



Natural variation in sensory-motor white matter organization influences manifestations of Huntington's Disease

Journal:	<i>Human Brain Mapping</i>
Manuscript ID	HBM-16-0290.R2
Wiley - Manuscript type:	Research Article
Date Submitted by the Author:	n/a
Complete List of Authors:	Orth, Michael; University of Ulm, Department of Neurology Gregory, Sarah; Wellcome Trust Centre for Neuroimaging, Scahill, Rachael; University College London, Institute of Neurology Mayer, Isabella; University of Ulm, Department of Neurology Minkova, Lora; University Medical Center Freiburg, Freiburg Brain Imaging Kloeppe, Stefan; University of Freiburg Seunarine, Kiran; UCL Institute of Child Health, Imaging and Biophysics Unit Boyd, Lara; University of British Columbia, Borowsky, Beth; CHDI Foundation Inc Reilmann, Ralf; George-Huntington-Institute Landwehrmeyer, Bernhard; University of Ulm, Neurology Leavitt, Blair; University of British Columbia, Department of Medical Genetics Roos, Raymund; Leiden University Medical Centre, Neurology Durr, Alexandra; Universite Pierre et Marie Curie, Paris, Department of Genetics and Cytogenetics Rees, Geraint; UCL Institute of Cognitive Neuroscience, Rothwell, John; University College London Institute of Neurology Langbehn, Douglas; University of Iowa, Departments of Psychiatry and Biostatistics Tabrizi, Sarah; University College London, Institute of Neurology
Keywords:	effective connectivity, cortical thickness, SEP, grip force, principal component analysis, biological trait

SCHOLARONE™
Manuscripts

1
2
3 **Natural variation in sensory-motor white matter organization influences manifestations of**
4
5 **Huntington's Disease**
6

7
8 Michael Orth MD¹, Sarah Gregory PhD², Rachael I Scahill PhD³, Isabella SM Mayer RA^{1,4}, Lora Minkova
9
10 PhD⁵, Stefan Klöppel MD⁶, Kiran K Seunarine PhD⁷, Lara Boyd PhD⁸, Beth Borowsky PhD⁹, Ralf
11
12 Reilmann MD¹⁰, G. Bernhard Landwehrmeyer MD, Blair R Leavitt MD¹¹, Raymund AC Roos MD¹²,
13
14 Alexandra Durr MD¹³, Geraint Rees PhD², John C Rothwell PhD⁴, Douglas Langbehn MD¹⁴, Sarah J
15
16 Tabrizi MD¹⁵, and the TRACK-On Investigators s
17

18
19 ¹Department of Neurology, Ulm University Hospital, Ulm, Germany
20

21
22 ²Wellcome Trust Centre for Neuroimaging, University College London, London, UK
23

24
25 ³HD Research Group, UCL Institute of Neurology, Queen Square, London, UK
26

27
28 ⁴Sobell Department of Motor Neuroscience and Movement Disorders, University College London
29
30 Institute of Neurology, Queen Square, London, UK
31

32
33 ⁵Department of Psychiatry and Psychotherapy; Freiburg Brain Imaging, University Medical Center,
34
35 Albert-Ludwigs-University Freiburg, Freiburg, Germany
36

37
38 ⁶Department of Psychiatry and Psychotherapy; Department of Neurology; Freiburg Brain Imaging,
39
40 University Medical Center, Albert-Ludwigs-University Freiburg, Freiburg, Germany
41

42
43 ⁷Developmental Imaging and Biophysics Section, UCL Institute of Child Health, London, UK
44

45
46 ⁸Centre for Brain Health, University of British Columbia, Vancouver, Canada
47

48
49 ⁹CHDI Foundation Inc., Princeton, NJ, USA
50

51
52 ¹⁰George-Huntington-Institute, Technology-Park Muenster, Muenster, Germany
53

54
55 ¹¹Center for Molecular Medicine and Therapeutics and Department of Medical Genetics, Child and
56
57 Family Research Institute, University of British Columbia, Vancouver, Canada
58

59
60 ¹²Department of Neurology, Leiden University Medical Centre, Leiden, Netherlands

1
2
3 ¹³APHP Department of Genetics, Groupe Hospitalier Pitié-Salpêtrière, and Institut du Cerveau et de la
4
5 Moelle, INSERM U1127, CNRS UMR7225, UPMC Université Paris VI UMR_S1127, Paris France
6

7 ¹⁴Departments of Psychiatry and Biostatistics, University of Iowa, Iowa City, IA, USA
8

9 ¹⁵Department of Neurodegenerative Disease, University College London, Institute of Neurology,
10
11 Queen Square, London, UK
12
13

14
15
16 **Contact for correspondence**

17
18 Michael Orth, M.D., Ph.D.

19
20 Department of Neurology, Ulm University Hospital

21
22 Oberer Eselsberg 45/1

23
24 89081 Ulm, Germany

25
26 e-mail: michael.orth@uni-ulm.de; Tel: +49-731 50063095; Fax: +49-731 50063082
27
28
29
30
31

32 Running head: Natural variation influences HD manifestations
33
34
35
36

37 **Keywords:** effective connectivity; cortical thickness; somatosensory evoked potentials; grip force;
38
39 principal component analysis; biological variation; biological trait
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Abstract

While the *HTT* CAG-repeat expansion mutation causing Huntington's disease (HD) is highly correlated with the rate of pathogenesis leading to disease onset considerable variance in age-at-onset remains unexplained. Therefore, other factors must influence the pathogenic process. We asked whether these factors were related to natural biological variation in the sensory-motor system.

In 243 participants (96 premanifest and 35 manifest HD; 112 controls) sensory-motor structural MRI, tractography, resting-state fMRI, electrophysiology (including SEP amplitudes), motor score ratings, and grip force as sensory-motor performance were measured. Following individual modality analyses we used principal component analysis (PCA) to identify patterns associated with sensory-motor performance, and manifest versus premanifest HD discrimination.

We did not detect longitudinal differences over 12 months. PCA showed a pattern of loss of caudate, grey and white matter volume, cortical thickness in premotor and sensory cortex, and disturbed diffusivity in sensory-motor white matter tracts that was connected to CAG repeat-length. Two further major principal components appeared in controls and HD individuals indicating that they represent natural biological variation unconnected to the HD mutation. One of these components did not influence HD while the other non-CAG driven component of axial versus radial diffusivity contrast in white matter tracts were associated with sensory-motor performance and manifest HD.

The first component reflects the expected CAG expansion effects on HD pathogenesis. One non-CAG driven component reveals an independent influence on pathogenesis of biological variation in white matter tracts and merits further investigation to delineate the underlying mechanism and the potential it offers for disease modification.

Introduction

In Huntington's disease (HD), the length of the expanded CAG tract in *HTT* explains about half of the variability of motor age-at-onset and is therefore the main determinant of biological events prior to clinical diagnosis (GeM-HD Consortium, 2015). The remaining variability is independent of CAG repeat length and reflects the modifying influence of genetic and environmental factors on the pathogenic process (GeM-HD Consortium, 2015). Structural differences in cerebral white and grey matter may be detectable as far as 15-20 years before onset of unequivocal signs of HD and have been reported using VBM and DTI (for a review see (Georgiou-Karistianis, et al., 2013); Thieben et al., 2002; Aylward, 2007; Rosas et al., 2008). Similar to structural abnormalities, cross-sectional task-based, or resting state functional MRI (fMRI) studies have documented widespread cortical and subcortical changes of brain function in manifest HD (Wolf, et al., 2014). TRACK-HD, a large longitudinal study combining clinical and structural imaging data from both premanifest and early manifest HD participants showed that clinical measures, e.g. motor and cognitive tasks, and volumetric imaging tracked disease evolution were associated with manifest HD. PREDICT-HD, a longitudinal study in premanifest HD (preHD), demonstrated that motor and cognitive task performance, and structural imaging (in particular putamen volume), improved predictions of motor diagnosis compared with models using *HTT* CAG repeat length and age.

Some of the effects reported in TRACK-HD and PREDICT-HD were independent of *HTT* CAG repeat length and age. This indicates that biological variation exists that is not related to the cause of HD but may be common in the population and, when present in an individual with the *HTT* CAG-repeat expansion, exerts an influence on HD pathogenesis. The identification of such traits could help reveal an interaction between the biology underlying the biological trait and the pathogenesis or manifestation of HD. The trait itself or the genetics underlying its variation could suggest a route to disease modification (genetic or pharmaceutical). Thus, HD-independent work on mechanisms underlying the trait could provide specific pathways/processes to be tested as candidates for HD modification. It remains unresolved which additional biological factors are associated with task

1
2
3 performance and clinical disease stage. We addressed this question in an *a priori* defined neuronal
4
5 network. We focused on sensory-motor circuits that involve brain areas for which macro-
6
7 (volumetric) and microstructural (DTI) abnormalities have consistently been reported in HD. These
8
9 include cortical somatosensory projections, abnormal in manifest disease the primary sensory
10
11 cortex, in which there is evidence of thinning prior to symptom onset, white matter changes assessed
12
13 using DTI, and SEPs where amplitudes of cortical components were reduced.
14
15

16
17 Most previous analyses – including analyses of the Track-HD data – have examined these measures
18
19 one at a time. The fact that differences to controls are expected for many of these in HD is a premise
20
21 of the present study in which we investigate the patterns by which they differ. Analysing data from
22
23 different modalities one at a time does not reveal these patterns. We aimed to first describe patterns
24
25 of the *HTT* CAG-repeat expansion associated pathology of the sensory-motor circuit using multimodal
26
27 measurements, i.e. sensory-motor network brain structure (VBM, sensory-motor cortex thickness
28
29 and DTI tractography), function (functional MRI and electrophysiology), and sensory-motor task
30
31 performance (grip force and motor score). We hypothesized that non-CAG-repeat driven factors
32
33 influence the pathogenic process and thus predict task performance and clinical disease stage and
34
35 examined whether these factors were related to natural biological variation in the sensory-motor
36
37 system.
38
39
40
41
42
43

44 **Methods**

45
46 We investigated sensory-motor clinical measures, and used neuroimaging and electrophysiological
47
48 techniques to investigate macrostructure, microstructure and network function. Following
49
50 independent single modality evaluation, we employed principal component analysis (PCA) as a
51
52 descriptive tool to identify data patterns using multimodal assessments. Finally, we asked whether
53
54 those PCA patterns (1) were associated with pathology that is also known to be linked to *HTT* CAG-
55
56 repeat expansion, (2) were able to discriminate between controls, preHD and early HD participants,
57
58
59
60

1
2
3 and (3) were associated with clinical measures of sensory-motor network performance (grip force
4
5 and UHDRS motor score).
6
7

8 9 **Participants**

10 All individuals who met baseline eligibility criteria for the TRACK-HD premanifest cohort in 2008 (for
11 inclusion/exclusion criteria see (Tabrizi et al., 2009) were eligible to participate in Track-On regardless
12 of current disease status. A premanifest gene carrier was either (a) an existing premanifest gene
13 carrier previously enrolled in TRACK-HD or (b) a newly recruited premanifest gene carrier with CAG
14 repeat length ≥ 40 and burden of pathology score $(\text{CAG}-35.5) \times \text{age} > 250$. All participants had to be
15 able to tolerate MRI, and sample donation, and control participants were age and gender frequency
16 matched to the premanifest gene carrier group.
17

18 Participants were assessed at baseline and 12-months at four study sites in London (61 participants;
19 25.1%), Paris (64; 26.3%), Leiden (60; 24.7%) and Vancouver (58; 23.9%). The study was approved by
20 local ethical committees, and written informed consent was obtained from each participant.
21

22 The United Huntington's Disease Rating Scale (UHDRS) motor part was administered at both visits.
23 Participants with a UHDRS diagnostic confidence score of 4 on the motor scale (criteria for clinical
24 diagnosis of early HD) at 12-months were defined as the early (diagnosed) group. The remaining HD
25 group was divided into preHD-A (further from predicted diagnosis age; ≥ 10.8 years at baseline) and
26 preHD-B (nearer; < 10.8 years) based on the survival analysis formula .
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47

