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Diffusion tensor imaging in dementia with Lewy bodies and Alzheimer's disease

Running title: Diffusion tensor imaging in dementia

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Abstract

Dementia with Lewy bodies (DLB) is a common form of dementia, with fewer memory deficits, and more visuo-perceptual problems than Alzheimer's disease (AD). We hypothesized that there would be disease specific alterations revealed by diffusion tensor imaging with AD showing temporal lobe and DLB more parietal changes. We recruited 15 people with AD, 16 with DLB, and 15 healthy control subjects of similar age. They were scanned on a 1.5T MRI system with diffusion tensor FLAIR imaging. Apparent diffusion coefficient (ADC) and fractional anisotropy (FA) maps were calculated, and data were analysed using predefined regions of interest (ROI) and also with SPM. We found a significant decrease in the FA map in a ROI in the parietal lobe (precuneus) of the DLB group. Using SPM we found increased ADC in the left temporal lobe of AD subjects compared to controls. There were no other significant differences between groups. We conclude that there are subtle changes visible with diffusion imaging in DLB and AD which may reflect disrupted connectivity and underlie observed perfusion changes in these disorders.

Abstract: 176 words

Key Words: MRI; Diffusion Tensor Imaging; dementia with Lewy bodies; Alzheimer's disease;

1. Introduction

Dementia with Lewy bodies (DLB) is a common form of dementia in older people, with clinical symptoms differing from Alzheimer's disease (AD) – people with DLB typically have fewer memory deficits, and more visuo-perceptual problems, fluctuating awareness, and parkinsonian motor features (McKeith et al., 2004). The underlying pathological substrates for these features are not fully understood.

Magnetic resonance (MR) diffusion weighted imaging (DWI) is a technique which allows images of the diffusion of water in the brain to be produced. Because of the highly structured nature of axons, water tends to diffuse along the direction of white matter tracts rather than perpendicular to them. DWI is a sensitive indicator of changes to the integrity of axons (Bammer and Fazekas, 2002). From DW images can be calculated the apparent diffusion coefficient, and the fractional anisotropy. The apparent diffusion coefficient (ADC) is a direction independent measure of the overall diffusivity of water. Damage to axons, or a decrease in cell density, increases the ADC as the barriers to water diffusion decrease. The fractional anisotropy (FA) depends on the relative diffusivity of water in different directions and varies from zero, where diffusion is equal in all directions, to 1, where diffusion occurs along a single direction. FA is high in regions of coherent white matter tracts (such as the corpus callosum) since the fibres all go in the same direction. A decrease in FA indicates water diffusing more isotropically – this may be caused by damage to the walls of the axons, allowing water to diffuse perpendicularly to the axon direction.

There have been a number of studies of diffusion imaging in Alzheimer's disease. The most consistent finding has been increased ADC in the temporal and parietal lobes (Kantarci et al.,

2001; Bozzali et al., 2002; Head et al., 2004), though some studies have found few differences between AD and comparable healthy subjects (Bozzao et al., 2001). The only study of diffusion imaging in dementia with Lewy bodies (Bozzali et al., 2005) found reduction in FA and increase in ADC in DLB compared to controls in most white matter regions of the brain that were examined. We are not aware of any studies which have directly compared DLB with AD.

The purpose of this work was to compare the diffusion parameters ADC and FA between people with AD, DLB, and subjects of a similar age without dementia.

Most previous studies have used T2 weighted diffusion images, though Kantarci used a diffusion sequence with FLAIR (Fluid attenuated inversion recovery) to reduce the signal from the cerebrospinal fluid (CSF), and found higher ADC in the hippocampus of people with Alzheimer's disease (Kantarci et al., 2002). Previous work (Bhagat and Beaulieu, 2004) has shown that the use of FLAIR DWI results in lower ADC and higher FA values in the brain due to the decreased influence of CSF in the images. Since people with dementia frequently have a degree of brain atrophy, and since we were interested in comparing the diffusion values of brain, we chose to use a FLAIR sequence to minimise the effect of atrophy and partial volume of CSF on the diffusion images.

