Boeve, 2005; McKeith et al., 2004).

#### **1.2.2.7 Autonomic dysfunction**

Autonomic dysfunction is relatively common in patients with DLB (O'Brien et al., 2006). Frequent clinical features include problems with bladder and bowel function and orthostatic hypotension (Horimoto et al., 2003). Syncope and falls are included as supportive features for the clinical diagnosis of DLB and may be contributed to by underlying autonomic dysfunction (McKeith et al., 2005).

### **1.2.3 Neuropathology**

#### **1.2.3.1 Lewy related pathology**

Definitive diagnosis of DLB rests on pathological verification of the presence of Lewy body pathology which includes Lewy bodies (LB) and Lewy neurites (LN), both of which contain aggregates of the synaptic protein,  $\alpha$ -synuclein. 'Classical LB' are typically found in the brainstem and have a hyaline core and pale halo (Figure 1.1). The 'Cortical LB' are composed only of filaments without an obvious core (Figure 1.2). The most specific method of detecting Lewy related pathology is using immunohistochemistry with anti- $\alpha$ -synuclein antibodies (Gomez-Tortosa et al., 2000).

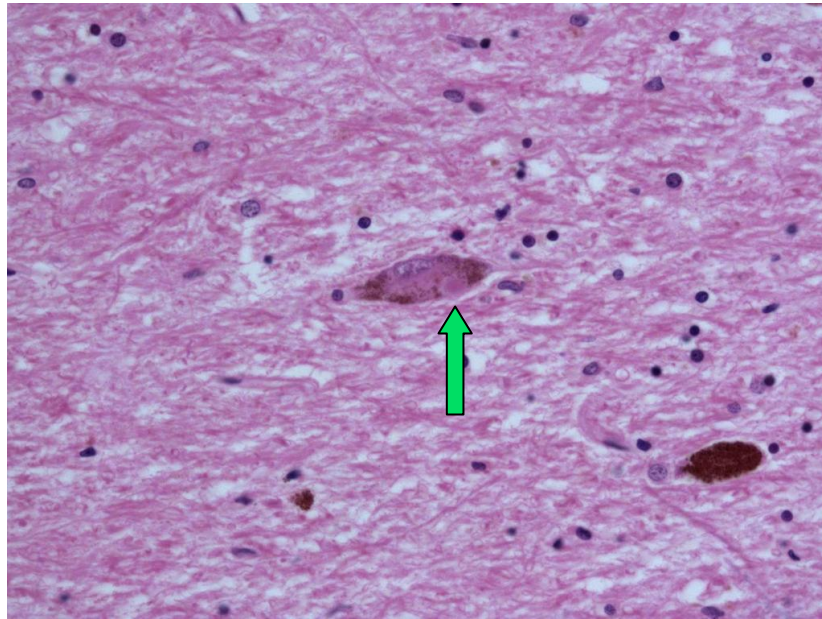


Figure 1.1. Classic Lewy body (green arrow) in the Substantia Nigra, X400 High Power Field, haematoxylin and eosin staining.\*

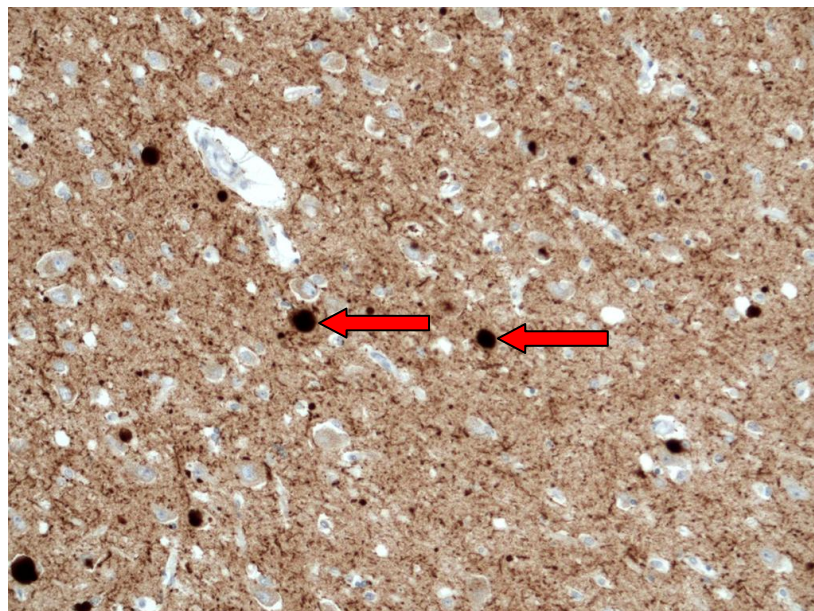


Figure 1.2. alpha-synuclein immunohistochemistry X200 High Power Field. Cortical Lewy bodies (red arrows) and diffuse Lewy neurites (brown staining background) in the cingulate gyrus.\*

\*Images represented in Figure 1.1 and 1.2 above were obtained from Dr Johannes Attems, Institute for Ageing and Health and have not been published elsewhere.

### 1.2.3.2 *Other pathological features of DLB*

Other pathological features associated with DLB include regional neuronal loss, particularly brainstem and the nucleus basalis of Meynert, plaques and neurofibrillary tangles and spongiform change (microvacuolation) which occurs predominantly in the visual association areas and temporal cortex (Kovari et al., 2009).

### 1.2.3.3 *The Braak Hypothesis*

Lewy body pathology is also seen in other degenerative disorders including Parkinson's disease and multiple system atrophy which share some of the clinical features of DLB and are referred to as synucleinopathies (Jellinger, 2003).

The accumulation of  $\alpha$ -synuclein within neuronal cells is associated with dysfunction and cell death although the process is not clear. Projection neurons with long, thin unmyelinated axons are more commonly affected, which suggest that the process of neuronal damage in Lewy body disease follows certain rules (Braak et al., 2004). Braak *et al.* (2003) proposed a scheme to stage Lewy related pathology in PD. They hypothesized that the accumulation of  $\alpha$ -synuclein begins in the dorsal motor nucleus of the vagus in the lower brainstem and progresses in an ascending fashion to the limbic and neocortex in a caudo-rostral distribution. It was suggested that the clinical features of the disease also mirror this, although with differing individual thresholds to manifest symptoms, and devised a pathological staging system to reflect this: (i) preclinical (Stages 1-2); (ii) early (Stages 3-4) and (iii) late (Stages 5-6).

It was therefore suggested that  $\alpha$ -synuclein pathology might behave in a "prion-like" manner, with between cell transfer of the abnormal protein, although without the infectivity properties of a prion disease (Angot et al., 2010). However, recent studies have found that up to 49% of DLB cases do not follow the distribution of the Braak hypothesis (Zaccai et al., 2008; Kovari et al., 2003; Gomez-Tortosa et al., 1999) and among AD cases with Lewy body pathology, 70% did not show lesions in the medulla oblongata (Jellinger, 2003).

Parkkinen *et al.* (2008) also found that more than half of their subjects with Braak stage 5-6 were unaffected by cognitive impairment or parkinsonism. This indicated that some people were able to tolerate a significant burden of synuclein pathology. This perhaps supports the view that the main pathological culprit is the small synaptic aggregates of



synuclein (Kramer and Schulz-Schaeffer, 2007) rather than Lewy bodies, which may potentially be neuroprotective (Tanaka et al., 2004).

#### ***1.2.3.4 Amygdala Lewy bodies***

$\alpha$ -synuclein aggregates are not specific to the Lewy body spectrum of disease. LB confined to the amygdala are seen in around 50% of cases of AD (Lippa et al., 2005; Popescu et al., 2004). Findings from neuropathological studies suggested the possibility that the presence of tau pathology may modify the distribution pattern of  $\alpha$ -synuclein with accumulation beginning in the amygdala if associated with tau pathology (Arai et al., 2001). Arguing against this however are (1) reports of  $\alpha$ -synuclein occurring in the amygdala with relative sparing of the substantia nigra in DLB cases with mild AD pathology (Tsuboi et al., 2007) and (2) the finding in one study that the progression pattern of Lewy related pathology was not associated with AD pathology (Zaccai et al., 2008).

#### ***1.2.3.5 The role of Alzheimer's pathology in DLB***

Most cases with cortical LB also show some associated Alzheimer pathology (tau-based neurofibrillary tangles and neuritic plaque) which modifies the DLB clinical syndrome. In cases where the burden of tau-based Alzheimer pathology is high, the key clinical features of DLB are likely to be obscured (McKeith et al., 2005). Therefore, the sensitivity of the clinical diagnostic criteria is much lower than in cases with more concomitant tau-based pathology highlighting the need for improved methods of case detection. The effect of diffuse amyloid plaques as opposed to neuritic plaques to cognitive impairment in DLB is less clear (McKeith et al., 2005).

#### ***1.2.3.6 Consensus criteria for the pathological diagnosis of DLB***

The pathological criteria for diagnosis of DLB were again revised in 2005 to help address some of the boundary issues between AD and DLB. The use of the National Institute of Ageing (NIA) – Reagan criteria for defining the significance of concurrent AD pathology in DLB was determined by consensus which require the presence of both tau-based neuritic plaques and neurofibrillary tangles and not diffuse amyloid plaques. Lewy body type pathology are assigned based on a semiquantitative scale (McKeith et al., 2005).

Reflecting the fact that DLB, like AD, is a clinico-pathological syndrome the pathological diagnosis was represented as a probability statement about “*the likelihood that the observed neuropathologic findings predict the clinical syndrome of DLB*”. That is, “*the likelihood that the observed neuropathology explains the DLB syndrome is directly related to the severity of the Lewy-related pathology and inversely related to the severity of concurrent AD type pathology*” (McKeith et al., 2005) (represented in Table 1.3).

	<b>Alzheimer Type Pathology</b>		
	NIA-Reagan Low (Braak stage 0-II)	NIA-Reagan Intermediate (Braak stage III-IV)	NIA-Reagan High (Braak stage V-VI)
<b>Lewy body type pathology</b>			
Brainstem predominant	Low	Low	Low
Limbic (transitional)	High	Intermediate	Low
Diffuse neocortical	High	High	Intermediate

Table 1.3. Assignment of the likelihood that the pathologic findings are associated with the DLB clinical syndrome, (McKeith et al., 2005).

### ***1.2.3.7 Clinicopathological Correlations***

#### *Global cognition*

Cognitive impairment has been found to correlate with the burden of cortical Lewy bodies in the frontal and temporal lobes in one study (Harding and Halliday, 2001).

#### *Visual hallucinations*

Interestingly, there are almost no LBs and only sparse LNs in the primary visual cortex but more numerous lesions have been reported in the visual association cortices, especially the posterior occipitotemporal gyrus. DLB cases with visual hallucinations were found to have increased Lewy body numbers in the parahippocampal gyrus and amygdala with early hallucinators having higher densities in the parahippocampal gyrus and inferior temporal cortex (Harding et al., 2002).

#### *Parkinsonism*

The striatum is an area typically affected in DLB and often results in motor parkinsonism. The Lewy body density in the substantia nigra has been found not to differ in DLB patients who develop parkinsonism early or later in the course of the disease (Gomez-Tortosa et al., 1999). Neuronal loss in the substantia nigra has been

observed in DLB, although neuronal loss in the putamen is perhaps less than in PD. Despite this, similar levels of dopamine deficiencies are seen in both DLB and PD and are sufficient to cause motor parkinsonism (Piggot et al., 1999).

#### *Cognitive fluctuation, sleep and autonomic dysfunction*

Attentional dysfunction and cognitive fluctuation are key features of DLB and are thought to be mediated in part, by cholinergic system degeneration (Ballard et al., 2001a). The nucleus basalis of Meynert, located in the substantia innominata is a major source of cholinergic innervation of the neocortex and is subject to greater neuronal loss in DLB than AD as well as extensive Lewy related pathology (Lippa et al., 1999). This may have an effect on attention as well as global cognitive function.

Neuronal loss has been reported in the pedunculopontine nucleus in Lewy body disease. This may contribute to the development of REM sleep behaviour disorder and, given the cholinergic inputs to the thalamus, an important structure in the maintenance of attention, may contribute to attentional problems in DLB (Boeve, 2010). Neuropathological correlates of autonomic dysfunction are likely to include neuronal loss and Lewy-related pathology in the autonomic nuclei of the brainstem and sympathetic and parasympathetic ganglia (Idiaquez and Roman, 2011).

#### **1.2.4 Neurochemistry**

##### ***Acetylcholine***

Cholinergic deficiencies (cholinergic neurons and choline acetyl transferase activity) in DLB are greater than those found in AD. Areas affected include neocortical areas as well as the basal ganglia, including the striatum and the pedunculopontine pathway that project to the thalamus (Francis and Perry, 2007). Postsynaptic muscarinic receptors have also been shown to be upregulated and more functionally intact in DLB compared with AD. This may, in part, explain why patients with DLB benefit from treatment with cholinesterase inhibitors (Francis and Perry, 2007; McKeith et al., 2000b). Nicotine binding is also reduced by as much as 70% in the substantia nigra in DLB. Similar reductions are seen in PD; however this seems mainly due to neuronal loss. In DLB patients, neuronal loss is less (40%) and suggests that the loss of cholinergic function precedes neuronal degeneration (Francis and Perry, 2007).

### ***Serotonin***

Lewy bodies occur at the dorsal raphe nucleus and reductions of serotonin levels have been reported in the striatum, neocortex and frontal cortex (Francis and Perry, 2007). Relatively higher binding to the 5HT transporter reuptake site in the parietal cortex has also been shown in DLB patients with major depression compared with those who are not depressed (Francis and Perry, 2007).

### ***Dopamine***

Moderate neuronal loss in the substantia nigra has been found in DLB, although it was less than in PD. Striatal dopamine concentration has also been found to be reduced almost as much as in PD and would be enough to account for the motor features of the disease (Piggot et al., 1999).

In PD there is loss of the dopamine transporter throughout the striatum. In DLB however, the loss is more significant in the caudal (posterior) striatum. Loss of the dopamine transporter in DLB can be measured *in vivo* by FP-CIT SPECT imaging which has been found to be reduced when compared with AD and has become a useful biomarker for distinguishing between the two conditions (O'Brien et al., 2004).

Postsynaptic dopamine receptor changes also differ between PD and DLB. In PD, the D2 receptors are upregulated in response to loss of the dopamine transporter which does not occur in DLB and are reduced in the caudal striatum when compared with controls (Piggot et al., 1999). Given that neuroleptics have dopamine receptor antagonist properties, this may explain the neuroleptic sensitivity reactions seen in DLB (Piggott et al., 2007; Aarsland et al., 2005). The thalamus also has D2 and D3 dopamine receptors which may influence cognitive fluctuation.

## ***1.2.5 Management of DLB***

The management of DLB is largely symptomatic and should be multi-disciplinary and involve consideration of both pharmacological and non-pharmacological strategies.

### ***1.2.5.1 Acetylcholinesterase inhibitors***

Given that cognitive impairment in patients with DLB has been correlated with cortical cholinergic dysfunction and reductions in choline acetyltransferase, a number of studies have assessed the impact of cholinesterase inhibitors.

McKeith *et al.* (2000b) reported therapeutic effects of rivastigmine from a multicentre, placebo-controlled double blinded trial in 120 patients with probable DLB. They found that the rivastigmine treated group were less apathetic, had less anxiety and they also had fewer delusions and hallucinations. Hallucinations and psychotic features resolved almost completely in over half the patients on rivastigmine. Decreased choline acetyltransferase activity in the temporal lobe has also been shown to correlate with the presence of visual hallucinations in DLB (Francis, 2009) and the alleviation of this symptom in response to cholinergic replenishment supports the association. Open label studies using galantamine, donepezil and tacrine in DLB also reported similar benefits but also found positive effects on cognition with a median MMSE improvement of 2.7 (Aarsland *et al.*, 2004). Although McKeith *et al.* (2000b) did not find a significant change in MMSE, later analysis showed that attention did improve (Wesnes *et al.*, 2002). The pedunclopontine nucleus is subject to degeneration in DLB and provides cholinergic input to the thalamus, an important structure for maintenance of attention. Given the improvement in attention with cholinesterase inhibitors, it is possible that this is an important component of attentional dysfunction and cognitive fluctuation in DLB.

The beneficial effects of these medications however, were lost after discontinuation, suggesting they were due to a symptomatic benefit rather than modification of the disease process (McKeith *et al.*, 2000b).

Over 75% of patients experience side effects particularly when starting cholinesterase inhibitors but they are usually of mild to moderate severity with approximately 10% of patients needing to stop the medication because of the side effects (Bhasin *et al.*, 2007).

Cholinesterase inhibitors now form the mainstay of treatment for cognitive symptoms and psychosis in DLB (Aarsland *et al.*, 2004).

#### **1.2.5.2 Memantine**

Memantine is an N-methyl D-aspartate (NMDA) receptor antagonist and has recently been trialled for use in DLB (Emre *et al.*, 2010; Aarsland *et al.*, 2009). Changes in markers of neuronal glutamatergic activity have been reported in DLB and memantine acts to block the effects of raised glutamate levels (Dalfo *et al.*, 2004). There have been two randomised controlled trials of memantine in DLB which found improvement in the 'clinical global impression of change' (CGIC) scale (Emre *et al.*, 2010; Aarsland *et al.*, 2009). In the larger study by Emre *et al.* (2010) they also reported improvement in the

Neuropsychiatric Inventory (NPI). In the DLB group, the improvements in the NPI were in the domains of delusions, hallucinations, sleep and appetite or eating. However, in contrast to Aarsland *et al.* (2009) the participants were not able to take cholinesterase inhibitors. Memantine did not worsen symptoms and was also well tolerated. Overall, memantine might have some mild beneficial effects in global clinical status and behavioural symptoms and may be a treatment option for some patients with DLB.

### ***1.2.5.3 Treatment of Psychotic symptoms***

Psychotic symptoms, particularly visual hallucinations and delusions, are common in patients with DLB with as many as two-thirds of patients experiencing quite prominent psychotic symptoms at some stage (McKeith *et al.*, 2004). Treatment however, presents a therapeutic challenge. In general, psychotic symptoms and behavioural problems in dementia are managed with neuroleptics (antipsychotics), but these drugs need to be used with caution in older people with dementia because of increased risk of stroke and stroke-like events and an increased mortality (Sacchetti *et al.*, 2010). In DLB the dangers are even more extreme as they can (and often do) worsen motor parkinsonism and precipitate fatal sensitivity reactions in up to 50% of patients with DLB (Aarsland *et al.*, 2005). Neuroleptic sensitivity reactions are only seen in patients with DLB and include sudden worsening of parkinsonism, confusion and impaired consciousness (Ballard *et al.*, 1998; McKeith *et al.*, 1992).

Adverse effects also occur with use of the newer antipsychotic agents with worsening of parkinsonism reported after olanzapine and risperidone (Poewe, 2005). There is currently only anecdotal evidence to suggest severe adverse reactions following use of clozapine (Burke *et al.*, 1998) despite its efficacy in psychosis associated with PD with no worsening of parkinsonism or global cognition (Weintraub and Hurtig, 2007). Therefore, cholinesterase inhibitors are the first choice and antipsychotics are reserved as second line therapy in DLB. Antipsychotic treatment in DLB should be initiated by specialists using atypical agents at low dose with frequent and careful monitoring.

### ***1.2.5.4 Parkinsonism***

Treatment of the motor features of parkinsonism in patients with DLB follows the same principles as in idiopathic Parkinson's disease. However, patients with the PIGD subtype are generally less responsive to levodopa when compared with the TD subtype (Sethi, 2008). Given that the PIGD subtype is more common in DLB, the overall

effectiveness of levodopa on motor disability would be expected to be less pronounced when compared with PD. Only limited studies have been undertaken investigating treatment response of motor features of DLB. Both Bonelli *et al.* (2004) and Molloy *et al.* (2005) showed a significant response to L-dopa in approximately one-third of subjects with DLB, less than in PD but still a reasonable proportion who benefit from treatment.

#### **1.2.5.5 Depression, REM sleep behaviour disorder and Autonomic failure**

Depression is common in DLB however, there are no studies assessing the effectiveness and safety of an agent targeting depression specifically in DLB. The choice of antidepressant is therefore based on patients without parkinsonism (Fernandez *et al.*, 2003).

Sleep disorders are common and difficult to effectively treat. The treatment of RBD with clonazepam should be considered when there is the potential for harm of the patient or their partner (Boeve, 2010).

Treatment of autonomic failure has not been specifically assessed in DLB. The options are therefore similar to those available for the general population. For orthostatic hypotension these include conservative measures, compression stockings, or therapeutic options such as fludrocortisone or midodrine ( $\alpha$ 1 agonist) (Gupta and Nair, 2008). Consideration should be given to referring to a multidisciplinary falls and syncope service for further falls risk factor modification and management (O'Brien *et al.*, 2006).

Anticholinergic medications are often prescribed for urinary tract symptoms of detrusor overactivity, which can occur in autonomic failure (Sahai *et al.*, 2006). However, the treating clinician should be aware of the potential for worsening cognition and consider more selective agents that do not cross the blood brain barrier such as trospium and darifenacin.

#### **1.2.6 Summary**

The clinical and pathological characteristics of DLB have been operationalised as far as is possible on the current evidence base to assist with accurate clinical diagnosis and also allow for more robust case detection of DLB for research purposes. However there remains significant pathological overlap between the different dementia subtypes, particularly the boundary between DLB and AD. Alzheimer pathology modifies the

clinical expression of DLB symptoms. Therefore, in cases of DLB with significant co-existent Alzheimer pathology, clinical assessment methods will not be sensitive enough to detect DLB. We need to develop techniques which will aid our understanding of the underpinnings of the clinical features of DLB and develop biomarkers to assist with improving the case detection of DLB.

### **1.3 Alzheimer's disease**

Alzheimer's disease is the most common cause of dementia (Blennow et al., 2006). As already discussed in section 1.2.3.5, there is significant overlap between AD and DLB with AD pathology modifying the clinical features of DLB. The focus of this section is to provide a summary of the clinical and pathological features of AD. This is not an exhaustive review but rather its aim is to provide a framework to assist in the discussion surrounding comparisons with DLB.

#### ***1.3.1 Clinical features***

AD is the leading cause of dementia and accounts for approximately 50-60% of dementia cases (Blennow et al., 2006). It is defined clinically by an insidious onset and progression of cognitive impairment. Diagnostic criteria require that two or more cognitive domains are affected (McKhann et al., 1984). In contrast to DLB, it is typically characterized by early and prominent impairment in episodic memory. Other signs include dysphasia, dyspraxia and agnosia along with other cognitive symptoms such as impaired orientation and judgement (Dubois et al., 2007; McKhann et al., 1984)

The criteria for the National Institute of Neurological and Communicative Diseases and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) are commonly used for the clinical diagnosis of AD (McKhann et al., 1984). The criteria for 'probable AD' rely largely on exclusion of other dementias.

#### ***1.3.2 Risk factors and associations***

Ageing is the most significant association factor. Findings from epidemiological studies suggest the concept of reduced brain reserve which includes reduced brain volume, low



educational or occupational level and reduced mental activity during late life (Blennow et al., 2006). Other risk factors include head injury and vascular risk factors (Blennow et al., 2006).

### **1.3.3 Genetics**

AD has both sporadic and familial forms. Familial AD is rare, with an estimated prevalence of less than 0.01% (Harvey et al., 2003). It is an autosomal dominant form of AD with disease onset before the age of 65 years. Causative gene mutations include mutation of the amyloid precursor protein (APP) gene on chromosome 21 and presenilin 1 (PSEN1) and presenilin 2 (PSEN2) genes (Blennow et al., 2006).

There is an association between apolipoprotein E (ApoE)  $\epsilon$ 4 allele and AD, accounting for most of the genetic risk in sporadic AD (Raber et al., 2004). ApoE acts as a cholesterol transporter in the brain and is less efficient than the other apolipoprotein forms in the neuronal repair process. Carrying the gene increases an individual's risk by lowering the age of onset by almost 10 years for each allele copy. Heterozygotes carry an approximately 3-fold increased risk of the disease and homozygotes, a 15-fold increased risk (Bekris et al., 2010). A number of other AD genes, each with a small effect compared to ApoE have recently been described (Hollingworth et al., 2011; Jones et al., 2010). Genes involved include clusterin (ApoJ) and those involved with synaptic function and inflammation (Hollingworth et al., 2011; Jones et al., 2010).

### **1.3.4 Pathological features**

The characteristic features of AD include senile or neuritic plaques and neurofibrillary tangles in medial temporal lobe structures and other cortical areas. This is often associated with neuronal and synaptic degeneration and loss.

#### ***Amyloid plaques***

The *amyloid cascade hypothesis* suggests that the  $\beta$ -amyloid ( $A\beta$ ) plaques begin from the abnormal processing of the amyloid precursor protein which leads to an imbalance between the production and clearance of  $\beta$ -amyloid from the cortex (Hardy and Selkoe, 2002). It has been suggested that the  $A\beta$  accumulation triggers the abnormal

accumulation of tau, although the underlying processes are unknown. This then results in neuronal and synaptic dysfunction and cell death which is linked with the clinical manifestations of dementia (Terry et al., 1991).

The finding that familial AD is associated with mutations in the APP gene itself or presenilin genes which encode protease subunits (gamma secretase) and people with Down's syndrome carry an extra APP gene (located on chromosome 21) and develop the plaques earlier in life provide support for this hypothesis.

### ***Neurofibrillary Tangles***

Neurofibrillary tangles (NFT) are composed of hyperphosphorylated tau protein. The normal function of tau is in the assembly and stabilization of neuronal cell microtubules. In AD, hyperphosphorylation begins intracellularly and tends to aggregate into insoluble fibrils and tangles (NFT) which impair neuronal function. Tau pathology begins in the transentorhinal region then hippocampus and amygdala and later, other cortical regions (Ballard et al., 2011). Interestingly, the deposition of NFT and the process of neurodegeneration follow a similar distribution. In contrast, the A $\beta$  plaques are extracellular and occur in a somewhat differing pattern to that of neurodegeneration, although with a degree of overlap.

Clinical features of AD have been found to be related to NFT, rather than plaque formation. However, neurodegeneration and specifically synaptic loss has been found to be the most closely associated with the clinical features of AD (Terry et al., 1991).

### ***1.3.5 Imaging changes***

Structural imaging using CT or MRI is important in the diagnosis of AD to exclude other causes such as subdural haematoma, tumours or extensive vascular lesions.

Cerebral atrophy can also be identified and is largely caused by the loss of neurons and synapses. The progression of atrophy correlates with cognitive decline (Savva et al., 2009). However, atrophy is not specific to AD, so there is significant overlap with other dementias.

Early degenerative change in AD occurs in the medial temporal lobe structures including the entorhinal cortex and hippocampus. MTL atrophy can be identified using

structural MRI and is associated with the presence and severity of AD neuropathology (Gosche et al., 2002; Jack et al., 2002), the clinical features of AD, and is useful for separating those with AD from healthy controls with a greater than 85% diagnostic accuracy (Scheltens et al., 2002).

FDG-PET is used to measure glucose metabolism, an indicator of synaptic activity (Jagust et al., 2007). AD is associated with reduced glucose uptake in the temporoparietal, posterior cingulate and precuneal regions (Jagust et al., 2007) with greater reductions being associated with severity of cognitive impairment (Jack et al., 2010; Minoshima et al., 1997). Reduced blood flow has also been found in these areas using  $^{99m}\text{Tc}$ HMPAO SPECT indicating the susceptibility of temporal and parietal regions to functional change in AD (Dougall et al., 2004).

Amyloid plaques can now be visualized *in vivo* using PIB (Pittsburgh Compound-B) PET enabling further insights into the pathogenesis of AD. In a longitudinal study of AD, mild cognitive impairment (MCI) and healthy controls using PIB-PET and structural MRI, Jack *et al.* (2009) found that the PIB rate of change was small and similar across groups and did not correlate with cognitive or functional measures. Whereas ventricular expansion rate differed by group (AD>MCI>normal) and correlated with cognitive and functional measures (Jack et al., 2009). These findings are supported by neuropathological data.

### **1.3.6 Therapeutics**

Cholinergic degeneration of the basal forebrain in AD can result in deficits in input into the hippocampus and entorhinal cortex, important structures for memory function. Cholinesterase inhibitors have been shown to have symptomatic benefits on cognition in AD with a treatment effect of 2.7 points on the Alzheimer's disease assessment scale – cognitive subscale (ADAS-cog) and 1.4 points on the MMSE. In terms of efficacy, all available cholinesterase inhibitors seem to be similar and a Cochrane Review concluded that they are efficacious in mild to moderate AD (Birks, 2006).

