White matter connections reflect changes in voluntary-guided saccades in pre-symptomatic Huntington’s disease

Stefan Klöppel,1,2 Bogdan Draganski,1 Charlotte V. Golding,3 Carlton Chu,1 Zoltan Nagy,1 Philip A. Cook,4 Stephen L. Hicks,3 Christopher Kennard,3 Daniel C. Alexander,5 Geoff J. M. Parker,6 Sarah J. Tabrizi7 and Richard S. J. Frackowiak1,8,9

1Wellcome Trust Centre for Neuroimaging, Institute of Neurology, UCL, London, UK, 2Department of Neurology, Neurozentrum, University Clinic Freiburg, Freiburg, Germany, 3Division of Neuroscience and Mental Health, Faculty of Medicine, Imperial College London, UK, 4Department of Radiology, University of Pennsylvania, Philadelphia, USA, 5Department of Computer Science, UCL, London, 6Imaging Science and Biomedical Engineering, University of Manchester Manchester, 7Department of Clinical Neurology, Institute of Neurology, UCL, London, UK, 8Département d’études cognitives, École Normale Supérieure, Paris, France and 9Laboratory of Neuroimaging, IRCCS Santa Lucia, Roma, Italy

Correspondence to: Stefan Klöppel MD; Department of Neurology; Breisacher Str. 64; 79106 Freiburg; Germany
E-mail: stefan.kloeppe1@uniklinik-freiburg.de

Huntington’s disease is caused by a known genetic mutation and so potentially can be diagnosed many years before the onset of symptoms. Neuropathological changes have been found in both striatum and frontal cortex in the pre-symptomatic stage. Disruption of cortico-striatal white matter fibre tracts is therefore likely to contribute to the first clinical signs of the disease. We analysed diffusion tensor MR image (DTI) data from 25 pre-symptomatic gene carriers (PSCs) and 20 matched controls using a multivariate support vector machine to identify patterns of changes in fractional anisotropy (FA). In addition, we performed probabilistic fibre tracking to detect changes in ‘streamlines’ connecting frontal cortex to striatum. We found a pattern of structural brain changes that includes putamen bilaterally as well as anterior parts of the corpus callosum. This pattern was sufficiently specific to enable us to correctly classify 82% of scans as coming from a PSC or control subject. Fibre tracking revealed a reduction of frontal cortico-fugal streamlines reaching the body of the caudate in PSCs compared to controls. In the left hemispheres of PSCs we found a negative correlation between years to estimated disease onset and streamlines from frontal cortex to body of caudate. A large proportion of the fibres to the caudate body originate from the frontal eye fields, which play an important role in the control of voluntary saccades. This type of saccade is specifically impaired in PSCs and is an early clinical sign of motor abnormalities. A correlation analysis in 14 PSCs revealed that subjects with greater impairment of voluntary-guided saccades had fewer fibre tracking streamlines connecting the frontal cortex and caudate body. Our findings suggest a specific patho-physiological basis for these symptoms by indicating selective vulnerability of the associated white matter tracts.

Keywords: presymptomatic Huntington’s disease; gene carriers; white matter; saccades

Abbreviations: AAL = automated anatomical labelling; BAs = Brodmann areas; DTI = diffusion tensor MR image; FA = fractional anisotropy; HD = Huntington’s disease; PSCs = pre-symptomatic gene carriers; SVM = support vector machine; VBM = voxel-based morphometry


Introduction

Huntington’s disease (HD) is an inherited neurodegenerative disorder resulting from an expanded CAG trinucleotide repeat in the huntingtin gene (HD Collaborative Research Group, 1993). Clinically, the onset of symptoms is usually defined by unequivocal chorea, which gene mutation carriers develop between the ages of 40 and 50. Years before the onset of typical symptoms, so-called
Progressive degeneration of the striatum (Aylward et al., 1996; Kirkwood et al., 2000; Reading et al., 2004; Paulsen et al., 2006). Progressive degeneration of the striatum (Aylward et al., 2000; Gutekunst et al., 2002; Aylward et al., 2004) and cortical changes, especially in the frontal cortex (Rosas et al., 2002; Rosas et al., 2005) have been identified as a likely cause. PSCs constitute an important model for the study of genetically caused neurodegenerative diseases as the mutation provides a way of unequivocally defining the pre-symptomatic and early stages when disease-modifying treatments are most likely to be beneficial (Paulsen et al., 2006).