The measured diffusion coefficient in the brain depends upon the diffusion weighting used. The majority of studies have utilised a diffusion weighting of $b = 1000 \text{ s.mm}^{-2}$. For values of b up to about 1000 s.mm^{-2} , the signal decreases in an approximately exponential manner with b . However, for higher b values, the signal decay becomes less rapid. It has been hypothesised that with higher b -values, more of the signal comes from water whose movement is restricted by the tissue structure, and is thus potentially more indicative of the brain tissue (DeLano and Cao, 2002). Yoshiura (Yoshiura et al., 2003) found greater

differences between AD and healthy controls for higher b values. Hence we collected data with b values of both 1000 and 4000 s.mm⁻²

In order to compare our findings with those of Bozzali et al (2002, 2005), we utilised the same regions of interest as in their work, anticipating similar findings. In particular, in view of the role of the medial temporal lobe in memory function, and of the parietal lobe in visuo-perceptual functioning, and given that temporal hypoperfusion is apparent in AD (Colloby et al., 2002; Pasquier et al., 2002), and parietal hypoperfusion more severe in DLB than AD (Colloby et al., 2002; Firbank et al., 2003a) we hypothesized that these perfusion deficits would be related to adjacent tissue damage, and that DWI would show temporal lobe abnormalities in the AD group, and more prominent parietal changes in the DLB group.

2. Methods

2.1 Subjects

We recruited 15 people with Alzheimer's disease and 16 with dementia with Lewy bodies, from clinical Old Age Psychiatry, Geriatric Medicine and Neurology Services. Fifteen healthy subjects of similar age were also recruited.

All subjects were aged over 60, had mild to moderate dementia (MMSE>10) and did not have contra-indications for MRI. All Alzheimer's disease subjects fulfilled criteria for probable AD according to NINCDS/ADRDA (McKhann et al., 1984). Dementia with Lewy body cases all met criteria for probable DLB according to the consensus criteria (McKeith et al., 1996). All diagnoses were made by consensus between experienced clinicians (JO'B, MSK, AT), a method we have previously validated against autopsy diagnosis (McKeith et al., 2000). Routine clinical workup for dementia included detailed physical, neurological and

neuropsychiatric examinations, including screening blood tests and CT scan. Additional assessments performed were of cognition (Cambridge Cognitive Examination (CAMCOG) (Roth et al., 1986)), mood (Cornell depression scale (Alexopoulos et al., 1988)), neuropsychiatric features (Neuropsychiatric inventory (NPI) (Cummings et al., 1994)), clinical fluctuation (Clinical Assessment of Fluctuation scale (Walker et al., 2000)) and motor features of parkinsonism (UPDRS subsection III (Fahn et al., 1987)).

Exclusion criteria included severe concurrent illness (apart from dementia for patients), space occupying lesions on MRI, and history of stroke. In addition, controls had no history of psychiatric illnesses.

2.2 MRI acquisition

Subjects were scanned on a 1.5T MRI system (Intera scanner; Philips, Eindhoven, the Netherlands). Images were acquired with T1 weighted volumetric sequence (1.5 mm cubic voxel; TR = 10 ms, TE = 4.6 ms, FA = 20°); FLAIR (5mm slices; TR = 6000 ms, TI = 2000 ms, TE = 100ms) and diffusion tensor FLAIR imaging with b value of 1000 s.mm⁻² (3mm slices. TR = 6000 ms, TI = 2000 ms, TE = 88 ms. 3 images of b=0 and 24 uniformly distributed diffusion weighted images with b = 1000 s.mm⁻²) and with b value of 4000 s.mm⁻² (6mm slices. TR = 6000 ms, TI = 2000 ms, TE = 130 ms. 3 images of b=0 and 24 uniformly distributed diffusion weighted images with b = 4000 s.mm⁻²). Field of view for the diffusion images was 240 mm; Matrix 94x128; Nominal pixel size 1.89x1.89 mm; SENSE factor = 2. Total scanning time was approximately 30 minutes.

2.3 Analysis

We used both FSL (Smith et al., 2004) (<http://www.fmrib.ox.ac.uk/fsl/fdt/index.html>) and SPM2 <http://www.fil.ion.ucl.ac.uk/spm/> software for analysis. FSL was used for processing the diffusion data, and SPM2 for segmenting the 3D sequence into grey and white matter, for spatially normalising data to MNI space, and performing statistical parametric mapping to compare groups. We performed region of interest (ROI) analysis using FSLview to draw a set of predefined regions on each subject. Regions of white matter hyperintensities (WMH) were segmented from the FLAIR images using in-house software (Firbank et al., 2003b). Briefly, this uses SPM to segment the brain from the FLAIR images, and then determines the volume of WMH by using an intensity threshold of 1.45 times the modal intensity for each slice.

