Disconnection as a mechanism for cognitive dysfunction in multiple sclerosis

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Disconnection of cognitively important processing regions by injury to the interconnecting white matter provides a potential mechanism for cognitive dysfunction in multiple sclerosis. The contribution of tract-specific white matter injury to dysfunction in different cognitive domains in patients with multiple sclerosis has not previously been studied. We apply tract-based spatial statistics (TBSS) to diffusion tensor imaging (DTI) in a cohort of multiple sclerosis patients to identify loci where reduced white matter tract fractional anisotropy (FA) predicts impaired performance in cognitive testing. Thirty-seven multiple sclerosis patients in remission (median age 43.5 years; Expanded Disability Status Scale range 1.5–6.5; 35 relapsing remitting, two secondary-progressive) underwent 3 T MRI including high-resolution DTI. Multiple sclerosis patients underwent formal testing of performance in multiple cognitive domains. Normalized cognitive scores were used for voxel-wise statistical analysis using TBSS, while treating age as a covariate of no interest. Permutation-based inference on cluster size ($t$, $P < 0.05$ corrected) was used to correct for multiple comparisons. Statistical mapping revealed differential patterns of FA reduction for tests of sustained attention, working memory and processing speed, visual working memory and verbal learning and recall. FA was not associated with frontal lobe function or visuospatial perception. Cognitively relevant tract localizations only partially overlapped with areas of high FLAIR lesion probability, confirming the contribution of normal-appearing white matter abnormality to cognitive dysfunction. Of note, tract localizations showing significant associations with cognitive impairment were found to interconnect cortical regions thought to be involved in processing in these cognitive domains, or involve possible compensatory processing pathways. This suggests that TBSS reveals functionally relevant tract injury underlying cognitive dysfunction in patients with multiple sclerosis.

Keywords: multiple sclerosis; cognitive impairment; magnetic resonance imaging; diffusion tensor imaging; disconnection

Abbreviations: BA = Brodmann area; CIS = clinically isolated syndrome; COWAT = Controlled Oral Word Association Test; DTI = diffusion tensor imaging; FA = fractional anisotropy; MRS = magnetic resonance spectroscopy; MTI = magnetization transfer imaging; NAWM = normal-appearing white matter; PASAT = Paced Auditory Serial Addition Test; ROI = region of interest; TBSS = tract-based spatial statistics.

Introduction

Cognitive dysfunction occurs in 43–65% of patients with multiple sclerosis and most commonly manifests as disturbances in recent memory, sustained attention, verbal fluency, conceptual reasoning and visuospatial perception (Rao et al., 1991). The pattern of cognitive decline is not uniform, and disease duration and physical disability correlate only weakly with cognitive impairment (Ron et al., 1991). The mechanism underlying cognitive impairment in multiple sclerosis has not been fully elucidated.

In recent years there has been renewed interest in the role of disconnection in the aetiology of higher brain dysfunction (Catani and ffytche, 2005). In this context disconnection may occur between cognitively important cortical or subcortical regions, either at the level of interconnecting white matter, or in cortical relays within association cortex. Quantitative imaging techniques, such as diffusion tensor imaging (DTI), have allowed study of the substrates underpinning models of connectivity, and the disconnection paradigm has broadened to include neurodevelopmental...
and neurodegenerative disorders (Catani, 2006).

Given the multifocal white matter pathology that occurs in multiple sclerosis, it is conceivable that cognitive impairment in multiple sclerosis is, at least in part, caused by disconnection. A disconnectionist model for cognitive impairment in multiple sclerosis could explain the involvement of multiple cognitive domains as a series of disconnection syndromes affecting different cognitive networks. Furthermore, the disconnection model has the appeal that it does not exclude other mechanisms shown to contribute to cognitive impairment in multiple sclerosis, such as grey matter pathology (Amato et al., 2004; Morgen et al., 2006).

The relationship between white matter injury and cognitive performance in multiple sclerosis has been studied previously. Frontal lesion volume has been shown to affect performance in tests of frontal lobe function (Arnett et al., 1994; Foong et al., 1997; Rovaris et al., 1998), and frontal and parietal lesion burden has been shown to correlate with performance on tests of complex attention and verbal working memory (Sperling et al., 2001). Additionally, it has been shown that injury to white matter which is not detectable by conventional MRI [within the so-called normal-appearing white matter (NAWM)] but which can be detected by techniques such as DTI, magnetization transfer imaging (MTI) and magnetic resonance spectroscopy contributes to cognitive dysfunction in multiple sclerosis patients (Rovaris et al., 1998; Pan et al., 2001; Rovaris et al., 2002; Cox et al., 2004).

