Functional Magnetic Resonance Imaging of Working Memory in Huntington's Disease: Cross-Sectional Data From the IMAGE-HD Study

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Abstract: We used functional magnetic resonance imaging (fMRI) to investigate spatial working memory (WM) in an N-BACK task (0, 1, and 2-BACK) in premanifest Huntington's disease (pre-HD, $n = 35$), early symptomatic Huntington's disease (symp-HD, $n = 23$), and control ($n = 32$) individuals. Overall, both WM conditions (1-BACK and 2-BACK) activated a large network of regions throughout the brain, common to all groups. However, voxel-wise and time-course analyses revealed significant functional group differences, despite no significant behavioral performance differences. During 1-BACK, voxel-wise blood-oxygen-level-dependent (BOLD) signal activity was significantly reduced in a number of regions from the WM network (inferior frontal gyrus, anterior insula, caudate, putamen, and cerebellum) in pre-HD and symp-HD groups, compared with controls; however, time-course analysis of the BOLD response in the dorsolateral prefrontal cortex (DLPFC) showed increased activation in symp-HD, compared with pre-HD and controls. The pattern of reduced voxel-wise BOLD activity in pre-HD and symp-HD, relative to controls, became more pervasive during 2-BACK affecting the same structures as in 1-BACK, but also incorporated further WM regions (anterior cingulate gyrus, parietal lobe and thalamus). The DLPFC BOLD time-course for 2-BACK showed a reversed pattern to that observed in 1-BACK, with a significantly diminished signal in symp-HD, relative to pre-HD and controls. Our findings provide support for functional brain reorganisation in cortical and sub-cortical regions in both pre-HD and symp-HD, which are modulated by task difficulty. Moreover, the lack of a robust striatal BOLD signal in pre-HD may represent a very early signature of change observed up to 15 years prior to clinical diagnosis. *Hum Brain Mapp* 35:1847–1864, 2014. © 2013 Wiley Periodicals, Inc.

Key words: working memory; Huntington's Disease; fMRI; DLPFC; N BACK

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INTRODUCTION

Working memory (WM) is one of the first cognitive functions to decline in Huntington's disease (HD) [Lawrence, et al., 1996, 1998; Lemiere, et al., 2002, 2004]. WM allows individuals to temporarily retain information in an active state and manipulate and recall this information for a limited period of time after storage [Baddeley, 1992]. The network of brain structures underpinning WM is well established, and includes fronto-parietal and striatal areas, supported by activity in the thalamus, insula, cingulate, and temporal gyri, as well as cerebellum [Ben-Yehudah et al., 2007; Callicott et al., 2003; Hester et al., 2004; McNab and Klingberg, 2008; Rypma and D'Esposito, 1999; Wolf and Walter, 2005]. Several studies have reported impairment in WM tasks in both premanifest HD (pre-HD) [Lemiere et al., 2002; Nehl et al., 2001] and early symptomatic HD (symp-HD) [Lawrence et al., 1996, 2000] participants, compared with controls.

Functional magnetic resonance imaging (fMRI) has gained impetus as a tool to investigate the neural correlates underlying cognitive domains known to be affected in HD (for reviews see Bohanna et al., 2008; Georgiou-Karistianis, 2009; Paulsen, 2009). In particular, Wolf et al. have conducted a number of elegant studies investigating the neural correlates of WM dysfunction in both pre-HD and symp-HD. For example, in the study by Wolf et al. [2007], hypo-activation of the left dorsolateral prefrontal cortex (DLPFC) was shown during performance of an event-related verbal WM task in pre-HD ($n = 16$), compared with controls ($n = 16$). Longitudinally, however, Wolf et al. [2011] reported no further loss of DLPFC activity in pre-HD. A further study investigated WM-related patterns of functional connectivity in pre-HD and controls, and revealed reduced connectivity in two distinct fronto-striatal and fronto-parietal networks in pre-HD [Wolf et al., 2008b]. In a subsequent study, reduced connectivity related to WM was also found to extend beyond fronto-striatal circuitry to include reduced connectivity in a left lateralized fronto-parietal network [Wolf et al., 2008a]. Wolf et al. [2009] further documented that as the disease progresses into manifest stages ($n = 12$ symp-HD), lower task-related activation in the left DLPFC, left inferior frontal gyrus, bilateral parietal cortex and the bilateral putamen is observed with increased WM load [Wolf et al., 2009], supporting a more widespread network of dysfunction in HD beyond fronto-striatal circuitry.

HD-related neuropathology, which most likely underlies WM dysfunction, is increasingly well described. For example, volume loss has been reported before and after onset of symptoms in the anterior cingulate gyrus, parietal lobe and striatum [Aylward et al., 2000, 2004; Douaud et al., 2006; Georgiou-Karistianis et al., 2013; Hobbs et al., 2009; Kassubek et al., 2004; Paulsen et al., 2006; Rosas et al., 2005; Rosas et al., 2008; Tabrizi et al., 2009], and the degree of atrophy is likely to impact the blood-oxygen-level-dependent (BOLD) signal response initially in pre-HD as part

of a compensatory response [Paulsen, 2009; Wolf et al., 2009]. Moreover, Sotrel et al. [1991] and Selemon et al. [2004] found cortical thinning of the DLPFC in symp-HD, while Rosas et al. [2005] reported that cortical loss of the DLPFC and anterior cingulate gyrus was associated with poorer performance on the Stroop Word test in pre-HD. Hobbs et al. [2011] reported an association between decreased posterior cingulate volume and worse performance on a visual WM task in pre-HD. White matter tracts connecting these various structures have also been shown to degenerate with disease progression [Aylward et al., 1998, 2011; Thieben et al., 2002]. Dumas et al. [2012] has observed more widespread microstructural deficits in white matter pathways of the prefrontal cortex in symp-HD. Increased diffusivity in frontal and parietal white matter, together with higher diffusivity in prefrontal afferents to the putamen has also been reported in HD [Douaud et al., 2009]. Moreover, Klöppel et al. [2008] reported reduction of DLPFC projections to posterior parts of the striatum in pre-HD, compared with controls. Other studies have reported that working memory performance in symp-HD is not associated with CAG repeat length but is strongly associated with disease duration [Finke et al., 2006].

In comparison to Wolf and colleagues, where pre-HD and symp-HD groups were studied separately, and with smaller sample sizes, here we report a cross sectional fMRI investigation of WM (via the N-BACK task) in a larger sample of both pre-HD ($n = 35$) and early symp-HD ($n = 24$) groups as part of an Australian based imaging study - IMAGE-HD (see Georgiou-Karistianis et al., 2013), a multimodal biomarker development study that complements large-scale multi-site studies [i.e., TRACK-HD – Tabrizi et al., 2011, 2012; PREDICT-HD – Paulsen et al., 2006]. Moreover, although the studies by Wolf et al. have utilized a verbal WM paradigm (consisting of letter stimuli), with a focus on lateral prefrontal areas and circuitry involving the lateral prefrontal cortex, here we sought to manipulate spatial location by presenting number stimuli (numbers 1–4) at one of four spatial locations to investigate further the functional neuroanatomy underlying these deficits. We selected this task as it has previously been shown to robustly activate the DLPFC, anterior cingulate, inferior parietal, temporal gyrus, thalamus, and striatum in healthy controls [Callicott et al., 2003]. These same regions have previously been shown to correlate with a decline in verbal WM in HD [Wolf et al., 2008a,b]. Our task provided an opportunity to not only validate and further investigate the underlying neuropathology of WM in HD, but to also extend the work by Wolf et al. to the spatial domain. We hypothesized that there would be no behavioral performance differences between the groups based on our predefined accuracy threshold (i.e., $\geq 70\%$). In the absence of behavioral differences, we hypothesized that pre-HD participants compared with controls, would show significant differences in the pattern of BOLD activation in caudate, putamen, anterior cingulate cortex, inferior parietal cortex and DLPFC, with either increased and decreased activation in these regions; symp-HD participants would

demonstrate more extensive widespread increased and decreased changes in the BOLD activation patterns than the pre-HD participants. We also sought to examine for the first time BOLD time-course data that enabled an investigation of possible differences in the temporal dynamics of activation between groups across the ROIs.