Memantine, a NMDA-receptor antagonist has shown some benefit on cognitive and behavioural symptoms in moderate to severe AD (Raina et al., 2008). Increased glutamatergic activity in AD is thought to impair neuronal function so NMDA-receptor antagonists are thought to protect the neurons from this without preventing

physiological activation. At this time, there is no evidence to support that memantine has any disease-modifying or neuroprotective properties (Raina et al., 2008).

Behavioural symptoms are relatively common in the later stages of AD. These include agitation, aggression and psychosis. Cholinesterase inhibitors are important to consider as they can help alleviate these symptoms. Atypical antipsychotic medications such as risperidone and olanzapine have been shown to have some benefit in reducing aggression, agitation and psychosis. Although patients with AD are not as vulnerable to neuroleptic sensitivity reactions, it is still important to introduce these medications with caution and ensure careful monitoring as they can cause extrapyramidal side effects and also increase a person's risk of cerebral events (Ballard and Corbett, 2010).

### ***1.3.7 Summary***

In summary, AD is characterized by early impairment of episodic memory. The pathological hallmarks include amyloid plaque and neurofibrillary tangles and resultant neurodegeneration with neuronal and synaptic destruction. The imaging features of atrophy, particularly in the medial temporal lobe structures reflect neurodegeneration and correlate with clinical measures of dementia. The functional imaging changes of temporal and parietal hypometabolism and hypoperfusion also reflect the clinical and pathological features of the disease.

The next chapter will focus on structural and functional MRI techniques and findings from MRI studies in Lewy body dementias.

## **Chapter 2 Magnetic Resonance Imaging in Lewy body dementias**

### **2.1 Introduction**

Lewy body dementias (LBD) include dementia with Lewy bodies (DLB) and Parkinson's disease dementia (PDD) and share common clinical and pathological features (Lippa et al., 2007). Given the similarities between DLB and PDD, as described in section 1.2.2.2, the separation between the two conditions has been determined by consensus based on the duration of parkinsonian features relative to the development of dementia; the requirement being less than 12 months for DLB (McKeith et al., 2004). Whilst PDD and DLB are not necessarily the same disorder, they may represent different points on the same, possibly continuous, spectrum of Lewy body disorders (Lippa et al., 2007).

Clinically differentiating DLB from other dementia subtypes can be equally difficult. The consensus criteria for DLB assist with improving the clinical diagnosis although it is limited by the low sensitivity (Litvan et al., 2003). It is therefore important to establish additional markers which, when combined with clinical assessment can improve diagnostic accuracy. Advanced MR techniques can provide a wealth of information including structural changes, microstructural changes through diffusion tensor imaging, tissue energetics and metabolism through spectroscopy and cerebral perfusion. MR neuroimaging in Lewy body dementias may assist with providing pathophysiological information as well as increasing the accuracy of ante-mortem diagnosis.

This chapter reviews the MRI literature in DLB and PDD to determine whether there are characteristic imaging changes that may assist with the diagnosis, and given the clinical and pathological similarities between the conditions, how imaging changes may provide further insight into the relationship between DLB and PDD.

### **2.2 Structural Magnetic Resonance Imaging**

Conventional MRI data has been used extensively in the study of neurodegenerative disorders to examine regional changes in tissue volume. Several methods are routinely used to analyse MRI data including visual inspection, region of interest (ROI) analysis or voxel based morphometry (VBM).

Visual rating and ROI analysis methods depend on the appropriate *a priori* choice of structures to be analysed while VBM performs a point by point analysis of each voxel within the image across whole data sets allowing unbiased and largely operator independent data analysis. All 3 methods have been used to investigate structural imaging changes in Lewy body dementias.

### 2.2.1 Cortical atrophy

A common finding in structural MR studies in dementia is whole brain atrophy when compared with control subjects, with widespread grey matter reduction in the frontal, temporal and parietal areas (Beyer et al., 2007; Ballmaier et al., 2004; Burton et al., 2002; Hashimoto et al., 1998) (See Table 2.1 on page 27).

Using VBM, Burton *et al.* (2004) compared subjects with PD (n=24), PDD (n=31), DLB (n=20), AD (n=35) and controls (n=39). They observed a diffuse pattern of grey matter atrophy affecting the temporal, parietal, middle and inferior frontal gyri and the occipital lobe in PDD when compared with control subjects, with no significant difference between the cortical atrophy profile in PDD and DLB. A VBM study by Beyer *et al.* (2007) in PDD (n=15), DLB (n=18), AD (n=21) and healthy controls (n=20) also found reduced grey matter in the temporal, parietal and occipital lobes. However, in contrast to Burton *et al.* (2004) the changes were more pronounced in DLB than PDD. The authors suggest several explanations for the differing result: (1) the clinical criteria for PDD used by Burton *et al.* (2004) required the presence of visual hallucinations or cognitive fluctuations, so the PDD subjects selected had a clinically similar phenotype to the DLB subjects, possibly increasing the likelihood of similar cerebral changes and (2) a shorter duration of illness in the PDD group than in the previous study which may be associated with more severe morphological changes. The DLB group included by Beyer *et al.* (2007) were also older; and, although not significant, may have influenced the differing results. However, the increased cortical amyloid deposition in DLB compared to PDD, reported in pathological and amyloid PET studies would be consistent with the finding of greater structural cortical change in DLB (Edison et al., 2008; Gomperts et al., 2008). Interestingly, a recent VBM study in PDD has reported greater cortical atrophy in patients who developed dementia early in the course of their disease (< 8years) compared with those who developed dementia later in the course of their illness (> 8 years) (Beyer and Aarsland, 2008). Although they did not include a DLB

comparison or control group, this finding is consistent with neuropathological data which found more severe plaques and cortical  $\alpha$  synuclein pathology in the group with shorter duration of PD prior to dementia (Ballard et al., 2006) and reinforces the heterogeneity amongst patients with PDD which, in small sample sizes, would influence the MR study findings.

Whitwell *et al.* (2007b) described what they term a signature MR pattern in DLB. Using both VBM and ROI analysis they studied subjects with DLB (n=72), AD (n=72) and controls (n=72) with careful matching for age, education and dementia severity. They found very little cortical grey matter loss, in contrast to the relatively diffuse pattern of grey matter loss observed by others (Beyer et al., 2007; Burton et al., 2004; Burton et al., 2002). However, they report scattered regions of cortical loss in frontal and parietal lobes, which is in keeping with other studies, but to a lesser degree. There was also a region of grey matter loss identified around the third ventricle, which may represent the hypothalamus. These findings were in contrast to AD, where a widespread pattern of grey matter loss was found, involving the medial temporal and temporoparietal regions. As with other MR studies, there was a large degree of overlap in results between individual subjects precluding the use of those MR methods as diagnostic scans. However, the study contributes useful information to patterns of cerebral atrophy in DLB as detected by MR and, in particular, how they differ from AD.

Occipital hypometabolism and hypoperfusion in DLB have been documented in PET and SPECT studies respectively (Minoshima et al., 2002; Lobotesis et al., 2001; Ishii et al., 1999). This led to interest into whether there were associated structural MR changes. Most volumetric studies using ROI analysis (Middelkoop et al., 2001) and VBM (Burton et al., 2004; Burton et al., 2002) did not find a significant occipital structural change in DLB. However, a VBM study comparing PD and PDD found that there was significant grey matter loss of the left occipital lobe (Burton et al., 2004). The left occipital gyrus was also found to have more atrophy in DLB compared with control subjects using VBM (Beyer et al., 2007).

Author	NC	DLB	PDD	AD	MRI	MRI Findings
Huber <i>et al.</i> (1989)	10	0	10 (10) <sup>b</sup>	0	1.5T visual image analysis	<i>PDD: brain surface area, ventricular measurements, supra- and infra-tentorial lesions were not significantly different to PD.</i>
Middelkoop <i>et al.</i> (2001)	24	23	0	25	1.0T semi-automated segmentation	<i>DLB: occipital lobe–no difference in raw or normalised volume compared with controls and AD.</i>
Burton <i>et al.</i> (2002)	25	25	0	30	1.0T VBM	<i>DLB: grey matter atrophy–diffuse involving temporal, frontal, parietal lobes and insular cortices. Occipital lobe–no grey matter loss.</i>
Ballmaier <i>et al.</i> (2004)	38	16	0	29	1.5T cortical pattern matching	<i>DLB: TL and orbitofrontal cortices–relative preservation compared with AD.</i>
Burton <i>et al.</i> (2004)	36	17	26 (31) <sup>b</sup>	28	1.5T VBM	<i>PDD: no significant differences to DLB. Grey matter atrophy: widespread, temporal lobes, occipital lobes, right frontal and left parietal lobe. Occipital grey matter atrophy compared with PD.</i>
Ramirez-Ruiz <i>et al.</i> (2005)	0	0	16/8 (13/11) <sup>b</sup>		1.5T VBM	<i>PDD: progressive grey matter loss predominantly in the hippocampal, temporal and occipital lobes.</i>
Summerfield <i>et al.</i> (2005)	13	0	16 (16) <sup>b</sup>	0	1.5T VBM	<i>PDD: cortical and subcortical grey matter atrophy–hippocampus, thalamus and anterior cingulate most affected compared with controls. Left superior temporal gyrus and right hippocampus atrophy compared with PD.</i>
Beyer <i>et al.</i> (2007)	20	18	15	21	1.5T VBM	<i>PDD: Grey matter atrophy–diffuse, less than DLB.</i>
Whitwell <i>et al.</i> (2007b)	72	72	0	72	1.5T VBM and ROI	<i>DLB: Grey matter atrophy–dorsal midbrain, hypothalamus and substantia innominata. Relative sparing of the hippocampus and temporoparietal cortex.</i>
Beyer&Aarsland (2008)	0	0	15	0	1.5T VBM	<i>PDD: group that developed dementia early had more areas of atrophy than the late dementia group–medial frontal gyrus, right precuneus, left inferior parietal lobule, superior frontal gyrus and middle temporal gyrus. In the late dementia group, there was atrophy in the inferior frontal gyrus and in unmodulated images, reduced grey matter in the insula.</i>

<sup>a</sup> number of subjects in another dementia comparison group

<sup>b</sup>PD comparison group

Table 2.1. Structural MRI studies – Cortical Atrophy.

### 2.2.2 Rate of cerebral atrophy

The rate of cerebral atrophy in DLB has been assessed in several longitudinal MR studies (Whitwell *et al.*, 2007a; Burton *et al.*, 2005; O'Brien *et al.*, 2001) and are summarised in Table 2.2. The overall brain atrophy rate in DLB has been reported to be 1.4% per year, slightly less than in AD (2% per year) but still 3 times the rate in similarly aged controls (O'Brien *et al.*, 2001). Whitwell *et al.* (2007a) reported a longitudinal MR study with pathologically confirmed cases of DLB (n=9), AD (n=12), mixed AD/DLB (n=13), controls (n=25) and other dementia subtypes.



Although there was a large amount of overlap in individual patient brain atrophy rates between the groups, a lower atrophy rate (0.4% per year) and ventricular expansion rate (4.8% per year) was reported in DLB compared with AD (atrophy 1.1% per year and ventricular expansion rate of 8.3% per year). The group with mixed AD/DLB pathology also had a greater atrophy rate than the DLB group, (1.3% per year, ventricular expansion 7.2% per year); a similar rate to that previously reported in DLB. Interestingly, the rate of atrophy in the pure DLB was not significantly different to the control group. The authors suggest that the subject group clinically diagnosed with DLB may contain subjects with mixed pathology and conclude that the technique may help to differentiate DLB from AD and be valuable for future disease modifying treatment trials (Whitwell et al., 2007a). In a longitudinal study (Burton et al., 2005) comparing PD (n=18), PDD (n=13) and controls (n=24), whole brain atrophy rates were higher in PDD (1.12% per year) than PD (0.31% per year) and controls (0.34% per year), similar to the rate in DLB reported by O'Brien *et al.* (2001) yet higher than reported in DLB by Whitwell *et al.* (2007a). It is possible that the atrophy reflected in the sample of PDD may be partly due to Alzheimer pathology rather than Lewy related pathology (Burton et al., 2005).

Author	NC	DLB	PDD	AD	MRI	MRI Findings: Cerebral Atrophy Rate (% per year)
O'Brien <i>et al.</i> (2001)	20	10	0	9 (9) <sup>a</sup>	1.0T boundary shift integral	DLB: 1.4 Controls: 0.5-0.7 AD: 2.0 VaD: 1.9
Burton <i>et al.</i> (2005)	24	0	13 (18) <sup>b</sup>	0	1.5T boundary shift integral	PDD: 1.12 PD: 0.31 Controls: 0.34
Whitwell <i>et al.</i> (2007a)	25	9	0	12 (13+12+5+5) <sup>a</sup>	1.5T semi-automated segmentation and manual tracing	DLB: 0.4 ventricular expansion rate: 4.8% per year, not significantly different to controls and less than AD.

<sup>a</sup> number of subjects in another dementia comparison group.

<sup>b</sup>PD comparison group

Table 2.2. Structural MRI studies – Rate of Cerebral Atrophy.

### 2.2.3 Medial temporal lobe

A robust MR finding in DLB and PDD is that of relative preservation of the medial temporal lobe when compared with Alzheimer's disease (Whitwell et al., 2007b; Tam et al., 2005; Burton et al., 2002; Barber et al., 2001; Barber et al., 1999a; Harvey et al., 1999) (see Figure 2.1, page 29 and Table 2.3, page 32).

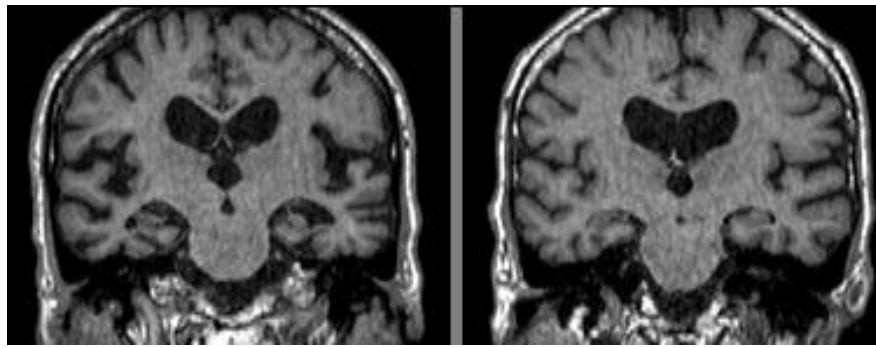


Figure 2.1. Coronal view illustrating relative preservation of the Medial Temporal Lobe and hippocampus in dementia with Lewy bodies (right) compared with Alzheimer's disease (left).

Barber *et al.* (1999a) used a standardised visual rating scale to grade medial temporal atrophy (MTA) in patients with DLB ( $n=26$ ), AD ( $n=28$ ), VaD ( $n=24$ ) and healthy volunteers ( $n=26$ ) and found that whilst MTA occurred more in all dementia groups compared with controls, there was less MTA in DLB compared with AD and VaD. The absence of MTA was highly specific for separating DLB from AD (100%) and VaD (88%), but with a much lower sensitivity (38%). This finding has been consistent in both ROI (Barber *et al.*, 2001; O'Brien *et al.*, 2001; Harvey *et al.*, 1999; Hashimoto *et al.*, 1998) and VBM (Beyer *et al.*, 2007; Burton *et al.*, 2004; Burton *et al.*, 2002) studies. This was supported by a prospective MRI study with pathological verification which found the pathological correlates of MTA include senile plaques and neurofibrillary tangles but not Lewy body associated pathology (Burton *et al.*, 2009).

The hippocampus itself, which forms part of the medial temporal lobe, is less atrophic in DLB than in AD (Whitwell *et al.*, 2007b; Barber *et al.*, 2001; Barber *et al.*, 2000a; Hashimoto *et al.*, 1998). Using VBM (Summerfield *et al.*, 2005; Burton *et al.*, 2004) and manual volumetric measurement techniques (Junque *et al.*, 2005; Tam *et al.*, 2005; Camicioli *et al.*, 2003) subjects with PDD have been found to have less hippocampal atrophy than AD but more than controls; a finding that is also consistent with the pathological overlap between PDD and DLB. A single study has reported contrary findings, with hippocampal volumes in PDD patients smaller than in AD (Laakso *et al.*, 1996), although the differences were not statistically significant. The authors suggest that this may, in part, be due to coexistent AD

pathology in the PDD group and this finding has not been replicated in other larger studies.

Sabattoli *et al.* (2008) studied hippocampal morphology using manual tracing of hippocampal boundaries and computer-assisted post processing in DLB (n=14), AD (n=28) and healthy control subjects (n=28). They found that in DLB subjects there was global hippocampal volume loss of 10-20% compared with the control group, less than in AD with a differing pattern of hippocampal atrophy. DLB was characterised by atrophy in the medial and lateral aspects of the hippocampal head (anterior CA1) and hippocampal midline (CA2-3 fields, subiculum and presubiculum). Whereas AD subjects had greater atrophy in the anterior and posterior aspects of the hippocampal tail (CA1 field and subiculum) with the authors suggesting differing underlying neuropathological substrates (Sabattoli *et al.*, 2008). Using a high resolution MRI sequence at a higher field strength (3T), and manual ROI analysis methods, Firbank *et al.* (2010) also found less atrophy in the subiculum and CA1 regions of the hippocampus in DLB (n=16) when compared to AD (n=16) although did not find any differences in the CA2 or CA3/4 regions. They also report a hypointense line visible on the images between CA1 and CA3/4 that was less distinct in AD than in DLB suggesting that it may represent disease related change. Using manual tracing (Bouchard *et al.*, 2008) and VBM (Ibarretxe-Bilbao *et al.*, 2008), hippocampal atrophy in PDD was found to primarily affect the head of the hippocampus with a similar pattern being observed in the older PD group (Bouchard *et al.*, 2008).

The entorhinal cortex which forms part of the MTL was found to be smaller in DLB, PDD and AD compared to control subjects using a manual segmentation technique (Kenny *et al.*, 2008). The percentage volume reduction was 14.7% in the PDD group, 19.9% in the DLB group and 21.9% in the AD group. Although the entorhinal cortex volume was the smallest in AD, it was not significantly smaller than other dementia groups, suggesting this may reflect a common mechanism (Kenny *et al.*, 2008).

Hanyu *et al.* (2005) used the MR technique of magnetisation transfer ratios (MTR) to detect regional differences in the white matter between AD, DLB and controls. The MTR measurement reflects the degree of interaction between mobile tissue water and macromolecular structures such as myelin. They found a significant difference in the hippocampal MTR, with the MTR of those with dementia being significantly lower

than in healthy controls (controls>DLB>AD). Interpretation of this reduction in MTR is consistent with loss of myelin and/or increased tissue water content associated with changes in cellular structure and may contribute to a differential diagnosis between DLB and AD (Hanyu et al., 2005).

Author	NC	DLB	PDD	AD	MRI	MRI Findings
Hashimoto <i>et al.</i> (1998)	27	27	0	27	1.5T semi-automated segmentation and manual tracing	DLB: hippocampal formation–relative preservation compared with AD.
Barber <i>et al.</i> (1999a)	26	26	0	28 (24) <sup>a</sup>	1.0T MTA visual rating scale	DLB: MTL–relative preservation. TL atrophy correlates with memory impairment and age.
Harvey <i>et al.</i> (1999)	0	9	0	11	0.5T visual rating and volumetric analysis	DLB: MTL–relative preservation
Barber <i>et al.</i> (2000a)	26	27	0	25 (24) <sup>a</sup>	1.0T ROI analysis semi-automated segmentation	DLB: larger temporal lobe, hippocampal and amygdala volume compared to AD. Ventricular volume increased compared with controls, although whole brain volume was relatively preserved.
Barber <i>et al.</i> (2001)	26	26	0	22	1.0T ROI analysis	DLB: MTL–relative preservation
Burton <i>et al.</i> (2002)	25	25	0	30	1.0T VBM	DLB: MTL–relative preservation compared with AD.
Ballmaier <i>et al.</i> (2004)	38	16	0	29	1.5T cortical pattern matching	DLB: TL–relative preservation (hippocampus, parahippocampus and amygdala) compared with AD.
Burton <i>et al.</i> (2004)	36	17	26 (31) <sup>b</sup>	28	1.5T VBM	PDD: no significant differences to DLB. MTL–relative preservation compared with AD.
Hanyu <i>et al.</i> (2005)	18	17	0	31	1.5T Magnetisation Transfer Ratios ROI	DLB: hippocampal MTR–lower hippocampal MTR than controls, higher MTR than AD (Controls>DLB>AD DLB).
Junque <i>et al.</i> (2005)	16	0	16 (16) <sup>b</sup>	0	1.5T Manual tracing ROI	PDD: hippocampal (20%) atrophy compared with controls. PD: hippocampal atrophy (10%) compared with controls (not statistically significant).
Summerfield <i>et al.</i> (2005)	13	0	16 (16) <sup>b</sup>	0	1.5T VBM	PDD: cortical and subcortical grey matter atrophy–hippocampus, thalamus and anterior cingulate most affected compared with controls. Left superior temporal gyrus and right hippocampus atrophy compared with PD.
Tam <i>et al.</i> (2005)	39	25	31 (33) <sup>b</sup>	31	1.5T Visual rating scale	PDD: MTA–relative preservation compared with AD, similar to DLB
Beyer <i>et al.</i> (2007)	20	18	15	21	1.5T VBM	PDD&DLB:MTL–relative preservation, compared with AD.
Whitwell <i>et al.</i> (2007b)	72	72	0	72	1.5T VBM and ROI	DLB: relative sparing of the hippocampus and temporoparietal cortex.
Bouchard <i>et al.</i> (2008)	44	0	13 (44) <sup>b</sup>	0	1.5T Manual tracing ROI	PDD: hippocampal atrophy predominantly in the head of the hippocampus.
Ibarretxe-Bilbao <i>et al.</i> (2008)	56	0	9 (16+ 19) <sup>b</sup>	0	1.5T VBM, ROI	PDD: grey matter loss involving entire hippocampus compared with controls. PD+visual hallucinations: hippocampal loss confined to the head of the hippocampus.
Sabbatoli <i>et al.</i> (2008)	28	14	0	28	1.0T manual tracing, radial atrophy mapping	DLB: hippocampus–10-20% volume loss (compared with controls) affecting regions CA1, dorsal midline aspect CA2-3 and subiculum and presubiculum. Atrophy less than AD.
Kenny <i>et al.</i> (2008)	37	20	30 (31) <sup>b</sup>	26	1.5T manual segmentation	DLB&PDD: total normalised entorhinal cortex volumes smaller in dementia (AD, PDD and DLB) compared with controls. Smaller entorhinal cortex volume in DLB compared with PD.
Burton <i>et al.</i> (2009)	0	23	0	11 (12) <sup>a</sup>	1.0T (N=20) 1.5T (N=26) visual rating	DLB: less hippocampal atrophy than AD on MRI in pathologically confirmed cases.
Firbank <i>et al.</i> (2010)	16	16	0	16	3.0T High resolution sequence manual ROI	DLB: less hippocampal atrophy in CA1 and subiculum compared to AD. Hypointense line between CA1 and CA3/4 less distinct in AD.

<sup>a</sup> number of subjects in another dementia comparison group, <sup>b</sup>PD comparison group

Table 2.3. Structural MRI studies – Medial temporal lobe structures.

#### 2.2.4 *Subcortical structures*

DLB and PDD are characterised by a pattern of subcortical structural changes and MRI study findings are summarised in Table 2.4. Nigrostriatal degeneration and dopaminergic loss, particularly of the dopamine transporter in the striatum occurs in LBD but not to a significant extent in AD (Piggot et al., 1999). This can be visualised using specific imaging ligands and nuclear medicine techniques. For example, using FP-CIT SPECT imaging reductions of 40-50% were documented in striatal structures in LBD (O'Brien et al., 2004). These changes were found to have a sensitivity of 78% and specificity of 90% in distinguishing DLB primarily from AD in a multicentre study and have been incorporated in the latest revision of the consensus criteria for DLB (McKeith et al., 2007; McKeith et al., 2005). It is important to consider how these functional dopaminergic changes may be related to structural and other MR changes. Striatal atrophy has been documented in LBD: putamen atrophy has been reported in PDD (Summerfield et al., 2005; Burton et al., 2004) and in DLB, relative to AD (Cousins et al., 2003). Although no significant structural differences were detected in the caudate nucleus in DLB (Almeida et al., 2003; Cousins et al., 2003; Barber et al., 2002), atrophy of the right caudate tail was reported in PDD in one VBM study (Burton et al., 2004). However, it seems very unlikely that the magnitude of atrophy seen (5-10% reduction in volume) is sufficient to explain the highly significant (up to 50%) reduction in dopamine transporter binding seen with FP-CIT SPECT.

Atrophy of the substantia innominata and dorsal midbrain in DLB (Hanyu et al., 2007; Whitwell et al., 2007b; Brenneis et al., 2004) has been documented, findings which are supported by the pathological involvement of the areas, including the nucleus basalis of Meynert (contained in the substantia innominata) (Lippa et al., 1999).

The thalamus has also been reported to be smaller in PDD (Summerfield et al., 2005; Burton et al., 2004). Atrophy of the amygdala in DLB was similar to AD in one study (Hashimoto et al., 1998) and two other studies have found no difference between DLB and controls (Burton et al., 2002; Barber et al., 2000a). A significant reduction in amygdala volume in PDD compared with controls has been reported in two studies using manual volumetric techniques (Bouchard et al., 2008; Junque et al., 2005).

Author	NC	DLB	PDD	AD	MRI	MRI Findings
Hashimoto <i>et al.</i> (1998)	27	27	0	27	1.5T semi-automated segmentation and manual tracing.	<i>DLB&amp;AD: amygdala atrophy comparable.</i>
Barber <i>et al.</i> (2000a)	26	27	0	25 (24) <sup>a</sup>	1.0T ROI analysis semi-automated segmentation	<i>DLB: larger amygdala volume compared to AD. Ventricular volume increased compared with controls, although whole brain volume was relatively preserved.</i>
Hanyu <i>et al.</i> (2001)	12	0	6 (11) <sup>b</sup>	(6) <sup>a</sup>	1.5T MTR, ROI	<i>PDD: lower MTR in the subcortical white matter including frontal and genu of the corpus callosum compared with controls.</i>
Barber <i>et al.</i> (2002)	25	26	0	21 (18) <sup>a</sup>	1.0T ROI	<i>DLB: caudate nucleus–no significant volumetric difference between dementia groups.</i>
Almeida <i>et al.</i> (2003)	35	0	20	27	1.5T volumetric segmentation	<i>PDD: caudate nucleus volume–no significant difference between controls and PD.</i>
Cousins <i>et al.</i> (2003)	37	14	0	27	1.5T volumetric, semi-automated and manual segmentation	<i>DLB: putamen–smaller compared with controls but not AD. When normalised to total intracranial volume reduced compared with controls and AD.</i>
Brenneis <i>et al.</i> (2004)	10	10	0	10	1.5T VBM	<i>DLB: basal forebrain atrophy compared with AD. Lateral prefrontal cortex and left premotor cortical atrophy compared with controls.</i>
Burton <i>et al.</i> (2004)	36	17	26 (31) <sup>b</sup>	28	1.5T VBM	<i>PDD: no significant differences to DLB. Grey matter atrophy: widespread, subcortical regions include right caudate tail and putamen and bilateral thalamus.</i>
Junque <i>et al.</i> (2005)	16	0	16 (16) <sup>a</sup>	0	1.5T Manual tracing ROI	<i>PDD: amygdala (21%) atrophy compared with controls. PD amygdala atrophy (11%) compared with controls, not statistically significant.</i>
Summerfield <i>et al.</i> (2005)	13	0	16 (16) <sup>b</sup>	0	1.5T VBM	<i>PDD: cortical and subcortical grey matter atrophy–hippocampus, thalamus and anterior cingulate most affected compared with controls.</i>
Wiltshire <i>et al.</i> (2005)	27	0	25 (24) <sup>b</sup>	16	1.5T ROI	<i>PDD (&amp;PD): callosal atrophy not significantly different to controls or AD. Dementia severity did not correlate with corpus callosal atrophy.</i>
Hanyu <i>et al.</i> (2007)	28	31	0	122 (34) <sup>a</sup>	1.5T ROI	<i>DLB: substantia innominata–smaller than AD.</i>
Whitwell <i>et al.</i> (2007b)	72	72	0	72	1.5T VBM and ROI	<i>DLB: grey matter atrophy–dorsal midbrain, hypothalamus and substantia innominata. Relative sparing of the hippocampus and temporoparietal cortex.</i>

<sup>a</sup> number of subjects in another dementia comparison group, <sup>b</sup>PD comparison group

Table 2.4. Structural MRI studies – Subcortical structures.

