Dorsolateral prefrontal cortex dysfunction in presymptomatic Huntington’s disease: evidence from event-related fMRI

Robert Christian Wolf,1 Nenad Vasic,1 Carlos Schönfeldt-Lecuona,1 G. Bernhard Landwehrmeyer2 and Daniel Ecker2

1Department of Psychiatry and 2Department of Neurology, University of Ulm, Leimgrubenweg 12, 89075 Ulm, Germany
Correspondence to: Robert Christian Wolf, Department of Psychiatry III, University of Ulm, Leimgrubenweg 12-14, 89075 Ulm, Germany
E-mail: christian.wolf@uni-ulm.de

Evidence from magnetic resonance imaging (MRI) suggests early structural and functional brain changes in individuals with the Huntington’s disease (HD) gene mutation who are presymptomatic for the motor symptoms of the disorder (pre-HD subjects). The objective of this study was to investigate the functional neuroanatomy of verbal working memory (WM) in pre-HD subjects. By means of event-related functional MRI, we studied healthy controls (n = 16) and pre-HD subjects (n = 16) with a parametric WM paradigm comprising three different WM load levels. Voxel-based morphometry (VBM) was used to control potentially confounding brain atrophy. Although WM performance did not significantly differ between pre-HD subjects and healthy controls, pre-HD subjects showed a significantly decreased activation of the left dorsolateral prefrontal cortex (DLPFC) at intermediate and high WM load levels. This region was not affected by early cortical atrophy, as revealed by VBM. Pre-HD individuals close to the onset of motor symptoms showed an increased activation of the left inferior parietal lobule and the right superior frontal gyrus compared with both pre-HD subjects far from symptom onset and healthy controls. In addition, the activation level in the left DLPFC was positively correlated with the UHDRS cognitive subscore in pre-HD subjects. Our findings demonstrate that early functional brain changes in pre-HD subjects may occur in the DLPFC before manifest cortical atrophy, and support a role of this region in the expression of clinical symptoms. Compensatory brain responses in pre-HD individuals may occur with closer proximity to the onset of manifest clinical symptoms.

Keywords: Huntington’s disease; presymptomatic; working memory; prefrontal cortex; functional MRI; VBM

Abbreviations: DLPFC = dorsolateral prefrontal cortex; HD = Huntington’s disease; fMRI = functional magnetic resonance imaging; VBM = voxel-based morphometry

Received April 24, 2007. Revised July 1, 2007. Accepted August 9, 2007. Advance Access publication September 13, 2007

Introduction
Huntington’s disease (HD) is an autosomal dominant inherited neurodegenerative disorder that is characterized by progressive motor dysfunction, psychiatric disturbances and cognitive dysfunction. The neuropathological hallmark in HD is a selective degeneration of subcortical and cortical neurons. The earliest neuropathologic changes are thought to occur in the striatum, while cortical neurodegeneration becomes evident with progression of the disorder (Rosas et al., 2004). Cognitive dysfunction is considered to be intrinsic to HD, with considerable variation within the affected cognitive domains over time. An impairment of attention, concentration, visuospatial processing and certain aspects of mnemonic functions can be observed during early stages of HD (Butters et al., 1978). However, HD patients exhibit deficits in executive functioning on tests tapping planning, problem solving, cognitive flexibility and the maintenance of a cognitive set at later stages of the disorder (Butters et al., 1978; Lange et al., 1995; Paulsen et al., 1995). Over time, memory deficits become more evident, including an impairment of both short- and long-term memory, declarative as well as procedural memory. In a meta-analysis of cognitive dysfunction in HD patients, verbal and visual delayed recall have been found to be most impaired in HD, followed by tests involving executive skills, attention and concentration (Zakzanis, 1998), primarily...
suggesting a memory deficit due to frontostriatal dysfunction.

The earliest manifestations of the disorder can be investigated in subjects who carry the HD mutation, but who remain presymptomatic for the motor disturbances (presymptomatic HD, pre-HD). There is growing evidence that striatal atrophy (Kipps et al., 2007), psychiatric symptoms (Nehl et al., 2001) and changes in psychomotor performance (Snowden et al., 2002) may precede the onset of motor dysfunction by several years. Moreover, cognitive function is impaired in pre-HD subjects, predominantly involving attention, behavioural inhibition and working memory (WM) functions (Nehl et al., 2001; Paulsen et al., 2001). It has been shown that WM performance in pre-HD subjects is correlated with the estimated time to symptom onset and the manifestation of depressive symptoms (Nehl et al., 2001). However, the functional neuroanatomy underlying these deficits in pre-HD subjects is largely unknown. Previous positron emission tomography (PET) studies investigating postsynaptic dopamine D$_2$ receptor binding in pre-HD subjects have shown a correlation of $[^{11}C]$-raclopride (RAC) binding in the striatum and executive performance (Lawrence et al., 1998). Moreover, H$_2$15O-PET during performance of a motor sequence learning task demonstrated abnormally increased activation of the orbitofrontal cortex and the mediodorsal thalamus in pre-HD individuals (Feigin et al., 2006). At present, a few studies have used the methodological advantages of functional magnetic resonance imaging (fMRI) to investigate the neuronal correlates of cognitive dysfunction (Kim et al., 2004; Paulsen et al., 2004; Reading et al., 2004), showing activation differences in several cortical and subcortical regions including the striatum, the thalamus, the anterior cingulate and the prefrontal cortex in pre-HD subjects compared with healthy controls. However, these findings are potentially limited by several methodological caveats, including small sample sizes and fMRI design characteristics. For instance, block-design paradigms include the analysis of incorrectly performed trials, thus potentially biasing between-group activation differences (Walter et al., 2007). Moreover, assuming that the differences in frontostriatal activation are dynamically related to task requirements in neuropsychiatric disorders (Callicott et al., 2003; Manoach, 2003), parametrically designed tasks may be better suited to test cognitive function and associated cerebral activation in a given study population.

