Voxel-wise analysis of diffusion weighted imaging reveals disruption of the olfactory tract in Parkinson’s disease

Christoph Scherfler,1 Michael F. Schocke,2 Klaus Seppi,1 Regina Esterhammer,2 Christian Brenneis,1 Werner Jaschke,2 Gregor K. Wenning1 and Werner Poewe1

Departments of 1Neurology and 2Radiotherapy, Innsbruck Medical University, Innsbruck, Austria

Correspondence to: Dr Christoph Scherfler, Department of Neurology, Innsbruck Medical University, Anichstrasse 35, A-6020 Innsbruck, Austria
E-mail: christoph.scherfler@uibk.ac.at

Diffusion weighted imaging (DWI) and the trace of diffusion tensor [Trace (D)], a marker of water molecule diffusivity, provide information on structural integrity of nervous tissues. To investigate structural lesions within the brain’s neuronal architecture in early stages of Parkinson’s disease, 12 patients with disease duration of 3.5 ± 1.5 years were studied with DWI. Data were compared with 12 age-matched healthy control subjects. To objectively localize focal changes of structural neuronal integrity without having to make an a priori hypothesis as to its location statistical parametric mapping (SPM) was applied to our DWI study. SPM localized significant increases of diffusivity in the region of both olfactory tracts in patients (P < 0.001). Trace (D) cut-off values for the voxel cluster of the olfactory tracts have been calculated from the subjects entered into SPM and applied to a total of 17 different individuals (9 patients with Parkinson’s disease, disease duration 3.1 ± 1.3 years and 8 age-matched healthy subjects). Out of 17 subjects, 16 subjects, i.e. 94%, were correctly discriminated with a sensitivity of 100% and a specificity of 88%. All patients with Parkinson’s disease were correctly classified and only one normal subject was classified as having the disease, underlining the high potential of this method to separate patients with the illness from healthy subjects. Increased diffusivity in the olfactory tract is in line with the well-established clinical finding of hyposmia in Parkinson’s disease. Whether DWI can be used as a marker to identify individuals at risk to develop this disease remains to be shown.

Keywords: Parkinson’s disease; diffusion weighted imaging; statistical parametric mapping; olfactory tract; trace of diffusion tensor

Abbreviations: ADC = apparent diffusion coefficient; DWI = diffusion weighted imaging; ROI = region of interest; SPM = statistical parametric mapping; Trace (D) = trace of diffusion tensor; UPDRS = Unified Parkinson’s Disease Rating Scale


Introduction

Conventional MRI provides high sensitivity in detecting macroscopic abnormalities occurring in neurological conditions such as cerebral vascular disease, brain tumours and multiple sclerosis. When applied to neurodegenerative diseases such as Parkinson’s disease or Alzheimer’s disease, its diagnostic value is limited to the exclusion of alternative possible diagnoses. Diffusion-weighted imaging (DWI) is a unique form of MR contrast that enables to quantify the motion of water molecules (Le Bihan 1991, 2003; Parker, 2004). Within the CNS, which is primarily organized in bundles of fibre tracts, the water molecules mainly move along these structures, whereas diffusion perpendicular to the fibre tracts is restricted by axonal cell membranes (Hajnal et al., 1991). Given that axonal cell membranes are altered by neurodegenerative processes including neuronal loss, multidirectional mobility of water molecules increases within the tissue architecture, which results in increased diffusivity. Quantification of diffusion is feasible by applying field gradients of different degrees of diffusion sensitization, allowing the calculation of the apparent diffusion coefficient (ADC) in tissue, a measure for diffusivity of water molecules in one direction (Pierpaoli et al., 1996). The trace of diffusion tensor Trace (D) is given by the average of ADCs measured in three orthogonal directions and is by definition independent from the fibre
Diffusion weighted imaging in idiopathic Parkinson’s disease

Methods

Subjects

Patients with Parkinson’s disease (11 women and 10 men) were recruited from referrals to the Movement Disorders Clinic at the Department of Neurology at Innsbruck Medical University. Only patients fulfilling established diagnostic criteria were eligible for the study (Hughes et al., 1992). Motor disability related to parkinsonism was assessed in all patients in off-drug states using part III of the Unified Parkinson’s Disease Rating Scale (UPDRS) and classified according to H&Y (Hoehn and Yahr, 1967; Fahn et al., 1987, Table 1). Signs of dementia, autonomic dysfunction, vertical gaze palsy or cerebellar signs were absent in all participants. In addition to those with severe white matter, vascular or space-occupying lesions within the cerebrum, thirteen patients were receiving regular L-dopa therapy. In addition, 18 were taking dopamine agonists. The 20 age-matched and sex-matched healthy subjects (9 women and 11 men) served as control group (Table 1). Twelve subjects of the control and patient cohort, were randomly assigned for SPM analysis. This allowed for explanatory voxel-by-voxel group comparison without requiring an a priori hypothesis. The remaining nine patients and eight control subjects served as test set to evaluate the sensitivity and specificity of the method described below. The study was approved by the Ethics Committee of the Innsbruck Medical University. Subjects’ written informed consent was obtained according to the Declaration of Helsinki.