METHODS

Participants

A total of 108 participants were originally recruited as part of IMAGE-HD (2008–2009), consisting of 36 early diagnosed symp-HD, 36 pre-HD and 36 healthy controls. As part of the complete protocol all individuals underwent a battery of HD-sensitive neurocognitive assessments and a subsequent imaging session, including MRI and fMRI acquisition. For the current study 17 participants were excluded, including 4 controls, 1 pre-HD and 12 symp-HD, on the basis of the following criteria: i) failure to perform

with $\geq 70\%$ behavioral accuracy during the 1-BACK condition [$n = 3$ controls (average accuracy = 48.9%); $n = 1$ pre-HD (average accuracy = 25%); $n = 9$ symp-HD (average accuracy = 36.3%)] (note all errors were made within the response time window), ii) movement of greater than 2 mm during the fMRI scan ($n = 3$ symp-HD), and iii) claustrophobia ($n = 1$ symp-HD). The sample therefore consisted of 90 participants (all able to perform the 1-BACK condition with $\geq 70\%$ accuracy), consisting of 32 healthy controls (11 males, 21 females; mean age = 42.1 ± 2.3), 35 pre-HD (14 males, 21 females; mean age = 41.8 ± 1.7) and 23 symp-HD (15 males, 8 females; mean age = 50.9 ± 1.6) participants. Demographic and clinical details for the 90 participants are included in Table I. Healthy controls were matched for age, gender and IQ (National Adult Reading Test 2nd edition, NART-2 [Nelson et al., 1992], an estimate of premorbid intellectual ability) to the pre-HD group. One-way ANOVAs revealed no significant differences in age or IQ scores between the pre-HD and controls. Moreover, there were no significant differences in IQ scores across any of the three

TABLE I. Demographic information, clinical measures and neurocognitive data of participants included in all 1-BACK and 2-BACK analyses

	Mean $\pm$ SD					
	1-BACK			2-BACK		
	Controls	Pre-HD	Symp-HD	Controls	Pre-HD	Symp-HD
n (sample sizes)	32	35	23	22	22	17
Gender (M:F)	11:21	14:21	15:8	10:12	10:12	11:6
Age (years)	42 ± 13 (24–72)	42 ± 10 (24–65)	$51 \pm 8^{***,+++}$ (37–70)	37 ± 8 (24–57)	42 ± 11 (24–65)	$52 \pm 8^{***,+++}$ (39–70)
IQ estimate	119 ± 10	117 ± 11	116 ± 11	122 ± 9	120 ± 10	115 ± 8
UHDRS	–	0.9 ± 1 (0–4)	$14 \pm 7^{+++}$ (6–30)	–	0.6 ± 1 (0–3)	$15 \pm 8^{+++}$ (6–30)
CAG repeats	–	42 ± 2	43 ± 2	–	42 ± 2	43 ± 2
Disease Burden Score	–	270 ± 53	$363 \pm 62^{+++}$	–	262 ± 57	$359 \pm 56^{+++}$
Estimated YTO	–	15 ± 8	–	–	17 ± 8	–
Duration of illness (years)	–	–	2.5 ± 1.5	–	–	2.3 ± 1.5
SDMT (number correct)	57 ± 10	$51 \pm 9^*$	$42 \pm 10^{***,+++}$	60 ± 10	$52 \pm 10^*$	$45 \pm 10^{***}$
STROOP (number correct)	111 ± 17	104 ± 17	$94 \pm 18^{**}$	115 ± 15	108 ± 16	$97 \pm 16^{**}$
FrSBe – total score	86 ± 25	93 ± 23	89 ± 17	85 ± 17	93 ± 19	91 ± 17
UPSIT (number correct)	34 ± 3	33 ± 5	$29 \pm 6^{***}$	35 ± 3	32 ± 6	$29 \pm 7^*$
Speeded Tapping (ms)	216 ± 38	245 ± 45	$315 \pm 78^{***,+++}$	207 ± 20	234 ± 41	$321 \pm 84^{***,+++}$
Self-paced Tapping – (1/SD ITI) 550 ms	25 ± 7	$20 \pm 8^{***}$	$12 \pm 3^{***,+++}$	27 ± 7	$21 \pm 6^{**}$	$12 \pm 3^{***,+++}$
Self-paced Tapping – (1/SD ITI) 333 ms	30 ± 8	$24 \pm 9^{***}$	$14 \pm 6^{***,+++}$	32 ± 7	$26 \pm 9^*$	$14 \pm 7^{***,+++}$
SCOPI – total OCD	83 ± 22	83 ± 25	88 ± 21	85 ± 22	86 ± 23	85 ± 21
HADS: A	5 ± 2.6	6.8 ± 3.4	5.4 ± 3.8	5.5 ± 2.7	7.3 ± 3.5	5.7 ± 3.9
HADS: D	2.5 ± 3.2	2.7 ± 3.0	2.1 ± 2.1	2.6 ± 2.3	2.6 ± 2.6	2 ± 2
BDI II	3.8 ± 3.8	$8.9 \pm 9.8^*$	6.3 ± 6.2	4.8 ± 4.8	8.3 ± 8.8	7.1 ± 6.5

SD, standard deviation; IQ, estimated full scale IQ, National Adult Reading Test (NART) error score; UHDRS, motor subscale score, Unified Huntington's Disease Rating Scale (pre-HD, UHDRS<5; symp-HD, UHDRS $\geq$ 5); CAG, cytosine-adenine-guanine (number of repeats >40 is full penetrance); Disease Burden Score (CAG-35.5)*age; YTO, years to onset; SDMT, Symbol Digit Modalities Test; STROOP, STROOP speeded word reading task (number of correct words); FrSBe, Frontal Systems Behaviour Scale; SCOPI, Schedule of Compulsions, Obsessions, and Pathological Impulses; HADS A, Hospital Anxiety and Depression scale – anxiety sub score; HADS D, Hospital Anxiety and Depression scale – depression sub score; BDI-II, Beck Depression Inventory score Version II; UPSIT, University of Pennsylvania Smell Identification Test (score out of 40); ITI, inter-tap interval. Δ Symp-HD or pre-HD versus controls:

* $P \leq 0.05$; $P \leq 0.01$; $P \leq 0.001$; Δ symp-HD versus pre-HD: $P \leq 0.01$; $P \leq 0.001$.

groups. The symp-HD group was significantly ($P < 0.01$) older than both the pre-HD and control groups. Fewer participants met the $\geq 70\%$ accuracy criterion in the high memory load condition (2-BACK). Although the sample size was reduced to 61 participants, the demographic and clinical profile remained relatively similar to the 1-BACK sample and the pattern of between-group differences remained the same. The 2-BACK sample therefore consisted of 22 controls (10 males, 12 females; mean age = 37 ± 8), 22 pre-HD (10 males, 12 females; mean age 42 ± 11) and 17 symp-HD (11 males, 6 females; mean age 52 ± 8) participants (see Table I).

