The Corticospinal Tract in Huntington’s Disease

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Huntington’s disease (HD) is characterized by progressive motor impairment. Therefore, the connectivity of the corticospinal tract (CST), which is the main white matter (WM) pathway that conducts motor impulses from the primary motor cortex to the spinal cord, merits particular attention. WM abnormalities have already been shown in presymptomatic (Pre-HD) and symptomatic HD subjects using magnetic resonance imaging (MRI). In the present study, we examined CST microstructure using diffusion tensor imaging (DTI)-based tractography in 30-direction DTI data collected from 100 subjects: Pre-HD subjects (n = 25), HD patients (n = 25) and control subjects (n = 50), and T2*-weighted (iron sensitive) imaging. Results show decreased fractional anisotropy (FA) and increased axial (AD), and radial diffusivity (RD) in the bilateral CST of HD patients. Pre-HD subjects had elevated iron in the left CST, regionally localized between the brainstem and thalamus. CST repeat length in conjunction with age, as well as motor (UHDRS) assessment were correlated with CST FA, AD, and RD both in Pre-HD and HD. In the presymptomatic phase, increased iron in the inferior portion supports the “dying back” hypothesis that axonal damage advances in a retrograde fashion. Furthermore, early iron alteration may cause a high level of toxicity, which may contribute to further damage.

Keywords: CAG repeats, corticospinal tract, DTI diffusion tensor imaging, Huntington’s disease, structural connectivity, tractography

Introduction

Huntington’s disease (HD) is a neurodegenerative autosomal dominant disorder caused by increased CAG repeats. This process leads to an abnormally increased synthesis of huntingtin (Htt), which is a protein that causes neuronal damage. Many studies have documented the deleterious effect of HD on the brain (for a review see Esmaeilzadeh et al. 2011). Furthermore, striatal damage is most often associated with the disease, even though the expression pattern of the mutated protein (mutant Htt) is expressed throughout the nervous system and periphery (Ehrlich 2012). It has also been shown that presymptomatic (Pre-HD) subjects may have brain atrophy years before the disease manifests. Changes in cerebral white matter (WM) and striatal volume are particularly sensitive in elucidating differences between Pre-HD groups and controls (Paulsen et al. 2010).

Although degeneration of striatal gray matter (GM) is the principal neuropathological feature of HD, there is increasing evidence that WM degeneration is present in both clinical and preclinical HD. Abnormalities in WM microstructure have been reported in postmortem studies of humans with HD (de la Monte et al. 1988; Halliday et al. 1998). This research was confirmed by in vivo studies with magnetic resonance imaging (MRI), which reported extensive WM volume loss (Aylward et al. 1998, 2012, Aylward, Nopoulos et al. 2011; Thieben et al. 2002; Rosas et al. 2003; Fennema-Notestine et al. 2004; Beglinger et al. 2005; Giarmiello et al. 2006; Paulsen et al. 2006; Jech et al. 2007; Squitieri et al. 2009; Tabrizi et al. 2009, 2011, 2012, 2013; Paulsen et al. 2010) and changes in WM microstructure (Mascalchi et al. 2004; Reading et al. 2005; Rosas et al. 2006; Bartzokis et al. 2007; Sritharan et al. 2010; Stoffers et al. 2010; Dumas et al. 2012; Matsui et al. 2014). Nevertheless, these previous macro- and microstructural studies focused on whole-brain WM or Region of Interest analyses. Only a few studies investigated a specific tract in its entirety, for example, the whole commissural fiber (Magnotta et al. 2009; Muller et al. 2011; Bohanna, Georgiou-Karistianis, Egan et al. 2011; Di Paola et al. 2012; Dumas et al. 2012), the motor corticostriatal circuit (Kloppel et al. 2008; Bohanna, Georgiou-Karistianis, Sritharan et al. 2011) or the WM pathway of the sensorimotor cortex (Dumas et al. 2012).

In previous works, we extensively investigated the connectivity of WM in HD by studying the biggest WM commissure, the corpus callosum (CC). In our first study (Di Paola et al. 2012), we found that early WM demyelination damage characterizes HD in the presymptomatic stages. Then in the advanced stages of the disease (first and second stages), the damage spreads considerably and involves the entire CC body and myelin and axonal damage are found simultaneously. Furthermore, we suggested that callosal damage progresses in a posterior–anterior direction. Subsequently, we investigated the different callosal tracts in more detail and found that the impairment seems to start in the motor and visual tracts and we confirmed it proceeds in a posterior–anterior direction (Phillips, Sanchez-Castaneda et al. 2013). Finally, we were interested in how early WM demyelination damage in HD relates to iron content. Our interest in iron was twofold: first, as iron accumulation in subcortical GM structures is implicated in HD (Bartzokis et al. 1999; Hilditch-Maguire et al. 2000), we were interested in knowing whether iron content follows the same pattern (accumulation) in WM; second, as iron is strictly related to myelin production, we wanted to determine whether there was a link between myelin damage and iron content in our HD data. As expected, our data showed that the early and heavily myelinated fibers of the isthmus were most affected by myelin damage. Specifically, we discovered that myelin breakdown starts in the presymptomatic stage of HD, but changes in iron content (reduction) manifest only in the early stages of HD (Di Paola et al., accepted). We explained the reduction as a failure in attempt to remyelination processes in the early stages of HD.

In the present study, we were interested in the the corticospinal tract (CST) for at least three reasons: it is a large WM tract with a well-known structure and function, and it is anatomically
linked to the basal ganglia, which is considered part of the hypothesized main brain target region of HD pathology. Structurally, the CST is composed by WM fibers, which are major descending tracts, with about half of them arising from the primary motor cortex (Kandel et al. 2000; Schultz 2001).

Functionally, the CST is the main motor pathway that conducts motor impulses from the brain to the spinal cord. The CST is crucial for voluntary movement (Kandel et al. 2000; Schultz 2001). Although the hallmark symptom of HD is purposeless, involuntary, choreic movements (Young et al. 1986; Folstein 1989), voluntary movement abnormalities are also present such as impaired precision on self-paced timing motor tasks in Pre-HD subjects (Rowe et al. 2010) and early eye movement disorder (Beenen et al. 1986; Blekher et al. 2006; Golding et al. 2006).