In this study, we chose to investigate WM function in pre-HD subjects compared to healthy controls. The neuropsychological concept of WM assumes that a limited capacity system, which temporarily maintains and stores information online, supports human thought processes by providing an interface between perception, long-term memory, executive function and actions requiring cognitive control (Goldman-Rakic, 1996; Baddeley, 2003). WM dysfunction is present during early stages of HD (Lemiere et al., 2004) and has been demonstrated in both patients with manifest HD and pre-HD subjects (Lawrence et al., 1996; Nehl et al., 2001; Montoya et al., 2006). Moreover, functional neuroimaging studies have shown that WM function and cognitive control in healthy control subjects is associated with the lateral prefrontal cortices and the striatum (Manoach et al., 1997; Rypma and D'Esposito, 1999; Wolf and Walter, 2005), while manipulation processes and increasing WM load are particularly associated with additional bilateral striatal and dorsolateral prefrontal input (Wolf and Walter, 2005). Given that frontostriatal circuits are vulnerable in both HD patients and pre-HD subjects, potential cognitive markers loading functionally on these brain regions should be particularly significant.

We used the advantages of event-related fMRI (Josephs and Henson, 1999) and a previously validated parametric verbal WM task known to illicit frontostriatal activity (Wolf and Walter, 2005; Wolf et al., 2006) in order to investigate WM-related activation in pre-HD subjects and healthy controls. Additionally, we sought to provide additional information about the studied pre-HD population by using a complementary behavioural assessment of other cognitive domains in addition to verbal WM. On the functional level, we were particularly interested in lateral prefrontal areas and the circuitry involving the lateral prefrontal cortex, since both structural and functional alterations within frontostriatal circuitry have been recognized to play a crucial role in the pathophysiology of the cognitive symptoms found in pre-HD and manifest HD (Montoya et al., 2006; Rosas et al., 2004). Given the non-linear relationship between cerebral activation and increasing cognitive load in healthy controls as well as in psychiatric populations (Callicott et al., 2003), we hypothesized that activation differences in prefrontal regions during the manipulation/maintenance of memoranda would preferentially occur at higher WM load levels.

Materials and methods

Subjects

We studied 16 right-handed subjects (Oldfield, 1971) who underwent genetic testing that confirmed CAG expansion of the HD gene (pre-HD subjects). Pre-HD participants with a history of other neurological disorder, learning disabilities, substance abuse or a concurrent axis I and II disorder according to DSM-IV criteria were excluded from the study. One pre-HD subject was medicated with citalopram (40 mg/d), but was not diagnosed to suffer from a major depressive episode according to DSM-IV criteria at the time of the fMRI scanning (R.C.W., N.V.). The remaining 15 pre-HD subjects were unmedicated. All pre-HD participants were screened for symptoms of manifest HD using the motor, cognitive and behavioural subscales derived from the UHDRS (Huntington Study Group, 1996); see Table 1 for a detailed description of demographics, CAG repeat length and test scores. In addition, the occurrence of depressive symptoms was rated by means of the Beck depression inventory (BDI) (Beck et al., 1961), where pre-HD subjects showed significantly increased
self-rating scores compared with healthy controls. However, the increased BDI scores in the pre-HD group were not indicative of clinical depression in the individual subject, since this was ruled out by a detailed psychiatric interview prior to scanning.

Ratings on the UHDRS motor scale confirmed that pre-HD participants were presymptomatic with low motor-abnormality scores. For all pre-HD subjects, the predicted age at the onset of motor symptoms was estimated by using the analysis previously reported by Langbehn et al. (2004) based on age and CAG repeat length. By using this model, pre-HD subjects were assigned to two subgroups based on the estimated proximity to manifest motor symptom onset, after performing a median split (median estimated time to motor symptom onset = 18.3 years). A significant difference between ‘far’ and ‘close’ pre-HD subjects was found with respect to the UHDRS behavioural score (two sample t-test, \( P < 0.05 \)). Although the mean UHDRS motor and cognitive scores and BDI differed between the two groups, this difference was not significant (Table 2). Compared with those in the far group, pre-HD subjects in the close group had a significantly larger mean CAG repeat length and less estimated years to symptom onset (\( P < 0.05 \)).

The healthy control group consisted of 16 right-handed subjects (5 female) matched for age, education and handedness. Control subjects, far and close pre-HD subjects did not differ with respect to age and years of education. Subjects with a neurological or psychiatric disorder, substance abuse or dependence were excluded. The project was approved by the local Institutional Review Board. Written informed consent was obtained from all participants following a complete description of the study.

### Cognitive tasks

#### Neuropsychological tests

A comprehensive neuropsychological test battery was administered to each subject. The battery consisted of eight tests, which assess alertness, divided attention, verbal and spatial WM, executive function and inhibition processes. Tonic and phasic alertness (tAL/pAL), as well as divided attention (DA) were measured using a computerized DA-test from a standardized test battery (Zimmermann and Fimm, 1994). Verbal and spatial WM tests included the digit and spatial span. WM maintenance functions were assessed by forward testing, and WM manipulation processes determined by backward testing (12 verbal and 9 spatial items, respectively, presented at 1 Hz). Executive function was measured using a computerized version of the Wisconsin Card Sorting Test (WCST) (Nelson, 1976). This WCST variant consisted of 48 cards and a maximum of five category switches. Inhibition was tested by a computerized version of the Stroop Word-Color Interference Test (Perlstein et al., 1998) based on randomized single trials (20 trials per colour and condition).