Scanning protocol

DWI and the T₁ weighted MRI were obtained on a 1.5 T whole-body MR scanner (Magnetom Vision, Siemens, Germany) and a circular polarized head coil, using a protocol identical to that described in a previous study by our group (Schocke et al., 2004). In brief, the dual-echo spin-echo sequence had a repetition time of 3500 ms, echo times of 22 and 90 ms, a slice thickness of 2 mm, a matrix of 256 × 256 pixels, and a field of view of 200 mm. This sequence was performed twice, providing 2 × 15 slices that were interleaved without any gap. DWI scans were acquired using a spin-echo type of echo-planar imaging sequence with diffusion-sensitizing gradients switched in slice direction and three different b-values (30, 300 and 1100 s/mm²). Sequential sampling of k-space was used with an effective echo time of 123 ms, a bandwidth of 1250 Hz/pixel, and an acquisition matrix of 128 × 128, which was interpolated to 256 × 256 during image calculation. The DWI sequence provided 20 consecutive slices with a slice thickness of 3 mm and a field of view of 230 mm. The acquisition time of each DWI sequence was 5 s. Trace (D) maps were routinely calculated by the commercially available software (Siemens, Erlangen, Germany) by fitting the logarithm of the signal intensity as a function of the gradient factor b over three different b values for each pixel and averaging the ADC maps measured in three orthogonal directions. In order to avoid CSF contamination and partial volume effects derived from small cistic lesions, we excluded all Trace (D) voxel values that were above a threshold of mean CSF Trace (D) – 2 SD, determined for each individual subject (Schocke et al., 2004). Results from an ROI analysis of DWI MRI scans from 13 patients and 8 control subjects included also in the present study have been already published by Schocke et al. (2004). Only pairs of DWI and T₁ weighted MRI scans performed consecutively were subjected to further analysis in the present study.

Data analysis

SPM was performed using the software package SPM2 (Wellcome Department of Cognitive Neurology, London, UK; Friston et al., 1995) implemented in Matlab 7.01 (Mathworks Inc., Sherborn, MA, USA). To achieve accurate spatial normalization T₁ weighted images were normalized onto the T₁ template in MNI (Montreal Neurological Institute). Subsequently, the resulting transformation parameters were applied to the patient’s corresponding Trace (D) image. A Gaussian kernel (4 × 4 × 4 mm) was then convolved with the

<table>
<thead>
<tr>
<th>Table 1 Demographic and clinical characteristics of patients with Parkinson’s disease and control subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
</tr>
<tr>
<td>------------------</td>
</tr>
<tr>
<td>Parkinson’s disease (n = 12) mean ± SD</td>
</tr>
<tr>
<td>Controls (n = 12) mean ± SD</td>
</tr>
<tr>
<td>Test set</td>
</tr>
<tr>
<td>Parkinson’s disease (n = 9) mean ± SD</td>
</tr>
<tr>
<td>Controls (n = 8) mean ± SD</td>
</tr>
</tbody>
</table>
spatially normalized parametric images to smooth them in order to accommodate inter-individual anatomic variability and to improve signal-to-noise for the statistical analysis. The obtained datasets allowed for categorical comparisons of mean Trace (D) values in analogous voxel regions of the brain volume between healthy volunteers and patients. Data clusters revealed by SPM showing significant differences of Trace (D) values between groups were transformed onto the individual Trace (D) images to obtain mean regional Trace (D) values. Student’s t-tests were applied for clinical data and SPM analysis. A linear regression analysis was performed in order to investigate whether regional Trace (D) values (independent variable) can be predicted by patients’ age, disease duration and UPDRS motor scores (dependent variables). Cut-off values of voxel clusters showing significant alterations between groups were determined as follows: (i) the mean and its confidence intervals (CIs) for each group were determined and (ii) the cut-off value was determined by averaging over the lower bound of the CI of one group and the upper bound of the other group. Sensitivity and specificity values were calculated by applying those cut-off values in order to discriminate the remaining individuals not subjected to the SPM analysis. The positive predictive values [PPV, i.e. likelihood of a person with the abnormal Trace (D) values having Parkinson’s disease] and the negative predictive values [NPV, i.e. likelihood of a person with the abnormal Trace (D) values not having Parkinson’s disease] were calculated. Data analysis and statistics were carried out on a Windows XP workstation (Pentium 4, Sony PCV-RS404) using a commercial software package: SPSS for Windows 12.0 (SPSS, Surrey, UK).