Finally, the CST is anatomically linked to the basal ganglia. The basal ganglia is believed to be a major component of the extrapyramidal motor system (Kandel et al. 2000). The extrapyramidal and pyramidal (or CST) systems are extensively interconnected and cooperate in controlling movement. Indeed, the motor actions of the basal ganglia are mediated in large part by the supplementary, premotor, and motor cortices via the pyramidal system (Schultz 2001).

Despite all of the above-mentioned connections between HD and the CST, few studies have identified alterations in the corticospinal pathway. Voxel-based morphometry studies (Fennema-Notestine et al. 2004; Della Nave et al. 2010) found reduced WM volume in the internal capsule of HD patients in different clinical stages of the disease. Diffusion tensor imaging (DTI) studies (Rosas et al. 2006; Magnotta et al. 2009; Stoffers et al. 2010) found a WM alteration (decreased fractional anisotropy [FA]) in the internal capsule of Pre-HD subjects and patients in the early stages (first and second) of HD. However, none of these previous works investigated the entire CST WM tract, but were interested only in WM changes in general. Examining the CST should increase our knowledge about the structural WM changes in HD patients beyond the well-known motor corticostriatal circuit. Furthermore, it seems important to investigate the role of the CST in HD motor symptoms.

To study the microstructure of the CST in HD, we adopted two different MRI approaches: DTI-based tractography and $T_2^*$-weighted MRI (iron sensitive) imaging. DTI is the MRI technique most frequently used to study WM fiber changes. It is a noninvasive technique that uses local water diffusion in the brain tissues to study microstructural aspects of WM anatomy (Pierpaoli et al. 2001). It has been used in numerous studies to investigate everything from the effects of age on WM in healthy subjects (Lebel et al. 2008; Phillips, Clark et al. 2013) to disease effects in Alzheimer’s disease (Di Paola et al. 2010) and schizophrenia (Phillips et al. 2009, 2011). Although the biological determinants of diffusion parameters (FA, axial diffusivity [AD], and radial diffusivity [RD]) are not yet fully understood (for an in-depth discussion of diffusion imaging see Jones et al. 2013), and interpretation should keep in mind the underlying WM anatomy and possible tract-specific neuro-pathology (Douaud et al. 2011), it is agreed that this approach is sensitive to microstructural tissue properties. Furthermore, DTI tractography allows the precise mapping of tract anatomy within subjects (Conturo et al. 1999; Ashburner and Friston 2000; Catani et al. 2002; Wakana et al. 2004). Tractography results agree well with postmortem definitions (Catani et al. 2003; Jellison et al. 2004).

We also used $T_2^*$-weighted volumes, which are sensitive to iron/ferritin (Cherubini, Peran, Caltagirone et al. 2009; Cherubini, Peran, Hagberg et al. 2009) to investigate the iron content of the CST. This is a valuable tool because, according to the HD “demyelination hypothesis” (Bartzokis et al. 2007), HD is related to myelin loss (myelin breakdown). Myelin breakdown is associated with increased density of the oligodendrocytes in the brain, which are involved in repairing myelin damage. As the oligodendrocytes have the highest iron content (Bartzokis et al. 2007), when they increase iron content also increases. Thus, an increase in iron content is associated with the remyelination process (Bartzokis et al. 2007). Although iron has been shown to accumulate abnormally in the striatum of HD patients (Sanchez-Castaneda et al. 2012), little work has been done to investigate iron levels in the WM of HD subjects. The few studies present in the literature show a nonunivocal picture of the regional iron content changes in HD, with no iron level differences in the callosal splenium but decreased iron levels in the frontal lobe WM (Bartzokis and Tishler 2000; Bartzokis et al. 2007), or reduced iron content in the callosal isthmus (Di Paola et al., accepted).

Additionally, we examined whether changes at level of the primary motor cortex, where the most part of the CST fiber arises, were present. The rationale behind this was that it could allow us to provide some evidence in support of the “Dying back” hypothesis in HD WM degeneration (see Han et al. 2010 for a review). In short, if axonal degeneration advances in a retrograde fashion then abnormalities in the CST should be detectable before changes in motor cortex occur. In order to examine the CST cortical target regions, we used a cortical thickness approach that would give us a picture of regionally specific cortex changes between groups. This approach has been used previously to find reduced thickness in the motor cortex in HD patients (Rosas et al. 2008). Furthermore, regional cortical thickness has been shown to correlate with the connectivity properties of regional WM tracts (Kochunov et al. 2011; Phillips et al. 2011).

Finally, we were interested in knowing whether changes at level of CST, were related with the motor function as well as with CAG repeat length and age. Indeed, although high CAG repeat length is the starting point for brain abnormalities in HD, it has been shown that the interaction of CAG with age, negatively effects the brain much more, as revealed by previous studies (Aylward, Mills et al. 2011; Matsui et al. 2014; Phillips, Sanchez-Castaneda et al. 2013).

Thus the main aims of our study were the following: 1) to explore CST WM microstructural changes that might account for motor impairment in a large cohort of subjects (25 HD patients, 25 Pre-HD and 50 healthy subjects), we predicted that Pre-HD subjects would show abnormalities in the CST which would be worse in the HD patients; 2) to investigate whether there is a correlation between motor impairment (motor scores data) and the CST fibers, we hypothesized that motor scores would be associated with CST fiber organization; 3) to explore whether CST microstructural changes are influenced by genetic factors, we expected that the CAG repeat length and its interaction with age, would be negatively associated with CST microstructure.
Methods

Subjects

Subject demographics and clinical assessments are outlined in Table 1. HD patients (n = 25) and Pre-HD subjects (n = 25) underwent a genetic test (abnormal CAG repeats ≥36) and were examined clinically by the same neurologist with expertise in HD. All individuals were assessed using the Unified Huntington’s Disease Rating Scale (UHDRS), which includes motor, cognitive, behavioral, and functional subscales (Kieburz et al. 1996). Each section consists of a multistep subscale. The motor section measures eye movements, limb coordination, time-persistence and movement disorders (such as rigidity, bradykinesia, dystonia, chorea, and gait disturbances). A higher score indicates greater motor impairment. The cognitive scale mainly evaluates executive function, assessed by a Phonetic Verbal Fluency test (Benton and Hamsher 1978), Symbol Digit Modalities Test (Smith 1975), and the Stroop interference test (Stroop 1935). The Phonetic Verbal Fluency evaluates an individual’s ability to retrieve specific information within restricted search parameters. Successful retrieval requires executive control over cognitive processes such as selective attention, selective inhibition, mental set shifting, internal response generation, and self-monitoring. The score is the number of corrected words produced in 60 s. The Symbol Digit Modalities test measures the time to pair abstract symbols with specific numbers. The test requires elements of attention, visuospatial processing, working memory, and psychomotor speed. The score is the number of correctly coded items from 0–110 in 90 s. The Stroop consists of 3 conditions: 1—Color Naming requires naming the colors; 2—Word Reading requires reading color words printed in black ink; 3—the Interference condition, naming the ink color of color words while inhibiting word reading. The number of correct responses in 45 s determines the score in each condition. The 3 conditions of the Stroop permit separation of the probable contribution of psychomotor speed from the executive function of response inhibition. Results for all the tests are reported as the raw number of correct responses. Higher scores indicate better cognitive performance.