### 2.2.5 White matter hyperintensities

Damage to the white matter is particularly evident in the ageing brain appearing as focal punctuate areas of hyperintense signal on T2-weighted MRI. Findings from studies investigating white matter hyperintensities (WMH) are summarised in Table 2.5. Using a standardised visual rating scale, WMH were shown to be more extensive in DLB when compared with control subjects but were similar to AD (Barber *et al.*, 2000c; Barber *et al.*, 1999b). However, using automated volumetric analysis Burton *et al.* (2006) found no significant difference between the amount of

WMH in the DLB, PDD and control subjects and greater amounts in AD. The authors suggest that the non-significance of the results may relate to a smaller sample size in their study and use of a different analysis technique. After one year they showed that WMH increase significantly in patients with AD, PDD and healthy controls, but not in those with DLB; and that the amount of WMH at baseline predicts progression, possibly indicating baseline susceptibility in subjects (Burton *et al.*, 2006). Interestingly, Beyer *et al.* (2006) using a visual rating scale, also found that there was no significant difference in WMH between PDD (n=16) and control (n=20) subjects. However, in contrast to other studies, they also included a PD group (n=19), which had significantly lower WMH score than both the PDD and control group. The reason for the difference is not entirely clear and may, in part, reflect the heterogeneous nature of PD combined with a small sample size. The authors raise the possibility of lower neurotransmitter levels in PD compared to controls leading to fewer WMH (Beyer *et al.*, 2006). Although, this would presumably apply to both PD and DLB and would not necessarily explain the lack of progression of WMH observed by Burton *et al.* (2006) in DLB contrasting with the progression of WMH observed in PDD.

WMH can be further divided into periventricular hyperintensities (PVH) which are adjacent to the ventricles and deep white matter hyperintensities (DWMH). The relationship between WMH and brain atrophy was studied by Barber *et al.* (2000b), who found that PVH rather than DWMH in AD and DLB correlated with increasing ventricular dilatation suggesting that PVH are associated with atrophy rather than cerebral ischaemia (Barber *et al.*, 2000b).

Author	NC	DLB	PDD	AD	MRI	MRI Findings
Barber <i>et al.</i> (2000b)	0	27	0	25	1.0T, semi-automated segmentation	<i>DLB: PVH increase associated with ventricular dilatation. DWMH associated with history of hypertension.</i>
Beyer <i>et al.</i> (2006)	20	0	16 (19) <sup>b</sup>	0	1.5T visual rating	<i>PDD: more WMH in the deep white matter and periventricular areas compared with PD subjects however not increased compared with controls.</i>
Burton <i>et al.</i> (2006)	33	14	13	23	1.5T volumetric analysis	<i>DLB: WMH baseline similar to controls, no significant increase in progression rate over 12 months. PDD: WMH increased progression rate compared with controls</i>

<sup>b</sup>PD comparison group

Table 2.5. Structural MRI studies – White matter hyperintensities.

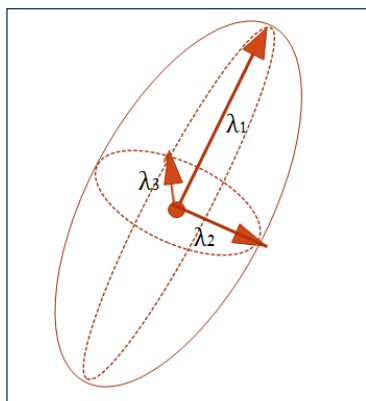


### 2.3 MR Diffusion Tensor Imaging (MR-DTI)

Diffusion weighted MR imaging measures the diffusional displacements of water molecules within the tissue on a timescale of milliseconds. Water movements at this level are dictated by true diffusion but also by interaction between moving water molecule and tissue structures at the cellular level, such as cell membranes and intracellular organelles (Le Bihan, 2007).

Diffusion measurements can also provide detail on tissue microstructure by utilising the anisotropic nature of diffusion in neuronal white matter tracts with water molecules diffusing further along the tracts than perpendicular to them (Le Bihan, 2007; Basser et al., 1994). This is represented by a diffusion ellipsoid at each voxel (see Figure 2.2), made up of three orthogonal eigenvectors and their component eigenvalues ( $\lambda_1$ ,  $\lambda_2$ ,  $\lambda_3$ ).  $\lambda_1$  (axial diffusivity) represents the eigenvalue of the direction of maximal diffusion and  $\lambda_2$  and  $\lambda_3$  are the eigenvalues representing the perpendicular plane and their average lengths are referred to as radial diffusivity. With neuronal degeneration the mean diffusivity,  $MD = \frac{1}{3}(\lambda_1 + \lambda_2 + \lambda_3)$  increases with the loss of structural barriers that normally restrict diffusion (also referred to as apparent diffusion coefficient, ADC). Also, diffusion becomes less directionally oriented with an associated reduction in fractional anisotropy (FA) (Assaf and Pasternak, 2008; Beaulieu, 2002) which is a scaled version of the standard deviation of  $\lambda_1, \lambda_2, \lambda_3$ ; scaled so that  $0 \leq FA \leq 1$ .

White matter tract changes that occur in dementia can potentially be detected as reflected in alterations diffusion tensor imaging measures.



Fractional Anisotropy =

$$\sqrt{\frac{(\lambda_1 - \lambda_2)^2 + (\lambda_1 - \lambda_3)^2 + (\lambda_2 - \lambda_3)^2}{2(\lambda_1^2 + \lambda_2^2 + \lambda_3^2)}}$$

Mean Diffusivity =

$$\frac{\lambda_1 + \lambda_2 + \lambda_3}{3}$$

Figure 2.2. Representation of the diffusion tensor ellipsoid:  $3 \times 3$  tensor matrix, eigenvalues  $\lambda_1$ ,  $\lambda_2$ ,  $\lambda_3$ .

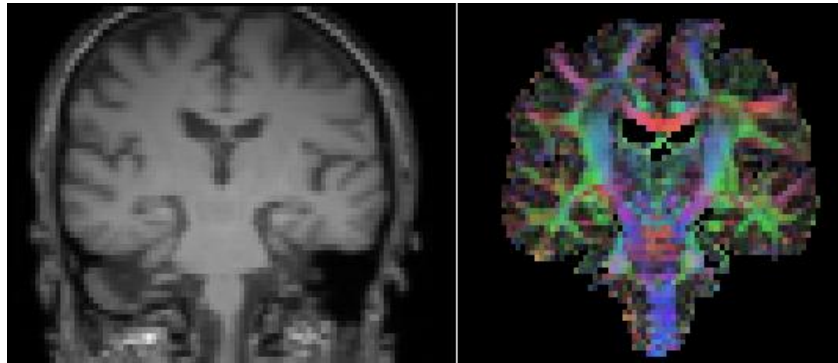


Figure 2.3. Coronal view illustrating Diffusion Tensor Imaging in dementia with Lewy bodies.

The colour coded fractional anisotropy (FA) map (right) encodes the degree of anisotropy by image intensity, while pixel colour shows the primary direction of diffusion (red: left–right, green: anterior–posterior, blue: superior–inferior). (Left) Structural scan.

DTI changes in LBD have been reported using conventional region of interest (ROI) or voxel based morphometry (VBM) methods (Kantarci et al., 2010; Lee et al., 2010b; Ota et al., 2009; Firbank et al., 2007a; Firbank et al., 2007b; Matsui et al., 2007; Bozzali et al., 2005). Some studies found widespread FA changes in comparison to healthy controls (Lee et al., 2010b; Bozzali et al., 2005) whilst others have found very little change (Kantarci et al., 2010; Firbank et al., 2007b). Bozzali *et al.* (2005) used this technique to investigate DLB (n=15) compared with a control group (n=36). Using ROI methods, they report DT-MRI changes in the corpus callosum and pericallosal areas (increased diffusivity and decreased FA values). White matter was also affected in the frontal, parietal and occipital areas in DLB patients with less prominent involvement of the temporal white matter. The caudate nucleus had DTI changes with increased diffusivity without an associated FA reduction. The authors suggest that the white matter changes found in DLB may reflect the pathophysiological process that eventually affect neurons in the association cortex (Bozzali et al., 2005). The findings were somewhat limited by the small sample size and lack of another type of dementia as a comparison group (Bozzali et al., 2005).

FA reductions have also been reported in the posterior cingulate and precuneal areas in DLB and PDD (Lee et al., 2010b; Firbank et al., 2007a; Firbank et al., 2007b;

Bozzali *et al.*, 2005). Firbank *et al.* (2007b) compared subjects with DLB (n=16), AD (n=15) and a healthy older control group (n=15) using a DT-MRI technique with fluid attenuated inversion recovery (FLAIR) to suppress the confounding signal from cerebrospinal fluid (CSF). Using a ROI analysis method they found that the DLB group had reduced FA in the precuneal area on ROI analysis. Given that hypoperfusion in this area has previously been found in DLB using SPECT (Firbank *et al.*, 2003), the authors hypothesised that the disrupted connectivity as demonstrated by a reduced FA in the area of the precuneus may result in medial parietal hypoperfusion (Firbank *et al.*, 2007b). Diffuse white matter changes found by Bozzali *et al.* (2005) were not replicated in this study. The authors suggested that the results by Bozzali *et al.* (2005) may have been influenced by brain atrophy as the use of diffusion sequence without FLAIR CSF suppression results in high ADC values from the CSF potentially increasing white matter ADC due to partial volume effects. Firbank *et al.* (2007b) included subjects with moderate white matter hyperintensities which may also have contributed to the differing results.

Further analysis on the same patient group (Firbank *et al.*, 2007b) found bilateral clusters adjacent to the posterior cingulate with reduced FA (Firbank *et al.*, 2007a). This correlated with global atrophy in AD and DLB. Given that the hippocampus, anterior and posterior cingulate and lateral parietal regions show functional connectivity, the authors conclude that dementia progression as measured by global atrophy is associated with disruption of the white matter connecting the regions (Firbank *et al.*, 2007a).

Matsui *et al.* (2007) also reported a reduction in the FA in the posterior cingulate (ROI) in a study of PDD subjects (n=11) compared with PD (n=26) and healthy controls (n=10). This finding, along with Firbank *et al.* (2007a) supports the notion that the posterior cingulate may play an important role in the pathological process of LBD.

The most consistent finding in DTI studies has been that of reduced FA in the region of the inferior longitudinal fasciculus (ILF) (temporo-occipital), although this area has also been found to be affected in AD (Kantarci *et al.*, 2010; Lee *et al.*, 2010b; Ota *et al.*, 2009; Bozzali *et al.*, 2005). Using tract based ROI methods Kantarci *et al.* (2010) reported reduced FA in inferior longitudinal fasciculus in DLB, which was similar to that reported in AD. In addition, they reported increased diffusivity in the

amygdala grey matter which, in contrast to AD, was not proportional to the degree of atrophy. Given that dysfunction of the visuo-amygdaloid pathway (via the inferior longitudinal fasciculus) has been implicated in visual hallucinations, they suggest that the disruption in the inferior longitudinal fasciculus as measured with DTI may be related to retrograde degeneration of the axonal projections (Kantarci et al., 2010). DTI findings in LBD have been variable. A combination of relatively small sample sizes, heterogeneity within cohorts and differing analysis methods make conclusions as to the DTI changes in DLB difficult. Consistent changes have been reported in the inferior longitudinal fasciculus, precuneus and posterior cingulate regions and highlight their potential importance in Lewy body dementias.

#### **2.4 Proton Magnetic Resonance Spectroscopy (<sup>1</sup>H-MRS)**

Proton MR spectroscopy provides information relating to brain metabolism by measuring the signal originating from protons attached to key biomolecules (Soares and Law, 2009). The MRS signal is much weaker than MRI due to the lower concentration of these biomolecules (mM compared to water protons at 110 Molar). To gain sensitivity MRS voxels are typically 1 to 8 cm<sup>3</sup> in volume (Soares and Law, 2009; Gaudin, 1995) and are usually only collected from a few pre-selected regions, limiting information about true regional differences. The brain proton spectrum includes metabolite peaks for 5 important compounds: (1) N-acetyl aspartate (NAA) is regarded as a marker of neuronal integrity and is reduced in neuronal dysfunction or loss. (2) Creatine (Cr) is related to general metabolism, it is assumed to be relatively constant and is therefore often taken as an internal reference level. (3) Choline (Cho) is seen as an indicator of membrane activity, and (4) myo-inositol (mI) is mainly contained in glial cells. (5) Glutamine/glutamate (Glx) metabolism occurs in neurons and glial cells and reduction may reflect glial cell or axonal impairment (Soares and Law, 2009; Gaudin, 1995). Table 2.6 summarises the findings of six <sup>1</sup>H-MRS studies identified in Lewy body dementias.

Author	Con-trols	Patient number			Area (voxel of interest)	DLB				PDD			
		DLB	PDD	AD		NAA/Cr	Cho/Cr	Glx/Cr	mI/Cr	NAA/Cr	Cho/Cr	Glx/Cr	mI/Cr
Molina <i>et al.</i> (2002)	11	12	0	0	WM: centrum semiovale	↓	↓	↓↔	NA				
					GM: parasagittal parietal cortex	↔	↔	↔	NA				
Summerfield <i>et al.</i> (2002)	13	0	14	0	lentiform/caudate nucleus					↔	↔	NA	↔
					bilateral occipital cortices					↓	↔	NA	↔
Kantarci <i>et al.</i> (2004)	206	20	0	121 (41/8) <sup>a</sup>	Right and left posterior cingulate gyrus and inferior precunej	↔	↑↔	↔	↔				
Griffith <i>et al.</i> (2008a)	12	0	12 (12) <sup>b</sup>	0	Posterior cingulate gyrus					↓	↔	NA	↔
Griffith <i>et al.</i> (2008b)	61	0	12	22	Posterior cingulate gyrus					↓	↔	↓	↔
Xuan <i>et al.</i> (2008)	8	8	0	0	Bilateral hippocampi	↓	↔	NA	NA				

↔ = unchanged, ↓ = reduced, ↑ = increased; WM=white matter, GM=grey matter; NA=not applicable  
<sup>a</sup> number of subjects in another dementia group, <sup>b</sup>PD comparison group

Table 2.6. <sup>1</sup>H-MRS Studies.

Using a single voxel spanning the right and left posterior cingulate gyri and inferior precunej, Kantarci *et al.* (2004) studied proton MRS in subjects with AD (n=121), DLB (n=20), VaD (n=8), frontotemporal lobar degeneration (n=41) and healthy controls (n=206). NAA/Cr levels were reduced in all dementias except for DLB suggesting posterior cingulate neuronal integrity in DLB as compared with other dementia subtypes (Kantarci *et al.*, 2004). This is however in contrast to the DTI findings (Firbank *et al.*, 2007a). Molina *et al.* (2002) also found that there was no change in the NAA/Cr in DLB (n=12) compared to healthy controls (n=11) using the parasagittal parietal cortex as a VOI. They acknowledged the limitation of using a single VOI potentially obscuring larger inter-group sized difference.

Griffith *et al.* (2008a) compared the spectroscopic profiles in PD (n=12), PDD (n=12) and controls (n=12) using a VOI located in the posterior cingulate gyrus. The PDD group had significantly reduced NAA/Cr when compared with both the PD and control groups with no detected change in Cho/Cr or mI/Cr. The same group also compared the proton MRS in PDD and AD, again using a VOI located in the posterior portion of the posterior cingulate gyrus (Griffith *et al.*, 2008b). A reduction in NAA/Cr was found in both PDD and AD suggesting neuronal dysfunction, a finding which contrasted with Kantarci *et al.* (2004) data for DLB subjects. A reduced Glu/Cr was also reported in PDD, differing from an increased mI/Cr in AD (Griffith *et al.*, 2008b).

In a proton MRS study by Xuan *et al.* (2008) in DLB (n=8) and healthy controls (n=8), the right and left hippocampi were chosen as VOI, an area that has been previously studied in AD, and a significant decrease in the NAA/Cr ratio was observed.

Summerfield *et al.* (2002) compared <sup>1</sup>H-MRS in subjects with PDD (n=14), PD (n=12) and controls (n=13). Using a single voxel technique, they selected an area of the basal ganglia and the occipital cortex. Subjects with PDD showed lower NAA/Cr values than in PD or controls without an associated change in mI/Cr in the occipital cortex suggesting neuronal dysfunction without glial involvement. The results are in contrast to those of AD: decreased NAA/Cr and increased mI/Cr in the occipital area (Soares and Law, 2009).

In summary, some but not all studies found relatively normal NAA/Cr ratios in DLB while in PDD, similar reductions to AD are seen. The differing voxels of interest, comparison groups and small sample size currently limit the generalisability of results.

## 2.5 Functional MRI (fMRI)

Functional MRI (fMRI) can be used to image regional brain activity. This technique relies on the fact that the magnetic state of haemoglobin (Hb) is oxygen dependent and changes from diamagnetic to paramagnetic as oxyhaemoglobin is deoxygenated (Jezzard *et al.*, 2001). The increased consumption of oxygen by neurons during activation is accompanied by a disproportionate supply of oxygenated blood so that there is a net reduction in deoxy-Hb downstream from the area of activation which leads to an increase in the MR signal (T2 or T2\* weighting), a process described as Blood Oxygenation Level Dependent (BOLD) contrast (Jezzard *et al.*, 2001).

As previously noted, SPECT and PET studies have reported changes in occipital blood flow and glucose metabolism respectively in DLB compared with AD (Minoshima *et al.*, 2002; Lobotesis *et al.*, 2001; Ishii *et al.*, 1999). However, the cause of this is not clear and may not relate to structural changes (Burton *et al.*, 2002; Middelkoop *et al.*, 2001). Greater deficits have been observed in the lateral occipitotemporal cortex compared with ventral and medial areas (Ishii *et al.*, 1999). In an exploratory fMRI study, Sauer *et al.* (2006) looked at task-related brain activity using a visual motion task as the lateral occipitotemporal probe and colour and face

discrimination task as the ventral occipitotemporal probe in 32 subjects: AD (n=10), DLB (n=9) and controls (n=13). Subjects were given task-related instructions and asked to respond using an alternative key, pressing as quickly and as accurately as possible. The motion task resulted in reduced activation in the lateral occipitotemporal area in DLB subjects compared with AD and controls and a trend toward a slower reaction time. Deficits were also evident in the face task (discriminating gender) with reduced ventral occipitotemporal activation and accuracy in the DLB group compared with AD and controls. The superior temporal sulcus (STS) is known to be atrophied in AD and to a lesser extent, in DLB (Gomez-Isla et al., 1999), and a greater resting blood flow in the right medial temporal lobe has also been documented in a SPECT study (Ishii et al., 1999). Sauer *et al.* (2006) found greater motion-related STS activation in DLB than AD suggesting that the slower reaction time in the motion task was less likely to be a result of non-specific effects such as fatigue. Since these fMRI studies rely on indirect detection of activity (the BOLD effect), any underlying changes in the microvasculature at the capillary level may confound interpretation of changes in activity measured in these studies (Sauer et al., 2006).

A recent study investigated the functional connectivity using the resting BOLD contrast (T2\* weighting) in DLB (n=15), AD (n=35) and controls (n=38) (Galvin et al., 2011). Using a precuneal seed, they found differing patterns of functional connectivity in DLB compared with AD. In DLB, there was increased connectivity in the putamen and inferior parietal cortex (dorsal attention network) and decreased connectivity in the medial prefrontal cortex, frontoparietal operculum (frontoparietal executive control networks) and primary visual cortex. The findings were somewhat limited by the sample size, with results reported uncorrected for multiple comparisons. However, they are suggestive of differences in the functional connectivity pattern between AD and DLB and warrant further investigation.

## 2.6 Conclusions

MR changes in DLB and PDD are similar and are characterised by a profile of atrophy predominantly affecting the subcortical structures with relative preservation of the medial temporal lobe structures which are very consistent with the characteristic clinical and cognitive features of the disease. These structural imaging

similarities provide support for the view that DLB and PDD represent points along a spectrum of Lewy body disorders. Relative preservation of the MTL compared with AD shows promise as a diagnostic marker in differentiating AD from DLB and has been incorporated into the revised criteria for the clinical diagnosis of DLB (McKeith et al., 2005). The rate of cerebral atrophy has potential as an outcome measure in therapeutic trials of putative disease modifying agents. However, further longitudinal studies looking at serial rates of atrophy are required before this can be fully established. In particular, as with the validation of any surrogate marker, a link between atrophy rate and some clinically significant outcome needs to be established for DLB, as has been done for AD (O'Brien et al., 2001).

It is not yet possible to form robust conclusions about the utility of other MR measures. A combination of inconsistent results reported between studies and a degree of overlap in MR measures between dementia subtypes currently limits the diagnostic utility of some imaging techniques. There are many factors which may contribute to the varying results including the heterogeneity in study populations with differing levels of education, age range and cognitive and functional deficits, all of which are inherent to any clinical study in dementia. Other factors may include differing MR techniques and analysis methods, recruitment strategies and the fact that clinical research, despite the application of well-validated clinical diagnostic criteria remains limited by a level of diagnostic uncertainty. It is also possible that a number of subjects have more than one type of dementia pathology, for example, PDD or DLB subjects may also have varying degrees of Alzheimer pathology, which would dilute the differences between the conditions. However, there is little doubt that DLB and PDD are different clinicopathological entities to AD. The challenge is discovering the link between the clinical presentation and a relatively non-invasive *in vivo* technique to refine the ante-mortem diagnosis thereby allowing patients access to appropriate prognostic information and treatment strategies. MR techniques offer a non-invasive method to study cerebral changes in more detail. MR studies to date have been single modality, mostly looking at structural change. Advanced MR techniques such as DTI offer more sensitive measures for detecting early and potentially preclinical changes.

The next chapter will describe the aims and hypotheses of the research undertaken in this thesis: *Structural and diffusion MRI in dementia with Lewy bodies. A comparison with Alzheimer's disease and normal ageing.*



## Chapter 3 Study aims, objectives and hypotheses

### 3.1 Aims

The main aim of this research was to use high field strength (3T) MRI to investigate structural and microstructural (diffusion imaging) changes in DLB, and to compare these findings both to healthy older controls and those with AD, a disease control. Our intention was to better understand neurobiological changes underpinning DLB, and investigate MR indices that may be helpful for both early and differential diagnosis of DLB, and subsequently show promise for monitoring disease progression.

### 3.2 Objectives and hypotheses

To examine and compare the regional volumetric grey matter (GM) changes in DLB compared to AD and healthy controls using voxel based morphometry analysis of the structural T1 weighted images acquired at 3T, and investigate the relationship of GM volume loss with clinical and neuropsychological measures.

- DLB will be associated with less grey matter atrophy than AD but more than controls. The pattern of regional GM atrophy will display a posterior (parieto-occipital) and subcortical predominance compared to controls. This will be in contrast to AD, which will be whole brain atrophy with prominent temporo-parietal GM volume loss.
- There will be less GM atrophy in the medial temporal lobe structures in DLB when compared with AD.

To examine and compare the diffusion tensor imaging changes in the white matter tracts of DLB patients compared to AD and healthy controls using the tract based spatial statistics method of analysis. To investigate the relationship between DTI indices and clinical and neuropsychological measures.

- There will be less white matter tract change in DLB than AD. In DLB the pattern of regional change in DTI indices will include the visual association

areas and subcortical structures, consistent with the neuropsychological profile. There will be less change in the white matter tracts of the medial temporal lobe structures in DLB than seen in AD.

- Impaired episodic memory will correlate with DTI change in medial temporal lobe structures in both AD and DLB and executive dysfunction will correlate with DTI change in a fronto-subcortical pattern in DLB.

## **Chapter 4 Clinical and Neuropsychological Characteristics**

### **4.1 Introduction**

As described in section 1.2.2.3, the pattern of neuropsychological impairment in DLB has been described as reflecting the combination of frontosubcortical dysfunction and the cortical cognitive deficits typically seen in AD (Janvin et al., 2006). This typically manifests in DLB as a dysexecutive syndrome with prominent visuo-perceptual problems and fluctuating attention (McKeith et al., 2004). Memory is also affected, but usually to a lesser extent than seen in AD, at least in the earlier stages of illness (McKeith et al., 2004). The pathological substrates for these specific cognitive features are less clear and possibly reflect differing contributions of neurochemical and morphological changes when compared with AD.

This chapter aims to characterise the clinical and neuropsychological features of a cohort of DLB participants in comparison to AD and healthy ageing. These subjects were recruited to undergo detailed MR imaging so that the specific aims and hypotheses which have been specified in Chapter 3 could be tested. Parameters which may separate the groups will be of particular interest as a means of developing a useful ‘probe’ for subsequent correlations with imaging changes in order to explore the potential pathological underpinnings of clinical features.

### **4.2 Methods**

#### **4.2.1 Subjects**

##### *Subjects with dementia*

Seventy one people with dementia over the age of 60 (both sexes) were recruited from a community dwelling population of patients referred to local Old Age Psychiatry, Geriatric Medicine or Neurology Services. The research was approved by the local ethics committee. All patients and where appropriate, their nearest relative provided written informed consent.

### *Control Subjects*

Thirty-five control subjects were recruited from among relatives and friends of subjects with dementia or people who volunteered via advertisements in local community newsletters. A detailed examination was undertaken to exclude those with evidence of dementia (from history or CAMCOG score < 80), depression (history and assessment) or a history of any other significant neurologic, physical or psychiatric disorder including drug and alcohol abuse.

#### **4.2.2 Assessment and Diagnosis**

Diagnosis was made following a detailed clinical assessment. This included review of clinical records, a psychiatric and medical history review and physical examination. For those with dementia, a standard dementia screen was also completed which included haematology and biochemistry analysis, B12 and folate concentrations, thyroid function tests, syphilis serology and CT brain. Diagnoses of AD and DLB were made in accordance with NINCDS/ADRDA (McKhann et al., 1984) and dementia with Lewy bodies consensus criteria (McKeith et al., 2005; McKeith et al., 1996) respectively by consensus agreement between three experienced raters (RB, JO'B and RW – as outlined in the declaration) blinded to all information from the research MRI scans.

#### **4.2.3 Clinical and Neuropsychological measures**

##### **4.2.3.1 Clinical Scales**

For subjects with dementia, neuropsychiatric features were assessed using the Neuropsychiatric Inventory (NPI), an informant-based scale that is the most widely used neuropsychiatric tool in dementia and covers all relevant behavioural domains. It includes questions to assess 10 behavioural disturbances occurring in dementia patients: delusions, hallucinations, dysphoria, anxiety, agitation/aggression, euphoria, disinhibition, irritability/lability, apathy and aberrant motor behaviour (Cummings et al., 1994). For each behaviour the presence followed by its frequency and severity were determined.

Cognitive fluctuations were assessed using the Clinician Assessment of Fluctuation (CAF), a carer-based questionnaire which rates the frequency (scale 0-4) and

severity (scale 0-4) of fluctuating cognition or consciousness over the 4 weeks prior to assessment, with 0 (and 16 by definition) representing no fluctuation and 12 representing a high degree of fluctuation (Walker et al., 2000b). The CAF is one of 2 fluctuation scales that have been validated in DLB and the only scale that has been validated against a biological standard (Ferman et al., 2004; Walker et al., 2000a; Walker et al., 2000b)

Function was assessed using the Bristol Activities of Daily Living scale. It is a carer based questionnaire related to 20 daily living activities, developed specifically for use in people with dementia (Bucks et al., 1996). It has been validated and is widely used (Bucks and Haworth, 2002).

The Geriatric Depression Scale was used to assess depressive symptoms in all subjects (Sheikh and Yesavage, 1986). The short version selected is a 15 item self-report (yes/no) specifically developed and validated for use in the elderly and people with dementia (Gerety et al., 1994; Norris et al., 1987).

Motor symptoms were assessed using the Unified Parkinson's Disease Rating Scale-III (motor subsection) as it is the most widely used and validated scale for assessment of motor features of Parkinson's disease (Ramaker et al., 2002; Fahn et al., 1987). Ballard *et al.* (1997) has also identified 5-items of the UPDRS-III as motor features that were independent of confounding by cognitive impairment, comprising (1) Tremor at Rest, (2) Bradykinesia, (3) Action Tremor, (4) Facial Expression and (5) Rigidity.

#### **4.2.3.2 Neuropsychological Assessment**

##### **Global Cognitive Measures**

Assessment of global cognitive measures in all subjects (AD, DLB and controls) included the Cambridge Cognitive Examination (CAMCOG) which incorporates the Mini-Mental State Examination (MMSE) (Folstein et al., 1975).

The National Adult Reading Test (NART) was completed by all participants (Nelson and Willison, 1991) which provided an estimate of pre-morbid intelligence and educational level.

### Attention – CDR System

Attention was assessed with the Cognitive Drug Research (CDR) computerised assessment system—see <http://www.cognitivedrugresearch.com/>. This computerised attentional battery was selected as it has been used in dementia and, in particular, has been validated for use in DLB and AD (Ballard et al., 2002; Wesnes et al., 2002; Ballard et al., 2001a). The attentional battery included Simple Reaction Time (SRT), Choice Reaction Time (CRT) and Digit Vigilance (Vig) (Table 4.1). The difference between CRT and SRT is referred to as Cognitive Reaction Time (CogRT).