2.4 Image segmentation and normalisation

The 3D T1 weighted images were segmented into CSF, and grey and white matter in native space, using the standard segmentation routines in SPM2. The fraction of grey and white matter was calculated as a proportion of intracranial volume (from the sum of GM, WM and CSF). Normalisation of the images proceeded by a two stage process. First, the T1 weighted images were aligned using an affine normalisation to the standard SPM T1 MNI [Montreal Neurological Institute] template. An average of all the aligned images was then calculated, and smoothed using a 10-mm FWHM isotropic Gaussian kernel. This image was then used as a custom template, and the T1 weighted images were spatially normalised to it using an affine transformation with 12 degrees of freedom together with a series of non-linear warps characterized by a set of $7 \times 8 \times 7$ basis functions (in the x, y, and z directions) - (SPM's standard non linear normalisation).

The segmented white matter images were transformed to MNI space using these normalisation parameters. The images were resliced with a voxel size of 2x2x2 mm. An

average of all the normalised WM segmented images was calculated, representing the proportion of WM in each voxel. A WM mask was generated by thresholding this averaged WM image at a level of 0.2. The WM mask was used to spatially limit the diffusion SPM analysis.

2.5 Diffusion calculation

Apparent diffusion coefficient (ADC) and fractional anisotropy (FA) maps were calculated from the DTI sequence using FSL. Separate maps were calculated for the $b=4000 \text{ s.mm}^{-2}$ and the $b=1000 \text{ s.mm}^{-2}$ cases. For each subject, all of the diffusion weighted images were aligned with the initial (zero diffusion weighting) image using the affine transform software in FSL. The data were reviewed manually, and any individual diffusion weighted images which were affected by movement artefacts were removed from the analysis. The diffusion parameters (mean diffusion, fractional anisotropy) were then calculated from the remaining aligned images – i.e. for some slices the ADC and FA were calculated from less than 24 diffusion direction images. For each subject there were 24 x 32 (diffusion directions x slices) individual diffusion weighted images - approximately 1 percent of these were affected by movement.

2.6 Region of interest analysis

A series of pre-defined regions of interest were drawn on each dataset by an operator blind to subject group. Regions were drawn in FSLview, in native image space, and in positioning the regions, the 3D image, initial (zero diffusion weighting) image and FA maps were viewed to

ensure that regions were positioned in white matter. The 3D images were spatially aligned (using FSL) to the diffusion images in order to account for any movement which might have occurred between image acquisitions. Regions were drawn in native space since it was felt that transformation of the images to standard space might have introduced blurring, and hence reduced the mean FA values, particularly for small WM tracts. Regions were drawn using the $b=1000 \text{ s.mm}^{-2}$ (3mm slice) data. The ROIs were transferred to the $b=4000 \text{ s.mm}^{-2}$ images following a linear registration of the two datasets. The mean ADC and FA on $b=1000$ and 4000 s.mm^{-2} in each ROI were calculated for each subject.

We drew ROIs in principal areas thought to be affected in dementia. Areas of white matter hyperintensities were avoided. For most regions, we used the ROI locations described by Bozzali (Bozzali et al., 2005). All regions were drawn on both left and right sides, and data averaged. These ROIs were: Putamen & head of Caudate – a square of 3x3 pixels on the two adjacent slices on which these structures were best depicted. Genu and splenium of corpus callosum – 7x2 pixels on three adjacent slices. Anterior and posterior peri-callosal area – a diamond of 13 pixels and square of 9 pixels respectively on the same slices as the corpus callosum ROIs. Parietal WM 3x3 pixels on two slices posterior to the central sulcus, on the most caudal slices on which it was visible. Frontal WM 3x3 pixels on two slices starting with the most cranial fully volumed lateral ventricle. Occipital WM 3x3 pixels on two slices starting with the most caudal slice on which the occipital horn of the lateral ventricle was visible. Temporal lobe 3x2 pixels on 2 slices, the more caudal of which is in level with the posterior commissure. On the internal capsule were placed 2 regions – anterior and posterior – each 2x4 pixels on the slice with both head of caudate and putamen best depicted. Thalamus 4x4 pixels on 3 slices cranial to the posterior commissure. In line with Bozzali, anterior and posterior data were averaged for the corpus-callosum, peri-callosal and internal capsule regions. The location of these regions is shown in figure 1. In addition to these

regions, we also included one in the white matter adjacent to the precuneus, and one in the WM temporal lobe adjacent to the hippocampus. Precuneus – 3x3 pixels on two slices, cranial-caudal position determined by the top of the parietal-occipital sulcus, and posteriorly by the bottom of the same sulcus (see figure 2). Medial temporal lobe 3x3 pixels on two slices half way between the anterior and posterior commissure, and lateral to the hippocampus (see figure 3). These regions were included since abnormalities in cerebral metabolism are frequently seen in these locations in DLB and AD respectively. Table 2 contains the approximate Talairach coordinates of the regions of interest, which were calculated by transforming the ROIs into MNI space using the transforms as calculated in section 2.4. All regions were drawn by the same operator (MJF) who was blinded to subject group. Reliability of the region drawing was verified by redrawing the regions on 6 randomly selected subjects after a period of at least 3 months using the same guidelines, but without viewing the previous regions. Over all ROIs, the root mean square distance between the initial and the redrawn regions was 3.7 mm (2 pixels) with maximum distance of 11 mm. There was a 44% overlap of voxels between the two sets of regions.