The behavioral section investigates the presence of depression, aggressiveness, obsessions/compulsions, delusions/hallucinations and apathy. A higher score indicates greater impairment. The functional assessments include the HD functional capacity scale (HDFCS), the independence scale and a checklist of common daily tasks. All 3 scales primarily investigate independence in daily life activities. The HDFCS is reported as the Total Functional Capacity (TFC) score (range 0–13) and is the only functional subscale with established psychometric properties (including inter-rater reliability and validity) based on radiographic measures of disease progression. Thus, the TFC score is used worldwide to determine patients’ HD stage. On the Independence Scale, the investigator indicates whether the patient can perform the task that evaluates independent level (range 10–100). The checklist (functional assessment) is summed by giving a score of 1 to all “yes” answers (range 0–25). Pre-HD are defined as subjects in whom the suspected clinical diagnosis is confirmed by DNA analysis, revealing (CAG)n expansion into the range characteristic of HD (>36 repeats), but who have not yet manifested HD symptoms, defined by a total motor score of <5 on the UHDRS and cognitive and behavioral assessment within the norm. The Disease Burden index, a measure of disease severity, was used with the previously described formula (age-×(CAG-35.5)), where CAG is the number of CAG repeats (Penney et al. 1997). A higher score reflects increased disease severity. The Mini Mental State Examination (MMSE) (Folstein et al. 1975), which measures global cognitive functioning, was administered to Pre-HD subjects and HD patients. A lower score indicates greater impairment.

Patients in advanced stages of the disease (Stages III and IV) and/or with traumatic brain injury or focal lesions were excluded. After clinical and neuroradiological examination, 20 out of the original 80 recruited patients were excluded from the initial sample based on the inclusion criteria: 10 were in advanced stages of the disease (Stages III and IV), 3 had traumatic brain injury or focal lesions, and 7 had MRI acquisitions with movement artifacts. Thus, 50 subjects (Pre-HD and HD) were included in the study. Fifty healthy subjects were recruited from the community.

Ethics Statement

The Santa Lucia Foundation Research Ethics Committee approved this study, which was designed in accordance with the principles of the Declaration of Helsinki. All participants had the cognitive capacity to understand the research protocol and gave their oral and written consent. The study was undertaken after the patients’ informed consent had been obtained. All procedures were performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Table 1

Sociodemographic and clinical characteristics of patients and control subjects

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Pre-HD (n = 25)</th>
<th>HD (n = 25)</th>
<th>Controls (n = 25)</th>
<th>Fisher’s exact test; F or t-test</th>
<th>df</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender male/female</td>
<td>16/9</td>
<td>14/11</td>
<td>30/20</td>
<td>0.367</td>
<td>2</td>
<td>0.833</td>
</tr>
<tr>
<td>Age (years ± SD)</td>
<td>37.44 ± 7.01</td>
<td>47.40 ± 14.53</td>
<td>42.88 ± 12.48</td>
<td>4.349</td>
<td>2</td>
<td>0.012ab</td>
</tr>
<tr>
<td>CAG repetition length</td>
<td>43.28 ± 2.17</td>
<td>46.68 ± 6.80</td>
<td>43.08 ± 4.21</td>
<td>0.879</td>
<td>1</td>
<td>0.350</td>
</tr>
<tr>
<td>MMSE</td>
<td>27.82 ± 3.23</td>
<td>24.97 ± 3.23</td>
<td>24.97 ± 3.23</td>
<td>0.129</td>
<td>1</td>
<td>0.721</td>
</tr>
<tr>
<td>UHDRS motor</td>
<td>8.00 ± 9.29</td>
<td>37.22 ± 13.18</td>
<td>37.08 ± 13.08</td>
<td>0.129</td>
<td>1</td>
<td>0.721</td>
</tr>
<tr>
<td>UHDRS cognitive</td>
<td>25.78 ± 42.34</td>
<td>142.65 ± 50.35</td>
<td>142.65 ± 50.35</td>
<td>0.129</td>
<td>1</td>
<td>0.721</td>
</tr>
<tr>
<td>UHDRS behavioral</td>
<td>7.67 ± 7.84</td>
<td>18.39 ± 9.13</td>
<td>18.39 ± 9.13</td>
<td>0.129</td>
<td>1</td>
<td>0.721</td>
</tr>
<tr>
<td>UHDRS functional</td>
<td>25 ± 5.87</td>
<td>17.91 ± 5.68</td>
<td>17.91 ± 5.68</td>
<td>0.129</td>
<td>1</td>
<td>0.721</td>
</tr>
<tr>
<td>TFC</td>
<td>13 ± 3.34</td>
<td>8.33 ± 2.37</td>
<td>8.33 ± 2.37</td>
<td>0.129</td>
<td>1</td>
<td>0.721</td>
</tr>
<tr>
<td>Independence Scale</td>
<td>99.8 ± 1.04</td>
<td>78.04 ± 12.49</td>
<td>78.04 ± 12.49</td>
<td>0.129</td>
<td>1</td>
<td>0.721</td>
</tr>
<tr>
<td>Disease Burden</td>
<td>292.3 ± 87.52</td>
<td>458.8 ± 104.75</td>
<td>458.8 ± 104.75</td>
<td>0.129</td>
<td>1</td>
<td>0.721</td>
</tr>
<tr>
<td>CST L FA mean ± SD</td>
<td>0.502 ± 0.023</td>
<td>0.476 ± 0.029</td>
<td>0.476 ± 0.029</td>
<td>0.129</td>
<td>1</td>
<td>0.721</td>
</tr>
<tr>
<td>CST L AD mean ± SD</td>
<td>1.246 ± 6.31E −05</td>
<td>1.31E −03 ± 5.40E −05</td>
<td>1.31E −03 ± 5.40E −05</td>
<td>0.129</td>
<td>1</td>
<td>0.721</td>
</tr>
<tr>
<td>CST L RD mean ± SD</td>
<td>5.64E −04 ± 4.84E −05</td>
<td>6.25E −04 ± 6.77E −05</td>
<td>6.25E −04 ± 6.77E −05</td>
<td>0.129</td>
<td>1</td>
<td>0.721</td>
</tr>
<tr>
<td>CST L iron mean ± SD</td>
<td>17.77 ± 0.88</td>
<td>18.37 ± 0.97</td>
<td>18.37 ± 0.97</td>
<td>0.129</td>
<td>1</td>
<td>0.721</td>
</tr>
<tr>
<td>CST R FA mean ± SD</td>
<td>0.484 ± 0.034</td>
<td>0.461 ± 0.030</td>
<td>0.461 ± 0.030</td>
<td>0.129</td>
<td>1</td>
<td>0.721</td>
</tr>
<tr>
<td>CST R AD mean ± SD</td>
<td>1.25E −03 ± 5.15E −05</td>
<td>1.32E −03 ± 5.54E −05</td>
<td>1.32E −03 ± 5.54E −05</td>
<td>0.129</td>
<td>1</td>
<td>0.721</td>
</tr>
<tr>
<td>CST R RD mean ± SD</td>
<td>5.82E −04 ± 5.85E −05</td>
<td>6.47E −04 ± 6.35E −05</td>
<td>6.47E −04 ± 6.35E −05</td>
<td>0.129</td>
<td>1</td>
<td>0.721</td>
</tr>
<tr>
<td>CST R iron mean ± SD</td>
<td>17.89 ± 0.87</td>
<td>18.21 ± 1.03</td>
<td>18.21 ± 1.03</td>
<td>0.129</td>
<td>1</td>
<td>0.721</td>
</tr>
</tbody>
</table>