All participants underwent a rigorous screening process prior to recruitment that included a comprehensive review of medical history. All participants were right handed (Edinburgh Handedness Test [Oldfield, 1971]) and were free from brain injury, neurological, and/or severe diagnosed psychiatric conditions (e.g., bipolar, psychosis), other than HD. Participants remained on their normal medication regime. Controls reported taking medications for vascular and heart conditions ($n = 2$) and selective serotonin reuptake inhibitor (SSRI) antidepressants ($n = 1$). Pre-HD participants reported taking medications for vascular and heart conditions ($n = 1$) and SSRI ($n = 4$) and noradrenergic and specific serotonergic antidepressants (NaSSA) ($n = 2$). Symp-HD participants reported taking medications for vascular and heart conditions ($n = 5$), SSRI ($n = 5$) and serotonin-norepinephrine reuptake inhibitor (SNRI) ($n = 2$) antidepressants (one participant taking SSRI was also taking a D_2/D_3 receptor antagonist), as well as anxiety/mood stabilizers ($n = 3$) and neuroleptic medications ($n = 3$). Only pre-HD and symp-HD participants underwent gene testing prior to enrolment to the study and CAG repeat length ranged from 39 to 50 (42 ± 2 for pre-HD; 43 ± 2 for symp-HD). All pre-HD and symp-HD participants were clinically assessed by a clinician (A.C. or P.C.) prior to enrolment and underwent a Unified Huntington's Disease Rating Scale (UHDRS) motor assessment. As per Tabrizi et al. (2009), inclusion in the pre-HD group required a UHDRS total motor score of ≤ 5 and the average group's estimated years to clinical onset was 15 ± 8 according to the formula based on the participant's age and number of CAG repeats on the expanded allele [Langbehn et al., 2004] with a mean disease burden score (DBS [Penney Jr et al., 1997]) of 270 ± 53 . Individuals in the symp-HD group all had a UHDRS motor score of >5 and the duration of diagnosed HD ranged from 0.42 to 5 years with a group mean DBS of 363 ± 62 .

A battery of neurocognitive tests were administered on the day of scanning that were selected based on their sensitivity in detecting differences between groups from previous large scale multi-site studies [Stout et al., 2011; Tabrizi et al., 2009]. The tests assessed visuomotor speed and attention (Symbol Digit Modalities Test, SDMT [Smith, 1982]), speeded reading (STROOP Word Test [Stroop, 1935]), odour recognition (University of Pennsylvania Smell Identification Test, UPSIT [Doty et al., 1984]) and

motor performance (speeded tapping and self-paced tapping tasks [Hinton et al., 2007; Paulsen, Zimbelman et al., 2004]). Participants completed behavioral questionnaires which included assessments of behaviors associated with frontal-striatal brain dysfunction, including executive function (Frontal Systems Behavior Scale, FrSBe [Grace and Mallory, 2001]) and psychiatric disturbances (Schedule of Obsessions, Compulsions and Psychological Impulses, SCOPI [Watson and Wu, 2005]; Hospital Anxiety and Depression Scale, HADS [Zigmond and Snaith, 1983]; Beck Depression Inventory Version II, BDI-II [Beck et al., 1996]). Scores on neurocognitive tests are shown in Table I for both 1-BACK and 2 BACK samples. Considering the 1-BACK sample, a one-way ANCOVA revealed that both pre-HD and symp-HD groups performed significantly differently ($P < 0.05$) to the control group in SDMT and self-paced tapping fast (3.00 Hz) and slow conditions (1.82 Hz). There were no significant between-group differences in HADS, SCOPI or FrSBe. Additionally, pre-HD participants scored significantly ($P < 0.05$) higher on the BDI-II, compared with controls; symp-HD participants scored significantly ($P < 0.05$) lower on STROOP ($P < 0.05$), UPSIT and speeded tapping ($P < 0.01$), compared with controls. Symp-HD also performed significantly worse on the SDMT ($P < 0.01$), UPSIT ($P < 0.05$) and speeded and self-paced tapping tasks ($P < 0.01$) compared with pre-HD individuals. For the 2-BACK participant characteristics, analysis revealed that both pre-HD and symp-HD groups performed significantly worse than controls on SDMT ($P < 0.05$ and $P < 0.001$, respectively) and self-paced tapping, fast ($P < 0.05$ and $P < 0.001$, respectively) and slow ($P < 0.01$ and $P < 0.001$, respectively) conditions. In addition, symp-HD participants scored significantly lower on STROOP ($P < 0.01$), UPSIT ($P < 0.05$) and speeded tapping ($P < 0.001$), compared with controls. Symp-HD also performed significantly ($P < 0.001$) worse than pre-HD participants on the speeded and self-paced tapping tasks.

The study was approved by the Monash University and Melbourne Health Human Research Ethics Committees and written informed consent was obtained from each participant in accord with the Helsinki Declaration.

Procedures

fMRI working memory experimental paradigm (N-BACK task)

Prior to scanning participants underwent a demonstration of the N-BACK task, followed by a series of practice trials to ensure that each participant was able to complete the task requirements. For imaging, participants were fitted with a head-coil. A mirror enabled visualization of task stimuli. Responses were made using an MR-compatible button box with four response buttons arrayed in a diamond-like orientation.

A modified version of the Callicott N-BACK paradigm (Callicott et al., 2003) was used, consisting of three

Figure 1.

Representation of the 0-BACK, 1-BACK and 2-BACK conditions and their expected responses denoted by the arrow. Participants only view a single stimulus diamond at a time. 0-BACK requires the participants to respond to the location of the number currently seen. 1-BACK requires the participants to encode the position of the current stimulus and to respond to the number seen one stimulus back. 2-BACK requires the participants to encode the position of the current stimulus and to respond to the number seen two stimuli back. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

conditions (0-BACK, 1-BACK and 2-BACK) administered in a block design with the 0-BACK interspersed between the 1-BACK and 2-BACK conditions. Each stimulus consisted of four circles arrayed in a diamond-like orientation (see Fig. 1), with one of the four circles containing a number from 1 to 4. Participants were required to respond using a diamond shaped button box by pressing the button positioned in the location containing the number (for the 0-BACK condition; no WM required), or the button positioned in the location that contained the number in the previous stimulus (1-BACK; low difficulty WM condition), or the button positioned in the location that contained the number two stimuli back (2-BACK; high

difficulty WM condition). The experiment was presented using a block design (i.e., 0-BACK, 1-BACK and 2-BACK each presented in separate blocks). The WM conditions (1-BACK and 2-BACK) were presented in 4 blocks each interspersed with the 0-BACK baseline condition (8 blocks), with each block duration of 30s. The order of presentation of each condition was randomized such that some participants were presented with four blocks of 0-BACK 1-BACK 0-BACK 2-BACK and some were presented with four blocks of 0-BACK 2-BACK 0-BACK 1-BACK. Each stimulus was presented for 750ms with an inter-stimulus interval (ISI) of 1500ms. Responses were therefore captured over the entire period encompassing stimulus presentation and ISI (total of 2250 ms).

Functional data acquisition

Structural and functional MR images were acquired with the Siemens Magnetom Tim Trio 3 Tesla MRI scanner (Siemens AG, Erlangen, Germany) and a 32-channel head coil at the Murdoch Childrens Research Institute (Royal Children's Hospital, Victoria, Australia). Echo planar images (EPI) were acquired in the axial plane (30 slices, 4mm slice thickness, 1.8mm x 1.8mm in-plane resolution, TE = 35ms, TR = 2250ms, flip angle = 90°). Two runs of 205 volumes were acquired totalling 410 volumes. High resolution T₁-weighted images were also acquired for registration (192 slices, 0.9mm slice thickness, 0.8mm x 0.8mm in-plane resolution, TE = 2.59 ms, TR = 1,900 ms, flip angle = 9°).