2.7 SPM analysis

Each subject's diffusion weighted image was spatially aligned with the same subject's 3D T1 weighted image using affine normalisation in SPM. The diffusion images were then spatially normalised using the normalisation parameters calculated from the 3D-T1 images as described above, and the ADC and FA maps resliced in normalised space with voxel size of 2x2x2 mm. One DLB subject and one control subject could not be normalised due to large ventricles and susceptibility artefact respectively, and these subjects were excluded from the SPM analysis.

The normalised FA and ADC maps were all smoothed with a 10mm filter. Given the ROI results, and the thinner slice width of the $b=1000 \text{ s.mm}^{-2}$ data, we just performed SPM analysis on this data. SPM comparisons were made of FA and ADC, using a 3 group ANOVA to investigate differences between controls and AD, and controls and DLB.

2.8 Statistics

The statistical package SPSS for Windows (version 11) was used for data analysis. Variables were compared between groups using ANOVA. Post hoc Sheffe tests were performed on all significant ($p < 0.05$) ANOVA comparisons. As in our previous studies, volumes of WMH were divided by whole brain volume, and analysed after a logarithmic transform, because of the highly skewed nature of the data (Firbank et al., 2003b). Data have been back transformed for presentation in the tables, which show geometric mean of WMH with the 1SD range. All statistical tests were two tailed and were regarded as significant at $P < 0.05$. All P values < 0.1 are quoted.

SPM results were thresholded at $P = 0.001$ uncorrected for multiple comparisons. They were regarded as significant if the cluster P -value corrected for multiple comparisons was less than 0.05. Coordinates from SPM were converted to Talairach space using the MNI2TAL transform (<http://www.mrc-cbu.cam.ac.uk/Imaging/Common/mnispace.shtml>). The Talairach daemon (Lancaster et al., 2000) was used to assign anatomical labels to voxel coordinates.

3. Results

As shown in Table 1, the groups were well matched for age & sex, and the dementia groups were comparable in cognitive scores. There were no significant differences in WMH volume

between the groups. Both dementia groups had significantly less grey and white matter than controls, but there was no difference between AD and DLB.

Figure 4 shows typical ADC maps calculated for the two diffusion values. Table 2 shows the mean ADC and FA values determined from the ROI analysis of the $b=1000 \text{ s.mm}^{-2}$ data.

There were no significant differences in ADC between groups. For FA, there was a significant difference between groups in the precuneus and in the peri-callosal area.

Table 2 also shows the FA and diffusion values for the high b value diffusion images. Again, there were no significant differences in the ADC values, but the FA was significantly different in the temporal and also again the precuneus region.

The SPM comparison of control vs AD showed a large significant cluster in the left temporal lobe where ADC was raised in AD (see figure 5; table 3). There were however, no significant differences between AD and controls in FA, or between the DLB group and controls in either FA or ADC. There were no differences between AD and DLB in the SPM analysis.

4. Discussion

The values of apparent diffusion coefficient and fractional anisotropy in the control subjects are similar to those measured by Bhagat (Bhagat and Beaulieu, 2004) with a comparable sequence on normal subjects of similar age. The ADC values of the controls are also similar to those of Bozzali (Bozzali et al., 2005).

In AD, we found increase of ADC in the temporal lobe, consistent with the known pathological processes in the temporal lobe. The temporal lobe changes were not in the

medial temporal region, but more lateral, in a similar location to the inferior longitudinal fasciculus and occipito-temporal projection system (Catani et al., 2003; Mori et al., 2005) which connect the parahippocampal gyrus and amygdala with the occipital lobe (Catani et al., 2003). It may be that atrophy of medial temporal grey matter structures in Alzheimer's disease is linked to disruption of the connecting white matter. In the comparison of DLB vs controls, we found using the ROI analysis, a region in the precuneus area, with reduced FA on both $b=1000 \text{ s.mm}^{-2}$ and $b=4000 \text{ s.mm}^{-2}$ data. There was no significant difference in any area on the SPM comparison of DLB vs controls. We have previously found that the posterior cingulate/precuneus region shows prominent hypoperfusion in DLB (Colloby et al., 2002), which is in excess of atrophy in the region (Firbank et al., 2003a). Our finding of low FA in the precuneus area is consistent with the medial parietal hypoperfusion being secondary to disrupted connectivity of the area. There are reciprocal connections (Cavanna and Trimble, 2006) between the precuneus and the lateral parietal lobes and the dorsolateral prefrontal cortex. These areas show hypoperfusion in DLB (Colloby et al., 2002; Firbank et al., 2003a), and in Parkinson's disease, the prefrontal cortex shows early hypoperfusion followed later by medial and lateral parietal hypoperfusion (van Laere et al., 2004).