HD, Huntington’s Disease; Pre-HD, gene-positive, without motor symptoms; SD, standard deviation; df, degrees of freedom; CAG, trinucleotide repeat number; MMSE, Mini Mental State Examination; UHDRS, Unified Huntington’s Disease Rating Scale; TFC, Total Functional Capacity; NA, not available; MMSE, missing data for 5 Pre-HD subjects and 5 HD patients; UHDRS motor, missing data for 2 Pre-HD subjects and 2 HD patients; UHDRS cognitive, missing data for 5 Pre-HD subjects and 2 HD patients; UHDRS behavioral, missing data for 4 Pre-HD subjects and 2 HD patients; UHDRS functional, missing data for 2 Pre-HD subjects and 2 HD patients; TFC, missing data for 2 Pre-HD subjects and 2 HD patients.

*Pre-HD < HD (when it refers to a cognitive scale comparison or CAG repetition; higher punctuations mean greater impairment).

†Student, Bonferroni correction.

‡Pre-HD > HD (when it refers to a cognitive scale comparison; higher punctuations mean lesser impairment).
consent. Proxies were available to sign the written informed consent for participants with disabling motor impairments, however, all subjects in the current study were capable of signing their own consent.

**MRI Data Acquisition**

All MRI data were acquired on a 3 T Allegra MRI system (Siemens, Germany) using a birdcage head coil. Scans were collected in a single session, with the following pulse sequences: 1) proton density and T2*-weighted double turbo spin echo (SE) acquired in transverse planes (time repetition [TR]: 4500 ms, time echo [TE]: 12 ms, time to inversion [TI]: 112 ms, field of view [FOV]: 230 × 172 mm, matrix: 320 × 240, slice thickness: 5 mm, number of slices: 24); 2) fluid-attenuated inversion recovery in the same planes as the SE sequence (TR/TE/TI: 8500/109/2000ms; FOV: 230 × 168 mm, matrix: 256 × 256, slice thickness: 5 mm, number of slices: 24); 3) T2*-weighted 3D images, with partitions acquired in the sagittal plane using a modified driven equilibrium Fourier transform (Deichmann et al. 2004) sequence (TE/TR/TI: 2/7.92/910 ms, flip angle: 15°, 1 mm3 isotropic voxels); and 4) diffusion-weighted images were also acquired using SE echo-planar imaging (TE/TR: 89/8500 ms, bandwidth: 2126 Hz/voxel, matrix: 128 × 128, 80 axial slices, voxel size: 1.8 × 1.8 × 1.8 mm) with 30 isotropically distributed orientations for the diffusion sensitizing gradients at a b value of 1000 s/mm2 and 6 b=0 images. Scanning was repeated 3 times to increase the signal-to-noise ratio.

Six consecutive T2*-weighted gradient echo-planar whole-brain volumes were acquired at different echo times (TE) (TEs: 6, 12, 20, 30, 45 and 60 ms; TR = 5000; bandwidth = 1116 Hz/vox; matrix size: 128 × 128 × 80; flip angle 90°; voxel size of 1.5 × 1.5 × 2 mm3).

Images were visually inspected for gross anatomical abnormalities by 2 experienced observers (a neuropsychologist expert in neuroimaging and a neuroradiologist) who were blinded to participants' identity. Images were also visually inspected for movement artifacts, which are a common source of concern when studying HD. As movement can compromise tracking, we excluded subjects who had excessive movement in their scans.

**DTI Processing**

Diffusion-weighted images were processed with FMRIB’s Software Library (FSL 4.1 [www.fmrib.ox.ac.uk/fsl/]). Images were corrected for eddy current distortion. The nondiffusion-weighted images were skull stripped using FSL’s brain extraction tool (BET) (http://www.fmrib.ox.ac.uk/fsl/bet/index.html) and used to mask all diffusion-weighted images (Smith 2002). A diffusion-tensor model was fitted at each voxel using Diffusion Toolkit, generating FA, AD, and RD maps. RD was defined as the average of the second and third eigenvalues of the diffusion tensor, whereas AD corresponded to the first eigenvalue.

**T2*-Weighted Images Processing**

T2*-weighted volumes were post processed according to previously published methods (Cherubini, Peran, Caltagirone et al. 2009; Cherubini, Peran, Hagberg et al. 2009; Peran et al. 2009). Briefly, the 6 T2*-weighted volumes were averaged to generate a mean T2*-weighted volume. A full affine 3D alignment was calculated between each of the 6 T2*-weighted volumes and the mean T2*-weighted volume. For each subject, we performed a voxel-by-voxel nonlinear least-squares fitting of the data acquired at the 6 TEs to obtain a mono-exponential signal decay curve. To facilitate analysis of the relaxation results, we considered the inverse of relaxation times, that is, relaxation rates R2* = 1/T2* × 1000.