48 **Clinical assessment and electrophysiology**

49 Participants were assessed clinically, including grip force analysis (Reilmann, et al., 2010), and *HTT*
50 CAG repeat length determined as described for the TRACK-HD study . At all four sites somatosensory
51 evoked potentials (SEP) were recorded following median nerve stimulation with surface electrodes,
52 and at three sites transcranial magnetic stimulation (TMS) was done as previously described using
53 established techniques . The protocol included M. abductor pollicis brevis (APB) hot-spot and motor
54
55
56
57
58
59
60

1
2
3 threshold determination, motor evoked potential latencies and amplitudes, input/output curves at
4
5 rest (110%, 130%, 150% resting motor threshold) and with pre-activation (125%, 150%, 175% active
6
7 motor threshold), and silent period determination.
8
9

10 11 **Neuroimaging**

12 ***Voxel based morphometry***

13
14
15 Voxel based morphometry data were acquired using previously validated protocols for multi-site use
16
17 on two different 3T MRI scanner systems (Philips Achieva at Leiden and Vancouver and Siemens TIM
18
19 Trio at London and Paris). Cortical thickness measures were generated for each participant using
20
21 Freesurfer version 5.3.0 applying default parameters and optimized for 3T data (Fischl and Dale,
22
23 2000). Measures were extracted from Brodmann areas: BA4a/4b (motor cortex, M1); BA6 (premotor
24
25 cortex, PMC) and BA3a/3b, BA1, BA2 (somatosensory cortex, S1)
26
27 <https://surfer.nmr.mgh.harvard.edu/fswiki/BrodmannAreaMaps>. All segmentations were visually
28
29 inspected for accuracy, blind to participant status.
30
31
32
33
34

35 ***Diffusion tensor imaging***

36
37
38 Diffusion-weighted images with 42 unique gradient directions ($b = 1000 \text{ sec/mm}^2$) were acquired
39
40 from both Siemens and Phillips scanners. Eight images with no diffusion weighting ($b = 0 \text{ sec/mm}^2$)
41
42 and one image with no diffusion weighting ($b = 0 \text{ sec/mm}^2$) were acquired from the Siemens and
43
44 Phillips scanners respectively. For the Siemens scanners, TE = 88ms and TR = 13s; for the Phillips
45
46 scanners, TE = 56ms and TR = 11s. Voxel size for the Siemens scanners was 2 x 2 x 2 mm and for the
47
48 Phillips scanners 1.96 x 1.96 x 2. 75 slices were collected for each diffusion-weighted and non-
49
50 diffusion weighted volume. The diffusion data were preprocessed using standard FSL pipelines . Data
51
52 were corrected for eddy current distortions, diffusion tensors fitted using DTFIT and all metrics
53
54 derived.
55
56
57
58
59
60

1
2
3 For DTI, the no-gradient (B0) image was then skull-stripped using the Brain Extraction Tool (BET) and
4 was manually examined and corrected. (For Siemens data B0 images were merged to create a mean).
5
6 Diffusion tensors were then fit to the corrected data using dtifit. FA, AD and RD values were derived
7 from the tensors. For registration, we first created a high quality T1 brain mask by combining and
8 dilating a thresholded segmented image (created using the VBM toolbox [http://www.neuro.uni-
9 jena.de/vbm/](http://www.neuro.uni-jena.de/vbm/)) with an eroded T1 mask from BET. The mask was then applied to the original, brain-
10 extracted, T1 image. The resultant T1 image was then registered to the B0 image using FLIRT
11 (Jenkinson and Smith, 2001). Within-voxel crossing fibers were modelled using a Bayesian
12 probabilistic method implemented in Bedpostx (Behrens, et al., 2007).
13
14

15
16 For fiber-tracking, pathways selected were between S1 and somatosensory thalamus; M1 and motor
17 thalamus; PMC and the motor thalamus and the corticospinal tract (CST), connecting M1 and the
18 cerebral peduncle. **For all regions except the cerebral peduncle, we used a regions of interest (ROI)
19 approach. The main advantage in using a single voxel is that the signal measured fully represents
20 the activity or structure within that voxel and is not impacted by the signal in neighboring voxels.
21 However, it is widely accepted that neighbouring voxels are likely to have a similar signal, be it
22 resting state activity or white matter microstructure. In fact, averaging over a ROI can significantly
23 increase the signal to noise ratio (SNR) compared to that of a single voxel. A single voxel, for
24 example, may be heavily impacted by the presence of other tissues or non-neuronal noise which
25 would lower the SNR and the quality of the extracted signal. The location of a single voxel can also
26 vary considerably between individuals. Using a cluster of voxels rather than an individual voxel
27 within a region would provide greater confidence that the 'nodes' used in the analyses were
28 located in the anatomical or functional region of interest. Furthermore, when defining a 'node'
29 based on the literature or a previous study, it is possible to use both standardised atlases or masks
30 to create a ROI. This ensures across-participant consistency in terms of location. It is important to
31 note, however, that for instance for DTI, effects can be masked or diluted by reverse-sense
32 changes in different voxels within a single ROI. There is evidence that, for example, FA can increase**
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 in some voxels due to selective degradation of one of two fibre populations, but decreases due to
4
5 the same effect in neighbouring voxels that contain only one fibre population (Groeschel et al,
6
7 2014). In averaging over a ROI it is, therefore, possible to mix voxels with differing effects, which
8
9 may be partially cancelled out on averaging. Finally, for DCM (see below) the timeseries extraction
10
11 was based on a principal components approach. Instead of signal averaging, we therefore
12
13 extracted the signal that explains the majority of the variance in that ROI. For the tractography
14
15 analyses, all metrics are weighted to ensure that those streamlines or fibres which contribute most
16
17 towards the formation of a tract are most represented.
18
19

20
21 ROI were created using the Anatomy Toolbox; thalamic regions for DTI and DCM analyses were
22
23 therefore identical. The cortical regions were, however, different from those used in the DCM
24
25 analyses as larger regions were more suitable for fibre-tracking. The cerebral peduncle region was
26
27 created using the FSL Montreal Neurological Institute template and the Johns Hopkins University
28
29 White Matter Labels atlas. All regions were defined in standard space and warped into native space
30
31 for each participant using the inverse deformation parameters that were outputted from the DARTEL
32
33 registration for the resting state fMRI images (see resting state fMRI methods section). Masks were
34
35 used to exclude any streamlines that tracked via the contralateral hemisphere or posteriorly to the
36
37 thalamus and peduncle and to ensure tracts did not extend beyond the white matter into grey
38
39 matter, CSF or dura.
40
41

42
43 For each participant, and each set of tracts, probabilistic tractography was then performed using
44
45 probtrackx. Connectivity distributions were generated from our seed regions in native space. The
46
47 resulting tract images were then warped into diffusion space using the FLIRT tool and overlaid onto
48
49 the B0 image for quality checking. FA, AD and RD values were extracted for each participant for each
50
51 tract.
52
53

54 ***Resting state functional MRI***

55
56
57
58
59
60

1
2
3 Resting-state fMRI data were collected using 48 continuously acquired ascending axial slices covering
4 the whole cortex and cerebellum (slice thickness: 2.8 mm, gap: 1.5 mm, in plane resolution 3.3 x 3.3
5 mm, field of view (FOV) 212 mm) with a T2*-weighted echo-planar imaging (EPI) sequence
6
7 (repetition time (TR) 3000 ms, echo time (TE) 30 ms, flip angle (FA) 80°). 165 volumes were acquired
8
9 in a single 8:20 min run. Preprocessing and subsequent statistical analyses were performed using
10
11 SPM8 (Wellcome Trust Centre for Neuroimaging, <http://www.fil.ion.ucl.ac.uk/spm>). Dynamic Causal
12
13 Modelling (DCM) using a stochastic framework, which models the randomness inherent within
14
15 resting state brain activity was then employed to investigate causal interactions between five
16
17 regions within the sensorimotor network. Model specification and estimation were conducted using
18
19 DCM10 (SPM12b Wellcome Trust Centre for Neuroimaging) and all connectivity parameters extracted
20
21 for further analysis.
22
23
24
25
26

27 For resting state fMRI, the first four EPI images were discarded to allow for steady state equilibrium.
28
29 The T1 scan was segmented into grey and white matter using the VBM8 toolbox
30
31 (<http://dbm.neuro.uni-jena.de/vbm/>) and deformation parameters extracted using DARTEL
32
33 (Ashburner, 2007). The segmented images were used to create an improved anatomical scan for co-
34
35 registration. Functional images were first realigned and field maps used for inhomogeneity
36
37 correction. EPI images were then co-registered to the new anatomical image and normalised using
38
39 the DARTEL deformation parameters. Finally the data were smoothed using a 6mm full-width at half-
40
41 maximum Gaussian kernel. Using a first level design consisting of smoothed images only, the
42
43 principal eigenvariate of the white matter and CSF time series were extracted from a single voxel
44
45 located within the pons (0, -24, -33) or lateral ventricle (-1, 45, 3) respectively. The white matter and
46
47 CSF signals were then included with movement regressors as nuisance covariates in a second GLM at
48
49 the individual level. The timeseries for each region within our model was then extracted using this
50
51 GLM. The model consisted of five regions within the sensory and motor networks within the left
52
53 hemisphere (dominant). Cortical regions were derived using co-ordinates from previous Track-HD
54
55 studies: S1 (-40, -34, 61); M1 (-40, -18, 60); and the PMC (-24, 0, 54). For the thalamic regions, see
56
57
58
59
60

1
2
3 DTI methods. For cortical regions, timeseries were extracted by placing an 8mm sphere around the
4
5 specified co-ordinates, localized to the nearest local maximum for peak activity, within a regional
6
7 anatomical mask generated by either the WFUPickatlas (Eickhoff, et al., 2005) or the Anatomy
8
9 Toolbox (Maldjian, et al., 2003). For the thalamic regions of interest, the principal eigenvariate of the
10
11 timeseries was extracted from the pre-defined mask. Connections between and within all regions
12
13 were modelled except the bidirectional connections between the two thalamic regions, the cortical
14
15 motor regions and the sensory thalamic region and the S1 and the motor thalamic composite region;
16
17 this totalled 17 connections. DCM specification and estimation was carried out with DCM10 in SPM
18
19 software (SPM12b; Wellcome Trust Centre for Neuroimaging, <http://www.fil.ion.ucl.ac.uk/spm>) and
20
21 connectivity parameters for each participant were extracted. The DCM estimation and model
22
23 convergence were examined using SPM-derived quality assurance.
24
25
26
27
28

29 **Statistical analysis**

30
31 We first inspected all data for implausible outliers (between 0 and 2% of data, depending on the
32
33 measure), which were either corrected or discarded after joint review with the subject-matter
34
35 investigators. We used log or square root transforms when appropriate to achieve approximate
36
37 residual normality, as is assumed by inference procedures.
38
39

40
41 For modeling group differences in outcomes among early HD, preHD-B, preHD-A, and controls, we
42
43 used general weighted least square (GWLS) regression with restricted maximum likelihood
44
45 estimation (Diggle, et al., 1994). The method accounts for repeated measure correlations among
46
47 participants with measures at two visits. Participants with only one observation were also included
48
49 however. This increases statistical power and ensures unbiased longitudinal analyses if visit 2 data is
50
51 missing at random.
52

53
54 Individual measures from the various modalities were treated, one-at-a-time, as outcome variables.
55
56 The main effects of interest were the above HD groups and their potential interaction with time
57
58 between visits ("time"). Longitudinal group effects were tested via the time interactions with group.
59
60

1
2
3 The "cross-sectional" effects of interest were mean group values, averaged over both visits. These
4
5 were estimated by a linear combination of main effects of group and group-by-time interaction, such
6
7 that estimated baseline and 12 month values were given equal weight. These averaged cross-
8
9 sectional comparisons reduce statistical noise due to factors such as measurement error and
10
11 accurate but irrelevant measurement of short term (e.g. day-to-day) fluctuations, leading to smaller
12
13 standard errors for group comparisons.
14
15