One of the aims of the study was to compare different b values of 1000 and 4000 s.mm^{-2} . There were some indications that the $b=4000 \text{ s.mm}^{-2}$ data was more sensitive – the significance of the FA in the precuneus region was greater in the higher b value data, and the temporal lobe region showed significant changes in the $b=4000 \text{ s.mm}^{-2}$ FA data. However, the $b=4000 \text{ s.mm}^{-2}$ data had much lower signal to noise levels, and we had to use double the slice thickness to obtain usable images.

Possible limitations of the study include the absence of postmortem diagnosis of subtype of dementia, which might have limited the difference between AD and DLB. However, we used strict criteria for diagnosis, which we have previously shown to have sensitivity and specificity for a clinical diagnosis of probable DLB of 0.83 and 0.95. (McKeith et al., 2000) We are thus confident in the accuracy of the diagnosis.

There were some differences between the SPM and ROI results - the ADC temporal lobe findings were not significant on the ROI analysis, although the $b=4000 \text{ s.mm}^{-2}$ data showed a reduced temporal FA in the two dementias. However, a disadvantage of ROI analysis is that only limited areas of the brain are sampled. The location of the temporal and medial temporal ROIs were outside of the SPM region of significant change (See table 2; Left medial temporal lobe ROI Talairach coordinates: -42, -18, -14). Since the SPM results showed a degree of left sided laterality, it is possible that we did not see this in the ROI analysis due to having averaged left and right regions. Some of the changes on the SPM analysis were located in the grey matter (see table 3), and it is possible some of the ADC change was due to atrophy in AD. Contrariwise, the precuneus FA was statistically significant on ROI, but not SPM analysis. We chose the location of the precuneus ROI since we had strong *a priori* expectations of change in this region, as it shows hypoperfusion on DLB. It may be that in preparing the images for SPM analysis – warping the images into standard space, and smoothing – that the precuneus region was blurred, and hence the statistical significance reduced.

We did not find striking differences between diffusion images in DLB and controls, and so did not replicate the finding of widespread abnormalities reported by Bozzali and colleagues, despite having subjects broadly matched for age and dementia severity, using regions of

interest in the same anatomical locations, and measuring similar ADC values in the control group. Possibly there is heterogeneity in the diffusion measurement - reports of diffusion imaging in AD have produced varied findings, for example some reports (Bozzao et al., 2001; Duan et al., 2006; Medina et al., 2006) found no difference in ADC in the temporal lobe of AD, whereas others (Bozzali et al., 2002; Head et al., 2004) all found significant changes. It may be that for diffusion imaging in dementia, the size effect is relatively small, and that the studies have included too few subjects to consistently see differences. Factors like white matter hyperintensities have a marked influence upon FA and ADC (Firbank et al., 2003b). We included people with moderate WMH, since WMH are frequently present in AD and DLB (Barber et al., 1999) whilst Bozzali excluded those with moderate WMH from their study in order to exclude vascular pathology.

Another explanation, however, is that the results of Bozzali were influenced by brain atrophy of the DLB group – they employed a T2 weighted diffusion sequence, which results in high ADC values of the CSF, and since they had a slice thickness of 5mm, partial volume effects may have increased the measured ADC in the brain, through the inclusion of CSF. Since DLB have greater atrophy than controls, the ADC would be preferentially increased in DLB. It would be instructive to compare FLAIR and T2 weighted DTI in the same patients.

In conclusion we found changes on the diffusion images indicative of damage to structures known to be affected in AD and DLB. Our results are consistent with the hypothesis that disrupted connectivity in DLB underlies some of the perfusion changes previously described in the disorder.

5. Acknowledgements

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5.1 Disclosure statement

No authors have any conflicts of interest to declare.

Table 1. Demographics, and brain volume data.