Iron images (R2*) were registered into subjects’ DTI space using FSL. flirt 12 degree-of-freedom transformation with the DTI B0 image used as the reference image. Registrations were visually inspected for accuracy.

**Tractography**

Tractography methods are outlined in more detail in Wakana et al. (2007), however, the tractography and region of interest (ROI) drawing was modified to use TrackVis, an interactive environment for fiber tracking reconstruction, display and analysis developed at the Harvard Medical School Martinos Center for Biomedical Imaging at Massachusetts General Hospital (www.trackvis.org). The fiber assessment by continuous tracking approach was used to reconstruct fiber paths. For details of the TrackVis tracking algorithm see Wedeen et al. (2008). A track angle threshold of 35° was used as well as an image mask based on the B0 image to restrict tracking to biologically plausible results.

It is important to note that tractography is still an emerging technology and has limitations, for example, the fiber bundles are not reconstructed directly; instead trajectories are calculated through the data, which are largely parallel to nerve fibers. Therefore, when interpreting diffusion data it should be kept in mind that we cannot yet ascertain categorically that scores are driven by a specific biological or physiological process (Jones et al. 2013).

Tractography of the CST was performed by manually drawing regions of interest on each individual’s FA color map. This approach has the advantage of avoiding potential normalization problems (Alexander et al. 2001), however, it presents its own problems because it relies on an expert to identify the tracts. This was carried out by a single expert (O.P.) who was blinded to the subject’s age, gender, and diagnosis. To determine intrarater reliability, fiber tracts were identified in 10 randomly chosen brain volumes. Reliability was assessed using the intraclass correlation coefficient (2-way mixed for intrarater). Statistical analyses were performed using SPSS 14.0. Excellent intrarater reliability was achieved for ROI placement, as determined by computing the intraclass correlation coefficients for tract volume and mean FA, AD, and RD (see Supplementary Table 1).

Region of interest placement was carried out according to Wakana et al. (2007). As mentioned before, the CST origins by a number of different cerebral regions (supplementary motor cortex, premotor area, somatosensory cortex, parietal lobe, and cingulate gyrus), descends through the cerebral brain and then runs through the midbrain to reach the spinal cord. In the present study, we focused on the tracts that arise from the primary motor cortex and we limited our attention to the portion running in the cerebral hemisphere, using a previously validated region of interest approach (Wakana et al. 2007). This had the advantage of not being susceptible to the confounds of voxel-based registrations (Alexander et al. 2001; Phillips et al. 2009, 2011). The first region of interest was placed on the axial slice at the level of decussation of the superior cerebellar peduncle. The second region of interest was designed to select only fibers going to the primary motor cortex, that is, the most ventral axial slice, which identified the cleavage of the primary motor cortex, was found and the tract bundle in the primary motor cortex was selected. Fibers that were clearly not part of the anatomical connectivity of the track were eliminated. FA, AD, RD, and iron were calculated for each subject by averaging all voxels over the entire tract, counting each voxel only once.

**CST and Primary Motor Cortex**

To determine whether the CST modifications were related to changes in the cerebral motor cortex, we ran a cortical thickness analysis. Cortical thickness was estimated using FreeSurfer software (FreeSurfer 5.1.0, http://surfer.nmr.mgh.harvard.edu/), a widely documented and automated program for the analysis of brain structure. The technical details of these procedures have been described in previous publications (Dale and Sereno 1993; Dale et al. 1999; Fischl, Sereno, Dale 1999, Fischl, Sereno, Tootell et al. 1999, Fischl et al. 2001, 2002, Fischl, Salat et al. 2004, Fischl, van der Kouwe et al. 2004; Fischl and Dale 2000; Segonne et al. 2004; Jovicich et al. 2006). Briefly, FreeSurfer local cortical thickness was measured by estimating the shortest distance between the position of spatially equivalent surface points on the pial surface and the gray-white matter boundary and vice versa and averaging these 2 values (Fischl and Dale 2000). A 10-mm full-width half-maximum Gaussian kernel was then applied to smooth the data.

**Regional CST Microstructural Properties**

To examine regional differences in microstructural properties along the CST, we subdivided the FMRIB58_FA standard-space FA template (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FMRIB58_FA) at the midsagittal slice to create 3 masks that would allow us to roughly assess the inferior, medial, and superior portion of each subject’s CST. The first division was at the apex of the cerebral peduncle, before the start of
the thalamus (Part 1—inferior), the second included the internal capsule (Part 2—middle), the third was drawn at the apex of the corpus callosum (Part 3—superior) (see Fig. 3, Part A). These masks were then transferred to each subject’s native space using the 12 degrees-of-freedom registrations between the FMRIB58 FA atlas and each subject’s B0 image. Then DTI parameters and iron level were calculated for each region.

Statistical Analysis
Demographic differences were assessed using chi-square independent sample t-tests or ANOVA, as appropriate.

To test for CST differences among groups, a multivariate analysis of variance (MANOVA) was applied to all 3 groups (healthy controls, HD patients, and Pre-HD subjects). Sex and age were included as covariates in the model. Then, contrasts were run to individuate significant differences between groups. Results were corrected using a false discovery rate (FDR) threshold P < 0.05.

To investigate whether CST changes in the course of pathology were related to motor and genetic aspects, we performed correlation analyses between CAG repeat length, Disease Burden, and UHDRS-motor score and track measures. We included the extracted CST values from both Pre-HD and HD subjects in the correlation analysis together with the above-mentioned genetic and clinical scores. Sex and age were included as covariates. To control for multiple comparisons, results were FDR corrected (P < 0.05).

To explore group differences in motor cortex thickness, we used Freesurfer’s surface group analysis tools (Qdec). The general linear model (GLM) was used to compare groups (Controls vs. Pre-HD, Controls vs. HD and Pre-HD vs. HD). Results were corrected for multiple comparisons using an FDR correction (P < 0.05).

To explore group differences in regional CST microstructural properties, a MANOVA was applied to all 3 groups (healthy controls, HD patients, and Pre-HD subjects) for the 3 CST portions (inferior, medial, and superior portion). Sex and age were included in the model. Contrasts were run to individuate the significant differences between groups in each portion of the CST. FDR corrected statistical outcomes were reported for P < 0.05.