16
17 Because we also attempted to measure longitudinal change over 1 year, one might question the
18
19 validity of using both the baseline and follow-up measures for a mean cross-sectional analysis. The
20
21 justification is empirical and due to the slow evolution of most HD phenomenon. Even for measures
22
23 that exhibit statistically significant longitudinal changes over one year, the magnitude of change is
24
25 quite small in comparison to the cross sectional changes exhibited between HD groups and controls.
26
27 This cross sectional difference is the net result of many years of longitudinal change (Tabrizi et al.,
28
29 2009; Tabrizi et al., 2013). We also note that this is an observational study. Within the broad ranges
30
31 defined by our groupings, the time of the baseline measurement is arbitrary. For measures that are
32
33 not subject to practice effects, there is no reason to prefer time 1 or time 2 as representative of
34
35 premanifest or control states.
36
37

38
39 We controlled for age at study entry, gender, study site (scanner) and education level, as well as their
40
41 interaction with time. For TMS thresholds, we additionally controlled for main effects of skull to
42
43 cortex (M1) distance and for Freesurfer measures of mean cortical thickness in the left Brodmann
44
45 areas B4a and B4b (the cortical areas stimulated). Some models also controlled for underlying HD
46
47 progression risk determined by CAG repeat length and age via the cumulative probability of onset
48
49 (CPO) statistic from a survival analysis of onset ages . Because variance of outcome measures
50
51 sometimes changes notably in early HD vs preHD vs controls, separate residual covariance was
52
53 estimated for each group. We used the Kenward-Rogers correction to estimate denominator degrees
54
55 of freedom (Kenward and Roger, 1997). All models were fit using Proc Mixed from SAS 9.4 (Littell, et
56
57 al., 2006). Within modalities (electrophysiology, DTI, DCM, etc.) we also calculated false discovery
58
59
60

1
2
3 rates (FDR), abbreviated by q , using the Benjamini and Hochberg method (Benjamini and Hochberg,
4
5 1995).

6
7
8 We created a composite grip force motor score from the sum of sample-standardized orientation
9
10 and position indices, collected while grip force heavy-load task was performed with the dominant
11
12 hand.

13
14
15 For the final steps of the statistical regression analyses, which involved composite scores created
16
17 from multiple variables, we addressed the issue of missing data by use of multiple imputation
18
19 (Schafer, 1997) done separately for cases and controls. MI was based on outcome measures at
20
21 baseline and follow-up (treated as separate variables) along with all demographic covariates,
22
23 including HD group and study site interactions with time. Five imputations were generated using Proc
24
25 MI and subsequent model inferences were adjusted using Proc MIAnalyze (SAS 9.4).

26
27
28 Principal component analysis (PCA) was performed on outcome variables of interest as identified by
29
30 their between-group differences in the GWLS regressions. The PCA input was the maximum
31
32 likelihood estimate of the correlation matrix, derived during the multiple imputation procedure. Non-
33
34 rotated component scores were then used in to model the relationships to grip force, UHDRS motor
35
36 score (Pearson correlations and linear models), and manifest versus premanifest HD discrimination
37
38 (logistic discrimination).
39

40
41
42 For the PCA, we selected measures from each of the modalities. We were guided by the results of
43
44 individual modality analyses, i.e. we chose measures with the greatest difference between controls
45
46 and HD. Some of the measures within a given modality were highly correlated, for instance the DTI
47
48 measures from all tracts or cortical thickness from all regions. Hence we only included for instance
49
50 diffusivity measures from 2 of the 4 tracts in the multi-model PCA. Overall, we included only 15
51
52 variables within the multimodal PCA to ensure representation of the different modalities was roughly
53
54 equal. We included VBM data (volumes of white matter, grey matter, and caudate), cortical thickness
55
56 data (BA6 (premotor cortex), BA3a+2 (somatosensory cortex)), diffusion metrics from tractography
57
58
59
60

1
2
3 (AD and RD of S1-thalamus and PMC-thalamus tracts), effective connectivity parameters from DCM
4 (PMC-thalamus and PMC- PMC connections), and SEPs (N20/P25 amplitude and N20 latency) into the
5 PCA (multimodal-PCA). In a second PCA that focused solely on DTI, we included RD and AD measures
6
7 from all 4 available DTI tracts. We were interested in the question of whether patterns in HD
8
9 participants differed from those in controls or were similar. For this reason, we did the PCAs
10
11 separately in HD and healthy controls.
12
13
14
15
16
17
18
19

20 Results

21 The total Track-On cohort comprised 131 HD (101 former TRACK-HD and 30 new premanifest) and
22
23 112 controls (79 former TRACK-HD and 33 new) recruited between April 16, 2012 and December 10,
24
25 2012 (Table 1). At the 12-month follow-up visit, 11 participants from the HD group and 7 from the
26
27 control group did not take part. The most common reasons for withdrawal were intolerance to MRI,
28
29 personal time constraints and the burden of the study day.
30
31
32

33 We defined *a priori* a total of 53 measures for analysis (17 DCM, 3 brain volume, 7 cortical thickness,
34
35 12 DTI, 14 electrophysiology; supplementary table 1). First we asked if there was detectable change
36
37 in any of the measures from baseline to the 12 month visit. Longitudinal change was small and not
38
39 statistically significant (data not shown). In contrast, cross-sectional differences among the groups,
40
41 averaged over time, were evident and are presented in table 2.
42
43

44 PCA was performed on all gene-carrier participants (combined HD and preHD), and, independently,
45
46 in controls because we were interested in comparing independently derived descriptive patterns
47
48 between the HD and the control groups.
49

50 In the combined HD group, multimodal-PCA reduced the dimensionality of the data to three
51
52 important dimensions or principal components (PCs) based on their eigenvalues (Table 2;
53
54 supplementary table 2). In HD, the first multimodal-PC explained 21% of the variance in the data. In
55
56 the multimodal-PC1 dimension, lower caudate, grey and white matter volumes, and lower BA6 and
57
58
59
60

1
2
3 BA1 and 2 cortical thickness, were associated with higher AD and RD of S1-thalamus and PMC-
4 thalamus tracts (Fig 1A). The first PC was the only multimodal-PC that correlated significantly with
5 thalamus tracts (Fig 1A). The first PC was the only multimodal-PC that correlated significantly with
6 the age-CAG cumulative probability of onset ($r=-0.58$, $p<0.0001$, Fig 1B). Further, this first
7 multimodal-PC showed association with sensory-motor network specific performance (grip force
8 orientation and position index; Fig 1C, Table 3). Multimodal-PC1 substantially mediated, and
9 improved upon, the predictive effect of age-CAG on grip force ($p=0.006$) such that there was no
10 residual association of age-CAG ($p=0.468$) after this PC was included in the model. Multimodal-PC1
11 was also associated with clinical motor score ($p=0.041$), and probability of manifest versus preHD
12 status ($p=0.003$, Fig 1D, Table 3). However, unlike for grip force associations, the influence of age-
13 CAG on clinical motor score or HD status remained significant, though weaker, when the first PC was
14 included in the model. This indicates that the first multimodal-PC does not completely mediate the
15 age-CAG relationship to motor score.
16
17
18
19
20
21
22
23
24
25
26
27
28
29

30 The second multimodal-PC in HD explained 15% of data variance (Fig 1A). Higher AD was correlated
31 with smaller SEP amplitudes and greater cortical thickness. Multimodal-PC2 was not predicted by age
32 and CAG length ($r=0.12$, $p=0.178$), nor was it associated with HD outcomes (data not shown). This
33 second PC may represent an element of biological variation unrelated to HD, an interpretation
34 supported by partial similarity to the first multimodal-PC in an independent PCA in healthy controls.
35 The patterns were similar except that volumes of grey and white matter and caudate also correlated
36 with the component in controls but not HD (Fig 1A; supplementary table 3). Consistent with their
37 known central role in the illness, these volumes instead contributed substantially to the first principal
38 component of variation among the HD groups.
39
40
41
42
43
44
45
46
47
48
49

50 The third multimodal-PC in HD, explaining 11% of data variance, revealed a dimension of contrast
51 between AD and RD (Fig 1A). The third PC was a substantial additional predictor of grip force score
52 ($p=0.036$), of UHDRS motor score ($p=0.007$) and of diagnosis ($p=0.021$, Fig 1E, Table 3). Caudate, grey,
53 and white matter volumes contributed very little to this third multimodal-PC, and its values were not
54 predicted by age and CAG length ($r=0.04$; $p=0.643$). By definition, this AD versus RD component is
55
56
57
58
59
60

1
2
3 uncorrelated with the more general brain structural effects of the first multimodal-PC, which are
4 driven by genetic HD load. Furthermore, multimodal-PCA of the normal controls revealed a similar
5 AD versus RD contrasting component that accounted for 12 % of the variance within the control
6
7 group (supplementary table 3). Therefore, higher AD compared to RD is associated with HD
8
9 pathogenesis in the presence of a CAG expansion mutation, but is also observed as natural variation
10
11 in healthy controls. It is independent of the unique HD brain structural effects that are related to *HTT*
12
13 CAG repeat-length.
14
15
16

17
18
19 We then asked if the relationship of AD and RD extended to the other white matter tracts that we
20
21 had measured but excluded from the initial PCA. There was a very high correlation of AD, and RD,
22
23 values among the four tracts. The finding was consistent in separate analyses of the controls (Figs 2A,
24
25 B) and the HD group (Figs 2D, E). This was further reflected in a PCA restricted to AD and RD values
26
27 from all 4 tracts (ADRD-PCA) and performed independently in healthy controls and HD participants.
28
29 Two dimensions (illustrated for controls in Fig 2C and for HD in Fig 2F) explained 94% of the
30
31 variability in controls and 90% of variability in HD participants (Fig 2G). ADRD-PC1 contains a
32
33 dimension of common correlation among all diffusivity measures while ADRD-PC2, consistent with
34
35 the multimodal PCA, reflects the contrast between AD and RD values (Fig 2G, Table 4). AD measures
36
37 were significantly correlated with grip force, but the RD measures were not (Fig 2H, Table 5). There
38
39 was no correlation between age and grip force performance in controls, and correlation of AD and
40
41 RD with grip force was not significantly affected if controlled for age. In HD participants, the patterns
42
43 were similar to controls. The correlation of both ADRD-PC1 and AD with grip force was stronger, and
44
45 the relationship of ADRD-PC2 and grip force was slightly stronger than in controls (Fig 2I, Table 5).
46
47
48
49
50
51
52

53 Discussion

54
55
56 In this study, we went beyond the analysis of data from single modalities one at a time to investigate
57
58 data patterns reflecting the structural and functional state of an *a priori* defined sensory-motor
59
60

1
2
3 circuit that is relevant for motor control. We aimed to delineate CAG repeat length dependent and
4
5 independent patterns that influence the pathogenic process before disease onset. We did not detect
6
7 longitudinal differences over 12 months. Cross-sectionally, in HD, dimension reduction using principal
8
9 component analyses revealed one dimension specific to its cause, i.e. *HTT* CAG repeat-length
10
11 expansion, that included a correlated loss of caudate, grey and white matter volume, cortical
12
13 thickness, and disturbed diffusivity in white matter tracts. Within our data, this component predicted
14
15 sensory-motor performance and a diagnosis of manifest HD. Two further dimensions were not
16
17 unique to HD but were also present in controls. The first of these dimension represented natural
18
19 variation in axonal diffusivity, cortical thickness and SEP amplitude but did not influence HD. The
20
21 second non-CAG dimension consisting of an AD versus RD contrast in white matter tracts did
22
23 influence HD in that it was an additional predictor of sensory-motor performance in HD and controls,
24
25 and a clinical diagnosis of HD. This pattern of diffusivity is consistent with naturally occurring
26
27 variability in a biological trait that influences HD manifestations.
28
29
30