Several neuropathological studies indicate a region-specific degeneration of pyramidal neurons projecting to the basal ganglia from Brodmann areas 8, 9 and 10 (de la Monte et al., 1988; Hedreen et al., 1991; Mann et al., 1993; Heinsen et al., 1994; Macdonald and Halliday, 2002; Selemont et al., 2004) with pyramidal neurons in adjacent area 46 showing far less degeneration (Selemont et al., 2004). While these studies usually focused on later stages of disease, neuroimaging studies have described cortical thinning in PSCs (Rosas et al., 2005). Because both cortex and striatum are affected there is likely to be disruption of fibres connecting these structures (Alexander et al., 1986), which may play a role in symptom development.

The hypothesis of impaired cortico-striatal connections has been explored by neuropathological examination (Fennema-Notestine et al., 2004) and magnetic resonance imaging (MRI) of the cerebral white matter. Since neuropathological studies indicate a relatively specific degeneration of cortical pyramidal cells, the study of their projections in the white matter is of primary relevance to the detection of early structural changes in PSC.

Although analyses of white matter on T1-weighted images have been reported (Thieben et al., 2002; Beglinger et al., 2005) the majority have used diffusion weighted and diffusion tensor imaging (DTI) (Mascalchi et al., 2004; Reading et al., 2005; Rosas et al., 2006; Seppi et al., 2006). Previous studies (Reading et al., 2005; Rosas et al., 2006) made voxel-by-voxel comparisons of fractional anisotropy (FA) values; we on the other hand were interested in whether there was a characteristic distributed pattern of white matter changes that differentiated PSCs from controls.

To this end, we used a support vector machine (SVM), a multivariate method used for the identification of consistent pattern differences, to identify and characterize regionally disrupted FA in PSCs. This method can identify patterns of abnormality that are not obvious in simple univariate analyses such as the categorical contrasts utilized in voxel-based morphometry (VBM—Ashburner and Friston, 2000). Similar multivariate methods have previously been applied to FA maps of patients with Alzheimer’s disease (Teipel et al., 2007).

In addition to their use for computing FA-maps, DTI data can be used to identify probable fibre orientations at each voxel. Deterministic tractography algorithms follow these fibre orientation estimates from voxel to voxel to generate streamlines representing anatomical connections between specified seed and target points in the brain (Mori et al., 1999). More recent ‘probabilistic’ tractography techniques (Parker et al., 2002; Behrens et al., 2003; Parker and Alexander, 2003; Parker and Alexander, 2005) aim to quantify the connectivity and the relative strengths of different routes between regions. Such methods have been extensively used and validated in both healthy and diseased individuals (e.g. Parker and Alexander, 2003; Parker and Alexander, 2005; Parker et al., 2005; Newton et al., 2006). We hypothesized that a regionally specific pattern of cortical degeneration involving pyramidal neurons would result in specific patterns of change in cortico-fugal connections. The detection of such changes in vivo could provide a sensitive index of progressive neurodegeneration in HD.

Material and methods

Twenty-five PSCs of the HD-gene mutation and 20 age- and gender-matched controls were studied. A neurologist experienced in HD assessed subjects with the Unified Huntington’s Disease Rating Scale (UHDRS) to confirm pre-symptomatic status prior to inclusion. Our PSCs all scored zero (normal neurological examination with no symptoms) or one (non-specific abnormalities on examination with no typical symptoms). Based on an algorithm suggested by Langbehn et al. (2004) and a threshold used by Feigin et al. (2006) the estimated onset time to development of symptoms in our group (at a 60% certainty level) ranged from 6 to 35 years (see Table 1 for full demographic and clinical details).