#### FMRI working memory task

We used a modified version Sternberg item recognition paradigm (Sternberg, 1966), which has been previously validated in healthy...
controls (Wolf and Walter, 2005; Wolf et al., 2006) (Fig. 1). During a stimulus period of 1500 ms, three capital grey letters appeared on a black screen. One, two or three of these letters would then become highlighted for 500 ms. Subjects were instructed that during a subsequent 6000 ms delay period they were to focus only on those letters which were highlighted, and to memorize the letters that followed them in the alphabet (manipulated set). By emphasizing the shifting of memoranda towards other letters of the alphabet, we thus introduced a manipulation component during the delay period. Low manipulation demand was characterized by one letter, which had to be identified as the one that followed next in the alphabet and had to be maintained for a short period of time (load level 1). Intermediate and high manipulation demands were characterized by two and three letters, respectively (load levels 2 and 3). Moreover, unlike the ‘traditional’ Sternberg item recognition paradigm, the stimulus period was held constant regardless of the WM load condition, in order to control for potentially confounding activation effects originating from the stimulus presentation phase. In the probe period of 2000 ms a lower case letter was presented, and subjects had to indicate whether this letter was or was not part of the manipulated set. The control condition displayed three grey Xs and required a stereotype button press in response to the presentation of a small x during the probe period. In this example, the probe (t) was part of the manipulated set (starting from the highlighted letters S and G).

**Functional data acquisition**

The functional data were acquired using a 3 T Magnetom ALLEGRA (Siemens, Erlangen, Germany) head magnetic resonance imaging (MRI) system. T2*-weighted images were obtained using echo-planar imaging in an axial orientation (TR = 2400 ms, TE = 35 ms, FOV = 192 mm, 64 × 64 matrix, 28 slices, slice thickness 4 mm, gap 1 mm). Stimuli were presented via LCD video goggles (Resonance Technologies, Northridge, CA, USA) and both reaction times (RT) and accuracy indices were recorded. Head movement was minimized using padded earphones. The fMRI protocol was a rapid event-related design with a pseudorandomized time jitter of 1.5 ± 0.5 TR inter-trial-interval. Trial duration was 10 s ± 2.4–4.8 s. Stimuli were pseudorandomized and counterbalanced for the relative appearance frequency of each letter per load, highlighted position and probe letter. The task design avoided the appearance of probes from recent negative trials in order to prevent proactive interference during retrieval (Jonides et al., 1998). Subjects performed three sessions in total, each including 28 trials, comprising 164 volumes (492 volumes in total). The first 8 volumes of each session were discarded to allow for equilibration effects.

**Volumetric MRI data acquisition**

As we were interested in identifying brain activation differences without the confound of local anatomical changes associated with cortical volume loss, we additionally investigated regional changes in brain volume using voxel-based morphometry (VBM); (Ashburner and Friston, 2000; Good et al., 2001). VBM is a user-independent, automated whole-brain analysis to detect structural differences between groups of subjects. This method involves voxel-wise comparisons of the probability of the presence of grey or white brain matter. VBM is an unbiased exploration of the whole brain, and it has been therefore an increasingly useful approach to the analysis of structural images of HD patients (Kassubek et al., 2005).

As for the functional data, VBM data were acquired using a 3 T Magnetom ALLEGRA (Siemens, Erlangen, Germany) head MRI system. The MRI parameters of the three-dimensional magnetization-prepared rapid gradient-echo (3D-MPRAGE) sequences were as follows: TE = 3.93 ms; TR = 2080 ms; TI = 1100 ms; FOV = 256; slice plane = axial; slice thickness = 1 mm; resolution = 1.0 × 1.0 × 1.0; number of slices = 256.

**Data analysis**

**Behavioural data analysis**

**Neuropsychological tests**

Performance measures were recorded as follows: (i) alertness (AL): mean reaction times (RT in ms) during tonic tAL (target presentation without alert) and phasic (pAL, target presentation after an alert) alertness; (ii) divided attention (DA): mean reaction times (RT in ms) of correctly identified targets and number of omitted targets (DA-om); (iii) digit span, forward and backward condition (DS-f and DS-b): number of correctly retrieved items; (iv) spatial span, forward and backward condition (SS-f and SS-b): number of correctly retrieved items; (v) WCST-p: number of perseverative errors (WCST-p), number of completed categories (WCST-cat) and adjusted switch costs following the procedure by Spitzer et al. (2001); WCST-sc, given in s; 6. Stroop-test: mean RT of correctly identified targets (Stroop-RT in ms) and error differences between incongruent and congruent conditions (Stroop-err).

Differences between controls and pre-HD subjects were assessed by calculating 13 separate t-tests (P < 0.05). In order to avoid a-error accumulation, all t-tests were Bonferroni-corrected (P < 0.0039).

**fMRI working memory task**

Task accuracy was recorded as percentage of correct responses during target and non-target trials as well as
RT of correctly performed trials. Changes in task accuracy and RT with increasing WM load were assessed separately using a repeated measures analysis of variance (ANOVA; \( P < 0.05 \)) with the factors group and load for accuracy and RT, followed by Scheffé’s test post hoc (\( P < 0.05 \)). Additionally, we performed planned \( t \)-tests for accuracy and RT for all WM conditions (\( P < 0.05 \)) between controls and pre-HD subjects.