Studies attempting to ‘map’ cognitive dysfunction to white matter injury using a voxel-wise statistical analysis are limited. Charil et al. (2003) studied a data set of 452 multiple sclerosis patients and used statistical parametric mapping to investigate the relationship between lesion location and cognitive performance. The use of conventional imaging in that study precluded assessment of the contribution of ultrastructural tissue injury within NAWM to cognition. In addition the mental component of the Expanded Disability Severity Score (EDSS) was used, which is a subjective, non-specific measure of cognitive function.

Despite these limitations a correlation between cognitive dysfunction and lesions at the grey–white matter junction of the associative, limbic and prefrontal cortices was identified. Ranjeva et al. (2005) studied patients with clinically isolated syndromes, finding that poorer performance during the Paced Auditory Serial Addition Test (PASAT), a test of processing speed and working memory was associated with MTR-defined abnormalities in the splenium of the corpus callosum, the right superior longitudinal fasciculus, the left Brodmann area (BA) 40 and right BA4.

DTI provides a three-dimensional representation of the magnitude of diffusion encapsulating its directional dependence. When applied to the brain, DTI is a powerful non-invasive technique for exploring cerebral ultrastructure. Fractional anisotropy (FA), a parameter derived from DTI data, characterizes the shape of the diffusion tensor ellipsoid within each voxel and hence provides a surrogate quantification of ultrastructural fibre organization (Basser and Pierpaoli, 1996). DTI examination of multiple sclerosis patients has revealed reduced FA in plaques, adjacent to plaques and to varying degrees in NAWM (Werring et al., 1999; Guo et al., 2002; Hasan et al., 2005; Kealey et al., 2005). Furthermore, Lin et al. (2007) have shown that reduced FA in the pyramidal tract NAWM correlates with pyramidal tract lesion burden. This was interpreted as suggesting that Wallerian degeneration secondary to macroscopic multiple sclerosis lesions leads to spatially remote FA reductions in regions connected by white matter tracts.

Tract-based approaches to co-registration have the potential to improve anatomical specificity when compared to conventional co-registration and lesion morphometric techniques. Tract-based spatial statistics (TBSS) (Smith et al., 2006), part of FSL (Smith et al., 2004), is a recently described technique allowing voxel-wise analysis of multi-subject diffusion tensor data. In TBSS the study group FA data are projected onto an alignment-invariant tract representation, or ‘mean FA tract skeleton’, followed by the application of voxel-wise cross-subject statistics.

We hypothesize that multiple sclerosis-related cognitive dysfunction results from a series of domain-specific disconnection phenomena. As such, disruption to critical white matter tracts will lead to reduced functional connectivity between cortico-cortical and cortico-subcortical cognitive processing regions, resulting in impairment to specific cognitive domains. To test this hypothesis, we use TBSS for voxel-wise statistical mapping of white matter tract injury (as quantified by FA) in a cohort of multiple sclerosis patients compared to control subjects, and then apply the TBSS technique to map white matter tract disruption which predicts performance during testing of multiple cognitive domains in a cohort of patients with multiple sclerosis.

**Methods**

**Subjects**

The study was approved by the National Health Service Local Research Ethics Committee for Nottingham and the University of Nottingham Research Ethics Committee. Forty-one patients with definite multiple sclerosis between the ages of 31 and 56 years (median 44.3) and 27 healthy controls were prospectively recruited from a local database of relapsing remitting multiple sclerosis (RRMS) patients. At the time of assessment and scanning three patients were found to have progressed to secondary progressive (SPMS) disease. Multiple sclerosis patients had not experienced relapse or required steroid treatment for at least 2 months prior to inclusion, and none were on immunomodulatory therapy. Participants did not have any other significant neurological, medical, psychiatric or cognitive disorder. All multiple sclerosis patients underwent neurological assessment using the EDSS at the time of participation in the study.