Results
Subjects’ Demographic Data
Pre-HD subjects and HD patients differed for age and CAG repetition length, but not gender. Furthermore, as expected, HD patients performed significantly worse on all measures assessed by the UHDRS and obtained a significantly higher score on Disease Burden (Table 1).

Diagnosis Findings
When HD patients and controls were compared, HD patients showed reduced FA in both the left and right CST and higher AD and RD. HD patients also exhibited reduced FA compared with Pre-HD subjects in both left and right CST and higher AD and RD. An examination of iron differences revealed a significant increase in the left CST of Pre-HD subjects compared with controls, with no differences in FA, AD, or RD. Statistical details are outlined in Table 2 (Figure 1).

Correlations
CAG repeat length correlated with CST diffusivity: left FA (r = −0.343, P < 0.017), AD (r = 0.291, P < 0.045), RD (r = 0.323, P < 0.025), and right FA (r = −0.304, P < 0.036), AD (r = 0.426, P < 0.003), and RD (r = 0.426, P < 0.003).

Disease Burden was further correlated with the left CST: FA (r = −0.387, P < 0.007), AD (r = 0.334, P < 0.020), and RD

Table 2
Tractography group comparisons

<table>
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Note: Significant FDR corrected results are in BOLD.

Figure 1. CST tractography and group comparisons. (A) Numbers 1–4 show tractography ROI placement on a single subject for the left and right CST. (B) Bar graphs show differences between CST FA, AD, and RD for the left and right hemisphere. The error bars represent the standard error mean (SEM).
UHDRS-motor scores were correlated with the left CST: FA ($r = -0.415, P < 0.005$), AD ($r = 0.414, P < 0.005$), RD ($r = 0.434, P < 0.003$) and right FA ($r = -0.356, P < 0.013$), AD ($r = 0.483, P < 0.001$), and RD ($r = 0.438, P < 0.003$) (Figure 2).

CST and Primary Motor Cortex

The primary motor cortex showed significantly reduced bilateral thickness in HD patients compared with controls. Cortical thickness was not significantly different between Controls and Pre-HD subjects or Pre-HD subjects and HD patients (see Supplementary Figure 1).

Figure 2. Significant motor and genetic correlations with CST FA, AD, and RD.
Regional CST microstructural properties
When HD patients and controls were compared, HD patients showed significantly reduced FA in both left and right CST Part 1 (inferior) and Part 3 (superior) and higher AD and RD across the whole tract bilaterally. There were no differences in iron (Figure 3).

Furthermore, HD patients showed reduced FA compared with Pre-HD subjects in the left CST Part 1 and right Part 3. HD patients also had higher AD compared with Pre-HD subjects in all parts of the left and right CST. Higher RD was seen in Parts 1 and 3 bilaterally and on the left in Part 3.

Pre-HD subjects showed increased RD in the right Part 2 compared with controls and elevated iron levels in Part 1 compared with HD patients and controls. Statistical details are outlined in Table 3.

Discussion
This study sought to characterize (at the microstructural level) the CST in Pre-HD subjects and HD patients compared with healthy controls and to determine whether CST microstructure is correlated with motor impairment (motor scores). Several major findings emerged from the study: 1) the CST is damaged globally in HD patients; 2) Pre-HD subjects present increased iron levels regionally localized in the inferior left part of the CST; 3) CST connectivity correlates with motor scores; 4) CAG repeat length in conjunction with age negatively effects the connectivity of the CST.

Postmortem imaging studies and animal models have repeatedly demonstrated the striking myelin breakdown and WM atrophy in HD (Bruyn and von Wolferen 1973; de la Monte et al. 1988; Bartzokis et al. 2007). DTI work has revealed connectivity impairments in the CC of Pre-HD subjects (Rosas et al. 2010; Di Paola et al. 2012), as well as the WM of the sensorimotor cortex (Dumas et al. 2012) and the motor corticostriatal circuit in HD patients (Bohanna, Georgiou-Karistianis, Sritharan et al. 2011). Further evidence of impaired

![Figure 3](https://academic.oup.com/cercor/article-abstract/25/9/2670/2926079)

**Figure 3.** Regional CST microstructural properties group comparisons. (A) Divisions on the FMRIB58 FA atlas to create regional masks. (B) Graphs show group differences between regional CST FA, AD, and RD for the left and right hemisphere.

<table>
<thead>
<tr>
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<th>Control versus HD</th>
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Note: FDR corrected statistical results at P > 0.05. Bold values indicated FDR corrected significant results.
myelin in HD comes from a DTI study on an HD mouse model (Xiang et al. 2011), which reported thinner myelin sheaths and increased myelin periodicity (i.e., increased nodes of Ranvier, which is used as an index of myelin compaction; in other words, the larger the periodicity the less compacted the WM).

We found decreased FA and increased AD and RD in the CST of HD patients, which globally indicates an abnormal change in the structural connectivity of this pathway. Reductions in FA might reflect decreased numbers of fibers or indicate reduced axonal myelination (Konrad and Winterer 2008). Furthermore, AD, which is a measure of how fast diffusion occurs in the preferred direction, has been shown to be sensitive to number of axons as well as their coherence (Takahashi et al. 2000). Increased AD is related to WM axonal atrophy likely associated with Wallerian degeneration; but in some cases it can be attributed to increased fiber organization (Hasan et al. 2007). RD is a measure of how fast diffusion occurs in the perpendicular direction. Increased RD is thought to reflect reduced myelination (Song et al. 2002; Concha et al. 2006; Schmierer et al. 2008). And although the biological role of this connectivity change is still not fully understood (Jones et al. 2013) and caution should be used in interpreting diffusion findings (Wheeler-Kingshott and Cercignani 2009), it is likely that demyelination and axonal damage occur with disease progression. Thus, our data (lower FA, higher AD and RD) suggest global damage (both at axonal and myelin levels) of the CST microstructure in the early stage of HD, with CST microstructure still globally spared in the presymptomatic stage (Pre-HD) (no differences in FA, AD, and RD when the whole tract was considered, increased RD only in the right Part 2 when CST was split into 3 parts). Worth noting, if both AD/ RD were increasing proportionally, it would be expected that there would be no detectable change in FA. However, the increase in RD was emphatic enough to lead to a decrease in FA. Our finding of altered CST WM is in line with the findings of previous WM DTI studies on Pre-HD subjects (Rosas et al. 2006; Magnotta et al. 2009; Stoffers et al. 2010). Nevertheless, although these studies were valuable, they provided only a limited assessment of the CST because they used a whole brain or region of interest approach, with limited DTI parameters. Interestingly, an earlier DTI study showed a selective degeneration in the subcortical gray matter which lead to an increase in FA because of the hypothesized increased orientation of the fibers (Douaud et al. 2009). Although our analysis of the CST also showed degeneration, our FA result was in the opposite direction, which emphasizes the importance of caution when interpreting diffusion data (Jones et al. 2013).