31
32 The *HTT* mutation is the main determinant of pathogenesis that leads to the structural and functional
33
34 neurodegeneration that underlies HD and the emergence of unequivocal clinical signs. We examined
35
36 this pathogenic process in that we investigated the structural and functional state in an *a priori*
37
38 defined sensory-motor circuit relevant for motor control. Presumably owing to the slow progression
39
40 of HD and perhaps also the sensitivity limits of the methods employed, there was no detectable
41
42 change in any of the sensory-motor modalities within the 12-month observation period. This may
43
44 appear inconsistent with the findings in the original TRACK-HD study at 12 months (Tabrizi et al.,
45
46 2011). It is important to note, though, that many of the measures that we used in the present study
47
48 were not part of TRACK-HD. It is therefore possible that e.g. electrophysiological measures or DTI are
49
50 less suitable for the detection of change over 12 months either because network brain function, or
51
52 white matter microstructure, do not change much or because measurement error for these
53
54 measures is large relative to the change that happens. In addition, in TRACK-HD most detectable
55
56 changes were observed only in the diagnosed, early HD group, which was both substantially larger
57
58
59
60

1
2
3 and more advanced in disease than in our newly diagnosed HD subgroup. It is therefore conceivable
4
5 that the ability to detect change over time depends on the measurements themselves, on the time
6
7 that elapsed between measurements, and also on the stage of HD. Cross-sectionally however, our
8
9 data confirmed that, compared with controls, in *HTT* gene expansion carriers white and grey matter
10
11 brain structure is diminished, and sensory afferent signal transmission is concomitantly slower and
12
13 less efficient. Furthermore, these differences were measurable before unequivocal motor signs of HD
14
15 emerged. Brain structure and electrophysiology remained abnormal following the emergence of
16
17 diagnostic motor signs. Further, in early HD, in addition to RD, AD was substantially worse and
18
19 effective connectivity from PMC to thalamus reduced. The analyses used to examine differences at
20
21 the individual modality level the patterns by which the data differ across modalities. We therefore
22
23 next employed multivariate analysis using PCA to reveal such data patterns. PCA reduced data to two
24
25 dimensions that were relevant for HD and one that represents natural variation that does not
26
27 influence HD. The first dimension relevant to HD contained a correlated loss of caudate, grey and
28
29 white matter volume, cortical thickness, and disturbed diffusivity in white matter tracts. These
30
31 changes increased with an increase of the age-CAG-length HD risk, indicating that structural
32
33 abnormalities relate to the primary cause of pathogenesis. For performance that is related to the
34
35 sensory-motor network, we show that the current structure of white and grey matter within the
36
37 network is a more relevant (and presumably more proximal) predictor than the key HD biological
38
39 variables, age and CAG-repeat length. Our data indicate that structural changes in white and grey
40
41 matter are relevant for sensory-motor task performance and are an integral part of HD biology.
42
43
44
45

46
47 We then examined whether we could identify biological variation that was independent of genetic
48
49 risk but still associated with sensory-motor performance and clinical category, i.e. preHD versus
50
51 manifest HD. PCA identified two dimensions not connected to CAG-repeat length in which data
52
53 variance was similar in controls and HD participants. One of these dimensions was not associated
54
55 with clinical status. It contrasted more AD with smaller SEP amplitudes and greater cortical thickness,
56
57 and, in controls, increased grey matter volume as well. In HD participants this dimension was not
58
59
60

1
2
3 related to age-CAG repeat length. The dimension seems to capture an element of natural biological
4
5 variation unrelated to HD.

6
7 Axonal damage is known to be associated with a loss of amplitude of evoked potentials so that it is
8
9 conceivable that variation of axonal microstructure relates to SEP amplitude. In addition, the
10
11 distance between the soma of the cell receiving sensory afferent inputs within S1 and the recording
12
13 electrode on the scalp can also influence SEP amplitude. With a thicker cortex that distance may be
14
15 slightly greater, and subsequently the SEP amplitude smaller.

16
17 A second dimension unrelated to age-CAG repeat length in both controls and HD participants
18
19 revealed differences between AD and RD in white matter tracts. This dimension was also, by
20
21 definition, uncorrelated with either the previous non-CAG-repeat dimension or with the primary
22
23 dimension related to CAG-repeat length found only in HD. This axial-radial DTI dimension was
24
25 independently associated with sensory-motor performance and a clinical diagnosis of HD. Somewhat
26
27 surprisingly, it was also associated with task performance in controls. The diffusivity contrast was
28
29 evident in all the tracts we analysed, and, thus, is not specific to a single white matter tract.

30
31 Therefore, the diffusivity patterns constitute a quantitative phenotype that reflects naturally
32
33 occurring variability in white matter tract biology. AD reflects water movement in tracts parallel to
34
35 the main fiber organization while RD measures water movement perpendicular to this organisation.
36
37

38
39 In animal models there is evidence suggesting that AD reflects axonal integrity and RD myelin
40
41 integrity. Myelin-producing glial cells and neurons form a unit in which axon integrity depends on
42
43 intact myelin and axons help maintain that myelin. In diseases primarily affecting white matter
44
45 myelin, such as multiple sclerosis, clinical signs seem to manifest when axonal damage arises from
46
47 the loss of myelin trophic support (Nave, 2010). Naturally occurring variability in the axon-myelin unit
48
49 in itself does not cause disease. However, with a genetically sensitized background such as in HD or
50
51 in other diseases naturally occurring variability in the make-up of the axon-myelin unit may influence
52
53 onset and the course of the disease. DTI diffusivity patterns need to be interpreted with caution, as
54
55 we cannot infer with confidence the anatomical significance based on the diffusivity changes we
56
57
58
59
60

1
2
3 observed in our participants. Nonetheless, given the prominent involvement of white matter in HD ,
4
5 and since mutant huntingtin has been implicated in oligodendrocyte function and axonal transport ,
6
7 our observational data suggest that it is worth exploring the role of the differential loss of myelin and
8
9 axons in HD pathogenesis further.
10

11
12 In contrast to brain structure, sensory-motor functional measures such as effective connectivity or
13
14 SEPs did not contribute significantly to models of clinical status. While some functional measures
15
16 differed significantly between preHD or early HD versus controls, task performance or clinical status.
17
18 One possible explanation could be the use of resting state measures. Brain activity differs
19
20 substantially when performing a task compared to when at rest even within our carefully chosen
21
22 sensory-motor network model. It is also possible that the precision of functional measurements is
23
24 inadequate to reliably detect relevant underlying phenomena.
25
26

27
28 In summary, we went beyond the analysis of single modality data one at a time to assess patterns of
29
30 structural and functional properties of the sensory-motor system in HD and healthy controls using
31
32 multivariate analysis. While there was very little, if any, change in these modalities over 12 months,
33
34 the cross-sectional analysis revealed a dimension of white and grey matter loss attributable to HD
35
36 biology that is associated with network specific task performance and clinical diagnosis. Our data
37
38 suggest that the microstructural anatomy influencing water diffusivity perpendicular to white matter
39
40 tracts, e.g. myelin, is already changed in preHD. In contrast, the anatomical compartment that
41
42 determines water diffusivity parallel to the tracts, e.g. the axon, is not substantially affected until
43
44 early HD. Although these patterns are amplified with HD, we also show that these measures of white
45
46 matter organization consistently vary among healthy controls across different white matter tracts.
47
48 Given the absence of an age-CAG correlation, this indicates that naturally occurring variability in
49
50 white matter tract microanatomy may represent a biological trait with a disease modifying influence
51
52 on the pathogenesis of HD. These findings need to be confirmed, e.g. examining more tracts with DTI
53
54 in conjunction with more task performance outcomes.
55
56
57
58
59
60

1
2
3 No disease modifying treatment is known that can delay the onset or slow the progression of HD,
4 although there are several promising approaches about to reach clinical testing (Wild and Tabrizi,
5 2014). These approaches directly target the production or regulation of the huntingtin protein.
6
7 However, there is still a need to identify other important disease-modifying biological factors in HD
8
9 as these may eventually provide additional drug development targets for this devastating disease
10
11
12
13 (GeM-HD Consortium, 2015).
14
15

16 17 **Acknowledgments**

18
19 The authors thank the Track-On study participants and their families, the CHDI/ High Q Foundation,
20
21 a not-for-profit organization dedicated to finding treatments for HD and Richard Frackowiak,
22
23 Christian Wolf, Christoph Kaller, Karl Friston, Nikolaus Weiskopf, Daniel S. O'Leary and Stephane
24
25 Lehericy for helpful advice. We thank Ray Young for his assistance with artwork.
26
27

28 29 **Author contributions**

30
31 Conception and design of the study: M.O., A.D., R.A.C.R., B.R.L., G.B.L., R.R., B.B., L.B., R.I.S., S.K.,
32
33 G.R., J.C.R., D.L. and S.J.T.
34

35 Acquisition and analysis of data: M.O., I.S.M.M., S.G., K.K.S., L.M., R.I.S. G.R. D.L.

36
37 Drafting of the manuscript, tables and figures: M.O., S.G.

38
39 All co-authors reviewed and critiqued the manuscript.

40
41 TRACK-ON investigators: Canada: Kate Brown, Joji Decolongon, Mannie Fan, Tamara Koren, Terri
42
43 Patkau (University of British Columbia, Vancouver, Canada). France: Celine Jauffret, Sabine Meunier,
44
45 Traian Popa (ICM Institute, Paris). Germany: Christian Sass, Nathalia Weber (GHI, Münster).
46
47 Netherlands: Omar Odish, Anne Schoonderbeek (Leiden University Medical Center, Leiden). UK:
48
49 Claire Berna, Helen Crawford, Mahaleskshmi Desikan, Davina J Hensman Moss, Nicola Z Hobbs,
50
51 Eileanoir Johnson, Gail Owen, Adeel Razi, Joy Read, Gionata Strigaro (University College London,
52
53 London). USA: Hans Johnson, Jim Mills (University of Iowa, Iowa).
54
55
56
57
58

59 **Conflicts of interest and financial disclosures**

1
2
3 This work was funded by the CHDI Foundation Inc., and the Wellcome Trust (grant to G.R.). The
4
5 authors have no conflicts of interest in relation to the material presented in the paper.
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Peer Review