**Neuropsychological test protocol**

All multiple sclerosis patients underwent a detailed neuropsychiatric test battery based on the Minimal Assessment of Cognitive...
Function in Multiple Sclerosis battery (Benedict et al., 2002). The tests and versions performed were as follows: 'Controlled Oral Word Association Test (COWAT)', a test of phonetic fluency (Benton et al., 1983); letters F, A and S were used. 'Benton Visual Retention Test (BVRT)', a test of visual perception, visual memory and visuoconstructive ability (Benton, 1974); Form C, administration A was used and the 'total correct' sub-score was used for the statistical analysis. PASAT, a test of sustained attention, working memory and processing speed; Rao’s adaptation of the Gronwall administration (Gronwall, 1977; Rao et al., 1991), form A, 3 s stimulus was used. 'Judgement of Line Orientation (JLO)', a test of visual perception and spatial processing (Benton et al., 1978); Form V was used. 'Delis-Kaplan Executive Function Score Sorting Test (DST)', a test of executive function (Delis et al., 2001); Card sets 1 and 2 (standard form) were used and the 'confirmed correct sorts' (DST-CS) and 'Free sorting description score' (DST–DS) sub-scores were used for the statistical analysis. 'California Verbal Learning Test version II (CVLT-II)', a test of verbal learning and memory (Delis et al., 2000); the standard form was used, and the sub-score for 'Short delay free recall' (CVLT-II sd), a measure of verbal learning and recall was used for the statistical analysis.

The protocol used varied from the full Minimal Assessment of Cognitive Function in Multiple Sclerosis battery in that the Symbol Digit Modality Test was not performed, and the BVRT was used in place of the Brief Visuospatial Memory Test. In addition the National Adult Reading Test (NART) was performed as an estimate of premorbid intelligence. All tests were administered by a single researcher (R.A.D.) during an interview on the day of the MRI scan.

Neuropsychological test scores were expressed as z-scores derived from published normative data. For the DST and CVLT-II, the normative data were obtained from the accompanying test manuals (Delis et al., 2000, 2001). Sources of normative data for the PASAT (Rao et al., 1991) and BVRT (Youngjohn et al., 1993) are detailed in the references. Full, appropriate normative data were not available for the JLO. Raw JLO scores were corrected for age and sex according to the adjustments recommended by Benton et al. (1978). NART score was converted to the Wechsler Adult Intelligence Scale – Revised Full-Scale IQ (WAIS-R FSIQ) using the formula of Nelson and Willison (1991). This was then used to give a predicted score for the COWAT using the formula of Crawford et al. (1992); deviation from the predicted score was expressed as a z-score.

**MRI protocol and image analysis**

MRI scanning was performed on a Philips Achieva (Philips, Eindhoven, NL) at 3 T. All patients underwent axial DTI (Single-shot diffusion weighted EPI, b = 1000 mm$^2$/s, 15 directions, TE = 56 ms, TR = 9700 ms, 2 × 2 × 2.5 mm$^3$ voxel size interpolated to 1 × 1 × 2.5 mm$^3$ voxels, 45 interleaved slices with no gap, four averages), axial FLAIR (TR = 11 000 ms, TE = 125 ms, TI = 2800 ms, Matrix 256 × 256, slice thickness 2.5 mm/0 mm gap, FOV = 256) and sagittal MPRAGE (TR = 7.5, TE = 2.2, Flip = 8, Matrix 256 × 256, voxel size = 0.8 × 0.8 × 0.8 mm, TFE factor = 236, FOV = 205). Intravenous gadolinium-chelate contrast material was not administered.

Post-processing of diffusion tensor data was performed using the FSL version 3.3 (Smith et al., 2004). Following eddy current correction using the FMRIB’s Diffusion Toolbox (FDT), non-brain voxels were extracted using the Brain Extraction Tool (Smith, 2002) with a brain extraction factor of 0.3. FA maps were generated using FDT. Individual FA maps were visually inspected for the presence of significant residual motion or other artefacts, which led to the exclusion of four of the multiple sclerosis patients and two control subjects from the TBSS analysis. The TBSS registration and tract skeletonization process was performed as described previously (Fig. 1A and B), using a lower threshold of FA of 0.2 to prevent inclusion of non-skeleton voxels (Smith et al., 2006). The skeletonized, fully non-linearly aligned FA data sets obtained using the TBSS process were then used for voxel-wise cross-subject statistical analysis.

T2-hyperintense lesion volume measurement was performed on the FLAIR images using the locally developed NeuRoi software package (Dr C. Tench, Department of Clinical Neurology, University of Nottingham, UK). This provides a semi-automated method for defining T2-hyperintense lesions using Sobel edge detection and non-maximum suppression. Mean lesion probability distribution images were created by first registering the brain-extracted FLAIR images to MNI152 space, and then applying the transformation matrix for each individual FLAIR image to the corresponding masked binarized lesion map. The co-registered lesion maps were averaged using FSLmaths tools to create a lesion distribution.