The tests were administered using a portable laptop computer placed at a standard distance from participants. The responses were recorded using a response pad with 2 buttons, one marked “no” and the other marked “yes”.

Task	Task Explanation	Variables Measured
Simple Reaction Time	Each time “yes” was presented on the screen, the participant was required to press the “yes” button as quickly as possible on the response pad provided.	Response time (msec)
Choice Reaction Time	Each time the word “yes” or “no” appeared on the screen, and the participant was required to press the corresponding “yes” or “no” button as quickly as possible on the response pad provided.	Response time (msec)
Cognitive Reaction Time	Difference between Choice Reaction Time and Simple Reaction Time CRT Mean – SRT Mean	Response time difference (msec)
Digit Vigilance	A digit was randomly selected and constantly displayed on the right of the screen. A series of digits were presented in the centre of the screen (80 per minute for 4 minutes) and the participant was required to press the “yes” button when the digit in the series matched the one on the right.	Response time (msec); percentage of accurate responses

Table 4.1. CDR Attentional Task Battery.

## **Executive Function**

### 1. CAMCOG executive function subscore

The CAMCOG executive function subscore (max 28) comprised category fluency (max 6), abstract thinking (max 8), ideational fluency (max 8) and visual reasoning (max 6).

### 2. Delis Kaplan executive function – verbal fluency tasks

As indicated in Chapter 1, DLB is characterised by a prominent dysexecutive syndrome. We therefore adopted the Delis Kaplan verbal fluency tasks which have been used in people with dementia and included: (1) letter fluency (FAS), (2) category fluency (animals/boys names) and (3) category switching (fruits/furniture) (Delis et al., 2001). Higher level functions required for these tasks include initiation, simultaneous processing, systematic retrieval and speed of processing.

## **Praxis**

Given the visuo-perceptual difficulties in DLB compared with AD, the CAMCOG Praxis subscore (max 12) was of interest as it includes tasks of visuo-spatial function – copying 3 figures (max 3) as well as clock drawing (max 3).

## **Memory**

### 1. CAMCOG memory subscale

This included assessment of recent and remote memory and new learning.

### 2. Episodic memory

The Hopkins Verbal Learning Test (HVLT) and Brief Visual Memory Task – Revised (BVMT), are both widely used and well-validated measures of episodic memory (Benedict, 1997; Benedict and Groninger, 1995; Brandt, 1991). In brief, the HVLT consisted of a 12 word list which was read aloud 3 times (3 trials). After each trial, the participant was asked to recall as many words as they could from the list (total recall). The participant was then asked to recall as many items from the list following a 20 minute delay (delayed recall). Following this, there was a recognition trial where 12 items and 12 distractors (items not on the original word list) were read

aloud and the participant was asked if the items were on the original word list and asked to respond “yes” or “no”. The BVMT structure was similar. It involved the participant viewing an A4 sheet of paper with 6 figures for 60 seconds. The sheet was then covered and the participant was asked to draw as many items as they could recall (1 point for each correct figure and 1 point for each figure correctly placed). This was repeated 3 times (total recall) and then after a 20 minute delay (delayed recall). The participant was then shown 6 of the original figures and 6 distractors (figures not on the original sheet), one at a time and asked if the figures were on the original sheet of figures ‘yes’ or ‘no’ (recognition trial).

In order to incorporate all aspects of the task, we derived a composite score of episodic memory (verbal and visual). Using Statistical Package for Social Sciences (SPSS, version 17), we normalised the scores of the cohort (AD, DLB and Controls) for each task component (total recall, delayed recall and recognition) creating a z-score. That is,  $z_i$  (task component) =  $\frac{x_i - \mu_{\text{cohort}}}{S_{\text{cohort}}}$ ; where  $i$  = study participant.

The episodic memory composite was then calculated by adding the normalised scores (z-scores) of the components of the HVLT and BVMT (equal weighting). Episodic Memory Composite =  $z_{\text{HVLT total recall}} + z_{\text{HVLT delayed recall}} + z_{\text{HVLT recognition (true positive + true negatives)}} + z_{\text{BVMT total recall}} + z_{\text{BVMT delayed recall}} + z_{\text{BVMT recognition (true positives + true negatives)}}$ .

#### 4.2.4 Statistics

Descriptive analyses of the group characteristics were completed using the statistical software, SPSS 17. Data was reviewed for normality by visual inspection of the histograms and using the Shapiro-Wilk test. Differences in demographic and clinical data were assessed using either t-tests, Analysis of variance (ANOVA), or rank-sum tests (Kruskal Wallis or Mann-Whitney U) as appropriate for continuous variables and  $\chi^2$  test for categorical data. Where post hoc analysis was required to test for group differences following ANOVA, a Tukey correction for multiple comparisons was applied to control for Type I errors. For each test statistic, a probability value  $p < 0.05$  was regarded as significant.



### **4.3 Results**

#### ***4.3.1 Subject characteristics***

One hundred and six participants were included in the analysis (36 AD, 35 DLB and 35 Controls). Seventy-one participants with dementia were recruited between November 2008 and September 2010 along with 35 age-matched healthy control subjects.

Thirty six subjects met the NINCDS/ADRDA criteria for probable AD (McKhann et al., 1984).

Thirty four subjects met the original consensus diagnostic criteria for probable DLB and one for possible DLB (McKeith et al., 1996) and all 35 DLB subjects fulfilled the revised consensus criteria for probable DLB (McKeith et al., 2005).

#### ***4.3.2 Subject demographics***

The demographic data for patients and control subjects are summarised in Table 4.2. Subject groups were well matched for age ( $F_{2,105}=0.804$ ,  $p=0.450$ ) and educational level ( $F_{2,104}=0.979$ ,  $p=0.379$ ). There were more men included in the DLB group, consistent with the male predominance reported in DLB but this difference did not reach statistical significance ( $\chi^2=3.85$ ,  $df=2$ ,  $p=0.146$ ). NART-FS was similar in the dementia groups and, as expected, lower than the control group ( $F_{2,103}=7.69$ ,  $p=0.001$ ; post hoc DLB vs AD,  $p=0.998$ ).

The AD and DLB group mean CAMCOG ( $t_{69}=0.58$ ,  $p=0.566$ ) and MMSE ( $t_{69}=0.71$ ,  $p=0.480$ ) scores were comparable suggesting a similar level of dementia severity. As expected these measures were significantly lower than the control group.

Characteristic	DLB (n=35)	AD (n=36)	NC (n=35)	p value
Age (years)	78.4±6.9	78.3±5.8	76.7±5.2	0.450*
M:F (% male)	27:8 (77%)	21:15 (58%)	20:15 (57%)	0.146§
Education (years)	10.8±2.6	11.1±3.5	11.7±2.6	0.379*
NART-predicted IQ	102.8±12.4	102.6±13.6	112.4±9.2	0.001*, 0.998**
Estimated dementia duration, months	40.6±20.6	52.6±27.4	NA	0.042†
MMSE (max 30)	20.3±5.3	19.5±4.4	29.1±1.0	0.480†
CAMCOG (max 107)	67.7±15.3	65.8±12.1	97.3±3.8	0.566†

Values expressed as mean ± 1SD (unless otherwise stated).

\* ANOVA – Controls, AD and DLB; \*\*post hoc AD vs. DLB

§Pearson's Chi-square test – Controls, AD and DLB;

† Student's t-test – AD vs. DLB

Table 4.2. Subject demographics.

### 4.3.3 Clinical features

#### 4.3.3.1 Dementia with Lewy bodies subject characteristics

The core clinical diagnostic features of the DLB group are summarised in Table 4.3.

##### *Visual Hallucinations*

Twenty-nine subjects had a history of visual hallucinations which were fully formed, occurring on more than one occasion and not attributable to medical factors, medications or advanced dementia. Using the NPI, we found that 26/33 (79%) had visual hallucinations in the 4 weeks prior to study assessment. An informant or carer was not available for 2 DLB subjects who had a history of hallucinations. Two DLB informants reported no visual hallucinations in the previous 4 weeks although the DLB subjects had a clear history of recurrent hallucinations prior to this. One DLB informant reported occasional visual hallucinations which did not meet the criteria for recurrent and fully formed visual hallucinations.

Clinical Features	Present
	Yes : No (%Yes)
<b>Core Features</b>	
Recurrent Visual Hallucination	29 : 6 (83%)
Cognitive Fluctuation	32 : 3 (91%)
Spontaneous Motor Parkinsonism	29 : 6 (83%)
<b>Supportive Features</b>	
REM sleep behaviour disorder	21 : 14 (60%)
Abnormal dopaminergic SPECT imaging*	22 (100%)
Neuroleptic sensitivity reactions	0 (0%)

\*Twenty-two patients had FP-CIT SPECT imaging prior to study inclusion, all were abnormal. 13 patients did not have FP-CIT SPECT imaging, which is not needed to make a diagnosis of probable DLB.

Table 4.3. Clinical features of the DLB cases.

### *Cognitive Fluctuation*

Cognitive fluctuations were present in the majority of DLB cases 32/35 (91%) as assessed by 2 independent raters (RW and JO'B) according to the consensus diagnostic criteria (Table 4.3). There were significantly more DLB carers who reported fluctuation 28/33 (85%) than AD carers 11/35 (31%) ( $\chi^2=23.10$ ,  $df=7$ ,  $p=0.002$ ), the results are represented in Figure 4.1 and Table 4.4. Of the 3 DLB subjects that were assessed as non-fluctuators by diagnostic rating, 2 did not have informants and the other was also reported by the informant not to fluctuate using the fluctuation scale. Therefore, there were 4 DLB subjects who were rated as fluctuators but reported by their informant not to have cognitive fluctuations in the 4 weeks prior to assessment as assessed by the fluctuation scale.

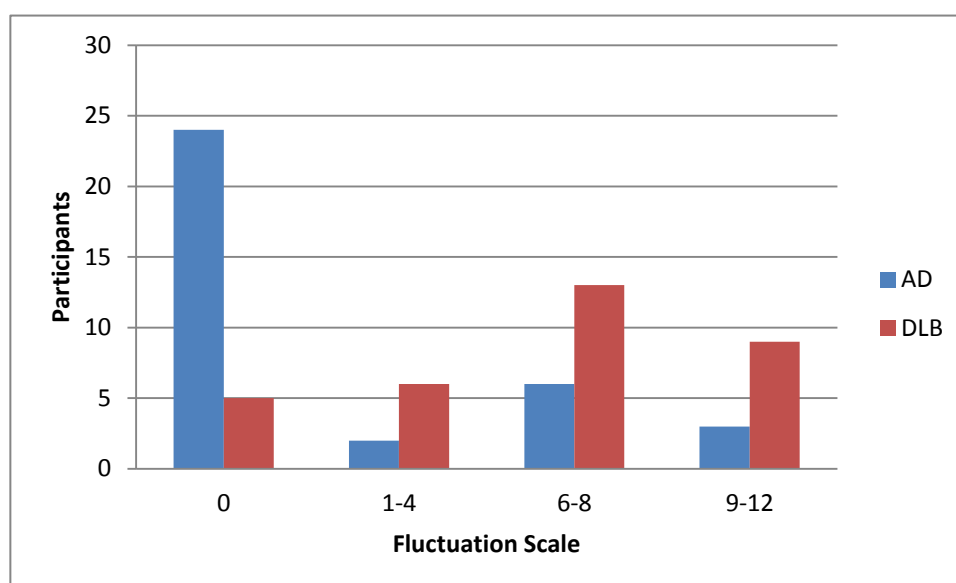


Figure 4.1. Clinician's Assessment of Fluctuating Cognition.

Characteristic	DLB n=33	AD n=35	p-value
Fluctuation Scale (max 16)	6.1±3.8	2.2±3.6	0.001*

Values expressed as mean ± 1SD

\*Rank sum test (Mann Whitney U) AD vs. DLB

Table 4.4. The Clinician Assessment of Fluctuation.

### *Motor parkinsonism*

Spontaneous motor parkinsonism, as defined by the UK Parkinson's Disease Society Brain Bank Criteria (Hughes et al., 1992) was present in 29 DLB cases, and 11 (31%) of the DLB participants were taking anti-parkinsonian medication. The estimated duration of parkinsonism was 31 months and the maximum duration was 72 months. The severity of parkinsonism was rated with the Unified Parkinson's Disease Rating Scale – III (UPDRS-III). The average UPDRS-III for the DLB group ( $\bar{x}=26.0\pm 10.7$ ) was higher than the control group ( $\bar{x}=2.0\pm 1.9$ ), and the AD group ( $\bar{x}=5.6\pm 4.3$ ) (DLB > AD;  $\Delta = 20.4$ , 95% CI: (16.6, 24.2);  $t_{69}=10.6$ ,  $p<0.001$ ). The '5-item UPDRS' was also significantly higher in the DLB than the AD group ( $\Delta = 9.7$ , 95% CI: (8.0, 11.4);  $t_{69}=11.3$ ,  $P<0.001$ ) represented in Table 4.5.

Characteristic	DLB n=35	AD n=36	Controls n=35	p-value
UPDRS III	26.0±10.7	5.6±4.3	2.0±1.9	<0.001†
5-item UPDRS	10.9±5.0	1.3±1.2	0.4±0.7	<0.001†

Values expressed as mean ± 1SD (unless otherwise stated).

† Student's t-test – AD vs DLB

Table 4.5. Group UPDRS scores and motor parkinsonism.

#### *REM sleep behaviour disorder*

Twenty-one DLB subjects (60%) met the International Classification of Sleep Disorders-II diagnostic criteria B for Rapid Eye Movement sleep behaviour disorder (AASM, 2005) as assessed by two independent raters (RW and JO'B).

#### *Dopaminergic imaging*

Twenty-two DLB subjects underwent dopaminergic SPECT imaging as part of their clinical diagnostic work-up prior to study inclusion. All 22 (100%) had abnormal images showing reduced striatum dopaminergic binding consistent with their diagnosis. The remaining 13 DLB subjects did not have dopaminergic imaging, as it was not judged to be clinically indicated.

#### *Neuroleptic sensitivity reactions*

None of the DLB subjects had a documented history of neuroleptic sensitivity reactions; 3/35 were prescribed neuroleptics whilst involved in the study (2 quetiapine and 1 aripiprazole).

#### **4.3.3.2 Clinical scales**

Results for the clinical assessment scales are shown in Table 4.6.

#### *Neuropsychiatric Symptoms*

As expected, the DLB group had more depressive features as measured by the Geriatric Depression Scale than the AD group ( $\bar{x}_{DLB}=6.2\pm3.7$ ,  $\bar{x}_{AD}=2.8\pm1.8$ ;

AD<DLB  $\Delta = 3.4$ , 95% CI: (1.8, 5.0); DLB, AD & NC:  $F_{2,105}=26.8$ ,  $p<0.001$ ). There was not a significant difference in the average GDS score between the AD and Control groups. Interestingly, despite higher rates of depressive symptoms, fewer DLB informants (14/33, 42%) reported depression than AD informants (21/35, 60%) and the average NPI depression score between AD and DLB was similar ( $\bar{x}_{DLB} = 1.58 \pm 2.85$ ,  $\bar{x}_{AD} = 1.69 \pm 1.89$   $t_{66}=0.19$ ,  $p=0.851$ ). Therefore, in DLB, informants were less likely to report depressive symptoms than the subject themselves and in AD, the informants were more likely to report depressive symptoms than the subject themselves.

The mean NPI score was higher in the DLB group, although, the difference was not statistically significant ( $\bar{x}_{DLB}=21.5 \pm 17.1$ ,  $\bar{x}_{AD}=16.8 \pm 11.9$ ;  $t_{66}=1.30$ ,  $p=0.197$ ). From the 10 symptoms measured: visual hallucinations were more common in DLB than AD; and disinhibition was reported more frequently in AD (31%) than in DLB (3%) ( $\chi^2=9.43$ ,  $df=1$ ,  $p=0.002$ ). Although delusions were reported slightly more frequently in DLB (39%) than AD (27%) as was apathy (DLB 64%, AD 50%), these difference were not significant.

### Function

The DLB group had slightly more overall functional impairment than the AD group as assessed by the Bristol ADL scale ( $\bar{x}_{DLB}=18.2 \pm 9.6$ ,  $\bar{x}_{AD}=14.1 \pm 7.2$ ,  $\Delta=2.04$ , 95% CI: (0.05, 8.2);  $t_{66}=2.02$ ,  $p=0.042$ ).

Clinical Scales	DLB n=35	AD n=36	Controls n=35	p-value
GDS (max 15)	6.2±3.7	2.8±1.8	1.7±2.2	<0.001*
NPI total	<sup>a</sup> 21.5±17.1	<sup>b</sup> 16.8±11.9	NA	0.197†
NPI: depression, Yes (% yes)	<sup>a</sup> 14 (42%)	<sup>b</sup> 21 (60%)	NA	0.491§
NPI: VH, Yes (% yes)	<sup>a</sup> 26 (79%)	<sup>b</sup> 3 (9%)	NA	<0.001§
NPI: delusions, Yes (% yes)	<sup>a</sup> 13 (39%)	<sup>b</sup> 10 (29%)	NA	0.346§
NPI: apathy, Yes (% yes)	<sup>a</sup> 21 (64%)	<sup>b</sup> 17 (50%)	NA	0.260§
NPI: disinhibition, Yes (% yes)	<sup>a</sup> 1 (3%)	<sup>c</sup> 11 (31%)	NA	0.002§
Bristol ADL	<sup>a</sup> 18.2±9.6	<sup>c</sup> 14.1±7.2	NA	0.042†

Values expressed as mean  $\pm$  1SD (unless otherwise stated).

\* ANOVA – Controls, AD and DLB;

§ Pearson's Chi-square test – Controls, AD and DLB;

† Student's t-test – AD vs DLB

<sup>a</sup> (n=33), <sup>b</sup> (n=34), <sup>c</sup> (n=35)

Table 4.6. Results of clinical scales by group.

**4.3.3.3 Other clinical variables***Vascular Risk Factors*

The vascular risk factor profile of all participants was similar (Table 4.7). A greater proportion of the DLB group were taking anti-platelet agents (51%) compared with controls (23%). This difference reached statistical significance between the DLB and Control group ( $\chi^2=6.12$ ,  $df=1$ ,  $p=0.013$ ), but not between the DLB and AD groups ( $\chi^2=1.13$ ,  $df=1$ ,  $p=0.288$ ).

	<b>DLB (n=35)</b>	<b>AD (n=36)</b>	<b>NC (n=35)</b>	<b>p-value</b>
<b><i>Vascular Risk Factors</i></b>				
Hypertension	11 (31.4%)	14 (39%)	18 (51.4%)	ns
Hypercholesterolaemia	11 (31.4%)	14 (39%)	16 (45.7%)	ns
Type 2 diabetes mellitus	4 (11.4%)	2 (5.7%)	3 (8.3%)	ns
Cardiovascular disease	8 (22.9%)	3 (8.3%)	3 (8.6%)	ns
<b><i>Smoking history</i></b>				
Current: Past: Never	2:15:17	2:15:19	2:21:12	ns
<b><i>Medications</i></b>				
Antihypertensives	19 (54.3%)	16 (44%)	19 (54.3%)	ns
Cholesterol lowering agents	11 (31.4%)	13 (36.1%)	14 (40%)	ns
Antiplatelet agents	18 (51.4%)	14 (38.9%)	8 (22.9%)	0.047, *ns,
Warfarin	1 (2.9%)	1 (2.8%)	3 (8.6%)	ns
Oral hypoglycaemic agents	1 (2.9%)	1 (2.8%)	1 (2.9%)	ns

Statistical tests -  $\chi^2$  across all groups;

\* $\chi^2$  between AD and DLB;

ns, not significant ( $p>0.05$ ).

Table 4.7. Vascular risk factors in all groups.

*Other medications taken by dementia subjects*

The majority of both AD (92%) and DLB (86%) groups were taking cholinesterase inhibitors. Fifteen (43%) DLB participants were taking anti-depressant medication compared with 9 (25%) of those with AD, although the difference was not statistically significant ( $\chi^2=1.82$ ,  $df=1$ ,  $p=0.177$ ). Very few subjects were taking antipsychotic medication: 3 DLB (9%) and 1 AD (3%) (Table 4.8).

<b>Medication</b>	<b>DLB (n=35)</b>	<b>AD (n=36)</b>	<b>p-value</b>
Cholinesterase Inhibitors	30 (86%)	33 (92%)	ns
Memantine	1 (3%)	2 (6%)	ns
Benzodiazepines	2 (6%)	0	ns
Antidepressants	15 (43%)	9 (25%)	ns
Antipsychotics	3 (9%)	1 (3%)	ns

Table 4.8. Medications taken by dementia subjects.

**4.3.4 Neuropsychological Assessment****4.3.4.1 Attention**

The task mean results were not normally distributed and, for each variable the correlation between mean and standard deviation was such that:  $0.64 \leq r \leq 0.95$ ,  $p < 0.001$ . We therefore transformed the data to carry out group comparisons. For SRT, CRT and Vig group mean data, we used a reciprocal transformation [SRT Mean<sub>T</sub> = 1/SRT Mean] and for CogRT mean, and group standard deviation data we used a logarithmic transformation [SRT SD<sub>T</sub> = log(SRT SD)].  $\chi^2$  was used to compare the differences in Vig accuracy. For all other group comparisons, ANOVA was used. For post hoc testing between AD and DLB a Tukey correction for multiple comparisons was applied. All results are represented in Table 4.9 and Figure 4.2.

As can be seen, the dementia groups had larger response times and greater variability in response times than controls in all CDR tasks. Within the dementia groups, the DLB group were also significantly slower than the AD group in all tasks. The CogRT time is the difference between CRT and SRT and, effectively accounts the motor response time, which is often slower in DLB. CogRT mean was also slower in



DLB than AD ( $F_{2,99}=21.47$ ,  $p<0.001$ , post-hoc  $\text{CogRT}_{\text{DLB}}>\text{CogRT}_{\text{AD}}$ ,  $p=0.029$ , corrected). The DLB group also had greater variability in response times than AD (SD) which reached statistical significance in all tasks suggestive of fluctuating levels of attention.

The accuracy rate in the vigilance task was also significantly lower in the DLB group than in the AD ( $\chi^2 =13.43$ ,  $df=4$ ,  $p=0.009$ ) and control groups ( $\chi^2=26.70$ ,  $df=4$ ,  $p<0.001$ ), consistent with more severe attentional deficits in DLB.

CDR Task	DLB (n=34)	AD (n=34)	Controls (n=35)	p-value
SRT Mean	692 (309)	484 (452)	320 (71)	<0.001*, 0.012**
SRT SD	309 (569)	139 (228)	64 (24)	<0.001*, 0.032**
CRT Mean	1104 (738)	779 (345)	517 (92)	<0.001*, 0.020**
CRT SD	477 (665)	259 (220)	99 (29)	<0.001*, 0.021**
CogRT Mean	<sup>b</sup> 477 (321)	<sup>c</sup> 335 (198)	197 (80)	<0.001*, 0.029**
CogRT SD	<sup>b</sup> 561 (870)	<sup>c</sup> 271 (226)	120 (30)	<0.001*, 0.011**
Vigilance Mean	<sup>a</sup> 608 (110)	520 (102)	424 (51)	<0.001*, 0.002**
Vigilance SD	<sup>a</sup> 133 (47)	104 (38)	62 (20)	<0.001*, 0.017**
Vig accuracy	<b>n=29</b>	<b>n=34</b>	<b>n=35</b>	<0.001 <sup>§</sup> , 0.009 <sup>§§</sup>
0-50%,	7 (24%)	2 (6%)	0	
51-75%	7 (24%)	1 (3%)	0	
76-99%,	7 (24%)	14 (41%)	3 (9%)	
100%	8 (28%)	17 (50%)	32 (91%)	

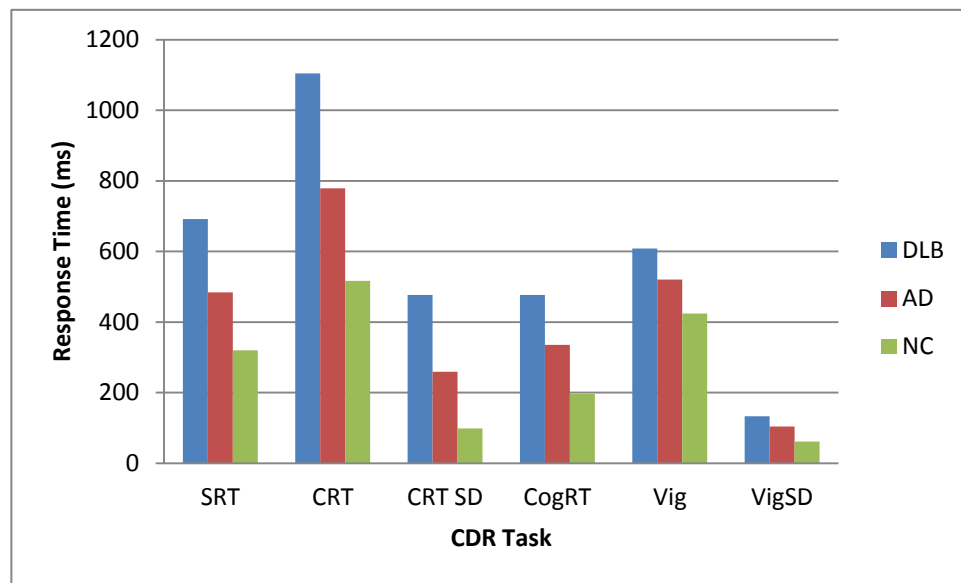
Values represented as mean (standard deviation), in milliseconds except for Vig accuracy, where values are represented as number of participants (percentage).

<sup>a</sup>n=29, <sup>b</sup>n=32, <sup>c</sup>n=33;

ANOVA on transformed variables – \*DLB, AD and Controls, with post hoc \*\*DLB vs AD group comparison;

$\chi^2$ –<sup>§</sup>DLB, AD, Controls and <sup>§§</sup>DLB vs AD.

Table 4.9. CDR Computerised attentional task: response by group.



Bar chart representing CDR task mean response time in milliseconds (ms) by group.

Figure 4.2. CDR Computerised attentional task: response by group.

#### 4.3.4.2 Praxis and Executive Function

The results of neuropsychological tests of praxis and executive function are represented in Table 4.10.

The CAMCOG praxis subscale includes 4 items involving visuo-spatial and visuo-constructive tasks: copying a pentagon, spiral, 3D house and clock face drawing. The other items relate to ideomotor (e.g. wave good-bye, brush teeth) and ideational praxis (placing paper in an envelope). Given the weighting toward visuo-spatial/visuo-constructive domains, it was not surprising that that DLB mean group performance was worse than the AD group ( $\bar{x}_{DLB} = 7.0 \pm 2.5$ ,  $\bar{x}_{AD} = 9.5 \pm 1.4$ ;  $\Delta = 2.5$ , 95% CI: (1.5, 3.4);  $t_{69} = 5.07$ ,  $p < 0.001$ ).

The CAMCOG executive function subscale includes abstract thinking, verbal and ideational fluency and visual reasoning. These domains are affected in both AD and DLB and were comparable in our dementia cohort ( $\bar{x}_{DLB} = 12.1 \pm 5.3$ ,  $\bar{x}_{AD} = 13.4 \pm 4.0$ ;  $t_{63} = 0.71$ ,  $p = 0.266$ ).

Verbal fluency tasks included the letter fluency, category fluency and category switching. The DLB group performance for the letter fluency task was significantly

worse than the AD group ( $\bar{x}_{DLB}=20.8\pm13.3$ ;  $\bar{x}_{AD}=27.5\pm14.3$ ;  $t_{67}=2.00$ ,  $p=0.049$ ) and slightly worse in the category fluency task although this did not reach statistical significance ( $\bar{x}_{DLB}=17.6\pm9.1$ ;  $\bar{x}_{AD}=20.1\pm8.5$ ;  $t_{67}=1.22$ ,  $p=0.227$ ). The category switching task performance between dementia groups was similar.

Task	DLB (n=35)	AD (n=36)	Control (n=35)	p-value (AD vs DLB)†
<i>Praxis</i>				
CAMCOG Praxis (max 12)	7.0±2.5	9.5±1.4	11.3±1.1	<0.001
<i>Executive Function</i>				
CAMCOG –EF (max 26)	12.1±5.3	13.4±4.0	22.4±2.7	0.266
Letter Fluency	<sup>a</sup> 20.8±13.3	<sup>b</sup> 27.5±14.3	41.0±11.1	0.049
Category Fluency	<sup>a</sup> 17.6±9.1	<sup>b</sup> 20.1±8.5	37.9±8.6	0.227
Category Switching	6.2±3.4	6.3±2.8	13.6±2.4	ns

Values expressed as mean ± 1SD (unless otherwise stated).