Image acquisition

Subjects were scanned on a 1.5T Sonata Scanner (Siemens, Erlangen, Germany). Diffusion weighted imaging was performed with an echo planar sequence with a double spin-echo module to reduce the effect of eddy currents (Reese et al., 2003). Each data volume consisted of 40 axial slices of 2.3 mm thickness, with no inter-slice gaps, and an acquisition matrix of 96 × 96 in thickness of view of 220 × 220 mm², resulting in 2.3 mm³ isotropic voxels (inter-slice temporal separation = 155 ms, TE=90 ms, flip angle 90°, fat saturation, bandwidth 203 Hz/Pixel). Interleaved slice

Table I Demographic details of subjects

<table>
<thead>
<tr>
<th></th>
<th>PSC (n = 25)</th>
<th>Control (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean; range)</td>
<td>39.6 26–54</td>
<td>35.2 26–58</td>
</tr>
<tr>
<td>Gender: f:m</td>
<td>13:12</td>
<td>9:11</td>
</tr>
<tr>
<td>Years to onset (mean; range)</td>
<td>19.0 6–35</td>
<td>NA</td>
</tr>
<tr>
<td>CAG-length (mean; range)</td>
<td>41.67 38–47</td>
<td>NA</td>
</tr>
<tr>
<td>UHDRS motor (mean; range)</td>
<td>0–8 2.60</td>
<td>NA</td>
</tr>
</tbody>
</table>
sampling was chosen to avoid cross talk between adjacent slices. Each DTI data set consisted of 61 high-diffusion weighted images \( (b=1000 \text{s/mm}^2) \), with diffusion gradients applied along 61 evenly distributed diffusion directions, obtained from the optimization procedure in (Jansons and Alexander, 2003), and seven additional images with minimal diffusion-weighting \( (b=100 \text{s/mm}^2) \) also with evenly distributed directions. We fit the diffusion tensor using the standard linear least squares fit to the log measurements (Basser et al., 1994). The fitting also provides an effective \( b=0 \) image. Data acquisition was cardiac gated to reduce motion artefacts due to pulsation of the cerebrospinal fluid (Wheeler-Kingshott et al., 2002). Diffusion data acquisition time was 22 min on average, depending on heart rate.

To facilitate spatial normalization and to perform a complementary study of grey matter changes (see Supplementary material) we also acquired 3D structural MR images from each subject using a T1-weighted MDEFT sequence (176 slices, 1 mm thickness, sagittal, phase encoding in anterior/posterior, FoV 224 × 256 mm\(^2\), matrix 224 × 256, TR = 20.66 ms, TE = 8.42 ms, TI = 640 ms, flip angle 25°, fat sat, bandwidth 178 Hz/Pixel) (Deichmann et al., 2004).

**Preparation of FA-maps**

DTI measures the passive self-diffusion characteristics of water. Water diffusion is less hindered parallel to the white matter fibres than perpendicular to them (Basser et al., 1994) and water moves further on average in the parallel direction. This allows the technique to provide information about white matter fibre orientation. The most common quantitative measure of white matter integrity is FA, a dimensionless scalar. It ranges from zero (in water under noise-free conditions), where the diffusion is isotropic, to one when diffusion is in a single direction only. Before tensor estimation, images were smoothed using a 2 mm FWHM kernel to improve signal to noise ratio. The three eigenvalues of the diffusion tensor were used to calculate the FA for each subject on a voxel-by-voxel basis (Pierpaoli and Basser, 1996; Pierpaoli et al., 1996) using the ‘Diffusion Toolbox II’ available on the SPM homepage (http://www.fil.ion.ucl.ac.uk/spm). The T1 images were segmented and normalized to standard space using a unified segmentation as implemented in SPM5 (Ashburner and Friston, 2005) running under MATLAB7 (Mathworks, Sherborn, MA, USA). After co-registering subject specific FA-maps and T1-images in their native space, the parameters estimated from normalization of T1 images to a standard anatomical template were applied to the appropriate subject’s FA-maps.

**Multivariate FA-analysis**

Normalized FA-maps were classified using an in-house implementation of a SVM running under MATLAB7. SVM-classification employs a two-step procedure. In a first training phase, the algorithm learns about the differences in the FA-maps from PSC and controls. Generalization of the trained set is assessed with a leave-one-out validation procedure. The trained SVM is then used to assign new FA-maps to the appropriate class on the basis of image characteristics alone. A more complete account of this method is provided as Supplementary material that references the relevant literature.