References

- 1
2
3
4
5 Abbruzzese, G., Dall'Agata, D., Morena, M., Reni, L., Favale, E. (1990) Abnormalities of parietal and
6 prerolandic somatosensory evoked potentials in Huntington's disease.
7 Electroencephalography and clinical neurophysiology, 77:340-6.
- 8 Ashburner, J. (2007) A fast diffeomorphic image registration algorithm. *NeuroImage*, 38:95-113.
- 9 Aylward E.H. (2007) Change in MRI striatal volumes as a biomarker in preclinical Huntington's
10 disease. *Brain Res Bull*, 30;72(2-3):152-8.
- 11 Behrens, T.E., Berg, H.J., Jbabdi, S., Rushworth, M.F., Woolrich, M.W. (2007) Probabilistic diffusion
12 tractography with multiple fibre orientations: What can we gain? *NeuroImage*, 34:144-55.
- 13 Behrens, T.E., Woolrich, M.W., Jenkinson, M., Johansen-Berg, H., Nunes, R.G., Clare, S., Matthews,
14 P.M., Brady, J.M., Smith, S.M. (2003) Characterization and propagation of uncertainty in
15 diffusion-weighted MR imaging. *Magnetic resonance in medicine : official journal of the*
16 *Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine*,
17 50:1077-88.
- 18 Benjamini, Y., Hochberg, Y. (1995) Controlling the False Discovery Rate: A Practical and Powerful
19 Approach to Multiple Testing. *Journal of the Royal Statistical Society, Series B*, 57:289-300.
- 20 Bremer, J., Baumann, F., Tiberi, C., Wessig, C., Fischer, H., Schwarz, P., Steele, A.D., Toyka, K.V., Nave,
21 K.A., Weis, J., Aguzzi, A. (2010) Axonal prion protein is required for peripheral myelin
22 maintenance. *Nature neuroscience*, 13:310-8.
- 23 Diggle, P.J., Liang, K.-Y., Zeger, S.L. (1994) *Analysis of Longitudinal Data*. Oxford. Clarendon Press.
- 24 Eickhoff, S.B., Stephan, K.E., Mohlberg, H., Grefkes, C., Fink, G.R., Amunts, K., Zilles, K. (2005) A new
25 SPM toolbox for combining probabilistic cytoarchitectonic maps and functional imaging data.
26 *NeuroImage*, 25:1325-35.
- 27 Fischer, M., Orth, M. (2011) Short-latency sensory afferent inhibition: conditioning stimulus intensity,
28 recording site, and effects of 1 Hz repetitive TMS. *Brain stimulation*, 4:202-9.
- 29 Fischl, B., Dale, A.M. (2000) Measuring the thickness of the human cerebral cortex from magnetic
30 resonance images. *Proceedings of the National Academy of Sciences of the United States of*
31 *America*, 97:11050-5.
- 32 Friston, K.J., Harrison, L., Penny, W. (2003) Dynamic causal modelling. *NeuroImage*, 19:1273-302.
- 33 Friston, K.J., Li, B., Daunizeau, J., Stephan, K.E. (2011) Network discovery with DCM. *NeuroImage*,
34 56:1202-21.
- 35 Genetic Modifiers of Huntington's Disease (GeM-HD) Consortium. (2015) Identification of Genetic
36 Factors that Modify Clinical Onset of Huntington's Disease. *Cell*, 162:516-26.
- 37 Georgiou-Karistianis, N., Scahill, R., Tabrizi, S.J., Squitieri, F., Aylward, E. (2013) Structural MRI in
38 Huntington's disease and recommendations for its potential use in clinical trials.
39 *Neuroscience and biobehavioral reviews*, 37:480-90.
- 40 **Groeschel, S., Tournier, J.D., Northam, G.B., Baldeweg, T., Wyatt, J., Vollmer, B., Connelly, A. (2014)**
41 **Identification and interpretation of microstructural abnormalities in motor pathways in**
42 **adolescents born preterm. *Neuroimage*. 15;87:209-19.**
- 43 Huang, B., Wei, W., Wang, G., Gaertig, M.A., Feng, Y., Wang, W., Li, X.J., Li, S. (2015) Mutant
44 huntingtin downregulates myelin regulatory factor-mediated myelin gene expression and
45 affects mature oligodendrocytes. *Neuron*, 85:1212-26.
- 46 Jech, R., Klempir, J., Vymazal, J., Zidovska, J., Klempirova, O., Ruzicka, E., Roth, J. (2007) Variation of
47 selective gray and white matter atrophy in Huntington's disease. *Movement disorders :*
48 *official journal of the Movement Disorder Society*, 22:1783-9.
- 49 Jenkinson, M., Smith, S. (2001) A global optimisation method for robust affine registration of brain
50 images. *Medical image analysis*, 5:143-56.
- 51 Kenward, M.G., Roger, J.H. (1997) Small sample inference for fixed effects from restricted maximum
52 likelihood. *Biometrics*, 53:983-97.
- 53 Kuwert, T., Noth, J., Scholz, D., Schwarz, M., Lange, H.W., Topper, R., Herzog, H., Aulich, A.,
54 Feinendegen, L.E. (1993) Comparison of somatosensory evoked potentials with striatal
55 glucose consumption measured by positron emission tomography in the early diagnosis of
56
57
58
59
60

- 1
2
3 Huntington's disease. *Movement disorders : official journal of the Movement Disorder Society*, 8:98-106.
- 4
5 Langbehn, D.R., Brinkman, R.R., Falush, D., Paulsen, J.S., Hayden, M.R. (2004) A new model for
6 prediction of the age of onset and penetrance for Huntington's disease based on CAG length.
7 *Clinical genetics*, 65:267-77.
- 8
9 Lefaucheur, J.P., Menard-Lefaucheur, I., Maison, P., Baudic, S., Cesaro, P., Peschanski, M., Bachoud-
10 Levi, A.C. (2006) Electrophysiological deterioration over time in patients with Huntington's
11 disease. *Movement disorders : official journal of the Movement Disorder Society*, 21:1350-4.
- 12
13 Li, J.Y., Conforti, L. (2013) Axonopathy in Huntington's disease. *Experimental neurology*, 246:62-71.
- 14
15 Littell, R.C., Milliken, G.A., Stroup, W.W., Wolfinger, R.D., Schabenberger, O. (2006) *SAS for Mixed*
16 *Models*. Cary, NC SAS Institute Inc.
- 17
18 Maldjian, J.A., Laurienti, P.J., Kraft, R.A., Burdette, J.H. (2003) An automated method for
19 neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets.
20 *NeuroImage*, 19:1233-9.
- 21
22 Matsui, J.T., Vaidya, J.G., Wassermann, D., Kim, R.E., Magnotta, V.A., Johnson, H.J., Paulsen, J.S.,
23 Investigators, P.-H., Coordinators of the Huntington Study, G. (2015) Prefrontal cortex white
24 matter tracts in prodromal Huntington disease. *Human brain mapping*, 36:3717-32.
- 25
26 Nave, K.A. (2010) Myelination and the trophic support of long axons. *Nature reviews. Neuroscience*,
27 11:275-83.
- 28
29 Orth, M., Rothwell, J.C. (2004) The cortical silent period: intrinsic variability and relation to the
30 waveform of the transcranial magnetic stimulation pulse. *Clinical neurophysiology : official*
31 *journal of the International Federation of Clinical Neurophysiology*, 115:1076-82.
- 32
33 Paulsen, J.S., Long, J.D., Ross, C.A., Harrington, D.L., Erwin, C.J., Williams, J.K., Westervelt, H.J.,
34 Johnson, H.J., Aylward, E.H., Zhang, Y., Bockholt, H.J., Barker, R.A. (2014) Prediction of
35 manifest Huntington's disease with clinical and imaging measures: a prospective
36 observational study. *The Lancet. Neurology*, 13:1193-201.
- 37
38 Reilmann, R., Bohlen, S., Klopstock, T., Bender, A., Weindl, A., Saemann, P., Auer, D.P., Ringelstein,
39 E.B., Lange, H.W. (2010) Grasping premanifest Huntington's disease - shaping new endpoints
40 for new trials. *Movement disorders : official journal of the Movement Disorder Society*,
41 25:2858-62.
- 42
43 Rosas H.D., Salat D.H., Lee S.Y., Zaleta A.K., Pappu V., Fischl B., Greve D., Hevelone N., Hersch S.M.
44 (2008) Cerebral cortex and the clinical expression of Huntington's disease: complexity and
45 heterogeneity. *Brain*, 131:1057-68.
- 46
47 Schafer, J.L. (1997) *Analysis of Incomplete Multivariate Data*. New York. Chapman & Hall.
- 48
49 Smith, S.M., Jenkinson, M., Woolrich, M.W., Beckmann, C.F., Behrens, T.E., Johansen-Berg, H.,
50 Bannister, P.R., De Luca, M., Drobnjak, I., Flitney, D.E., Niazy, R.K., Saunders, J., Vickers, J.,
51 Zhang, Y., De Stefano, N., Brady, J.M., Matthews, P.M. (2004) Advances in functional and
52 structural MR image analysis and implementation as FSL. *NeuroImage*, 23 Suppl 1:S208-19.
- 53
54 Song, S.K., Sun, S.W., Ju, W.K., Lin, S.J., Cross, A.H., Neufeld, A.H. (2003) Diffusion tensor imaging
55 detects and differentiates axon and myelin degeneration in mouse optic nerve after retinal
56 ischemia. *NeuroImage*, 20:1714-22.
- 57
58 Song, S.K., Sun, S.W., Ramsbottom, M.J., Chang, C., Russell, J., Cross, A.H. (2002) Dysmyelination
59 revealed through MRI as increased radial (but unchanged axial) diffusion of water.
60 *NeuroImage*, 17:1429-36.
- 61
62 Tabrizi, S.J., Langbehn, D.R., Leavitt, B.R., Roos, R.A., Durr, A., Craufurd, D., Kennard, C., Hicks, S.L.,
63 Fox, N.C., Scahill, R.I., Borowsky, B., Tobin, A.J., Rosas, H.D., Johnson, H., Reilmann, R.,
64 Landwehrmeyer, B., Stout, J.C. (2009) Biological and clinical manifestations of Huntington's
65 disease in the longitudinal TRACK-HD study: cross-sectional analysis of baseline data. *The*
66 *Lancet. Neurology*, 8:791-801.
- 67
68 Tabrizi S.J., Scahill R.I., Durr A., Roos R.A., Leavitt B.R., Jones R., Landwehrmeyer G.B., Fox N.C.,
69 Johnson H., Hicks S.L., Kennard C., Craufurd D., Frost C., Langbehn D.R., Reilmann R., Stout
70 J.C.; TRACK-HD Investigators. (2011) Biological and clinical changes in premanifest and early

- 1
2
3 stage Huntington's disease in the TRACK-HD study: the 12-month longitudinal analysis.
4 *Lancet Neurol*, 10:31-42.
- 5 Tabrizi, S.J., Scahill, R.I., Owen, G., Durr, A., Leavitt, B.R., Roos, R.A., Borowsky, B., Landwehrmeyer,
6 B., Frost, C., Johnson, H., Craufurd, D., Reilmann, R., Stout, J.C., Langbehn, D.R. (2013)
7 Predictors of phenotypic progression and disease onset in premanifest and early-stage
8 Huntington's disease in the TRACK-HD study: analysis of 36-month observational data. *The*
9 *Lancet. Neurology*, 12:637-49.
- 10 Thieben M.J., Duggins A.J., Good C.D., Gomes L., Mahant N., Richards F., McCusker E., Frackowiak
11 R.S. (2002) The distribution of structural neuropathology in pre-clinical Huntington's disease.
12 *Brain*, 125:1815-28.
- 13 Tobin, J.E., Xie, M., Le, T.Q., Song, S.K., Armstrong, R.C. (2011) Reduced axonopathy and enhanced
14 remyelination after chronic demyelination in fibroblast growth factor 2 (Fgf2)-null mice:
15 differential detection with diffusion tensor imaging. *Journal of neuropathology and*
16 *experimental neurology*, 70:157-65.
- 17 Topper, R., Schwarz, M., Podoll, K., Domges, F., Noth, J. (1993) Absence of frontal somatosensory
18 evoked potentials in Huntington's disease. *Brain : a journal of neurology*, 116 (Pt 1):87-101.
- 19 Weaver, K.E., Richards, T.L., Liang, O., Laurino, M.Y., Samii, A., Aylward, E.H. (2009) Longitudinal
20 diffusion tensor imaging in Huntington's Disease. *Experimental neurology*, 216:525-9.
- 21 Wild, E.J., Tabrizi, S.J. (2014) Targets for future clinical trials in Huntington's disease: what's in the
22 pipeline? *Movement disorders : official journal of the Movement Disorder Society*, 29:1434-
23 45.
- 24 Wolf, R.C., Sambataro, F., Vasic, N., Depping, M.S., Thomann, P.A., Landwehrmeyer, G.B., Sussmuth,
25 S.D., Orth, M. (2014) Abnormal resting-state connectivity of motor and cognitive networks in
26 early manifest Huntington's disease. *Psychological medicine*, 44:3341-56.
- 27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Tables

Table 1: Demographic and clinical characteristics of study participants by subgroup. Disease burden was calculated as age X (CAG_n – 35.5). Education was classified according to the International Standard Classification of Education (ISCED). Data are given as means (SD).

Table 2: Principal component analysis with data from all modalities. In independent analyses, in HD participants and healthy controls the first 3 principal components (PC) explain about 50% of the variance.