† Student's t-test – AD vs DLB

<sup>a</sup>(n=34), <sup>b</sup>(n=35)

Table 4.10. Praxis and executive function task results by group.

#### 4.3.4.3 Memory

The results of neuropsychological tests of memory are represented in Table 4.11.

The CAMCOG Memory subscale includes assessment of recent and remote memory as well as new learning. The dementia groups performed significantly worse than the control group. When the dementia groups were compared, the DLB group performed significantly better than the AD group in this task, suggestive of less impaired memory function. Memory was further assessed with the verbal and visual measures of episodic memory using the HVLT and BVMT (Table 4.11). The total recall was similar in both AD and DLB groups and significantly lower than the controls. Sixteen DLB participants (45.7%) were able to recall at least one item after a 20 minute delay, compared with only 4 AD (11.4%) participants, and they also had greater retention as evidenced by higher scores on the recognition trial ( $\Delta=3.8$ , 95% CI: (2.47, 5.06);  $t_{67}=5.79$ ,  $p<0.001$ ). The DLB group also performed better than

the AD group in the BVMT with improved mean total recall ( $\Delta=1.37$ , 95% CI: (0.35, 2.40);  $t_{41,9}=2.23$ ,  $p=0.031$ ), retention (DLB 58.8%, AD 34.3%) and recognition ( $\Delta=1.87$ , 95% CI: (0.95, 2.79);  $t_{66}=4.06$ ,  $p<0.001$ ) with a higher recognition trial discrimination index (DI) ( $\Delta=1.87$ , 95% CI: (0.46, 2.79);  $t_{66}=1.87$ ,  $p<0.001$ ). Scores for each of these components: total recall, delayed recall and recognition for both HVLТ and BVMT were then normalised and added to form an episodic memory composite score for all groups (higher score indicating a better performance). The episodic memory composite was significantly lower in the AD group when compared with the DLB group ( $\Delta=2.84$ , 95% CI: (1.61, 4.07);  $t_{69}=4.61$ ,  $p<0.001$ ) and, as expected, both dementia groups had significantly lower scores than the controls group ( $\bar{x}_{AD} = -4.45 < \bar{x}_{DLB} = -1.61 < \bar{x}_{NC} = 6.25$ ,  $F_{2,100} = 163.5$ ,  $p<0.001$ ).

Task	DLB (n=35)	AD (n=36)	Control (n=35)	p-value (DLB vs AD)*
CAMCOG Memory (max 27)	15.0±4.7	9.7±4.0	23.6±1.6	<0.001 <sup>†</sup>
HVLТ total recall (max 36)	10.8±4.8	10.1±5.0	25.9±4.6	ns <sup>†</sup>
HVLТ delayed recall (>0)	16 (46%)	4 (11%)	34 (97%)	0.010 <sup>§</sup>
HVLТ recognition (max 24)	18.1±2.9	14.4±2.5	22±1.9	<0.001 <sup>†</sup>
BVMT Total (max 36)	<sup>a</sup> 6.9±6.7	<sup>b</sup> 4.1±2.5	18.8±6.5	0.027 <sup>†</sup>
BVMT delayed recall (>0)	<sup>a</sup> 20 (59%)	<sup>b</sup> 12 (34%)	35 (100%)	0.041 <sup>§</sup>
BVMT recognition (max 12)	<sup>c</sup> 9.2±2.1	<sup>b</sup> 7.3±1.7	11.0±1.0	<0.001 <sup>†</sup>
BVMT recognition DI	<sup>c</sup> 3.2±2.1	<sup>b</sup> 1.3±1.7	4.9±1.1	<0.001 <sup>†</sup>
Episodic Memory Composite	<sup>c</sup> -1.6±3.2	<sup>b</sup> -4.5±1.5	6.2±2.6	<0.001 <sup>†</sup>

Values expressed as mean ± 1SD;

Values for HVLТ and BVMT delayed recall (>0) represent, number (percentage) of participants with a result greater than zero.

\*AD and DLB group comparisons were all significantly different to controls.

<sup>§</sup>  $\chi^2$  – Controls, AD and DLB;

<sup>†</sup> Student's t-test – AD and DLB

<sup>a</sup> (n=34), <sup>b</sup> (n=35) <sup>c</sup> (n=33)

Table 4.11. Results of the memory tasks by group.

## 4.4 Discussion

We recruited a large and well characterised group of representative DLB and AD subjects as well as a healthy older comparison group. Groups were matched for key demographic variables such as age, sex and level of education. Between the DLB and AD groups, dementia severity was comparable although the reported duration of illness was shorter in DLB than AD (average of 41 months compared with 53 months;  $p=0.042$ ).

### 4.4.1 Clinical features

The visual processing system in DLB remains an area of intense research interest given the prominent disruptions which manifest as recurrent visual hallucinations and visuo-perceptual dysfunction (McKeith et al., 2004). It seems likely that there is a link between the presence of visual hallucinations and visuo-perceptual change in DLB. This was supported by a study by Mosimann *et al.* (2004) that found patients with DLB and visual hallucinations had more impaired visual perception than the DLB patients without hallucinations. We did not find a difference in the CAMCOG praxis subscale between the DLB group with hallucinations and those without. However, there were only 7 DLB subjects without hallucinations making the comparison difficult. Recurrent visual hallucinations were present in the majority of our DLB group (83%), similar to the reported rate in other study cohorts (McKeith et al., 2004). Visuo-spatial and visuo-constructive deficits were also observed in the DLB group with the CAMCOG praxis subscale score being significantly worse than in AD.

Parkinsonism is one of the core diagnostic features of DLB and was present in most of the DLB group (83%). The UPDRS III score was also significantly higher (mean 26) than in the AD and control group. Although the UPDRS score for the AD group was slightly higher than the controls, this did not reach statistical significance. The development of motor parkinsonism in DLB is a result of dopaminergic deficiency with reduction of the striatal dopamine transporter (Piggot et al., 1999). Interestingly, 60% (23/35) of the DLB cohort had dopamine transporter imaging (100% positive) as part of their clinical care prior to their recruitment into the study. This strongly supports that the majority of the DLB cases had striatal dopaminergic deficiency, a feature that is characteristic of DLB and rare in AD (O'Brien et al., 2004).

While some variation in cognitive performance and function is not uncommon in all dementias, pronounced variations in attention and cognitive performance is characteristic of DLB (Walker et al., 2000b). Fluctuation is characterised by periodic shifts in attention or arousal, though this remains difficult to assess and accurately quantify (McKeith et al., 2004). In this study, cognitive fluctuations were present in 91% of DLB cases as assessed by the clinical diagnostic raters which is consistent with the reported 80-90% rate in all DLB cases (McKeith et al., 1996). A similar rate was also found using the Clinician Assessment of Fluctuation, a carer based questionnaire (Walker et al., 2000b). Interestingly, results of the CDR attentional battery were also in accordance with this. The DLB group had significantly slower cognitive reaction times, choice reaction times, higher vigilance scores with lower accuracy rates and greater variability (SD) in response times in all tasks compared to the AD group. This was consistent with more impaired attention and fluctuation in DLB subjects (Ballard et al., 2002). It is possible that other factors such as parkinsonism or depression may have contributed to these findings. However, the cognitive reaction time, which effectively controls for the motor response component by subtraction of the SRT from CRT, was also significantly slower in DLB than AD. Although this could represent a slowing of cognitive processing speed, the significant differences in variability (SD) and deficits in accuracy indicate widespread attentional problems in DLB compared with AD (Ballard et al., 2002).

Attention involves a complex network of anterior and posterior cortical regions. The thalamus is also a key area involved in maintaining consciousness and organising and processing information between subcortical and cortical structures (Schiff, 2008). The association between fluctuation and increased thalamic perfusion in DLB highlights its potential importance in the underlying pathological process (O'Brien et al., 2005). The cholinergic system, particularly the basal forebrain system is also one of the key modulating features providing major cholinergic input to the thalamus and cortical regions, with significant deficits found in DLB (Lippa et al., 1999; Perry et al., 1993). Cholinesterase inhibitors have been shown to improve attentional function in DLB (McKeith et al., 2000b) and it is also important to note that 86% of the DLB group were prescribed cholinesterase inhibitors but still had quite profound attentional deficits.

Neuropsychiatric features are more common in DLB than AD (Ballard et al., 1999), and not surprisingly, the most common in our DLB cohort was visual hallucinations. Delusions and apathy were frequently reported in both the AD and DLB groups with symptoms being more common in DLB, although this did not reach statistical significance. The DLB group also had significantly more depressive symptoms as assessed by the geriatric depression scale and more DLB participants were also taking antidepressant medication. This was an expected finding, as depression frequently occurs in DLB, and has been incorporated into the consensus criteria as a supportive feature of the disease (McKeith et al., 2005).

The burden of vascular disease is an important consideration in the study of dementia as it can contribute to cognitive decline as well as gait disturbance (O'Brien et al., 2003). Our study groups were reasonably well matched in terms of vascular risk factors. The DLB group were more likely to be taking anti-platelet therapy, which may be partly attributable to the slightly higher incidence of reported cardiovascular disease. All dementia subjects had a CT brain scan prior to study entry and had no evidence of major cerebrovascular disease.

#### ***4.4.2 Neuropsychological changes***

Executive function broadly refers to the control of aspects of attention and future planning (Metzler-Baddeley, 2007). DLB patients often have more severe executive dysfunction (dysexecutive syndrome) when compared with AD of similar dementia severity (McKeith et al., 2004) which was reflected in our study cohort. The DLB group performed significantly worse in the letter (phonemic) fluency task than the AD group, though the average category (semantic) fluency scores were comparable. The finding of reduced letter fluency and similar category fluency in DLB when compared with AD has been a consistent finding (Metzler-Baddeley, 2007). Suggested reasoning for this is that the reduced category fluency in AD relates to impaired semantic memory, which does not affect their performance in the letter fluency task. In DLB however, it is hypothesised that verbal fluency task deficits are related to frontal-subcortical network dysfunction which affect all verbal fluency tasks equally (O'Brien et al., 2006). Disruption in the function of this network may be due to reduction or loss of the dopamine projections from the striatum to the frontal cortex. Lewy body pathology may also have an influence and studies have

reported Lewy body formation in the anterior cingulate and subcortical areas including the striatum (Duda, 2004).

The memory of the DLB subjects was significantly impaired relative to the controls, but relatively preserved when compared with AD. While the HVLT total recall was similar, the recall rate following a delay was significantly higher in DLB compared to AD as was recognition, a finding consistent with other studies (O'Brien et al., 2006). The DLB group also performed better than AD subjects on the visual memory task (DLB 59% vs AD 34%,  $p=0.027$ ). However, the difference in delayed recall between the DLB and AD groups was not quite as striking as the difference in the HVLT delayed recall task (DLB 46% vs AD 11%,  $p=0.010$ ). This may have, in part, been due to impaired visual processing in the DLB group. The episodic memory composite score (comprised of the sum of z scores for 6 individual tests) was significantly higher in DLB than AD suggesting that the AD group had more impaired episodic memory. Impaired episodic memory in AD is an early and prominent feature of the disease and correlates with hippocampal atrophy (Jack et al., 2010). Medial temporal lobe structures including the hippocampus atrophy in DLB when compared with controls but is to a lesser extent than seen in AD with relative preservation of medial temporal lobe structures being an established feature of DLB (McKeith et al., 2005). AD pathology is also usually present in DLB in varying degrees (Firbank et al., 2010; McKeith et al., 2005). It is therefore possible that impaired episodic memory in DLB reflects the burden of AD pathology and medial temporal lobe atrophy in DLB.

This study represents a large and well characterised group of DLB and AD participants. Importantly the methods that we utilised for diagnosis were robust and have been shown to be highly specific in prospective validation studies (McKeith et al., 2000a). A potential limitation of the study is that not all participants were able to complete all of the neuropsychological testing. This was usually in participants with moderate to severe dementia. This was a particular problem with the CDR vigilance task where 6 DLB participants were not able to complete the task. This may therefore bias the results. However, a poorer performance in this DLB subgroup would imply that the difference in the CDR Vigilance task between AD and DLB would be even greater than reported. Some studies report findings in mild disease or, with a MMSE > 16 'cut-off' which helps address this problem to some extent. However, it also



excludes investigating the spectrum of disease. Instead, we have reported all of the available data for the cohort, which is especially important when correlating with the imaging data.

#### **4.5 Summary**

DLB and AD result in similar levels of global cognitive deficits when compared with normal ageing with significant overlap in some cognitive domains such as learning. However, there were distinct and significant differences in the cognitive profile between the conditions. DLB was characterised by cognitive slowing with fluctuating levels of attention, impaired executive function and visuo-spatial and visuo-constructive deficits. Memory was impaired in both AD and DLB. However, in DLB, the deficits in delayed recall and recognition were less than observed in AD. This pattern of neuropsychological impairment in DLB is consistent with a predominantly subcortical pattern of pathological change with varying degrees of Alzheimer pathology, which manifests in the more AD-like cortical syndrome of impairment, with deficits in episodic memory.

The clinical and neuropsychological features of this cohort are characteristic of the DLB and AD clinical syndromes. This is an important consideration when investigating group differences in imaging parameters and may help to provide a better understanding of the *in vivo* pathological differences between the conditions. In the next chapter, we investigate the structural grey matter changes in DLB compared to AD and controls. Given the neuropsychological profiles of our DLB and AD groups, we expect to find less atrophy in the medial temporal lobes structures in DLB than in AD. Other areas of interest include the subcortical structures and, given the visuo-perception difficulties, the parieto-occipital regions.

## **Chapter 5 Structural magnetic resonance imaging changes in dementia with Lewy bodies**

### **5.1 Introduction**

As discussed in Chapter 2, structural MRI has been used extensively to investigate patterns of grey matter (GM) atrophy in AD but to a much lesser extent in DLB. AD is characterised by a pattern of generalised cortical atrophy with particular GM loss of the medial temporal lobe structures and temporoparietal association cortices (Whitwell et al., 2008; Jack et al., 1992). Visual rating and volumetric studies have shown that subjects with DLB have relative preservation of the medial temporal lobe (MTL) when compared with AD (Whitwell et al., 2007b; Barber et al., 1999a) (see section 2.2.3). Other reported results have been less consistent. Some studies have found atrophy affecting subcortical regions such as the putamen, substantia innominata and dorsal midbrain (see section 2.2.4) along with overlap of affected areas in AD such as atrophy of the amygdala (Hanyu et al., 2007; Whitwell et al., 2007b; Cousins et al., 2003).

Voxel based morphometry (VBM) performs a voxel-level analysis of tissue concentration between subject groups. It is an objective and largely operator independent method of MRI data analysis (Ashburner and Friston, 2000). Findings from other VBM studies comparing the structural grey matter changes in DLB and AD have varied (Takahashi et al., 2010a; Beyer et al., 2007; Whitwell et al., 2007b; Burton et al., 2004; Burton et al., 2002). Some have reported a diffuse pattern of cortical GM loss whilst another has reported relatively little. The pattern of subcortical structural change is also unclear. VBM analysis methodology continues to be improved and refined and the ‘Diffeomorphic anatomical registration through exponentiated Lie algebra’ (DARTEL) image registration algorithm has recently been introduced. DARTEL provides a more sophisticated mathematical framework than the standard unified segmentation approach (Ashburner, 2007). This has been incorporated into the Statistical Parametric Mapping (SPM) toolbox (<http://www.fil.ion.ucl.ac.uk/spm>) and is now in widespread use. DARTEL offers more accurate inter-subject registration of brain images, thereby improving the localization and sensitivity of VBM studies (Ashburner, 2007).

With the use of higher resolution imaging (3T) and the improved VBM-DARTEL analysis tool in a large group of well characterised DLB subjects we aimed to further

define MRI patterns of GM atrophy in DLB that may assist in differentiating it from AD. We also aimed to investigate correlations between GM loss and neuropsychological measures to help understand the *in vivo* pathological basis for clinical features in patients with dementia.

## 5.2 Study specific methods

Subject recruitment and assessment methods were described in section 4.2.

### 5.2.1 MRI data acquisition

Subjects underwent T1-weighted MR scanning on a 3T MRI system (Intera Achieva scanner, Philips Medical Systems, Eindhoven, Netherlands) with an 8-channel receiver head coil within 2 months of the neuropsychological assessment. The sequence was a standard T1-weighted volumetric sequence covering the whole brain (3D MPRAGE, sagittal acquisition, 1 mm isotropic resolution and matrix size of 240 (anterior-posterior)  $\times$  216 (superior-inferior)  $\times$  180 (right-left); repetition time (TR) = 8.3 ms; echo time (TE) = 4.6 ms; SENSE factor = 2; flip angle = 8°). The acquired volume was angulated such that the axial slice orientation was standardised to align with the AC-PC line.

### 5.2.2 VBM-DARTEL

The pre-processing steps are represented in Figure 5.1. VBM analysis was performed using SPM8 (<http://www.fil.ion.ucl.ac.uk/spm>) and MATLAB 7.9 (Math-Works, Natick, MA, USA). Subject images were visually inspected for any artefacts or gross anatomical abnormalities. MR images were then segmented into grey matter (GM), white matter (WM) and cerebrospinal fluid (CSF) using the standard unified segmentation model in SPM8 (Ashburner and Friston, 2005). GM templates were generated from all subjects using DARTEL (Ashburner, 2007). The subjects GM data were spatially normalised and warped in DARTEL and transformed to Montreal Neurological Institute (MNI) space (<http://www.mni.mcgill.ca/>). Images were then Jacobian scaled (similar to modulation) to ensure that relative volumes of GM were preserved following the spatial normalisation procedure and then smoothed with an 8mm full-width at half-maximum (FWHM) isotropic Gaussian kernel. After spatial pre-processing, the smoothed, modulated, normalised GM datasets were used for statistical

analysis. Total intracranial volume (TIV) of each subject was calculated by summing the total tissue probability of grey matter, white matter and CSF from probability maps generated during the initial segmentation step.

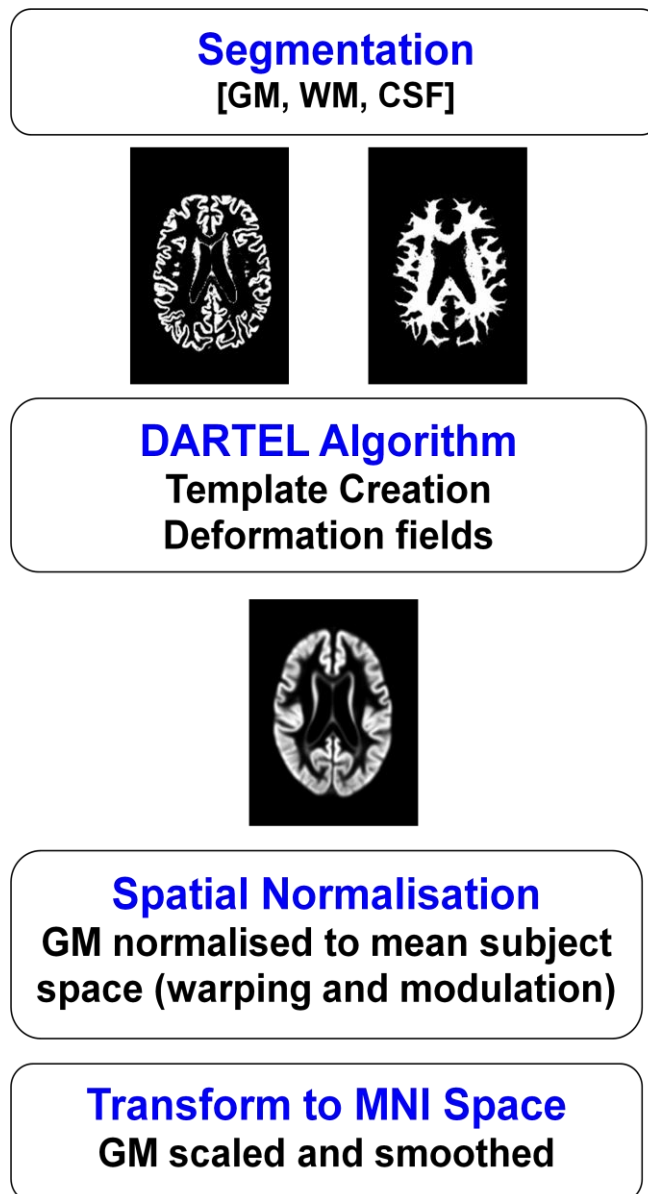


Figure 5.1. VBM-DARTEL pre-processing steps.

### 5.2.3 Statistical Analysis

Regional volume differences between groups were assessed using the SPM8 General Linear Model based on random Gaussian field theory (Friston et al., 1995). An absolute threshold mask of 0.05 was used for GM analyses. Age and TIV were included in the design matrix as nuisance covariates in all VBM analyses.

Analyses were also performed to investigate the effects of GM loss and cognitive measures (CAMCOG, CAMCOG memory subscale, CAMCOG praxis subscale and MMSE) in subjects with AD and DLB, separately and combined as a single dementia group.

Significant effects were assessed using an uncorrected threshold of  $p \leq 0.001$  and the family-wise error (FWE) threshold of  $p_{\text{FWE}} \leq 0.05$ , corrected for multiple comparisons. Only voxel level significance was considered. The cluster centres were converted from MNI space to Talairach coordinates using the programme GingerALE version 2.0.4 (<http://brainmap.org/ale/>). The anatomical location was then determined using Talairach daemon (<http://www.talairach.org/>).

## 5.3 Results

### 5.3.1 Subject characteristics

One hundred and six subjects completed the MRI scan and clinical assessment (35 DLB, 36 AD, 35 Controls). The MR data for two AD subjects were excluded, one due to motion artefact and the other due to inaccuracy in the segmentation step noted on visual inspection.

### 5.3.2 Subject demographics

The demographic data for all subjects is discussed in section 4.3.2 and an abbreviated version of the results is represented in Table 5.1.

Characteristic	DLB (n=35)	AD (n=36)	NC (n=35)	p value
Age y	78.4±6.9	78.3±5.8	76.7±5.2	ns*
MMSE	20.3±5.3	19.5±4.4	29.1±1.0	ns†
CAMCOG Memory (max 27)	15.0±4.7	9.7±4.0	23.6±1.6	<0.001†
CAMCOG Praxis (max 12)	7.0±2.5	9.5±1.4	11.3±1.1	<0.001†
UPDRS III	26.0±10.7	5.4±4.3	2.0±1.9	<0.001†

Values expressed as mean ± 1SD

\*ANOVA – DLB, AD, Controls; †Student's t-test – AD vs DLB

Table 5.1. Demographics, clinical and cognitive measures

### 5.3.3 Total grey matter, white matter and intracranial volumes across groups

The average GM, WM and TIV across groups are represented in Table 5.2. As expected, the mean TIV was similar across groups ( $F_{2,103}=1.55$ ,  $p=0.217$ ). The AD group had significantly lower volumes of GM than DLB and controls and lower WM volume than controls. There were no significant differences in the WM volume between DLB and AD or DLB and controls.

Volume (cm <sup>3</sup> )	DLB n=35	AD n=34	NC n=35	p value*
GM	558.8±52.4	529.7±56.5	580.3±48.4	$F_{2,103}=8.07$ , $p=0.001$ †
WM	437.3±88.9	412.0±47.6	455.0±57.6	$F_{2,103}=3.55$ , $p=0.032$ §
TIV	1533.7±165.2	1469.4±156.0	1495.0±135.2	$F_{2,103}=1.55$ , $p=0.217$

All values are represented as mean ± 1SD

Post Hoc tests: †Con > AD, DLB ( $p \leq 0.09$ ); DLB > AD ( $p=0.02$ ).

§Con > AD ( $p=0.009$ ); Con vs. DLB, AD vs. DLB (ns).

Table 5.2. Grey matter, white matter and total intracranial volumes across groups.

### 5.3.4 Differences in grey matter volume between DLB and Controls

As illustrated in Figure 5.2, clusters of reduced grey matter volume were observed in temporal, parietal and occipital areas ( $p < 0.001$ , uncorrected) in DLB patients relative to controls. Regions of significant difference ( $p < 0.05$ , corrected) were seen bilaterally in the parahippocampal gyrus, amygdala, superior temporal gyrus, uncus and the right caudate tail, represented in Figure 5.2 (B) and summarised in Table 5.3 (a).

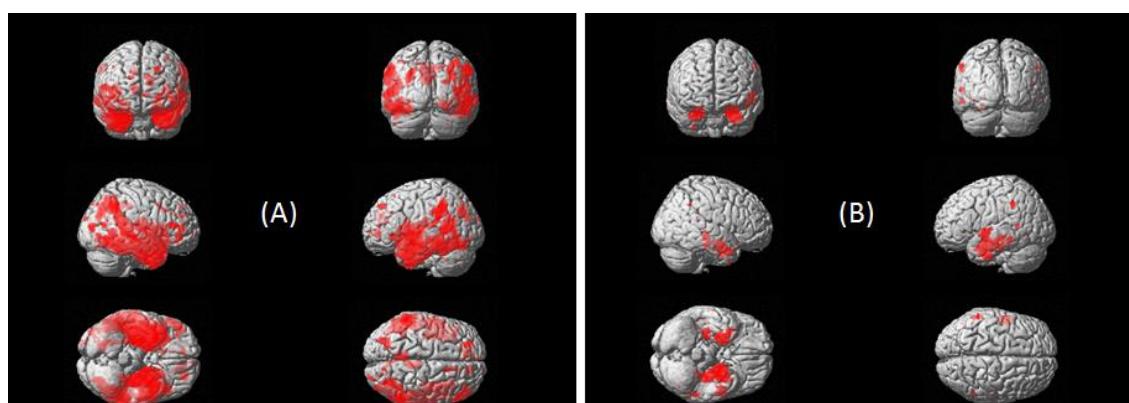


Figure 5.2. Three-dimensional surface renders showing the patterns of cortical GM loss in DLB (red) compared to controls; (A)  $p < 0.001$ , uncorrected and (B)  $p < 0.05$ , FWE-corrected.

### 5.3.5 Differences in grey matter volume between AD and Controls

Clusters of grey matter atrophy in AD were diffuse and extensive involving bilateral frontal, temporal, parietal and occipital lobes when compared with controls as shown in Figure 5.3 (A) ( $p < 0.001$ , uncorrected) and Figure 5.3 (B) ( $p < 0.05$ , FWE-corrected).

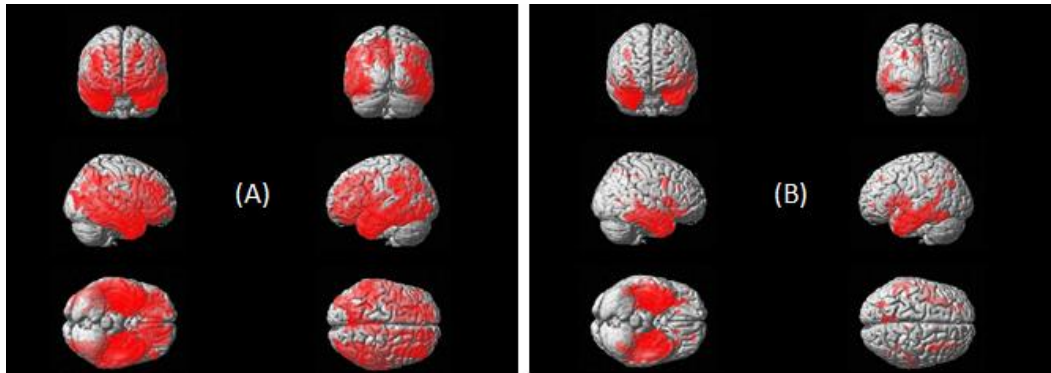


Figure 5.3. Three-dimensional surface renders showing the patterns of cortical GM loss in AD (red) compared to controls; (A)  $p < 0.001$  and (B)  $p < 0.05$ , FWE-corrected.



### 5.3.6 Differences in grey matter volume between DLB and AD

There was less atrophy in the medial temporal lobe structures in DLB when compared with AD, which was more pronounced on the left. Areas of significant GM volume loss in AD when compared to DLB included the left hippocampus and bilateral parahippocampal gyri ( $p < 0.05$ , corrected) (Table 5.3(b) and Figure 5.4). Examples of T1 images in AD and DLB are represented in Figure 5.5.