Data Analysis

Behavioral data analysis

Percentage of correct responses and RTs were recorded for each subject and group means and standard deviations for all groups are included in Table II. We imposed an accuracy threshold so that we could more meaningfully investigate underlying functional activation differences across groups, providing confidence that the observed neural responses actually reflect accurate WM performance. Therefore all individuals were required to meet an accuracy

TABLE II. N-BACK performance accuracy and response times (RT) for participants included in the behavioral and fMRI analyses. Median and range also included

		Mean ± SD (median, range)		
		Controls	Pre-HD	Symp-HD
	n (0-BACK, 1-BACK)	32	35	23
	n (2-BACK)	22	22	17
Accuracy (% correct)	1-BACK	93 ± 9(97, 40)	91 ± 7(93, 22)	91 ± 11(93, 55)
	2-BACK	88 ± 9(90, 29)	87 ± 9(87.5, 29)	82 ± 7(84.4, 24)
Response Time (x10 ² msec)	0-BACK	6.1 ± 1.5(5.7, 7)	6.6 ± 1.6(6, 6)	7.6 ± 1.4(7.6, 6)**
	1-BACK	5.5 ± 2.3(4.6, 8)	5.8 ± 2.5(5, 11)	6.3 ± 2.3(6, 10)
	2-BACK	5.4 ± 2.5(4.6, 9)	5.3 ± 2.7(4.6, 11)	6.3 ± 2.4(5.9, 8)

SD, standard deviation; ΔControls versus symp-HD: **P < 0.01.

threshold of $\geq 70\%$ in order to be included in any of the analyses (behavioral and fMRI). Of the 91 participants that met the accuracy cut-off for the 1-BACK condition (and were included in both the behavioral and fMRI 1-BACK versus 0-BACK analysis), a number did not meet this criterion with the more difficult 2-BACK condition and their data were excluded from the behavioral and fMRI 2-BACK versus 0-BACK analysis (see Table II for sample sizes included in each analysis). Statistical analyses to test for between group differences across each condition separately were performed using a one-way ANCOVA, covarying for age (SPSS 16.0 for Mac, Cary, NC). Bonferroni correction was used for post-hoc analyses ($P < 0.05$). Age was used consistently across all analyses as a covariate to ensure that age differences did not affect results.

fMRI spatial pre-processing

To ensure confident and reliable estimates of neural activity within groups known to differ in localized neural (particularly striatal) volume, special attention and a stringent spatial pre-processing method was applied prior to statistical modeling, using SPM8. Functional images within each session (205×2) were initially aligned and movement parameters estimated for each participant, followed by fMRI registration to individual T1 weighted scan in MNI space. Spatial normalization parameters were then estimated from T1 scans, allowing for increased degree of localized volume and shape corrections (largely to account for striatal neurodegeneration) via “SPM8 Segment” function (spatial regularization = 0.02, discrete cosine transform warp frequency cutoff of 22). This approach applies estimated tissue probability maps within a unified model additionally accounting for individual variability in neural structure and B0 field inhomogeneities [Ashburner and Friston 2005]. Functional images were then normalized to MNI space (2mm isotropic voxels) and each participant was carefully checked for normalisation accuracy. Finally, to account for small residual differences in peri-ventricular boundary between groups, all participants’ fMRI scans were masked by a probabilistic estimate of CSF and skull derived from the group averaged symp-HD baseline structural scan, ensuring all functional and structural scans were matched, voxel for voxel throughout the brain prior to statistical modelling. In the case of the caudate, we have ensured that both caudate-CSF and caudate-white matter boundaries are now matched across participants (see Supporting Information Fig. 1). Finally, functional images were smoothed with 4 mm FWHM.

fMRI statistical modeling

Statistical modeling of individual session, subject, and group responses during N-BACK were conducted with FMRIB Software Library (FSL) Expert Analysis Tool (FEAT) in FSL version 5.98 (FMRIB, Oxford, UK). Spatial pre-processing included only high-pass temporal filtering (cut off = 150s), with identity matrices generated for the required subject2standard.mat files (as all fMRI data were already nor-

malized and in MNI space). Individual session general linear models included convolved (block design) regressors for the 1-BACK and 2-BACK conditions, and for block switch instructions (with 0-BACK condition forming an unmodelled implicit baseline). Contrasts of regressors modelling neural response during 1-BACK and 2-BACK (relative to 0-BACK) were estimated for each session and participant. Estimates of N-BACK neural responses for each participant at each time-point were generated only from sessions where behavioral performance was $\geq 70\%$ accuracy, within separate 2nd level group models at each time-point. Group (3^{rd} -level) statistics were estimated while accounting for age (via a single mean centred regressor) using FMRIBS’s Local Analysis of Mixed Effects type 1 and 2 (FLAME 1+2, FSL). The resulting Z statistic images were thresholded at a significance level of $Z > 2.33$ with a cluster probability threshold of $P < 0.05$ to correct for multiple comparisons. Peak activation voxels were identified using probabilistic structure-specific masks derived from the semi-automated structural segmentation of standard T1 weighted images (i.e., Harvard-Oxford Atlas). These masks were thresholded and cropped to ensure there were no overlaps between adjacent masks. The masks comprised a broad range of regions which are represented in Tables III–VI.

Time-courses data analysis

We have included time-course data to identify whether signal change across the course of the task is different between groups. ROIs were generated from clusters showing significant between-group differences in regions known to activate the WM network. These regions comprised the anterior cingulate gyrus, insula, superior and inferior parietal lobe (specifically, posterior supramarginal gyrus), caudate, putamen, globus pallidus and thalamus. Despite the lack of significant group differences in the DLPFC in the voxel-wise analysis, we included this structure in our time-course analysis since it was strongly activated in all groups and conditions and also given its important role in WM. In all cases, except for the DLPFC, the ROIs were created by masking the activation maps of the relevant contrasts with the Harvard-Oxford Atlas derived masks used for the identification of peak activation voxels. Regarding the DLPFC we used the map of the mean activation across the three groups, which we masked with the relevant standard anatomical mask. The mean time-course for each condition and group was then estimated for all ROIs by extracting from each participant’s filtered functional spatially normalized data the percentage BOLD signal change over the duration of the task. If the voxel-wise activation map showed differences in the relevant structure bilaterally, then the time-course data were collapsed across hemispheres. The effects of Group, Time (12 levels) and their interaction were modelled using a cross-sectional time-course regression that treated subjects as random effects. The covariates of no interest in all analyses were mean-centred. In order to guard against violations of normality and homogeneity of

TABLE III. Brain regions showing significant between-group differences in BOLD signal during 1-BACK condition at $P < 0.05$ (corrected)

Regions		Controls > Pre-HD				Controls > Symp-HD			
		Peak voxel				Peak voxel			
		<i>x</i>	<i>y</i>	<i>z</i>	Z score	<i>x</i>	<i>y</i>	<i>z</i>	Z score
IFG (pars opercularis)	L	−50	12	−2	2.40	—	—	—	—
Frontal operculum	L	−48	10	−2	2.85	—	—	—	—
	R	42	14	4	3.00	—	—	—	—
Central operculum	L	−42	0	16	3.87	—	—	—	—
	R	64	−14	14	3.81	—	—	—	—
Anterior insula	R	—	—	—	—	30	20	−2	4.00
Middle-to-posterior insula	L	−42	−1−2	4	3.08	—	—	—	—
	R	38	8	6	4.09	—	—	—	—
Precentral gyrus	L	−46	2	20	3.19	−34	−24	54	3.37
Postcentral gyrus	L	−58	−10	18	3.14	−50	−28	56	4.23
	R	60	−16	22	3.46	—	—	—	—
Temporal pole	L	−50	8	−6	4.02	42	14	−20	2.95
Heschl's gyrus	L	−54	−16	6	3.67	—	—	—	—
	R	52	−10	6	2.43	—	—	—	—
Planum temporale	L	−54	−34	14	3.83	—	—	—	—
	R	56	−22	12	4.16	—	—	—	—
MTG (temporo-occipital)	L	—	—	—	—	−48	−62	0	3.15
Temporo-occipital fusiform cortex	R	28	−48	−10	3.33	—	—	—	—
Lingual gyrus	R	18	−60	−8	3.52	—	—	—	—
Lateral occipital cortex	R	—	—	—	—	34	−78	12	2.76
Caudate	L	−12	12	−4	3.90	—	—	—	—
	R	10	18	2	3.30	—	—	—	—
Putamen	L	−20	18	−8	3.35	—	—	—	—
	R	18	6	−6	3.47	—	—	—	—
Pallidum	L	−22	−4	−6	3.08	—	—	—	—
	R	16	6	−4	3.21	—	—	—	—
Cerebellum	R	—	—	—	—	28	−50	−24	4.62

The coordinates of peak voxels are in MNI space where *x*, *y*, and *z* represent the medial-lateral, anterior-posterior, and superior-inferior orientations, respectively. L, left; R, right; IFG, inferior frontal gyrus; MTG, middle temporal gyrus.

variance, regressions were bootstrapped 5000 times. Finally, a small number of extreme scores (residual ≥ 5) were removed. All ROI statistical analyses were performed in Stata 11 (StataCorp, 2009).