**Fig. 1** (A) Mean FA image (MNI coordinate $z = 96$). (B) Mean TBSS tract skeleton with lower FA display threshold of 0.2, overlaid on the mean FA image. Colour scale shows skeletal voxels with lower FA as red and higher FA as yellow. (C) As for (B) with mean FLAIR lesion probability distribution thresholded at 10% (blue).
probability distribution which was thresholded at 10% (i.e. voxels which contain macroscopic ‘lesion’ in 10% of subjects) (Fig. 1C).

Volumetric analysis of the MPRAGE images was performed using JIM 3.0 software (Xinapse Medical Systems, Thorpe Waterville, UK). Brain extraction and segmentation was performed using the semi-automated Brain Finder tool which provided measurements of total intracranial volume, parenchymal brain volume, CSF volume and parenchymal brain fraction.

Statistical analysis
Prior to the voxel-wise analysis, group comparison was made between the whole-brain TBSS skeleton mean FA for multiple sclerosis patients and controls. Normality of distribution of the data was assessed by the Shapiro–Wilk test, following which group comparison was performed by ANCOVA, treating age and parenchymal brain fraction as covariates.

The voxel-wise statistical analysis employed is based on a non-parametric approach utilizing permutation test theory with a standard general linear model design matrix. This approach has the advantage that it allows inference on the statistical maps when the null distribution is not known and provides an easily implementable solution to the multiple testing problem (Nichols and Holmes, 2007). The permutation testing was performed using the program Randomise, part of FSL, which uses Monte Carlo permutation testing where $n=5000$, to generate the random permutations. Group comparison between multiple sclerosis patients and controls was performed for the conditions ‘voxel FA for controls > voxel FA for multiple sclerosis patients’, and the converse statement ‘voxel FA for multiple sclerosis patients > voxel FA for controls’. The output was thresholded at cluster level and thus corrected for multiple comparisons using the null distribution of the maximum (across image) cluster size ($t > 2, P < 0.05$).

For the multiple sclerosis patients, inter-subject voxel-wise correlation was performed between skeletal voxel FA and the neuropsychological test $z$-scores, while treating age as a covariate of no interest. As premorbid IQ was felt to be a potential confounding variable, the analysis was repeated treating WAIS-R FSIQ as a covariate of no interest. Furthermore, the relationship between cognitive performance and overall burden of disease (as measured by EDSS) was explored by repeating the TBSS analysis of normalized PASAT $z$-scores while treating EDSS score as a covariate of no interest. Again the output was thresholded at cluster level and thus corrected for multiple comparisons using the null distribution of the maximum (across image) cluster size ($t > 2, P < 0.05$). Significant skeleton voxels were overlaid onto the mean FA template to allow anatomical localization by visual inspection. Reference was made to an MRI-based atlas of white matter tracts to describe affected locations (Mori et al., 2005).

As a test of the validity of the tract localizations demonstrated by TBSS and to visually assess for outlier effects, region of interest (ROI) analysis was performed. Two ROIs were defined on the TBSS skeleton, one at a site where significant correlations with PASAT $z$-scores were seen (posterior left cingulate) and one at a site where no significant correlations with PASAT $z$-score were seen (left parahippocampal white matter). The MN1152 space coordinates of the boundaries of the left posterior cingulate ROI were $x = 98$ to $104$, $y = 86$–$105$, $z = 104$–$111$ and of the left parahippocampal white matter were $x = 113$–$120$, $y = 95$–$114$, $z = 39$–$59$. The ROI was applied to each individual MS subject’s FA skeleton and the mean FA and standard deviation was calculated for each ROI using FSLstats. The mean ROI FA values at each site were used separately as variables in a partial correlation with PASAT $z$-score, while controlling for age and EDSS.

Results
For four of the 41 multiple sclerosis subjects the DTI data were degraded by motion artefact. Hence 37 multiple sclerosis subjects had usable DTI data and cognitive data. DTI data were degraded from 2 of 27 control subjects and hence 25 control data sets were finally included. The characteristics of the subjects included in the TBSS analysis are shown in Table 1.

Neuropsychological testing
The multiple sclerosis patient group cognitive profile is shown in Table 2. Overall the group had a mild degree of cognitive impairment, with performance in all tests falling within 1 SD of the normative group mean. A significant difference was found between multiple sclerosis patients and the normative data for performance in the COWAT and PASAT. The poorest performance was seen in the PASAT, COWAT and BVRT, where the group means were 0.6, 0.5 and 0.4 SD below the normative data mean, respectively. Performance in the JLO is not expressed as $z$-scores, but the group mean (from raw scores corrected for age and sex) showed no group impairment with the group median equaling the maximum score possible. Premorbid IQ was estimated using the WAIS–R FSIQ scores derived from NART. Scores ranged from 81 to 123 with a median of 102.7.