In this study, we were chiefly interested in the image voxel pattern that characterizes the difference between our groups of interest (PSCs and normals). This information was obtained from the SVM during training (see Supplementary material). We report the percentage of times FA-images were assigned to their correct diagnostic category along with sensitivity and specificity values (considering a correctly categorized PSC as a true positive). We also report the \( P \)-value obtained from simulations that gives the probability threshold at which classification is not significantly different from random.

**Probabilistic tracking**

DTI data from all 45 subjects were used to map cortico-striatal connections with probabilistic tractography. Tractography was performed in subject-specific native space while both seed and target areas were defined in standard anatomical space to allow bias-free placement, unaffected by experimenter judgements about anatomical correspondences. We first describe the creation of seed and target maps in standard (MNI) space followed by a description of the tracking algorithm, the generation of spatial transforms and finally, the statistical analysis.

**Cortical seed areas**

As previously suggested (Thottakara et al., 2006) we used Brodmann areas (BAs) (Brodmann, 1909) in the frontal lobe (BAs 4, 6, 8, 9, 10, 11, 44, 45 and 46) to define seed points. The BAs were defined with a template in standard space, available with MRlcro software (MRlcro by C. Rorden; www.mricro.com; Rorden and Brett, 2000), that subdivides the frontal lobes into 18 BA-based regions (nine in each hemisphere) (Fig. 1 top row). Each image containing a BA was transformed from standard into subject-specific space (see below). Only voxels with FA values >0.2 were used as seeds to make sure they were close to the grey:white matter border. Depending on brain size this procedure resulted in around 6000 seed points per subject.
Striatal target areas

Anatomical boundaries of the caudate and putamen were defined with the automated anatomical labelling (AAL) template in MRcro (Tzourio-Mazoyer et al., 2002). A further subdivision was made with a vertical plane through the anterior commissure, motivated by differential connectivity of its anterior and posterior parts (Lehericy et al., 2004a; Lehericy et al., 2004b). The result was four target areas within the basal ganglia of each hemisphere (anterior/posterior caudate and putamen) (Fig. 1, bottom row). The figure illustrates that the posterior caudate does not include the tail of the caudate. We therefore refer to this target area as the ‘caudate body’.

Fibre tracking

The Probabilistic Index of Connectivity (PICo) algorithm (Parker and Alexander, 2003; Parker and Alexander, 2005; Parker et al., 2005) was used to trace the white matter tracts for each subject. This method involves repeated iterations of a streamline process using Monte Carlo methods to exploit inherent uncertainty in the orientation of the principal diffusion direction(s) defined for each image voxel. It is implemented in the free Camino software package (http://www.cs.ucl.ac.uk/research/medic/camino; Cook et al., 2006). The procedure provides maps of the probability of a connection from a seed point to a chosen target area (Parker and Alexander, 2003; Parker and Alexander, 2005; Parker et al., 2005). Here we follow a previously outlined method (Parker and Alexander, 2003; Parker and Alexander, 2005; Parker et al., 2005), which was also used by Newton et al. (2006), but with minor refinements described in Cook et al. (2004). First, we used the algorithm of Alexander et al. (2002) to identify fibre crossings. Where crossings were identified these were parameterized using a mixture of two tensors; in all other parenchymal voxels a single tensor model was applied. Before tracking, probability density functions (PDFs) are generated from the diffusion tensor(s) for each voxel, providing voxel-wise estimates of confidence in fibre tract orientation. With each iteration, a streamline is propagated through the multi-tensor field sampled from PDFs for that iteration. Stopping criteria prevent biologically implausible curvature of streamlines (>180° on the scale of a single voxel) or attempts to transit non-white matter voxels (FA-value <0.1). One thousand iterations were used to identify each connection route. Each seed point, its coordinate and the probability of reaching a given target area were used for further analysis.

Spatial transforms between native DWI space and standard MN1 space

Spatial transformations are required to convert the seed and target mask from standard space to subject-specific native space. As described above they were estimated after first co-registering a T1 structural brain scan from each subject with the subject’s FA-map in native space. This co-registered structural image was used as a template to which the seed and target mask were transformed using a non-linear normalization procedure implemented in SPM5.