Table 3: PCA (on data from all modalities) **association with** grip force position and orientation composite score, UHDRS motor score and diagnosis status, i.e. premanifest versus manifest Huntington's disease. Abbreviations. ln(OR) = natural log of odds ratio; CPO = cumulative probability of onset based on age and CAG length CPO mediated (%) = 1 – (t value of CPO in CPO-only model/ CPO t value in multivariate model); (a) per 10% increase in CPO; for diagnosis state log odds ratio per 10% increase in CPO (b) PC coefficients are per standard deviation.

Table 4: Correlations of observed axial and radial diffusivity in independent PCAs in controls and Huntington's disease participants.

Table 5: Correlations of grip force with axial (AD) and radial diffusivity (RD) in independent principal component analyses (PCAs) in controls and Huntington's disease participants. Abbreviations: CST: cortico-spinal tract. M1: motor cortex. S1: somatosensory cortex. PMC: premotor cortex.

Supplementary Table 1: Individual modality results of variables included in principle component analysis. There is significant global white and grey matter loss across all clinical HD groups, but most pronounced in the early HD group. Cortical thickness in regions that are important in sensory-motor integration circuits was similar for preHD-A and controls. However, both the PMC (BA6) and S1 (BA2, BA3a) were significantly thinner in preB and in early HD than in controls. In all tracts FA, AD and RD were similar in the preHD-A group and controls. In preHD-B, RD, but not AD, was significantly increased in all four tracts. In the early HD group, in all four tracts RD and AD were greater than in

1
2
3 controls. Effective connectivity from PMC to motor thalamus was significantly reduced in the early
4
5 HD group. SEP N20 latencies and N20/P25 amplitudes of somatosensory evoked potentials were
6
7 abnormal in preHD and manifest HD. The early HD group had a much higher position-index and a
8
9 higher orientation index in grip force experiments.

10
11 Data are means of visit 1 and visit 2 data (95% CI) or estimates of the differences between the means
12
13 of visit 1 and visit 2 data in HD groups and controls (95% CI; p value; q value). All estimates are
14
15 adjusted for age, sex, and study site. Abbreviations. WM: white matter; GM: grey matter; FA:
16
17 Fractional Anisotropy; RD: Radial Diffusivity; AD: Axial Diffusivity; q results from corrections for
18
19 multiple comparisons using false positive discovery rates. ^sVolumes are relative to total intracranial
20
21 volume. * in $\text{mm}^2 \text{s}^{-1} 10^{-3}$; sqr: square root.

22
23
24
25 **Supplementary Table 2:** Principal Component (PC) Analysis correlations of the observed variables
26
27 from all modalities with PCs in Huntington's disease participants. Correlations of a magnitude greater
28
29 than 0.400 are highlighted in bold. Abbreviations: M1: motor cortex; S1: somatosensory cortex; PMC:
30
31 pre-motor cortex. GM: grey matter. WM: white matter. AD: axial diffusivity. RD: radial diffusivity.

32
33
34
35 **Supplementary Table 3:** Principal Component (PC) Analysis correlations of the observed variables
36
37 from all modalities with PCs in control participants. Correlations of a magnitude greater than 0.400
38
39 are highlighted in bold. Abbreviations: M1: motor cortex; S1: somatosensory cortex; PMC: pre-motor
40
41 cortex. GM: grey matter. WM: white matter. AD: axial diffusivity. RD: radial diffusivity.

Figure legends

Figure 1. Multimodal principal component analysis. **A.** Heat map of correlation coefficients of each modality with dimensions derived from principal component analysis done independently in healthy controls and HD participants. The first multimodal principal component (PC) in HD contains higher axial (AD) and radial diffusivity (RD) in S1-Thalamus (Thal) and PMC-Thalamus tracts; less cortical thickness in the PMC (BA6) and S1 (BA3a; BA2); and less total brain grey matter (GM), white matter (WM) and caudate volume. Multimodal-PC3 in HD reflects the difference between axial and radial diffusivity. Multimodal-PC2 in HD and PC1 in controls show a similar pattern of thicker cortex, higher axial diffusivity and lower SEP amplitudes. **B.** HD multimodal-PC1 scores negatively correlate with cumulative probability of onset (CPO) and grip force orientation and position index (**C**; pre HD blue dots, manifest HD red dots). **D.** Multimodal-PC1 scores distinguish manifest HD (red dots) from preHD participants (blue dots; the Y-axis just separates HD and controls). **E.** Multimodal-PC3 is a substantial additional predictor of manifest HD ($p=0.021$) improving the separation of manifest HD (red dots) and preHD participants (blue dots). Abbreviations: PMC-ThM: effective connectivity PMC to motor thalamus parcellation; PMC-PMC: effective connectivity PMC to PMC; S1-M1: effective connectivity S1 to motor cortex.

Figure 2. Principal component analysis with axial and radial diffusivity (ARD-PCA). In controls, axial diffusivity values (**A**), or radial diffusivity values (**B**), from different tracts are highly correlated. **C.** ARD principal component (PC) 1 reflects that greater, or smaller, axial diffusivity is associated with greater, or smaller, radial diffusivity. There is additional variability in the relationship of axial and radial diffusivity, which is reflected in ARD-PC2. In HD participants, the relationship between axial diffusivity (AD) (**D**), or radial diffusivity (RD) (**E**), in the two tracts is similar to controls. **F.** Manifest HD participants (red dots) have higher than average axial and radial diffusivity values and higher axial relative to radial diffusivity values (grey triangle) than preHD (blue dots). **G.** A principal component analysis with axial and radial diffusivity values from all 4 tracts done independently in healthy controls and HD participants reveals that PC1 and PC2 explain 94% of data variability in

1
2
3 controls and 90% in HD participants. Heat maps of correlation coefficients show that in controls and
4
5 HD participants in PC1 axial and radial diffusivity are positively correlated while PC2 reflects the
6
7 difference between axial and radial diffusivity. In controls (**H**) and HD participants (**I**) higher axial
8
9 diffusivity is associated with higher grip force orientation and position index scores. NB. C and F
10
11 contain approximate representations of the PCs relative to paired, observed AD and RD measures.
12
13 Abbreviations: M1: motor cortex; S1: somatosensory cortex; CST: cortico-spinal tract; PMC: pre-
14
15 motor cortex.
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Peer Review

Variable	Control N=112	preHD A N=41	preHD B N=55	preHD all N=96	Early HD N=35
Gender N (%F)	67 (59.8)	24 (58.5)	26 (47.3)	50 (52.1)	19 (54.3)
Age	48.1 (10.7)	40.4 (8.8)	44.0 (9.0)	42.4 (9.0)	45.3 (8.4)
Education	3.9 (1.0)	4.0 (1.0)	4.0 (1.0)	4.0 (1.0)	3.9 (0.9)
CAG Repeat Length	--	42.3 (2.2)	43.4 (2.3)	42.9 (2.3)	43.5 (2.5)
Disease burden	--	257.2 (30.7)	330.0 (39.8)	298.9 (51.1)	348.2 (61.6)
Motorscore	1.3 (1.6)	5.4 (3.3)	5.0 (3.8)	5.2 (3.6)	12.6 (7.1)

Table 1. Demographic and clinical characteristics of study participants by subgroup.

For Peer Review

		Controls	preHD-A versus controls	preHD-B versus controls	Early HD versus controls
Brain volume (VBM)					
Caudate ^s		0.5155 (0.5049 to 0.5261)	-0.05 (-0.07 to -0.04; p<0.0001; q<0.0001)	-0.10 (-0.12 to -0.08; p<0.0001; q<0.0001)	-0.15 (-0.17 to -0.13; p<0.0001; q<0.0001)
White matter ^s		0.3176 (0.3128 to 0.3224)	-0.01 (-0.02 to -0.0003; p=0.04; q=0.07)	-0.01 (-0.02 to -0.003; p=0.004; q=0.006)	-0.02 (-0.03 to -0.01; p<0.0001; q<0.0001)
Grey matter ^s		0.4512 (0.4443 to 0.4581)	-0.01 (-0.02 to 0.0001; p=0.05; q=0.07)	-0.01 (-0.02 to -0.003; p=0.01; q=0.01)	-0.03 (-0.04 to -0.01; p<0.0001; q<0.0001)
Cortical thickness (mm)					
Premotor cortex (BA6)		2.6852 (2.6590 to 2.7114)	0.001 (-0.05 to 0.05; p=0.97; q=0.97)	-0.08 (-0.12 to -0.04; p<0.0001; q=0.0003)	0.09 (-0.14 to -0.05; p<0.0001; q=0.0006)
Somatosensory cortex (BA2)		2.2353 (2.2099 to 2.2607)	0.01 (-0.04 to 0.05; p=0.72; q=0.97)	-0.04 (-0.07 to -0.01; p=0.02; q=0.04)	-0.06 (-0.11 to -0.01; p=0.02; q=0.03)
Somatosensory cortex (BA3a)		1.6850 (1.6598 to 1.7102)	-0.03 (-0.07 to 0.01; p=0.17; q=0.97)	-0.04 (-0.07 to -0.009; p=0.01; q=0.04)	-0.07 (-0.11 to -0.03; p=0.0006; q=0.002)
Somatosensory cortex (BA1)		2.3187 (2.2852 to 2.3522)	-0.00269 (-0.05110 to 0.04572; p=0.9122; q=0.9723)	0.02364 (-0.1051 to -0.01144; p=0.0152; q=0.0354)	-0.07925 (-0.1543 to -0.00420; p=0.039; q=0.0545)
Somatosensory cortex (BA2)		2.2353 (2.2099 to 2.2607)	0.008070 (-0.03636 to 0.05250; p=0.7180; q=0.9723)	-0.04265 (-0.07976 to -0.00554; p=0.0247; q=0.0433)	-0.06331 (-0.1143 to -0.01234; p=0.016; q=0.0279)
Motor cortex (BA4a)		2.6629 (2.6244 to 2.7014)	0.01463 (-0.05047 to 0.07972; p=0.6557; q=0.9723)	-0.02544 (-0.07921 to 0.02833; p=0.3506; q=0.4090)	-0.08815 (-0.1535 to -0.02284; p=0.009; q=0.0210)
Motor cortex (BA4b)		2.4495 (2.4052 to 2.4938)	0.03572 (-0.03568 to 0.1071; p=0.3224; q=0.9723)	0.04015 (-0.01765 to 0.09794; p=0.1718; q=0.2405)	-0.00184 (-0.07559 to 0.07192; p=0.96; q=0.96)
DTI tractography					
Motor cortex – motor thalamus	FA	0.4757 (0.4694 to 0.4820)	-0.01 (-0.02 to 0.002; p=0.11; q=0.22)	-0.01 (-0.02 to -0.003; p=0.01; q=0.02)	-0.00036 (-0.01 to 0.01; p=0.95; q=0.95)
	RD*	0.511 (0.506 to 0.517)	0.0011 (0.0028 to 0.02; p=0.01; q=0.11)	0.019 (0.010 to 0.027; p=0.0001; q=0.0001)	0.017 (0.007 to 0.026; p=0.001; q=0.002)
	AD*	0.713 (0.708 to 0.717)	0.021 (-0.01 to 0.016; p=0.11; q=0.22)	0.011 (-0.028 to 0.025; p=0.12; q=0.13)	0.033 (0.018 to 0.048; p<0.0001; q=0.0002)
Premotor cortex – motor thalamus	FA	0.4923 (0.4856 to 0.4990)	-0.01 (-0.02 to 0.002; p=0.10; q=0.22)	-0.01 (-0.02 to -0.002; p=0.02; q=0.04)	-0.00285 (-0.01467 to 0.008970; p=0.63; q=0.71)
	RD*	0.494 (0.488 to 0.499)	0.010 (0.0016 to 0.019; p=0.02; q=0.11)	0.017 (0.0086 to 0.026; p=0.0001; q=0.0004)	0.018 (0.0084 to 0.028; p=0.0006; q=0.001)
	AD*	1.121 (1.112 to 1.131)	-0.0015 (-0.02 to 0.013; p=0.80; q=0.89)	0.0089 (-0.005 to 0.023; p=0.21; q=0.21)	0.032 (0.015 to 0.048; p=0.0003; q=0.0008)