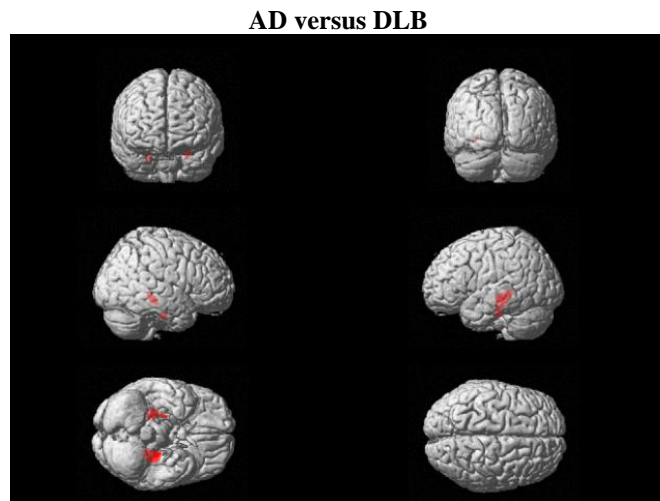
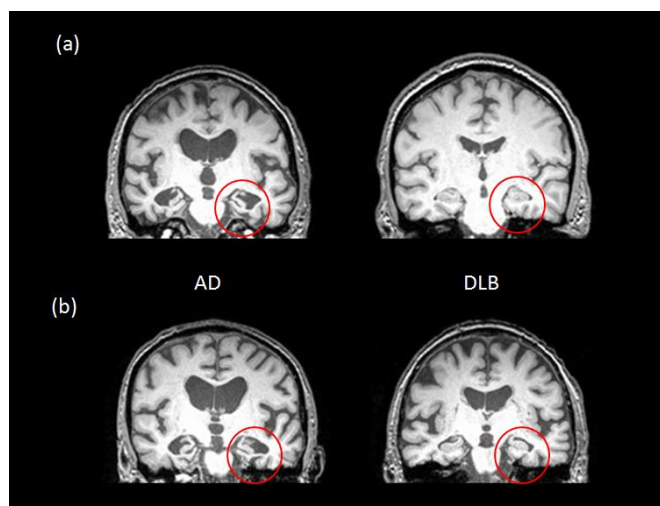


Figure 5.4. Three-dimensional surface renders showing the areas of greater cortical GM loss in AD (red) compared to DLB ( $p < 0.05$ , FWE-corrected).



Radiological view

Figure 5.5. Examples of coronal T1 images demonstrating medial temporal lobe atrophy in AD on the left and preservation of the medial temporal lobe structures on the right in DLB in (a) mild dementia and (b) moderate dementia.

The left MTL structures are circled in red.

### 5.3.7 Clinical correlations in DLB and AD

A positive correlation was observed between the CAMCOG memory subscale and regions of grey matter atrophy in temporal lobe structures (left>right) in the combined AD, DLB group and is represented in Figure 5.6. Table 5.3 (c) shows areas of significance. A correlation was observed in the DLB group alone but was strengthened by the inclusion of the AD group. However, the correlation was negligible in the AD group alone. There was no significant correlation found between regions of grey matter atrophy and the CAMCOG praxis subscale or global cognitive measure (CAMCOG and MMSE) in either AD or DLB subject groups.

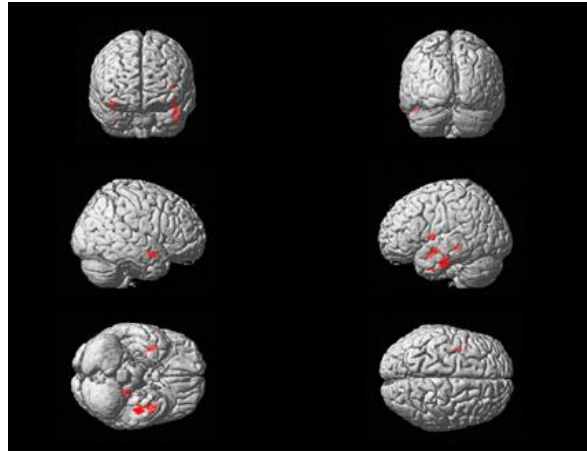


Figure 5.6. Three-dimensional surface renders showing the areas of greater cortical GM loss in AD (red) compared to DLB ( $p < 0.05$ , FWE-corrected).

Voxel-level ( $p_{\text{FWE-Corr}}$ )	Extent (k)	t, z	MNI Coordinates (x,y,z) (mm)	Anatomical Region (Brodmann Area)
<b>(a) Controls &gt;DLB</b>				
<0.001	3932	7.4, 6.3	-29, -9, -15	Left amygdala
0.001	1518	6.4, 5.6	30, -4, -29	Right amygdala
<0.001	3932	7.3, 6.2	-21, 0, -18	Left parahippocampal gyrus (34)
0.001	1518	6.3, 5.6	24, 2, -24	Right parahippocampal gyrus (34)
<0.001	3932	6.7, 5.8	-29, -7, -38	Left uncus (36)
0.027	1518	5.3, 4.8	35, -4, -36	Right uncus (36)
0.001	3932	6.3, 5.6	-36, 2, -32	Left superior temporal gyrus (38)
<0.001	3932	7.7, 6.5	-36, -22, -12	Left hippocampus
0.003	407	6.0, 5.3	35, -27, -6	Right caudate tail
<b>(b) DLB &gt; AD</b>				
0.001	982	6.4, 5.6	-30, -30, -12	Left parahippocampal gyrus
0.004	982	5.9, 5.2	-24, -25, -29	Left parahippocampal gyrus (35)
0.001	316	6.2, 5.5	24, -31, -9	Right parahippocampal gyrus (27)
0.001	316	6.2, 5.4	26, -28, -12	Right parahippocampal gyrus (28)
0.001	982	6.3, 5.5	-26, -36, -6	Left hippocampus
<b>(c) Correlation with CAMCOG memory subscale</b>				
0.002	103	6.1, 5.4	-20, -34, -8	Left parahippocampal gyrus (27)
0.015	221	5.4, 4.9	-44, -16, -32	Left fusiform gyrus (20)
0.043	96	5.1, 4.7	-36, 12, 4	Left insula (13)
0.004	221	5.8, 5.2	-48, -16, -26	Left subgyral grey matter (20)
0.004	159	5.8, 5.2	41, 3, -18	Right superior temporal gyrus (38)

(a) DLB v control; (b) AD vs DLB and (c) correlation with the CAMCOG memory subscale in dementia (DLB, AD). For each peak, the table shows voxel-level significance ( $p_{\text{FWE-Corr}}$ ), spatial extent (k), t and z scores, MNI coordinates and anatomical region

Table 5.3. Location and peak significance of GM volume reduction using VBM-DARTEL.

## 5.4 Discussion

In a VBM study using the DARTEL registration algorithm comparing patterns of GM loss in DLB and AD, patients with DLB showed a pattern of GM loss involving the temporal, parietal, occipital and subcortical structures when compared with controls. Areas of significant differences included bilateral parahippocampal gyri, amygdala and uncus; left hippocampus and superior temporal gyrus and right caudate tail. These structures were also involved to a much greater extent in the AD group as part of widespread GM loss. These findings are largely in keeping with a VBM study by Whitwell *et al.* (2007b). Although, other VBM studies have demonstrated a more diffuse cortical GM loss in DLB (Beyer *et al.*, 2007; Burton *et al.*, 2002). In a VBM study by Burton *et al.* (2002), diffuse cerebral changes were reported in DLB, including frontal areas. We did not find significant changes in the frontal regions in DLB. This may, in part, be explained by the differing levels of dementia severity between studies. In Burton's study, the DLB group was more impaired (mean MMSE=13.3) compared with our cohort (MMSE=20.3) (Burton *et al.*, 2002). It is possible that as disease progresses in our DLB cohort, areas of frontal atrophy may become apparent and longitudinal investigation is warranted to determine whether this is the case. Beyer *et al.* (2007) also found widespread changes in DLB, however, in contrast to our study, the results were of unmodulated data and reported tissue concentration rather than volume, making comparisons difficult.

We found relative preservation of the medial temporal lobe structures in DLB compared with AD. This is in agreement with other MRI studies (Whitwell *et al.*, 2007b; Burton *et al.*, 2002; Barber *et al.*, 1999a) and pathological studies (Burton *et al.*, 2009; Lippa *et al.*, 1998) and is consistent with the relative sparing of episodic memory in DLB and the medial temporal structures (hippocampus and amygdala) underlying this. Our findings again highlight the importance of this observation as a potential discriminator between AD and DLB and they further validate its incorporation in the revised DLB criteria (McKeith *et al.*, 2005).

Other studies have reported a pattern of subcortical structural change in DLB (Whitwell *et al.*, 2007). Our study found GM loss of the right caudate tail in DLB when compared to controls. Nigrostriatal dysfunction and dopaminergic loss, particularly of the dopamine transporter in the striatum occurs in DLB, but not to a significant extent in AD (Piggot *et al.*, 1999). This can be visualised via nuclear medicine techniques using

specific imaging ligands. Using FP-CIT SPECT, reductions of 40-50% have been documented in DLB (O'Brien et al., 2004). It is important to consider how these functional dopaminergic changes may be related to structural changes. In our cohort of DLB patients, 22 had FP-CIT SPECT imaging as part of their clinical diagnostic work-up prior to study inclusion. Of the subjects that had FP-CIT SPECT imaging, all were abnormal indicating reduced dopamine transporter uptake (see Table 4.3). Other studies have not found significant differences in caudate volume using ROI and semi-automated segmentation analysis methods (Cousins et al., 2003; Barber et al., 2002). Interestingly, in a VBM study, Burton *et al.* (2004) reported atrophy of the right caudate tail in Parkinson's disease dementia but not in the DLB comparison group. In a MRI study, using manual segmentation, Cousins *et al.* (2003) reported reduced putamen volume in DLB (approx 10-15%) compared to healthy controls. When the values were normalised to TIV they found that the volume loss was also more pronounced in DLB compared with AD. Along with striatal dopamine transporter deficiencies, striatal dopamine D2 receptors are also reduced (Piggot et al., 1999). So although there is some intrinsic striatal pathology which may account for a degree of structural atrophy, it is unlikely that at this level of atrophy it would account for the substantial loss in dopaminergic activity (Duda, 2004; O'Brien et al., 2004).

We identified an association between the CAMCOG memory subscale and atrophy in the medial temporal lobe structures in the combined AD and DLB group that was significant when corrected for multiple comparisons. Areas included the left parahippocampal gyrus, left subgyral grey matter, left fusiform gyrus and right superior temporal gyrus. The association was observed in the DLB group although strengthened by combining the AD group. The CAMCOG memory subscale is a combination of recent and remote memory and new learning with scores ranging from 0-27. The DLB group performed better than the AD group on this test, which is in keeping with the differing cognitive profiles between the conditions and would suggest that memory dysfunction in DLB is associated with GM atrophy in these regions. This correlation was not detected in AD alone, but there may be a number of reasons for this. Given the early and significant dysfunction of episodic memory in AD, many of the neuropsychological tests have a floor effect, making it difficult to detect correlations even where they may exist (large type II error probability).

In contrast to the memory assessments, the DLB group performed worse on the CAMCOG praxis subscale when compared with AD, which again is consistent with the expected neuropsychological profile of DLB of more impaired visuo-spatial processing and praxis (McKeith et al., 2005). One possible reason for this involves dysfunction of the parieto-occipital areas. The parieto-occipital changes in DLB have been demonstrated in functional imaging studies with hypometabolism and hypoperfusion in PET and SPECT studies respectively (Lobotesis et al., 2001; Ishii et al., 1998). We observed a pattern of GM loss preferentially affecting these areas as compared with frontal region (Figure 5.2). This was in contrast to AD which showed a more extensive and global pattern of cortical atrophy (Figure 5.3). However, we did not find significant areas of GM atrophy in the occipital areas ( $p < 0.05$ , FWE-corrected) in DLB, or significant differences between DLB and AD. In a VBM study of patients comparing DLB and Parkinson's disease dementia, Beyer *et al.* (2007) reported reduced tissue concentration of the left occipital gyrus in the DLB group. However, volumetric studies using ROI (Middelkoop et al., 2001) and VBM (Whitwell et al., 2007b; Burton et al., 2002) did not find significant occipital structural changes. Our study suggests that some neuronal loss in the parieto-occipital areas does occur, although it did not reach statistical significance when corrected for multiple comparisons, nor does it appear more pronounced than in AD. It seems more likely that the characteristic neuropsychological changes in DLB may reflect neuronal or synaptic dysfunction rather than neuronal loss. Our results also support previous studies suggesting that underlying structural changes are not sufficient to explain the functional metabolic abnormalities in the posterior parietal and occipital cortex which are seen in DLB but not AD.

The strengths of this study include a large and well-characterised group of patients with probable DLB. In addition, the dementia groups were well matched for age, level of education and dementia severity. We were therefore able to report results corrected for multiple comparisons using the family-wise error, which is in contrast to some other studies. This means there is a high likelihood that our findings represent true differences between the groups, although, the potential disadvantage with using this method is the possibility that we may have missed, or underestimated the degree of true MRI differences between the groups (type II error).

In conclusion, we have demonstrated a pattern of GM loss affecting the temporal, parietal, occipital and subcortical structures in DLB when compared with controls.

These changes were generally less extensive than seen in AD. In particular, we have shown that there was significantly less medial temporal lobe atrophy in the DLB group when compared to AD. This finding, along with pathological validation studies, suggests that degree of medial temporal atrophy may be a more useful imaging biomarker than previously thought. Relative preservation of the MTL structures has been included as a supportive feature in the revised consensus criteria for DLB and our findings support its inclusion.

The next chapter will focus on the white matter tract changes in DLB compared to AD and healthy ageing using diffusion tensor imaging. Given the patterns of grey matter atrophy in DLB, we expect to see more diffuse white matter tract abnormalities. Accordingly, we focus on the potential clinical correlations to provide further insight into the pathological changes in DLB.

## Chapter 6 Diffusion tensor imaging in dementia with Lewy bodies

### 6.1 Introduction

We have found that DLB is characterised by a pattern of relative structural preservation of the medial temporal lobe on MRI when compared to AD, a finding consistent with others (Whitwell et al., 2007b; Barber et al., 1999a). Other reported changes have been less consistent. This is in contrast to AD where the temporo-parietal pattern of grey matter atrophy is well established and also corresponds to the clinical and functional imaging features of the disease (Jack et al., 2010; Whitwell et al., 2008; Jack et al., 1992).

As discussed in Chapter 2, diffusion tensor imaging (DTI) is an MR technique that is commonly used to provide information about the integrity of white matter. DTI utilises the anisotropic nature of diffusion in neuronal white matter tracts with water molecules diffusing more freely along the tracts than perpendicular to them (Le Bihan, 2007; Basser et al., 1994). This is represented by a diffusion ellipsoid at each voxel, made up of three orthogonal eigenvectors and measures of their lengths, the component eigenvalues. With neuronal degeneration the mean diffusivity, MD increases with the loss of structural barriers that normally restrict diffusion and diffusion becomes less directionally oriented. This is also associated with a reduction in fractional anisotropy (FA), a measure of the standard deviation in diffusion along the 3 principal directions (Assaf and Pasternak, 2008; Beaulieu, 2002).

Previous studies investigating the DTI changes in DLB have used conventional ROI or VBM methods (Kantarci et al., 2010; Lee et al., 2010b; Ota et al., 2009; Firbank et al., 2007a; Firbank et al., 2007b; Bozzali et al., 2005). Only Lee *et al.* (2010b) and Kantarci *et al.* (2010) DTI studies were acquired at 3 Tesla. Some studies found widespread FA changes in comparison to healthy controls (Lee et al., 2010b; Bozzali et al., 2005) whilst others have found very little change (Kantarci et al., 2010; Firbank et al., 2007b). The most consistent finding has been of reduced FA in the region of the inferior longitudinal fasciculus (ILF) which provides a connection between the temporal and occipital lobes. However, this area was also found to be affected in AD (Kantarci et al., 2010; Lee et al., 2010b; Ota et al., 2009; Bozzali et al., 2005). FA reductions have also been reported in the posterior cingulate and precuneal areas (Lee et al., 2010b; Firbank et al., 2007a; Firbank et al., 2007b;



Bozzali et al., 2005). More recently, Kantarci *et al.* (2010) reported significantly lower FA and increased MD in the amygdala which, in contrast to AD, was not proportional to the degree of atrophy. DTI therefore shows promise as a useful imaging technique to provide important pathological insights into the disease process and, in particular, how it differs from AD. However, a combination of relatively small sample sizes, heterogeneity within cohorts and differing analysis methods make conclusions as to the DTI changes in DLB difficult.

DTI analysis using ROI and VBM methods have limitations. The placement of ROI is operator dependent with the inherent problem of reproducibility. Partial volume effects can also be problematic with varying amounts of CSF or grey matter included in the ROI, particularly in subjects with brain atrophy. The relatively operator-independent VBM style of analysis addresses some of these problems although it too has its own inter-subject registration and spatial smoothing issues (Smith et al., 2006). Tract based spatial statistics (TBSS) is a relatively novel methodological algorithm designed to combine the benefits of a relatively operator-independent VBM style of analysis with the tract based information gained from tractography and has already been successfully applied to studies of other dementias (Smith et al., 2006).

In this study we applied the TBSS analysis methods to investigate the pattern of white matter tract change in DLB, in comparison to AD and aged-matched controls. We anticipated that there would be significant FA reduction and MD increase in the temporal and parietal regions with generally less change than observed in AD, particularly in the medial temporal lobes. By correlating the DTI changes with clinical features, we aimed to provide further insights into the potential pathological features underpinning the characteristic clinical and cognitive features observed.

## **6.2 Study specific methods**

Subject recruitment and assessment methods are detailed in section 4.2.1–4.2.3.

### **6.2.1 MRI data acquisition**

Subjects underwent MR scanning on a 3T MRI system (Intera Achieva scanner, Philips Medical Systems, Eindhoven, Netherlands) with an 8-channel receiver head coil within 2 months of the study assessment.

### *Diffusion tensor imaging*

DTI images were acquired using a Pulsed Gradient Spin Echo (PGSE) sequence and multi-slice single shot EPI readout, with TE = 71 ms and TR = 2524 ms. The image volumes were angulated such that the axial slice orientation was standardised to align with the AC-PC line with 2 mm in-plane resolution, 6 mm slice thickness and matrix size of 128 (anterior – posterior)  $\times$  128 (right – left)  $\times$  24 (superior – inferior). The scan was accelerated with SENSE factor of 2 in the Anterior-Posterior direction, and reconstructed with the CLEAR algorithm. Diffusion weighting was achieved by applying diffusion sensitising gradient pulses with measurements made in 16 directions with a b value of 1000 s.mm<sup>-2</sup>.

#### **6.2.2 DTI preprocessing and analysis**

The Functional MRI of the Brain (FMRIB) software library (FSL) program was used to process and analyse the raw DTI data (Smith et al., 2004) accessed at [www.fmrib.ox.ac.uk/fsl](http://www.fmrib.ox.ac.uk/fsl).

To correct for the distorting effect of eddy currents, we adapted the approach of Shen *et al.* (2004) and used an affine registration, in FSL's FLIRT (FMRIB's Linear Image Registration Tool) to register pairs of diffusion weighted images together. The eddy corrected diffusion weighted images were then registered with a rigid body registration to the b=0 s.mm<sup>-2</sup> image. The MD and FA images were calculated using FSL tensor analysis of the aligned diffusion weighted images at each brain voxel.

Voxelwise statistical analysis of the FA data was then carried out using Tract based spatial statistics (TBSS) (Smith et al., 2006). The steps involved are represented in Figure 6.1. All subjects' FA data were aligned to a 1mm isotropic FA target image in standard space (FMRIB58\_FA) using the nonlinear registration tool FNIRT (Andersson et al., 2007a; Andersson et al., 2007b) which uses a b-spline representation of the registration warp field (Rueckert et al., 1999). We visually checked the data to verify the accuracy of the nonlinear transformation. A mean FA skeleton representing the centres of all tracts common to the group was created by (1) averaging the warped FA images and then (2) thresholding at FA>0.2 to exclude skeleton voxels which may contain grey matter (Figure 6.1). Each subject's aligned FA data was then projected onto the mean FA skeleton, which accounted for residual

misalignments between subjects after the initial nonlinear registration. The resulting data was fed into voxelwise cross-subject statistics. Data for MD were also projected onto the mean skeleton by using non-linear registration and projection vectors from the FA images.

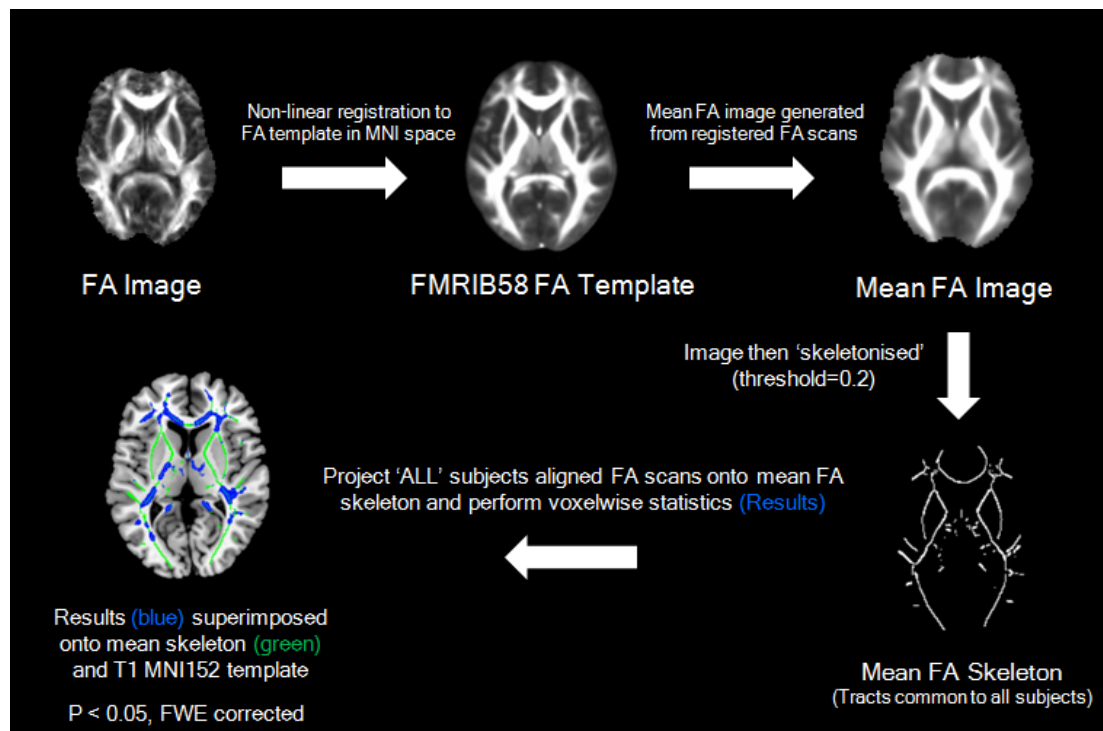


Figure 6.1. Representation of the pre-processing steps involved in tract based spatial statistics method of analysis.

### 6.2.3 White matter hyperintensities

Identification of white matter lesions generally requires T2 weighted and T2-FLAIR imaging. As part of the study, a more detailed scan protocol was collected in every subject providing additional data, which is not presented in this thesis. Study duration prevented collection of FLAIR scans, however the protocol did include acquisition of quantitative scans to measure tissue T1 and T2 with isotropic 2mm resolution. These scans were used (Drs Benjamin Aribisala & Michael Firbank) to generate FLAIR weighted images, which could be used in conjunction with the high resolution T1 weighted scans (Chapter 5 – section 5.2.1) to identify and visually rate the burden of white matter hyperintensities in the cohort.

Using the Fazekas scale, modified by Coffey (Coffey et al., 1989; Fazekas et al., 1987) white matter hyperintensities (WMH) were rated by consensus between 2 experienced raters (Drs Sean Colloby and Robert Barber), blinded to the subject diagnosis. In addition, frontal and non-frontal WMH burden was also visually rated. We did not exclude subjects with WMH as this is common in normal ageing and dementia (Barber et al., 1999b).

#### **6.2.4 Statistics**

##### *Demographic, clinical and cognitive measures*

Descriptive analyses of the group characteristics were completed using the statistical software software, SPSS 17 as detailed in section 4.2.4.

##### *DTI*

Differences in DTI indices in the DLB and AD groups were compared to controls using a permutation-based, non-parametric, two-sample, unpaired t-test in the FSL randomise programme (included in FSL). Age was included in the design matrix as a nuisance covariate in all analyses. Regression analysis was used to investigate the effects of clinical and neuropsychological measures on DTI parameters with both age and MMSE included as covariates. This was to control for the effects of global cognition on the association. We generated 5000 permutations of the data to test against at threshold levels (i)  $p < 0.05$  uncorrected for multiple comparisons (ii)  $p < 0.05$  family-wise (FWE) corrected which controls the rate of Type 1 errors for all voxels collectively and (iii)  $p < 0.005$  FWE corrected. Cluster like structures were enhanced using the threshold-free cluster enhancement algorithm (TFCE) (Smith and Nichols, 2009). The regions showing significant differences between groups were located and labelled anatomically by mapping the statistical map to the JHU DTI WM atlas in MNI space and Talairach atlas within the FSL atlas toolbox. The results of analyses are overlaid onto the MNI template image and mean FA skeleton (green) (see Figure 6.1).

## 6.3 Results

### 6.3.1 Subject characteristics

One hundred and six subjects completed the MRI scan and clinical assessment (35 DLB, 36 AD, 35 Controls) as outlined in section 4.2. The MR data for three subjects were excluded due to motion artefact (1 AD, 1 DLB, 1 Control).

### 6.3.2 Subject demographics

The demographic data for patients and control subjects are presented in section 4.3.2. An abbreviated version is represented in Table 6.1 below.

Characteristic	DLB (n=35)	AD (n=36)	NC (n=35)	p value
Age y	78.4±6.9	78.3±5.8	76.7±5.2	ns <sup>§</sup>
MMSE (max 30)	20.3±5.3	19.5±4.4	29.1±1.0	ns <sup>†</sup>
Episodic Memory Composite Score	-1.6±3.2	-4.5±1.5	6.2±2.6	<0.001 <sup>†</sup>
Letter Fluency (FAS)	20.8±13.0	27.5±14.3	41.0±11.1	0.038 <sup>†</sup>
UPDRS III	26.0±10.7	5.4±4.3	2.0±1.9	<0.001 <sup>*</sup>

Values expressed as mean ± 1SD.

<sup>\*</sup> ANOVA – Controls, AD and DLB; <sup>§</sup> Pearson's Chi-square test – Controls, AD and DLB

<sup>†</sup> Student's t-test – AD vs. DLB

Table 6.1. Demographics, clinical and cognitive measures.

### 6.3.3 White matter hyperintensities

The WMH scores were similar across all groups (Table 6.2). As expected, there was a trend toward less WMH in the control group when compared with AD and DLB. When comparing the frontal vs. non-frontal WMH change, there were no significant group differences and a trend toward less WMH burden in non-frontal (posterior) regions in the DLB group.

	<b>DLB (n=35)</b>	<b>AD (n=36)</b>	<b>Control (n=35)</b>	<b>p value<sup>§</sup></b>
<b>Periventricular Hyperintensities</b>				0.066
Absent	13 (37%)	8 (22%)	21 (60%)	
Caps	8 (23%)	8 (22%)	6 (17%)	
Smooth Halo	4 (11%)	7 (20%)	3 (9%)	
Irregular and extending into deep white matter	10 (29%)	13 (36%)	5 (14%)	
<b>Deep White Matter Hyperintensities</b>				0.074
Absent	12 (34%)	9 (25%)	15 (43%)	
Punctate foci	15 (43%)	7 (19%)	8 (23%)	
Beginning confluence of foci	3 (9%)	11 (31%)	7 (20%)	
Large confluent areas	5 (14%)	9 (25%)	5 (14%)	
<b>Subcortical Grey Matter</b>				0.637
Absent	16 (46%)	15 (42%)	18 (52%)	
Punctate	12 (34%)	15 (42%)	11 (31%)	
Multipunctate	4 (11%)	6 (16%)	4 (11%)	
Diffuse	3 (9%)	0	2 (6%)	
<b>Frontal Lobe Hyperintensities</b>				0.332
Absent	14 (40%)	12 (33%)	21 (60%)	
Punctate foci	14 (40%)	13 (36%)	8 (23%)	
Beginning of confluence of foci	5 (14%)	7 (19%)	3 (8.5%)	
Large confluent area	2 (6%)	4 (11%)	3 (8.5%)	
<b>Non Frontal Lobe Hyperintensities</b>				0.286
Absent	16 (46%)	11 (31%)	16 (46%)	
Punctate foci	11 (31%)	7 (19%)	8 (22%)	
Beginning of confluence of foci	3 (9%)	9 (25%)	7 (20%)	
Large confluent area	5 (14%)	9 (25%)	4 (12%)	

Results represented as Number (percentage);

<sup>§</sup>Pearson's Chi-square test – Controls, AD and DLB

Table 6.2. White matter hyperintensity burden across groups.