Maps of target-specific connection probability were created (one from each sub-region used for targeting, combining homologous areas from both hemispheres) that contain information about the probability with which each seed point connects to the four defined sub-regions of the basal ganglia. This was done subject by subject. We then used the inverse spatial normalization parameters to transform each subject’s target-specific images into standard space. The average localization across all subjects allowed us to identify cortical areas that contribute most to streamlines projecting to each part of the basal ganglia. We were also able to spatially localize differential connectivity between PSCs and controls. No statistical analysis was attempted on these images as the analysis of probabilities in native space was considered a more rigorous and reliable measure.

Statistical analysis of probabilities

We hypothesized that degeneration of specific frontal-basal ganglia fibres would be reflected by a reduction in the percentage of streamlines out of all frontal cortico-fugal streamlines to specific targets. We tried to avoid reporting an apparent reduction in streamlines in patients due to potentially increased head movement during scanning. Since increased head movement would affect all fibres and connections, we decided to compare the percentages of total frontal cortico-fugal streamlines to each of the four striatal subregions thus minimizing this potential problem.

All statistical analyses were based on measures obtained in native space, eschewing normalization back into standard space. In our primary analysis, we tested for a reduction in the percentage of streamlines in PSC compared to controls. This was done separately for all of the four subregions of the basal ganglia using one-tailed t-tests. A Bonferroni correction was applied to adjust the critical P-value. For completeness, we also tested for any increases in the PSC group compared to controls. To exclude possible confounds resulting from differences of size between the basal ganglia target-regions or between cortical seed regions in the two groups, we performed two additional ANCOVAs. The first three-way ANCOVA tested for an interaction between percentage of total seed points within a BA (reflecting its size) and group (GROUP: two levels—PSC and controls; BAs: nine levels—BA 4, 6, 8, 9, 10, 11, 44, 45, 46; and HEMISPHERE: two levels—right and left; with AGE as a covariate). A similar ANCOVA tested for an interaction between the size of target region and hemisphere with gene status (GROUP: two levels—PSC and controls; TARGET REGIONS: four levels—anterior and posterior caudate and putamen; and HEMISPHERE: two levels—right and left; with AGE as a covariate).

Areas with a reduced percentage of streamlines in PSCs identified in our primary analysis were subjected to two post hoc analyses. We hypothesized that in PSCs the percentage of streamlines reaching basal areas would fall the shorter the estimated years to onset. We therefore tested for a negative correlation between the percentage of total frontal cortico-fugal connections and the striatal area found in the initial analysis with age. To exclude the possibility that any observed effects were directly related to age and not to progressing neurodegeneration we also tested for an interaction between the age correlation and gene status (i.e. absence or presence of the huntingtin mutation) as previously suggested (Muhlau et al., 2007). This was done in a regression model with the percentage of fibres to the left caudate body (detected in our primary analysis) as the dependent factor. Gene status and age were entered as independent variables.

Recording of eye movements

The results of our fibre tracking analysis indicated a reduction of fibres connecting regions that are involved in the controls of...
voluntary saccades. We therefore attempted a correlation analysis of subject-specific impairment of this type of saccade with the extent of streamline reduction. A subgroup of 14 PSCs (mean age 39.8; range 28–53; 8 female) were included. Data on 12 PSCs have been presented earlier (Golding et al., 2006). As outlined previously (Golding et al. 2006) subjects were tested using a video-based pupil tracker (Eyelink II, SR Research, Canada) with a sampling rate of 250 Hz and a spatial resolution of 0.5°. Voluntary-guided saccades were tested with two visual targets (each subtending 5°) arranged on a computer monitor along the horizontal meridian at +15, 0 and –15. After fixating the centre for 500 ms, an arrow appeared, pointing to either a left or right target. Participants were instructed to move their eyes immediately in the direction of the cue and hold their gaze at the peripheral target. Saccadic latencies were quantified off-line using custom-made software running under MATLAB. The interval between acquisition of eye movement data and MRI scanning was up to 2 years.

We expected a correlation between greater impairment of voluntary-guided saccades and a lower percentage of all frontal cortico-fugal fibres reaching the caudate body (from both hemispheres). In accordance with our previous study (Golding et al. 2006), we used variability of saccadic latency as the index of impairment; an increase in variability indicating a greater deviation from the normal population.