Somatosensory cortex – Sensory thalamus	FA	0.4784 (0.4725 to 0.4843)	-0.005 (-0.02 to 0.006; p=0.35; q=0.5)	-0.01 (-0.02 to 0.00001; p=0.05; q=0.06)	0.003064 (-0.00682 to 0.01294; p=0.53; q=0.66)
	RD*	0.517 (0.511 to 0.522)	0.0082 (-0.001 to 0.017; p=0.08; q=0.22)	0.018 (0.0096 to 0.026; p=0.0001; q=0.0001)	0.014 (0.005 to 0.023; p=0.003; q=0.004)
	AD*	1.140 (1.131 to 1.149)	0.0048 (-0.0018 to 0.018; p=0.46; q=0.57)	0.016 (0.0015 to 0.030; p=0.03; q=0.04)	0.036 (0.021 to 0.052; p<0.0001; q<0.0001)
Cortico-spinal tract	FA	0.5254 (0.5200 to 0.5308)	-0.004 (-0.01 to 0.005; p=0.39; q=0.52)	-0.01 (-0.02 to -0.001; p=0.04; q=0.05)	0.002152 (-0.00797 to 0.01227; p=0.67; q=0.71)
	RD*	0.479 (0.474 to 0.484)	0.0055 (-0.0025 to 0.014; p=0.17; q=0.31)	0.015 (0.0074 to 0.023; p=0.0002; q=0.0004)	0.013 (0.0038 to 0.022; p=0.006; q=0.008)
	AD*	1.175 (1.167 to 1.184)	0.000086 (-0.01 to 0.01; p=0.99; q=0.99)	0.010 (0.0028 to 0.024; p=0.1219; q=0.13)	0.034 (0.019 to 0.050; p<0.0001; q=0.0002)
Dynamic causal modelling					
Premotor cortex – Motor thalamus		0.1853 (0.1470 to 0.2237)	-0.04206 (-0.1015 to 0.01743; p=0.1631; q=0.6930)	-0.06057 (-0.1239 to 0.002718; p=0.0604; q=0.5137)	-0.09966 (-0.1542 to -0.04507; p=0.0005; q=0.0093)
Premotor cortex self-connection		-0.03134 (-0.03444 to -0.02823)	-0.00374 (-0.00855 to 0.001060; p=0.1246; q=0.6930)	-0.00828 (-0.01657 to 0.000006208; p=0.0502; q=0.5137)	-0.00818 (-0.01465 to -0.00170; p=0.0145; q=0.1227)
Electrophysiology					
SEP N20 latency (ms)		19.81 (19.60 to 20.01)	0.02 (-0.33 to 0.38; p=0.89; q=0.94)	0.54 (0.15 to 0.93; p=0.007; q=0.04)	0.70 (0.23 to 1.16; p=0.004; q=0.04)
SEP N20/P25 amplitude (sqr mV)		1.87 (0.66 to 3.09)	0.07 (-0.13 to 0.27; p=0.46; q=0.84)	-0.31 (-0.48 to -0.14; p=0.0006; q=0.01)	-0.27 (-0.43 to -0.11; p=0.001; q=0.03)
Grip force					
Orientation index (log)		1.46 (1.39 to 1.54)	0.09 (-0.03416 to 0.21; p=0.15; q=0.27)	0.06 (-0.05711 to 0.18; p=0.31; q=0.31)	0.49 (0.3394 to 0.65; p<0.0001; q<0.0001)
Position index (log)		0.47 (0.40 to 0.54)	0.07 (-0.05639 to 0.19; p=0.27; q=0.27)	0.10 (-0.00314 to 0.20; p=0.057; q=0.17)	0.58 (0.4269 to 0.73; p<0.0001; q<0.0001)
Composite index (log)		1.93 (1.80 to 2.07)	0.16 (-0.08384 to 0.40; p=0.20; q=0.27)	0.16 (-0.05186 to 0.37; p=0.14; q=0.21)	1.07 (0.7757 to 1.37; p<0.0001; q<0.0001)

Supplementary Table 1. Individual modality results of variables included in principle component analysis.

HD				Controls		
PC	Eigenvalue	%variance explained	cumulative	Eigenvalue	%variance explained	cumulative
1	3.102	20.7	20.7	3.432	22.9	22.9
2	2.267	15.1	35.8	2.353	15.7	38.6
3	1.703	11.3	47.1	1.850	12.3	50.9
4	1.442	9.7	56.8	1.103	7.4	58.2
5	1.084	7.2	64.0	1.081	7.2	65.5
6	0.995	6.6	70.6	0.968	6.5	71.9
7	0.891	6.0	76.6	0.901	6.0	77.9
8	0.795	5.3	81.9	0.829	5.5	83.4
9	0.704	4.7	86.6	0.706	4.7	88.2
10	0.531	3.5	90.1	0.581	3.9	92.0
11	0.522	3.5	93.6	0.446	3.0	95.0
12	0.468	3.1	96.7	0.335	2.2	97.2
13	0.211	1.4	98.1	0.229	1.5	98.8
14	0.161	1.1	99.2	0.128	0.9	99.6
15	0.125	0.8	100	0.059	0.4	100

Table 2: Principal component analysis with data from all modalities.

	Regression coefficient	Standard error	T value	CPO mediated (%)	p
Grip force					
CPO only (a)					
Intercept	-0.3831	0.1560	-2.46		0.015
CPO (a)	-0.1462	0.0498	2.93		0.004
PC1 added					
Intercept	-0.1156	0.1812	-0.64		0.525
CPO (a)	-0.0442	0.0606	0.73	75.1	0.468
PC1 (b)	-0.1742	0.0614	-2.84		0.006
PC3 added					
Intercept	-0.0939	0.1767	-0.53		0.596
CPO (a)	-0.0357	0.0602	0.59	79.9	0.555
PC1	-0.1805	0.0617	-2.93		0.005
PC3	-0.1519	0.0700	-2.17		0.036
UHDRS motor score					
CPO only (a)					
Intercept	1.853	0.154	12.02		<.0001
CPO (a)	-0.273	0.049	5.55		<.0001
PC1 added					
Intercept	2.043	0.178	11.46		<.0001
CPO (a)	-0.201	0.060	3.35	39.6	0.001
PC1 (b)	-0.124	0.060	-2.06		0.041
PC3 added					
Intercept	2.068	0.173	11.95		<.0001
CPO (a)	-0.191	0.059	3.25	41.4	0.002
PC1	-0.131	0.058	-2.24		0.027
PC3	-0.180	0.065	-2.77		0.007
Premanifest versus manifest Huntington's disease					
CPO only (a)					
	ln(OR)				
	0.679	0.147	4.61		<.0001
PC1 added					
CPO (a)	0.422	0.172	2.54	44.9	0.011
PC1 (b)	0.627	0.211	2.98		0.003
PC3 added					
CPO (a)	0.444	0.172	2.58	44.0	0.010
PC1	0.653	0.206	3.17		0.002
PC3	0.496	0.214	2.32		0.021

Table 3: PCA (on data from all modalities) multivariate model of grip force position and orientation composite score, UHDRS motor score and diagnosis status, i.e. premanifest versus manifest Huntington's disease.

Variable	Controls		Huntington's Disease	
	PC1	PC2	PC1	PC2
M1 – Thal AD	0.785	-0.598	0.793	-0.577
S1 – Thal AD	0.812	-0.531	0.784	-0.548
CST AD	0.797	-0.544	0.782	-0.542
PMC – Thal AD	0.747	-0.625	0.770	-0.581
M1 – Thal RD	0.749	0.631	0.706	0.666
S1 – Thal RD	0.770	0.562	0.626	0.662
CST RD	0.741	0.595	0.644	0.702
PMC – Thal RD	0.634	0.718	0.619	0.684

Table 4: Correlations of observed axial and radial diffusivity in independent PCAs in controls and Huntington's disease participants.

Grip force correlation	Controls		Huntington	
	Correlation coefficient	p	Correlation coefficient	p
PCA1 mean	0.273	0.0065	0.381	<.0001
PCA2 mean	-0.168	0.0978	-0.201	0.0352
M1 – Thalamus AD	0.342	0.0006	0.420	<.0001
S1 – Thalamus AD	0.338	0.0007	0.407	<.0001
CST AD	0.277	0.0058	0.408	<.0001
PMC – Thalamus AD	0.291	0.0036	0.410	<.0001
M1 – Thalamus RD	0.087	0.3946	0.136	0.1577
S1 – Thalamus RD	0.136	0.1825	0.077	0.422
CST RD	0.070	0.4921	0.127	0.1857
PMC – Thalamus RD	0.075	0.4629	0.103	0.2864

Table 5: Correlations of grip force with axial (AD) and radial diffusivity (RD) in independent principal component analyses (PCAs) in controls and Huntington's disease participants.

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12	PC13	PC14	PC15
SEP amplitude	0.242	-0.412	-0.282	0.185	0.100	0.174	0.552	-0.426	0.266	-0.055	0.225	0.073	0.039	0.027	-0.007
SEP latency	-0.228	0.077	-0.154	0.166	0.645	0.575	-0.139	0.196	-0.129	0.182	0.141	0.128	0.013	-0.004	-0.015
PMC – Thalamus	0.334	0.126	0.389	-0.505	-0.040	-0.017	0.151	0.402	0.431	0.169	0.180	0.176	0.019	0.015	0.038
PMC – PMC	0.240	0.280	0.039	-0.591	0.344	0.116	0.437	-0.064	-0.168	0.029	-0.323	-0.226	0.026	-0.030	0.018
S1 - M1	-0.229	0.149	-0.111	0.178	-0.589	0.637	0.144	0.156	0.112	0.008	-0.262	0.007	0.028	0.037	0.003
S1 – Thalamus AD	-0.450	0.630	-0.439	0.080	-0.014	-0.238	0.205	0.101	0.120	0.062	0.001	0.083	0.012	-0.169	-0.195
PMC– Thalamus AD	-0.398	0.588	-0.513	0.214	0.079	-0.235	0.142	0.134	0.075	0.041	0.064	-0.119	-0.016	0.148	0.199
PMC– Thalamus RD	-0.460	0.326	0.649	0.165	-0.048	0.032	0.271	-0.155	-0.143	0.109	0.081	0.065	-0.299	0.031	-0.010
S1 – Thalamus RD	-0.406	0.277	0.701	0.358	0.081	-0.084	0.129	-0.048	-0.038	-0.024	0.007	-0.015	0.323	0.027	-0.002
BA6 thickness	0.732	0.554	-0.027	0.015	-0.021	0.039	-0.155	-0.134	0.022	0.098	0.055	-0.106	0.009	0.242	-0.168
BA3a thickness	0.532	0.592	-0.067	-0.023	-0.016	-0.002	-0.084	-0.260	-0.117	-0.083	-0.143	0.483	0.029	-0.039	0.096
BA2 thickness	0.578	0.564	0.139	0.170	-0.085	0.234	-0.127	-0.076	0.085	-0.103	0.252	-0.299	-0.021	-0.200	0.071
GM volume	0.577	-0.200	-0.140	0.172	-0.300	-0.112	0.257	0.178	-0.466	0.364	0.148	0.014	0.064	-0.040	0.019
WM volume	0.471	-0.174	0.126	0.571	0.222	-0.150	-0.051	-0.016	0.318	0.313	-0.352	-0.045	-0.058	-0.054	0.023
Caudate volume	0.570	-0.049	0.082	0.389	0.169	-0.060	0.255	0.456	-0.093	-0.435	-0.049	0.050	-0.069	0.033	-0.041

Supplementary Table 2: Principal Component (PC) Analysis correlations of the observed variables from all modalities with PCs in Huntington's disease participants.