	Control N=15	AD N=15	DLB N=16	Statistical comparison
Age mean (SD)	75 (8)	76 (7)	76 (7)	$F_{2,43} = 0.0; P = 1.0$ §
Sex F/M	6/9	5/10	7/9	$\chi^2 = 0.4; P = 0.8$ ¶
Disease duration: months	-	35 (14)	42 (18)	$t = -1.3; P = 0.2$ ‡
MMSE	28.3 (2.1)	19.1 (4.8)	19.1 (4.5)	$t = 0.0; P = 1.0$ ‡
CAMCOG	97 (4)	67 (11)	66 (15)	$t = 0.2; P = 0.8$ ‡
UPDRS	0.9 (1.4)	5.1 (6.0)	30.6 (16.0)	$t = -5.8; P < 0.001$ ‡
Cornell	ND	4.3 (4.6)	8.3 (5.2)	$t = -2.3; P = 0.03$ ‡
NPI hallucinations	ND	0 (0)	3.3 (3.3)	$t = -3.8; P = 0.001$ ‡
NPI total	ND	8.6 (10)	20 (19)	$t = -2.0; P = 0.052$ ‡
WMH % of brain (1SD range)	0.3 (0.1 – 1.0)	0.5 (0.2 – 1.4)	0.6 (0.1 – 3.2)	$F_{2,43} = 0.9; P = 0.4$ §
Intercranial grey matter fraction	0.41 (0.03)	0.36 (0.03)	0.38 (0.04)	$F_{2,42} = 9.5; P < 0.001$ +
Intercranial white matter fraction	0.23 (0.02)	0.21 (0.03)	0.21 (0.03)	$F_{2,42} = 6.3; P = 0.004$ +
Taking cholinesterase inhibitors	0/15	14/15	12/16	$\chi^2 = 1.9; P = 0.2$ *

WMH = white matter hyperintensity volume. WMH data are geometric mean with 1SD range

MMSE = Mini mental state exam. CAMCOG = Cambridge Assessment of Mental Disorders in the Elderly. UPDRS = Unified Parkinson's Disease Rating Scale (motor subscore).

Cornell = Cornell depression score. NPI = Neuropsychiatric inventory.

* Pearson Chi squared test for AD vs DLB

¶ Pearson Chi squared 3 group

‡ Student t test for AD vs DLB df=29

§ 3 group ANOVA

+ 3 group ANOVA with Scheffe Post hoc:

GM Con > AD $p=0.001$. Con > DLB $P=0.02$. AD vs DLB ns

WM Con > AD $p=0.01$. Con > DLB $P=0.02$. AD vs DLB ns

CSF Con < AD $p<0.001$. Con < DLB $P=0.004$. AD vs DLB ns

Table 2. Region of interest analysis. Diffusion parameters and Talairach coordinates of each region. FA values are mean (SD). ADC values are mean (SD) *10⁻⁴ mm²s⁻¹