Volumetric analysis

We analyzed the relationship between percent BOLD signal change and the volume measurements of the caudate and putamen. These structures were segmented from the T1 images using a semi-customised procedure based on SPM8 routines, whereby standardized subcortical masks from the Harvard-Oxford Atlas were optimized to best fit the standard (MNI) whole brain template (Georgiou-Karistianis et al. 2013). Particular care was placed on accurate identification of the peri-ventricular CSF boundary in the caudate, as it is highly sensitive to HD status and progression. Individual structural scans were coregistered to MNI space, and normalization parameters were derived using the SPM8 segmentation routine with permissive warping regularisation. ROIs were then eroded using a combination of the SPM8

probabilistic spatial estimate of CSF, and the voxel intensity distributions drawn from individual CSF and WM voxels within each scan. Volume measures were generated by warping the resulting masks back into subject space. Mean volumes were then calculated for each group and compared after correcting for age and total tissue volume (gray plus white matter).

Correlation analyses

Correlations between BOLD signal change and volume were estimated after controlling for the effects of DBS and age. Moreover, partial correlations were used to explore the relationships between the mean percent BOLD signal change of ROIs and the clinical measures (i.e., UHDRS, DBS) in pre-HD and symp-HD. The ROIs were the same as in the time-course analysis, but in this case were generated by masking the mean activation maps separately from each of the 1-BACK and 2-BACK conditions across the three groups with the corresponding standard structural masks. The mean percent BOLD signal change during each

TABLE IV. Brain regions showing significant between-group differences in BOLD signal during 2-BACK condition at $P < 0.05$ (corrected)

Regions		Controls > Pre-HD				Controls > Symp-HD				Pre-HD > Symp-HD			
		Peak voxel				Peak voxel				Peak voxel			
		<i>x</i>	<i>y</i>	<i>z</i>	Z score	<i>x</i>	<i>y</i>	<i>z</i>	Z score	<i>x</i>	<i>y</i>	<i>z</i>	Z score
SMC	L	-10	-2	46	4.85	-2	-14	56	3.85	-	-	-	-
	R	8	-4	56	3.90	6	-12	62	3.07	-	-	-	-
IFG (pars opercularis)	R	-	-	-	-	52	22	10	3.41	-	-	-	-
Posterior orbitofrontal cortex	R	18	16	-16	3.46	42	22	-10	3.94	-	-	-	-
Frontal operculum	L	-42	12	0	2.79	-	-	-	-	-	-	-	-
	R	38	12	6	3.20	32	16	12	2.47	-	-	-	-
Central operculum	L	-42	0	10	4.53	-60	-10	10	3.44	-	-	-	-
	R	50	-18	20	4.59	54	-16	18	3.88	-	-	-	-
Anterior insula	R	-	-	-	-	30	14	8	3.40	-	-	-	-
Middle-to-posterior insula	L	-38	-12	-8	4.82	-36	-18	10	3.08	-	-	-	-
	R	40	-8	-10	3.75	-	-	-	-	-	-	-	-
Paracingulate gyrus	L	-4	12	42	2.93	-2	10	44	2.57	-	-	-	-
	R	8	16	40	3.30	6	18	38	3.12	-	-	-	-
Anterior cingulate gyrus	L	-8	-2	46	4.44	-2	0	44	4.06	-	-	-	-
	R	10	12	34	4.39	10	14	34	5.35	-	-	-	-
Posterior cingulate gyrus	L	-8	-26	44	3.57	-4	-18	46	3.34	-	-	-	-
	R	4	-22	26	3.59	4	-16	42	4.05	-	-	-	-
Precentral gyrus	L	-28	-20	62	4.05	-28	-22	56	3.79	-	-	-	-
	R	52	0	36	5.41	62	6	26	3.25	-	-	-	-
Postcentral gyrus	L	-66	-10	24	4.10	-42	-30	62	4.19	-	-	-	-
	R	60	-16	24	4.28	60	-16	22	3.95	-	-	-	-
Anterior supramarginal gyrus	L	-62	-22	30	3.48	-58	-24	32	3.55	-	-	-	-
	R	58	-24	24	4.18	58	-26	40	3.77	-	-	-	-
Posterior supramarginal gyrus	L	-54	-42	20	2.71	-62	-46	16	3.44	-	-	-	-
	R	66	-42	14	4.79	66	-42	12	4.20	-	-	-	-
SPL	L	-44	-46	56	3.36	-	-	-	-	-	-	-	-
Temporal pole	L	-44	8	-14	4.03	-	-	-	-	-	-	-	-
	R	44	10	-18	3.71	-	-	-	-	-	-	-	-
Heschl's gyrus	L	-54	-18	6	3.92	-52	-20	10	3.51	-	-	-	-
	R	50	-16	10	3.69	48	-20	12	2.64	-	-	-	-
Planum temporale	L	-54	-40	16	4.10	-58	-20	8	3.21	-	-	-	-
	R	58	-26	16	4.32	56	-26	14	3.68	-	-	-	-
MTG (temporo-occipital)	L	-48	-62	4	3.62	-48	-62	2	4.72	-	-	-	-
	R	60	-44	12	3.83	52	-52	8	4.19	-	-	-	-
ITG (temporo-occipital)	L	-42	-56	-4	3.51	-46	-64	-14	2.82	-46	-64	-16	2.30
	R	44	-54	-10	3.23	-	-	-	-	-	-	-	-
Lateral occipital cortex	L	-46	-76	26	4.50	-46	-74	26	4.36	-	-	-	-
	R	48	-68	16	3.38	44	-74	16	3.40	-	-	-	-
Caudate	L	-10	8	-4	5.50	-10	14	8	4.00	-	-	-	-
	R	10	10	-2	4.85	14	-10	20	4.02	-	-	-	-
Putamen	L	-16	12	-2	4.62	-18	18	-8	3.97	-	-	-	-
	R	16	10	-2	5.69	24	6	0	3.80	-	-	-	-
Pallidum	L	-12	6	-2	3.98	-14	6	-4	3.20	-	-	-	-
	R	16	8	-2	4.77	20	0	-6	3.14	-	-	-	-
Thalamus	L	-14	-20	4	4.44	-14	-8	16	3.76	-	-	-	-
	R	4	-16	6	4.18	8	-2	12	4.33	-	-	-	-
Cerebellum	L	-4	-62	-18	4.48	-14	-58	-16	4.77	-30	-72	-24	4.45
	R	20	-62	-24	4.22	30	-58	-24	5.29	32	-56	-26	4.24

The coordinates of peak voxels are in MNI space where *x*, *y*, and *z* represent the medial-lateral, anterior-posterior, and superior-inferior orientations, respectively. L, left; R, right; SMC, supplementary motor cortex; IFG, inferior frontal gyrus; SPL, superior frontal gyrus; MTG, middle temporal gyrus; ITG, inferior temporal gyrus.

condition was then extracted from every participant's normalized filtered functional images (Featquery – FEAT Version 5.98). We controlled for age when correlating with DBS. Correlations with UHDRS were covaried for DBS and age.

Effect Size Analysis

To assess the sensitivity of the BOLD signal response in caudate and putamen (as revealed by the mean level of activation across the entire time-course), compared with volume atrophy in the same structures, we estimated standard regression coefficients (β) for the contrasts pre-HD versus controls, and between symp-HD versus controls across these two measures and for both 1-BACK and 2-BACK conditions.