**Analysis of functional MRI data**

**Single subject (1st level) analyses**

All functional data analyses were performed with SPM2 (Wellcome Department of Cognitive Neurology, London) and MATLAB 7.0 (MathWorks, Natick, MA, USA). The functional images were corrected for slice timing differences and for motion artefacts, then spatially normalized to the SPM2 EPI standard template of \( 3 \times 3 \times 3 \) mm\(^3 \) voxels. All images were spatially smoothed with a 9 mm full width at half maximum isotropic Gaussian kernel. Single subject analyses and group comparisons were performed within the framework of the general linear model (Friston et al., 1995a) using the canonical-hrf function as a predictor to estimate the haemodynamic response function of each event. For single subject (1st level) analyses, only correct trials were included, i.e. incorrectly performed trials were removed. These trials were pooled and used as individual regressors of no interest for each subject. We modelled stimulus and target periods as separate regressors, thus obtaining a lower degree of event correlation relative to the delay period. To test for differential activation during delay, this interval was modelled for each condition as one event occurring at the beginning of the delay phase, spanning a period of 6000 ms until the appearance of the probe letter. The images of these contrasts were entered into a fixed effects model for each subject (Friston et al., 1995b) and adjusted for global effects. Low frequency drifts were removed via a high-pass filter using low frequency cosine functions with a cut off of 137 s. High frequency drifts were removed via a Gaussian low-pass filter of 4 s. For each subject regionally specific main effects of load were calculated for the delay period of each condition (control condition, load\(_{n+1}\)) using linear contrasts.

**Between-group (2nd level) comparisons**

To account for inter-individual variance and in order to generalize inferences (Holmes and Friston, 1998), we conducted random-effects analyses on the 2nd level. Activation differences between healthy controls (\( n = 16 \)) and pre-HD subjects (\( n = 16 \)) during the delay period were assessed by an analysis of variance (ANOVA) by entering the specific contrasts for the four conditions of each subject (Wolf and Walter, 2005; Walter et al., 2007). A non-sphericity correction was conducted for all analyses. Since we found significantly higher BDI-scores in pre-HD subjects, we included this individual data as a nuisance variable. To control for potentially confounding effects of age, this information was included as 2nd nuisance variable in all analyses. For within-group comparisons, we contrasted each of the three load levels (i.e. 1, 2 or 3 manipulated letter(s)) with the control condition. For group-by-condition interactions, we calculated the following contrasts: \((\text{load} - \text{control condition})_{\text{controls}} > (\text{load} - \text{control condition})_{\text{pre-HD subjects}}\) \((\text{load}_{n+1} - \text{load}_n)_{\text{controls}} > (\text{load}_{n+1} - \text{load}_n)_{\text{pre-HD subjects}}\) and vice versa. An \( a \) priori significance threshold of \( P < 0.001 \) (uncorrected at the voxel level, corrected for spatial extent at \( P < 0.05 \)) was chosen for the main effect of group and all group-by-load interactions.

For further characterization of the fMRI signal change with increasing WM load, we extracted the mean effect parameters (corresponding to the percent signal change difference relative to the control condition) per subject and WM load at the most significantly activated voxel in the prefrontal cortical region emerging from the 2nd level between-group comparisons. In order to illustrate the activation pattern in a 2nd region of interest, we further extracted the mean effect parameters in the bilateral caudate nucleus. This structure was identified using a 2nd level conjunction analysis between healthy controls and pre-HD subjects, including a regions-of-interest analysis brain mask (Maldjian et al., 2003).

Additionally, we explored brain activation differences between far and close pre-HD subjects (\( n = 8 \), respectively) and healthy subjects (\( n = 16 \)) using the same analysis of covariance (ANCOVA) model and the contrasts described above. Due to the reduction of the sample size, a less conservative significance threshold of \( P < 0.005 \), uncorrected at the voxel level with a spatial contiguity criterion of 25 voxels (Forman et al., 1995), was chosen for the main effect of group and all group-by-load interactions. We report all anatomical regions and denominations according to the atlases of Talairach and Tournoux (1988) and Duvernoy (1999). Coordinates are maxima in a given cluster according to the standard MNI-template. Given the dearth of current research examining clinical correlates with neuronal functioning in pre-HD subjects, we conducted exploratory correlation analyses between CAG repeat length, estimated time to symptom onset and UHDRS subscores with the BOLD response using the appropriate scores and the extracted mean effect parameters per subject and WM load at the most significantly activated voxel in the cortical regions emerging from the between-group comparisons.

**Analysis of volumetric MRI data**

All VBM data analyses were performed with SPM2 and MATLAB 7.0. The images were analysed using the optimized VBM methods, as previously described in detail by Ashburner and Friston (2000) and Good et al. (2001). We used an extension of SPM2, the VBM tools written by C. Gaser (http://dbm.neuro.uni-jena.de/vbm). In brief, a study-specific whole brain template and grey/white matter
prior images were created from all participants. Using these customized template and priors, each participant’s original image was spatially normalized and segmented into grey and white matter, according to the optimized VBM protocol. The images were resliced with $1.0 \times 1.0 \times 1.0 \text{ mm}^3$ voxels. This procedure yielded modulated and unmodulated types of grey and white matter images. Modulated data were used for the group comparison of voxel-wise grey matter volume differences (GMV), i.e. for comparisons of an absolute amount of tissue type within a region (Ashburner and Friston, 2000). The resulting GM images were smoothed with a Gaussian kernel of 8 mm full width at half maximum (FWHM), on which all between-group analyses were performed. We used volumetric VBM to make the results more comparable with the extant literature. To identify the brain regions of GMV reduction in pre-HD subjects relative to the healthy controls, ANCOVA were performed using SPM2. The global GM volumes were included as a nuisance covariate in all analyses. These analyses yielded statistical parametric maps (SPMs), based on a voxel-level height threshold of $P < 0.001$ (family wise error corrected for multiple comparisons). As for the functional data, all anatomical regions and denominations are reported according to the atlases of Talairach and Tournoux (1988) and Duvernoy (1999). Coordinates are maxima in a given cluster according to the standard MNI-template implemented in SPM2.