### 6.3.4 Fractional Anisotropy

#### 6.3.4.1 Comparison of FA between DLB and Controls

In comparison to controls, the DLB subjects TBSS results for reduced FA are represented in Figure 6.2 ( $p < 0.05$ , corrected). Change was identified in the white matter affecting predominantly the parieto-occipital areas. This included the precuneal region and bilateral cingulate gyri.

Other areas affected included the frontal lobe white matter tracts in the region of the right anterior corona radiata, left precentral gyrus and a cluster involving the left superior longitudinal fasciculus.

The area of reduced FA in the temporal lobe was in the region of the posterior thalamic radiation, which included the optic radiation and extended into the right occipital lobe. A cluster in the cuneus was also identified bilaterally, an area that was not affected on the right in the AD group when compared with controls. The tracts potentially involved included the inferior longitudinal fasciculus, inferior fronto-occipital fasciculus and anterior thalamic radiation.

Areas of reduced FA in the corpus callosum included clusters in the right genu and splenium and left body and splenium.

Radiological view

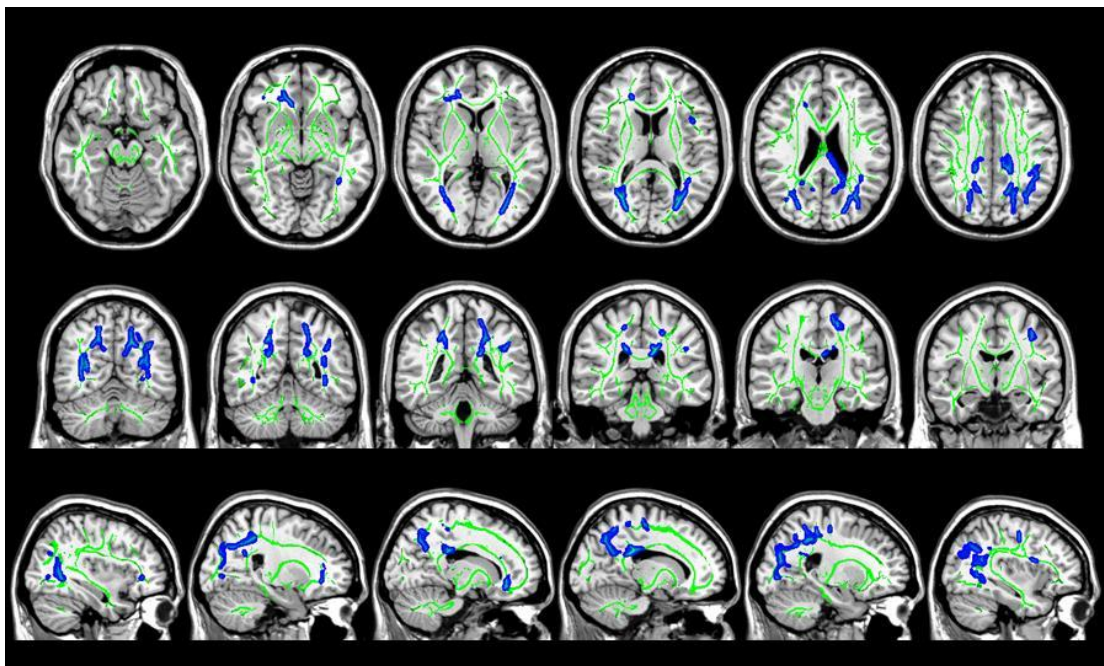


Figure 6.2. TBSS statistical maps for reduced FA in dementia with Lewy bodies. The areas of significant differences are represented in blue, overlaid onto the MNI template image and mean FA skeleton (green).

#### **6.3.4.2 Comparison of FA between AD and Controls**

In the AD group, areas of reduced FA compared to controls were more widespread than observed in DLB compared to controls and are represented in Figure 6.3 at  $p < 0.05$ , corrected. These included clusters in the temporal lobes, parieto-occipital areas as well as bilateral frontal lobes. In the frontal lobes, areas of reduced FA were extensive and included bilateral medial and superior frontal gyri and subgyral white matter.

In the parietal lobes, the areas of reduced FA were in the bilateral precuneal area with an extensive right sided cluster. The tracts involved in these areas included association fibres: superior longitudinal fasciculus and cingulum and projection fibres: anterior thalamic radiation.

Clusters of reduced FA were found in bilateral temporal lobes, with the left being more affected than the right. A large cluster was found in the left parahippocampal gyrus, extending to the posterior thalamic radiation. Other parts of the temporal lobe that were affected included bilateral middle temporal gyrus and subgyral white matter, right uncus and right superior temporal gyrus.

Areas of reduced FA in the occipital lobes were bilateral and included the middle occipital gyri, cuneus and subgyral white matter with changes being more extensive on the left. FA reduction in the corpus callosum was extensive and included the genu, body and splenium bilaterally.



Radiological view

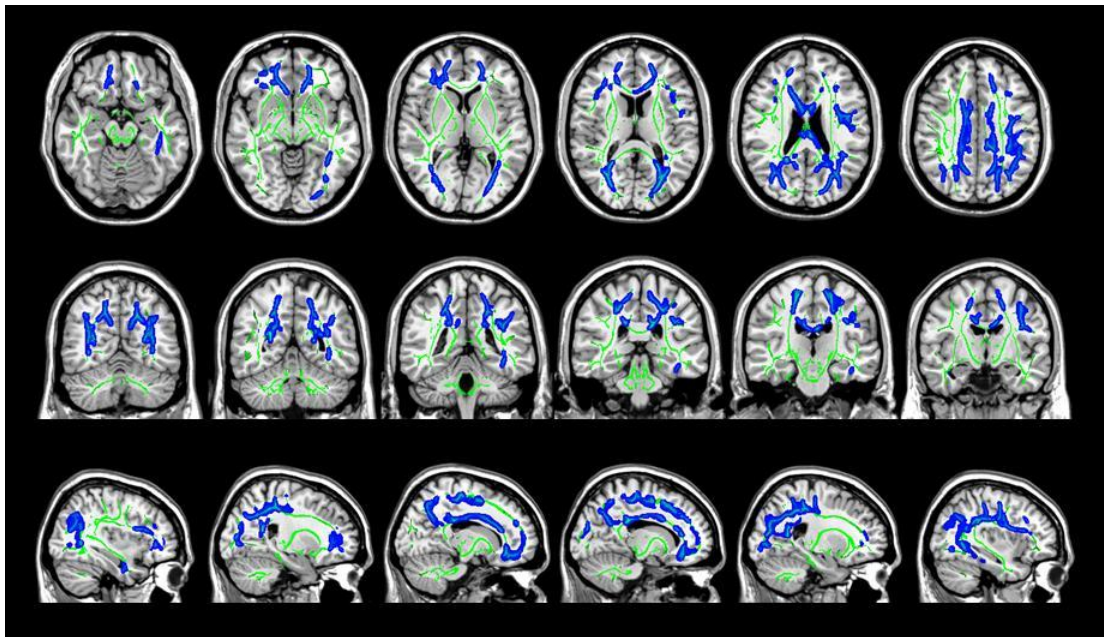


Figure 6.3. TBSS statistical maps for reduced FA in Alzheimer's disease. Areas of significant differences are presented in blue, overlaid onto the MNI template image and mean FA skeleton (green).

Generally, the pattern of reduced FA was more widespread in AD than DLB when compared with controls. In DLB, there was relatively little involvement of the temporal lobes apart from the posterior thalamic radiation (optic radiation) and in contrast, there was significant involvement of the parieto-occipital white matter tracts.

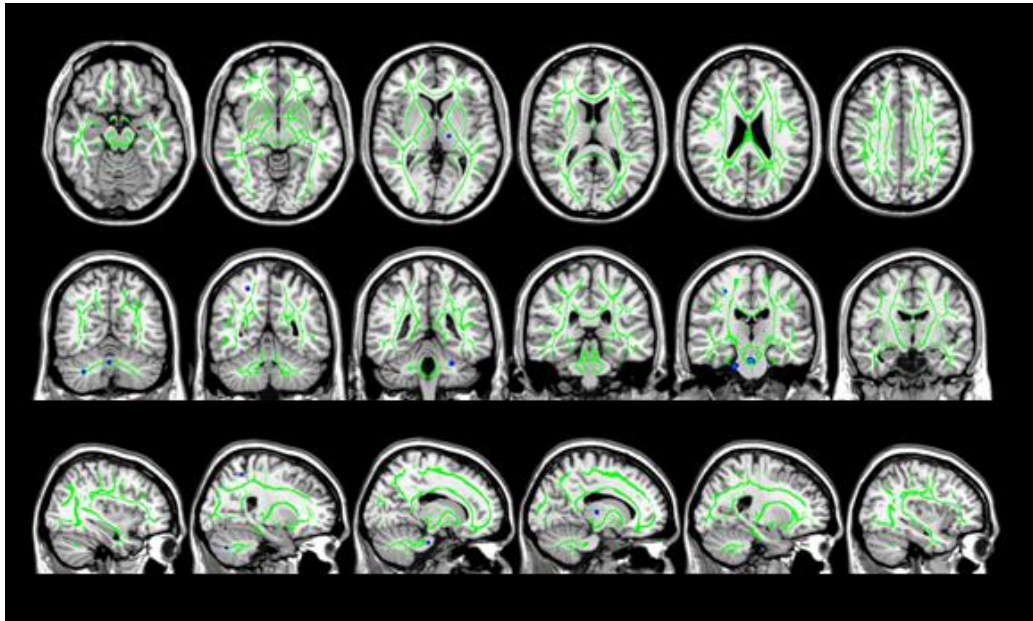
#### 6.3.4.3 Comparison of FA between DLB and AD

Figure 6.4 (a) demonstrates areas of reduced FA in DLB compared to AD ( $p < 0.05$ , uncorrected) and these results are summarised in Table 6.3 (a) (page 94). There were small clusters of significantly reduced FA in the pons and left thalamus in DLB. Other areas included the right precentral gyrus and right cerebellum.

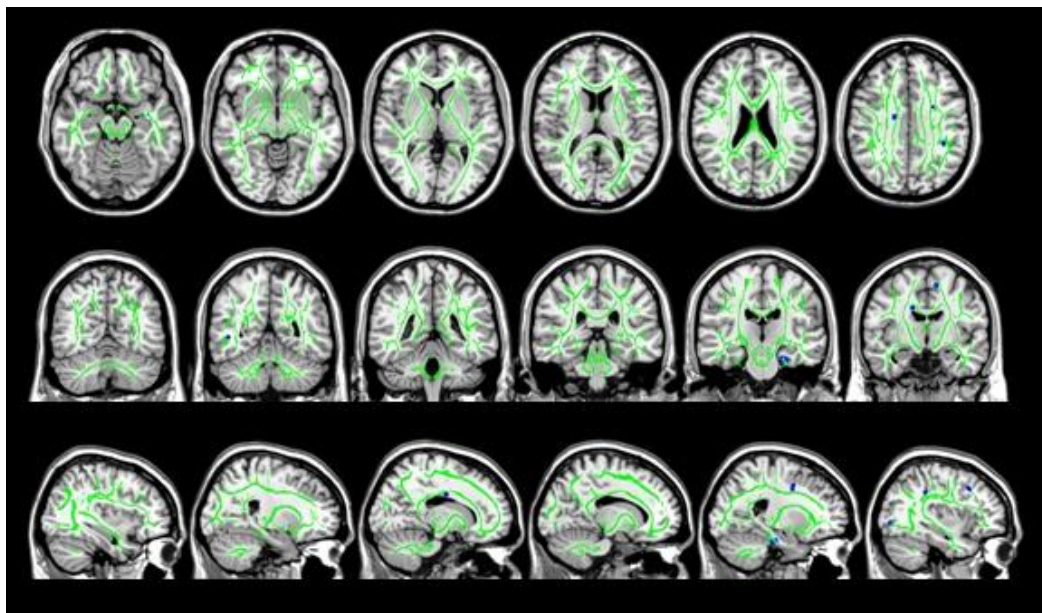
Areas of reduced FA in AD compared to DLB ( $p < 0.05$ , uncorrected) are shown in Figure 6.4 (b) and summarised in Table 6.3 (b) (page 94). These included clusters in the left and right frontal lobes and left parietal lobe. In the temporal lobes, small

clusters were seen in the parahippocampal gyri. Other areas include a cluster in the fornix and body of right corpus callosum.

(a) Areas of FA reduction: DLB v AD



(b) Areas of FA reduction: AD v DLB



Radiological view

Figure 6.4. Represents the results of the comparison between FA reduction in (a) DLB v AD and (b) AD v DLB. The areas of significant differences are represented in blue, overlaid onto the MNI template image and mean FA skeleton (green).

Anatomical Region	MNI Coordinates (x, y, z)			p value
<b>(a) AD vs DLB</b>				
Frontal lobe L Subgyral white matter	-27	4	39	0.019
Frontal lobe L Middle frontal gyrus	-33	7	40	0.007
Frontal lobe R Medial frontal gyrus	-10	-9	58	0.002
Parietal lobe L Supramarginal gyrus	-38	-37	36	0.023
Temporal lobe L Parahippocampal gyrus	-25	-23	-25	0.040
Temporal lobe R Parahippocampal gyrus	30	1	-32	0.047
Temporal lobe R Superior temporal gyrus	42	6	-25	0.042
Occipital lobe L Inferior occipital gyrus	-35	-81	2	0.031
Fornix Column and body	0	-1	7	0.046
Body of corpus callosum R	16	-9	33	0.024
<b>(b) DLB vs AD</b>				
Occipital lobe R Subgyral white matter	30	-55	-1	0.036
Brainstem R Pons	2	-24	-27	0.041
Brainstem L Pons	-1	-23	-27	0.043
Thalamus L	-14	-18	6	0.004

Table 6.3. Location and significance of Fractional Anisotropy (FA) reduction across groups. The table shows voxel level significance, MNI coordinates and anatomical region.

Examples of the quantitative differences in FA between AD and DLB at the voxel level are represented in Figure 6.5. These include:

- (a) left medial temporal – cingulum (MNI coordinates, mean  $\pm$  SD, t-score, df, p-value; -25, -23, -25, AD =  $0.30 \pm 0.06$ , DLB =  $0.33 \pm 0.06$ ,  $t = 2.1$ ,  $df = 67$ ,  $p = 0.02$ ),
- (b) fornix (0, -1, 7, AD =  $0.23 \pm 0.05$ , DLB =  $0.26 \pm 0.07$ ,  $t = 2.2$ ,  $df = 67$ ,  $p = 0.02$ ),
- (c) left thalamus – anterior thalamic radiation (-14, -18, 6, AD =  $0.29 \pm 0.04$ , DLB =  $0.26 \pm 0.03$ ,  $t = 2.7$ ,  $df = 67$ ,  $p = 0.005$ ), and
- (d) right occipital – inferior longitudinal fasciculus (30, -55, -1, AD =  $0.23 \pm 0.05$ , DLB =  $0.21 \pm 0.03$ ,  $t = 1.8$ ,  $df = 57$ ,  $p = 0.03$ ).

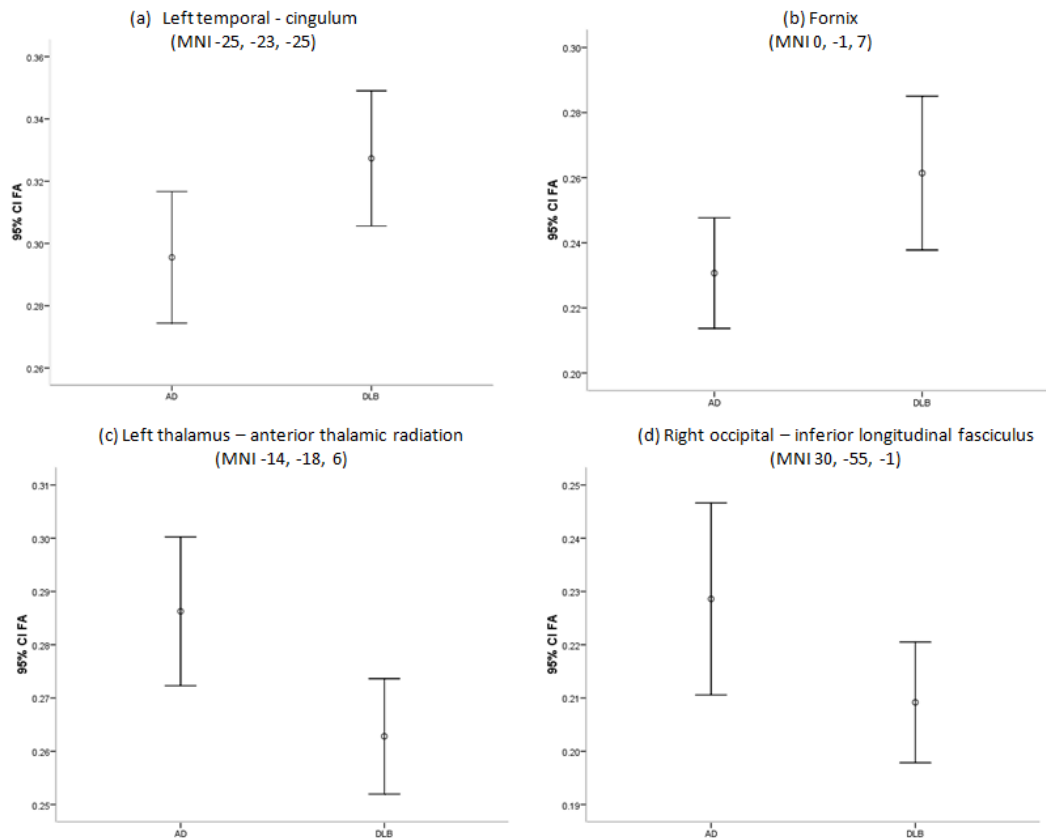


Figure 6.5. Graphical representation of the mean Fractional Anisotropy (FA) within a white matter tract voxel in AD and DLB. Areas include: (a) left temporal, (b) fornix, (c) left thalamus and (d) right occipital.

### **6.3.5 Mean Diffusivity**

#### **6.3.5.1 Comparison of MD between DLB and Controls and AD and Controls**

The results for increased MD in the dementia groups compared to controls were far more extensive than observed for reduced FA in both groups. The results of the analyses are shown in Figure 6.6 at  $p < 0.005$ , corrected.

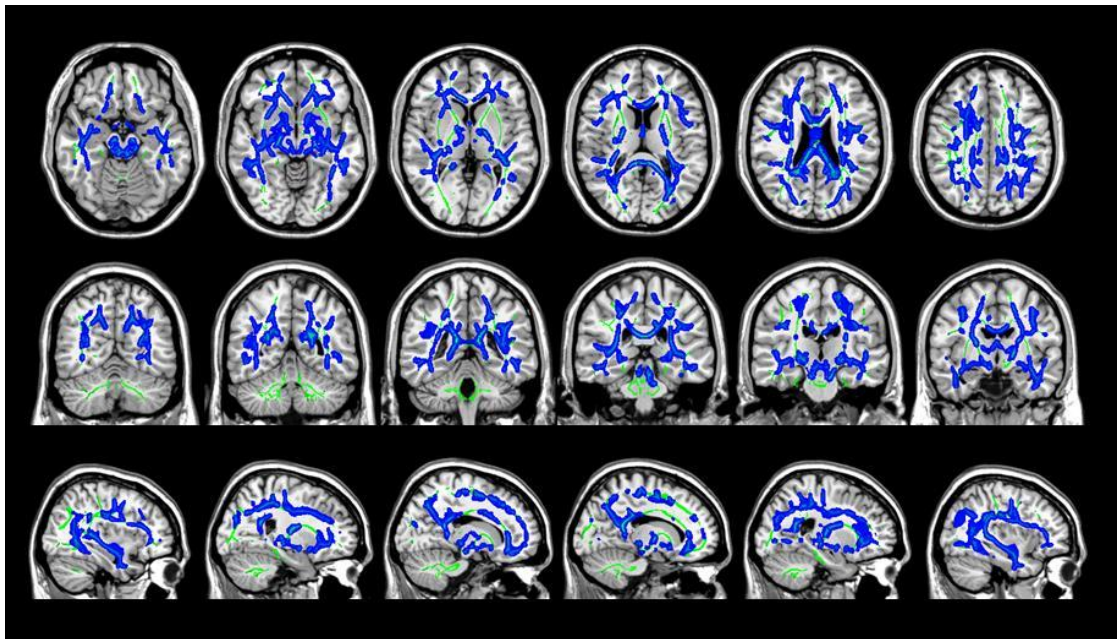
In comparison to controls, areas of significant MD increase in DLB were global and included areas of the brainstem, thalamus, cingulate, temporal, parieto-occipital and frontal lobes and are shown in Figure 6.6 (a). These areas were also affected in the AD group when compared with controls, represented in Figure 6.6 (b).

In the DLB group, there was significant MD increase in the thalamus and precuneal areas. Although these were also affected in AD, they were part of a more widespread pattern of loss. In AD there was also more involvement of the medial temporal lobe structures.



Radiological view

(a) DLB &gt; Controls



(b) AD &gt; Controls

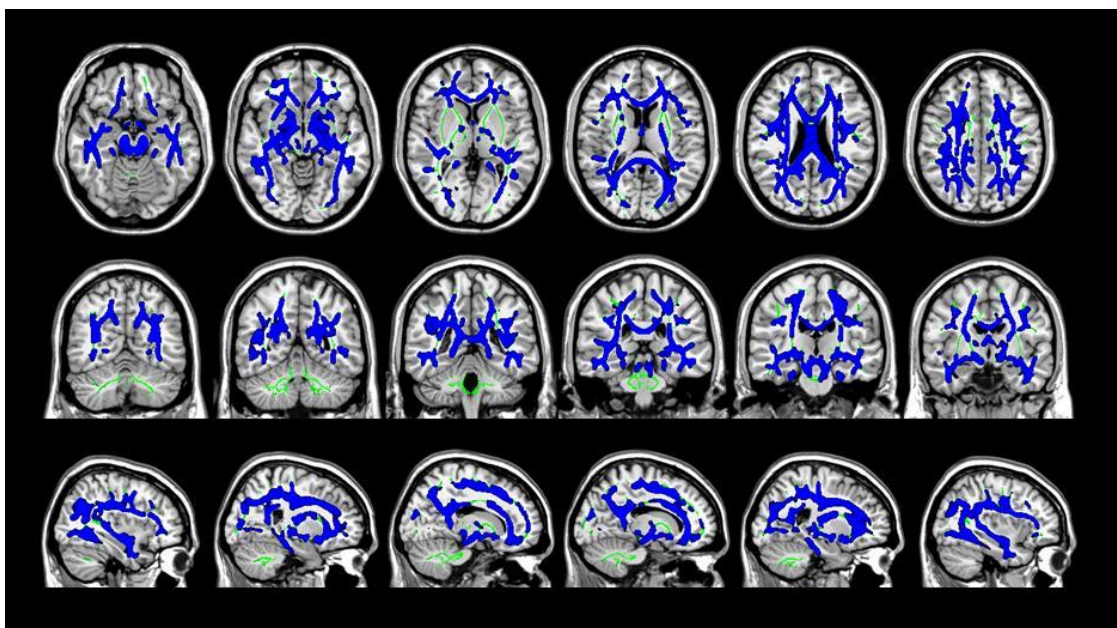


Figure 6.6. TBSS statistical maps for increased MD in (a) dementia with Lewy bodies and (b) Alzheimer's disease. Areas of significant differences ( $p < 0.005$ , corrected) are represented in blue overlaid onto the MNI template image and mean FA skeleton (green).

### **6.3.5.2 Comparison of MD between DLB and AD**

Areas of increased MD in the AD group compared with the DLB group was seen in bilateral temporal lobes ( $p < 0.05$ , uncorrected). There was a small cluster of increased MD in the right pons in the DLB group when compared to AD.

## **6.3.6 Clinical Correlations**

### **6.3.6.1 Episodic Memory**

We determined the correlations of MD with the episodic memory composite score in DLB and AD groups, controlling for the effects of global cognition and age by including them in the design matrix as covariates. These correlations are represented in Figure 6.7, and were significant at  $p < 0.05$ , uncorrected.

A correlation between impaired episodic memory and increased MD was regionally similar in AD and DLB, and included clusters in bilateral parahippocampal gyri (hippocampal cingulum) and left cingulate gyrus (frontal). In DLB, there was also a small cluster in the right thalamus.

We did not find a correlation between FA and episodic memory in AD or DLB.

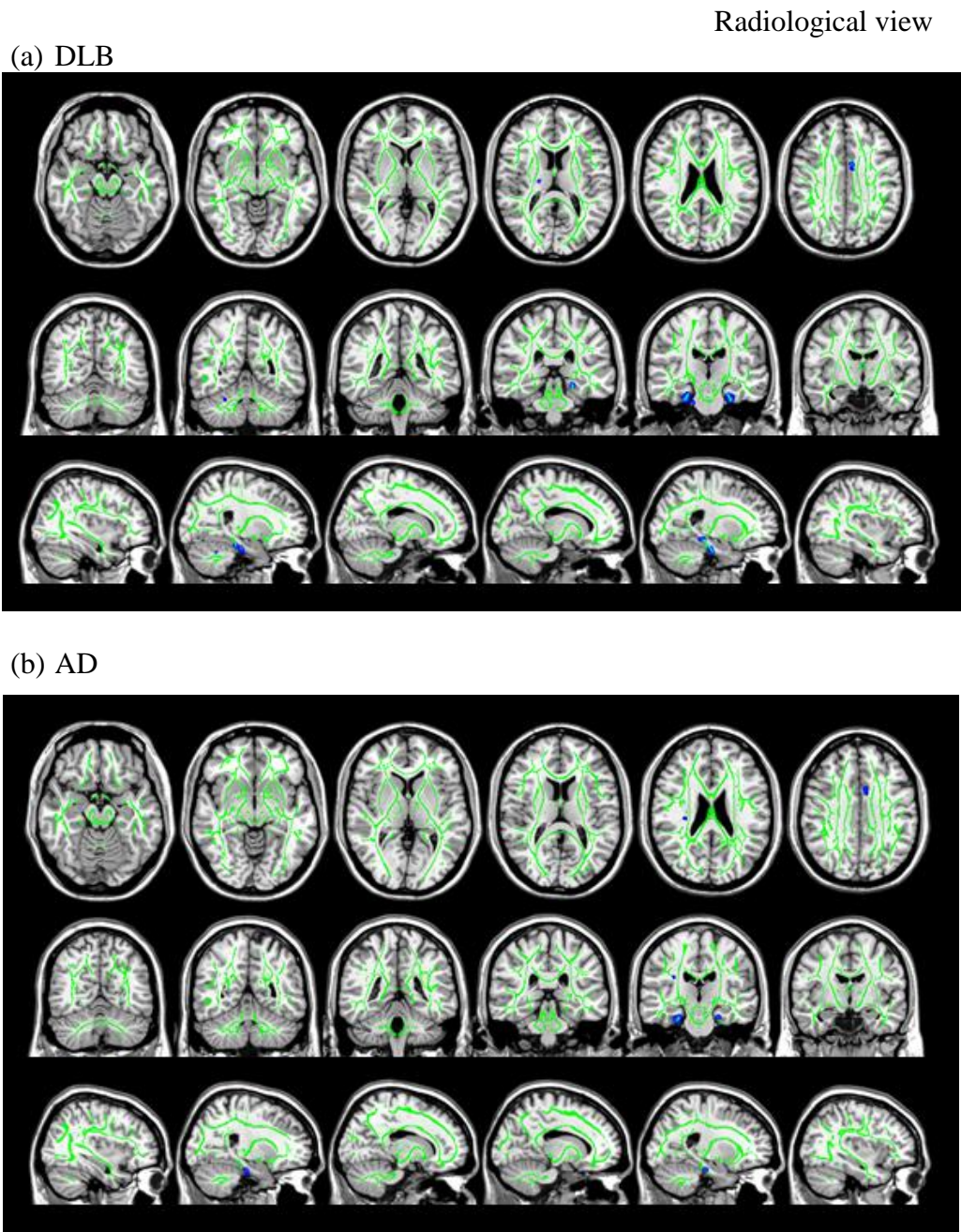


Figure 6.7. Represents the relationship between Mean Diffusivity (MD) and Episodic Memory in (a) DLB and (b) AD. The areas that correlate significantly are represented in blue, overlaid onto the MNI template image and mean FA skeleton (green).