To ensure that we are not looking at a non-specific change of eye movements we also tested for a correlation similar to the one above but with variability of reflexive saccades as the dependent measure. Disrupted connections from the frontal cortex to the caudate body should not affect this type of eye movement.

Results
Support vector classification
Figure 2 displays the voxels that were most influential in making a binary separation between PSCs and controls. Voxels in which higher FA values favoured PSC group membership were found bilaterally in the putamen and pallidum. Differentiating voxels formed a ring around each structure possibly reflecting a change of their borders. A third differentiating region lay in the occipital pole.

Voxels in which lower FA values were indicative of PSC group membership were found in the corpus callosum, predominantly in more anterior regions. Other relevant voxels were scattered across large parts of the brain without regional specificity. Correct assignment into the PSC group was made in 82.2% of subjects (sensitivity 88%, specificity 75%). The probability of such results occurring by chance is low ($P<0.001$). Incorrectly assigned scans came from three younger PSCs classified as controls (mean age correctly classified PSCs: 41.1 years; misclassified PSCs: 28.7 years). There were no significant age differences between correctly classified and misclassified controls ($P>0.5$).

Fibre tracking
As shown in Fig. 3 streamlines reaching the anterior part of the caudate originated in orbito-frontal cortex (upper left panel) whereas streamlines to anterior putamen came from more lateral areas (lower left panel). Similarly, posterior parts of the striatum were reached from more dorsal parts of the frontal cortex (right panels).

No interaction between the size of BA and group (i.e. PSC versus controls) nor between the size of target areas with group were found ($P>0.2$).

$T$-tests revealed a significantly lower percentage of streamlines reaching the caudate body in PSCs ($t=2.94$; $P=0.0025$; Fig. 4). This difference remained significant when PSCs were split at the median into groups close (<14.7 years) and far from estimated onset of typical HD-symptoms ($P=0.015$ and $P=0.023$, respectively). The same remained true when testing for a reduction in the percentage of streamlines to the right and left caudate body separately ($P=0.023$ and $P=0.0045$, respectively).
Streamlines reaching other sub-regions of the striatum were not significantly changed \((P>0.2)\).

In post hoc analyses, we found a significant negative correlation between the percentage of streamlines reaching the left posterior caudate and age in PSCs \((P=0.021; R^2=0.17)\) but not in controls or in the right hemisphere in either of the two groups. The regression model with the percentage of fibres to the left caudate body from both groups as the dependent variable revealed a significant factor, group \((t=2.39; P=0.021)\), thus formally confirming a differential correlation in PSCs and controls (i.e. absence of a correlation in controls and a negative correlation in PSCs).

**Correlational analysis**

We found a significant negative correlation \((r=-0.50; P=0.034; \text{Fig. 5})\) between the variance of voluntary-guided saccade latencies and the percentage of fibres reaching the caudate body. No significant correlation with the variability of reflexive saccade latencies was observed \((P>0.2)\).

**Discussion**

Fibre tracking shows that there is a smaller percentage of total frontal cortico-fugal streamlines reaching the caudate body in the PSC group than in non-carriers. This is most prominent in the left hemisphere where we found a negative correlation with age in PSCs and a significant interaction between gene-status and age. A negative linear correlation between subject-specific impairment of voluntary-guided saccades and percentage of streamlines to the caudate body indicates the role of the affected fibres in the control of this type of eye-movement. In the FA analysis, we detected patterns of voxel change that separated PSCs from controls in putamen and the anterior part of the corpus callosum.

We will first discuss some methodological issues and then the significance of our findings for an understanding of the pathophysiology of pre-symptomatic stages of HD.

**Methodological considerations**

The localization of seed point-projections to different sub-regions of the striatum is consistent with previous studies (Alexander et al., 1986; Lehericy et al., 2004a, Lehericy et al., 2004b). Note that the colour coding in Figs. 3 and 4 reflects relative probabilities of reaching an indicated target area and not their statistical significance. All statistical inferences were based on results from analyses in subject-specific native brain space.