**Results**

**Behavioural results**

**Neuropsychological results**

Compared with healthy controls, pre-HD subject’s task performance was worse during verbal WM maintenance, as measured by the digit span. However, this difference did not survive the Bonferroni correction for multiple comparisons. No differences were found for variables measuring, alternness, divided attention, verbal WM manipulation, spatial WM, card sorting and behavioural inhibition (see Table 3 for detailed results of the statistical analysis). When contrasted separately, far and close pre-HD subjects did not significantly differ from healthy controls on any cognitive domain. Moreover, far and close participants did not significantly differ from each other on any cognitive task; however, there was a trend for increased RT during the pAL task in the close pre-HD group (238.8 ± 29.7 ms versus 211.5 ± 25.1 ms; $P < 0.07$).

**FMRI working memory task**

In both groups, we found increasing RT with increasing load ($F(3, 90) = 217.59, P = 0.0001$); Fig. 2. Pre-HD subjects were slower than healthy controls; however, this difference was not significant ($F(1, 30) = 1.3785, P = 0.2496$). A significant group by load interaction was not found ($F(3, 90) = 0.7573, P = 0.5210$). For accuracy, we observed a significant linear decline in accuracy with increasing load in both groups ($F(3, 90) = 32.947, P = 0.0001$); Fig. 2. Pre-HD subjects exhibited slightly lower accuracy than healthy controls; however, this difference did not reach statistical significance ($F(1, 30) = 0.9273, P = 0.3433$). A significant group by load interaction was not found ($F(3, 90) = 1.0737, P = 0.3644$). Although close pre-HD subjects showed a trend to increased RT and worse task accuracy compared to far pre-HD subjects, this difference did not reach statistical significance with respect to both accuracy ($F(1, 14) = 1.8268, P = 0.1979$) and RT $F(1, 14) = 0.6513, P = 0.4331$) across all WM load levels.

**Table 3** Results of the neuropsychological assessment (two sample t-test, $P < 0.05$)

<table>
<thead>
<tr>
<th>Test</th>
<th>Healthy controls ($n = 16$)</th>
<th>Pre-HD subjects ($n = 16$)</th>
<th>Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean SD</td>
<td>Mean SD</td>
<td>T-value</td>
</tr>
<tr>
<td>tAL</td>
<td>220.0 ms 296</td>
<td>226.6 ms 28.5</td>
<td>-0.54</td>
</tr>
<tr>
<td>pAL</td>
<td>215.0 ms 35.9</td>
<td>225.2 ms 30.1</td>
<td>-0.87</td>
</tr>
<tr>
<td>DA</td>
<td>636.2 ms 84.4</td>
<td>649.5 ms 71.4</td>
<td>-0.48</td>
</tr>
<tr>
<td>DA-om</td>
<td>0.7 0.9</td>
<td>1.4 1.4</td>
<td>-1.56</td>
</tr>
<tr>
<td>DS-f</td>
<td>10.4 1.7</td>
<td>9.0 2.2</td>
<td>2.05</td>
</tr>
<tr>
<td>DS-b</td>
<td>7.9 1.7</td>
<td>6.9 3.0</td>
<td>1.38</td>
</tr>
<tr>
<td>SS-f</td>
<td>8.1 1.3</td>
<td>7.2 2.3</td>
<td>1.13</td>
</tr>
<tr>
<td>SS-b</td>
<td>7.2 1.4</td>
<td>6.9 2.6</td>
<td>0.49</td>
</tr>
<tr>
<td>WCST-P</td>
<td>0.6 1.3</td>
<td>0.5 1.1</td>
<td>0.16</td>
</tr>
<tr>
<td>WCST-cat</td>
<td>6.0 0.0</td>
<td>5.7 1.0</td>
<td>1.07</td>
</tr>
<tr>
<td>WCST-sc</td>
<td>2.4s 2.0</td>
<td>2.1 1.4</td>
<td>0.55</td>
</tr>
<tr>
<td>Stroop-RT</td>
<td>978 ms 58.7</td>
<td>75.6 ms 73.5</td>
<td>0.95</td>
</tr>
<tr>
<td>Stroop-err</td>
<td>2.7 2.5</td>
<td>2.4 3.6</td>
<td>0.29</td>
</tr>
</tbody>
</table>

tAL = tonic alertness; pAL = phasic alertness; DA = divided attention; DS-f = digit span, forward condition; SS-f = spatial span, forward condition; WCST-P = number of perseverative errors; WCST-sc = switch costs; WCST-cat = number of achieved categories; Stroop-RT = Stroop-effect, reaction time; Stroop-err = Stroop-effect, number of errors. See text for a detailed description of the cognitive tasks, the statistical analysis and significance levels.

* indicates that this difference did not survive the Bonferroni correction for multiple comparisons.
Functional imaging results

Load effects within groups

Both groups showed a main effect of WM load in a widely distributed cortical and subcortical network including the bilateral dorsolateral prefrontal cortex (DLPFC; (Brodmann areas (BA) 9, 46), ventrolateral prefrontal cortex (VLPFC; BA 44, 45, 47), the left anterior prefrontal cortex (BA 10) the premotor cortex, the supplementary motor area, the bilateral parietal cortex, the bilateral striatum and the cerebellum (figures and detailed coordinates are available on request).

Between-group differences

The 2nd level ANCOVA (healthy controls \( n = 16 \), pre-HD subjects \( n = 16 \)) revealed a significant main effect of group and WM load in the left middle frontal gyrus (BA 9; \( x = -36, \ y = 30, \ z = 36; \ Z = 5.47 \)) only. Group-by-load interaction contrasts showed no activation differences between healthy controls and pre-HD subjects at load level 1 (L1), i.e. when subjects manipulated one letter. At load level 2 (L2), pre-HD individuals showed decreased activation of the left middle frontal gyrus (BA 9) only. At load level 3 (L3), this left dorsolateral prefrontal region was again confirmed to be significantly less activated in pre-HD subjects than in healthy controls (Fig. 3, Table 4). The left middle frontal gyrus was the only brain region showing any between-group activation differences in these analyses.