Iron levels have only been investigated in a few HD imaging studies. While iron accumulation has been confirmed in GM in HD patients (Bartzokis et al. 1999; Hilditch-Maguire et al. 2000), as well as Pre-HD subjects (Sanchez-Castaneda et al. 2012). The three previous studies in the literature on iron and WM in HD describe regionally specific changes, with no iron level differences in the callosal splenium as opposed to decreased iron levels in the frontal lobe WM of HD patients (Bartzokis and Tishler 2000; Bartzokis et al. 2007), and reduced iron content in the callosal isthmus of HD patients (Di Paola et al., accepted).

In the present study, we found an increase in iron in the left CST of Pre-HD subjects. But HD patients did not have higher levels of iron, which indicates a change in WM microstructure between the pre-disease and the disease stage. Iron content is associated with remyelination (Bartzokis et al. 2007) and increased density of oligodendrocytes (the brain cells with the highest iron content), which attempt to repair myelin damage. According to Bartzokis et al. (2007), the HD brain may be continually trying to remyelinate in a losing attempt to compensate for the disease-related myelin loss. In HD, these remyelination processes may successfully compensate during younger years, which usually correspond to the beginning of the pathology or to the years before onset (Pre-HD stage), but eventually begin failing in older years as brain myelin volume continues to grow and the maintenance of this expanding volume becomes increasingly difficult. This is similar to what happens in healthy older individuals (Bartzokis et al. 2012).

Thus, taken together our results indicate that iron accumulates in the CST many years before disease onset. We can hypothesize that the higher iron level is related to a higher than normal level of oligodendrocytes in Pre-HD subjects due to the attempt to repair/remyelinate (Bartzokis et al. 2007). This is in line with previous reports of iron levels in Pre-HD subjects. In fact, extremely high numbers of oligodendrocytes have been reported years before symptom appearance (Myers et al. 1991; Sotrel et al. 1991; Gomez-Tortosa et al. 2001). In an attempt to repair the ongoing damage to WM, the oligodendrocytes maintain the connectivity of the CST at functional levels, as indicated by normal FA, AD and RD in Pre-HD subjects. Myelin repair may be seen as positive at this point in the disease. On the other side, increased iron levels likely add to oxidative stress. This happens because increased iron levels cause neuronal excitotoxicity by promoting free radical toxicity (Youdim et al. 2004, Bartzokis et al. 2007). Free radical toxicity can cause damage to biomolecules, lipids, proteins, and DNA (Jomova and Valko 2011). The free radical level may reach a threshold point of toxicity and contribute to the loss of connectivity (lower FA, higher AD, and RD) we found in HD patients. Recently, an HD mouse model showed that an increase in intra-neuronal labile iron pool lead to an increased susceptibility of iron-associated oxidative stress (Chen et al. 2013).

Our finding of increased iron in the left CST is consistent with the iron level asymmetry described in normal aging and movement disorders (Supprian et al. 1997, Steen et al. 2000, Xu et al. 2008; Langkammer et al., 2010). It is also in line with the leftward-biased GM loss present in the striatum of HD patients (Muhlau et al. 2007). This leftward asymmetry of brain iron has been ascribed to motor lateralization and the co-localization of dopaminergic neurons and iron (Xu et al. 2008). However due to the inconsistent of the leftward pattern of iron asymmetry result in HD, future studies need to be performed to determine whether this is an important finding to understand the HD pathology or merely an occasional result.

We can imagine the progression of HD WM changes by examining the modifications of iron levels and myelin integrity and connectivity of the CST found in our group. First, some mechanism must damage the CST tract before disease onset, as revealed by iron alterations occurring in the presymptomatic stage. The elevated iron level suggests that some repair attempt is in action. This keeps the CST at a high connectivity level in Pre-HD (no changes in other DTI parameters when the whole tract was considered). However, the mechanism causing damage continues and, at this point in the disease, the iron accumulation may even contribute to the WM degeneration by altering the whole microstructure of the tract (changes in all DTI parameters in HD stages). Longitudinal studies and studies
of juvenile HD are needed to confirm the findings of our study and gain an even deeper understanding of the role of increased WM iron levels in the pathogenesis of HD. At this point, however, the underlying cause of the early myelin damage is still unclear.

In the present study, we also investigated the brain changes at the level of the GM regions (motor cortex), where the WM of the CST mainly originates. It is still not clear whether WM changes occur after, before, in tandem or independently of changes to the GM regions. This would be important to know, because the integrity of the WM tracts is likely associated with the GM regions they are connected to. Indeed, understanding when and where changes occur first could help us better understand the progression of HD.

Regional cortical thickness has been shown to correlate with the connectivity properties of regional WM tracts (Kochunov et al. 2011; Phillips et al. 2011). In agreement with previous studies (Rosas et al. 2006; Stoffers et al. 2010), we found that the cortical thickness of the motor cortex is reduced in HD patients (i.e., they have less GM compared with healthy controls). As our results show some changes (higher iron level) in the CST of Pre-HD subjects, but not significant motor cortex thickness reduction in Pre-HD, we suggest that WM damage may precede GM changes in the motor cortex. This hypothesis is in agreement with previous studies pointing in this direction in HD patients (Bartzokis et al. 2007) and Pre-HD subjects (Nopulous et al. 2010). In any case, a future longitudinal investigation is needed to resolve this question.