One multiple sclerosis subject had acquired alexia without agraphia. This individual was able to complete the neuropsychological testing with the exception of the DST and NART, both of which require intact reading ability. Hence this subject was excluded from analyses where WAIS-R FSIQ (derived form the NART score) was used and from the analysis of DST and COWAT $z$-scores.

Table I Characteristics of the included participants

<table>
<thead>
<tr>
<th></th>
<th>Multiple Sclerosis patients ($n = 37$)</th>
<th>Healthy controls ($n = 25$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M:F ratio)</td>
<td>I:2.4 43.5 (range 31.1–56.3, IQR 35.3–497)</td>
<td>I:2 36.4 (range 28.2–55.3, IQR 31.5–43.3)</td>
</tr>
<tr>
<td>Ethnic Group</td>
<td>Caucasian = 36, Afro-Caribbean = 1</td>
<td>Caucasian = 25</td>
</tr>
<tr>
<td>Hand dominance</td>
<td>R = 34, L = 3</td>
<td>R = 21, L = 4</td>
</tr>
<tr>
<td>Type of MS</td>
<td>RRMS = 35, SPMS = 2</td>
<td>NA</td>
</tr>
<tr>
<td>Multiple sclerosis duration (years)</td>
<td>10.5 (range 3.3–28.0, IQR 6.3–14.8)</td>
<td>NA</td>
</tr>
<tr>
<td>Median EDSS</td>
<td>3 (range 1.5–6.5, IQR 2.4 to 4)</td>
<td>NA</td>
</tr>
<tr>
<td>Median T2-lesion volume (ml)</td>
<td>6.3 (range 0.1–30.3, IQR 2.8–12.3)</td>
<td>NA</td>
</tr>
<tr>
<td>NA = Not Applicable.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
TBSS analysis

Comparison between multiple sclerosis patients and controls

A small but significant difference in the whole-brain TBSS skeleton mean FA was demonstrated between patients and controls by ANCOVA (0.427 versus 0.445, $F = 5.7$, $P = 0.020$), while treating age and parenchymal brain fraction as covariates. Neither age nor atrophy exerted a covariate effect which is in line with suppression of CSF partial volume effects due to the imposed FA threshold in the skeletonization process.

In the voxel-based group comparison, multiple areas were identified where reduction in skeleton FA in multiple sclerosis patients versus controls reached significance, including the corpus callosum, the inferior longitudinal fasciculi and inferior fronto-occipital fasciculi bilaterally, the bodies and tails of the fornices bilaterally, the posterior corona radiata bilaterally and in the left cerebral peduncle (Fig. 2). To some extent these overlapped with the distribution of macroscopic lesions but tract FA reductions were also seen where probability of macroscopic lesion was <10%. No areas were identified where reduction in skeleton FA in the control versus multiple sclerosis patients reached significance.

Table 2 Group profile for cognitive performance

<table>
<thead>
<tr>
<th>Test</th>
<th>Subscore</th>
<th>Cognitive variable</th>
<th>Mean score</th>
<th>SD</th>
<th>Sig. a</th>
</tr>
</thead>
<tbody>
<tr>
<td>COWAT</td>
<td></td>
<td>Verbal fluency</td>
<td>$-0.5^b$</td>
<td>1.2</td>
<td>$P = 0.01$</td>
</tr>
<tr>
<td>BVRT</td>
<td></td>
<td>Visual memory, visuoconstructive ability</td>
<td>$-0.4^b$</td>
<td>1.4</td>
<td>NS</td>
</tr>
<tr>
<td>PASAT</td>
<td></td>
<td>Processing speed, working memory, attention</td>
<td>$-0.6^b$</td>
<td>1.3</td>
<td>$P = 0.01$</td>
</tr>
<tr>
<td>DST CS</td>
<td></td>
<td>Problem-solving ability</td>
<td>$-0.2^b$</td>
<td>0.9</td>
<td>NS</td>
</tr>
<tr>
<td>DST DS</td>
<td></td>
<td>Conceptual reasoning</td>
<td>$-0.3^b$</td>
<td>1.0</td>
<td>NS</td>
</tr>
<tr>
<td>CVLT-II</td>
<td>slow</td>
<td>Verbal learning and recall</td>
<td>$-0.2^b$</td>
<td>1.3</td>
<td>NS</td>
</tr>
<tr>
<td>JLO</td>
<td></td>
<td>Visual spatial perception</td>
<td>Range 13–30</td>
<td>Median 30</td>
<td>IQR 24–30</td>
</tr>
</tbody>
</table>

a Obtained by one-sample t-test against a normative mean value of 0. b mean Z-scores. NS = Not significant at the $P \leq 0.05$ level