### 6.3.6.2 Letter Fluency

#### *Relationship between FA and Letter fluency*

The relationship between FA and letter fluency in DLB was significant ( $p < 0.05$ , corrected) and is represented in Figure 6.8. The correlation involved predominantly bilateral frontal, parietal and subcortical structures. In particular, the precentral gyrus, anterior cingulate, the precuneus and striatal structures were associated. An example of this correlation is represented in Figure 6.9. FA values from a voxel in the middle frontal white matter tract (MNI 23, 33, 25) is plotted against the letter fluency task ( $r = 0.42$ ,  $df = 29$ ,  $p = 0.009$ ). This correlation was not observed in AD.

Radiological View

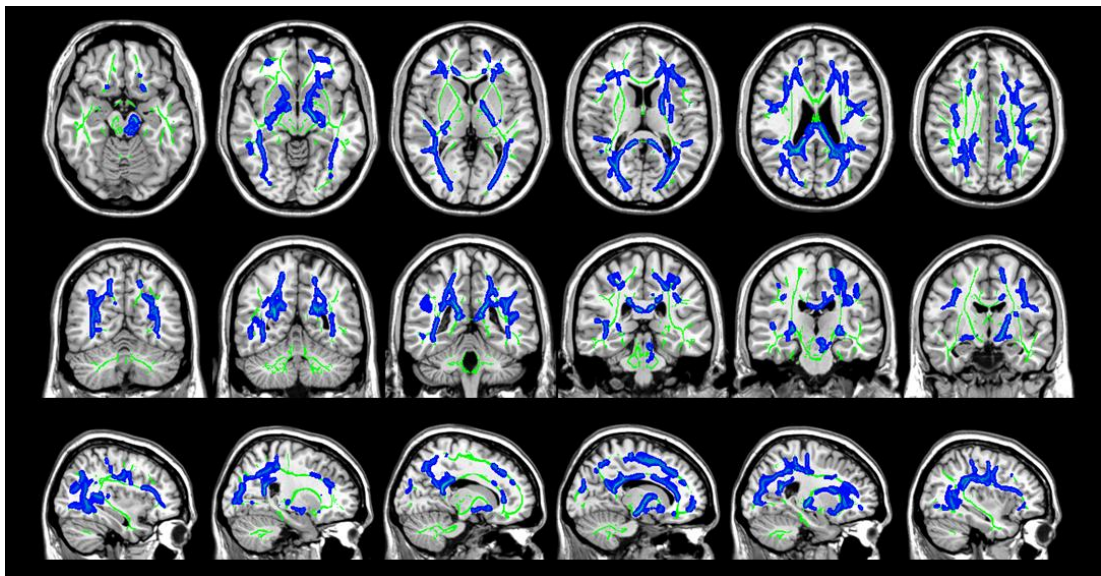


Figure 6.8. Represents the relationship between Fractional Anisotropy and Letter fluency in DLB. The areas that correlate significantly are represented in blue, overlaid onto the MNI template image and mean FA skeleton (green).

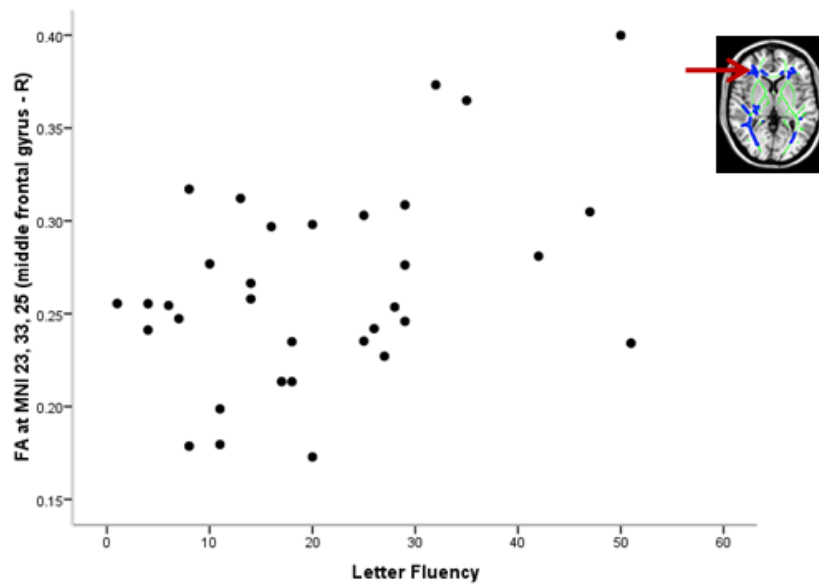


Figure 6.9. Fractional Anisotropy at MNI 23, 33, 25 (location is represented in the axial image by the red arrow) plotted against Letter Fluency in DLB.

### *Relationship between MD and Letter Fluency*

A relationship between MD and letter fluency was observed in DLB and, to a lesser extent in AD. In DLB, the areas were subcortical, including areas of the brainstem and striatum ( $p < 0.05$ , corrected). White matter tracts of the precuneus (parietal), precentral gyrus (frontal) and corpus callosum (parietal) were also associated (Figure 6.10). In AD, the relationship between MD and letter fluency was also observed in the white matter tracts of the parietal lobes extending to involve some of the frontal white matter tracts.

## Radiological View

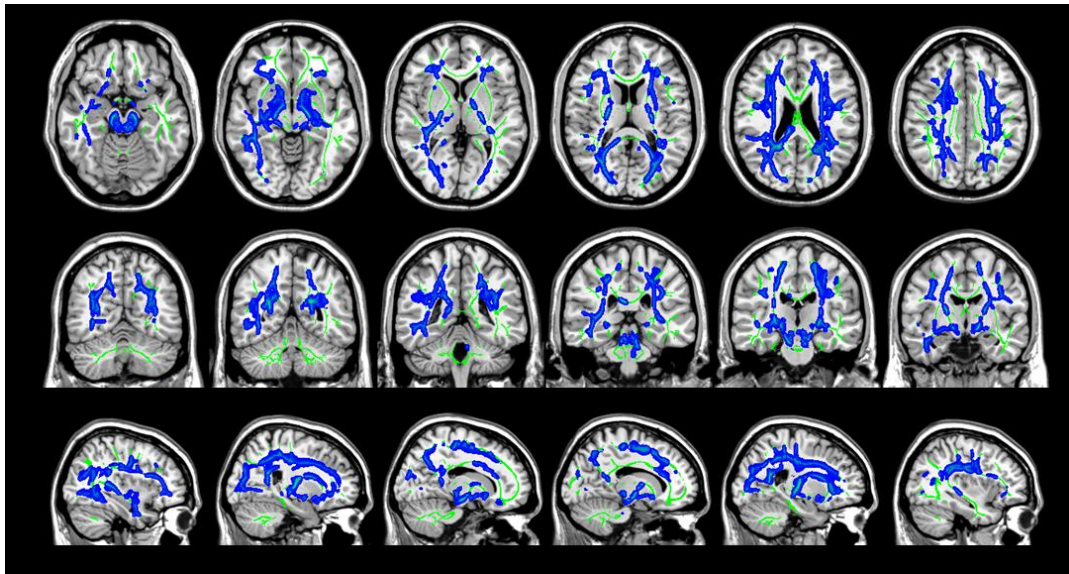


Figure 6.10. Represents the relationship between MD and Letter fluency in DLB. The areas that correlate significantly are represented in blue, overlaid onto the MNI template image and mean FA skeleton (green).

### 6.3.6.3 Motor Parkinsonism in DLB

#### *Relationship between FA and UPDRS-III*

The relationship between FA and UPDRS III in DLB was investigated. We found a correlation between higher UPDRS scores and reduced FA in subcortical structures including the thalami, pons and medulla as well as the precentral gyrus. Other small clusters were found in the occipital, parietal and temporal lobes, which included the precuneus and postcentral gyrus, represented in Table 6.4.

Anatomical Region	MNI Coordinates (x, y, z)			p value
<b>(a) DLB</b>				
Occipital R	33	-77	13	0.007
Occipital R (cuneus)	11	-86	26	0.009
Occipital L (cuneus)	-15	-80	19	0.009
Frontal L	-19	-40	55	0.009
Frontal L (precentral gyrus (BA 4))	-19	-20	65	0.008
Frontal R	25	42	8	0.009
Parietal L (precuneus)	-15	-69	45	0.009
Postcentral gyrus L	-24	-34	61	0.008
Temporal L	-51	-37	-16	0.009
Temporal L (parahippocampal gyrus)	-40	-24	-20	0.008
Thalamus R	5	-8	6	0.006
Thalamus L	-6	-25	16	0.014
Fornix	0	3	4	0.004
Pons R	7	-33	-27	0.010
Pons L	-4	-31	-27	0.008
Medulla R	2	-37	-43	0.007
Medulla L	-1	-37	-41	0.009

Table 6.4. Locations and significance of the correlation between FA and the UPDRS-III in DLB. The table shows voxel level significance ( $p < 0.05$ , uncorrected) MNI coordinates and anatomical region.

## 6.4 Discussion

We used Tract Based Spatial Statistics to investigate the patterns of white matter tract DTI changes in DLB. The main findings of this study were (i) DLB was associated with a significant FA reduction in white matter tracts of the temporo-occipital and parietal lobes, anterior and posterior cingulate and body and genu of the corpus callosum. The changes in MD were more widespread with increased MD also found in the subcortical structures including the pons and thalamus. (ii) The pattern of FA reduction in AD was more extensive and included white matter tracts of the frontal, temporal, parietal and occipital lobes. (iii) When DTI changes between DLB and AD were compared directly, DLB was associated with lower FA values in the pons and thalamus and increased MD in the right pons. In AD, areas of reduced FA and increased MD were found bilaterally in the medial temporal lobes compared with DLB. (iv) There was a significant correlation between DTI change and letter fluency in DLB and UPDRS-III correlated with FA reduction in the subcortical and cortical white matter tracts, including motor areas. The burden of white matter hyperintensities was found to be similar between dementia groups suggesting that the differing patterns of white matter tract change as measured with DTI was unlikely to be completely due to WMH.

We found a pattern of posterior predominance in the DTI changes in DLB with involvement of the parieto-occipital areas and temporal regions with relative sparing of the frontal lobes which are broadly in keeping with other DTI studies (Lee et al., 2010a; Ota et al., 2009; Bozzali et al., 2005).

These areas are important for visual information processing; the dorsal and ventral visual streams are hypothesised to connect the primary visual cortex in the occipital lobe to the parietal and temporal lobes respectively (Goodale and Milner, 1992). Given that characteristic features of DLB are recurrent visual hallucinations as well as early visuo-spatial and visuo-perceptual deficits, it is of great interest that these areas were preferentially affected (McKeith et al., 2005). The ventral visual stream involves the connection from the primary visual cortex in the occipital lobe via the inferior longitudinal fasciculus to the inferior temporal gyrus and parahippocampal gyrus, areas that have also been found to be associated with Lewy related pathology and visual hallucinations in pathological studies (Harding et al., 2002).

Previous DTI studies using ROI methods have found reductions of FA in the precuneus, occipital areas and inferior longitudinal fasciculus (ILF) in DLB (Lee et al., 2010b; Firbank et al., 2007b; Bozzali et al., 2005). SPECT studies have also reported precuneal and occipital hypoperfusion (Colloby et al., 2002) and PET studies have reported occipital hypometabolism (Imamura et al., 1997); without associated structural grey matter loss of sufficient magnitude to explain this (Middelkoop et al., 2001). Therefore, it is possible that the functional imaging changes reflect damage to the microstructural white matter connections, supporting the notion that DLB is a disease predominantly caused by neuronal synaptic dysfunction rather than loss.

We found reduced FA in the posterior cingulate in both AD and DLB which is in agreement with other DTI studies (Lee et al., 2010b; Firbank et al., 2007a). In one DTI study, reduced FA in the region of the posterior cingulate was associated with global cortical atrophy (Firbank et al., 2007a) and in AD has also been found to correlate with hippocampal volume (Xie et al., 2005). SPECT studies have shown posterior cingulate hypoperfusion in DLB and AD (Firbank et al., 2003; Colloby et al., 2002) and interestingly, amyloid deposition in the posterior cingulate and parietal regions (precuneus and lateral) were related to visuo-spatial impairment in DLB (Gomperts et al., 2008). Our findings of microstructural change in the posterior cingulate in DLB, an area that may be vulnerable as a result of high metabolic demands and amyloid deposition (Gomperts et al., 2008; Buckner et al., 2005), highlight its importance in the pathological process of DLB.

The thalamus organises and processes information between the subcortical and cortical structures. These include relaying information to the visual association cortices from the retina via the lateral geniculate nucleus and striatal structures to motor cortex. It is also a key area in maintaining consciousness (Schiff, 2008). When comparing areas of reduced FA between AD and DLB, we found reduced FA in the left thalamus in DLB. There were also clusters of increased thalamic MD in DLB when compared to controls.

Structural imaging studies have not found significant change in the thalamus in DLB (Burton et al., 2002) whereas functional imaging studies using SPECT have reported increased thalamic perfusion and a pattern of covariance with occipital

hypoperfusion in DLB (Shimizu et al., 2008). Increased thalamic perfusion has also been shown to be associated with an increase in fluctuation (O'Brien et al., 2005). It is possible that microstructural change causes an alteration in the neurotransmitter and/or receptor status with resultant perfusion changes in DLB. Multi-modal studies of perfusion and diffusion imaging in DLB will be required to help address this.

We found more extensive microstructural change in the temporal lobes in AD, particularly in the medial temporal lobe structures. This is consistent with findings from other studies, the known pathological change in the area and the early and characteristic impairment of episodic memory (Jack et al., 2010; Scola et al., 2010). This differs from DLB where episodic memory is usually not as profoundly affected and relative preservation of the medial temporal lobe in structural imaging has been well established (McKeith et al., 2005). Interestingly, we also found that the episodic memory composite score and medial temporal white matter tract change appear to be associated in both AD and DLB. Although this was significant at  $p < 0.05$ , uncorrected, it suggests a common underlying mechanism affecting the episodic memory performance in both groups. The DLB group performed better than the AD group on tests of episodic memory, showed less DTI change in the medial temporal lobe structures and that the medial temporal lobe change correlated with episodic memory in both groups. This suggests that microstructure change in this region may be related to Alzheimer pathology.

AD and DLB pathology commonly coexist, and possibly interact, although mechanisms for this are not yet understood (Crews et al., 2009). From clinico-pathological data, the more AD tangle pathology (i.e. Braak stage V or VI) that exists, the less likely the patient is to manifest the core clinical features of DLB even when a lot of Lewy related pathology is present (McKeith et al., 2005). So, whilst it is likely our cohort had some degree of AD pathology, they all met the clinical criteria for probable DLB, so were therefore unlikely to have a high degree of AD pathological burden.

DLB patients usually have more difficulty with tasks involving executive function rather than memory when compared with AD. This is thought, in part, to be due to the impairment in striatal function and dopaminergic deficiency given the importance of the fronto-striatal network in executive function. This network connects the pre-

frontal cortex and striatum via the thalamus with its function mediated by dopamine (Tekin and Cummings, 2002). However, the functional connections involved in tasks of executive function are much more complex with some studies indicating that input from parietal, occipital and temporal lobes is required, depending on the task demand (Woo et al., 2010; Collette et al., 2006).

We found a significant relationship between reduced FA and increased MD and the letter fluency task in DLB, which involved frontal and parietal lobes, the anterior cingulate and subcortical structures including the brainstem and striatum. There was also a correlation between reduced FA in the white matter tracts of the right prefrontal cortex and the letter fluency task.

Given that both age and MMSE were included in the analysis design as covariates, these findings suggest that impaired verbal fluency may be associated with change predominantly in the frontal, parietal and subcortical brain microstructure in DLB and not driven by global cognitive impairment. In AD, although the task performance was impaired, it was not as strongly correlated with change in the white matter tracts and no correlation was found with reduced FA. It is possible that executive dysfunction in AD, as assessed by the letter fluency task, is not strongly associated with microstructural change and may be better represented by other imaging modalities such as volumetric or perfusion techniques. However, the differences suggest that there may be differing pathological mechanisms underpinning executive dysfunction in AD and DLB.

The UPDRS-III scale, a measure of the severity of motor features of parkinsonism correlated with reduced FA in white matter tracts of the motor cortex as well as subcortical structures. Motor parkinsonism in DLB is largely a result of abnormalities in the basal ganglia with degeneration of dopaminergic neurons and striatal dopamine deficiency, although the motor cortex also contributes via thalamic projections. The parietal and occipital areas have also been found to be activated during motor tasks in patients with parkinsonism suggesting a compensatory mechanism (Galvan and Wichmann, 2008).

PET studies in Parkinson's disease have demonstrated a positive correlation between UPDRS and metabolic change in the primary motor cortex and supplementary motor areas (Nagano-Saito et al., 2004) and longitudinal studies have also reported a



progressive increase in the metabolic activity of subcortical structures and motor cortex (Huang et al., 2007). Interestingly, a SPECT study which compared DLB patients with and without parkinsonism found that those with parkinsonism had reduced blood flow in the primary and supplementary motor areas compared to those without (Takahashi et al., 2010b). A relatively small DTI study using VBM analysis techniques in PD reported significant FA reduction in the supplementary motor areas and anterior cingulate (Karagulle Kendi et al., 2008). These findings highlight the importance of these areas in the pathogenesis of parkinsonism and, along with our findings perhaps suggests a more complex mechanism for the motor symptoms in DLB with disruption in the white matter in these compensatory or ‘secondary’ regions leading to more prominent motor features.

An important consideration in this study is the impact of WMH which can alter DTI parameters. It would be instructive to investigate this relationship further with volumetric techniques given we did not exclude cases with WMH. Using a visual rating scale however, we found that the dementia groups were similar, which provides further support that the differences we observed were unlikely to purely be a function of WMH burden.

In conclusion, DLB was characterised by a predominantly posterior pattern of altered diffusivity with generally less change than observed in AD. The involvement of the visual association area, thalamus and significant correlations with executive tasks suggest the importance of microstructural change in the pathogenesis of the disease. DTI may be a useful technique to investigate early, and possibly pre-clinical, changes in DLB.

The next chapter will provide a summary and discussion of the main findings of the neuropsychological, structural imaging and diffusion imaging changes in dementia with Lewy bodies and highlight important areas for future work.

## Chapter 7 Conclusions and future studies

With the increasing prevalence of dementia, a better understanding of the neurobiological changes underpinning DLB is a vital step forward in order to improve early diagnosis, differential diagnosis and inform development of novel treatment options. This was the primary motivation for the study.

### 7.1 Summary of main findings

The first part of the thesis is concerned with the clinical and neuropsychological features of our cohort of AD and DLB subjects. As expected, DLB was characterised by a subcortical pattern of neuropsychological impairment with attentional and executive dysfunction and less impaired memory compared to AD, consistent with the notion that subcortical change predominates in DLB. The visual system was also impaired with the majority of DLB cases reporting visual hallucinations and having difficulty with visuo-perceptual based tasks.

The main finding of the structural GM analysis was of relative preservation of medial temporal lobe structures in DLB compared to AD. This was highly significant in the bilateral parahippocampal gyri and left hippocampus ( $p < 0.05$ , corrected) and consistent with the neuropsychological profile observed of relatively preserved new learning (compared to AD). There was much less regional volumetric GM loss in DLB than AD. Affected areas included the temporal, parietal, occipital and subcortical structures in DLB although only reached statistical significance when corrected for multiple comparisons in the temporal regions (including amygdala) and caudate tail. Therefore, at a similar level of dementia severity, there was more atrophy in AD than DLB, which suggests that there are differing pathological mechanisms underpinning the clinical and neuropsychological features of the conditions.

Microstructural changes in DLB were therefore investigated using diffusion tensor imaging. As expected, changes in the white matter tracts were less extensive in DLB than in AD. In DLB, the regional pattern of change (FA) compared with controls had a posterior predominance affecting the visual association areas and subcortical structures which, by contrast to the structural changes, were all highly significant, ( $p < 0.05$ , corrected).

The letter fluency task was used as an executive function probe and we found a significant pattern of correlation ( $p < 0.05$ , corrected) in DLB affecting frontal, parietal and subcortical structures, a pattern not observed in AD, highlighting the potential differing pathological mechanisms between the diseases.

## 7.2 Conclusions

### *Cortical atrophy*

Cerebral GM atrophy is largely caused by the loss of neurons and synapses. In contrast to DLB, neurodegeneration and particularly synaptic loss has been found to be associated with the clinical features of AD (Terry et al., 1991). Our finding of less global GM loss in DLB compared to AD was consistent with another large VBM study (Whitwell et al., 2007b). Given that the dementia groups were well matched for age, dementia severity and level of education, it indicates that DLB and AD have differing pathological substrates underpinning the clinical features with AD resulting in more neuronal and synaptic loss than DLB. Furthermore, medial temporal lobe atrophy on structural imaging has been associated with the presence and severity of AD neuropathology (Gosche et al., 2002; Jack et al., 2002) and is a useful biomarker in separating AD from healthy controls (Scheltens et al., 2002). We found significantly *less atrophy in the medial temporal lobes structures* in DLB when compared to AD, which was also consistent with the neuropsychological profile observed of relatively preserved new learning in the DLB group.

AD pathology commonly coexists in cases of DLB. Large amounts of AD neuritic pathology can mask the characteristic features of DLB (McKeith et al., 2005). Given that our DLB cohort were selected based on their clinical phenotype – having at least 2 out of 3 of the core clinical features, they were less likely to have large amounts of concomitant AD neuritic pathology. It is therefore possible that the relatively little structural GM loss observed in DLB may reflect the lesser burden of AD pathology in this group.

### ***Subcortical structures***

The thalamus organises and processes information between the subcortical and cortical structures with one of its functions being maintenance of consciousness. Similar to other studies, we did not find significant structural change in the thalamus in DLB (Whitwell et al., 2007b; Burton et al., 2002). However, we found small areas of reduced FA in the thalamus in DLB compared to AD, and we also found increased thalamic MD in DLB compared to controls. Functional imaging studies using SPECT have reported increased thalamic perfusion and a pattern of covariance with occipital hypoperfusion (Shimizu et al., 2008) and increased thalamic perfusion has also been shown to be associated with cognitive fluctuation in DLB (O'Brien et al., 2005). Given these findings, along with the prominent attentional deficits and the cognitive fluctuation observed in DLB, further studies are warranted to investigate thalamic changes in more detail using DTI ROI or tractography methods in combination with perfusion imaging.

### ***Visual association areas***

Given the early and prominent visuo-perceptual dysfunction in DLB, the finding of significant FA change preferentially affecting the parieto-occipital white matter tracts (part of the visual association areas), with relative sparing of the frontal white matter tracts was of interest. The ventral visual stream involves a connection from the primary visual cortex in the occipital lobe via the inferior longitudinal fasciculus to the inferior temporal gyrus and parahippocampal gyrus, areas which have been found to be associated with Lewy related pathology and visual hallucinations in pathological studies (Harding et al., 2002). Other DTI studies have found changes in the precuneus, inferior longitudinal fasciculus and occipital areas (Kantarci et al., 2010; Firbank et al., 2007b; Bozzali et al., 2005) and functional imaging studies using SPECT (Colloby et al., 2002) and PET (Imamura et al., 1997) have also reported precuneal and occipital hypoperfusion and occipital hypometabolism respectively without significant structural GM loss (Middelkoop et al., 2001). It is therefore possible that the functional imaging changes observed reflect damage to the white matter tracts and potentially contribute to the visuo-perceptual dysfunction observed in DLB. Further ROI studies of the dorsal and ventral visual streams

investigating the diffusion, perfusion and metabolic changes in combination along with clinical and neuropsychological correlates may help to address this.

### ***Executive function***

DLB was associated with more prominent executive dysfunction than AD. This was thought to be at least partly mediated by the striatal dopaminergic deficiency in DLB given the importance of the fronto-striatal system in executive function. Our finding of a significant correlation between letter fluency and FA and MD white matter tract change in DLB highlight the potential differing pathologic mechanisms between AD and DLB. It suggests that DTI may be a more sensitive imaging modality than structural T1 imaging to detect pathological change in DLB. Indeed, further studies are required to address this. In particular, longitudinal studies to review areas more vulnerable to white matter tract change, and pathological verification studies to help address what causes the observed diffusion tensor imaging changes observed.

### **7.3 Study strengths and limitations**

Strengths of the study include a large and well-characterised group of DLB subjects. It is one of the larger DLB cohorts to complete an MRI study, which therefore increases the chance for detecting differences between groups. The clinical and neuropsychological scales selected have also been highly cited and validated for use in dementia.

The VBM DARTEL method of structural imaging analysis is a sensitive algorithm for detecting regional GM loss (Ashburner, 2007). It is automated, relatively operator independent and examines the whole brain without the need for *a priori* region of interest. To ensure that the probability of incorrectly rejecting the null hypothesis for the large number (family) of statistical tests associated with voxelwise analyses do not exceed  $\alpha = 0.05$ , correction methods that control for the family-wise error rate (FWE) were used. However, these correction methods are conservative with the inherent higher rates of false negatives, and the potential to miss a true difference in GM volume loss between groups. So whilst the differences we reported were highly significant, other areas of difference could have been missed.

TBSS combines the strengths of a relatively operator independent, whole brain, VBM style of analysis with the tract-based information usually gained from tractography (Smith et al., 2006). This represents the first study using this method of analysis in DLB. The DTI performed was 16-directions. We could have improved the signal-to-noise ratio by incorporating more directions into the protocol as has been shown in other studies (Landman et al., 2007). A reduction in the slice thickness may have improved the spatial resolution and finally, the addition of a T2 weighted FLAIR image would have allowed for a more accurate volumetric assessment of white matter hyperintensities and therefore incorporation into the model of regional DTI change.

The reliance on the clinical diagnosis rather than pathological confirmation is a potential limitation as it is with all antemortem neuroimaging studies. However, the methods used for case detection have been well validated and found to have high specificity in prospective studies with autopsy validation (McKeith et al., 2000a). Study subjects have also been approached to register for brain tissue donation, which will enable future neuropathological correlational studies.

#### **7.4 Future directions**

DLB is a complex disease process that affects a number of different neuronal systems. Although the mechanisms are not entirely understood, it seems that the likely pathological substrate is aggregates of synaptic protein  $\alpha$  synuclein resulting in neuronal dysfunction and eventual loss. Subcortical structures seem more vulnerable with a variety of functional and structural imaging changes observed, in a differing pattern to that seen in AD. In order to develop disease biomarkers we need to establish a link between the clinical features observed and the *in vivo* imaging changes.

##### ***Structural imaging***

In DLB, cortical areas are potentially affected by a combination of impaired neuronal projections from subcortical structures and directly by varying degrees of AD pathology. One method to explore this further would be to investigate the differing patterns of structural covariance between DLB and AD.

Longitudinal imaging studies can also assist in providing insight into more susceptible regions of change in DLB, and how the pattern may differ from AD. The rate of atrophy also has potential as an outcome measure in therapeutic trials and establishing a link between atrophy rate and a clinically significant outcome. In the first instance, a longitudinal study using VBM-DARTEL method of analysis comparing the regional atrophy rate in AD and DLB would be of interest.

### ***Multi-modal imaging***

Another approach would be to incorporate information from differing imaging modalities. Areas of interest would include the posterior (parieto-occipital) and thalamic areas. These are key areas involved in visuo-perceptual and attentional function and our DTI findings in DLB indicate that further analysis is warranted. In particular, using a ROI method, data from diffusion and perfusion imaging techniques could be combined in a multivariate model along with clinical variables, to better understand the *in vivo* functional changes in DLB. This could be investigated using SPECT or PET in combination with DT-MRI. However, the main advantages for multi-modal MRI studies combining structural imaging, DTI and perfusion (using arterial spin labelling) are that all data can be acquired during a single session and registration issues are less problematic.

### ***Sub-group analysis***

It is likely that there were varying degrees of associated AD pathology in the DLB group which may be reflected in the degree of episodic memory dysfunction and GM volume loss, particularly hippocampal atrophy. To provide more insight into the pathophysiological mechanisms of DLB it would be instructive to divide the DLB group on the basis of hippocampal volume, a surrogate measure of AD pathology and further review any patterns of DTI change in the DLB group with particular focus on the relationship with executive function tasks and motor symptoms. This has the potential to explore the imaging changes characteristic of DLB pathology without the effect of AD ‘diluting’ the differences between the groups.

### ***Clinical aspects***

Important aspects of clinical imaging studies that need to be considered are a well-defined patient group with robust methods for characterisation of the core clinical

features. Adequate and well validated methods for testing of attention and fluctuation, visual perception and motor features is of particular importance so that they can be used reliably as probes for correlation with imaging changes. In addition, dopamine transporter imaging, amyloid PET and genetic markers (e.g. ApoE4) would provide better characterisation of the groups.

*Neuropathological correlational studies*

Finally, neuropathological correlation studies are needed to assist in understanding the pathological substrates of imaging changes in DLB. This has been clearly demonstrated by others and has been central to our understanding of the basis of medial temporal atrophy and amygdala changes in DLB (Burton et al., 2011; Burton et al., 2009).



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