Results
Patient groups did not differ in age, UPDRS part III and H&Y stages at the time of MRI examination. SPM analysis localized significant increases of Trace (D) values at the base of the frontal lobes corresponding to the anatomical location of the olfactory tracts of patients when compared with control subjects ($P < 0.001$, corrected for multiple comparisons; Fig. 1 and Table 2). Mean Trace (D) values of the olfactory tracts in the Parkinson’s disease patient and control group were $1.03 \pm 0.17 \times 10^{-3} \text{ mm}^3/\text{s}$ and $0.73 \pm 0.11 \times 10^{-3} \text{ mm}^3/\text{s}$, respectively. No significant decreases in Trace (D) values were detected in the patient group versus the control group. Linear regression analysis revealed no significant correlations ($r^2 = 0.17$) between Trace (D) values of the olfactory tract and patients’ age ($r = 0.37$), disease duration ($r = 0.13$) and UPDRS motor scores ($r = 0.1$). The cut-off value of the voxel clusters of the olfactory tracts detected by the SPM analysis was $0.78 \times 10^{-3} \text{ mm}^3/\text{s}$, implying that a Trace (D) value of $>0.78 \times 10^{-3} \text{ mm}^3/\text{s}$ is indicative of a diagnosis of Parkinson’s disease and a Trace (D) value of $<0.78 \times 10^{-3} \text{ mm}^3/\text{s}$ is indicative for a healthy control subject. Overall, 16 out of 17 independently analysed subjects, i.e. 94.1% were correctly classified. None of the patients was wrongly classified into the control group. Out of eight healthy subjects, seven were correctly classified given a sensitivity of 1 and a specificity of 0.88 for the cut-off level of $0.78 \times 10^{-3} \text{ mm}^3/\text{s}$. The PPV was 0.9 and the NPV was 1 (Table 3).

Discussion
We have applied SPM to spatially normalized parametric Trace (D) images and localized highly significant signal

![Image](https://example.com/image.png)

**Table 2** Between-group SPM findings showing the locations of significant increases of Trace (D) values in Parkinson’s disease patients versus healthy control subjects

<table>
<thead>
<tr>
<th>Trace (D) Brodmann area</th>
<th>Cluster size (mm$^3$)</th>
<th>Talairach co-ordinates</th>
<th>T-value</th>
<th>P-values (corr.)</th>
<th>Height threshold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right olfactory tract</td>
<td>1264</td>
<td>10 36 -27</td>
<td>6.26</td>
<td>0.001</td>
<td>0.01</td>
</tr>
<tr>
<td>Left olfactory tract</td>
<td>1528</td>
<td>-7 27 -27</td>
<td>5.07</td>
<td>0.001</td>
<td></td>
</tr>
</tbody>
</table>

![Image](https://example.com/image.png)
Diffusion weighted imaging in idiopathic Parkinson’s disease

Table 3 Diagnostic classification matrix, based on DWI using a Trace (D) cut-off level of $0.78 \times 10^{-3}$ mm$^2$/s determined for both olfactory tracts

<table>
<thead>
<tr>
<th>Clinical classification (n)</th>
<th>Predicted group by regional Trace (D) measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls</td>
</tr>
<tr>
<td>Controls (n = 8)</td>
<td>7 (87.5%)</td>
</tr>
<tr>
<td>Parkinson’s disease (n = 9)</td>
<td>0</td>
</tr>
</tbody>
</table>

The classification of subjects’ Trace (D) of both olfactory tracts with respect to their clinical diagnosis has been calculated. Rows represent the clinical diagnosis and columns the diagnosis predicted by Trace (D) values. Bold style indicates correct diagnosis.

Changes in both olfactory tracts of a cohort of Parkinson’s disease patients, which could not have been predicted by visual inspection or ROI analysis of DWI images (Fig. 1). Indeed, by applying an ROI approach focusing on the basal ganglia including the putamen, caudate nucleus, thalamus, the substantia nigra, the pons, as well as the parietal grey and periventricular white matter our group has previously failed to detect regional signal changes in patients when compared with a group of healthy volunteers (Schocke et al., 2004). While ROI-based techniques depend on a priori assumptions of the size and shape of the region to be evaluated, no a priori hypothesis regarding the localization of the DWI signal is required by SPM, provided that spatial normalization of the individual Trace (D) image is accurate. To achieve precise image normalization individual T$_1$-weighted images featuring higher spatial resolution compared with Trace (D) images were rendered onto the T$_1$ template in MNI space and the resulting transformation parameters were convolved with the subject’s corresponding Trace (D) image.