PASAT

Significant ($P \leq 0.05$) correlations were identified between normalized PASAT scores and tract FA at the following locations (Fig. 3A and B): in the body and splenium of the corpus callosum, the parieto-occipital radiations of the forceps major, the left cingulum, the right inferior longitudinal fasciculus running into the left temporal lobe and tracts within the parietal regions including the parietal portion of the left superior longitudinal fasciculus, and part of the parietal arcs of the arcuate fasciculi bilaterally. The distribution of

Fig. 2 Skeletal voxels where patient FA < control FA, $P \leq 0.05$; Cluster-based thresholding corrected for multiple comparisons. Uncorrected for the effects of age. Background: greyscale mean FA map with mean FLAIR lesion probability distribution thresholded at 10% shown in blue.
correlations was similar when premorbid IQ estimate (WAIS-R FSIQ) was treated as a covariate of no interest.

To further explore the relationship of confounding variables the TBSS analysis was repeated for the PASAT z-scores whilst controlling for overall multiple sclerosis severity as measured by the EDSS. The same output threshold and significance level were applied as previously. The distribution overlapped with that for PASAT when EDSS was not controlled for, but limited to the left side of the body and splenium of the corpus callosum radiating into the left parieto-occipital region, the left cingulum and the parietal arc of the left arcuate fasciculus.

Significant correlation was found between the mean FA of skeletal voxels in the left posterior cingulate ROI and PASAT z-scores (partial correlation controlling for age and EDSS, $R_{\text{partial}} = 0.51$, $P = 0.002$). The scatterplot between left posterior cingulate ROI mean FA and PASAT z-score (Fig. 4A) shows that the association is not dominated by outliers, including the SPMS patients (Fig. 4, red dots). Consistent with the TBSS results, the left parahippocampal ROI showed no correlation with PASAT z-score (Fig. 4B).

**BVRT**

Significant ($P \leq 0.05$) correlations were identified between BVRT z-scores and tract FA at the following locations: the body and splenium of the corpus callosum bilaterally, the parietal and occipital projections of the forceps major bilaterally, the left inferior longitudinal fasciculus running into the temporal lobe, the left arcuate fasciculus, the left cingulum, including the anterior portion and the tail of the left fornix. Involvement of white matter of the right parietal and medial right occipital lobes which was not classifiable.
as named tracts was also seen. When premorbid IQ was controlled for, a very similar distribution was seen, with stronger effects in the right temporal white matter tracts (Fig. 5).

**CVLT-II**
Significant \((P < 0.05)\) correlations were identified between CVLT-II sdf \(z\)-scores and tract FA at the following locations: the body and splenium of the corpus callosum, the parietal and occipital projections of the forceps major bilaterally, the parietal portion of the left superior longitudinal fasciculus, the left inferior longitudinal fasciculus and arcuate fasciculus, the left posterior cingulum and the left fornix running posteriorly from the left mesial temporal lobe. The distribution of correlations was similar when premorbid IQ was controlled for, with the exception that the correlations in the body of the corpus callosum and left cingulate white matter were not significant at the \(P \leq 0.05\) threshold (Fig. 6).

**Other cognitive tests**
No significant \((P \leq 0.05)\) correlation was identified between tract FA and adjusted COWAT, JLO, DST CS or DST DS scores using permutation-based inference (cluster size \(t > 2\)).

**Discussion**
Using tract-based FA regression analysis we were able to map the anatomical pattern of white matter tract involvement in multiple sclerosis brains where ultrastructural fibre integrity predicts impaired performance in specific cognitive domains. Differential, although partially overlapping, patterns of tract FA reduction were demonstrated for tests of sustained attention, working memory and processing speed, visual working memory and verbal learning and recall. The partial dissociation of cognitively relevant white matter involvement demonstrated by TBSS from areas of high lesion probability confirms that NAWM abnormality makes an important contribution to cognitive dysfunction in multiple sclerosis (Zivadinov et al., 2001; Rovaris et al., 2002).