RESULTS

Behavioral Results for the N-BACK Task

One-way ANCOVAs revealed no significant between-group differences in percentage accuracy in any of the conditions (see Table II). For RTs, one-way ANCOVAs showed a significant difference only between controls [$(6.1 \pm 1.5) \times 10^2$ ms] and symp-HD [$(7.6 \pm 1.4) \times 10^2$ ms] in the 0-BACK condition [$F(2, 86) = 5.19, P < 0.01$]; no other significant between-group differences were noted.

fMRI Voxel-Wise Results for the N-BACK Task

Overall, both WM conditions (1-BACK and 2-BACK) activated a large network of regions widespread throughout the brain and common to all three groups (Figs. 2 and 3). In addition, there were significant BOLD signal activation differences between groups despite no significant behavioral differences. In particular, the groups significantly differed in terms of activations throughout the WM network. Here we report significant results at $P < 0.05$, corrected.

Brain activation during 1-BACK compared with 0-BACK conditions

Similar patterns of BOLD signal increase were seen in all groups during the 1-BACK in comparison to the 0-BACK condition (Fig. 2A–C; Supporting Information Table I). Common regions of activation in all groups were widely distributed in cortical areas including bilateral activation in the orbito-frontal cortex, DLPFC, inferior, and superior frontal gyrus and anterior insula. Medial activation across all groups was also observed in the mid-anterior and posterior cingulate. In addition, shared activations were seen in the parietal lobe, precuneus, lateral occipital lobe and middle and inferior temporo-occipital cortex. Pre-HD exhibited the same pattern of activation as controls except no significant activation was observed in the planum temporale and, subcortically, the caudate (on the

left hemisphere), the putamen, pallidum, or thalamus. Symp-HD also had a similar pattern of response but the spatial extent was more reduced and significant activity was absent from anterior orbito-frontal cortex, right central operculum and inferior temporo-occipital cortex, and subcortically, from the striatum, thalamus and cerebellum.

Between-group differences indicated significant BOLD signal increases in controls, compared with pre-HD, in a range of structures including left inferior frontal gyrus, middle and posterior insula, pre and postcentral gyrus, temporal pole, temporo-occipital cortex, lateral occipital cortex, caudate, putamen and pallidum. Controls, in comparison with symp-HD, demonstrated significantly increased activation in right posterior orbito-frontal cortex, right anterior insula, pre and postcentral gyrus, temporal pole, temporo-occipital cortex, lateral occipital cortex and cerebellum (Table III; Fig. 2E,D). No significant differences were observed for the comparison between pre-HD and symp-HD. Voxel-wise results revealed no significant between group differences in the DLPFC or in the striatum between symp-HD and controls.

Brain activation during 2-BACK compared with 0-BACK conditions

Regions activated during the 2-BACK compared to the 0-BACK were generally similar to those seen during the 1-BACK condition (Fig. 3A–C; Supporting Information Table 2). Overall, controls showed more spatially extended significant BOLD signal increases than the pre-HD or symp-HD groups. Common cortical responses amongst the groups included bilateral activation in the superior frontal gyrus, DLPFC, supplementary motor cortex, inferior frontal gyrus, anterior and posterior orbitofrontal cortex, anterior insula and medially in the mid-anterior and posterior cingulate as well as paracingulate gyri. Other structures that exhibited a neural response across the three groups included pre and postcentral gyri, anterior and posterior supramarginal gyri, superior parietal lobe, precuneus, temporo-occipital cortex and lateral occipital cortex. Significant cerebellar activation was seen in controls and pre-HD but it was completely absent in symp-HD. Controls also showed increased BOLD signal bilaterally in the caudate, putamen, pallidum and thalamus, and so did pre-HD. Once again, symp-HD failed to show significant responses in these same structures.

Group differences between controls and pre-HD, and between controls and symp-HD were observed in the inferior frontal gyrus, right posterior orbito-frontal cortex, insula (middle and posterior for the contrast controls > pre-HD and right anterior for the contrast controls > symp-HD), anterior and posterior cingulate gyrus, paracingulate gyrus, pre and postcentral gyri, anterior and posterior supramarginal gyrus, and temporo-occipital and lateral occipital cortex. The same significant group differences were also observed subcortically in the caudate, putamen, pallidum, thalamus, and cerebellum. Cerebellar

Figure 2.

Brain regions showing BOLD signal increase during the 1-BACK condition in comparison to the 0-BACK baseline condition. The main effects seen in controls (A), pre-HD (B), and symp-HD (C). Group differences are shown for controls versus pre-HD

(D) and controls versus symp-HD (E). Axial slices are $z = -6$ to 44; sagittal slices are $x = 44$ (A–C), 12 (D), and 40 (E). Images are in 2 mm MNI space in radiological orientation (right hemisphere is at the left side of the image).

activity was also higher in pre-HD, compared with symp-HD. Moreover, there was a significant difference in BOLD signal activity between pre-HD and symp-HD in the inferior temporo-occipital cortex (Table IV; Fig. 3D,E). Voxel-wise results revealed no significant between group differences in the DLPFC.

Time-Course Results for the N-BACK Task

Percent signal change along time-courses in all ROIs across all groups during both conditions was significantly different from 0. During the 1-BACK condition a significant group effect was observed in the right ante-

rior insula, caudate, putamen, and pallidum. There was no significant effect for the DLPFC (pairwise comparisons were however performed for the DLPFC given its important role in the WM network). Pairwise tests contrasting the three groups revealed significant differences in the DLPFC between pre-HD and symp-HD; in DLPFC there was a significant difference between the pre-HD and symp-HD groups. While the mean DLPFC BOLD signal change across the entire time-course in controls was not significantly different from that in symp-HD participants, it was significantly different at specific time points throughout the time-course and did not differ from pre-HD. Group by Time interactions were seen in

Figure 3.

Brain regions showing BOLD signal increase during the 2-BACK condition in comparison to the 0-BACK baseline condition. The main effects seen in controls (A), pre-HD (B), and symp-HD (C). Group differences are shown for controls versus pre-HD

(D) and controls versus symp-HD (E). Axial slices are $z = -6$ to 44; sagittal slices are $x = 44$ (A–C) and 8 (D), and 12 (E). Images are in 2 mm MNI space in radiological orientation (right hemisphere is at the left side of the image).

all regions except the caudate. During the 2-BACK condition, group differences were found in DLPFC, anterior cingulate, insula, and left superior parietal lobe, right anterior supramarginal gyrus and, subcortically, in the caudate and thalamus. The main effect of group in the putamen exhibited a trend toward significance ($P < 0.06$) only, but pairwise tests revealed a significant difference between controls and pre-HD. Group by Time interactions were observed in the right posterior supramarginal gyrus, caudate, putamen and thalamus (see Fig. 4 for examples of group time-courses for the DLPFC, caudate and putamen for 1-BACK and 2-BACK conditions).

Correlation With Volume Measures

Volumetric analysis revealed that caudate and putamen volumes were significantly reduced in pre-HD and symp-HD, relative to controls (see Supporting Information Table 3). There were also significant volume reductions in these same structures in symp-HD, compared with pre-HD. During the 1-BACK condition, the correlation analysis of the percent BOLD signal change with the volume of the caudate revealed a negative association for both HD groups when analyzed together ($r = -0.32$, $P < 0.01$); this effect was stronger for the pre-HD group only ($r = -0.41$, $P < 0.01$), but was not significant in the symp-HD group.

Figure 4.

Time-course graphs of ROIs showing significant group effects and Group by Time interactions during the 1-BACK and 2-BACK conditions in comparison to the 0-BACK baseline condition. Shown are time-courses for the dorsolateral prefrontal cortex (DLPFC), caudate, and putamen. A significant main effect of Group is indicated by a “+” sign on the top right corner of a plot; a Group by Time interaction is indicated by a “x” sign on the top right corner of a plot; pairwise differences between

groups are represented by the letters a, b, and c next to the relevant time-course: a, Δ Pre-HD versus Symp-HD; b, Δ Pre-HD versus Controls; c, Δ Symp-HD versus Controls; * Δ Pre-HD versus Controls; ^ Δ Symp-HD versus Controls; ° Δ Pre-HD versus Symp-HD. Significance for all tests is $P < 0.05$. Time-courses are statistically compared starting at 0 sec. Images are in radiological orientation (right hemisphere is at the left side of the image).