Our findings of higher diffusivity at the base of the frontal lobes of a cohort of patients identified by SPM comprises a volume of ~1300 mm$^3$ paramedian on each side and is best explained by disruption of fibres of the olfactory tracts, which is in line with recent neuropathological observations showing extensive extranigral changes in Parkinson’s disease, including the olfactory bulb and related portions of the anterior olfactory nucleus (Pearce et al., 1995; Braak et al., 2003). Trace imaging has been employed in order to reveal neurodegenerative changes in the white matter of a cohort of Parkinson’s disease patients. The application of diffusion tensor imaging, another DWI approach focusing on fibre tracking, might provide additional information on the contribution of white matter disconnectivity to the imaging pattern detected.

Although the pathological hallmark of clinically established Parkinson’s disease is a degeneration of the nigro-striatal projection, neither by SPM nor by previously reported ROI analysis increases in diffusivity have been detected in this brain area. We suspect that, in contrast to the bundle-like configuration of the olfactory tract, neuronal loss and disruption of fibre tracts within the heterogeneously composed architecture of the substantia nigra pars compacta, and the fanlike shape of the nigro-striatal pathway are unlikely to be depicted by the reported resolution matrix necessary for DWI (Damier et al., 1999). Since this is the first DWI study to characterize abnormalities of diffusivity in patients by applying an investigator-independent statistical approach no a priori assumptions of signal alterations in distinct brain areas were made. Although the patients in the present study were not formally tested to determine if they had olfactory deficits, it has been well documented that patients exhibit marked olfactory disturbances that include impairments of odour detection, differentiation and identification and are present in 75–90% of patients with Parkinson’s disease (Ansari and Johnson 1975; Ward et al., 1983; Doty et al., 1988). Profound deficits in olfactory function have been also demonstrated at the earliest clinical stages of Parkinson’s disease and were shown to be correlated with the decline in striatal dopamine transporter availability but not with clinical criteria such as patients’ age, disease duration and UPDRS motor scores (Doty et al., 1992; Lehrner et al., 1995; Tissingh et al., 2001; Siderowf et al., 2005). In accord with this finding no correlation between motor criteria and regional Trace (D) values of the olfactory tract in our cohort of mild to moderate patients was evident indicating that the time course of developing motor impairments and the time course of neuropathological processes within the anterior olfactory system is divergent (Braak et al., 2003; Siderowf et al., 2005).

The domain of structural MRI has so far been the differentiation of Parkinson’s disease from symptomatic parkinsonism related to normal pressure hydrocephalus or vascular aetiology and from degenerative diseases as atypical parkinsonian disorders (Schulz et al., 1999; Brenneis et al., 2003; Eckert et al., 2004; Schocke et al., 2004). Following the identification of an imaging pattern differentiating a cohort of patients from age-matched healthy subjects DWI was challenged to separate patients and healthy subjects. Trace (D) cut-off values for the voxel cluster of the olfactory tracts identified by SPM have been calculated and applied to a total of 17 subjects, 9 patients and 8 healthy control subjects. Out of 17 independently analysed subjects, 16 subjects, i.e. 94.1%, were correctly discriminated with a sensitivity of 100% and a specificity of 88%. All patients were correctly classified, and only one normal subject was misclassified as having the disease. Increased diffusivity in the region of the olfactory tracts in clinically normal individuals is of particular interest. Braak et al. (2003) have proposed α-synuclein pathology in the olfactory bulbs and tracts as one of the interest sites of pathological changes in their staging system of Parkinson’s disease, occurring well before nigral pathology or clinical signs have developed. In keeping with this proposal, Ponsen et al. (2004) have found an increased risk for the development of clinically overt Parkinson’s disease in asymptomatic first-degree relatives of patients who had marked olfactory dysfunction as compared with those without. In accord with clinical and neuropathological observations we propose to...
study the potential of DWI as another screening test to identify at risk subjects developing the illness.

Disruption of olfactory tracts in a cohort of patients with mild to moderate Parkinson’s disease has been identified by DWI. Whether diffusivity in the olfactory tract is altered in pre-clinical Parkinson’s disease and correlated with conventional clinical measures of disease severity remains to be shown. Based on current pathological concepts regarding the involvement of the olfactory pathway in the earliest stages of the disease prior to nigral pathology (stage 1 according to Braak et al., 2003), this might well be so and DWI would add an observer-independent and quantitative measure to identify at risk subjects.

Acknowledgement
The study was funded by the Austrian Federal Ministry of Science and Transport (GZ 70038/2 PR 4/98).

References