TBSS provides a technique for co-registering likely white matter tracts to allow multi-subject voxel-wise statistical analysis. During co-registration TBSS attempts to deform an individual’s natural white matter tract ’skeleton’ (or at least, the voxels most likely to lie at the centre of the natural skeleton) to a common skeleton. This allows tissue injury (as measured by FA) occurring in white matter tracts to be assigned to a ‘tract’ location. The individual tract skeletons are constructed regardless of localized reductions in

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**Fig. 4** (A) Location of the left posterior cingulate ROI (red) drawn on the mean tract skeleton (blue, thresholded at tract voxel FA \(> 0.2\)), with scatterplot demonstrating a relationship between ROI mean FA and PASAT \(z\)-score (red dots—SPMS patients). (B) Location of the left parahippocampal white matter ROI, with corresponding scatterplot showing no relationship with PASAT \(z\)-scores.
**Fig. 5** Skeletal voxels (red) which show significant correlation with BVRT z-scores, with age treated as a covariate of no interest. Cluster-based thresholding corrected for multiple comparisons, \( P < 0.05 \). Background as for Fig. 2.

**Fig. 6** Axial images demonstrating skeletal voxels (red) which significantly correlate with CVLT-II sdf z-scores, treating age as a covariate of no interest. Cluster-based thresholding corrected for multiple comparisons, \( P < 0.05 \). Background as for Fig. 2.
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FA; hence, white matter tracts which are markedly disrupted can still be evaluated as part of the TBSS skeleton.

The use of FA allows the functional impact of both macroscopically abnormal white matter and microscopic NAWM multiple sclerosis lesions involving specific tracts to be investigated. As well as detecting direct multiple sclerosis-related ultrastructural injury, DTI-derived measures showing reduced NAWM integrity may reflect Wallerian degeneration secondary to spatially remote lesions in connected white matter tracts (Lin et al., 2007). The TBSS output for the neuropsychological tests overlaid onto the lesion probability maps demonstrate cognitively relevant tract localizations at sites where lesion probability was low, consistent with a contribution of NAWM abnormality to cognitive dysfunction. The results also support the notion that TBSS is sensitive for functionally relevant white matter abnormality, as opposed to simply detecting those tracts in which lesion burden is greatest. By mapping cognitively relevant tract abnormality in this way, we gain insights into the anatomical substrates underpinning performance in testing of different functional domains.

Mapping of PASAT performance has previously been performed using MTI by Ranjeva et al. (2005) in 18 patients with clinically isolated syndromes. Worse PASAT performance mapped to the splenium of the corpus callosum, the right superior longitudinal fasciculus, left BA40 and right BA4. Our findings thus overlap with those of the MTI study, with correlations observed for the splenium and the left-sided tracts underlying BA40. The latter may be of particular relevance, given the putative location of the phonological loop involving the BA40 and the left inferior frontal gyrus (BA44). The left parietal tract involvement that we have demonstrated could thus interfere with the interaction in the left hemispheric network involved in auditory working memory. In contrast to Ranjeva et al., we found involvement of the left SLF as opposed to the right, and failed to find involvement of tracts running to the right BA4 region. The prominent involvement of the left SLF is particularly interesting given the recent findings of Karlsgodt et al. (2008), who demonstrated correlation between working memory performance and left SLF integrity using TBSS in patients with early schizophrenia and healthy controls.

Several fMRI studies have shown compensatory cortical activity during PASAT in multiple sclerosis patients, and it is interesting to note that the pattern of tract involvement that we have demonstrated suggests the functional relevance of disruption of these compensatory mechanisms. Mainero et al. (2004) have shown increased fMRI-detected cortical activity during the PASAT in multiple sclerosis patients whose PASAT performance was comparable to healthy controls. In their study of 22 multiple sclerosis patients increased activation was identified in the prefrontal and inferior parietal cortices bilaterally, temporal cortices bilaterally (although predominantly right-sided), with further foci in the right SMA and right anterior cingulate. Staffen et al. (2002) studied 21 multiple sclerosis patients during a visual version of the PASAT and found a different pattern of activation between multiple sclerosis patients and controls, despite the fact that PASAT scores were not significantly different between the two groups. Both groups showed activations in the right frontal area. The multiple sclerosis group also showed activation in the left BA39 area. The involvement of tracts running into the inferior parietal regions (including left BA39) demonstrated by our data could thus result in loss of the inferior parietal compensatory activity and hence deterioration in PASAT performance. Likewise, the involvement of the right inferior longitudinal fasciculus could interfere with the predominantly right temporal cortical compensatory activity demonstrated by Mainero et al. (2004).