In this study, we split the pyramidal tract into 3 segments to evaluate the CST regionally as well as globally (see Fig. 3). In Pre-HD, our regional results showed an accumulation of iron in the left inferior portion (called Part 1) of the CST (between the brainstem and the thalamus). We hypothesized (see above) that the increase in iron might reflect an accumulation of oligodendrocytes attempting to repair axonal damage. The “Dying back” hypothesis in HD WM degeneration (see Han et al. 2010 for a review), can be used to explain our results of the regional CST microstructural analysis. According to this hypothesis, striatal and corticostriatal projection neurons in HD undergo normal development, retaining normal connectivity and functionality prior to the disease state. Affected neurons then begin to exhibit signs of synaptic and axonal alterations early in the disease process and even in the presymptomatic stages. Axonal degeneration steadily advances in a retrograde fashion and cell death appears much later in the course of HD. Thus, our data on CST changes seem to be in line with striatal and corticostriatal projection neuron degeneration, that is, damage and (eventually) repair mechanisms start from the bottom and move up the axon towards the cell body. In support of the “Dying back” pattern of neuronal degeneration, we did not find atrophy at the cortical level in Pre-HD, which, if present, can be considered an indirect measure of cell death. However, this was present in HD patients, indicating damage was downstream of changes to the WM. The alterations to the CST in the Pre-HD stage likely impaired the synaptic connectivity which has been shown to eventually result in the loss of trophic support (Zuccato and Cattaneo 2007) and cell death via apoptosis (Vis et al. 2005). Furthermore, analysis of the regional CST microstructural properties showed no changes in FA across the groups in Part 2 (middle), which may be partly due to the nature of the CST tract in that segment, where the fibers are more coherently oriented.

In HD, the main neurons affected in the striatum and the cortex have been described as having “selective neuronal vulnerability” or “differential neural vulnerability” (see Han et al. 2010 for a review). It is not known whether the CST neurons are also “selectively/differentially” vulnerable in HD.

Unlike many large WM tracts in the brain, the role of the CST is relatively well understood. It connects the primary motor cortex to the brainstem and is critical for voluntary movement. Focal acute damage to the CST causes a motor deficit in the contralateral arm and leg. Chronic damage to the CST causes progressive slow muscle weakness, atrophy and spasticity in the contralateral parts of the body in which the motor neurons are damaged (Schultz 2001). Consequently, problems in the CST have negative effects on motor abilities (Puig et al. 2013). We tested this assumption by correlating CST connectivity with motor scores and found a strong correlation (see Fig. 2), in accordance with our understanding of the tract. That is, higher connectivity is associated with greater motor ability and lower connectivity with poorer motor ability. The correlation between motor scores also supports our interpretation of the diffusivity differences between patients and controls. This is because there is a current debate over the precise biological meaning of diffusion measures (FA/AD/RD) (Jones et al. 2013). As these measures are correlated with decreased motor abilities, we feel that it is fair to characterize the differences between patients and controls as connectivity impairments and not simply abnormal diffusivity.

High CAG repeat length is the starting point for brain abnormalities in HD, but the path between genotype and actual brain damage is not well documented and is likely closely linked with age. For example, previously, we found that the connectivity of the corpus callosum was linked to Disease Burden (a measure of disease severity) (Phillips, Sanchez-Castaneda et al. 2013). Also, striatal atrophy, like clinical progression, may occur faster with higher CAG repeat length (Aylward, Mills et al. 2011). In the present paper, independent of age, we found a weak connection between high CAG repeat length and brain abnormality: high CAG repeats correlated with high connectivity impairment. This genetic basis for WM alterations was not found in two previous diffusion CC studies (Rosas et al. 2010; Bohanna, Georgiou-Karistianis, Sritharan et al. 2011), where CAG repeat length did not correlate with diffusivity measures. This may have to do with the increased sensitivity of tractography to elucidate the microstructural changes in WM or variations in subjects’ demographics. Note that although CAG repeat length and CST connectivity are significantly correlated, this is not the complete picture. Rather, Disease Burden, is more closely correlated and suggests the contribution of other factors. This fits with the current understanding of HD, that is, mutant htt likely contributes to reaching a threshold of toxicity and once the threshold is reached the progression primarily depends on mutation-independent factors (Persichetti et al. 1996; Bartzokis et al. 1999, 2007). A recent paper by Matsui et al. (2014), found a similar result where patients with higher levels of CAG repeats and age had increased mean diffusivity (MD) and RD in the inferior and lateral prefrontal cortex WM. In all, these findings strongly support the idea that CAG repeat length in conjunction with age, negatively affects WM.

Before drawing conclusions, some limitations of the present study must be acknowledged. First, longitudinal studies need to be performed to investigate the evolution of the CST.
changes in HD over time. Second, studies using a voxel-based kernel approach (Dukart et al. 2013; Gaser et al. 2013) are required to measure age effects independent of the disease, and to describe expected age and symptom severity related changes in HD relative to a baseline provided by healthy aging. This will allow for a better control of the effect of age, which has a nonuniform anatomical distribution across the brain. Third, larger Pre-HD and HD samples need to be collected to better assess the influence of CAG repeats. Fourth, a group of Pre-HD soft signs should be included in future studies to further investigate the presymptomatic stage of HD. The Pre-HD soft signs can indeed be considered an intermediate pathological stage between presymptomatic subjects (Pre-HD) and symptomatic patients (HD). Fifth, the $R_2^*$ measurement is sensitive to increases in tissue iron, which increase $R_2^*$ value (Yablonskiy and Haacke 1994); and it is also sensitive to increases in the amount of tissue water, which reduces the $R_2^*$ and may mask changes in iron content. Our DTI findings in HD patients indicate tissue damage during disease progression, which would lead to higher levels of water. The increase in water could be enough to lower the $R_2^*$ level so that iron measurements between patients and controls are not different even though the tissue microstructure is very different.

Conclusions
In this study, we have presented evidence that the CST, which is the brain’s main motor fiber, is damaged in HD patients. The CST WM tract is also damaged in the Pre-HD stage, but likely an active repair mechanism (as indicated by increased iron) helps keep the tract at a normal level. However, this repair seems to fail with disease progression and CST damage becomes extensive (as demonstrated by the alteration of all DTI parameters). Similar to what happens in striatal and cortical projection neurons, this damage may occur in a bottom-up “Dying back” fashion. Thus, the CST WM is not just abnormal in HD patients but is actively damaged by some factor closely associated with CAG repeat length before disease onset. Indeed, CAG repeat length in conjunction with age negatively affects the connectivity of the CST, which suggests there is a genetic component for connectivity. Finally, CST connectivity is correlated with motor scores, which further emphasizes the important functional role of the tract.

Supplementary Material
Supplementary material can be found at: http://www.cercor.oxfordjournals.org/.

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References


Di Paolo M, Phillips OR, Sanchez-Castaneda C, Di Pardo A, Maglione V, Caltagirone C, Sabatini U, Squitieri F. Reduced iron content in Huntington Disease corpus callosum Hum Brain Mapp, accepted.