	Control	AD	DLB	Anova <i>P</i> value
Putamen : ±24, 8, -1				
Mean ADC (b = 1000 s.mm ⁻²)	7.1 (0.3)	7.4 (0.4)	7.3 (0.7)	0.3
Mean FA (b = 1000 s.mm ⁻²)	0.20 (0.02)	0.21 (0.02)	0.21 (0.03)	1
Mean ADC (b = 4000 s.mm ⁻²)	5.8 (0.3)	5.9 (0.4)	5.9 (0.4)	0.4
Mean FA (b = 4000 s.mm ⁻²)	0.27 (0.03)	0.26 (0.04)	0.26 (0.04)	0.9
Caudate : ±14, 13, 10				
Mean ADC (b = 1000 s.mm ⁻²)	7.5 (0.3)	7.5 (0.4)	7.6 (0.5)	0.9
Mean FA (b = 1000 s.mm ⁻²)	0.19 (0.01)	0.21 (0.02)	0.20 (0.02)	0.2
Mean ADC (b = 4000 s.mm ⁻²)	6.3 (0.3)	6.3 (0.2)	6.4 (0.3)	0.8
Mean FA (b = 4000 s.mm ⁻²)	0.24 (0.02)	0.25 (0.03)	0.24 (0.03)	0.7
Corpus Callosum –				
Genu : 0, 26, 6				
Splenium : 0, -37, 17				
Mean ADC (b = 1000 s.mm ⁻²)	8.6 (0.4)	8.7 (0.5)	8.9 (0.8)	0.2
Mean FA (b = 1000 s.mm ⁻²)	0.78 (0.04)	0.77 (0.03)	0.77 (0.04)	0.5
Mean ADC (b = 4000 s.mm ⁻²)	5.4 (0.2)	5.4 (0.3)	5.6 (0.4)	0.3
Mean FA (b = 4000 s.mm ⁻²)	0.67 (0.03)	0.66 (0.03)	0.64 (0.05)	0.4
Pericallosal area –				
Anterior : ±20, 36, 5				
Posterior : ±21, -51, 18				
Mean ADC (b = 1000 s.mm ⁻²)	8.4 (0.5)	8.7 (0.6)	8.8 (1.0)	0.18
Mean FA (b = 1000 s.mm ⁻²)	0.48 (0.06)	0.44 (0.06)	0.41 (0.07)	0.024
Mean ADC (b = 4000 s.mm ⁻²)	5.1 (0.3)	5.3 (0.4)	5.4 (0.6)	0.3
Mean FA (b = 4000 s.mm ⁻²)	0.43 (0.05)	0.40 (0.05)	0.39 (0.06)	0.079
Parietal : ±24, -36, 42				
Mean ADC (b = 1000 s.mm ⁻²)	7.8 (0.4)	7.9 (0.4)	8.2 (0.9)	0.19
Mean FA (b = 1000 s.mm ⁻²)	0.46 (0.03)	0.44 (0.03)	0.43 (0.04)	0.15
Mean ADC (b = 4000 s.mm ⁻²)	4.6 (0.3)	4.6 (0.3)	4.9 (0.5)	0.06
Mean FA (b = 4000 s.mm ⁻²)	0.39 (0.05)	0.36 (0.04)	0.36 (0.04)	0.18
Frontal : ±18, 43, 14				
Mean ADC (b = 1000 s.mm ⁻²)	7.8 (0.3)	8.0 (0.3)	7.8 (0.4)	0.13
Mean FA (b = 1000 s.mm ⁻²)	0.40 (0.04)	0.39 (0.05)	0.40 (0.04)	0.8
Mean ADC (b = 4000 s.mm ⁻²)	4.9 (0.2)	5.0 (0.2)	5.0 (0.3)	0.17
Mean FA (b = 4000 s.mm ⁻²)	0.36 (0.03)	0.35 (0.03)	0.35 (0.05)	0.7
Occipital : ±19, -85, 7				
Mean ADC (b = 1000 s.mm ⁻²)	8.2 (0.5)	8.6 (0.5)	8.4 (0.8)	0.2
Mean FA (b = 1000 s.mm ⁻²)	0.33 (0.04)	0.31 (0.03)	0.33 (0.05)	0.3
Mean ADC (b = 4000 s.mm ⁻²)	5.0 (0.2)	5.1 (0.3)	5.1 (0.5)	0.6

Mean FA (b = 4000 s.mm ⁻²)	0.29 (0.05)	0.28 (0.04)	0.29 (0.04)	0.9
Temporal : ±45, -22, 0				
Mean ADC (b = 1000 s.mm ⁻²)	7.6 (0.3)	7.9 (0.3)	7.7 (0.4)	0.15
Mean FA (b = 1000 s.mm ⁻²)	0.40 (0.04)	0.37 (0.04)	0.38 (0.05)	0.2
Mean ADC (b = 4000 s.mm ⁻²)	4.8 (0.5)	5.1 (0.5)	5.2 (0.7)	0.1
Mean FA (b = 4000 s.mm ⁻²)	0.35 (0.04)	0.31 (0.03)	0.30 (0.04)	0.010 *
Thalamus : ±14, -24, 7				
Mean ADC (b = 1000 s.mm ⁻²)	8.1 (0.2)	8.2 (0.2)	8.3 (0.5)	0.3
Mean FA (b = 1000 s.mm ⁻²)	0.30 (0.02)	0.31 (0.03)	0.31 (0.03)	0.7
Mean ADC (b = 4000 s.mm ⁻²)	6.1 (0.2)	6.0 (0.3)	6.0 (0.4)	0.6
Mean FA (b = 4000 s.mm ⁻²)	0.31 (0.02)	0.31 (0.04)	0.31 (0.04)	1
Internal Capsule –				
Anterior : ±17, 16, 1				
Posterior : ±19, -8, 5				
Mean ADC (b = 1000 s.mm ⁻²)	7.6 (0.3)	7.6 (0.3)	7.8 (0.8)	0.3
Mean FA (b = 1000 s.mm ⁻²)	0.62 (0.03)	0.62 (0.03)	0.62 (0.05)	1
Mean ADC (b = 4000 s.mm ⁻²)	4.8 (0.2)	4.9 (0.2)	5.0 (0.5)	0.5
Mean FA (b = 4000 s.mm ⁻²)	0.56 (0.02)	0.56 (0.02)	0.57 (0.04)	0.9
Medial Temporal: ±42, -18, -14				
Mean ADC (b = 1000 s.mm ⁻²)	8.3 (0.4)	8.7 (0.4)	8.5 (0.6)	0.16
Mean FA (b = 1000 s.mm ⁻²)	0.44 (0.04)	0.42 (0.02)	0.42 (0.04)	0.4
Mean ADC (b = 4000 s.mm ⁻²)	5.5 (0.3)	5.6 (0.3)	5.5 (0.4)	0.4
Mean FA (b = 4000 s.mm ⁻²)	0.36 (0.02)	0.35 (0.03)	0.36 (0.03)	0.6
Precuneus : ±20, -46, 37				
Mean ADC (b = 1000 s.mm ⁻²)	8.0 (0.6)	8.5 (0.7)	8.7 (1.2)	0.12
Mean FA (b = 1000 s.mm ⁻²)	0.45 (0.06)	0.42 (0.06)	0.37 (0.06)	0.009 *
Mean ADC (b = 4000 s.mm ⁻²)	5.0 (0.4)	5.2 (0.3)	5.3 (0.3)	0.3
Mean FA (b = 4000 s.mm ⁻²)	0.37 (0.06)	0.38 (0.06)	0.30 (0.06)	0.001 *