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12	PC13	PC14	PC15
SEP amplitude	-0.531	0.080	-0.012	0.251	-0.130	0.547	0.390	0.023	0.297	0.127	0.172	0.206	-0.059	0.022	0.001
SEP latency	-0.304	-0.011	-0.018	-0.367	0.283	0.495	-0.453	0.490	-0.039	-0.001	0.008	-0.025	-0.003	0.015	-0.006
PMC – Thalamus	0.352	-0.077	0.459	-0.524	0.186	-0.205	0.269	0.088	-0.112	0.388	0.226	0.118	-0.010	-0.008	-0.001
PMC – PMC	0.204	0.255	0.122	0.086	0.619	0.400	0.065	-0.509	-0.228	0.042	-0.044	-0.091	-0.005	-0.017	0.001
S1 - M1	0.315	0.070	-0.124	0.403	0.534	-0.177	0.349	0.480	-0.038	-0.194	0.072	-0.002	0.068	0.018	0.012
S1 – Thalamus AD	0.321	0.765	-0.434	-0.167	-0.049	0.029	0.086	0.049	0.147	0.063	-0.039	-0.004	0.112	-0.166	-0.125
PMC– Thalamus AD	0.291	0.712	-0.499	-0.201	-0.049	-0.002	0.103	0.038	0.094	0.177	-0.146	-0.070	-0.022	0.148	0.126
PMC– Thalamus RD	0.223	0.498	0.734	0.153	-0.226	0.126	-0.056	0.132	-0.028	-0.053	-0.039	-0.009	0.080	-0.164	0.116
S1 – Thalamus RD	0.289	0.647	0.600	0.115	-0.165	0.021	-0.031	0.088	-0.128	-0.097	-0.066	0.037	-0.111	0.179	-0.107
BA6 thickness	0.854	-0.182	-0.173	-0.073	-0.066	0.124	0.068	0.069	0.043	-0.139	0.047	-0.064	-0.369	-0.096	0.011
BA3a thickness	0.715	-0.204	-0.132	0.156	-0.323	0.255	-0.043	0.013	-0.118	0.082	0.347	-0.254	0.157	0.065	-0.012
BA2 thickness	0.778	-0.094	-0.245	-0.101	-0.050	0.123	-0.152	-0.118	-0.134	-0.227	0.021	0.422	0.110	0.030	0.026
GM volume	0.435	-0.595	0.045	0.109	-0.138	0.231	0.228	0.177	-0.132	0.254	-0.448	0.001	0.059	-0.005	-0.028
WM volume	0.452	-0.271	0.400	-0.299	0.146	0.066	0.091	-0.106	0.590	-0.223	-0.077	-0.096	0.104	0.058	-0.007
Caudate volume	0.483	-0.004	0.043	0.463	0.217	-0.145	-0.483	-0.008	0.322	0.369	0.017	0.088	-0.042	0.000	-0.006

Supplementary Table 3: Principal Component (PC) Analysis correlations of the observed variables from all modalities with PCs in control participants.

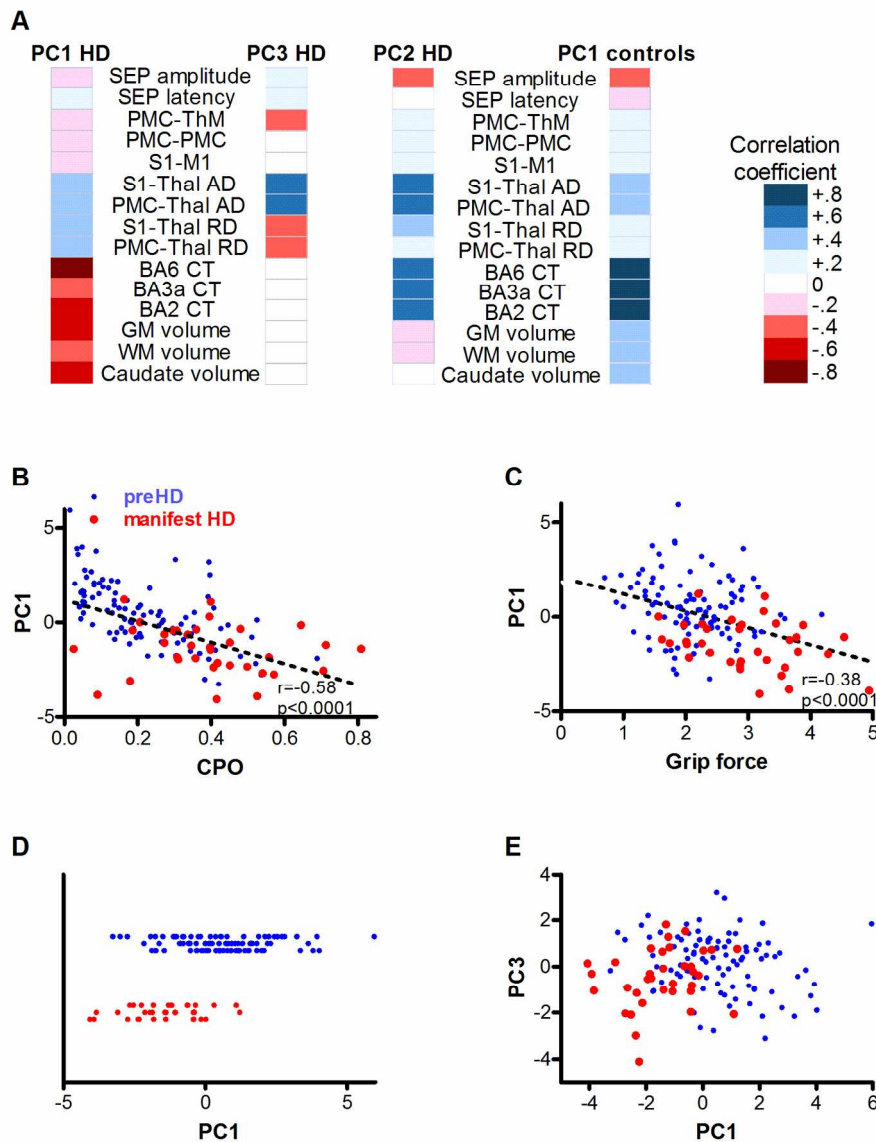


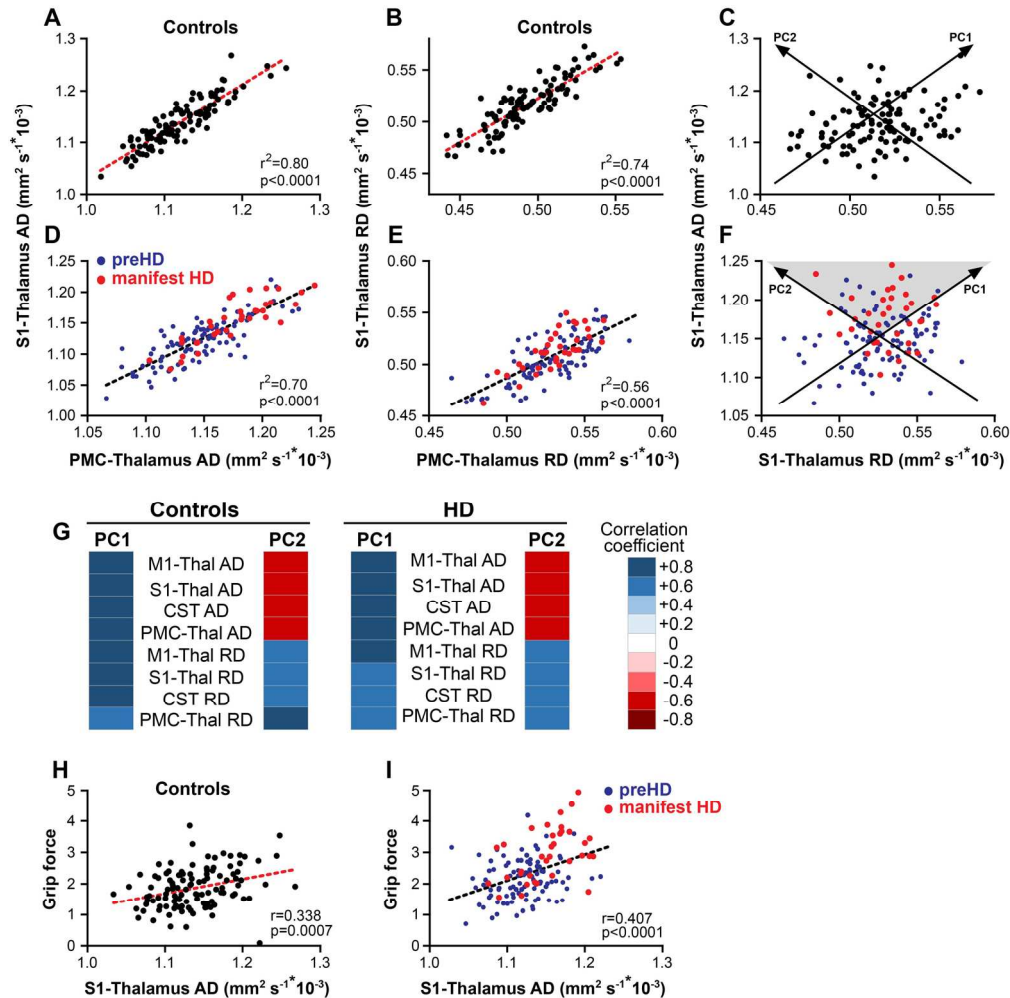
Figure 1. Multimodal principal component analysis. A. Heat map of correlation coefficients of each modality with dimensions derived from principal component analysis done independently in healthy controls and HD participants. The first multimodal principal component (PC) in HD contains higher axial (AD) and radial diffusivity (RD) in S1-Thalamus (Thal) and PMC-Thalamus tracts; less cortical thickness in the PMC (BA6) and S1 (BA3a; BA2); and less total brain grey matter (GM), white matter (WM) and caudate volume. Multimodal-PC3 in HD reflects the difference between axial and radial diffusivity. Multimodal-PC2 in HD and PC1 in controls show a similar pattern of thicker cortex, higher axial diffusivity and lower SEP amplitudes. B. HD multimodal-PC1 scores negatively correlate with cumulative probability of onset (CPO) and grip force orientation and position index (C; pre HD blue dots, manifest HD red dots). D. Multimodal-PC1 scores distinguish manifest HD (red dots) from preHD participants (blue dots; the Y-axis just separates HD and controls). E. Multimodal-PC3 is a substantial additional predictor of manifest HD ($p=0.021$) improving the separation of manifest HD (red dots) and preHD participants (blue dots). Abbreviations: PMC-ThM: effective connectivity PMC to motor thalamus parcellation; PMC-PMC: effective connectivity PMC to PMC; S1-M1:

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

effective connectivity S1 to motor cortex.

160x195mm (300 x 300 DPI)

For Peer Review



Principal component analysis with axial and radial diffusivity (ADRD-PCA). In controls, axial diffusivity values (A), or radial diffusivity values (B), from different tracts are highly correlated. C. ADRD principal component (PC) 1 reflects that greater, or smaller, axial diffusivity is associated with greater, or smaller, radial diffusivity. There is additional variability in the relationship of axial and radial diffusivity, which is reflected in ADRD-PC2. In HD participants, the relationship between axial diffusivity (AD) (D), or radial diffusivity (RD) (E), in the two tracts is similar to controls. F. Manifest HD participants (red dots) have higher than average axial and radial diffusivity values and higher axial relative to radial diffusivity values (grey triangle) than preHD (blue dots). G. A principal component analysis with axial and radial diffusivity values from all 4 tracts done independently in healthy controls and HD participants reveals that PC1 and PC2 explain 94% of data variability in controls and 90% in HD participants. Heat maps of correlation coefficients show that in controls and HD participants in PC1 axial and radial diffusivity are positively correlated while PC2 reflects the difference between axial and radial diffusivity. In controls (H) and HD participants (I) higher axial diffusivity is associated with higher grip force orientation and position index scores. NB. C and F contain approximate representations of the PCs relative to paired, observed AD and RD measures. Abbreviations: M1: motor cortex; S1: somatosensory cortex; CST: cortico-spinal tract; PMC: pre-motor cortex.

170x169mm (300 x 300 DPI)