No significant associations were observed during the 2-BACK condition.

Correlation With Clinical Measures

Partial correlations controlling for DBS and age revealed several significant correlations between percent BOLD activation and genetically linked indices of HD. Specifically, UHDRS was negatively associated with percent BOLD signal change in the anterior insula ($r = -0.45$, $P < 0.01$), the anterior cingulate gyrus ($r = -0.31$, $P < 0.05$) and posterior supramarginal gyrus ($r = -0.27$, $P < 0.05$) in all HD and symp-HD ($r = -0.58$, $P < 0.01$, $r = -0.49$, $P < 0.05$ and $r = -0.44$, $P < 0.05$, respectively) considered separately. Signal change in left SPL of symp-HD and in putamen of pre-HD was also

negatively correlated with UHDRS ($r = -0.57$, $P < 0.01$ and $r = -0.43$, $P < 0.01$, respectively). During the 2-BACK condition, we observed a negative association between BOLD signal change in the caudate and DBS in all HD ($r = -0.34$, $P < 0.05$) and pre-HD ($r = -0.38$, $P < 0.05$). We also found DBS to be negatively correlated with pre-HD DLPFC percent BOLD signal change in DLPFC and with putamen and pallidum percent signal change in symp-HD ($r = -0.44$, $P < 0.05$ and $r = -0.60$, $P < 0.01$, respectively). A strong negative correlation was also found between signal change in the anterior insula and UHDRS of all HD ($r = 0.45$, $P < 0.01$), which became stronger when considering symp-HD on their own ($r = -0.62$, $P < 0.05$). The BOLD response in the anterior cingulate gyrus of symp-HD was also negatively correlated with UHDRS ($r = -0.59$, $P < 0.05$).

Effect Sizes

Analysis of effect sizes indicated that group differences were overall more pronounced in volume measures, compared with the mean level of activation across the entire BOLD time-course (see Supporting Information Table IV). The largest effect size was observed during 2-BACK in the caudate volume for the contrast symp-HD versus controls ($\beta = -1.57$), which was significantly different ($P < 0.001$) from the same contrast in BOLD signal response ($\beta = -0.67$). This finding was mirrored during 1-BACK but the effect sizes were smaller ($\beta_{\text{volume}} = -1.33$, $\beta_{\text{BOLD}} = -0.51$; $P < 0.001$). No significant difference between effect sizes across modalities was observed in the caudate for pre-HD versus controls during either 1-BACK or 2-BACK. Putamen volume was significantly more predictive than BOLD signal response at discriminating between pre-HD versus controls, and between symp-HD versus controls during both 1-BACK and 2-BACK (pre-HD versus controls, $P < 0.01$; symp-HD versus controls, $P < 0.001$, respectively).

DISCUSSION

In support of our hypothesis, results demonstrated different patterns of activity in the WM network in both pre-HD and symp-HD groups, compared with controls, defined by both increased and decreased activations. The main findings relating to both the voxel-wise and time-course analyses will be highlighted below. Specifically, during the 1-BACK condition, voxel-wise BOLD signal activity was significantly reduced across a number of regions from the WM network, including left inferior frontal gyrus, caudate, putamen, and pallidum in the pre-HD group, compared with controls; symp-HD, compared with controls, showed no significant striatal differences, but exhibited reduced activity in the right anterior insula and cerebellum. The BOLD time-course analysis, however, showed significantly reduced activity in the caudate in both symp-HD and pre-HD, compared with controls. Moreover, although there were no DLPFC voxel-wise BOLD signal differences between groups, the BOLD time-course analysis exhibited significantly increased activation in symp-HD, relative to both pre-HD and controls (the latter two groups did not differ). The pattern of reduced voxel-wise BOLD activity in pre-HD and symp-HD, compared with controls, became more insidious during 2-BACK affecting the same structures as in 1-BACK (with striatum, insula and cerebellum now discriminating between both HD groups and controls), but also a larger number of regions, such as the anterior cingulate gyrus, parietal lobes and thalamus. Caudate activation was significantly reduced in pre-HD and further diminished in symp-HD, relative to controls, as evidenced by the time-course analysis. Moreover, although there were no DLPFC voxel-wise BOLD signal differences between groups, the time-course for 2-BACK showed a reversed pattern to that

observed in 1-BACK, with a significantly diminished signal in symp-HD, relative to pre-HD and controls (the latter two groups did not differ).

The lack of robust striatal activation evident in both pre-HD and symp-HD suggests a likely functional compromise of the WM network. Although sensory processing of stimuli, and thus cortical activity, occurs in pre-HD and symp-HD groups, the diminished subcortical functional activation suggests a possible disconnection of the WM regions. Wolf et al.'s [2008a] finding of significantly reduced connectivity in pre-HD between cortical and subcortical regions during WM performance, involving left DLPFC, anterior cingulate and putamen, provides some evidence of this likely disconnection. Interestingly, and contrary to our results, Wolf et al. [2007, 2009] found no functional impairment in the caudate in their sample of pre-HD and symp-HD, respectively, compared with controls. The authors suggested that this may be due to increased variability of caudate volume and activation extent during symp-HD stages, or to compensatory mechanisms associated with volumetric abnormalities [Wolf et al., 2009]. The caudate differences between our findings, and those of Wolf et al., may be due to differences in sample sizes and/or the properties of the WM tasks. Our time-course results for the striatum, and in particular the caudate, were obtained in the context of a stringent spatial pre-processing method aimed specifically at reducing volume variability in our sample. In our study, the voxel-wise results revealed a significant difference in the striatum between pre-HD and controls during 1-BACK (but no difference between symp-HD and controls), and between both pre-HD and symp-HD groups and controls during 2-BACK. Since we optimized the spatial pre-processing methods to reduce biases introduced by atrophy changes, it is likely that the smaller symp-HD sample substantially diminished the required power to detect significant activation and therefore group differences in this contrast. Note also that our voxel-wise analysis failed to detect a number of other differences between symp-HD and controls that were present for the comparison between pre-HD and controls. The BOLD time-course analysis demonstrated greater sensitivity for detecting functional differences between symp-HD and control groups in the striatum during 1-BACK, compared with the BOLD voxel-wise analysis. This is not surprising as ROI analysis increases the signal-to-noise ratio.

Overall, two general temporal patterns were revealed by the time-course analysis in controls: (1) a pattern of early transient activity in the anterior cingulate gyrus, caudate, putamen, and thalamus; and (2) a pattern of extended activity throughout the task, present in DLPFC, right anterior insula, left SPL, and right posterior supramarginal gyrus and globus pallidus. This combination of transient and sustained activity has been previously reported in studies investigating the temporal dynamics of WM in healthy controls [Ashby et al., 2005; Cohen et al., 1997; Courtney et al., 1997; McNab and Klingberg, 2008; Yantis et al., 2002]. In particular, sustained activity in DLPFC may

reflect the dynamic maintenance of information in WM, whereas transient activity in the striatum may correspond to a context-dependent gating mechanism thought to selectively update the contents of WM in response to the changing conditions under which maintenance is required [Frank et al., 2001; Houk and Wise, 1995]. In this light, the departure of this pattern, observed in both HD groups, suggests maintenance is potentially starting to be affected in symp-HD and context-dependent updating may have already been disrupted in both pre-HD and symp-HD.

Cortical hyper-activation of DLPFC in symp-HD has been previously reported as evidence of a possible compensation mechanism [Paulsen et al., 2004]. This suggests that in symp-HD the DLPFC may modulate its activity depending on task demands. For example, despite no differences in performance during intermediate WM load (i.e., 1-BACK), and no BOLD voxel-wise differences in the DLPFC between groups, we found evidence of increased functional activation in the DLPFC BOLD time-course analysis in symp-HD, relative to both pre-HD and controls. However, at a higher WM load (i.e., 2-BACK), our results indicate that DLPFC BOLD time-course activity in symp-HD shows a diminished signal compared to pre-HD and controls, suggesting an inability to keep up with the increased level of demand. This finding is also in accord with Wolf et al. [2007, 2009], who demonstrated hypo-activation in the left DLPFC with a higher WM load, and with our recent study suggesting that in symp-HD, as task difficulty increases beyond capacity, prefrontal activity diminishes [Gray et al., 2013]. Moreover, the successful recruitment of compensatory prefrontal responses was associated with reduced disturbances within cognitive-affective domains in symp-HD [Gray et al., 2013]. In this study, the clinical relevance of the hypo-activation in the DLPFC is further supported by the finding that the mean percent BOLD signal change in this structure was negatively correlated with DBS during 2-BACK in pre-HD, suggesting that the trend toward signal reduction may commence prior to symptom onset despite an ability to maintain optimum WM performance.