As shown by the mean activation level in this region, both groups exhibited a linear activation relationship with increasing WM demand. However, pre-HD subjects exhibited a significantly lower degree of brain activation across all WM load levels (Fig. 3). The contrasts (pre-HD subjects > controls) did not yield any significant activation differences at the initially chosen threshold of \( P < 0.001 \). Furthermore, as shown by the mean activation level in the bilateral caudate nucleus, both groups showed a linear relationship between WM load and brain activation (Fig. 6, supplementary data available online). Decreased activation of the striatum in the pre-HD group was not found, even when the significance threshold was lowered to \( P < 0.005 \), uncorrected for spatial extent.
Group-by-load interaction contrasts for healthy controls, far and close pre-HD subjects revealed the following activation differences: Compared with both far and close pre-HD subjects, the control group showed an increased activation of the left middle frontal gyrus both at L2 and L3. Compared with healthy controls, close pre-HD subjects showed an increased activation of the left inferior parietal lobule (BA 40) and the right superior frontal gyrus (BA 8) at L2 and L3. Compared with far pre-HD subjects, close pre-HD subjects showed significantly more activation of the left inferior parietal lobule (BA 40) at L2. At L3, close pre-HD subjects showed significantly more activation of the left inferior parietal lobule (BA 40) and the right superior frontal gyrus (BA 8) compared with far pre-HD subjects (Fig. 4; see also Table 4 for details on stereotaxic coordinates and Z-values). The group-by-load interaction contrasts (far pre-HD subjects > controls) and (far pre-HD subjects > close pre-HD subjects) did not yield any significant activation differences.

**Correlations with clinical measures**

A significant positive correlation was found between the activation size in the left DLPFC (BA 9; x = −36, y = 30, z = 36) at load level 3 and the UHDRS cognitive score (r = 0.515, P < 0.05; Fig. 5). Using data brushing and Grubbs’ test (P < 0.05), we identified one potential outlier, which was subsequently removed in a recalculation of this analysis. However, the correlation between prefrontal activation and the UHDRS cognitive score remained significant (r = 0.521, P < 0.05). There were no significant results when correlating the estimated years to symptom onset, the BDI score and the UHDRS motor and behavioural scores with the activation size in the left DLPFC.

**Table 4** Brain regions showing activation differences between healthy controls and pre-HD subjects during the delay period

| Table 4 Brain regions showing activation differences between healthy controls and pre-HD subjects during the delay period |
|---|---|---|---|---|---|---|---|
| Controls > all pre-HD subjects<sup>a</sup> | | | | | | | |
| Anatomical region | x | y | z | Z | Number of activated voxels | |
| Load level 2 | Left middle frontal gyrus (BA 9) | −36 | 30 | 33 | 4.32 | 51 |
| Load level 3 | Left middle frontal gyrus (BA 9) | −36 | 30 | 36 | 5.22 | 101 |
| Controls > far pre-HD subjects<sup>b</sup> | | | | | | | |
| Anatomical region | x | y | z | Z | Number of activated voxels | |
| Load level 2 | Left middle frontal gyrus (BA 9) | −36 | 33 | 33 | 3.86 | 83 |
| Load level 3 | Left middle frontal gyrus (BA 9) | −36 | 33 | 33 | 4.14 | 96 |
| Controls > close pre-HD subjects<sup>b</sup> | | | | | | | |
| Anatomical region | x | y | z | Z | Number of activated voxels | |
| Load level 2 | Left middle frontal gyrus (BA 9) | −36 | 27 | 33 | 4.06 | 25 |
| Load level 3 | Left middle frontal gyrus (BA 9) | −36 | 27 | 33 | 4.24 | 91 |

Results of the between-group ANCOVA, group-by-load interaction effects. <sup>a</sup>P < 0.001 (uncorrected at the voxel level, P < 0.05 corrected for spatial extent). <sup>b</sup>P < 0.005 (uncorrected at the voxel level, spatial contiguity criterion of 25 voxels). X, y and z are Talairach coordinates of the most significant centre of activation within an activated cluster. Z = Z-value; BA = Brodmann area.
Differences in grey matter volume between pre-HD subjects and healthy controls

No grey matter volume changes in either direction were found at the chosen threshold of P < 0.001 (corrected for multiple comparisons). For exploratory purposes, we lowered the significance threshold to P < 0.005. At this level of significance, reduced grey matter volume was found in the right caudate nucleus, the right putamen, the anterior cingulate cortex (BA 32) and the right inferior parietal lobule (BA 40) in the pre-HD group compared to healthy controls. Grey matter volume changes in the left middle frontal gyrus (BA 9) were not detected.

Discussion

In this study we investigated attention, WM and executive function, as well as WM-related brain activation in pre-HD subjects using event-related fMRI. With respect to attention, verbal and spatial WM, executive function and inhibition, pre-HD subjects showed an unimpaired pattern of task performance compared with healthy controls. Although fMRI task performance did not significantly differ between pre-HD subjects and healthy controls, pre-HD subjects exhibited significantly lower task-related activation in the left DLPFC with increasing WM demand. Activation in this region was unbiased by cortical atrophy changes as revealed by VBM, and predicted cognitive performance as assessed by the UHDRS cognitive subscore.