We measured relative percentages of frontal cortico-fugal streamlines to sub-regions of the basal ganglia. As percentages add up to 100 a reduction to one sub-region is necessarily associated with a relative increase to one or more of the others. Relative percentage changes very likely represent degeneration-related fibre loss in PSCs rather than new fibre formation to other regions. A possible alternative explanation is differential developmental in the context of a huntingtin mutation resulting in a complex and heretofore unrecognized reorganization of fibres from frontal cortex to sub-cortical regions. This scenario is relatively unlikely because of the two observed negative correlations (with estimated years to onset and with variability of voluntary-guided saccades).

The interpretation of the result of a multivariate analysis, such as that we employed, is different to that of a mass-univariate approach, like VBM (Ashburner and Friston, 2000). VBM analyses images voxel by voxel and therefore the importance of a voxel for image differentiation can be viewed in isolation. A comparison of our results with previous univariate approaches (Reading et al., 2005; Rosas et al., 2006) reveals similar patterns of change, though the results were descriptive, without correction for multiple comparisons across the whole brain, or achieved

---

*Fig. 4* Figure displaying cortical areas from which more fibres reached the caudate body in controls than PSCs.

*Fig. 5* Correlation between percentages of fibres and the caudate body out of all frontal cortico-striatal fibres with individual voluntary saccade variability.
significance only for experimenter-defined regions of interest. Our method avoids the danger of spuriously significant differences resulting from a high number of statistical tests, as there is no independent analysis of all voxels in isolation. On the other hand, P-values for differential individual voxel values cannot be provided.

Pathophysiology in PSCs

Our study provides evidence for a region-specific involvement of cerebral white matter. The caudate body contributes to the oculo-motor loop connecting cortex and basal ganglia (Alexander et al., 1986). The main cortical input to the caudate body comes from the frontal eye fields (FEFs) (Cui et al., 2003; Wiesendanger et al., 2004). The FEFs lie in BA 8 (dark red and labelled in Fig. 1) in the precentral sulcus at its intersection with the superior frontal sulcus (Rosano et al., 2002). Our finding of reduced streamlines between these regions is intriguing because of their role in the control of eye movements (Leigh and Kennard, 2004; Pierrot-Deseiligny et al., 2004) and because pathological eye movements are one of the earliest clinical signs in HD (Beenen et al., 1986; Blekher et al., 2006; Golding et al., 2006). The examination of both saccades and ocular pursuit form an important part of the standard clinical examination battery (UHDRS; Huntington Study Group, 1996). Voluntary-guided saccades are part of purposeful behaviour whereas reflexive saccades result from involuntary attention to novel stimuli (Leigh and Kennard, 2004). As reviewed by Pierrot-Deseiligny et al. (2004), studies in humans and monkeys indicate a crucial role for the FEFs in generating voluntary but not reflexive saccades. The findings of this study indicate a correlation between individual impairment of voluntary saccades and the degeneration of fibres between FEFs and caudate body. Reflexive saccades, which do not involve the FEFs (Leigh and Kennard, 2004), did not correlate with any streamline reduction, thus lending support for the specificity of our results.

A limitation of our study is that no data on the subject-specific localization of the FEFs are available. A localization based on functional imaging data would be preferable and should increase sensitivity. Similarly, the relatively long interval between MRI-scanning and acquisition of eye-movement data may have attenuated sensitivity. The fact that we were still able to detect a significant correlation argues for the relative robustness of this finding.

It seems surprising that fibre loss is more prominent in the left hemisphere and that a correlation with age is also confined at this stage in the disease to the left. We are not the first to report a left-sided pathological bias in pre-symptomatic HD. Previous studies using standard univariate VBM found a similar pattern of left-hemispheric predominance (Thieben et al., 2002; Muhlau et al., 2007). There is also a spectroscopic study that shows an early increase of lactate, a marker of degeneration, in the left striatum (Jenkins et al., 1998). VBM studies in early affected patients (Kassubek et al., 2004; Kassubek et al., 2005; Douaud et al., 2006) suggest that the left-sided pattern is a feature of the pre-symptomatic phase of the disease.