The clinical significance of other functional measures reported in this study was illustrated by correlations with genetically linked markers of disease progression. In the pre-HD group, and during 1-BACK performance, there was a significant correlation between mean percent BOLD signal change and the caudate volume, suggesting that reduced BOLD signal may be associated with early volumetric loss. Moreover, we observed a negative association between mean BOLD response in the caudate and DBS in both pre-HD and symp-HD groups. In addition, decreased mean signal in the right anterior insula, left SPL and right posterior supramarginal gyrus correlated with higher UHDRS scores. Previous studies have suggested that the anterior insula and the parietal lobe play an inhibitory role in WM [Bunge et al., 2001; Garavan et al., 2002; Hester et al., 2004; McNab and Klingberg, 2008]. HD-related hypo-activation in the parietal lobe has been repeatedly

reported [Clark et al., 2002; Deckel et al., 2000; Goldberg et al., 1990; Wolf et al., 2007, 2009]. Wolf et al. [2009] linked this to WM load in the context of decreased activity in the network including the prefrontal cortex, putamen and cerebellum, which is thought to subserve manipulation of memoranda during WM.

The few reported fMRI studies in HD have produced variable findings (for reviews see [Esmaeilzadeh et al., 2010; Georgiou-Karistianis, 2009; Paulsen, 2009]). In symp-HD there have been reports of both hyper-activation [Clark et al., 2002; Dierks et al., 1999; Georgiou-Karistianis et al., 2007; Kim et al., 2004; Wolf et al., 2008a; Wolf et al., 2008] and hypo-activation [Kim et al., 2004; Saft et al., 2008; Wolf et al., 2008a,b] in tasks examining cognitive function and attention. A similar pattern of mixed findings has been reported in pre-HD [Hennenlotter et al., 2004; Paulsen, Nehl et al., 2004; Paulsen, Zimbelman et al., 2004; Reading et al., 2004; Saft et al., 2008; Wolf et al., 2007; Zimbelman et al., 2007]. These findings suggest that the pattern of functional changes during both pre-HD and symp-HD stages is highly complex and may depend on many factors such as task type, presence or absence of atrophy, stage of disease, level of performance (normal or otherwise) and task difficulty. It has been proposed that hyper-activation of BOLD responses may occur when people are more than ten years before HD onset, followed by hypo-activation as individuals become close to onset, resulting finally in hyper-activation during symp-HD [Saft et al., 2008]. Indeed Wolf et al. [2007] showed that pre-HD individuals close to onset exhibited an increased activation of the left parietal lobule and right superior frontal gyrus compared with pre-HD far from onset and controls, in the absence of group differences in performance. The results reported here, of hyper- and hypo-activation in DLPFC, clearly illustrate how highly complex the pattern of functional changes can be. In the present study, the pattern of activation seems to depend on task load (as reported previously, see Wolf et al., 2009), as well as disease stage and severity. In addition, the variability in activation patterns across studies may indicate that there are indeed critical points during the pre-HD neurodegenerative process, involving the onset or worsening of more than one pathological process (e.g., axon or myelin degeneration, neuronal dysfunction or death) [Bohanna et al., 2008; Georgiou-Karistianis, 2009], which is likely to impact on the pattern of results.

The current available evidence suggests that functional deficits in multiple cortical and subcortical regions in HD extend well beyond the volumetric abnormalities [Ciarra-miello et al., 2006; Paulsen et al., 2006; Rosas et al., 2006; Sritharan et al., 2010]. For example, early dysfunction of lateral prefrontal and cingulate regions has been identified in pre-HD stages whereas compensatory responses involving more posterior brain regions may occur closer to the onset of manifest clinical symptoms [Saft et al., 2008; Wolf et al., 2007]. Here we found that group differences in BOLD response, as revealed by time-course analysis, implicating both pre-HD and symp-HD, were present in

caudate and putamen together with volumetric differences. This suggests that brain imaging methods offer a sensitive means to document early change in HD, even during optimum WM performance [Bohanna et al., 2008; Georgiou-Karistianis, 2009; Nguyen, Bradshaw, Stout, Croft, and Georgiou-Karistianis, 2010; Paulsen, 2009]. However, our effect size analysis suggests that volume change (in caudate and putamen) may be more predictive than BOLD signal response as an early biomarker. More research is warranted with larger groups, including pre-HD participants further from estimated symptom onset.

A number of important factors should be considered in light of our results. For example, we imposed a $\geq 70\%$ accuracy threshold to investigate underlying functional activation differences across groups with no significant behavioral differences. All pre-HD participants had knowledge of their genetic status, which may have affected overall task performance. Also, care should be taken when interpreting the results due to possible effects of medication status on the pattern of results. Although we report functional differences across groups, if functional group differences reflect longitudinal processes that contribute to deteriorating WM in HD, it would be important to explore whether the greatest functional differences are observed in the worst performers. Note for the 1-BACK condition in particular, the number of individuals performing below accuracy threshold was very small to be able to examine differences across groups meaningfully. Although an analysis of this type was beyond the scope of this paper, it would be an important consideration for future research. The reduced number of participants on the 2-BACK relative to the 1-BACK condition may have influenced the pattern of functional activation regarding the absence of significant within and between group effects. Note, however, that the composition of the subgroups in terms of demographic, clinical and neurocognitive characteristics, across each of the conditions, remained similar. When comparing changes in caudate and putamen volumes across groups, we only corrected for the differences in brain tissue size by using total gray and white matter as covariates. This may have potentially under corrected for differences in total intracranial volume between groups. A further point is that the "HD-gene carrier state" may confer some non-progressive baseline functional activation difference compared with healthy individuals. A limitation of HD research has been that between group striatal functional differences may be confounded by structural differences in both pre-HD and symp-HD [Tabrizi et al., 2009]. This paper, however, represents a significant contribution in this regard as we developed a stringent spatial pre-processing methodology, which successfully reduced volume variability, especially in periventricular areas. While a residual effect of volume differences cannot be fully ruled out, the influence of structural differences on the pattern of functional activity has been significantly reduced. The time-course data, taken together with correlations between mean percent BOLD signal change and measures of clinical severity, provide new evi-

dence to suggest that the regional pattern of neural activity during task performance is different in HD gene carriers compared with healthy controls. This pattern of activity changes further as individuals move into the post-diagnosis period. More research into ROI time-courses is required if we are to understand the precise mechanisms underlying regional changes in fMRI neural activity in HD.

In conclusion, we report for the first time that WM task demands reveal differences in functional BOLD activation in both pre-HD and symp-HD groups, compared with healthy controls, using both voxel-wise and time-course approaches. These functional brain changes have been observed in the absence of WM performance differences across groups. Our findings, taken together with converging evidence from previous studies by Wolf et al., provide support for functional brain reorganisation in cortical and subcortical regions in both pre-HD and symp-HD, which may become further compromised as more complex tasks place greater demands on the WM network and as individuals move into the post-diagnosis period. Although functional connectivity [Thiruvady et al., 2007; Wolf et al., 2008a,b] may provide further insights relating to the diminished activation across cortical and subcortical regions, our findings, together with the lack of robust striatal activation, represent a very early signature of brain reorganization observed on average up to 15 years prior to clinical diagnosis. In the future we aim to determine the longitudinal sensitivity of these WM functional changes in HD.

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