A number of previous studies have addressed the question whether cognitive deficits are present in pre-HD subjects before overt motor symptoms (Lawrence et al., 1996; Snowden et al., 2002). However, the variability in cognitive performance in pre-HD subjects has been highlighted (Lundervold and Reinvang, 1995), while manifest cognitive deficits may occur only in pre-HD subjects approaching the motor symptom onset of the illness. In our study, pre-HD individuals were not significantly impaired with regard to attention, verbal and spatial WM, executive function and inhibition, nor did they differ from healthy controls with respect to RT and accuracy during the fMRI task. Thus, in terms of cognitive function, we studied a well preserved pre-HD sample with a median estimated time to motor symptom onset of 18.3 years, where manifest WM and executive deficits might not be present at this early stage. Currently available data further suggests that cognitive impairment may not evolve uniformly: while subtle changes in psychomotor speed may be present several years before the clinical onset, deficits in WM and executive function develop close to the time to motor symptom onset (Nehl et al., 2001; Snowden et al., 2002). However, the pattern of cognitive deficits in pre-HD subjects may vary with time, as shown by previous longitudinal studies on cognitive function in pre-HD subjects. In a study by Lemiere and co-workers, pre-HD were impaired in tests measuring verbal and spatial span, learning and delayed recall as well as conditional associative learning, indicating that some cognitive functions may deteriorate over time, while other cognitive domains do not significantly deteriorate prior to motor symptom onset (Lemiere et al., 2004). Thus, the current pre-HD sample warrants further investigation over time.
Although striatal degeneration is considered to be the neuropathological hallmark of HD, recent studies suggest that brain degeneration in HD extends beyond the striatum, involving extrastriatal and cortical regions in presymptomatic and early stages of the disease (Kassubek et al., 2005; Rosas et al., 2005; Paulsen et al., 2006; Rosas et al., 2006). The involvement of cortical regions in HD has been of particular interest, since huntingtin protein aggregates have been found to concentrate in cortical neurons (Ferrante et al., 1997; Sapp et al., 1997; Gutekunst et al., 1999) to a greater extent than in striatal regions. In pre-HD individuals, pathology of the frontal cortex has been shown to occur with regard to cellular morphology (DiProspero et al., 2004), cortical thickness (Rosas et al., 2005) and metabolism (Ciarmiello et al., 2006). More recently, FMRI has been previously used to probe both cortical and subcortical function in pre-HD subjects, suggesting that activation of lateral and orbitofrontal regions might be altered in this population (Paulsen et al., 2004; Reading et al., 2004). In the first fMRI study in 7 pre-HD subjects, Reading et al. (2004) found hypoactivation of the left anterior cingulate cortex (BAs 24 and 32) in pre-HD subjects despite unimpaired task performance, as well as decreased volume of the caudate nucleus. In conjunction, left anterior cingulate dysfunction was discussed as a specific abnormality of corticostriatal circuits in pre-HD individuals, which may be evident several years before the onset of motor symptoms. Moreover, several prefrontal regions including the bilateral middle frontal gyrus have been reported to show a reduced activation in both pre-HD subjects and HD patients during performance of a serial RT (Kim et al., 2004).

In our study, left DLPFC hypoactivation in the pre-HD group was found with increasing WM load, i.e. at load levels 2 and 3, which required an increasing manipulation of verbal stimuli. This prefrontal region was the only cortical area showing activation abnormalities over the entire sample despite unimpaired task performance. The left DLPFC is part of a WM-related neuronal network associated with manipulation and maintenance of memoranda (D’Esposito et al., 1998; D’Esposito et al., 2000; Wolf and Walter, 2005). Apart from its role in the central executive subsystem of WM (Baddeley, 2003), the DLPFC has been implicated in the exerion of cognitive control, in the sustaining of cognitive capacity and in the regulation of impulsive behaviour (Miller and Cohen, 2001; Finn et al., 2002). Our results further support a role of the left DLPFC in the expression of clinical symptoms, since hypoactivation of this region predicted cognitive achievement, as measured by the UHDRS cognitive subscore. Interestingly, lower activity of the left DLPFC was also found in virtually the same prefrontal region in an independent HD patient sample studied with fMRI and the cognitive activation task employed in this study (Wolf et al., unpublished data), suggesting that along with overt symptoms of HD, left DLPFC hypoactivation might increasingly have an impact on manifest executive and WM dysfunction in early and advanced stages of HD.

Of note, decreased activation of the left DLPFC in pre-HD individuals occurred only at intermediate and high WM processing levels, and not during the low WM condition, thus providing evidence for a functional deficit of the left DLPFC related to increasing cognitive demand. Moreover, aberrant function of the left DLPFC in our study occurred in a very early pre-HD group (median estimated time to motor symptom onset = 18.3 years), suggesting that a functional impairment of this region may occur prior to overt atrophy, or to atrophy processes below the detection threshold of VBM. The finding of decreased left DLPFC activation along with relatively unimpaired task performance further suggests an early neuronal signature of cortical dysfunction in pre-HD subjects prior to manifest motor symptoms, psychiatric symptoms and manifest cognitive impairment.

Previous fMRI studies in pre-HD have shown both decreased (Reading et al., 2004) and increased (Paulsen et al., 2004) activation of the cingulate cortex in pre-HD subjects, without reporting functional abnormalities of the DLPFC. However, this may be due to sample size differences, or due to the fact that these studies tested other cognitive domains than WM function. Nevertheless, lower DLPFC activity in pre-HD subjects has been found in a recent study investigating motor sequence learning (Feigin et al., 2006), suggesting that DLPFC dysfunction in pre-HD might be related to mnemonic control processes subserved by this prefrontal area. However, a domain-specific impairment of cortical regions in pre-HD individuals has not been directly investigated at present, and has to be elucidated in future studies.