* Scheffe post hoc

Precuneus FA (b=1000) DLB < Control ($P = 0.01$)

Pericallosal FA (b=1000) DLB < control ($P = 0.024$)

Temporal FA (b=4000): Con > AD $p = 0.05$; Con > DLB $P = 0.017$

Precuneus FA (b=4000): Con > DLB $p = 0.009$; AD > DLB $P = 0.004$

Table 3. Significant clusters in the comparison of ADC between AD and controls. For each cluster, the table lists the local maxima voxels (at least 8 mm apart) as listed by SPM.

cluster	cluster	voxel	x,y,z	Location
<i>P</i>	size	T	(Talairach)	
< 0.001	1387	4.66	-51, -59, -5	Left Occipital Lobe Middle Occipital Gyrus Grey Matter
		4.52	-53, -53, -7	Left Temporal Lobe Middle Temporal Gyrus White Matter
		4.34	-53, -26, -7	Left Temporal Lobe Middle Temporal Gyrus White Matter
		4.26	-55, -13, -25	Left Temporal Lobe Fusiform Gyrus White Matter
		4.15	-53, -30, -9	Left Temporal Lobe Middle Temporal Gyrus White Matter
		4.05	-57, -33, 3	Left Temporal Lobe Middle Temporal Gyrus Grey Matter
		3.97	-42, -34, -18	Left Temporal Lobe Fusiform Gyrus Grey Matter
		3.78	-42, -5, -30	Left Temporal Lobe Inferior Temporal Gyrus Grey Matter
		3.74	-38, -68, -7	Left Occipital Lobe Sub-Gyrus White Matter
		3.67	-48, -36, -17	Left Temporal Lobe Fusiform Gyrus White Matter
		3.61	-38, -3, -25	Left Temporal Lobe Sub-Gyrus White Matter
		3.6	-32, -3, -25	Left Temporal Lobe Sub-Gyrus White Matter
		3.36	-42, -49, -14	Left Temporal Lobe Fusiform Gyrus White Matter
		3.34	-55, -44, 8	Left Temporal Lobe Middle Temporal Gyrus Grey Matter
0.049	427	4.88	-6, -82, -3	Left Occipital Lobe Lingual gyrus Grey Matter
		4.62	-14, -86, 21	Left Occipital Lobe Cuneus Grey Matter
		4.18	-10, -89, 8	Left Occipital Lobe Cuneus White Matter

Figure Legends

Figure 1. Axial sections showing the location of ROIs in a) occipital; b) thalamus and temporal; c) thalamus, putamen, anterior and posterior limbs of the internal capsule; d) caudate, genu and splenium of the corpus callosum, along with peri-callosal areas; e) frontal; f) parietal regions.

Figure 2 The precuneus region of interest in axial and sagittal views. The frontal ROI is also depicted on both views, and the sagittal shows the caudate ROI in addition.

Figure 3 The medial temporal WM region of interest in axial and coronal views.

Figure 4 Typical apparent diffusion coefficient maps for $b = 1000 \text{ s.mm}^{-2}$ (left image) and $b=4000 \text{ s.mm}^{-2}$ (right image)

Figure 5 Areas with increased apparent diffusion coefficient in AD vs controls. SPM thresholded at $P < 0.001$ uncorrected for multiple comparisons and overlaid onto the mean structural image. The top left slice is at MNI coordinate $Z = -40 \text{ mm}$, and subsequent slices are every 10mm. Images are in neurological format, with subject left on the left side .

Fig 1

Fig 2

Fig 3

Fig 4

Fig 5

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