In our multivariate FA analysis, we find differences between PSCs and controls in the putamen and globus pallidus as well as anterior parts of the corpus callosum. This finding corresponds to previous voxel-based univariate FA analyses that report similar FA changes in PSCs and early HD patients in the same structures (Rosas et al., 2006). The reduction in FA observed in the corpus callosum could well reflect a degeneration of inter-hemispheric connections in line with reported deterioration in cognitive tasks involving inter-hemispheric transfer (Brandt et al., 2002). FA increases in grey matter are more difficult to interpret. FA values in grey matter are lower than in white matter. Degeneration leading to a simple shift of the grey:white boundary might explain such FA-increases in the PSC group. Our data support this notion by differentiating voxels in the form of a ‘rind’ around the putamen. However, we did not observe such a pattern for the caudate nucleus.

Although not the main focus of our study, Fig. 2 indicates that there are additional features in occipital cortex that serve to separate PSCs from controls. Although visual impairment is not a typical clinical feature of HD, spectroscopic studies have also indicated abnormalities of the occipital cortex (Jenkins et al., 1993; Jenkins et al., 1998). Numerous differentiating voxels are found scattered widely in the cortex without any obvious region-specific pattern. One can speculate that a global change in brain shape related to degeneration could provide an explanation. It should be noted that the pattern of correlated changes in white matter integrity we find is very specific and provides sufficient information to assign 82% of scans to the correct group. The fact that PSCs misclassified as controls were younger than the rest of their group is not surprising as it reflects fewer pathological changes in the brain.

Taken together our findings suggest a distributed pattern of white matter changes. It is not surprising that degeneration of the specific tract remains undetected in our as well as previous FA analyses (Reading et al., 2005; Rosas et al., 2006). The white matter between cortex and striatum contains many different and crossing fibre-systems. FA is an aggregate measure of diffusion in voxels that has proven a sensitive measure when prominent fibre systems (e.g. the pyramidal tract) are affected by disease (Sach et al., 2004; Thomalla et al., 2004). We consider fibre tracking and the multivariate analysis of FA we use in this study as complementary analyses. Whereas the first method aims at detection of changes in specific tracts, the latter tests for specific changes across the whole brain. The changes of FA in the anterior corpus callosum detected by our and previous studies (Rosas et al., 2006) may therefore reflect the involvement of other currently undetected, tract-specific changes. The negative results of our complementary analysis of grey matter from T1-weighted images of the same
subjects (see supplementary material) suggest a higher sensitivity for diffusion-weighted imaging. This could stem from relatively specific involvement of pyramidal cells in the frontal cortex that result in degeneration of the underlying white matter. A possible advantage of the implementation of fibre tracking we propose is that all analyses are done in subject-specific native brain space. This could be of particularly importance if one considers the great variability of gyriﬁcation in the frontal lobes.

Our study presents an exploratory approach attempting to detect region-speciﬁc changes. The fully automated methods we used helped to explore the whole white matter space, eschewing any experimenter biased a priori selection of small regions or tracts of interest. For particular clinical questions, the methods we present can be tailored to any speciﬁc tract under study. Such regional precision could involve semi-automated methods that potentially avoid diﬃculties with spatial normalization in the study of neurodegenerative diseases. We suggest that subject-speciﬁc localization of the FEFs (using functional imaging) should yield greater sensitivity for the detection of changes in connecting ﬁbre tracts. A longitudinal design following degeneration would also clarify whether such a measure provides a useful and sensitive biomarker for HD (Paulsen et al., 2006).

Acknowledgements

We would like to thank Maggie Burrows, Susie Henley, Rachel Taylor, Tom Warner and Edward Wild for their help with the recruitment. Furthermore, we would like to thank John Ashburner for helpful suggestions on SVM classiﬁcation. This work was supported by the Wellcome Trust (grant 075696 2/04/2 to R.S.J.F. and S.J.T.), by the Engineering and Physical Sciences Research Council (grant GR/T22858/01 to D.C.A. and P.A.C.; grant GR/T02669/01 to G.J.M.P.) and by the High-Q Foundation (grant to C.K.). Funding to pay the Open Access publication charges for this article was provided by the Wellcome Trust.

References


Brodmann K. Vergleichende lokalisationslehre der grosshirnrinde in ihren prinzipien dargestellt auf grund des zellenbaues. Leipzig: Johann Ambrosius Barth; 1909.