The precise neuronal mechanisms of DLPFC dysfunction in pre-HD individuals are unclear at this stage of research. It has been shown that subtle deficits in cortical neurophysiology and morphology can precede the occurrence of clinical symptoms and neuronal degeneration in both animal models (Laforet et al., 2001; Hickey and Chesselet, 2003) and humans (Gutekunst et al., 1999). Moreover, recent data in pre-HD individuals suggest early metabolic changes (Ciarmiello et al., 2006) and altered membrane turnover (Gomez-Anson et al., 2007) of the frontal cortex. Furthermore, post-mortem studies indicate that loss of synaptic proteins in prefrontal BA 9 may at least partially contribute to aberrant neurotransmission of this region (DiProspero et al., 2004). Yet in a neurodegenerative disease eventually leading to cell death, even subtle alterations of frontal metabolism and neurotransmission may disrupt lateral prefrontal circuitry and prefrontal BOLD response in pre-HD subjects at an early stage, leading to hypoactivation of the left DLPFC during cognitive performance.Interestingly, both far and close pre-HD subjects exhibited lower activation of the left DLPFC at intermediate and high WM load levels, indicating that aberrant activation of this region does not differentiate...
Prefrontal cortex dysfunction in presymptomatic HD

Brain (2007), 130, 2845–2857

between far and close pre-HD individuals at this stage. We speculate that prefrontal dysfunction in pre-HD might occur early during presymptomatic stages of the disease, being persistent over time, possibly worsening with striatal degeneration. This hypothesis is consistent with research suggesting that huntingtin is expressed throughout brain development (Bhide et al., 1996), and with findings indicating that the pathologic process in HD develops linearly from birth (Penney et al., 1997). However, the precise physiologic properties of prefrontal dysfunction and their impact on prefrontostriatal circuits still need further clarification, and cannot be derived from the data provided by this study.

Along with subcortical hypoactivation, increased activation of cortical regions in pre-HD subjects compared with healthy controls has been demonstrated in several cognitive activation studies using both PET (Feigin et al., 2006) and fMRI (Reading et al., 2004). In a recent PET study for instance, activation responses in pre-HD were abnormally increased in the left mediodorsal thalamus and the orbitofrontal cortex individuals during sequence learning (Feigin et al., 2006). Although striatal activation differences were not observed in pre-HD subjects, the authors hypothesized that an enhanced activation of these pathways might compensate for caudate degeneration and for an impairment of lateral prefrontal-striatal circuits. In a previous fMRI study, the observation of simultaneous hyperactivation of the cingulate cortex along with striatal activation has been interpreted as compensatory cortical activation in far pre-HD subjects, in contrast to pre-HD individuals, which were close to motor symptom onset (Paulsen et al., 2004). In our study however, although the caudate nucleus exhibited subtle grey matter alterations, bilateral activation of the caudate nucleus was similar between healthy controls and pre-HD individuals. Moreover, both far and close pre-HD subjects did not differ from controls with respect to activation of the caudate, indicating that both groups were primarily characterised by cortical dysfunction. This was true even when the significance threshold was lowered to P < 0.01.

In this study, close pre-HD subjects showed an increased activation of the left inferior parietal lobule and the right superior frontal gyrus compared with both far pre-HD subjects and healthy control participants. Increased parietal, as well as superior frontal activation was found at intermediate high load WM levels, i.e. at load levels 2 and 3. Of note, both pre-HD groups showed similar accuracy compared with healthy controls during the fMRI task, suggesting that even in the presence of abnormal prefrontal and parietal activation, pre-HD individuals were able to maintain normal cognitive performance levels. It has been previously suggested (Reading et al., 2004; Rosas et al., 2004) that with progressive neuronal degeneration, pre-HD individuals may increasingly rely on neuronal compensation mechanisms in order to maintain optimal cognitive performance. Our finding of inferior parietal hyperactivation in close pre-HD subjects, but not in far pre-HD individuals, are in accordance with previous results showing hyperactivation of the parietal cortex in both pre-HD (Feigin et al., 2006) and HD patients (Dierks et al., 1999; Georgiou-Karistianis et al., 2007). The inferior parietal lobule is known to be involved in WM storage processes (Baddeley, 2003), and may have been used to a greater extent in close pre-HD subjects in order to achieve a task performance similar to healthy controls. However, aberrant activation in frontoparietal areas in pre-HD subjects does not necessarily imply an isolated cortical pathology of these regions at this stage, since the DLPFC is interconnected with ventrolateral prefrontal, parietal, and striatal regions (Alexander et al., 1986). It has been shown that white matter alterations occur early in pre-HD (Paulsen et al., 2006; Rosas et al., 2006), which might impair both functional and anatomical connectivity. Indeed, a recent fMRI study demonstrated that compromised functional interactions between prefrontal regions in HD patients (Thiruvady et al., 2007). However, the hypothesis of disrupted frontoparietal and frontostriatal connectivity in pre-HD subjects was not tested in our study and remains to be elucidated by further research.

In conclusion, we have demonstrated a load-dependent hypoactivation of the left DLPFC in pre-HD subjects with well-preserved cognitive function. As shown by VBM, left DLPFC activation differences were not biased by volumetric changes in this region. Left DLPFC hypoactivation was associated with lower UHDRS cognitive scores, suggesting that left DLPFC function may represent an early neurobiologic marker of neuronal dysfunction in pre-HD subjects. Longitudinal assessment of presymptomatic carriers of the HD gene mutation may allow the monitoring of prefrontal and parietal changes over time, and their relationship to the onset of manifest clinical symptoms.

Supplementary material
Supplementary material is available at Brain online.

Acknowledgements
This study was supported by the European Huntington’s Disease Network (EHDN) and by PREDICT HD. The authors would like to thank Georg Grön (Department of Psychiatry III, Ulm) for insightful comments during the preparation of this manuscript. We are grateful to Beate Englet and Sebastian Satzinger for their assistance with data collection.

References


Walter H, Vasic N, Höse A, Spitzer M, Wolf RC. Working memory dysfunction in schizophrenia compared to healthy controls and patients with depression: evidence from event-related fMRI. NeuroImage 2007; 1551–61.