Given that the PASAT involves a component of sustained attention, the involvement of the left cingulum is notable. The anterior cingulum is known to play an important role in the executive control of attention, while the posterior cingulum has spatial attention and orienting functions (Hirono et al., 1998; Small et al., 2003). Although some left anterior cingulate involvement was observed, the posterior cingulate was more extensively involved. It should be noted, however, that TBSS demonstrates the tract within the cingulum as opposed to the cingulate cortex per se, and hence the involvement of the posterior cingulum may disrupt the temporal and parietal connections of the anterior cingulate via the cingulate tract bundle.

Less data are available regarding the activation patterns and impact of localized tract injury on BVRT and CVLT-II performance. BVRT is predominantly a test of short-term visual memory. The hypothetical ‘visuospatial scratchpad’ is believed to be located in the temporoparietal regions, particularly on the right (Baddeley, 1992), and the predicted involvement of tracts subserving these regions is confirmed by the TBSS study.

The CVLT-II sdf provides a measure of verbal learning and recall. The involvement of the left fornix running posteriorly from the left mesial temporal lobe is thus notable, given the known role of the dominant (usually left) mesial temporal region in verbal learning (Powell et al., 2005). In health, the degree of symmetry of perisylvian language pathways has been shown to relate to verbal recall (Catani et al., 2007). Although extreme left lateralization was found in the majority of subjects, Catani et al. found verbal recall based on semantic association was better in subjects with a more symmetrical pattern. In the current TBSS study bilateral (but more prominently left-sided) perisylvian white matter involvement has been demonstrated for the TBSS analysis of CVLT-II sdf scores. This may be particularly relevant as CVLT-II sdf performance is enhanced if the semantic associations within the word list are identified by the subject.

Performance during the CVLT is known to be affected by frontal lesions, particularly left posterior dorsolateral frontal...
region and the posterior medial frontal region, due to impairment of organizational structure to the new learning (Alexander et al., 2003). We failed to demonstrate direct involvement of the frontal lobe tracts (with the exception of the anterior corpus callosum). However, involvement of the left arcuate fasciculus and superior longitudinal fasciculus could impair temporo-frontal and parieto-frontal connectivity, respectively.

The two tests of frontal lobe function, the DST and COWAT, failed to show any significant TBSS correlations. The reason for this is unclear, but may reflect the more variable localization of function within the frontal lobes. This relative dispersion of function would limit the ability of the TBSS technique to localize relevant tract injury. We also failed to demonstrate the expected parietal localization of tract injury for the JLO, a test of visuospatial perception. The negative findings for both the JLO and the DST are likely to reflect the low spread of data in a small sample resulting in insufficient statistical power to detect relationships.

The distribution of statistically significant tract-based FA reductions in multiple sclerosis patients compared to controls showed only partial overlap with the visible T2 lesions. There was a double dissociation with large areas of high T2 lesion probability but no significant tract FA reduction in the centrum semiovale, and several tracts with reduced FA but <10% lesion probability, such as fornices, corpus callosum and fronto-temporal white matter. A further notable result from the comparison with healthy controls is that many of the tracts showing associations with cognitive performance did not co-localize with between-group statistical tract abnormality. For example, the TBSS output for PASAT showed correlations with the left cingulate white matter tract and the left superior longitudinal fasciculus which were not identified as being significantly different during the comparison of multiple sclerosis patients and controls.

An important consideration which is as yet unresolved is the degree to which normal inter-subject variations in tract FA influence cognitive performance. The assumption underlying the proposed hypothesis is that the TBSS localizations demonstrated relate to pathology, but physiological variation may account in part for the findings. In an attempt to partially control for inherent variability we repeated the TBSS analysis of cognitive performance controlling for premorbid IQ, and found a similar pattern of tract localizations for the cognitive tests. However, domain-specific differences in white matter organization are more difficult to control for. For example, the heterogeneity in laterization of perisylvian language networks recently demonstrated by (Catani et al., 2007) has been shown to relate to verbal memory performance. This inherent structural variability in health has the potential to confound the results of the TBSS study, but could also explain the fact that significant tract localizations were identified at sites where no difference between the patient and control groups were found. Thus the localizations demonstrated cannot necessarily be assumed to be the result of disease-specific alterations to white matter structure.

In summary, the application of TBSS to map cognitively relevant tract disorganization has been demonstrated in a cohort of multiple sclerosis patients, and thus provides insights into the anatomical substrates underlying functional connectivity. Many of the localizations of white matter injury can be shown to be consistent with the known functional anatomy of processing within the specific cognitive domain studied, and in the case of PASAT performance, the localization of injury could result in disconnection of compensatory mechanisms previously shown to occur in multiple sclerosis patients in fMRI studies.

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References


